Agriculture and Food

AIMS Agriculture and Food, 3 (2): 120–134.
DOI:10.3934/agrfood.2018.2.120
Received: 16 April 2018
Accepted: 14 June 2018
Published: 25 June 2018

http://www.aimspress.com/journal/agriculture

Research article

Soil management practices affect arbuscular mycorrhizal fungi propagules, root colonization and growth of rainfed maize

Methuselah Mang'erere Nyamwange[1], Ezekiel Mugendi Njeru[1,*], Monicah Mucheru-Muna[2] and Felix Ngetich[3]

[1] Department of Microbiology, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya
[2] Department of Environmental Sciences, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya
[3] University of Embu, Department of Land and Water Management, P. O. Box 6-60100, Embu, Kenya

* **Correspondence:** Email: njeruezek@gmail.com.

Abstract: Agronomic management practices influence beneficial soil biota, especially arbuscular mycorrhizal fungi (AMF). AMF colonizes about eighty percent of land plants, promoting absorption of essential nutrients and crop growth. Here, a 5-year field experiment was carried out in Central Kenyan Highlands to determine the effect of tillage, mulching and inorganic fertilizers on the number of infective AMF propagules in the soil, mycorrhizal root colonization of maize and uptake of P and N from the soil. The study involved conventional and minimum tillage systems, mulching using dried maize stovers and inorganic fertilizers (120 kg N/ha). The experiment was set up in randomized complete block design and replicated thrice. The number of infective AMF propagules decreased in the following order; V4 stage ($p < 0.0001$), V6 stage ($p < 0.0001$), maize harvest ($p = 0.0076$) and before maize planting ($p = 0.0061$). Minimum tillage + mulch + no NP fertilizer (ZRO) treatment recorded the highest number of infective AMF propagules with an average of 90 propagules g^{-1} of soil whereas conventional tillage + mulch + NP fertilizer (CRF) and conventional tillage + no mulch + NP fertilizer (CWF) treatments recorded the lowest number of AMF propagules with an average of 1.33 propagules g^{-1} of soil. Besides, AMF colonization of maize roots at V4, V6 and harvest stages was significantly affected by tillage ($p < 0.0001$), mulch ($p = 0.0001$) and fertilizer ($p < 0.0001$). Results at juvenile stage showed a strong positive correlation between AMF colonization and shoot P ($r = 0.933$, $p < 0.0001$) and N ($r = 0.928$, $p < 0.0001$). These findings demonstrate a strong effect of agronomic management practices on soil AMF propagules which subsequently affected root colonization and uptake of essential nutrients such as P and N.

Keywords: arbuscular mycorrhizal fungi; root colonization; soil management practices; P uptake; maize

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are beneficial soil biota that form associations with roots of about eighty percent of land plants, including agricultural crops [1]. AMF increase the plant-root absorptive surface area, enabling plants to get access to a variety of essential nutrients from the soil [2]. Phosphorous (P) and nitrogen (N) are the essential nutrients which plants acquire as a result of AMF colonization [3], although these beneficial fungi can also increase uptake of K, Zn, Cu and nitrates from the soil [4]. In return, the AMF acquire plant-derived C which is necessary for their growth [5].

In addition, AMF provide other agroecosystem services such as carbon sequestration and formation of good soil aggregation [3,6]. AMF are associated with glomalin related soil protein which binds together soil particles, increasing the stability of the soil against agents of erosion [7]. Also, plants colonized by AMF have enhanced resistance to biotic and abiotic stresses [8] as well as increased tolerance to water stress, especially in areas with limited water supply [1]. As a result, AMF communities are essential in enhancing sustainable agriculture because of their contribution to increased crop production and soil fertility [3].

Soil management practices like tillage, crop rotation, fertilizer application and crop protection affect AM fungi symbiosis, particularly in arable lands [9,10]. Tillage is a common soil management practice used in modern agriculture mainly to control weeds, prepare seedbeds prior to planting and incorporate cover crops, pesticides as well as fertilizers [11]. Additionally, tillage can also be used to minimise the occurrence of plant diseases and enhance decomposition of crop residues [12]. However, deep tillage can lead to disruption of AMF extraradical hyphal network, thereby contributing to low levels of AMF active propagules in the soil [9]. Consequently, conventional tillage practices reduce the abundance of AMF and other beneficial soil microorganisms when compared with conservation tillage practices [1]. However, most studies on impacts of conventional tillage on AMF involve the use of high-level mechanization, and there have been no studies which have been done to elucidate the impact of low-cost manual tillage practiced by smallholder farmers on AMF communities [13]. This study involved the use of low-cost manual tillage practices such as hand hoes in preparation of land.

Conservation tillage involves use and management of crop residues which cover about 30% of the soil surface, thereby reducing soil erosion and degradation [12]. Moreover, conservation tillage restores the fertility status of the soil, lowers soil temperature, minimizes application of chemical fertilizers, and increases water retaining capacity of the soil [14]. Therefore, conservation tillage practices enhance establishment of more AMF in the soil [9]. Forms of conservation tillage include minimum tillage, no-till and reduced tillage [11]. No-till involves cultivation of crops without subjecting the soil to any form of tillage while minimum tillage is cultivation of crops with little soil disturbance without turning the soil over [15].

