Journal of Agriculture and Ecology Research International
1(1): 1-17, 2014; Article no. JAERI.2014.001

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Soil Fungal and Bacterial Populations in White Lupin (*Lupinus albus*) - Maize (*Zea mays* L) Cropping System Amended With Minjingu Phosphate Rock

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JJL and RNO jointly designed the study and wrote the protocol. Author JJL performed the statistical analysis and wrote the first draft of the manuscript. Both authors managed the analysis, literature searches and addressed subsequent reviewer comments and suggestions for improvement. Both authors read and approved the final manuscript.

Original Research Article

Received 23rd April 2014
Accepted 20th May 2014
Published 19th June 2014

ABSTRACT

Aims: To determine fungal and bacterial populations under white lupin (*Lupinus albus*) - maize (*Zea mays* L) cropping system amended with Minjingu Phosphate Rock (MPR).
Study Design: A randomized complete block design with four replicates was used. Treatments were; (i) control i.e. fallow (F) – maize (M) rotation with triple super phosphate fertilizer (TSP) applied (M_{TSP} - F), (ii) fallow - maize rotation with MPR applied (M_{MPR} - F), (iii) white lupin (L) – maize rotation with MPR applied (M_{MPR} - L) and (iv) maize/white lupin intercrop with MPR applied (M/L_{MPR} - F).
Place and duration of study: The experiment was conducted in Njoro sub-County, Kenya during the long (LRS) and short rain seasons (SRS) of 2010 and 2011.

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Methodology: Population of bacteria and fungi were determined at seedling, flowering and maturity stages of crop development by serial dilution plate method (Johnson and Curl, 1972).
Results: Significantly higher bacterial population was recorded in M_{TSP}- F at maize seedling and 50% flowering in LRS of 2010 and 2011. At maturity, treatments M/L_{MPR} – F in LRS of 2010 and M/L_{MPR} – F and M_{MPR}- L in LRS of 2011 had significantly higher population. In the SRS of both years, bacterial population was significantly higher in M_{TSP}- F and M/L_{MPR} – F at all sampling periods. In the LRS of 2010, fungal population was significantly higher in M_{TSP}-F at maize seedling and in M_{TSP}- F and M/L_{MPR} – F at 50% flowering and maturity. In the LRS of 2011, fungal population was significantly higher in M/L_{MPR} – F followed by M_{MPR}- L at all maize growth stages. During the SRS of both years fungal population was significantly higher in M_{MPR}- L across all sampling periods. Positive correlation between fungal and bacterial populations was found at termination of experiment.
Conclusion: White lupin-maize cropping system with application of MPR increased soil bacterial and fungal population, an indication of improved soil health and hence cropping system sustainability.

Keywords: Cropping system; maize; microorganism; phosphate rock; phosphorus; soil health.

1. INTRODUCTION

Soil health is the capacity of a soil to function as a vital living system, to sustain plant and animal productivity, maintain or enhance water and air quality and promote plant and animal health. Soil organisms and biotic parameters such as abundance, diversity, food web structure or community stability meet the criteria for useful indicators of soil quality and are biological indicators of soil health [1,2]. Healthy soil teems with both microscopic and larger organisms [3].

Soil organisms regulate a wide range of processes that are critical for plant growth and crop productivity [4]. Fungi and bacteria are responsible for the decomposition of plant residues and the release of plant nutrients [5]. Organic matter decomposition underpins all other soil functions [6]. Therefore, the balance of fungi and bacteria in the soil is important for its optimal functioning [6]. Soil organisms are highly dynamic and react rapidly to changes in environmental conditions and land management practices [7, 8]. Cropping systems such as rotations can impact soil microbial populations, diversity, richness, and community composition [9].

Previous studies in the central rift valley province of Kenya have focused on sustainable increase of maize yield, the country's staple [10], using low cost inputs [11, 12). The extent to which fungal and bacterial populations vary with use of Minjingu Phosphate Rock (MPR), a low cost input, in white lupin -maize cropping systems has not been previously examined. White lupin (*Lupinus albus*) is a crop that has not been previously planted in the central rift valley province. It is, however, a potential crop that can enhance the dissolution of MPR through acidification of the rhizosphere. MPR has limited dissolution in soil [13]. White lupin responds to P deficiency by producing special root structures called cluster roots [14]. These roots strongly acidify the surrounding rhizosphere and exude organic anions, mostly citrate and malate, which are responsible for the release bound forms of P from sparingly soluble inorganic and organic compounds [15,16]. Qualitative and quantitative analysis of microbial

activities are the key factors for productivity and sustainability of soil's health for maintenance of crop production [17]. Further, the ratio of fungi to bacteria can be used to detect detrimental changes in the soil and to prevent further degradation [6].

The objective of the current study was to determine population of fungal and bacterial numbers under lupin -maize cropping systems with application of MPR.

2. MATERIALS AND METHODS

2.1 Site

The experiment was conducted in a farmer's field in Njoro sub- County, Kenya (longitude 35°23' and 35°35' East and Latitude 0°13' and 1°10' south; 2200 m asl) for four seasons; long (LRS) and short (SRS) rain seasons of 2010 and 2011. The mean annual rainfall received in the area ranges between 840 to 1000 mm. The rainfall distribution is bimodal in nature with the LRS occurring from March to August and SRS from September/October with peaks in April and November, respectively. The mean air temperature is 15.9℃ [18]. The respective total rainfall amounts received in 2010 and 2011 were 918 mm and 982 mm while the minimum and maximum air temperature were17.6 and 19.1°C, respectively. The soils are well drained, dark reddish in colour and are classified as mollic Phaozems [19].

The initial chemical and physical characteristics (Table 1) of the top (0 to 0.15 m) soil layer prior to the commencement of the experiment were; neutral in pH (pH water 6.4), moderate in organic C (15 g kg^{-1}), high in total N (3.5 g kg^{-1}), low in Olsen extractable P (14.2 mg kg^{-1}) and exchangeable bases (cmol kg^{-1}); Ca (6.5), Mg (0.72) and K (1.42), and clay loam in texture (%); sand (36), silt (29.6), and clay (34) [20].

