Journal of Agricultural Science; Vol. 8, No. 12; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Nodulation, Nutrient Uptake and Yield of Common Bean Inoculated with *Rhizobia* and *Trichoderma* in an Acid Soil

Alice M. Mweetwa[1], Gwen Chilombo[1] & Brian M. Gondwe[1]

[1] Department of Soil Science, School of Agricultural Sciences, University of Zambia, Lusaka, Zambia

Correspondence: Alice M. Mweetwa, Department of Soil Sciences, School of Agricultural Sciences, University of Zambia, Box 32379, Lusaka, Zambia. Tel: 260-971-803-918. E-mail: alicemweetwa@yahoo.com

Received: September 1, 2016 Accepted: October 8, 2016 Online Published: November 15, 2016
doi:10.5539/jas.v8n12p61 URL: http://dx.doi.org/10.5539/jas.v8n12p61

Abstract

Common bean is an important source of protein, fat, carbohydrate, vitamins and minerals for both human beings and livestock. In Zambia, common bean is produced mostly by smallholder farmers whose current yields fall far short of the potential 2 t/ha due to various challenges. Among the biophysical constraints, poor soil fertility and acidity pose the greatest challenges. This study investigated the individual and dual inoculation of *Rhizobia* and *Trichoderma* to common bean in a phosphorus deficient, acid soil. Soil in which the common bean was grown was characterized for selected chemical properties before planting and at harvest using standard laboratory procedures. Soils were amended with nitrogen and phosphorus fertilizer at the rates of 100 kg N and 80 kg P_2O_5 per ha; 1 g/kg of seed of *Trichoderma harzianum*; 100 g/kg of seed of *Rhizobium tropici*; or a combination of both at the recommended rates at planting. Nodulation and nodule effectiveness were determined at 51 days after planting. Nitrogen and phosphorus accumulation in the above ground biomass, biomass and grain yields were determined at maturity. To determine differences among soil amendments, data were analysed using Analysis of Variance and Least Significant Difference at 95% confidence limit. Relationships among parameters were determined using correlation analysis. The results showed that amending soils with inorganic N at high rates can depress nodulation even in the presence of high levels of inorganic phosphorus. Inoculating common bean with *Rhizobia* and *Trichoderma* either singly or in combination increases nodule number and effectiveness per plant but may not result in higher nitrogen and phosphorus accumulation, or in an increase in subsequent biomass or grain yields. The low phosphorus accumulation in *Trichoderma* inoculated plants observed in this study needs to be investigated further by studying the extent of colonization and the accompanying changes in root volume.

Keywords: *Rhizobia*, *Trichoderma,* co-inoculation, common bean, nitrogen, phosphorus

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food grain legume in the world (Beebe, 2009; Nakitto, Muyona, & Nakimbungwe, 2015). It is an important source of protein, fat, carbohydrates, vitamins and minerals for humans and livestock (Beebe, Gonzalez, & Rengifo, 2000). Protein content in most varieties is high and averages 25%. The crop is rich in the amino acids tryptophan and lysine, which are not typically found in the staple maize crop. Common bean therefore, provides a good alternative to animal protein to meet minimal daily requirements. In addition, common bean provides other health benefits that include weight control, reduced proneness to diabetes, colon cancer and heart problems (Nyau, 2014).

Current crop production in Zambia covers over 85,000 hectares of land mostly in the Northern, North western and Eastern provinces of Zambia (CSO, 2012). These provinces cover parts of agro-ecological regions II and III of the country, with average seasonal rainfall between 800 and 1000, and more than 1000 mm, respectively. In Zambia, the crop is grown mostly at subsistence and small-scale levels, with only a small portion being grown by commercial farmers. While the potential yield of most varieties is 2 t/ha, the average yield is only 0.58 t/ha (Hamazakaza et al., 2014). Constraints to common bean production have been cited as being the use of unimproved varieties, and attack by pests and diseases. Pests and diseases alone account for 25-50% of the total yield losses (Mweetwa, 2011). In addition, poor soil fertility prevalent in the common bean production areas limits its productivity. Specifically, the leaching of bases in high rainfall areas (agro-ecological region III) results in an accumulation of hydrogen and aluminium ions in the soils. The resulting acidity has both direct and indirect effects on plant growth; common bean grows well in soils with pH ranging from 6.5 to 7.5 (Izquierdo, 1990). On the other hand, common bean is particularly sensitive and highly susceptible to physical and chemical environmental stresses compared to other legumes (Kabahuma, 2013) making the crop very nutrient demanding (da Silva et al., 2014).