Use of organic materials enhance survival of AMF propagules and colonized roots in the soil [14]. Organic materials release water-soluble C which stimulates AMF growth in the soil [16]. High-input agricultural systems like the application of inorganic fertilizers have negatively affected

AMF symbioses [9] especially use of soluble mineral fertilizers have contributed to reduction in diversity of AMF and other beneficial microorganisms in the soil [10]. Inorganic fertilization increases available soil P which suppresses AMF in the soil [16]. Additionally, use of inorganic fertilizers results in poor spore production by AMF communities in the soil [3,10].

In this study, we tested the hypotheses that tillage, mulching and inorganic fertilization influence soil mycorrhizal infection potential, root colonization and growth of maize. The specific objectives were to monitor the effect of tillage, mulching and inorganic fertilization on (1) soil AMF propagules (2) maize AMF root colonization at different growth stages, and (3) its relationship with growth and shoot P and N concentration.

2. Materials and methods

2.1. Experimental site

Experimental fields werelocated at Kirege Primary School (S 00°20’07.0”; E 037°36’46.0”), Chuka Division in Tharaka-Nithi County, Kenya. The study site lies at an altitude of 1526 metersabove sea level on the Eastern slopes of Mt. Kenya. The site is characterized by annual precipitation of between 900–1400 mm and annual mean temperature of 20 °C. The rainfall is bimodal with long rains from March to June and short rains from October to December [17]. The experimental fields were part of a field experiment which was established in April 2013. The soil is Humic Nitisol with the following physicochemical properties; clay 72%, silt 20%, sand 8%, pH (water) 4.75, total N (Kjeldahl method) 0.02%, organic carbon 0.15% and available P 0.01%. The study site is mainly a maize growing area with each household having an average farm size of 1 acre [18]. Other food and horticultural crops grown include beans (*Phaseolus vulgaris L.*), sweet potatoes (*Ipomoea batatas L.*), irishpotatoes (*Solanum tuberosum L.*), kales (*Brassica oleracea L.*), onions (*Allium cepa L.*) and tomatoes (*Solanum lycopersicum L.*) [19].

2.2. Experimental design

The study was set up as a randomized complete block design with eight treatments which were replicated three times. The soil management practices tested included;

(1) Conventional tillage + mulch + NP fertilizer (CRF),
(2) Conventional tillage + mulch + no NP fertilizer (CRO),
(3) Conventional tillage + no mulch + NP fertilizer (CWF),
(4) Conventional tillage + no mulch + no NP fertilizer (CWO),
(5) Minimum tillage + mulch + NP fertilizer (ZRF),
(6) Minimum tillage + mulch + no NP fertilizer (ZRO),
(7) Minimum tillage + no mulch + NP fertilizer (ZWF), and
(8) Minimum tillage + no mulch + no NP fertilizer (ZWO).

In conventional tillage, plots were hand hoed to a depth of about 0.15 m at the beginning of the season and weeded if required using a hand hoe. In minimum tillage, manual uprooting of weeds was also done in the course of the season to reduce soil disturbance. For residue retention treatments, maize stovers from the previous cropping season were broadcasted at the rate of 3 Mg ha^{-1}, a week after emergence. Inorganic fertilizers used in this study were NP (23:23) and triple superphosphate

(TSP). Nitrogen was applied at a rate of 120 kg N ha^{-1} in split whereby 60 kg N ha^{-1} was applied as the starter fertilizer whereas the remaining 60 kg N ha^{-1} was applied by top dressing with urea 30 days after planting. TSP fertilizer was used to supply phosphorous at a rate of 90 kg P ha^{-1} in plots which did not receive NP fertilizer. The whole experimental trial included the 24 plots measuring 7.0 m by 7.0 m with 1 m and 2 m wide alleys separating plots within a block and between the blocks respectively.

2.3. Soil sampling

Soil was sampled at 0–20 cm depth from each plot, pooled together and then sieved using a 2 mm sieve to remove plant debris and other course materials. There were four sampling times; before maize planting (1st November 2016), when the maize was at V4 stage (22nd November 2016), when the maize was at V6 stage (3rd January 2017) and at harvesting stage of the maize (28th March 2017). The soil samples were placed in well-labelled polythene bags and transported to the laboratory for determination of physical and chemical properties of the soil. The soil was then used in the greenhouse for AMF propagules enumeration.

2.4. Maize crop management

The test crop was maize (*Zea mays L.*) which is commonly grown in the study area. Maize was planted on 7th April 2013 for the first cropping season and grown in the subsequent four years. Three maize seeds were planted per hill at a spacing of 0.75 m by 0.50 m between and within rows, respectively. The plants were thinned to 2 plants per hill 14 days after emergence to give the recommended plant density of 53,333 plants ha^{-1} [17].

2.5. Enumeration of infective AMF propagules

Estimates of infective AMF propagules (spores, hyphae and colonized roots) were determined by Most Probable Number (MPN) method [20]. Thirty g of the site soil was diluted serially with 270 g of sterilized sand in a 10-fold dilution series. Five levels were performed with 5 replicate tubes in each dilution to yield a five by five MPN matrix for every soil sample. Sand was sterilized by autoclaving at 121 °C for 60 minutes. Seeds of bermuda grass (*Cynodon dactylon*) were sown into all tubes and watered as required. Seven days after germination, the grass was thinned to four plants per tube. In the greenhouse, completely randomized design was used. After 4 weeks, the grass was harvested, roots washed and stained with 0.05% trypan blue stain in lactic acid. Tubes containing mycorrhizal colonization (positive tubes) in different dilutions were recorded, and the number of infective AMF propagules in the soil calculated [21].