Table 1. Selected chemical and physical properties of the soil

Property	Unit	Soil depth (cm)		
		0-15	15-30	30-60
pH (H_2O)	-	6.4	6.3	6.0
organic C	g kg^{-1}	15	13	12
available P	(mg kg^{-1})	14.2	11.3	8.2
Total N	g kg^{-1}	3.5	2.4	2.7
Ca	(cmol kg^{-1})	6.5	2.7	3.1
Mg	(cmol kg^{-1})	0.72	0.83	0.42
K	(cmol kg^{-1})	1.42	0.89.0	0.56
sand	%	36.0	34.0	32.0
clay	%	34.0	40.0	40.0
silt	%	29.6	25.6	27.6
Textural class	-	Clay loam	Clay loam	Clay loam

2.2 Experimental Design and Treatments

The experiment was laid out in a randomized complete block design with four replications. The plot sizes measured 3.75 m × 4.8 m. Space for foot path (0.5 m) between the plots and blocks (1m) was provided. The treatments were; (i) control i.e. fallow (F) – maize (M) rotation with triple super phosphate fertilizer (TSP) applied (M_{TSP} - F), (ii) fallow - maize rotation with

MPR applied (M_{MPR} - F), (iii) white lupin (L) – maize rotation with MPR applied (M_{MPR} - L) and (iv) maize / white lupin intercrop with MPR applied (M/L_{MPR} - F).

2.3 Land Preparation and Application of Inputs

Land was prepared manually using hand hoes. MPR was incorporated to a depth of 0 - 0.15 m along the planting furrows two weeks before planting. TSP was applied at planting by banding. Both P sources were applied at the rate of 60 kg P ha^{-1}. MPR was applied only once during the entire experimental period while TSP was applied twice i.e. at planting of maize in the LRS of 2010 and 2011. Nitrogen was applied at the rate of 75 kg N ha^{-1} as calcium ammonium nitrate (CAN) fertilizer to all plots, split into two applications; 45 and 30 kg N ha^{-1} at planting and at topdressing (a month after planting), respectively.

2.4 Planting Operations

Long rain season: Maize (*Zea mays* L., Hybrid, 513) was sown during the LRS of 2010 and 2011 in all treatments, at 75 cm × 30 cm spacing. Two maize seeds were sown into each planting hole and thinned to one plant 30 days after sowing (DAS). In M/L_{MPR} – F treatment, two white lupin (*Lupinus albus*) seeds were sown between the rows of maize i.e. one row of white lupin between two rows of maize in the LRS of 2010 and 2011. Thinning to one plant (maize and white lupin) was done a month after sowing.

Short rain season: white lupin was sown at the rate of two seeds per hole at spacing of 75 × 30 cm in the treatment M_{MPR}- L during the SRS of both 2010 and 2011. Thinning to one plant per hole was done a month after sowing. The M_{TSP}- F, M/L_{MPR} – F and (M_{MPR} –F) were left under a weedy fallow in the SRS of both years.

2.5 Management of Residues

White lupin residues and weeds were chopped into 5-20 cm small pieces spread across the plots and incorporated in soil to a depth of 15 cm during land preparation for maize planting, using hand hoes. Maize residues were removed from field after harvest of grains.

2.6 Soil and Plant Sampling

Composite soil samples for determination of initial physical and chemical properties (Table 1) were collected from three profile pits (0-60 cm depth) before application of treatments, put in polythene bags and sealed. Thereafter soil samples were collected from the top soil (0-20 cm) at seedling, 50% flowering and maturity stages of maize (LRS) and white lupin (SRS) for enumeration of soil fungi and bacteria. Soil samples from the weedy fallow in the SRS were collected at the same time period as lupin samples. The samples were obtained randomly from four locations in each plot between the plants within a row and bulked to get one composite sample. The samples were put in sterile polythene bags and sealed with rubber bands. In the laboratory, roots were removed from soil by sieving. Samples for analysis of initial physical and chemical properties were air dried in the laboratory while those for microbial analysis were stored at 4℃ and analysed within 3 days.

2.7 Soil Physical and Chemical Analysis and Enumeration of Fungi and Bacteria

Air - dried soils sieved through 2 mm mesh were analyzed for pH (Soil: H_2O), texture, total N, organic carbon, and available P according to standard procedures [21]. Exchangeable bases (K, Ca and Mg) were extracted with 1.0 M-ammonium acetate at pH 7 and measured by atomic adsorption spectrophotometry. Fungi and bacteria were enumerated by serial dilution plate method [22] using potato dextrose agar (PDA) for fungi and nutrient agar (NA) media for bacteria. The inoculated petri plates were incubated in a sterile culture room at 25˚ ± 1℃. Colony forming units (CFU) were estimated by counting the number of colonies under a digital counter, after seven days for fungi and two days for bacteria. Number of bacteria and fungi in 1 gm of soil was calculated using the following formulae:

$$\frac{\text{Number of colonies}}{\text{amount plated} \times \text{dilution}} = \text{Number of bacteria/fungi (CFU)/g soil}$$

2.8 Statistical Analysis

Analysis of variance (ANOVA) was used to detect statistical variation in fungal and bacterial population in the different treatments. The SPSS software appropriate for a randomized complete block design was used [23]. Tukey's Honestly Significant Difference (*P*=.05) was used for mean separation.

3. RESULTS AND DISCUSSION

3.1 Soil Bacterial population

3.1.1 Influence of season and stage of crop growth

Population of bacteria increased from maize seedling to 50 % flowering and declined at maturity in treatments; M_{TSP}- F (control), M_{MPR} –F and M_{MPR}- L, in LRS and in M/L_{MPR} – F, M_{TSP}- F and M_{MPR} –F in SRS of both 2010 and 2011 (Table 2).

In both years, bacterial numbers declined at 50 % flowering in M/L_{MPR} – F in LRS and M_{MPR}- L in SRS followed by a slight increase towards maturity (Table 2). A paired sample t test showed that the number of bacteria was significantly (*P*=.05) higher in the LRS of 2011 than SRS of 2011 at seedling (t=0.043) and maturity (t=0.03). There were no significant differences in bacterial numbers in the LRS and SRS of 2010.

