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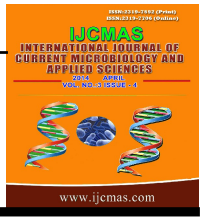

Original Research Article

Response of microbial populations, soil available, P and yield of Lupin (*Lupinus albus L cv. Amiga*) to application of Minjingu phosphate rock - A green house study

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Keywords

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A B S T R A C T

The objective of the current study was to determine soil fungal and bacterial populations, available P and uptake, and yield of white lupin (*Lupinus albus)* under varying P rates. The source of P was minjingu phosphate rock (MPR). Soil for the study was obtained from Egerton University – Njoro agricultural field Twelve pots arranged in a randomized complete block design with three replicates were set up under glass house conditions. Treatments comprised three P rates; control (0 mg P pot^{-1}), 60, 120 and 180 mg P pot^{-1}(corresponding to 0, 30, 60 and 90 kg P ha^{-1}). Plant and soil samples were collected at 28, 56 and 103 days after sowing (DAS). Number of fungi and bacteria colony forming units (CFU g^{-1} dry soil), soil available P and uptake, Dry Matter (DM) of shoots and pods and lupin seed yield were determined. Number of bacteria increased progressively from 28 to 103 DAS. Fungal numbers increased from 28 to 56 DAS and declined at 103 DAS. At 28 DAS, bacterial and fungal numbers were significantly higher ($P<0.05$) in 60 and 90 kg P ha^{-1} treatments. At 56 DAS, bacteria population was higher in control and 30 kg P ha^{-1} while fungal numbers were higher in 90 and 60 kg P ha^{-1} treatments. At final harvest of lupin (103 DAS) lowest numbers of both organisms were observed in control treatments. The control (0 kg P ha^{-1}) had lower levels of available P in soil at all periods of sample collection. Soil available P and uptake by lupin was higher in the 60 and 90 kg P ha^{-1} treatments at all sampling periods. P uptake increased from 28 to 56 DAS and declined at 103 DAS. Lupin DM, pod weight and seed yield were significantly higher in the 60 and 90 kg P ha^{-1} treatments. To optimize lupin production and its subsequent integration in the maize based cropping systems of Njoro, application of MPR at a rate of 60-90 kg P ha^{-1} is a feasible

Introduction

Phosphorus (P) is an important macronutrient for plant growth (Shen et al., 2011). Its deficiency is common in several areas of East Africa, and is aggravated by rising costs of inorganic P fertilizer (Rao et al., 2002; Onwonga et al., 2013). To alleviate P deficiency and improve maize performance, in the central rift valley highlands of Kenya, application of minjingu phosphate rock (MPR) is a

cheaper alternative to the expensive triple super phosphate fertilizer (Onwonga et al., 2013). MPR is the predominant type of phosphate rock (PR) deposit in Eastern Africa with sufficient quantity and reactivity cum potential for direct application (Okalebo et al., 2007). It is however characterized by low solubility in water (Thuita et al., 2005). In the study by Onwonga et al. (2013), solubilization of MPR was achieved by the acidic nature of the study soils (pH in water solution <5.0) and with the application of manure, conditions necessary for PR solubilization (Okalebo et al., 2007). In non acid soils, such as found in Njoro sub-County in Kenya, or in the absence of manure, other alternatives of solubilizing MPR must be explored particularly those involving leguminous plant species.

Certain plant species exhibit mechanisms localized in the rhizosphere that allow for efficient use of P through dissolution of PR (Arcand and Schneider, 2006; Okalebo et al., 2007). One such crop is white lupin (*Lupinus albus* L.). The legume forms special root structures called cluster or proteoid roots in response to P deficiency (Shane and Lambers 2005). The roots strongly acidify the surrounding rhizosphere and secrete large amounts of organic acids, mainly citrate (Weisskopf et al., 2006) and this enables them access sparingly available nutrients such as phosphate (Weisskopf et al., 2011). Soil organisms are sensitive to chemical changes in the surrounding soil (Alexander, 1977; Beauregard et al., 2010). Bacteria and fungi are the dominant decomposers in soil (Waring et al., 2013). A microbial community of fungal: bacterial dominance indicates both its response to environmental change and its impact on ecosystem function (Strickland and Rousk, 2010). Understanding changes in microbial population at different growth stages of white lupin under different rates of P from MPR is therefore of considerable interest.