In Zambia, most small-scale producers of common bean do not typically use chemical fertilizers to enhance crop productivity. While chemical fertilizers are readily available and may provide immediate answers to current limiting nutrient levels in the soil, they tend to be expensive and in the long-term, cause soil acidification and pollution of soils and water. This has resulted in the promotion of more sustainable agricultural practices such as the use of organic fertilizers to address poor soil fertility. Organic fertilizers have the advantage of improving soil physical properties, acting as substrates for beneficial soil microorganisms, buffering soil reaction, controlling erosion and as well as controlling plant parasitic nematodes and fungi (Gupta, 2011; Panda, 2011) in addition to supplying plant nutrients to the soil. The challenge usually of using organic fertilizers, such as animal and green manures, is that they tend to be bulky and of variable quality, and nutrient release may also be slow. This, coupled with competition for alternative uses on most small-scale farms, limits their use as single solutions to limiting soil nutrients.

As a legume, common bean forms symbioses with a broad range of *Rhizobia* that biologically fix nitrogen in the nodules. Several factors influence nitrogen fixation in legumes; these include soil nutrient conditions, temperature and moisture (Kabahuma, 2013). In biological nitrogen fixation, phosphorus is critical for nodule formation and functioning as well as for specific nitrogenase activity (Kouas, Labidi, Debez, & Abdelly, 2005). In Zambia, regions of common bean production tend to be deficient in phosphorus and high in acidity, this limits biological nitrogen fixation and productivity of the crop. In addition, common bean crop productivity is often limited by nitrogen deficiency despite its ability to form symbiotic relationships with nitrogen fixing *Rhizobia* (Rodino, Santalla, De Ron, & Drevon, 2005); unlike other legumes, common bean is a poor nitrogen fixer; this is attributable to some genetic factors and its sensitivity to physical and environmental stresses (Yadegari & Rahmani, 2010).

The use of bio-fertilizers such as *Rhizobia* and other plant-growth promoting bacteria or fungi have been suggested to result in increased growth and subsequent yield in many crop species including legumes (Stajkovic et al., 2011). *Rhizobia* inoculation alone to seeds or application to the soil at planting has shown varied results in terms of stand count after emergence, stand count at harvest, biomass yield, number of nodules per plant, biological nitrogen fixation and grain yield of common bean (Musandu & Joshua, 2001; Mweetwa et al., 2014a). On the other hand, co-application of *Rhizobia* with other microorganisms such as *Pseudomonas flourescens*, *Bacillus* and *Azospirillum lipoferum* has been reported to result in improved dry weight, phosphorus uptake, nodulation, nitrogen fixation, enzyme activity, protein content, yield and yield components (El-Katany, 2010; Stajkovic et al., 2011; Yadegari & Rahmani, 2010; Samavat, Samavat, Mafakheri, & Shakour, 2012). The benefits of co-inoculating *Rhizobia* with other microorganisms are derived from the ability of these microorganisms to make available to the symbiosome essential nutrients such as phosphorus, to enhance water uptake by the host plant, to produce plant growth regulators and to inhibit some soil-borne plant pathogens through anti-fungal activity and siderophore production (Sharma, Jadega, Kataria, Anamika, & Kumar, 2014; Tatarani, Dichio, & Xiloyannis, 2012; Yadegari & Rahmani, 2010; Izquierdo, 1990).

Trichoderma species are filamentous fungi of the ascomycota known to exist in many soil types of the World (Tatarani et al., 2012). Many species of the *Trichoderma* genus are known to control various plant pathogens through a number of chemiotropic mycoparasitic interactions (Sharma et al., 2014). In addition, *Trichoderma* have been reported to enhance uptake of nutrients and water through stimulated production of lateral roots, and to promote plant growth and biomass yield through the stimulation of the production of several natural plant growth hormones (Tatarani et al., 2012). Dual inoculation of *Trichoderma* and *Rhizobia* to legumes has been reported with conflicting results in terms of the nature of the interactions between the two organisms, and their combined effect on nodulation, biomass production and grain yield. Inhibition of *Rhizobia* growth by *Trichoderma in vitro* has been attributed to competition for nutrients, antibiosis and lysis (Jayaraj & Ramabadran, 1999); on the other hand, compatibility, even synergism has been reported by others.

In view of the potential benefits to biological nitrogen fixation by enhanced phosphorus uptake through *Trichoderma*-plant interactions, a study was undertaken to investigate dual inoculation of *Rhizobia* and *Trichoderma harzianum* to common bean grown in a phosphorus deficient acid soil. This paper reports the effect of singly or co-inoculating *Rhizobia* and *Trichoderma harzanium* on nodulation, nutrient uptake, biomass production and grain yield of common bean under greenhouse conditions.