Increase in bacterial numbers at 50% maize flowering in treatments M_{TSP}- F, M_{MPR} –F and M_{MPR}- L during the LRS of 2010 and 2011 may partly be attributed to higher quantities of root exudates released by maize roots at this stage of growth compared to seedling and maturity. The exudates provided carbon for bacterial growth and metabolism. Root exudates are complex mixtures of carbon-containing compounds (rhizodeposits) with sugars, amino acids, and organic acids being released in largest quantities [24]. Quantity and composition of root exudates is influenced by plant developmental stage [25]. In a study on characterization of root exudates at different growth stages by rice cultivars, exudation rates were, in general, lowest at seedling stage, increased until flowering but decreased at maturity [26]. Release of rhizodeposits from plant roots influences diversity and composition of rhizosphere bacterial

communities [27]. The exudates released by maize roots are readily utilized C sources by most bacteria [28]. They include; glutamate - a strong bacterial attractant [29], ribitol and glucose.

Provision of carbon and nutrients from incorporated lupin residues in the SRS of 2010 may have additionally contributed to increased number of bacteria during the LRS of 2011 in the M_{MPR}- L treatment at 50% flowering of maize. In a litterbag decomposition experiment on effect of crop residue management practices on size of microbial community that regulates residue decomposition, mass loss from lupin residue (75%) was nearly twice that of either barley (42%) or wheat (38%) straw after the first 90 days of decomposition [30]. The differences in residue mass loss were also reflected in significantly greater populations of bacteria and fungal hyphae on residues of lupin than on wheat or barley straw.

The increase in bacterial numbers at second sampling in weedy fallow plots (M/L_{MPR} – F M_{TSP}- F and M_{MPR} –F) in the SRS of 2010 and 2011 which coincided with 50% flowering of lupin (M_{MPR} –L plot) may have been partly due to conducive microclimate attributable to adequate ground cover by weeds. Microclimate (soil moisture and temperature) is a factor that determines the environmental conditions for soil organisms [31]. Microclimate protects soil organisms from soil temperature variation and drought stress [31]. Microclimate and fertilizer (amendment) influences size and structure of microbial biomass [32]. In a study on impact of *Chrysanthemoides monilifera* weed on coastal ecosystem processes, soil macrofauna occurred in higher abundance and were detected more frequently in the weedy areas [33]. This was credited to the change in microclimate within *C. monilifera* infestations, which were moister and darker.

Low bacterial numbers in M_{MPR} –L treatment at 50% flowering of lupin during the SRS and in M/L_{MPR} –F at 50% flowering of maize during the LRS of both years may have been due to acidification of the rhizosphere by white lupin in response to the applied insoluble MPR [34]. Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil [35]. White lupin forms special root structures called cluster or proteoid roots which release citrate in response to P deficiency [36]. Fastest secretion and strong acidification of the rhizosphere occurs at the mature stage of cluster roots [14]. In a study on microbial degradation of citrate produced by white lupin, total bacterial abundance decreased significantly at mature stage of cluster roots [14]. Neutral to slightly alkaline conditions favour bacterial growth [37].

Higher bacterial numbers in the LRS of 2011 than SRS of 2011 at seedling and maturity stages of maize growth could partly be attributed to higher rainfall received in the former season. In an experiment to determine soil microbial biota response to long term changes in rainfall, strong seasonal dynamics in composition of bacterial communities were observed [38]. Similarly, strong seasonal population shifts have been reported in the rhizosphere of maize [39].

Differences in amounts and types of exudates released by different crops in the two seasons may have also caused variation in bacterial numbers between seasons. Composition of root exudates is influenced by plant species [40]. Bacterial communities in root-associated habitats respond with respect to density, composition, and activity to the abundance and great diversity of organic root exudates, eventually yielding plant species-specific microfloras which may also vary during plant development stages [41].

The LRS was planted with maize mainly and an enrichment of several populations of bacteria in the rhizosphere of maize has been reported by other workers [39]. In an experiment to detect the impact of soil enrichment of soluble organic C on bacterial community structure, bacterial densities increased with application of maize exudate solutions [42]. The resident plants in the SRS were lupin and weeds in the weedy fallow plots. Acidification of the rhizosphere by lupin exudates may have caused lower bacterial numbers. White lupin excretes large amounts of citric and malic acids when exposed to P starvation [43]. The low P content recorded is due to the fact that TSP was applied only in the LRS (M_{TSP}- F) and had been taken up by maize whereas MPR in M/L_{MPR} –F and M_{MPR}- F treatments was insoluble in water [34]. Weisskopf et al. [14] reported that citrate released by white lupin caused strong acidification and reduced the abundance of bacteria. Bacteria are affected by low pH [37].

Differences in abundance of microorganisms in rhizosphere of plant species and even varieties within species have been reported by other workers and are attributed to root-derived substrates [44,45]. Lower bacterial numbers in weedy fallow plots in the SRS of 2011, may partly be attributed to depletion of nutrients by weeds. Weed seedlings emerge yearly from the vast reservoir of viable weed seeds in soils cultivated to crops [46]. In an experiment on seeding densities and weed management practices on nutrient uptake by crop and weeds, unchecked weeds caused depleted N, P and K [47]. The type of exudates by weeds may have also repelled soil bacteria. Allelochemicals are produced by some weeds [48].

3.1.2 Influence of P sources and season

3.1.2.1 Long Rain Season (LRS)

Significantly higher bacterial numbers (*P*=.05) were found in treatment M_{TSP}- F at seedling and 50% flowering of maize in both LRS of 2010 and 2011 (Table 2). Significantly higher bacterial numbers were observed in M/L_{MPR} – F during the LRS of 2010 and in M/L_{MPR} – F and M_{MPR}- L during the LRS of 2011 at maize maturity,

Significantly higher bacterial numbers (*P*= .05) in the M_{TSP}- F treatment at maize seedling in the LRS of both years may be attributed to availability of P to microorganisms from readily available highly soluble TSP fertilizer. Thuita et al. [34] studying the solubility and availability of P from phosphate rocks found higher values of available P in the control TSP treatment at 20 days after planting. P is an essential element for all living organisms as it plays a key role in most life processes [49]. Bacteria require mineral nutrients and have high cellular requirements for both nitrogen and phosphorus, relative to carbon [50].