2.6. Plant sampling

Maize plants were sampled from every plot for determination of AMF root colonization at V4 stage (22nd November 2016), V6 stage (3rd January 2017) and at maize harvest (28th March 2017). Four maize plants were uprooted from every plot to obtain the whole root system. Sampled plants were placed in labelled polyethene bags and transported to the laboratory for analyses. Roots were processed for AMF colonization while the shoots were oven-dried to a constant weight and preserved for N and P analyses.

2.7. Determination of maize shoot P and N

Dried maize plants from each subplot were analysed for shoot N and P using wet digestion method [22]. Shoot P was determined using spectrophotometry method at a wavelength of 880 nm after the development of blue colouration [23] while shoot N was determined using Kjeldahl method [24].

2.8. Assessment of mycorrhizal root colonization

Finer and fibrous roots from the sampled maize crops were cleared and washed free of the soil particles and placed in falcon tubes [25]. Ten percent potassium hydroxide was added to the roots in falcon tubes and placed in a waterbath at 80 ℃ for 15 minutes. After the heat treatment, roots were rinsed with tap water and five percent hydrochloric acid added and allowed to acidify the roots for 10 minutes. Staining of the roots was done using 0.05% trypan blue stain in lactic acid at 80 ℃ for 15 minutes. Ten percent lactic acid was added to destain the roots. The percentage of mycorrhizal root colonization was assessed under dissecting microscope at x40 magnification using gridline intersect method [26].

2.9. Data analyses

Data was tested for homogeneity of variance by Bartlett test. The percentage data was arcsine ($\sqrt{x}$) transformed to fulfill assumptions of ANOVA. Data reported in figures and tables were back transformed. Data on percentage of mycorrhizal root colonization and numbers of infective AMF propagules was analyzed by two-way ANOVA and wherever feasible means separated by Tukey's Honest Significance Difference (HSD) at $p < 0.05$. The relationship between mycorrhizal root colonization and shoot N and P was tested by Pearson's Product Moment correlation. All statistical analyses were performed using SAS (version 9.0) software.

3. Results

3.1. Effects of tillage, mulch and inorganic fertilizers on number of infective AMF propagules

Minimum tillage treatments produced the highest number of infective AMF propagules at V4, V6 and harvest stages compared to conventional tillage treatments. The number of infective AMF propagules was highest at juvenile stage ($p < 0.0001$), followed by V6 stage ($p < 0.0001$), harvest stage ($p = 0.0076$) and before maize planting ($p = 0.0061$). The highest number of AMF propagules in the soil was recorded in ZRO treatment with an average of 90 propagules g^{-1} of soil whereas CRF and CWF treatments recorded the lowest number of AMF propagules with an average of 1.33 propagules g^{-1} of soil (Figure 1).

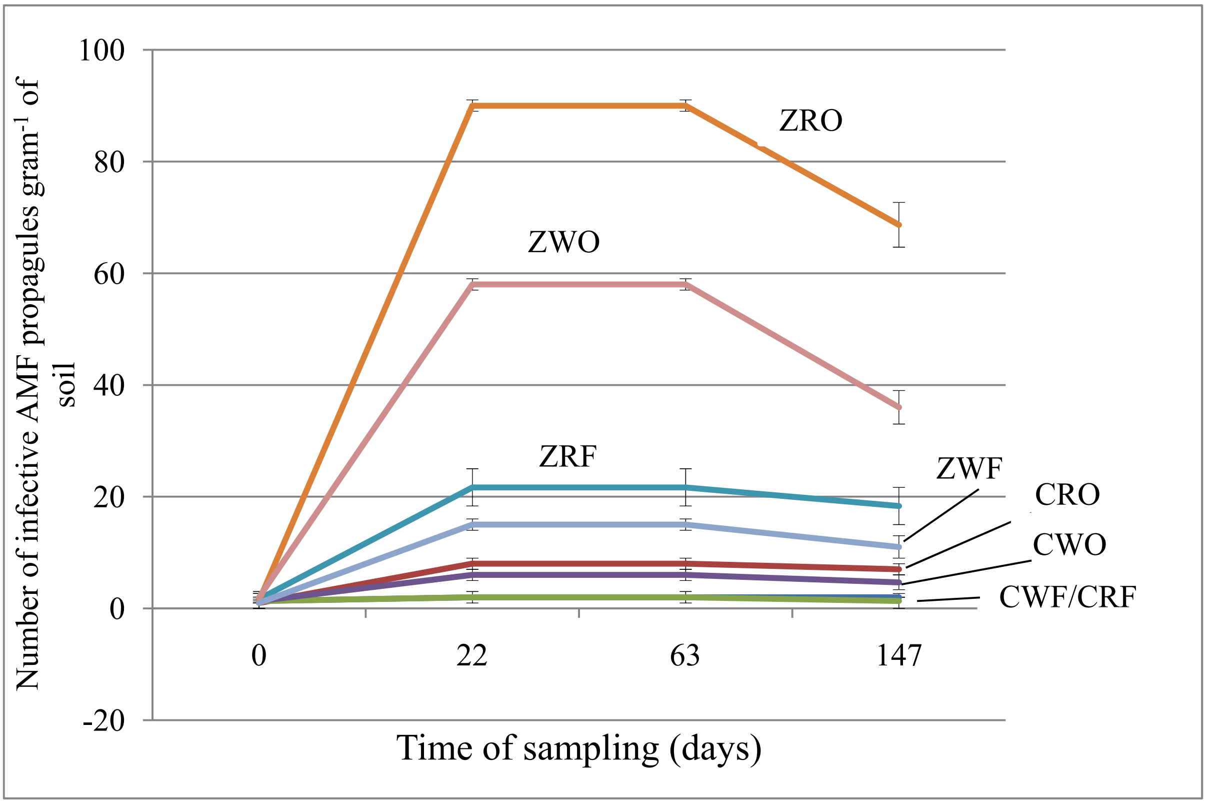

Figure 1. Number of infective AMF propagules as influenced by tillage, mulch and inorganic fertilizers. Time of sampling (days) were; 0 days: before maize planting, 22 days: maize at V4 stage, 63 days: maize at V6 stage: 147 days: maize at harvest. Vertical bars represent ± standard error.CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment.

