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Effect of no-till and residue retention on fungal composition and population in maize-bean intercrop

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Abstract

This study determined the effect of *Zea mays* L. and *Phaseolus vulgaris* L. intercrop under no-till with residue retention (Conservation Agriculture- CA) on composition and population of soil fungal species. Soil samples were collected from 64 farms, half of which had tested CA and the other half had tested Conventional Tillage (CT) for five years. Half of CA and CT farms were in Upper Midland (UM 3), the other half in Lower Midland (LM 4). Samples were analyzed for fungal microbial population, composition and soil fertility. In CT land was dug by hand held hoe and residue removed. CA resulted in increase of soil fungal populations of 56% and 113% in LM 4 and UM 3 respectively as compared to CT. Fungal species belonging to 11 genera were detected. Total fungal, *Penicillium* and *Colletotrichum* CFUs/g soil were significantly higher in CA than in CT. Soil populations of *Aspergillus* and *Fusarium* did not change significantly but their relative composition changed due to changes in soil populations of other fungal genera. Total fungal CFUs/g soil were significantly correlated to *Penicillium* ($p \leq 0.01$) and *Macrophomina* ($p \leq 0.05$) CFUs/g soil. There was no significant difference between CA and CT on crop diseases.

Keywords: *Conservation agriculture-sustainable intensification, Maize-bean intercrop, Aspergillus, Fusarium, Fungal microbial population*

1. Introduction

Maize is the most important food crop in Kenya with per capita consumption of 103 kg per year (Abate *et al.,* 2015). It is grown by 98% of the rural farm households (Kenya, 2011). Smallholder farmers in East-Africa commonly intercrop maize with grain legumes to maximize utilization of land and labor, and attain larger crop yields (Mucheru-Muna *et al.,* 2010). Legumes supplement maize the staple food as an important dietary protein source and as a source of income for households before maize is harvested. In five counties of Kenya where legumes are commonly grown, maize was intercropped with legumes by an average of 72% of small scale farm households (Muricho *et al.,* 2011). The current average yield of maize in Kenya is approximately 2.0 t/ha (http://www.kalro.org/Maize, Accessed on February 10, 2020) as compared to potential yields of over 6.0 t/ha (Abate *et al.,* 2015). The yield of beans in Kenya in 2007 was less than 0.6 t/ha which has remained at

that level since 1960s (Katungi *et al.,* 2009) as compared to potential yield of 2.2 t/ha (Karanja *et al.,* 2008). The low farmers' yields have been attributed mostly to declining soil fertility and low adoption of recommended agronomic practices (Muricho *et al.,* 2011). Many countries including Kenya are promoting Conservation Agriculture (CA) as opposed to Convention Tillage (CT) to reverse increasing soil degradation (Friedrich *et al.,* 2012). However, farmers who implemented CA in Kenya reported higher incidences of infection of their crops by diseases and insect pests which they attributed to CA practice (KALRO-Annual Report, 2015). It is possible that residue left on the soil surface in CA may have harboured and transferred fungi and other pests to the next season. Most of the fungi are beneficial or benign and only 8% of the fungal species cause diseases and are responsible for 85% of all the plant diseases (Pernerzny *et al.,* 2017). Most diseases in cereals including maize are caused by fungi (Oerke, 2006). Saprophytic fungi are the largest group of fungi (Hou *et al.* 2012) and these together with bacteria (Berg and McClaugherty, 2003) are considered critical to organic matter decomposition (95% of the matter) and nutrient cycling. Several of these saprotrophic fungal species including those in *Aspergillus* (Tekaia and Latge, 2005; and Amaike and Keller, 2011) and *Fusarium* genera (Karim *et al.,* 2016) are also pathogenic. In addition, species of certain types of moulds (fungi) can produce mycotoxins on numerous foodstuffs such as cereals, dried fruits, nuts and spices (WHO, 2018).

Mycotoxins that present concern to human health and livestock include aflatoxins, and ochratoxin A produced by *Aspergillus* species, ochratoxin A and patulin produced by *Penicillium* species, fumonisins, zearalenone and nivalenol/deoxynivalenol produced by *Fusarium* species (ibid). The major concern in Kenya is the contamination of maize by mycotoxins, mainly aflatoxins and fumonisins (Kang'ethe *et al.* 2017; Mutiga *et al.,* 2015; and Bii *et al.* 2012) produced by *Aspergillus* and *Fusarium* mould respectively.

This research determined if there was significant change in the population and/or the composition of major fungal species especially those that may produce mycotoxins in the soil of the farms that had continuously implemented CA for five years as compared to those that had implemented CT. A structured questionnaire was used to capture farmers' perceptions on the disease incidence.

The null hypothesis that was tested was: - The soil population of fungal genera in CA and conventional tillage of non-rotational *Zea mays* L.-*Phaseolus vulgaris* L. intercrop is not different.

2. Materials and methods

This study was carried out on farms that had been randomly selected and had tested the productivity of *Zea mays* L and *Phaseolus vulgaris* L intercrop system under CA and CT for five years since 2010.

These farms were located in humid (Upper Midland- UM 3) and sub humid (Lower Midland– LM 4) Agro-Ecological Zones (AEZ) of Meru, Embu and Tharaka Nithi Counties of Kenya (Figure 1). Thirty three of the farms were located in UM 3 and 31 in LM 4. Thirty three farms had tested CA and 31 had tested CT for five years.

These counties have well drained soils, very deep, dusky red to dark reddish brown and friable clay (Jaetzold *et al.,* 2006). The rainfall in the humid AEZ is between 1,000 and 1,250 mm per year while in the sub humid AEZ the rainfall is between 780 and 900 mm per year. The average temperatures are 18°C to 21°C and 21°C to 24°C for the humid and sub humid AEZs respectively (Jaetzold *et al.,* 2006).

Soil samples for microbial and soil fertility analyses were collected in August 2015 from each of the 64 farms that had participated in the trials. Each farm was considered a replicate. Soil samples were collected from the top 0 -3 cm of soil between crop rows using a 100 cm x 25 cm quadrant and a hand held shovel after removing crop debris on top of the soil. Soil from four sites on each plot were bulked in a plastic basin, thoroughly mixed and subsamples of 1kg obtained and packaged in 2 kg size khaki bags and immediately taken to KALRO Embu for air drying. Equipment used for sampling was disinfected after sampling in each farm with 4% sodium hypochlorite diluted to 0.05% with water. After drying, samples were taken to KALRO Kabete where each sample was divided into two. One part of sample was subjected to fungal microbial analysis and the other part to soil fertility analysis. Soils for fungal microbial analysis were analyzed for total fungal Colony Forming Units (CFUs) and the CFUs of the fungal genera that were detected in the soil. The second sub sample of soil was analyzed for total organic carbon, pH, total nitrogen, phosphorus, potassium, calcium, magnesium, manganese, copper, ferrous, zinc and sodium.