Significantly higher bacterial numbers in M_{TSP}- F treatment at 50% maize flowering in the LRS of both years may partly be attributed to abundance of root exudates which was a source of C to the bacteria. Root exudates and rhizo-deposits form the substrates for rhizosphere bacteria [51]. Root exudates from maize attract plant beneficial rhizobacteria [52]. Higher bacterial numbers in M/L_{MPR} – F treatment in the LRS of both years and M_{MPR}- L treatment during the LRS of 2011 at maize maturity may be attributed partly to the continual availability of P to bacteria due to high residual effect of MPR. In an experiment on effect of combining organic residues with minjingu phosphate rock on sorption and availability of phosphorus and maize production in acid soils of western Kenya, MPR applied alone in the first season gave a significant residual effect [53]. In another experiment on use of MPR to

improve P availability of soil in Morogoro Tanzania, a continual release of P from MPR was similarly observed and was attributed to its long lasting residual effect [54].

Increased extractability of P from MPR by white lupin grown as an intercrop or in rotation with maize in M/L_{MPR} – F and M_{MPR}- L treatments, respectively may have occurred. White lupin plants (*Lupinus albus* L.) have a great ability of mobilizing the sparingly soluble P through changing rhizosphere processes, particularly by citrate exudation [55,43]. P deficiency induces cluster root formation in white lupin [43]. Mature cluster roots secrete greater amounts of carboxylates, mainly citrate that strongly acidifies the rhizosphere [43]. Solubilization, soil extraction and uptake of phosphate into the plant occur mainly at this mature stage of cluster root development [14]. White lupin has been found to effectively utilize P from PR particularly when soils are deficient in P or in response to Al toxicity [56].

The incorporated lupin residues may have also solubilized MPR. Ikerra et al. [54] reported that combining tithonia with MPR increased labile P more intensely than applying MPR alone. Incorporated lupin residues in the intercrop or from lupin grown in the previous short rain seasons, additionally supplied carbon and nutrients also causing the increase in number of bacteria at maturity in the M/L_{MPR} – F treatment during the LRS of both years and M_{MPR}- L treatments in the LRS of 2011. Senescent maize leaves and roots may have also supplied C to the bacteria and causing an increase in their number. Plant material incorporated into soil forms hot spots of high biological activity until the substrate is consumed [57]. Plant residues are the largest source of C entering the soil [58]. The residues enter the soil as dead and decaying above ground biomass, senescent root tissue, sloughed root cells and exudates [58]. A study on soil biota and lupin residue decomposition showed that loss in mass of lupin residues was gradual and it coincided with large numbers of microfauna [59]. In another experiment on effect of crop residue management on microbial properties a greater population of bacteria and fungal hyphae were found on residues of lupin than barley or straw [30].

The lupin residues incorporated and senescent maize leaves may have also contributed to increased bacterial numbers through improvement in physical properties of soil through build up of soil organic matter (SOM). SOM is a function of organic matter inputs (residues and roots) and litter decomposition [60]. Its composition and breakdown rate affect the soil structure and porosity; the water infiltration rate and moisture holding capacity of soils; the diversity and biological activity of soil organisms; and plant nutrient availability [60, 61].

3.1.2.2 Short Rain Season (SRS)

In the SRS of both years, bacterial numbers were significantly higher in M/L_{MPR} – F and M_{TSP}- F treatments across sampling periods (Table 2). Lowest numbers were registered in M_{MPR} –F (weedy fallow) and M_{MPR} –L treatments in both years (Table 2).

The higher bacterial number in M/L_{MPR} –F may partly be attributed to supply of P due to residual effect of MPR. Although MPR is insoluble [62], white lupin residues incorporated after harvest of grain may have enhanced solubilization of MPR and availed P to the bacteria. Solubility of MPR is enhanced in low pH and P limiting soils, in the rhizosphere of vigorously growing legumes and with the application of organics [62]. According to Mclenaghen et al. [63] the combined effect of legume growth (in terms of improved P utilization from PR and subsequent organic matter additions (green manure incorporation) on crop growth, depicts lupin's capacity to solubilise the sparingly soluble (reactive) PR. The enrichment of soil N through biological nitrogen fixation by intercropped white lupin or

incorporated residues from the prior LRS may have also caused increase in bacterial numbers. BNF values of 60-210 kg N ha^{-1} have been reported in white lupin [64]. The higher bacterial numbers in weedy fallow in M_{TSP}- F and M/L_{MPR} –F treatments may also be attributed to C supply from the senescent maize leaves of the LRS.

The M_{MPR} –F treatment had significantly lower (*P*=.05) bacterial numbers in the SRS of both years probably as a result of unavailability of P as MPR fertilizer is not soluble in water. The continuous cropping of maize with no legume planted may have resulted in depletion of soil nutrients. Monocropping practice depletes soil nutrients over short period of time as a result of continuous cropping on the same piece of land [65]. In a study on impact of cropping system on nutrient uptake by maize, maize crop was found to deplete soil nutrients [66].

The significantly lower bacterial numbers in M_{MPR} –L treatment during the SRS of both years can be attributed to acidification of the rhizosphere by white lupin in response to the insoluble P source (MPR).

3.2 Soil Fungal Population

3.2.1 Influence of season and stage of crop growth

Fungal numbers increased from seedling to 50% flowering and declined at maturity in all treatments in the LRS and M_{MPR}- L in the SRS of both years (Table 3).

During the SRS of 2010 and 2011, the fungal numbers gradually declined with sampling periods in treatments M_{TSP}- F, M_{TSP}- F and M/L_{MPR} – F which were under weedy fallow (Table 3). The t test values for comparison of fungal numbers between the LRS of 2010 and SRS of 2010 were 0.05, 0.038 and 0.192 with the corresponding values for LRS of 2011 and SRS of 2011 being 0.019, 0.49 and 0.017. T-tests failed to reveal statistically significant differences in fungal numbers between SRS of 2010 and LRS of 2010 at crop maturity only. Fungal numbers were generally higher in the second year (Table 3).