2. **Materials and Methods**

2.1 Site Description, Soil Collection and Characterization

Greenhouse experiments were conducted at the University of Zambia, 15°24′ South and 28°19′ East. Soils used in the study were collected from the Liempe University of Zambia Farm (15°24′ South and 28°28′ East). This site was specifically selected for soil collection because soils in that location are known to be acid and because the land had been left fallow for a number of seasons.

Soils were collected to a depth of 20 cm from ten (10) random spots and mixed thoroughly to make a composite sample. Soil intended for chemical characterization was air-dried and passed through a 2-mm sieve. Soil was then characterized for reaction (pH) and exchangeable bases. Soil reaction was determined in 0.01 M Calcium chloride using a soil: solution ratio of 1:2.5 using glass calomel combination electrode connected to a pH meter. The ammonium acetate method (Thomas, 1982) was used to determine exchangeable bases of the soil. These soil characteristics were also measured at harvest to determine the effects of the different soil amendments.

2.2 Fertilizer and Inoculation Treatments and Experimental Setup

The study was conducted as a greenhouse experiment during the 2013/14 cropping season. Common bean seeds of the common variety Lyambai were planted in pots with the following fertilizer or inoculation treatments: unamended controls; nitrogen and phosphorus fertilizer application to the soil at planting (100 kg N/ha and 80 kg P_2O_5/ha, respectively); 1 g/kg seed of *Trichoderma harzanium* (Eco-T); 100 g/kg seed of *Rhizobium tropici* (Biofix); a combination of *Trichoderma harzanium* and *Rhizobium tropici* as one application. Microbial inoculants were applied to the seeds just before planting. Each treatment was replicated four times and arranged in a completely randomized design (CRD). Four seeds were sown in each pot (5 kg soil) and thinned to one plant at 14 days after emergence. In order to determine both nodulation and yield of common bean due to these amendments, two sets of experiments were setup exactly as described above.

2.3 Determination of Nodulation and Nodule Effectiveness

At 51 days after planting, plant stems were cut just above the soil level. The remaining below ground biomass was rinsed under running water to remove soil particles from the roots. Sieves were placed at the bottom of the biomass while rinsing to capture any nodules that could have detached during the processing. Nodules from each plant were physically counted from the roots to determine number per plant. In addition, nodule effectiveness was determined by selecting four equal sized nodules; nodules were sliced open with a sharp surgical razor to expose their centre. Nodule colour was then scored as follows: pink and red nodules were scored as effective, while white, brown and/ or green nodules were scored as ineffective. Nodule effectiveness was then calculated as a percent.

2.3 Determination of Nitrogen and Phosphorus Uptake

At maturity, plants were cut just above the soil level. The harvested above ground biomass was dried and weighed and oven dried at 60 °C for 72 hours. Using the modified Kjedahl and Dry Ashing methods, tissue nitrogen and phosphorus were determined (Doll & Lucas, 1975; Kalra & Maynard, 1991).

2.4 Determination of above Ground Biomass and Grain Yield

Above ground biomass yield was determined by cutting the plant stems just above the soil, oven drying at 60 °C to constant weight and weighing;while the grain yield was determined by weighing the shelled pods and as well as the actual seed after shelling.

2.5 Data Management and Statistical Analyses

Effects of different amendments were compared using analysis of variance (ANOVA). Means were compared at 95% confidence using Least Significance Difference (LSD). Other relationships between parameters were made using correlation analysis in Excel Windows software.

3. Results

3.1 Effect of N and P Fertilizer, Rhizobium tropici and Trichoderma harzianum on Number of Nodules per Plant and Nodule Effectiveness in Common Bean

The number of nodules across amendments ranged from 0 to 228 per plant and was significantly affected by the type of amendment. The application of inorganic nitrogen and phosphorus did not yield any nodules, while inoculating with *Rhizobia* and *Trichoderma* resulted in more nodules per plant than the unamended plants. The number of nodules per plant was not significantly different between the single *Rhizobia* and *Trichoderma* treatments with 197 and 189 nodules per plant, respectively (Figure 1). The most number of nodules was observed in co-inoculated plants (228 nodules per plant).