Figure 1: Location of 64 trial farms in Embu, Tharaka-Nithi and Meru counties of Kenya
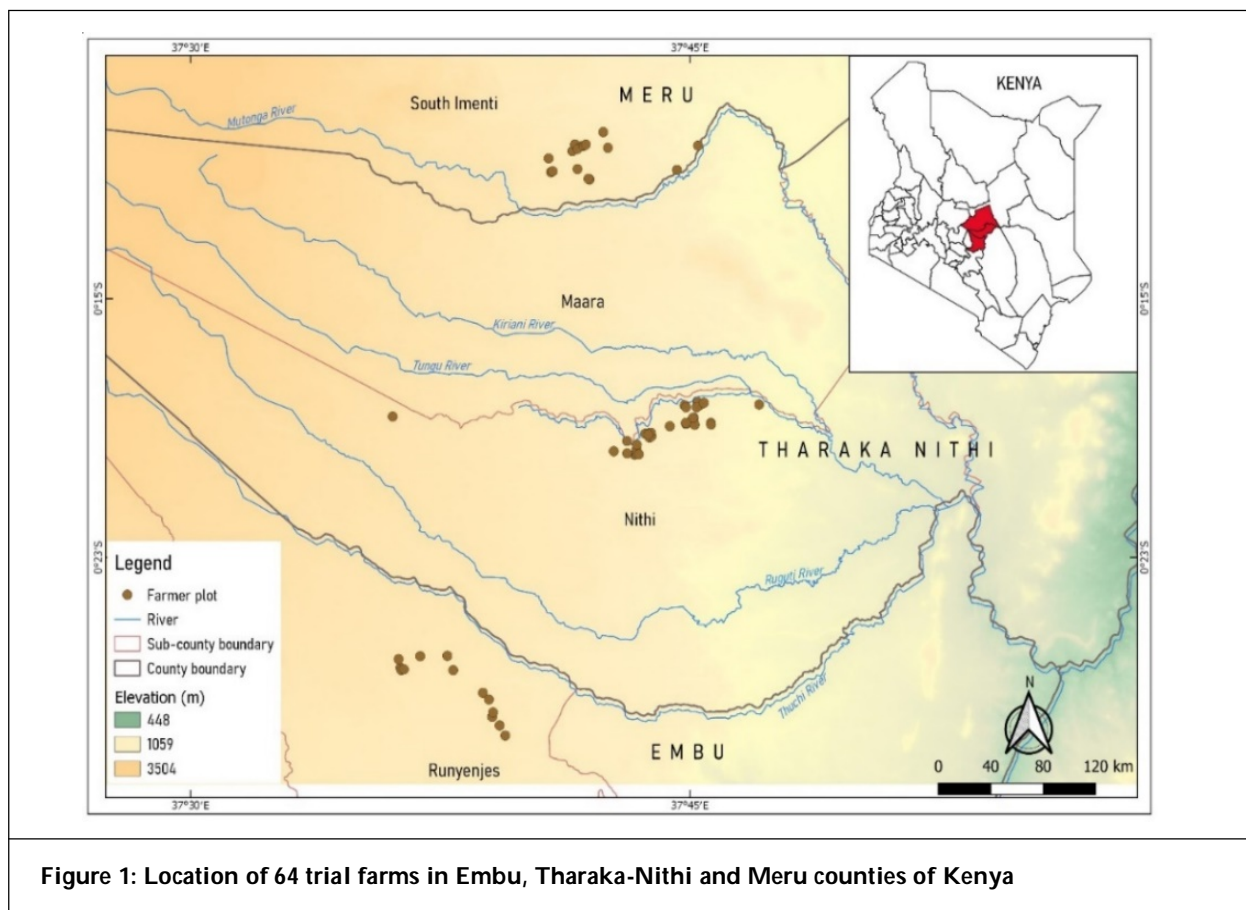
The plots that were sampled had been managed as follows: "CA had involved growing of crops on untilled land, retention of at least 75% of crop residue on the soil surface of the plots where crops were grown and control of weeds by herbicides. Before planting or immediately after planting but before seedling emergence, farmers had sprayed their CA plots with Weedal 480 SL (41% Glyphoshate), a broad spectrum, systemic and non-selective herbicide from Dow Chemical Company of India. Weedal 480 SL had been used at the manufacturer's recommended rates. Maize had been planted at a spacing of 75 cm between rows and 30 cm within rows with one seed per hill. Fertilizer had been applied at the rate of 60 kg /ha for P_2O_5, and 23.4 kg/ha for N at planting time, followed by 36.6 kg/ha of N as topdressing four weeks after maize seedling emergence. Diammonium Phosphate (DAP) was the source of P_2O_5 and N at planting time and Calcium Ammonium Nitrate (CAN) the source of N at topdressing. A row of beans had been planted between maize rows at an inter-row spacing of 10 cm with one bean seed per hill. Beans had received 20 kg/ha of N from CAN that was applied at planting time. Basagran (containing 480 g/l sodium bentazone), a pre-emergence herbicide from BASF Chemical Company of Germany, had been sprayed on CA plots at manufacturer's recommended rates before crop emergence. The CA practice had been tested against CT in which farmers planted their maize and beans as intercrop after digging their land with a hand held hoe. Crops had been weeded two times to control weeds. In CT plots, the same rates of fertilizers had been used but crop residue had been removed.

3. Soil fungal extraction

To extract fungi from soil, samples were pulverized under aseptic conditions and passed through a 2 mm sieve to remove any plant debris. 10 g of each sample were weighed into separate sterile 250 ml Erlenmeyer flasks. Ninety ml of sterile distilled water were added (FAO, 2013). Flasks containing samples were covered with sterile aluminum foil and shaken in batches of eight flasks for 30 min in an IKA Labortechnik horizontal shaker, from Germany, at 180 strokes per minute. Samples were allowed to settle for 5 min. 10 ml of supernatant of each sample aliquots were placed into sterile 25 ml McCartney bottles using sterile pipettes. These samples formed the first dilution of 10^{-1}. Further factor ten dilutions were prepared by adding 1 ml of previous dilution to 9 ml of distilled sterile water using micropipette fitted with disposable tips. Bottles were closed with screw caps and shaken in a vortex mixer (Winn Vortex Genie test tuber mixer from Netherlands) for 30 sec.