Changes in fungal numbers from seedling to maturity in all treatments during the LRS may have been partly due to varying amounts of nutrients such as C available to fungi at the different plant developmental stages. The exudates released may have been lower at seedling and maturity and hence lower number of fungi than at 50 % flowering which had higher release of exudates. Exudates are highly variable with growth stage [67]. The release of organic substances by roots influences nutrient availability in the rhizosphere and indirectly acts on soil microorganisms [39]. Increases in specific carbon substrates and/or signaling compounds support an increased fungal population load [68]. A field study on composition and dynamics of fungal population in bulk and rhizosphere soil of maize, fungal populations underwent pronounced changes during the development of the plant [39]. This was attributed to changing root morphology and root exudation patterns during plant development [69]. A study on qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development showed that younger maize plants exuded considerably higher amounts of ^{14}C labeled organic substances per g root dry matter than older ones [25]. Neumann et al. [43] also reported that faster release of citrate occurred in mature cluster white lupin roots.

Table 2. Population of bacteria (CFU $\times 10^{-5}$ g^{-1} dry soil) at different sampling times and treatments

Treatment	2010						2011					
	LRS			SRS			LRS			SRS		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
M_{TSP}- F	3.9^{a}	5.5^{a}	1.5^{b}	3.1^{a}	3.4^{a}	3.1^{a}	4.1^{a}	5.3^{a}	1.7^{b}	3.3^{a}	3.5^{a}	0.7^{b}
M_{MPR} –F	3.1^{b}	4.9^{b}	1.8^{b}	2.5^{b}	2.8^{b}	1.9^{c}	2.2^{c}	2.6^{b}	1.1^{c}	1.9^{c}	2.1^{c}	0.4^{b}
M_{MPR}- L	3.3^{b}	4.7^{b}	1.7^{b}	2.9^{b}	1.1^{c}	1.2^{c}	3.1^{b}	3.2^{b}	2.2^{a}	2.4^{b}	1.1^{d}	1.3^{a}
M/L_{MPR} – F	2.6^{c}	1.9^{c}	2.6^{a}	3.3^{a}	3.5^{a}	2.9^{b}	2.7^{b}	1.4^{c}	2.4^{a}	2.5^{b}	2.7^{b}	1.2^{a}

Key: LRS = long rain season; SRS= short rain season; S1 = seedling; S2 = 50% flowering; S3 = maturity. Means in a column followed by the same letter are not significantly different at P=.05, using the Tukey mean separation procedure

Table 3. Population of fungi (CFU $\times 10^{-5}$ g^{-1} dry soil) at different sampling times and treatments

Treament	2010 LRS			2010 SRS			2011 LRS			2011 SRS		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
M_{TSP}- F	4.9^{a}	6.9^{a}	2.4^{a}	1.3^{b}	0.5^{b}	0.1^{b}	4.4^{d}	5.6^{c}	1.5^{b}	1.9^{c}	1.7^{d}	0.9^{c}
M_{MPR} –F	4.1^{c}	5.3^{b}	1.8^{b}	1.3^{b}	0.4^{b}	0.2^{b}	5.2^{c}	4.6^{d}	1.6^{b}	2.5^{c}	2.3^{c}	0.5^{d}
M_{MPR}- L	4.2^{c}	5.1^{b}	1.4^{b}	2.4^{a}	4.2^{a}	2.1^{a}	6.1^{b}	6.9^{b}	2.5^{a}	5.1^{a}	5.4^{a}	2.1^{a}
M/L_{MPR} – F	4.4^{b}	7.1^{a}	1.5^{b}	1.4^{b}	0.5^{b}	0.4^{b}	7.2^{a}	10.1^{a}	2.4^{a}	3.7^{b}	3.6^{b}	1.5^{b}

Key: LRS = long rain season; SRS= short rain season; S1 = seedling; S2 = 50% flowering; S3 = maturity. Means in a column followed by the same letter are not significantly different at P=.05, using the Tukey mean separation procedure

The lower fungal numbers at seedling and maturity may also be attributed to lack of vegetative cover and nutrient depletion, respectively. In a study on fungal population and diversity in organically amended soil, low fungal population was found at the cultivation of maize and was attributed to lack of vegetation and organic amendment input [70]. Swer et al. [70] also reported lower fungal population in the pre-harvest of maize and attributed same to insufficient or depletion of nutrient availability for the fungi due to crop nutrient uptake

The gradual decline in fungal numbers in weedy fallow during the SRS of 2010 and 2011 may partly be attributed to reduced nutrients available to fungi due to uptake by weeds. In a study on influence of weeds on soil nutrient dynamics, weeds took up nutrients from soil with resultant increased concentration in aboveground tissue [71].

Higher fungal populations in the LRS than SRS can partly be attributed to rhizodeposition and microclimate modification by maize due to a longer growth cycle. Maize was mainly grown in the LRS while the SRS constituted white lupin and weedy fallow. Swer et al. [70] reported higher fungal species during the maize crop cycle than french bean and attributed it to provision of adequate plant cover by maize which created favourable microclimatic conditions for the profuse growth and sporulation of the fungal species. Higher fungal populations in the second year can as well be attributed to provision of C and improvement of soil physical properties due to SOM build up by incorporated white lupin residues, senescent maize leaves and/or weeds from the previous year.

In an experiment to determine fungal population and diversity in organically amended agricultural soils of India, higher fungal species diversity and richness was observed in plant compost (PC) amended plot [70]. They reported that utilization of weeds and other crop residues (in the form of PC) from the field act as a good source of organic fertilizer both for the population and diversity of the fungal species. Microbial activity occurs at a faster rate when maximum organic matter and favourable conditions are available [70].

3.2.2 Influence of P sources and season

In the LRS of 2010, at seedling stage of maize growth, fungal numbers were significantly higher (P=.05) in the M_{TSP}- F treatment (Table 3). At 50% flowering and maturity in the LRS of 2010, the numbers were significantly higher (P=.05) in the M_{TSP}- F and M/L_{MPR} – F treatments. In the LRS of 2011, the fungal numbers were higher in the M/L_{MPR} – F followed by M_{MPR}- L treatment at all stages of maize growth. During the SRS of 2010 and 2011, the fungal population was higher in the M_{MPR}- L treatment at all sampling periods (Table 3).