Further, there is need for information on P rate from MPR required for optimal white lupin production. Such knowledge will be important in the likely introduction of the legume and MPR as P fertilizer source into the maize based cropping systems of Njoro. The objective of the current study was therefore to determine soil bacterial and fungal population, available P and uptake and white lupin yield under varying rates of MPR in a green house study.

Materials and Methods

General

A pot trial was conducted under glasshouse conditions with natural light from January 4^{th,} to April 10th, 2012 at Egerton University, Njoro Kenya. The average monthly temperatures varied between 19-21° C during the day and 14-18° C at night. Soil for the study was obtained from Egerton University agricultural field and had the following physical and chemical characteristics; neutral in pH (pH water 6.4), moderate in organic C (14 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) (Landon, 1991).

Treatments, experimental design and experimental set up

Treatments comprised; a control (0 mg P pot^{-1}), and three P rates; 60, 120 and 180 mg P pot^{-1} (corresponding to 0, 30, 60 and 90 kg P ha^{-1}) on weight basis of 2000 000

kg soil ha^{-1}. The source of P was MPR. Four kilogram air dried soil was mixed with MPR as per outlined treatments. Treated soils were then placed into twelve plastic pots of 25 cm in diameter and 23 cm in height. A randomized complete block design with three replicates was used with each replicate consisting of four pots. Seeds were surface sterilized for 20 min in H_2O_2, rinsed with sterile water and germinated. Two seedlings with fully expanded leaves of the first pair were transplanted to each pot, as the seed represents a significant source of P that may be used potentially under conditions of low P availability (Buhler et al. 2003).i.e. the remainder of the seed was removed from each seedling using a scalpel before transplanting. No mineral nutrients other than P applied. Soil was watered to field capacity (15% w/w) twice a week to replace water losses by evapotranspiration. Weeds were hand pulled as they emerged and left in the pots to decompose.

Soil and plant sampling

About 70 kg top soil (0-15cm) was collected from 15 locations at the agricultural research field site (longitude $35^o23'$ and $35^o35'$ East and Latitude $0^o13'$ and $1^o10'$ south) of Egerton University, Kenya. The soils were composited by mixing thoroughly and then transported to the laboratory. Approximately 20 kg of soil was used for determination of soil initial physical and chemical properties and 48 kg for setting up of pot experiments respectively. During lupin growth in the pot experiment, plant and soil samples were collected at three growth stages: vegetative stage i.e. 28 days after sowing (DAS)], 50% flowering (56 DAS), and at final harvest (103 DAS) when leaves turned yellow. Soil samples were divided into two as per the analysis to be done i.e. measurement of available P and enumeration of fungi and bacteria.

Samples for microbial analysis were put in sterile polythene bags and sealed with rubber bands while those for available P analysis were put in separate polythene bags and sealed. In the laboratory, roots were removed from soil by sieving. Samples for analysis of initial physical and chemical properties and available P (from experimental pots) were air dried in the laboratory while those for microbial analysis stored at 4°C and analysed within 3 days. Appropriate sterile materials were used and sterile conditions maintained throughout the microbial study. Plant samples were taken by cutting the plants two cm above the soil level with a sharp knife. At harvest, pods (which contained seeds) were separated from stem. Shoots collected at the three plant growth stages were rinsed of any adhering soil particles using distilled water, placed in paper bags and oven-dried at 70°C for 24 hours. Dry weight of shoots was determined and a portion ground in a plant grinder with a 2mm sieve, and analyzed for total P to determine nutrient uptake. Pods were shelled manually to obtain seeds. Pods were sun dried and their dry weight determined. Seed yield per plant was also determined.