3.1. Determination of total fungal Colony Forming Units (CFUSs) per gram of soil

To determine total fungal CFUs, 100 μl of respective sample dilutions were surface plated in triplicates under aseptic conditions on sterile 90 mm diameter petri dishes containing 15 ml Potato Dextrose Agar (PDA) a general purpose non-selective media from Difco.

3.2. Preparation of PDA media

Nineteen point 5 g of PDA powder was weighed and put into 500 ml sterilizing bottle followed by addition of 484 ml of distilled water. Contents were shaken until PDA dissolved and sterilized at 121°C for 30 min. After cooling to 50°C 10 ml of 5 g of streptomycin sulphate in 100 ml and 6 ml of 1 g neomycin sulphate in 100 ml distilled and sterile water were added to control gram +ve and –ve bacteria. Fifteen ml of PDA media was dispensed into petri dishes and allowed to set under sterile conditions for five days before use. Samples were spread evenly using sterile L shaped glass rod. Samples were incubated under 12 h cycles of light and dark at room temperature for 7 to 10 days after which fungal CFUs were counted and recorded. Dilutions with colony counts in the range of 25 to 100 per plate were considered for plate counts to avoid large errors of estimate at the lower plate counts (Sutton, 2011). In addition plates with differences of less than 20% of the mean for the triplicates (Sutton, 2011) and at least two plates per sample were used for the total fungal CFUs.

3.3. Determination of relative composition at genus level of the fungi found in soil

Colonies from plates with less than 50 CFUs were presumptively differentiated into different genera based on macro and micro-morphological characters (Kidd *et al.*, 2016; Ismail *et al.* 2015; Leslie and Summerell, 2006; Barnett and Hunter, 1987; and Nelson *et al.* 1983). Mycelia from the edge of the different colonies were sampled with a sterile needle and re-inoculated in triplicates into fresh petri dishes containing half strength PDA media and incubated at 12 h cycles of light and dark for seven days. Colonies were examined to ensure that they were not mixed. Mycelia from macro- and micro-morphologically pure colonies were used to generate single spore colonies in water agar using the procedure of Leslie and Summerell (2006).

4. Soil chemical analysis

Total organic carbon, pH, total nitrogen, phosphorus, potassium, calcium, magnesium and manganese, copper, ferrous, zinc and sodium were determined using the methods in Hinga *et al.* (1980).

5. Data analysis

Data for soil fungal populations was subjected to tests for outliers using box and whisker plots, and test for normality of distribution using skewness and kurtosis, Kolmogorov-Smirnov and Shapiro-Wilk tests for normality, in Statistical Package for Social Scientists (SPSS) for Windows, Version 20.0 (2011). Armonk, NY: IBM Corp. Survey data was analyzed using descriptive statistics in SPSS. Inferential statistical analyses including correlation and analysis of variance were carried out using SPSS and GenStat for Windows 15th Edition (2012), VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

6. Results

Among the households that participated in this study, 49 were men headed and 15 were women headed (Table 1). These farmers had been selected randomly in 2010, divided into two groups who tested the productivity of maize and beans in intercrop under CA and CT respectively.

6.1. Normality of distribution of different fungal species CFUs under CA and CT tillage methods

Visual examination of data for the CFUs per gram of soil of different fungal species showed high variability. Data was therefore subjected to SPSS Explore procedure to investigate the normality of distribution. The results of this analysis are given in a box and whisker diagram (Figure 2), that showed that there were many samples that were in Turkey's outer (extreme outliers) ring and in Turkey's inner (outliers) ring. No data for fungal species CFUs/g of soil passed test of normality for Shapiro-Wilk test. Only *Aspergillus* CFUs under CT and *Colletotrichum* CFUs under CA passed the Lilliefors corrected Kolmogorov-Smirnov tests for normality of distribution.

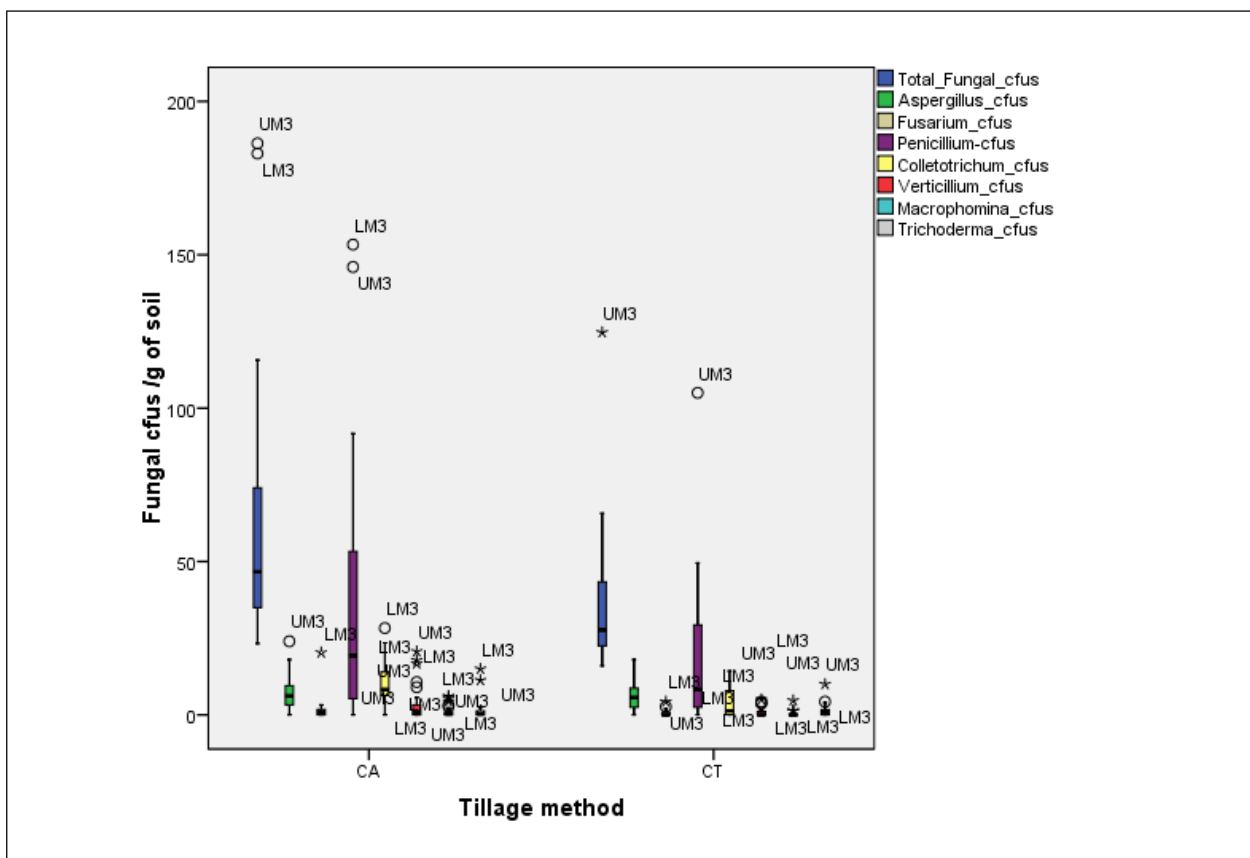| Table 1: Trial collaborating farmers by gender of household head					
Agro-Ecological Zone			**Tillage method**		**Total**
			CA	**CT**	
LM 4	Household Head	Man	13	10	23
		Woman	3	5	8
	Sub Total		16	15	31
UM 3	Household Head	Man	13	13	26
		Woman	4	3	7
	Sub Total		17	16	33
Total	Household Head	Man	26	23	49
		Woman	7	8	15
	Total		**33**	**31**	**64**