Nodule effectiveness ranged from 25 to 88% and was highest in plants co-inoculated with *Rhizobia* and *Trichoderma* (Figure 1). Single inoculations with *Trichoderma* or *Rhizobia* did not significantly increase nodule effectiveness compared to the unamended controls. In summary, co-inoculating common bean seeds at planting with *Trichoderma* and *Rhizobia* gave the greatest response of both nodule number per plant and percent effectiveness, while single inoculations only significantly increased nodule number per plant and not nodule effectiveness.

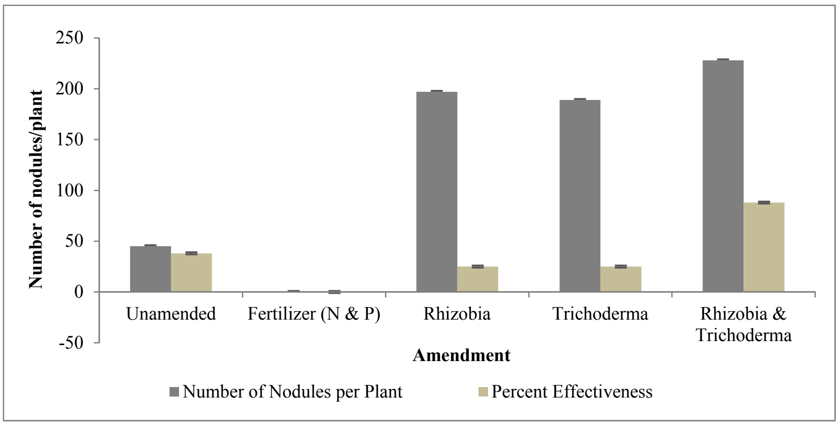

Figure 1. Number of nodules and nodule effectiveness of common bean plants in soils amended inorganic fertilizer, *Rhizobia* and *Trichoderma*

3.2 Nitrogen and Phosphorus Accumulation in Common Beans

Applying inorganic nitrogen and phosphorus resulted in the most nitrogen and phosphorus accumulation in plants at harvest than in the control plants and those treated with *Trichoderma*, *Rhizobia,* and *Rhizobia* and *Trichoderma*. Among the bio-fertilized, *Trichoderma* inoculated plants accumulated the least phosphorus, but did not differ significantly from the unamended control plants, plants inoculated with *Rhizobia,* and *Rhizobia* and *Trichoderma* (Table 1). Compared to the controls, amending soils with inorganic fertilizers resulted in approximately 11 fold increase in P accumulation, while *Rhizobia,* and *Rhizobia* and *Trichoderma* inoculation resulted in approximately 3 fold increases in P accumulation.While there was an increase in the total nitrogen in plants singly or co-inoculated with *Rhizobia* and *Trichoderma*, this increase was not significantly different from the control (Table 1).

Table 1. Nitrogen and phosphorus accumulation in common bean plants supplied with inorganic N and P, and inoculated with *Rhizobia* and *Trichoderma* at planting

Amendment	**Total Nitrogen (mg/plant)**	**Total Phosphorus (mg/plant)**
Unamended	90 b	8.89 c
Fertilizer (N & P)	780 a	110 a
Rhizobia	160 b	30 b
Trichoderma	170 b	15.7 bc
Rhizobia & *Trichoderma*	140 b	26.8 b
LSD	**192**	**14.9**
CV%	**2.13**	**2.15**
p-value	***0.034***	***0.0001***

There was a strong and positive relationship between N and P accumulation in plants (r^2 = 0.972) as well as between number of nodules per plant and N accumulation (r^2 = 0.714) as shown in Figure 2.

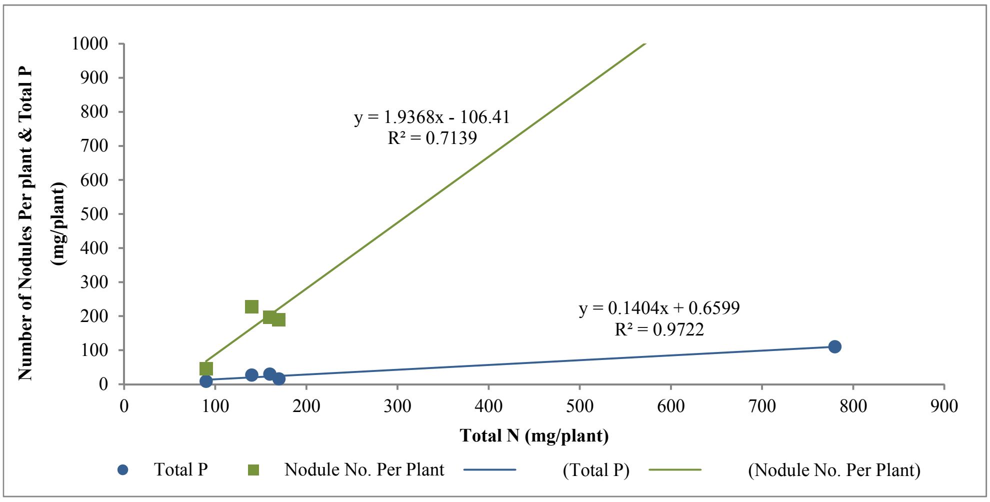

Figure 2. Relationship between Total P at harvest, number of nodules per plant at 51days after sowing and nitrogen accumulation in plants at harvest