The significantly higher fungal population in the M_{TSP}- F treatment at seedling stage in the LRS of 2010 may have been due to availability of P from the highly soluble TSP fertilizer [33] a substrate for soil microorganisms [72]. In contrast, lower fungal numbers in M_{MPR} –F, M_{MPR}- L, M/L_{MPR} –F at maize seedling during the LRS of 2010 may be attributed to P unavailability in soil due to insolubility of applied MPR [34].

Higher fungal numbers in M_{MPR}- L and M/L_{MPR} –F treatments at 50% flowering and maturity of maize in the subsequent LRS of 2011 is attributable to availability of P through solubilization of MPR by lupin. The release of citrate by white lupin and/or incorporation of white lupin residues may have solubilized MPR. Legumes have been shown to increase the dissolution and utilization of PR compared with non-legumes mainly due to rhizosphere processes [73]. Exudation of high amounts of citrate in white lupin has the advantage of being effective in mobilization of a wider range of sparingly soluble P sources such as acid

soluble Ca-P [74]. In a study on increasing PR solubility, white lupin solubilized the sparingly soluble (reactive) PR [63]. This may also explain high fungal numbers in the M_{MPR}- L treatments in the SRS of 2010 and 2011. Swer et al. [70] found significant correlations between fungal population and soil P.

3.3 Correlations between fungal and bacterial populations

The correlation between number of fungi and bacteria was -0.95 at 50% flowering of lupin in the SRS of 2010 and 0.97 at the termination of the experiment in the SRS of 2011. Negative correlation at 50% flowering indicates that as the population of fungi increased bacterial numbers were declining. This could have been as a result of acidification of the rhizosphere by citrate released by white lupin in response to low P in soil. White lupin produces cluster roots in response to P deficiency. The roots strongly acidify the surrounding rhizosphere and exude organic anions, mostly citrate and malate [43]. Bacteria are sensitive to low pH unlike fungi which are versatile and can flourish in wide pH ranges [37]. Positive correlation between fungi and bacteria at the termination of the experiment shows that both organisms increased and none was suppressed. This could be attributed to provision of nutrients such as P from MPR and availability of C and nutrients from organic matter due to residue incorporation and senescent maize leaves from the previous seasons. In a study on fertilizer and manure effects on soil microbes, organic matter increased total soil culturable microbial counts including bacteria, fungi, and actinomycetes [75].

4. CONCLUSION

Bacteria and fungi populations were influenced by stage of crop development, season, input use and cropping systems. Significantly higher bacterial numbers (P=.05) were found in M_{TSP}- F treatment at seedling and 50% flowering of maize in both LRS of 2010 and 2011. At maturity, significantly higher bacterial numbers were observed in M/L_{MPR} – F treatment during the LRS of 2010 and M/L_{MPR} – F and M_{MPR}- L treatments during the LRS of 2011. During the SRS of both years, bacterial numbers were significantly higher in M/L_{MPR} – F and M_{TSP}- F treatments across sampling periods. During the LRS of 2010, at seedling stage of maize growth, fungal numbers were significantly higher (P=.05) in M_{TSP}- F treatment. At 50% flowering and maturity in the LRS of 2010, the numbers were significantly higher (P=.05) in the M_{TSP}- F and M/L_{MPR} – F treatments. In the LRS of 2011, the fungal numbers were higher in M/L_{MPR} – F followed by M_{MPR}- L treatment at all stages of maize growth. During the SRS of 2010 and 2011, fungal population was higher in M_{MPR}- L at all sampling periods.

Number of fungi and bacteria increased in white lupin - maize cropping system. This was due to provision of carbon and nutrients by lupin residues and supply of P by MPR. Positive correlation was found between fungal and bacterial population at termination of the experiment. This indicated that both organisms increased and none was suppressed. Increase in microbial populations is thus a good indicator of soil health and by extension sustainability of the newly introduced white lupin –maize cropping system with use of MPR.

Determination of soil enzymes, soil pH changes and fungal and bacterial strains is recommended. The species of resident fungi in this study were not identified as to whether or not they were phosphate solubilizing microorganisms. This is a subject of further investigation.

ACKNOWLEDGEMENTS

The authors acknowledge the National Commission for Science Technology for funding the study. We thank Department of Crops Horticulture and Soils, Egerton University, for providing laboratory space for microbial analysis.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Doran JW, Zeiss MR. Soil health and sustainability: managing the biotic component of soil quality. Appl Ecol. 2000;15(1):3-11.
2. Pankhurst C, Doube BM, Gupta VVSR. 1997. Biological indicators of soil health. CAB International. UK; 1997;451.
3. Bot A, Benites J. The importance of soil organic matter, Key to drought-resistant soil and sustained food production. FAO Soils Bullet; 2005
4. Roberts DP, Dery PD, Yucel I, Buyer JS. 2000. Importance of pfkA for rapid growth of Enterobacter cloacae during colonization of crop seeds. Appl Environ Microbiol. 2002;66:87–91.
5. Das M, Royer TV, Leff LG. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Appl Environ Microbiol. 2007;73:756–767.
6. Claassens S. The fungal-bacterial ratio for soil health. 2013; Accessed 1^{5th} April 2014. Available: http://www.farmers weekly.co.za.
7. Brussard L, Kuyper T W, Didden WAM, de Goede RGM, Bloem J. Biological soil quality from biomass to biodiversity – Importance and resilience to management stress and disturbance. In Schjønning P, Elmholt S, Christensen BT, editors. Challenges in Modern Agriculture. CABI, Wallingford. 2004;139-161.
8. Winding A, Hund-Rinke K, Rutgers M. The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxic Environ Safety. 2005;62:230-248.
9. Ng JP, Hollister EB, Gonzalez-Charvez MCA, et al. Impacts of Cropping Systems and Long-Term Tillage on Soil Microbial Population Levels and Community Composition in Dryland Agricultural Setting. ISRN Ecology, vol. 2012, Article ID 487370, 11 pages, 2012. doi:10.5402/2012/487370ISRN.i:10.5402/2012/487370
10. Wekesa E, Mwangi W, Verkuijl H, Danda K, De Groote H. Adoption of maize production technologies in the coastal lowlands of Kenya. Mexico, D.F. CIMMYT; 2003.
11. Lelei JJ, Onwonga RN, Freyer B. Organic based nutrient management strategies: Effect on soil nutrient availability and maize (*Zea mays* L.) performance in Njoro, Kenya. African J Agric Res. 2009;4(2):092-099.