3.2. Maize mycorrhizal colonization at different growth stages

Fertilizer ($p < 0.0001$), mulch ($p = 0.0001$) and tillage ($p < 0.0001$) significantly affected mycorrhizal colonization at juvenile, V6 and maize harvest stages. At juvenile stage, the percentage of mycorrhizal colonization ranged from 41.33% for CWF treatment to 67.33% for ZRO treatment (Table 1). Similarly, at V6 stage ZRO treatment recorded the highest percentage of mycorrhizal colonization of 59% while CWF treatment recorded the lowest percentage of mycorrhizal colonization of 31.33%. Moreover, the same trend was observed at maize harvest stage (Table 1). At all stages, there was no significant interaction between the treatments.

Table 1. Percentage of mycorrhizal colonization of maize roots as influenced by mulch, tillage and inorganic fertilization, and their interactions.

Treatments	**Percentage of mycorrhizal colonization**		
	V4 stage	**V6 stage**	**Harvest**
ZRO	67.33 (3.71) a	59.00 (3.51) a	51.00 (3.51) a
ZRF	59.33 (0.67) b	50.67 (0.88) b	42.33 (1.20) b
ZWO	51.00 (0.58) cd	41.33 (0.33) cd	30.67 (0.68) cd
ZWF	44.00 (0.58) e	33.67 (1.20) ef	23.33 (1.22) e
CRO	53.33 (0.33) bc	43.33 (0.88) c	34.33 (0.88) c
CRF	47.67 (0.33) cde	39.33 (0.33) cde	30.00 (0.58) cde
CWO	45.67 (0.33) de	34.33 (0.34) def	25.33 (0.67) de
CWF	41.33 (0.67) e	31.33 (0.88) f	23.00 (0.58) e
Tillage			
Conventional tillage	47.00 (1.31) b	35.08 (1.42) b	25.17 (1.35) b
Minimum tillage	55.42 (2.77) a	46.17 (2.99) a	36.83 (3.31) a
Mulch			
Mulch	56.92 (2.34) a	48.08 (2.40) a	39.42 (2.55) a
No mulch	45.50 (1.09) b	37.17 (1.17) b	27.58 (0.99) b
Fertilizer			
NP fertilizer	48.08 (2.09) b	38.75 (2.28) b	29.67 (2.39) b
No NP fertilizer	54.33 (2.54) a	44.50 (2.83) a	35.33 (3.00) a
P values for main factors and their interactions			
Tillage	<0.0001	0.0021	0.0201
Mulch	0.0001	0.0001	<0.0001
Fertilizer	<0.0001	0.0301	0.0011
Tillage × mulch	0.1004	0.5523	0.0601
Tillage × fertilizer	0.2246	0.0613	0.0601
Mulch × fertilizer	0.5638	0.6865	0.4379
Tillage × mulch × fertilizer	0.9339	0.9355	0.8756

*Note: The mean standard errors are presented in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's HSD test). CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment

3.3. Growth of maize, P and N uptake at juvenile stage

Maize shoot dry matter was significantly influenced by tillage ($p < 0.0301$), mulch ($p = 0.0071$) and fertilizer ($p = 0.0301$). CRF treatment recorded the highest shoot dry matter with an average of 5.78 g $plant^{-1}$ whereas ZWO treatment recorded the lowest shoot dry matter with an average of 2.21 g $plant^{-1}$.

Phosphorous concentration in maize shoots at juvenile stage was significantly influenced by tillage ($p = 0.0401$), mulch ($p = 0.0049$) and fertilizer ($p = 0.0361$). Interestingly, ZRO treatment recorded the highest P concentration of 9.96 mg kg^{-1} on average whereas CWF treatment recorded the lowest P concentration of 4.55 mg kg^{-1} in average. Moreover, N concentration in maize shoots at juvenile stage was also significantly influenced by tillage (p = 0.0231), mulch (p = 0.0001) and fertilizer (p = 0.0081). The highest N concentration was recorded in ZRO treatment (3.37 g $100g^{-1}$) whereas the lowest N concentration was recorded in CWF treatment (1.32 g $100g^{-1}$) (Table 2).

Remarkably, AMF root colonization significantly affected P concentration ($r = 0.933$, $p < 0.0001$) and N concentration ($r = 0.928$, $p < 0.0001$) at juvenile stage of maize. Besides, there was a strong positive correlation between AMF colonization and shoot P and N concentration (R2 = 0.87, $p < 0.0001$; R2 = 0.8619, $p < 0.0001$ respectively) at juvenile stage (Figures 2 and 3).

Table 2. Shoot dry matter (DM), P and N concentration of maize plant at V4 stage as influenced by mulch, tillage and inorganic fertilization, and their interactions.