Figure 2: Box and whisker diagram showing extreme (star) and outlier (oval) of fungal species

After removal of four outlier farms (Figure 3), three with very high fungal CFUs/g soil and one with very low fungal CFUs/g of soil, total fungal, *Penicilium*, *Aspergillus* and *Colletotrichum* CFUs/g of soil under both CA and CT passed the z-test (Kim 2013; Ghasemi and Zahediasl 2012) for normality of distribution. In addition, *Aspergillus* CFUs/g of soil under both CA and CT and *Colletotrichum* CFUs /g of soil under CA passed the Lilliefors corrected Kolomogorov-Smirnov tests for normality. These genera were found to be the most frequently detected on the trial farms and also had higher population densities in CFUs/g of soil.

Data showing a moderate departure from normality or data from a large enough sample (greater than 40) can usually be used in parametric procedures without loss of integrity (Elliott and Woodward, 2007). Due to the fact that not all fungal species had normality of distribution, data was subjected to both inferential and descriptive statistical analysis.
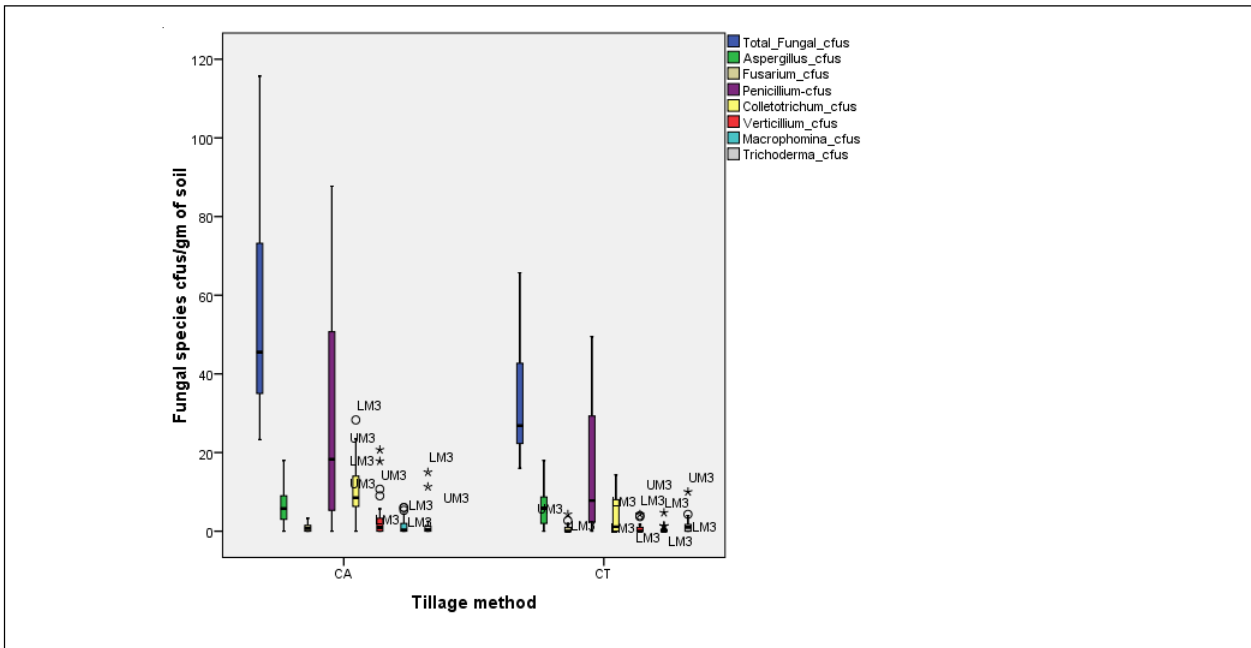