Soil and plant analysis

Air-dried soils sieved through 2 mm mesh were analyzed for pH (Soil: H_20; 1:2.5), texture, total N, organic carbon and available P according to standard procedures (McLean, 1982). Exchangeable bases (K, Ca and Mg) were extracted with 1.0 M-ammonium acetate at pH 7 and measured by atomic adsorption spectrophotometry (©Analyticjena). The

total P in ground shoot samples was assayed using the vanado-molybdate method as described by Okalebo et al. (2002). Total P uptake was calculated at the three maize growth stages using the following formulae [Peterburgski, 1986];
Total nutrient uptake (kg ha^{-1}) = nutrient concentration (P/100) x dry matter yield (kg ha^{-1}). This was converted to mg g^{-1} dry matter.

Enumeration of soil microbes

A 1:10 dilution was prepared by weighing 1 gm of soil into 10 ml sterile distilled water and shaken thoroughly to mix the soil and water. All tubes were capped to prevent addition of bacteria and fungi from the air. A 1:100 dilution was prepared by pipetting One mL of soil suspension into another tube of 9 mL of distilled sterile water and mixed thoroughly by shaking. A 1:1000 dilution was prepared by again pipetting 1 ml of the suspension into another 9 mL test tube and shaken thoroughly. 1 mL of the 1:1000 dilution was aseptically pipetted onto the surface of nutrient agar for growth of bacteria. A disinfected spreading rod was used to spread the liquid uniformly on the medium. Viable cells of the bacteria were assessed from the plates incubated at 30±0.1^{o} after 48 hours incubation.

The same procedure was followed for enumerating fungal colonies with the exception of medium used to grow fungi and period of incubation. Potato dextrose agar (PDA) was used and plates incubated for seven days. The microbial communities (bacteria and fungi) were enumerated under a digital colony counter as colony forming units (CFU) g^{-1} dry soil on the plates. Number of bacteria and fungi in 1 gm of soil were calculated using the following formulae:

$$\text{Number of colonies} = \frac{\text{Number of bacteria/fungi (c.f.u)/g soil}}{\text{Amount plated} \times \text{dilution}}$$

Statistical analysis

All data were subjected to analysis of variance (ANOVA) to determine statistical variation in treatment effects on measured parameters. Where F- values were significant, Tukey's Honestly Significant Difference was used for mean separation at the 5% probability level. All statistical analyses were performed using SPSS software (SPSS, 1999). Correlations between available soil P and P uptake by lupin were measured using Pearson's correlation coefficient by comparing their means.

Results and Discussion

Bacterial and fungal numbers as influenced by application of MPR with progression of lupin growth

Bacterial and fungal numbers in relation to lupin growth

Number of bacteria and fungi (CFU g^{-1} dry soil) varied with stage of lupin growth (Fig. 1). Number of bacteria increased progressively from 28 to 103 DAS. Fungal numbers increased from 28 to 56 DAS and declined at 103 DAS (Fig. 1).

Increase in microbial numbers from 28 to 56 DAS can be attributed to availability of soil carbon in form of sloughed off root cells and exudates. Plant roots are an active component of soil creating the rhizosphere (Micallef et al. 2009). The rhizosphere is characterized by a significant increase in number and activity of soil microorganisms due to exudation of carbon from roots primarily as mucilages,

simple hexose sugars, and organic anions (Jones et al. 2009; Weisskop et al., 2011). Rhizodeposition is more intense when plants are growing actively (Micallef et al.. 2009). In a study on utilization of root mucilage from pea by rhizobia bacteria (Knee et al., 2001), bacteria grew on mucilage to cell densities of three- to 25-fold higher than controls with no added carbon source, with cell densities of 1 to 15% of those obtained on an equal weight of glucose. In a study to determine the relative importance of roots exudates on fungal composition and diversity, Broeckling et al. (2008) reported that exudates were capable of maintaining fungal community. It is estimated that 2–4% of C from rhizodeposition i.e. root exudates and other organic substances released by plant roots during plant growth, remains adsorbed on clay minerals and SOM, while 0.8–3.2% is incorporated into rhizosphere microorganisms (Van Ginkel et al., 2000).