Treatments	Shoot DM (g $plant^{-1}$)	P (mg kg^{-1})	N (g $100g^{-1}$)
ZRO	2.43(0.03) e	9.96 (0.12) a	3.37 (0.13) a
ZRF	4.32 (0.65) b	8.30 (0.22) c	2.79 (0.18) bc
ZWO	2.21 (0.25) f	8.83 (0.39) b	3.11 (0.28) ab
ZWF	4.11 (0.38) bc	8.03 (0.07) c	2.43 (0.22) cd
CRO	3.56 (0.39) d	6.71 (0.48) d	2.35 (0.86) d
CRF	5.78 (0.63) a	5.48 (0.44) e	1.79 (0.08) e
CWO	3.86 (0.33) c	6.39 (0.74) d	2.11 (0.37) de
CWF	5.67 (0.67) a	4.55 (0.18) f	1.32 (0.27) f
Tillage			
Conventional tillage	4.21 (0.31) a	4.37 (0.42) b	2.33 (0.85) b
Minimum tillage	2.97 (0.23) b	7.39 (1.02) a	3.02 (0.32) a
Mulch			
Mulch	4.04 (0.34) a	9.38 (0.50) a	3.22 (0.22) a
No mulch	3.02 (0.09) b	4.96 (0.19) b	2.03 (0.09) b
Fertilizer			
NP fertilizer	5.34 (1.09) a	4.45 (0.33) b	1.21 (2.39) b
No NP fertilizer	2.51 (0.54) b	8.56 (0.83) a	3.01 (0.07) a
P values for main factors and their interactions			
Tillage	<0.0001	0.0401	0.0231
Mulch	0.0071	0.0049	0.0001
Fertilizer	0.0301	0.0361	0.0081
Tillage × mulch	0.1704	0.5556	0.0691
Tillage × fertilizer	0.2996	0.1612	0.0645
Mulch × fertilizer	0.5638	0.6945	0.9379
Tillage × mulch × fertilizer	0.9342	0.9305	0.2359

*Note:The mean standard errors are presented in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's HSD test). CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment.

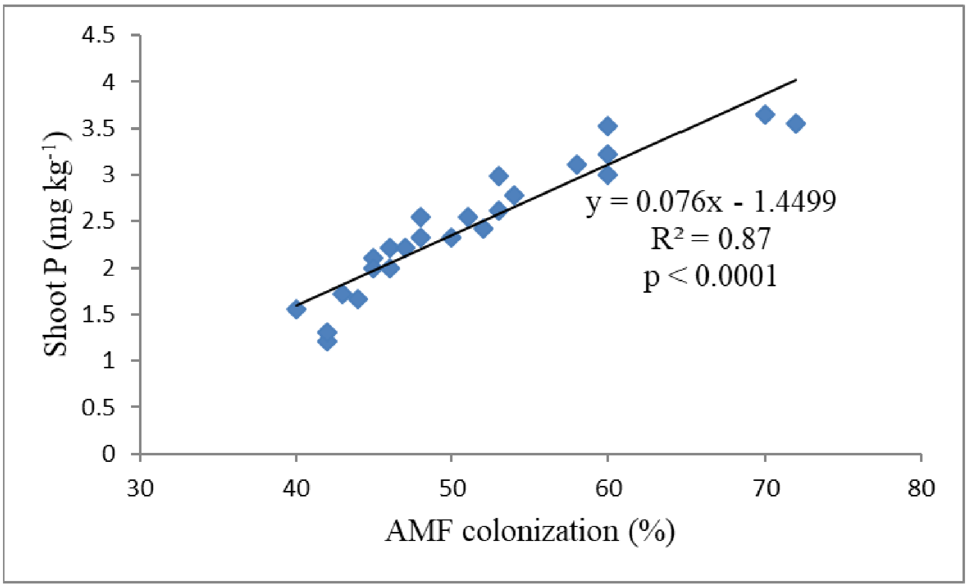

Figure 2. The relationship between AMF root colonization and shoot P on maize at juvenile stage.

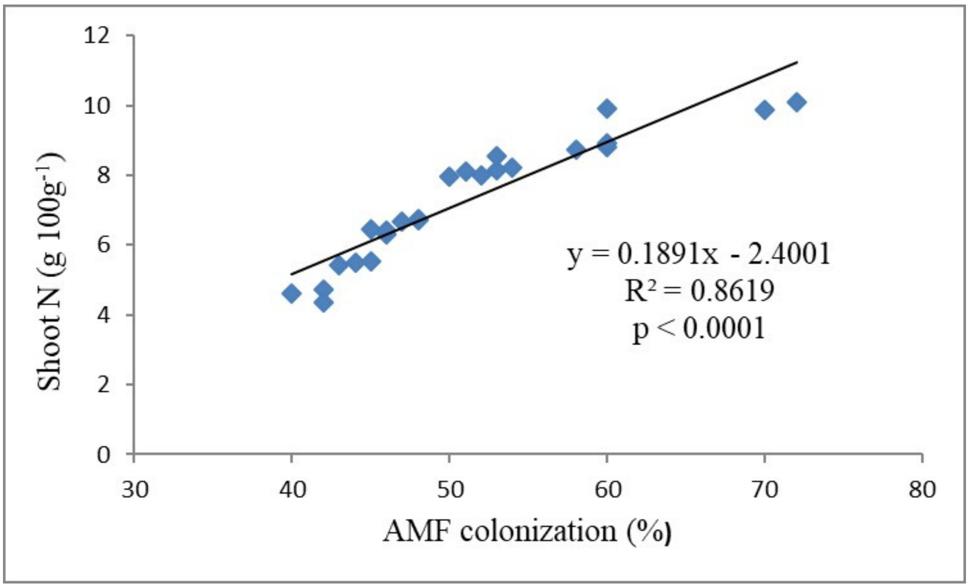

Figure 3. The relationship between AMF root colonization and shoot N on maize at juvenile stage.