Figure 3: Box and whisker diagram after removal of 4 outlier farms

6.2. Effect of tillage method on the prevalence of different fungal genera on trial farms

Results were subjected to both descriptive and inferential statistical analysis. The frequency of detection of the different fungal species on the trial farms is shown on Figure 4. *Aspergillus* was detected on all farms that tested CA and *Penicillium* on all farms that tested CT. *Rhizopus* was not detected on farms that tested CA in LM 4 and UM 3 respectively while *Perenospora* was not detected on the farms that tested CT in LM 4. Chi-square analysis on the frequency of detection of fungal species showed that only *Macrophomina* was statistically significantly different between CA and CT at p = 0.05. There was no difference in prevalence between CA and CT for the other fungal species. The mean populations of fungal species in CA and CT trial farms was analyzed (Table 2). The trend was that total fungal species were higher in CA than CT and in UM 3 than LM 4. The CA trial farms resulted in increase in total soil fungal population from 39.4 x 10^4 in CT-UM 3 to 61.5 x 10^4 in CA-UM 3 and 22.6 x 10^4 in CT-LM 4 to 48.2 x 10^4 in CA-LM 4 an increase of 56% and 113% respectively.
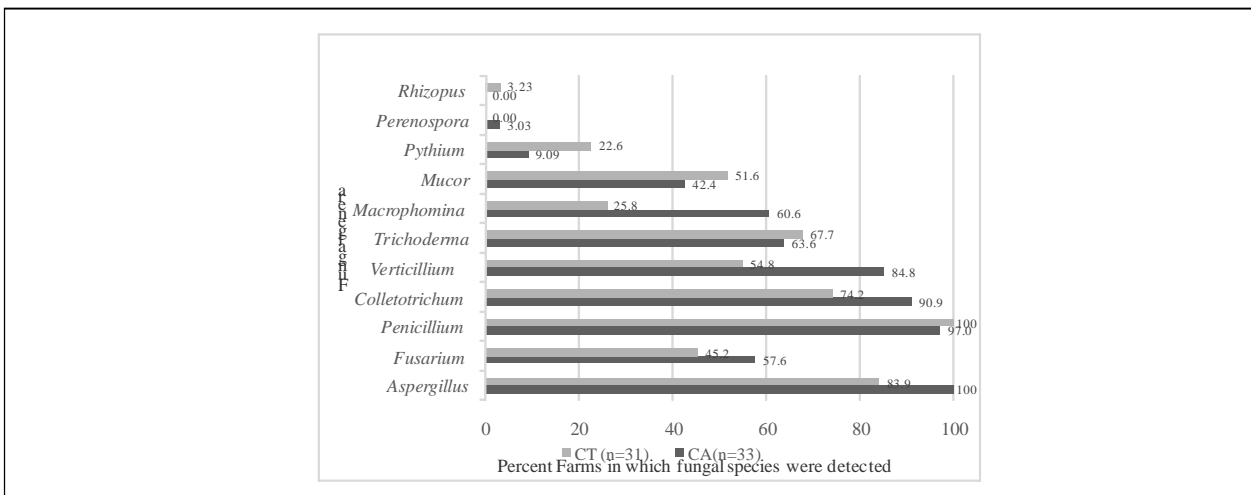

Figure 4: Percent number of farms in which various fungal species were detected

6.3. Effect of tillage method on the soil population of different fungal genera on trial farms

The fungal species that showed normality of distribution of CFUs/g of soil (total fungal, *Penicillium, Aspergillus,* and *Colletotrichum*) were subjected to analysis of variance of unbalanced design using Genstat regression to determine if the main effect and interaction effects for tillage and agro ecological zones on the fungal genera were statistically significant.

The results of analyses showed that there were no significant interaction effects between tillage method and agro ecological zones on the total fungal CFUs {$F_{(1, 56)} = 0.09, p = 0.764$}, *Penicillium* CFUs {$F_{(1, 56)} = 0.01, p = 0.908$}, *Colletotrichum* DFUs {$F_{(1, 56)} = 0.07, p = 0.799$} and *Aspergillus* CFUs {$F_{(1, 56)} = 0.02, p = 0.895$}. There was a significant effect of tillage method {$F_{(1, 56)} =16.85, p < 0.001$} and agro-ecological zone {$F_{(1\ 56)} = 6.42, p = 0.014$} on total fungal CFUs; of tillage method {$F_{(1, 56)} = 7.10, p = 0.01$} and agro-ecological zone {$F_{(1, 56)} = 9.68, p = 0.003$} on *Penicillium* CFUs; significant effect of tillage method {$F_{(1, 56)} =14.44., p < 0.001$} and non-significant effect of agro-ecological zone {$F_{(1, 56)} = 0.28, p = 0.601$} on *Colletotrichum* CFUs. Tillage method {$F_{(1, 56)} = 0.21, p = 0.645$} and agro-ecological zone {$F_{(1, 56)} = 0.04, p = 0.837$} did not have significant effects on *Aspergillus* CFUs.
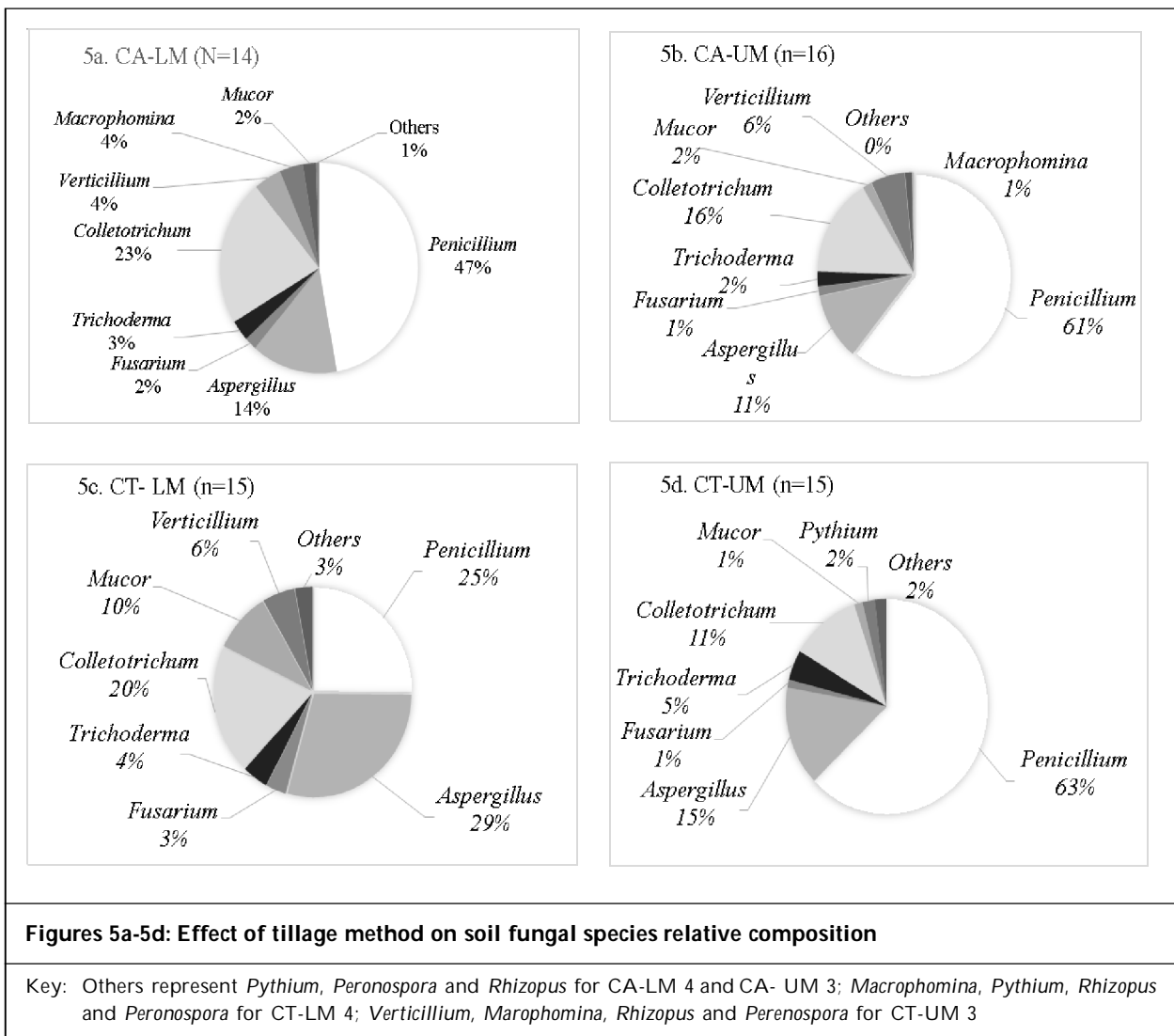
The null hypothesis was rejected for the population of total fungal, *Penicillium,* and *Collectotrichum* CFUs/g of soil but failed to be rejected for *Aspergillus* CFUs/g of soil.