Lower bacterial numbers at 56 than 103 DAS may be attributed to rhizosphere acidification caused by citrate released from mature root clusters of white lupin. The release of citrate was in response to low available P soil as MPR is insoluble in water (Okalebo et al., 2006). Root secretions contribute to formation of an important adaptive mechanism to phosphorus starvation, by which the plant can alter its microenvironment and subsequently affect phosphate availability in the rhizosphere (Li et al., 1997). In white lupin high quantities of citrate and protons are excreted, at the mature stage of cluster roots leading to drastic rhizosphere acidification (Weisskopf et al., 2006; Neumann et al., 1999). Weisskopf et al. (2006) further reported significant decrease of total bacterial abundance in a study on microbial degradation of citrate, at the mature stage of cluster roots

Tolerance of fungi to acidity may partly explain the higher fungal numbers at 56 DAS than 103 DAS. A study comparing soil fungal/bacterial ratios in a pH gradient showed that low-pH soils had a fungal/bacterial ratio of almost 9, while at pH 7 the ratio was 2 (Baath and Anderson, 2003). In a study on bacterial and fungal communities across a pH gradient, Rousk et al. (2009) reported that relative abundance of fungi was unaffected by soil pH. They reported a fivefold increase in fungal growth with lower soil pH.

Soil pH and nutrients availability are main drivers of soil microbial community composition (Waldrop et al., 2006). Increase in available P in soil from solubilized MPR (Table 2) and rhizodeposition by white lupin may have additionally caused higher fungal numbers at 56 DAS. Fungi are heterotrophic organisms depending on exogeneous C for growth (Jones et al., 2004). A study on regulation of fungal composition by roots exudates suggested that root exudates play an active role in shaping fungal community (Broeckling et al., 2008). In a study on the impact of phosphorus fertilization to fungal diversity, changes in soil P fertility associated with fertilization was attributed to shifts in fungal communities (Beauregard et al., 2010). The lower fungal numbers at 103 than 56 DAS may be attributed partly to declining root exudates with aging of the plant and low/declining organic matter content in the soil. As plants age, there is a slowing down of release of exudates as plants reach the end of their life cycle (Micallef et al. 2009). Most organic matter originates from plant tissues (Bot and Jose, 2005). It takes plant growth to develop most soil organic matter. In the pot experiments there was one cycle of plant growth and lupin residue was not incorporated into soil, and therefore lack

of soil organic matter build up. Continual addition of decaying plant residues to soil surface contributes to biological activity and carbon cycling process in the soil (Bot and Jose, 2005). Breakdown of soil organic matter and root growth and decay also contribute to these processes (Bot and Jose, 2005).

The reduction in fungal numbers at 103 DAS may also be linked to production of antifungal compounds by lupin. Many plant secondary metabolites are known to have anti fungal properties (Broeckling et al., 2008). Isoflavanoids released by lupin, stimulated by low pH levels in the rhizosphere of cluster roots, have antibiotic properties (Wojlaszek et al., 1993). White lupin produces large amount of different isoflavonoids (Katagiri et al., 2000) and some of them act as antifungal compounds (Wojtaszek and Stobiecki 1997; Bednarek et al. 2003). Weisskopf et al. (2006) showed that white lupin isoflavonoids induced sporulation in several fungal strains. Higher bacterial numbers at 103 DAS than 56 DAS may be attributed to higher colonization of old root clusters due to reduced competition from fungi. Many fungi and bacteria compete for the same resources (Rousk et al., 2008). In a study on cluster root function of P deficient white lupin, application of citric acid exogenously was 90% recoverable in senescent clusters (Neumann et al., 1999). There was little effect of microbial degradation of citric acid due to higher microbial colonization rate at the surface of older roots (Foster, 1986).