3.3 Biomass and Grain Yield of Common Bean Due to N and P Fertilizer, Rhizobia and Trichoderma Application

Applying inorganic nitrogen and phosphorus to the soil resulted in the highest biomass and grain yields compared to the unamended and bio-fertilized common bean crops (Figure 3). While grain yield increased by 314, 163 and 277%, respectively from the unamended controls with *Rhizobia*, *Trichoderma*, and *Rhizobia* and *Trichoderma* application, respectively, these increases were not statistically significant. On the other hand, biomass yield increased significantly from the control by 747, 187, 23 and 230% with fertilizer, *Rhizobia*, *Trichoderma*, and *Rhizobia* and *Trichoderma* application, respectively. Inoculation with *Trichoderma* alone did not result in any significant increase in biomass yield from the unamended control (Figure 3).

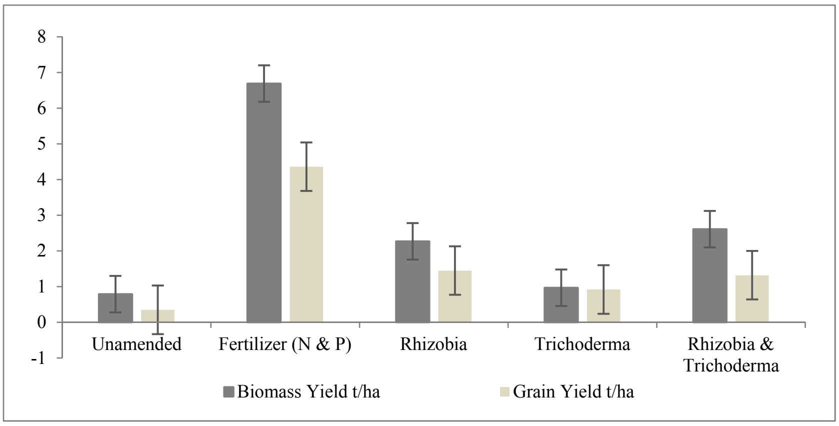

Figure 3. Biomass and grain yield (t/ha) of common bean supplied with inorganic N and P, and inoculated with *Rhizobia* and *Trichoderma*

Grain yield was positively correlated with the amounts of accumulated phosphorus and nitrogen by the plant with r^2 values of 1 and 0.97, respectively. On the other hand, there was a positive but weak relationship between grain yield and number of nodules per plant ($r^2 = 0.21$).

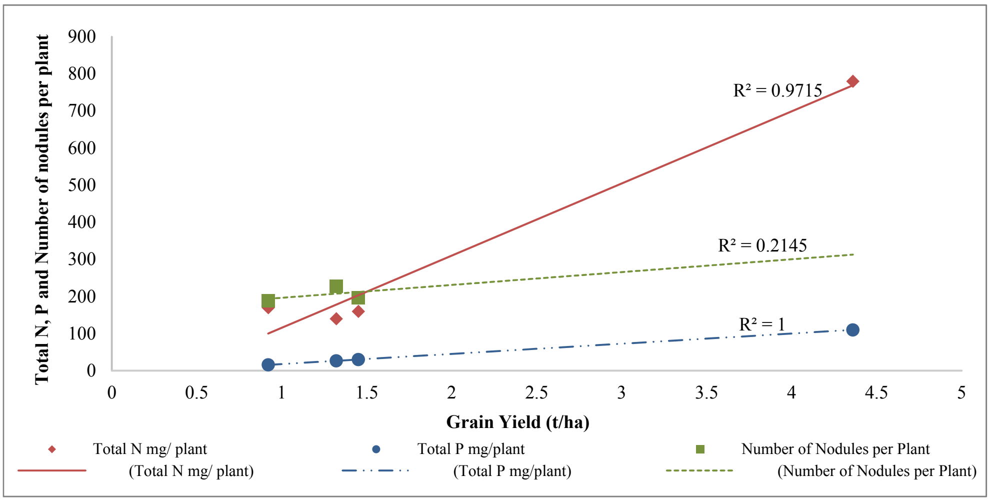

Figure 4. Relationship between Total N & P (mg/kg), number of nodules per plant, and grain yield (t/ha)