Fishers Least Significant Difference (LSD) tests showed that CA resulted in significantly higher total fungal, *Penicillium* and *Colletotrichum* CFUs than CT in both UM 3 and LM 4 AEZs. Agro-ecological zone UM 3 had significantly higher total fungal and *Penicillium* CFUs than LM 4.

6.4. Relative composition of fungal species

Aspergillus as a percent of total fungal CFUs was higher for CT than CA and ranged from 11% for CA in UM 3 to 29% for CT in LM 4 (Figures 5a-5d). However, the population density of *Aspergillus* remained similar for the two tillage methods and AEZs at about 6 x10^4 CFUs per gram of soil (Table 2). The population of *Fusarium* in CFUs/g of soil in comparison to total fungal population was low in both CA and CT (Figures 5a-5d).

Table 2: Effect of tillage method on mean soil population (CFUs x 10^4 per gram) of different fungal species

Tillage method	Total fungal species	Penicillium	Aspergillus	Fusarium	Trichoderma	Colletotricum	Verticillium	Macrophomina	Pythium	Perenospora	Rhizopus	Mucor
CA LM 4 Mean (n=14)	48.2	22.7	6.76	0.96	1.56	10.8	2.16	1.85	0.14	0.09	0	1.04
CA UM 3 Mean (n=16)	61.5	37.4	6.67	0.89	1.46	9.61	3.56	0.73	0.13	0.04	0.02	0.97
CT LM 4 Mean (n=15)	22.6	5.65	6.6	0.75	0.97	4.5	1.22	0.3	0.18	0	0.18	2.15
CT UM 3 Mean (n=15)	39.4	24.7	5.89	0.47	1.8	4.41	0.27	0.4	0.78	0.07	0	0.53
Overall Mean	42.93	22.61	6.48	0.77	1.45	7.33	1.80	0.82	0.31	0.05	0.05	1.17

Figures 5a-5d: Effect of tillage method on soil fungal species relative composition

Key: Others represent *Pythium, Peronospora* and *Rhizopus* for CA-LM 4 and CA- UM 3; *Macrophomina, Pythium, Rhizopus* and *Peronospora* for CT-LM 4; *Verticillium, Marophomina, Rhizopus* and *Perenospora* for CT-UM 3

6.5. Pearson's correlation matrix between fungal genera CFUs

Pearson's correlation matrix (Table 3a.) showed that total fungal, had a highly significant ($p \leq 0.01$) positive correlation to *Penicillium* and a significant positive correlation ($p \leq 0.05$) to *Macrophomina. Trichoderma* was highly significantly ($p \leq 0.01$) positively correlated to *Pythium. Colletotrichum* was significantly ($p \leq 0.05$) positively correlated to *Macrophomina* and *Macrophomina* highly significantly ($p \leq 0.01$) positively correlated to *Peronospora. Aspergillus* and *Fusarium* genera were not significantly correlated to other fungal genera.

6.6. Pearson's correlation matrix between different fungal genera CFUs and soil fertility factors

Aspergillus had a significant ($p \leq 0.05$) negative correlation to soil copper (Table 3b) but *Fusarium* was not significantly correlated to any of the soil fertility factors. Total fungal CFUs were significantly ($p \leq 0.05$) positively correlated to soil phosphorus and iron contents while *Penicillium* was significantly ($p \leq 0.05$) positively correlated to iron and significantly ($p \leq 0.05$) negatively correlated to potassium. *Colletotrichum* and *Verticillium* had positively significant ($p \leq 0.01$) correlation to potassium and phosphorus respectively.

6.7. Farmers' perceptions on ear rots of maize in CA and CT trial farms

Perceptions of farmers on incidences of ear rots in maize in CA and CT trial farms were captured in a formal questionnaire. Their responses were analyzed using chi-square. There was no significant difference in diseases between CA and CT plots according to women and men household head perceptions. The chi-square statistic was 0.8103. The *p*-value was .368042. The result was not significant at $p < .05$.

Table 3a: Pearson's correlation matrix between different fungal CFUs

Fungal Species CFUs (n=60)	Total fungal	Penicillium	Aspergillus	Fusarium	Trichoderma	Colletotricum	Verticillium	Macrophomina	Mucor	Pythium	Perenospora	Rhizopus
Total fungal		.928**	0.09	-0.02	0.07	0.15	0.21	.285*	-0.18	-0.09	0.06	-0.12
Penicillium			-0.05	-0.12	-0.05	-0.12	0.06	0.19	-0.17	-0.16	-0.04	-0.09
Aspergillus				0.16	0.04	-0.19	0.08	-0.07	-0.13	-0.16	0.08	0.15
Fusarium					-0.01	0.18	-0.10	0.10	0.01	0.04	-0.07	-0.14
Trichoderma						0.12	-0.17	-0.12	-0.09	.389**	0.04	-0.01
Colletotrichum							0.10	.319*	-0.10	0.15	0.11	-0.19
Verticillium								0.05	-0.18	-0.10	0.24	-0.03
Macrophomina									-0.19	-0.10	.356**	-0.10
Mucor										-0.06	-0.12	-0.08
Pythium											-0.06	-0.04
Perenospora												0.00
Rhizopus												