4. Discussion

4.1. Number of infective AMF propagules

The number of infective AMF propagules in the soil decreased in the following order; V4 stage, V6 stage, maize harvest and before maize planting. This could be ascribed to the fact that AMF species are biotrophic, thus senesce in the absence of host crops [27]. At V4 stage, the secretion of compounds by maize is higher than the other stages because the tissues are actively growing. These compounds may include nutrients and energy sources required by AMF to grow and sporulate [28]. Consequently, the high number of AMF propagules in the soil at V4 stage colonizes a large number of roots promoting the acquisition of essential nutrients from the soil which enhance plant growth. As the plant develops towards maturity, the quantity of these compounds secreted to the plant rhizosphere decreases [27] thus decreasing the number of infective AMF propagules obtained at V6 and harvest stages.

Tillage is a common practice used by farmers to control weeds, enhance crop residues decomposition, preparation of seedbeds prior to planting and also the incorporation of fertilizers into the soil [11,29]. However, previous studies on the impact of tillage on AMF mainly involved high level mechanization in conventional tillage [11,30]. By contrast, this study involved low cost manual tillage using hand hoe in conventional tillage showing that the number of infective AMF propagules were higher in minimum tillage than in conventional tillage system. Most AMF communities are found in the topsoil [12], therefore soil manipulation using low cost manual tillage like hand hoe can disrupt the hyphal networks of these fungi resulting in less number of AMF infective propagules in conventional tillage system. Minimum tillage is a form of conservation tillage which involves little soil manipulation, thus enhancing establishment of more AMF communities in the soil [3,31].

4.2. Mycorrhizal colonization of maize roots

AMF form symbiotic associations with about eighty percent of land plants, enhancing absorption of essential nutrients from the soil and protection against biotic and abiotic stresses [6]. However, the effectiveness of AMF symbiosis is influenced by the type of agronomic practices used by farmers [9]. In this work application of NP fertilizer treatment recorded lower percentage of mycorrhizal colonization compared to no application. Application of fertilization, especially soluble inorganic fertilizers negatively effects establishment of AMF communities in the soil [10].Mineral fertilizers increase soil acidity which reduces the numbers of viable AMF spores in the soil [1,32] and also increase amount of P in the soil which inhibit production of spores by AMF [33]. The soil pH was acidic, partly because of addition of NP fertilizers and these negatively affected AMF communities, leading to low colonization of maize roots in NP fertilizer treatment. Organic materials such as mulch increase the amount of water-soluble C in the soil, water retention capacity and fertility status of the soil [34,35]. Therefore, there are more AM fungi communities in soils containing organic inputs which can lead to higher percentage of mycorrhizal root colonization in mulch treatment than in no mulch treatment.

The hyphal fragments of AMF communities tend to lose their viability when they are exposed to well aerated soils [36]. Conventional tillage loosens soil particles and breaks extraradical hyphal network of AMF communities, thereby reducing AMF root colonization [33]. In this study, conventional tillage recorded lower percentage of mycorrhizal colonization compared to minimum tillage. This could be ascribed to better AMF hyphal network development in minimum tillage treatment which favoured more root colonization [9]. In addition, conventional tillage using a hand hoe disrupted extraradical hyphae of AMF communities in the soil resulting in less AMF root colonization in conventional tillage soils. These findings showed that juvenile stage recorded the highest percentage of mycorrhizal colonization compared to V6 and maize harvest stages. At juvenile stage, the plant produces greater quantity of beneficial exudates rich in carbon which are essential for sporulation of AMF in the soil [28]. Increase in AMF communities lead to more colonization of maize roots at V4 stage. The quantity of these exudates decreases as the plant develops from seedling to silking stage [27]. As a result, percentage of mycorrhizal colonization was lower at V6 than at harvest stages.

4.3. Growth of maize, uptake of P and N at juvenile stage

Nutrients in the soil must be available in sufficient amounts for the plants to grow optimally [37]. Natural reserves of most essential plant nutrients occur in forms which are unavailable to plants [38]. As a result, fertilizers are applied to supplement essential plant nutrients in the soil [39]. In this study, maize shoot dry matter was higher in NP fertilizer compared to no application. Application of inorganic fertilizers releases nutrients which are required for rapid growth of the shoot [8]. NP fertilizers contain nutrients which are soluble in the soil, thus readily available to plants for use even in small quantities [10,39]. Organic materials also contain nutrients required for plant growth. However, nutrients in organic inputs occur in balanced quantities and this is essential for making the plant to be healthy [15]. NP fertilizer treatment recorded higher shoot dry matter than mulch treatment because the concentration of nutrients in organic materials such as mulch is lower compared to concentration of nutrients in inorganic fertilizers [39].

Mulch treatment recorded highest concentration of maize shoot P and N whereas conventional tillage and NP fertilizer recorded lowest concentration of maize shoot P and N. Organic inputs enhance AMF colonization which subsequently improves uptake of P and N from the soil [16]. Organic materials also ameliorate soil structure which increases the growth of plant roots, enabling plants to absorb substantial amounts of beneficial nutrients like P and N from the soil [40]. On the other hand, conventional tillage breaks extraradical hyphae of AMF [36], reducing the extent of maize root colonization by these beneficial soil microorganisms. Consequently, there were low P and N concentrations in maize crops grown in conventional tillage soils. Inorganic fertilization inhibits plant root colonization by AM fungi because of the acidification of the soil [9]. Inorganic fertilizers also increase available P in the soil which suppresses establishment of AMF [10]. Therefore, low AMF colonization in conventional tillage and inorganic fertilizer treatments led to little uptake of P and N from the soil.