3.4 Changes in Soil Reaction and Exchangeable Bases Due to N and P Fertilizer, Rhizobia and Trichoderma Application

Soil pH declined from the initial 5.8 to 5.35, 5.11, 4.39, 5.19 and 4.18 in the unamended soils and soils amended with inorganic fertilizer, *Rhizobia*, *Trichoderma*, and *Rhizobia* and *Trichoderma* combined. The pH and Mg were lowest in soils to which *Rhizobia* was added either as a single inoculant or in combination with *Trichoderma*. The addition of *Rhizobia* appeared to depress pH and magnesium levels.

Calcium levels in soils amended with fertilizer, *Trichoderma*, and *Rhizobia* and *Trichoderma* were comparable to the levels at the start of the experiment (no differences). Unamended soils and soils to which *Rhizobia* was added alone had lower levels of calcium compared to the initial calcium levels. Amending soils with fertilizer resulted in the lowest levels of potassium at harvest.

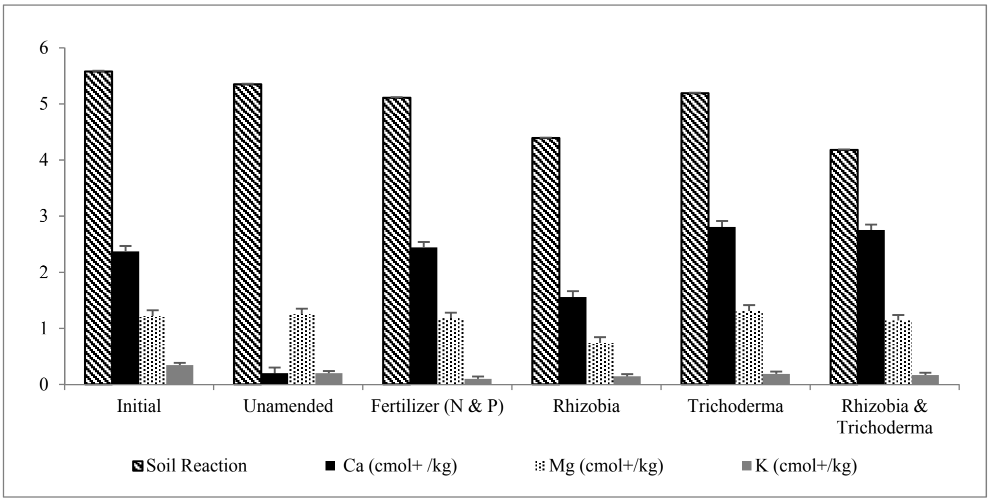

Figure 5. Changes in selected soil chemical properties due to to N and P fertilizer, *Rhizobia* and *Trichoderma* application

4. Discussion

The ability of legumes to form nodules has been attributed to many soil and biological factors that include levels of mineralizable N, levels of available P, soil reaction in the form of pH, type and vigour of legume, *Rhizobia* populations and their effectiveness in infecting and nodulating the host (Mohammadi, Shohrabi, Gholamreza, Khalesro, & Majidi, 2012).

Phosphorus plays a significant role in legume nodulation through its ability to enhance root development and proliferation thereby, affording the *Rhizobia* more sites for infection and initiation of nodule formation. For this reason, addition of phosphorus to the soil has been reported to result in enhanced nodulation of common bean in particular and other legumes, in general. Attar, Blavet, Selim, Abdelhamid, and Drevon (2012) reported higher nodule numbers in plants fertilized with phosphorus; in which case fertilization with 45 and 90 kg P_2O_5 per ha resulted in 31 and 37 nodules per plant, respectively compared to 20 in unfertilized plants. In addition, Muthamia, Kimani, Chemining'wa, and Esilaba (2015) also reported that phosphorus nutrition has a strong influence on the nodulation of common bean, with unfertilized plants having much lower numbers of nodules even in the presence of microbial inoculants. Adding phosphorus to the soil in other forms such as manure, has also been shown to have a positive effect on nodulation (Otieno et al., 1997). Our study on the other hand, reports a complete absence of nodules with the application of 80 kg P_2O_5/ha. We attribute the lack of nodulation to the addition of high levels of inorganic nitrogen at planting (100 kg N/ha). Application of nitrogen in high amounts to common bean at sowing has previously been reported to reduce nodule number, nodule fresh weight and nitrogenase activity (Izquierdo, 1990; Muller & Pereira, 1995; Otieno et al., 2007). Inorganic sources of nitrogen in the form of nitrate and ammonium are preferred by both the *Rhizobia* and legume hosts to biologically fixed dinitrogen which is energetically costly to acquire. Therefore, when levels of soil nitrogen are sufficient, nodulation is inhibited and existing nodules can degrade (Otieno et al., 1997). On the other hand, small starter doses of inorganic nitrogen have been shown to stimulate nodule initiation (Musandu & Joshua, 2001).