Table 3b: Pearson's correlation matrix between different fungal CFUs and different soil fertility factors

Fungal Species CFUs (n=60)	Soil pH	Total N %	Total Org Carbon %	Phosphorus ppm	Potassium me%	Calcium me%	Magnessium %	Manganese me%	Copper ppm	Iron ppm	Zinc ppm	Sodium me %
Total fungal	-.200	.004	.014	.298*	-.227	-.239	.189	.092	.138	.282*	.137	-.140
Penicillium	-.159	.007	.020	.251	-.319*	-.171	.209	.051	.214	.307*	.237	-.171
Aspergillus	.095	-.134	-.125	.028	-.104	-.011	.035	.080	-.265*	.081	-.170	-.038
Fusarium	.046	-.026	-.040	-.054	.228	-.050	-.151	.032	-.094	-.111	-.029	.152
Trichoderma	.013	-.086	-.083	-.155	.004	-.108	-.021	-.151	.058	-.084	.059	-.036
Colletotrichum	-.124	.118	.111	.059	.421**	-.144	-.080	.246	-.155	-.196	-.153	.155
Verticillium	-.177	-.008	-.014	.384**	-.113	-.160	.064	-.104	.031	.205	-.152	-.103
Macrophomina	-.091	-.012	-.031	-.091	-.007	-.210	.150	.208	-.078	-.117	-.103	-.006
Mucor	.072	.121	.107	.073	.135	.306*	-.051	-.153	.241	.117	-.010	.135
Pythium	-.159	-.131	-.127	-.114	-.036	-.214	-.180	-.060	-.090	-.116	-.011	-.045
Perenospora	-.238	.101	.106	-.101	-.137	-.216	.119	.000	-.067	.036	-.037	-.108
Rhizopus	.085	.053	.053	-.058	.038	.164	.109	-.031	-.168	-.093	-.184	.016

Note: ** Correlation is significant at the 0.01 level (2-tailed); and * Correlation is significant at the 0.05 level (2-tailed).

7. Discussion

Some plant-pathogenic fungi have quite a broad host range, but most are highly limited in the range of plant species or even cultivars that they cause disease (Li *et al.* 2020; and Borah *et al.* 2018). Plant type and soil type, are drivers of microbial community structure (Garbeva *et al.* 2004), where different fungi may be more associated with certain crop or plant species.

Fungal species that are pathogenic to specific crops would be expected to be more prevalent in fields where these crops are grown or were previously grown. Incidence of *Fusarium* head blight was higher when susceptible wheat was planted after a crop of corn or wheat as compared to when it was planted after a crop of soya bean (Dill-Macky and Jones, 2000).

The percent number of farms in which different fungal species were detected ranged widely from 3.03 % to 100% with only four genera of *Aspergillus, Penicillium, Colletotrichum* and *Verticillium* being detected in more than 70% of the farms. There was significant increase in prevalence of *Macrophomina* in CA as compared to CT. There was no significant difference between CA and CT for the other fungal species.

Population density in CFUs/g of different fungal species followed similar trend as that of prevalence in which *Penicillium, Colletotrichum, Aspergillus amd Verticillium* had the highest values in that decreasing order. Depending on the tillage method and AEZ, *Penicillium* alone was between 25% and 63% of all the fungal species.

In a study on comparison of fungal communities in long-term no-till (NT) plots to adjacent conventionally tilled (CT) plots, in US dryland Pacific Northwest wheat cropping systems, Sharma-Poudyal *et al.* (2017) reported that some of the fungal genera were more abundant in NT while others were more abundant in CT. *Fusarium, Mortierella, Penicillium, Aspergillus,* and *Macroventuria* were genera that were abundant but these fungi did not show any trends between NT and CT. In eastern Kenya, the population in CFUs/g of soil of total fungal and *Penicillium* had increased in CA as compared to CT unlike what was reported by Sharma-Poudyal *et al.* (2017) for *Penicillium. Fusarium* and *Aspergillus* were found not to be significantly different between CA and CT similar to what was reported by Sharma-Poudyal *et al.* (2017).

Residue forms substrate for saprotrophic fungi. In Kenya, maize and bean residue was removed from the CT plots to be used as livestock feed. However the maize and bean residue under the soil in form of roots was not removed in CT trials. This residue may have been adequate for *Fusarium* and *Aspergillus* but not *Penicillium* resulting in significantly higher population of Penicillium in CA as compared to CT. In addition *Penicillium* population in eastern Kenya soils was found to be several times that of *Fusarium* and *Aspergillus* which implies that *Penicillium* would have required more crop residue to proliferate. In the Sharma-Poudyal *et al.* (2017) experiment, wheat straw residue in CT was ploughed into the soil using chisel plough and in RT residue was left on the soil surface. Total amount of residue in both CA and CT plots was the same and all of it was available to the fungal species whether on the soil surface or below the soil surface.

In the current study, *Penicillium* and *Aspergillus* were found to have the highest population densities similar to what was reported by Njeru *et al.* (2016). However, *Fusarium* reported by Njeru *et al.* (2016) as having the third highest population density, was found in this study to have one of the lowest population densities.

Fusarium is a fungal genus that can be found in warmer areas such as eastern Kenya and also in cold areas such as Narok (Proctor DL (ed.) 1994). Narok as reported by Njeru *et al.* (2016) is an area where a lot of wheat is grown with many farmers using their own saved seed, planting wheat in consecutive seasons and rotating wheat with another cereal. Under such circumstances *Fusarium* which is mainly a small cereal disease pest would be expected to be more prevalent than in eastern Kenya. In eastern Kenya, maize was the most important crop planted by most farmers as an intercrop with beans. Intercrops usually have lower disease infection rates (Bockus and Shroyer, 1998) and this may explain the low level of *Fusarium* in the area in addition to the crop species.

In work reported by Muthomi *et al.* (2012), fungal genera (CFUS/g) isolated from soil in different AEZ of three different wheat growing Districts in Kenya were found to be *Alternaria, Fusasrium, Penicillium, Epicoccum* and *Trichoderma* in that order of decreasing frequency.

In the current study *Trichoderma* in addition to *Penicillium* had high population densities similar to what was reported by Muthomi *et al.* (2012). The fact that one study involved wheat while the other involved maize may have contributed to some of the differences that were observed on some of the fungal species populations.