There was a positive linear correlation between AMF root colonization and shoot P concentration. The hyphae networks of AMF communities increase the surface area for absorption of nutrients from the soil which contribute to increased levels of shoot P in plants colonized by these microorganisms [41]. Hyphal networks of AMF species also serve as an extension of maize roots and bypass the P-depletion zone to absorb more P [38]. The findings obtained in this study are congruent to those obtained by [41,42] who reported high levels of shoot P in plants colonized by AMF.

Nitrate or ammonium are the chief inorganic forms in which N is acquired by most plants [43]. Inadequate concentrations of N in the soil can limit growth of both soil microorganisms and plants [44]. Correlation analysis also showed that there was a positive linear correlation between AM fungi root colonization and N concentration in maize shoots at juvenile stage. AMF communities in the soil play a pivotal role in mineralization of N in the soil, enabling plants to obtain substantial amounts of this nutrient [44]. Arbuscular mycorrhizal fungi produce extensive hyphae networks which explore the soil and mineralize more N for the host plants [45]. Some AMF in the soil also produces extracellular enzymes which help in breakdown of organic materials which contain N, making it available for plant uptake [44]. There is always competition for mobile N by plants and microorganisms but the latter tend to outcompete the plants because of their ability to get access to micropores in the soil [45]. However, plants colonized by AMF can acquire nutrients from the soil especially N because of the extensive hyphae of these soil microorganisms [36]. The findings of this study are in agreement with those obtained by [6], who reported increased uptake of N following better AMF colonization.

5. Conclusions

Our experimental findings show that minimum tillage and mulching positively affected the numbers of AMF propagules in the soil. Inorganic fertilization and conventional tillage using low cost manual tillage such as hand hoe negatively affected numbers of infective AMF propagules, reducing the benefits provided to plants by AMF communities. In this study, application of tillage, mulching and inorganic fertilizers also affected colonization of maize roots at V4, V6 and harvest stages. Mulching and minimum tillage enhanced colonization of maize roots which subsequently increased uptake of more P and N from the soil, enhancing better growth of maize. However, inorganic fertilization negatively affected maize root colonization by AMF. This study points out that conservation agricultural practices such as minimum tillage and mulch should be used as agronomic practices because they favour establishment of AMF communities and associated agroecosystem services. Further investigations will provide the correct balance for incorporation of conventional and conservation agricultural practices to increase crop production as well as enhance protection of beneficial soil microorganisms such as AMF.

Acknowledgements

This study was supported by International Atomic Energy Agency (IAEA, Research Contact Number 16916) under the project titled 'Relationships between mulch retention and crop performances, resource use efficiency, soil organic carbon sequestration and environmental quality' and the Department of Microbiology in Kenyatta University. The authors also wish to thank Mr Antony Njagi for his assistance in management of the field experiments and collection of samples.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Turrini A, Sbrana C, Avio L, et al. (2016) Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize. *Biol Fert Soils* 52: 642–653.
2. Njeru EM, Bocci G, Avio L, et al. (2017) Functional identity has a stronger effect than diversity on mycorrhizal symbiosis and productivity of field grown organic tomato. *Eur J Agron* 86: 1–11.
3. Mader P, Edenhofer S, Boller T, et al. (2000) Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biol Fert Soils* 31: 150–156.
4. Cavagnaro TR, Jackson LE, Six J, et al. (2005) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant Soil* 282: 209–225.

5. Verzeaux J, Nivelle E, Roger D, et al. (2017) Spore density of arbuscular mycorrhizal fungi is fostered by six years of a no-till system and is correlated with environmental parameters in a silty loam soil. *Agron* 7: 38.
6. Njeru EM, Avio L, Sbrana C, et al. (2014) First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. *Agron Sustain Dev* 34: 841–848.
7. Borie F, Rubio R, Rouanet JL, et al. (2006) Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chile Ultisol. *Soil Tillage Res* 88: 253–261.
8. Nyaga J, Jefwa JM, Muthuri CW, et al. (2015) Arbuscular mycorrhizal fungi with different soil fertility amendment practices in agricultural landscapes of Kenyan Highlands. *Nutr Cycl Agroecosys* 103: 229–240.
9. Martinez TN and Johnson NC (2010) Agricultural management influences propagule densities and functioning of arbuscular mycorrhizas in low- and high- input agroecosystems in arid environments. *Appl Soil Ecol* 46: 300–306.
10. Wang FY, Hu JL, Lin XG, et al. (2011) Arbuscular mycorrhizal fungal community structure and diversity in response to long-term fertilization: a field case from China. *World J Microb Biot* 27: 67–74.
11. Alguacil MM, Lumini E, Roldan A, et al. (2008) The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecol Appl* 18: 527–536.
12. Kabir Z (2005) Tillage or no-tillage: Impact on mycorrhizae. *Can J Plant Sci* 85: 23–29.
13. Oruru MB and Njeru EM (2016) Upscaling arbuscular mycorrhizal symbiosis and related agroecosystems services in smallholder farming systems. *Biomed Res Int* 2016: 4376240.
14. Verbruggen E, Roling WFM, Gamper HA, et al. (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol* 186: 968–979.
15. Roldan A, Salinas-Garcia JR, Alguacil M, et al. (2007) Changes in soil sustainability indicators following conservation tillage practices under subtropical maize and bean crop. *Soil Tillage Res* 93: 273–282.
16. Bedini S, Avio L, Sbrana C, et al. (2013) Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. *Biol Fert Soils* 49: 781–790.
17. Jaetzold R, Schmidt H, Homet ZB, et al. (2007)Farm management handbook of Kenya: Natural conditions and farm information (2nd ed., Vol. 11/C). Nairobi: Eastern Province, Ministry of Agriculture/ GTZ.
18. Mugwe J, Mugendi D, Mucheru-Muna M, et al. (2009) Determinants of the decision to adopt intergrated soil fertility management practices by smallholder farmers in Central highlanda of Kenya. *Exp Agr* 45: 61–75.
19. Mburu SW, Koskey G, Kimiti JM, et al. (2016) Agrobiodiversity conservation enhances food security in subsistence-based farming systems of Eastern Kenya. *Agriculture and Food Security* 5: 19.
20. Lehman RM, Taheri WI, Osborne SL, et al. (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. *Appl Soil Ecol* 61: 300–304.
21. Porter W (1979) The 'most probable number method' for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Aust J Soil Res* 17: 515–519.
22. Mohidin H, Hanafi M, Rafii Y, et al. (2015) Determination of optimum levels of nitrogen, phosphorus and potassium of oil palm seedlings in solution culture. *Bragantia* 74: 247–254.