Relation between MPR application rates and soil bacterial and fungal numbers

At 28 DAS bacterial numbers were significantly higher ($P<0.05$) in 90 kg P ha^{-1} treatment than 60 kg P ha^{-1} rate (Fig. 1). Least bacterial numbers were found in control (0 kg P ha^{-1}) and 30 kg P ha^{-1} treatments. Treatment effects on fungal population at 28 DAS was similar to bacteria. Fungal numbers were higher at higher P application rates in the order 60 > 90 > 30 > 0 kg P ha^{-1} (Fig. 1). At 56 DAS, bacteria and fungi responded differently to MPR rates used. The bacteria population was higher in lower rates of P (control and 30 kg P ha^{-1}). Least bacterial numbers were found in the 60 and 90 kg P ha^{-1} treatments. For fungi, their numbers were significantly higher in the higher P rates in the order 60> 90>30>0 kg P ha^{-1} (Fig. 1). At final harvest of lupin (103 DAS) the number of bacteria and fungi were significantly higher with MPR application than the control (Fig. 1). Higher fungal and bacterial numbers in 60 and 90 kg P ha^{-1} treatments than control and 30 kg P ha^{-1} treatments at 28 DAS may be attributed to increased availability of P in soil (Table 2). Available P in soil was greater with increasing P rate (Table 2). Phosphorus is an important macronutrient for all living organisms (Marschner, 1995). In a study on phosphorus fertilization impacts on soil fungal and bacteria diversity involving different P rates, change in soil P fertility with application of fertilizer was associated with shifts in the composition of fungal and bacterial communities (Beauregard et al., 2010).

Despite higher levels of available P in soil with application of 60 and 90 kg P ha^{-1} at 56 DAS (Table 2) the number of bacteria was low. This may be attributed to acidification of the rhizosphere by citrate released form mature lupin root clusters. Mature lupin cluster roots strongly acidify the rhizosphere by citrate exudation (Neumann et al., 1999). Li et al. (1997)

reported that proteoid roots can decrease the pH of rhizosphere soil to as low as pH 3.6. In a calcareous soil (20% $CaCO_3$), proteoid roots of white lupin (Lupinus albus) acidified rhizosphere from pH 7.5 to 4.8 (Dinkelaker et al., 1989). Bacteria are sensitive to low pH (Rousk et al., 2010). Indirect liming effect of MPR due to its high calcium content (Zin et al., 2005) may have been unlikely in the short duration of the experiment. In a long term field experiment (2002-2007) investigating effect of phosphate rock on soil nutrient dynamics, values of pH increased gradually from 2004 to 2007 for PR treatments (Danso et al., 2010). Higher number of bacteria in control and 30 kg P ha^{-1} treatments at 56 DAS the pH decline from its initial alkaline value may have not been drastic. This may be attributed to lower secretion of citrate in control and 30 kg P ha^{-1} or there may have been fewer cluster roots in these treatments. This is however a subject for further investigation.

The higher fungal numbers with higher P application rates at 56 DAS may be attributed to increased availability of P in soil (Table 2) coupled with fungal tolerance to soil acidity. Phosphorus is essential for fungal growth (Herrera-Estrella et al., 2012). P in its oxidized form is incorporated into many of the biomolecules in a plant or fungal cell, such as to provide genetic material, membranes, and molecular messengers, among others (Herrera-Estrella et al., 2012). In a study on bacterial and fungal communities across a pH gradient the relative abundance of fungi was unaffected by pH and fungal diversity was only weakly related with pH (Rousk et al., 2010).

The higher number of bacteria and fungi in 30, 60 and 90 kg P ha^{-1} treatments than control at 103 DAS may be as a result of higher availability of P in soil (Table 2). In the control treatment, initial available P in soil (Table 2) was utilized by lupin for growth and may partly explain the low microbial numbers in this treatment at all sampling periods. P is an important plant macronutrient (Schachtman et al., 1988) and plants derive P needs from soil (Marschner, 1995). Both organic and inorganic forms of phosphorus are important sources of phosphorus for plant growth (Obaga et al., 2002).