Soil fertility factors such as organic matter content, nitrate and extractable phosphorus correlated with the density of *Aspergillus*, *Fusarium* spp., and total fungi in Mississippi Delta soils (Zablotowicz *et al.,* 2007). The highest populations of *A. flavus* were associated with soils containing higher organic matter, especially in sites under a no-tillage management (Zablotowicz *et al.,* 2007).

In the current study, total fungal population, *Penicillium* and *Colletotrichum* were significantly higher in CA than CT. There was no significant difference in the population of *Aspergillus* between CA and CT. *Aspergillus* was only negatively correlated to copper at alpha value of 0.05 unlike the findings in Mississippi Delta soils (Zablotowicz *et al.,* 2007).

In other work reported in Ghana, significant differences ($p < 0.05$) were observed in the *A. flavus* density and distribution within and across three agro ecologies noted for major maize production (Dadzie *et al.* 2019). In Kenya there was no significant difference observed in *Aspergillus* density between LM 4 and UM 3. However, the focus in Ghana was on *A. flavus* while in Kenya it was all the *Aspergillus* species and this may have contributed to the differences observed depending on how each *Aspergillus* species behaved. In addition, the climatic range between LM 4 and UM 3 in Kenya is narrower than the climatic range between Savannah and Rain forest zone in Ghana in which the study in Ghana was carried out and this may also have contributed to the differences observed.

Percent available C, N and soil pH did not significantly influence *A. flavus* density in Ghana. Soil carbon, nitrogen and pH had no significant effect to the population of *Aspergillus* in Kenya similar to what was reported by Dadzie *et al.* (2019) for Ghana.

Aspergillus as a percentage of total fungal species in soil in eastern Kenya varied widely but the population density in CFUs/g of soil in the two tillage methods and the two AEZ was not statistically significantly different at alpha value of 0.05. Population of *Aspergillus* was quite similar under the two tillage methods in the two AEZs. *Fusarium* population in CFUs/g of soil was slightly higher in CA than CT and in the sub-humid LM 4 AEZ than the humid UM 4 AEZ (Table 2).

Fusarium and *Aspergillus* were the most frequently isolated fungi from Eastern Kenya in maize, soil and mill dust samples collected from farmers and traders (Muthomi *et al.,* 2009). In the current study *Aspergillus* was detected on all the farms that tested CA and on 83.9% of the farms that tested CT while *Fusarium* was detected on 57.6% and 45.2% of the farms that tested CA and CT respectively (Figure 4). Results from the two studies agreed with each other closely probably due to similar environmental and agricultural conditions of where the two researches were carried out.

In South Texas, USA, under reduced tillage, corncobs were reported to be a major source of *A. flavus* inoculum. Corncobs from the previous season contained, on average, over 190 times more *A. flavus* propagules than soil from the same field (Garcia and Cotty, 2004). There was no significant difference in the incidence of *A. flavus* strain S, on corncobs and soil (Garcia and Cotty, 2004). In this study there was no significant difference in *Aspergillus* population between CA and CT similar to the trend reported for *A. flavus* strain S in Texas, USA. In Texas corn residue in both CA and CT must have been the same amount, only which in CT it was buried during ploughing and in CA it was left on the surface. In eastern Kenya the maize and beans crops residue in CT was removed as feed to livestock; the crop roots that were left may have been adequate substrate for *Aspergillus* and *Fusarium* resulting in no significant difference between CA and CT.

Colletotrichum and two *Fusarium* species, *F. graminearum* and *F. osxysporum* are in the list of "Top 10 fungal plant pathogens for Molecular Plant Pathology" (Dean *et al.* 2012) as scientifically and economically most important in terms of the impact of the disease they cause.

Colletotrichum causes several diseases in plants including anthracnose on bean caused by *C. lindemuthianum* (Kiptoo *et al.,* 2020) and maize anthracnose caused by *C. graminicola* (Matiello *et al.,* 2012) which can infect all maize plant parts, with the most frequent symptom being leaf blight. In the current study the population of *Colletotrichum* was second highest after *Penicillium* and was significantly higher in CA than CT. *Fusarium graminearum* is the major cause of maize stalk, ear and seed rots (Liu *et al.* 2013; and Munkvold 2003) and Fusarium ear blight of small grains (Liu *et al.* 2013; and Parry *et al.* 1995). *Fusarium oxysporum* species complex is usually associated with bean wilt or 'bean yellows', a disease that caused serious crop losses of one of the most popular climbing bean varieties in the Great Lakes Region (Spence, 2003). Root rot of corn is caused by

numerous *Fusarium* species, but most commonly by *F. oxysporum* (https://cropwatch.unl.edu/plantdisease/ corn/fusarium-root-rot retrieved on 12.10.19). In the results being reported, population of *Fusarium* was between 1% and 3% of total fungal population in CA and CT.

Chi-square analysis of farmers' responses on the effect of ear rots on maize under CA and CT showed that there was no significant difference between the two tillage methods. The occurrence and dominance of antagonist fungal species such as *Trichoderma*, *Penicillium* and *Aspergillus* species might have aided in antagonizing the pathogenic species and reduced the disease severity that the pathogenic fungi can inflict on the crop plants studied (Swer *et al.* 2011). In addition several fungal and bacterial species act as biological control agents of root pathogens and, in general, contribute to the maintenance of soil health (Corsi and Muminjanov, 2019) thereby reducing the disease incidences to the extent that there was no difference between CT and CA as observed by the trial participating farmers.

8. Conclusion

Several publications have given different results on the populations of fungal genera under CA and CT. Two of these fungi, the *Aspergillus* and *Fusarium* which cause diseases to several crops and also produce mycotoxins that are harmful to plants, livestock and human beings are of greatest concern in Kenya. Results from this study showed no significant difference in the population density of *Aspergillus* in CA and CT trial farms under the maize—beans intercrop system. Several studies involving other crop systems have given similar results. Results of this study showed that the population of *Fusarium* in the maize – bean intercrop system was quite low as compared to the other fungal genera. Continuous mono-cropping of maize and beans for five years did not result in any significant difference in the disease incidence between CA and CT according to farmers' perceptions. CA as compared to conventional tillage is considered a more sustainable farming practice for crop production by many farmers and many countries both developed and developing. Based on the results of this study, CA can be promoted to achieve the benefits of CA based sustainable intensification of crop production without the risk of increased disease incidences.

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