Because of the role that phosphorus plays in nodulation, number of nodules is severely restricted when it is limiting (Kouas et al., 2005). While low soil P may limit the numbers of nodules initiated, it does not directly influence functional characteristics nor restrict nitrogenase enzyme activity (Kouas et al., 2005). It must, however, still be noted that phosphorus plays a major role in determining the energy budget of the symbiosome in the form of ATP.

The current study reports significantly increased numbers of nodules with *Rhizobia* alone, *Trichoderma* alone, and *Rhizobia* co-inoculated with *Trichoderma* compared to the control. This increase could be attributed to both direct and indirect interactions between the host plant with *Rhizobia* and *Trichoderma*. Indirectly, *Rhizobia* and *Trichoderma* play some roles in modifying the fertility of the soil mainly through their participation in the decomposition of organic matter, mineral nutrient transformations such as phosphorus solubilisation (Mweetwa et al., 2014b) and modification of soil reaction. Directly, *Trichoderma* increases uptake of nutrients and water due to its ability to increase contact of the plant with the rhizospheric soil through enhanced plant lateral root production and mycelial extensions from the roots (Samolski, Rincón, Pinzón, Viterbo, & Monte, 2012; Pereira et al., 2014). This increases the surface area from which the plant can absorb nutrients required for the formation of nodules and for their functioning. In this paper, we report a 4 fold increase in the number of nodules per plant due to inoculation with *Trichoderma* alone. *Rhizobia* on the other hand, infect the host root system and therefore, directly have an influence on the nodulation process. Inoculating legume seeds at planting with *Rhizobia* has been shown to increase number of nodules per plant (Mweetwa et al., 2014a). Our results show a 4 fold increase in the number of nodules per plant and corroborate with earlier findings that inoculating common bean with *Rhizobia* can cause a significant increase in the number of nodules per plant (Otieno et al., 1997). It must be noted, however, that inoculation with *Rhizobia* may not always result in enhanced nodulation due to many other factors; these include the presence of many but ineffective *Rhizobia* strains; the ensuing competition between the introduced and the indigenous strains; poor quality of the inoculum and unfavourable soil conditions (Mcloughlin & Dunican, 1985; Montealegre & Graham, 1996; Mohammadi et al., 2012). This study shows that there is added value with respect to number of nodules per plant as well as their effectiveness when *Rhizobia* and *Trichoderma* are co-inoculated compared to when they are applied singly. The co-inoculation, brings together both the direct and indirect influences of the individual microbial symbionts.

Total nitrogen and phosphorus accumulated in common bean plants differed significantly among amendments. Inorganic N and P fertilization provided high amounts of available forms of these nutrients explaining why significantly higher amounts were accumulated during plant growth. On the other hand, inoculating with *Rhizobia* or *Trichoderma* alone or together did not result in significantly higher amounts of total nitrogen at harvest. Our results agree with earlier reports that have shown that while inoculating groundnuts, cowpea and soybean with *Rhizobia* resulted in increased nodule number and fresh weigh per plant, there was no corresponding increase in total nitrogen accumulated at harvest (Mungai & Karubiu, 2010; Mweetwa et al., 2014a). The observations in this study suggest that apart from nodule number, other nodule factors such as nodule efficiency play a crucial role in influencing the amount of total accumulated N at harvest (Mungai & Karubiu, 2010). In addition, inoculation with *Rhizobia* potentially introduces moderately effective strains that occupy the nodules, resulting in unimproved nitrogen accumulation or biomass yield (Singleton & Tavares, 1986).