12. Onwonga RN, Lelei J, Freyer B, Friedel JK, Mwonga SM, Wandahwa P. Low cost techniques for enhancing N and P availability and maize (*Zea mays* L.) performance on acid soils. World J Agr Sci. 2008;4(S):862-873.
13. Haque I, Lupwayi NZ, Ssali H. Agronomic effectiveness of unacidulated and partially acidulated Minjingu rock phosphates on *Stylosanthes guianensis.* Trop Grassl. 1999;33:159–164.
14. Weisskopf L, Abou-Mansour E, Fromin N, Tomasi N, Santelia D, Edelkott I, et al. White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. Plant Cell Environ. 2006;29:919–927.
15. Gilbert GA, Knight JD, Vance CP, Allan DL. Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant Cell Environ. 1999;22:801–810.
16. Wang BL, Shen JB, Zhang WH, Zhang FS, Neumann G. Citrate exudation from white lupin induced by phosphorus deficiency differs from that induced by aluminum. New Phytol. 2007;176:581–589.
17. Tilak KVBR, Ranganayaki N, Pal K, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN. Diversity of plant growth and soil health supporting bacteria. Curr Sci. 2005;89:136-150.
18. Jaetzold R, Schimdt H, Hornetz B, Shisnaya C. Farm Management Handbook of Kenya. Natural Conditions and Farm Mannegement Information. Volume IIA. Nairobi Kenya. 2007;319.
19. FAO-UNESCO Soil map of the world. Revised legend. World resources. Report 60, FAO, Rome; 1990.
20. Landon, JR. Booker Tropical Soil Manual. A Handbook for soil survey and agricultural land evaluation in the tropics and subtropics. Longman Scientific and Technical Essex, New York. 1991;474.
21. McLean E. Methods of Soil Analysis. In Black, C.A, editor. Agron. No. 9. Part II. Am Soc Agron, Madison, Wisconsin, USA. 1982;978-998.
22. Johnson LF, Curl EA. Methods for Research on the Ecology of Soil-Borne Plant Pathogens. 426 So. Sixth St., Minneapolis, MN 55415: Burgess publishing Company; 1972.
23. SPSS. Statistical package of the social sciences vol. 10.0. SPSS Inc., Chicago, Illinois; 1999.
24. Farrar J, Hawes M, Jones D, Lindow S. How roots control the flux of carbon to the rhizosphere. Ecology. 2003;84:827-837
25. Gransee A, Wittenmayer L. Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. Plant Nutr Soil Sci. 2000;163,4:381-385.
26. Aulakh MS, Wassmann R, Bueno C, Kreuzwieser J, Rennenber H. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol. 2001;3(2):139–148.
27. Dennis PG, Miller AJ, Hirsch PR. Are root exudates more important than other sources of rhizodeposits in determining the structure of rhizosphere bacterial communities? FEMS Microbiol Ecol. 2010;72:313–327.
28. Carvalhais LC, Dennis, PG, Fedoseyenko D, Hajirezaei MR, Borriss R, von Wirén R. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. Plant Nutr Soil Sci. 2010;000,1–9.
29. Barbour WM, Hattermann DR, Stacey G. Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. Appl Environ Microbiol. 1991;57:2635–2639.

30. Cookson WR, Beare MH, Wilson PE. Effects of prior crop residue management on microbial properties and crop residue decomposition. Appl Soil Ecol. 1998;7:179-188.
31. Martius C, Höfer C, Garcia MVB, Römbke J, Förster B, Hanagarth W. Microclimate in agroforestry systems in central Amazonia: does canopy closure matter to soil organisms? Agrofor. 2004;60:291-304.
32. Moore JM, Klose S, Tabatabai MA. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biol Fert Soils. 2000;31:200–210.
33. Lindsay EA, French K. The impact of the weed *Chrysanthemoides monilifera* ssp. rotundata on coastal leaf litter invertebrates. Biol Inv. 2004;8(2):177-92.
34. Thuita MN, Okalebo JR, Othieno CO, Kipsat MJ, Bationo A, Sanginga N, Vanlauwe B. An attempt to enhance solubility and availability of phosphorus from phosphate rocks through incorporation of organics in western Kenya. Proc African Crop Science Conference. 2005;7:1021-1027. Printed in Uganda.
35. Tokuda S, Hayatsu M. Nitrous oxide emission potential of 21 acidic tea field soils in Japan. Soil Sci Plant Nutr. 2002;47:637-642.
36. Shane M, Lambers H. Cluster roots: a curiosity in context. Plant and Soil. 2005;274:101–1.
37. Rousk J, Brookes PC, Baath E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. App Env Microbiol. 2009;149(3):1589-1596.
38. Cruz-Martinez K, Suttle, KB, Brodie EI, Power ME, Anderson GI, Banfield JF. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME J. 2009;3:738–744.
39. Gomes, NCM, Heuer H., Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K. Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil. 2001;232:167–180.
40. Lesuffleur F, Paynel F, Bataille MP, Le Deunff E, Cliquet JB. Root amino acid exudation: Measurement of high efflux rates of glycine and serine from six different plant species. Plant Soil. 2007;294:235–246.
41. Bowen GD, Rovira AD. The rhizosphere, the hidden half of the hidden half. In Waisel Y, Eshel A, Kafka U, editors. Plant Roots, the hidden half. Marcel Dekker, Inc. New York. 1991;641-629.
42. Baudoin E, Benizri E, Guckert A. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol Biochem. 2003;35:1183-1192.
43. Neumann G, Masonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E. Physiological aspects of cluster root function and development in phosphorus deficient white lupin (*Lupinus albus* L.). Ann of Bot. 2000;85:211-234.
44. Lijeroth E. Bååth E. Bacteria and fungi on roots of different barley varieties (*Hordeum vulgare L*.). Biol Fertil Soils. 1988;7:53-57.
45. Kowalchuk GA, Buma DS, De Boer W, Klinkhamer PGL, Van Veen JA. Effects of above-ground species composition and diversity on the diversity of soil-borne microorganisms. Anton Van Leeuwen. 2002;81:509–520.
46. Kremer RJ, Begonia MFT, Stanley L, Lanham ET. Characterization of rhizobacteria associated with weed seedlings. Appl Environ Microbiol. 1990;56:1649–1655.
47. Moorthy BTS, Mitra BN. Influence of Seeding Densities and Weed Management Practices on Nutrient Uptake by Crop and Weeds in Upland Rice Ecosystem. Inst Agric Anim Sci. 1990;11:89-93.

48. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol. 2006;57:233–266
49. Shahab S, Ahmed N. Effect of various parameters on the efficiency of zinc phosphate solubilization by indigenous bacterial isolates. Afr J Biotechnol. 2008;7(10):1543-1549.
50. Fagerbakke KM, Heldal M, Norland S. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. Aquat Microb Ecol. 1996;10:15–27.
51. Rengel Z. Genetic control of root exudation. Plant Soil. 2002;245:59-70.
52. Neal AL, Ahmad S, Gordon-Weeks R, Ton J. Benzoxazinoids in root exudates of maize attract *Pseudomonas putida* to the rhizosphere. PLoS ONE. 2012;7(4):e35498. doi:10.1371/journal.pone.0035498.
53. Kifuko MN, Othieno CO, Okalebo JR, Kimenye LN, Ndungu KW, Kipkoech AK. Effect of combining organic residues with minjingu phosphate rock on sorption and availability of phosphorus and maize production in acid soils of Western Kenya. Exper Agric. 2007;43:51-66.
54. Ikerra ST, Sem E, Mrema JP. Combining *Tithonia diversifolia* and Minjingu phosphate rock for improvement of P availability and maize grain yields on a chromic acrisol in Morogoro, Tanzania. In Bationo A, Waswa B, Kihara J, Kimetu J, editors. Advances in Soil Fertility Management in Sub-Saharan Africa: Challenges and Opportunities, Springer, Germany. 2007;333-344.
55. Veneklaas EJ, Stevens J, Cawthray GR, Turner S, Grigg AM, Lambers H. Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil. 2003;248:187–197.
56. Arcand MM, Schneider KD. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. Anais da Acad Bras de Ciên. 2006;78(4):791-807.
57. Kandeler E, Tscherko D, Spiegel H. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a chernozem under different tillage management. Biol Fertil Soils. 1999;28(4):343-351.
58. Carter MR, Stewart BA. Structure and Organic Matter Storage in Agricultural Soils. Advances in Soil Science. Lewis Publishers, CRC Press, Boca Raton, FL, USA. 1996;477.
59. van Vlieta J, Guptab VVSR, Abbotta LK. Soil biota and crop residue decomposition during summer and autumn in south-western Australia. Appl Soil Ecol. 2000;14:111–124.
60. Bot A, Benites J. The importance of soil organic matter, Key to drought-resistant soil and sustained food production; FAO Soils Bullet; 2005.
61. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. FEMS Microbiol Ecol. 2003;43:35–43.
62. Okalebo JR, Othieno CO, Woomer PL, Karanja NK, Semoka JRM, Bekunda MA, Mugendi DN, Muasya, RM, Bationo A, Mukhwana EJ. Available technologies to replenish soil fertility in East Africa, Nutr Cycl Agroecosys. 2006;76:153-170.
63. Mclenaghen, RD, Randhawa PS, Condron LM, Di HJ. Increasing phosphate rock availability using a lupin green manure crop. Super Soil: Proc 3 Australian New Zealand Soils Conference, University of Sydney, Australia; 2004.
64. Herridge DF. Biological nitrogen fixation: Techniques to enhance. Encycl Soil Sci. 2006;163-173.

65. Akintokun OO, Adetunji MT, Akintokun PO. Phosphorus availability to soybean from an indigenous phosphate rock sample in soil from southwest Nigeria. Nutr Cycl Agro Ecosy. 2003;65:35-42.
66. Sangakkara UR, Richner W, Schneider MK, Stamp P. Impact of intercropping beans (*Phaseolus vulgaris* L.) and sunhemp (*Crotalaria juncea* L.) on growth, yields and nitrogen uptake of maize (*Zea mays* L.) grown in the humid tropics during the minor rainy season. Maydica. 2003;48(3):233-238.
67. Prosser J, Rangel-Castro JI, Killham K. Studying plant–microbe interactions using stable isotope technologies. Curr Opin Biotechol. 2006;17:98–102.
68. Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM. Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol. 2008;74:738-744.
69. Brimecombe MJ, de Leij FA, Lynch JM. The effect of root exudates on rhizosphere microbial populations. In Pinton E, Varanini Z, Nanniperi R, editors. The rhizosphere: biochemistry and organic substances at the soil-plant interface. Springer, Netherlands. 2001;95–140.
70. Swer H, Dkhar MS, Kayang H. Fungal population and diversity in organically amended agricultural soils of Meghalaya, India. Organ Sys. 2011;6(2):3-12.
71. Blank RR, Young JA. Influence of three weed species on soil nutrient dynamics. Soil Sci. 2004;169(5):385-397.
72. Jayaprakash GM, Vinaya SGJ, Singaracharya MA. Replica plate screening method for detecting phosphatase activityin Basidiomycetesusing 1-naphthyl phosphate as a chromogenic substrate, Sci World. 2008;3:13-15.
73. Vanlauwe B, Diels J, Sanginga N, Carsky RJ, Deckers J, Merckx R. Utilization of rock phosphate by crops on a representative toposequence in the Northern Guinea savanna zone of Nigeria: response by maize to previous herbaceous legume cropping and rock phosphate treatments. Soil Biol Biochem. 2000;32:2079-2090.
74. Dinkelaker B, Römheld V, Marschner H. Citric acid excretion and precipitation of calcium in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell and Environ. 1989;12:285–292.
75. Reilly K, Cullen E, Lola-Luz T, Stone D, Valverde J, Gaffney M, Brunton N, Grant J, Griffiths BS. Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. J Sci Food Agric. 2013;93(15):3700-9.

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