Soil available P as affected by stage of lupin growth and MPR application

Soil available P declined with progression of lupin growth and development. The control had lower levels of available soil P at 28, 56 and 103 DAS (Table 2). Soil available P was higher in the 90 kg P ha^{-1} followed by 60 kg P ha^{-1} treatments at all sampling periods (Table 2)

Decline in available P with crop growth was due to uptake by lupin and microbial immobilization. P is one of the essential elements required for normal growth and development of plants (Marschner, 1995). Most crops take up majority of the nutrients during periods of vegetative growth (Mengel, 1996). Soil microorganisms act as sink and source of P and mediate key processes in soil P cycle such as P mineralization and immobilization (Oberson and Jones, 2005).

Available P was low in control treatment because no P input was added to soil and initial P in soil had been taken up by lupin for growth. Sources of phosphorus include inorganic or mineral P, and organic P sources like animal manure, compost and green manure (Ojiem, 2006) or low cost materials such as PRs (Waigwa et al., 2003).

Lupin however solubilized MPR resulting into increased availability of P in MPR applied treatments over control. Plant and microbial mechanisms can effectively extract P from PR and release it into soil solution or into the labile fraction of soil (Arcand and Schneider, 2006). White lupin plants (Lupinus albus L.) have a great ability of mobilizing the sparingly soluble P through changing rhizosphere processes, particularly by citrate exudation (Veneklaas et al., 2003; Neumann et al., 2000). P deficiency induces cluster root formation in white lupin (Neumann et al., 2000). Mature cluster roots secrete greater amounts of carboxylates, mainly citrate that strongly acidifies the rhizosphere (Neumann et al. 2000). Solubilization, soil extraction and uptake of phosphate into the plant occur mainly at this mature stage of cluster root development (Weisskopf et al., 2006). White lupin has been found to effectively utilize P from PR particularly when soils are deficient in P or in response to Al toxicity (Arcand and Schneider, 2006). Gardner et al. (1983) reported that the proteoid roots of P-deficient lupin plants secreted large quantities of citric acid. They reported that adding varying amounts of citrate resulted into release of large amounts of inorganic phosphate from insoluble phosphated ferric hydroxide. The higher available P content at higher P rates than control can be attributed to solubilization of MPR by citrate released from lupin root clusters (Neumann et al., 1999). The addition of PR to soil increases total soil P with the potential to replenish labile P and plant-available P (Arcand and Schneider, 2006).

Plant P uptake as affected by MPR application and sampling times.

P uptake by lupin increased from 28 to 56 DAS and declined at 103 DAS. Lowest uptake was found in the control treatment at all sampling periods. At 28 DAS highest uptake of P was observed with application of 90 kg P ha^{-1} followed by 60 and 30 kg P ha^{-1} (Table 3).

No significant differences in P uptake occurred in the latter two treatments at 28 DAS. At 56 DAS, highest uptake was found in the treatments with higher P rates (90 and 60 kg P ha^{-1}). At 103 DAS, highest uptake levels were found in the 90 kg ha^{-1} application rate with no significant differences observed in the other treatments (Table 3). The correlation (r^2) between soil available P and uptake was significant at 28, 56 and 103 DAS (0.948, 0.920 and 0.975, respectively). Higher P uptake at 56 DAS can be attributed to requirement of this element in the symbiotic nitrogen fixation process. Ojiem (2006) reported that P was essential for N_2 fixation by common bean. At 56 DAS, lupin was at 50% flowering, a stage in which BNF was close to maximum values. Symbiotic nitrogen fixation is maximum at flowering (Voisin et al., 2003). Declined P uptake at 103 DAS in all treatments may have been due to transfer of nutrients to the reproductive part of the plant such as seeds. Developing seeds are net importers of organic and inorganic nutrients (Patrick and Offler, 2000). Kisetu and Teveli (2013) observed low P and N contents in green gram plant tissues and attributed it to increase in number of pods per plant and the number of seeds per pod. They reported that most of the N and P nutrients contained in Minjingu Mazao fertilizer were transported to actively growing parts of the plant for formation of pods and for seeds production. Lower uptake in the control treatment at all sampling periods can be attributed to low available P in soil (Table 3) The higher P uptake in the