23. Kissel ED and Sonon LS (2008) Soil test handbook for Georgia (Vol. 1).
24. Okalebo JR, Gathua WK and Woomer PL (2002) Laboratory methods of soil and plant analysis: a working manual second edition. Sacred Africa, Nairobi.
25. Phillips J and Hayman D (1970) Improved procedures for clearing roots and staining parastic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society* 55: 158–161.
26. Giovannetti M and Mosse B (1980) An evaluation technique for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol* 84: 489–500.
27. Cavaglieri L, Orlando J and Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. *Microbiol Res* 164: 391–399.
28. Johnston-Monje D, Lundberg DS, Lazarovits G, et al. (2016) Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. *Plant Soil* 405: 337–355.
29. Reji PM, Feng Y, Githinji L, et al. (2012) Impact of no-tillage and conventional tillage systems on soil microbial communities. *Applied and Environmental Soil Science* 2012: 1–10.
30. Kabir Z, O'Halloran IP, Fyles JW, et al. (1998) Dynamics of the mycorrhizal symbiosis of corn (*Zea mays* L.): effects of host physiology, tillage practice and fertilization on spatial distribution of extra-radical mycorrhizal hyphae in the field. *Agricult Ecosys Environ* 68: 151–163.
31. Oehl F, Sieverding E, Ineichen K, et al. (2003) Impact of land use intensity on species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Appl Environ Microb* 69: 2816–2824.
32. Nakhro N and Dkhar MS (2010) Populations and biomass carbon in paddy field soil. *Agron J* 9: 102–110.
33. Klikocka H, Narolski B, Klikocka O, et al. (2012) The effect of soil tillage and nitrogen fertilization on microbiological parameters of soil on which spring Triticale is grown. *Pol J Environ Stud* 21: 1675–1685.
34. Arihara J and Karasawa T (2000) Effects of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Sci Plant Nutr* 46: 43–51.
35. Mariela FP, Pavel MEI, Manuel SRL, et al. (2016) Dehydrogenase and mycorrhizal colonization: Tools for monitoring agroecosystem soil quality. *Appl Soil Ecol* 100: 144–153.
36. Bilalis DJ and Karamanos AJ (2010) Organic maize growth and mycorrhizal root colonization response to tillage and organic fertilization. *J Sustain Agr* 34: 836–849.
37. Habibzadeh Y (2015) The effects of arbuscular mycorrhizal fungi and phosphorous levels on dry matter production and root traits in cucumber (*Cucumis sativulus L.*). *African Journal of Environmental Science and Technology* 9: 67–70.
38. Ryan MH, Van Herwaarden AF, Angus JF, et al. (2005) Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation of arbuscular mycorrhizal fungi. *Plant Soil* 270: 275–286.
39. Adediran JA, Taiwo LB, Akande MO, et al. (2004) Application of organic and inorganic fertilizer for sustainable maize and cowpea yields in Nigeria. *J Plant Nutr* 27: 1163–1181.
40. Gosling P, Hodge A, Goodlass G, et al. (2006) Arbuscular mycorrhizal fungi and organic farming. *Agricult Ecosys Environ* 113: 17–35.
41. Hajiboland R, Aliasgharzad N and Barzeghar R (2009) Influence of arbuscular mycorrhizal fungi on uptake of Zn and P by two contrasting rice genotypes. *Plant Soil Environ* 55: 93–100.

42. Oruru MB, Njeru EM, Pasquet R, et al. (2018) Response of a wild-type and modern cowpea cultivars to arbuscular mycorrhizal inoculation in sterilized and non-sterilized soil. *J Plant Nutr* 41: 90–101.
43. Asghari HR and Cavagnaro TR (2012) Arbuscular mycorrhizas reduce nitrogen loss via leaching. *Plos One* 7: 1–5.
44. LeighJ, Hodge A and Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytol* 181: 199–207.
45. Veresoglou SD, Chen B and MC Rillig (2012) Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol Biochem* 46: 53–62.