treatments 60 and 90 kg P ha^{-1} at 28 and 56 DAS and in the 90 kg ha^{-1} treatment at 103 DAS was due to higher soil available P content in the soil (Table 3). The significant correlations between available P in soil and uptake by lupin indicates that soil available P influenced P uptake by lupin. The production of citrate by lupin root clusters (Neumann et al., 1999) led to solubilization of MPR and consequently increased the available P content in soil.

Legumes have been shown to increase dissolution and utilization of phosphate rock P compared with non-legumes mainly due to rhizosphere processes (Horst et al., 2001; Kamh et al., 1999; Vanlauwe et al., 2000a). In a study on P uptake in grain legumes and changes in P pools in the rhizosphere, depletion of labile P pools (resin P and $NaHCO_3$-Pi) was greatest in the rhizosphere of faba bean, while sparingly available P pools, particularly residual P, were most strongly depleted in the rhizosphere of white lupin (Mat Hassan, et al., 2010).

The use of lupin green manure to increase rock phosphate availability has been found to significantly increase lupin P uptake and McLenaghen et al. (2004) attributed this to increased P availability from PR dissolution This was enhanced by the legume due to higher Ca uptake and acidification of the rhizosphere or extraction of native non-available P by the lupin GM extracting native non-available P.

Yield and yield components of lupin as affected by MPR application

Lupin DM yield, pod dry weight and seed yield were significantly higher ($P<0.05$) at higher P application rate (60 and 90 kg P ha^{-1}) than control and 30 kg P ha^{-1} treatments (Table 4).

The higher pod weight, seed and DM yields obtained at higher P application rates can be attributed to higher P uptake due to higher available P in soil (Table 2).

Kisetu and Teveli (2013) in a screen – house pot experiment to assess the response of green gram (Vigna radiata L.) to application of Minjingu Mazao fertilizer (31% P_2O_5) on a neutral Olasiti soil, showed that the number of pods and seeds increased from 3-6 and 7-9, respectively, in treatments 40 to 160 mg per 4 kg soil of fertilizer applied. Similarly, the tissue N and P increased with treatment levels. The increase in number of pods per plant and seeds per pod signified the role of N and P in protein synthesis in leguminous plants like green gram. They also observed low responses at low (< 80 mg per 4 kg soil) and high (> 320 mg per 4 kg soil) rates of Minjingu Mazao fertilizer applied. The supply of N through biological nitrogen fixation by lupin legume may have contributed to increased yield and yield components of lupin. Herridge (2006) reported BNF values of 60-210 kg N ha^{-1} in white lupin.

Fig.1 Number of bacteria and fungi (CFU $\times 10^{-5}$ g^{-1} dry soil) under different MPR rates and sampling times

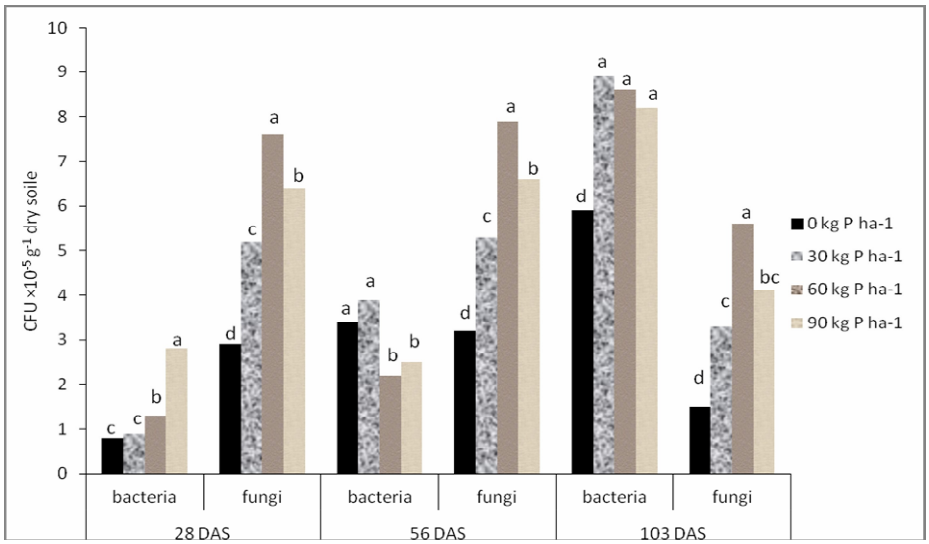

Table.2 Soil available P under different MPR rates and sampling times

P rates (kg P ha^{-1})	Soil available P (mg kg^{-1})		
	28 DAS	56 DAS	103 DAS
0	14.5^{d}	3.3^{c}	2.3^{c}
30	19.2^{c}	3.7^{c}	2.7^{c}
60	28.1^{b}	6.3^{b}	4.3^{b}
90	36.3^{a}	7.7^{a}	8.3^{a}

Key: DAS = days after sowing. Means in a column followed by the same letter are not significantly different at P<0.05, using the Tukey mean separation procedure

Table.3 Plant P uptake under different MPR rates and sampling times

P rates (kg P ha^{-1})	P uptake (mg P g^{-1} DM)		
	28 DAS	56 DAS	103 DAS
0	1.6^{c}	1.2^{b}	0.1^{b}
30	2.2^{b}	2.3^{b}	0.1^{b}
60	2.3^{b}	2.7^{a}	0.2^{ab}
90	3.1^{a}	3.8^{a}	0.3^{a}

Key: DAS = days after sowing; DM = dry matter weight. Means in a column followed by the same letter are not significantly different at P<0.05, using the Tukey mean separation procedure

Table.4 Lupin pod dry weight, DM and Grain yield under different MPR rates and sampling times

P rates (kg P ha^{-1})	Pod dry weight (g $plant^{-1}$)	Grain Yield (g $plant^{-1}$)	DM (g $plant^{-1}$)
0	3.1^{b}	5.2^{c}	1.5^{c}
30	3.6^{b}	6.1^{b}	1.7^{b}
60	5.1^{a}	6.8^{a}	2.1^{a}
90	5.8^{a}	6.9^{a}	2.5^{a}

Key: DAS = days after sowing; GY = grain yield; DM = dry matter weight. Means in a column followed by the same letter are not significantly different at $P<0.05$, using the Tukey mean separation procedure

Conclusion and recommendations

Stage of lupin growth and MPR application rate influenced number of fungi and bacteria in soil. Number of bacteria increased from 28 to 103 DAS whereas fungal numbers increased from 28 to 56 DAS and declined at 103 DAS.

Bacteria numbers were lower at 56 DAS than 103 DAS, despite the increase in rhizodeposition. At 28 DAS bacterial and fungal numbers were significantly higher ($P<0.05$) at 60 and 90 kg P ha^{-1}. At 56 DAS, bacteria population was higher at lower rates of MPR (control and 30 kg P ha^{-1}) while fungal numbers were higher at higher MPR rates. At final harvest of lupin (103 DAS) lowest numbers of both organisms were observed in the control. Soil available P and uptake, yield and yield components of lupin were higher at higher MPR application rates. To optimize lupin production in the mollic Phaeozems, of Njoro sub-County. MPR application rates of 60-90 kg P ha^{-1} is a feasible strategy. Application of 60-90 kg P ha^{-1} to lupin resulted in increased available P in soil and consequently higher soil fungal and bacterial numbers, P uptake, pod yield, lupin DM and seed yield of white lupin. Incorporation of Lupin, with potential to solubilize MPR, into the maize based cropping systems of Njoro is therefore a practicable trajectory for enhanced N and P availability. Characterization of bacterial and fungal species and, mechanisms and microbial processes for P mobilization of MPR is recommended for further study.

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