In this study, total nitrogen accumulated was strongly and positively correlated with total phosphorous accumulated ($r^2 = 0.97$). This relationship was deemed important because of the role of phosphorus in nodule function and subsequent nitrogen fixation and accumulation. Unlike nitrogen accumulation, total phosphorus accumulation was significantly higher in microbial amendments than in the unamended. However, results did not show any added value in phosphorus accumulation by inoculating with *Trichoderma* alone or in the presence of *Rhizobia* when compared to *Rhizobia* inoculation alone. This result was rather unexpected; being relatively immobile in the soil, phosphorus acquisition can be enhanced by increasing the contact of root surface area with the soil. While the extent to which *Trichoderma* colonises and responds to the plant varies among species, the hyphae of the fungi when in symbiosis with the host plant have the potential to greatly increase the absorbing surface area of the roots and to increase the amount of phosphorus taken up by the plant (Rubio et al., 2014). In addition, *Trichoderma* has been shown to provide an alternative phosphorus uptake pathway which contributes to the total phosphorus plant uptake. This unexpected observation in this study requires further investigation by looking at the extent of *Trichoderma* colonization and the accompanying changes in root volume and span.

Biomass yield varied across amendments with the highest being observed in plants treated with inorganic P and N fertilizers. This observation has been previously reported by Otieno et al. (1997) and Mungai and Karubiu (2010). Biomass yield was positively and strongly correlated with both total N and P accumulation in the plants ($r^2 = 0.90$ and $r^2 = 0.98$, respectively; data not shown). Phosphorus and nitrogen as plant nutrients are important in plant metabolism as components of chlorophyll and amino acids; they are therefore key in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in plants. This explains why plants that had accumulated the most P and N also had the most biomass yield. In this study, inoculation with *Rhizobium* either alone or in combination with *Trichoderma* resulted in significant increases in biomass yield; this observation is incongruent with earlier reports of non-responsiveness of biomass yield to *Rhizobia* inoculation in other legumes (Mweetwa et al., 2014a).

In the current study, inoculating plants with *Trichoderma* alone, *Rhizobia* alone and *Rhizobia* with *Trichoderma* resulted in non-significantly higher grain yields than the unamended plants. Application of *Rhizobia* alone has previously been shown not to significantly influence common bean grain yields (Musandu & Joshua, 2001; Otieno et al., 1997); our results corroborate these earlier reports. In this study, grain yield was positively and strongly correlated with nitrogen accumulation ($r^2 = 0.97$); therefore, the low grain yield was attributed to the poor responsiveness of total nitrogen accumulation to the various microbial amendments.

Growth of legumes is typically known to depress soil pH due to their high uptake of bases such as calcium, magnesium and potassium in order to meet plant development demands (Liu, Lund, & Page, 1989).The results of this study show a decline in soil pH at harvest across all the amendments. Potassium levels in the soils at harvest had declined from the initial 0.35 cmol+/kg soil in all cases including the unamended soils, while calcium levels declined from their original levels in soils amended with *Rhizobia* alone and the unamended soils. Magnesium levels were generally unchanged by the time of harvest except for soils amended with *Rhizobium* alone. The decline in pH observed across all treatments can therefore be attributed to uptake of bases as indicated by lower soil solution concentrations. While *Trichoderma* has been suggested to buffer pH fluctuations in the soil, this was not true for soils amended with *Trichoderma* alone or in combination with *Rhizobia*.

In conclusion, amending soils with inorganic N at high rates can depress nodulation even in the presence of high levels of inorganic phosphorus which is typically beneficial for nodule initiation and development. Inoculating common bean with *Rhizobia* and *Trichoderma* either singly or in combination can enhance nodule number per plant but may not result in higher nitrogen and phosphorus accumulation, or in an increase in subsequent biomass or grain yields. The poor phosphorus accumulation in *Trichoderma* inoculated plants observed in this study needs to be investigated further by studying the extent of colonization and changes in root volume.

References

Attar, H. A., Blavet, D., Selim, E. M., Abdelhamid, M. T., & Drevon, J.-J. (2012). Relationship between phosphorus status and nitrogen fixation by common beans (*Phaseolus vulgaris* L.) under drip irrigation. *International Journal of Environmental Sciences and Technology, 9*, 1-13. http://dx.doi.org/10.1007/s13762-011-0001-y

Beebe, S. (2009). Improvement of common bean for mineral nutritive content at CIAT. In R. M. Welch, & I. Cakmaka (Eds.), *Impacts of agriculture on human health and nutrition* (Vol. 1). Encyclopaedia of Life Support Systems (EOLSS). Retreived May 9, 2016, from http://www.eolss.net/Eolss-sampleAllCpater.aspx

Beebe, S., Gonzalez, A. V., & Rengifo, J. (2000). Research on trace minerals in the common bean. *Food Nutr. Bulletin, 21*, 387-91.

CSO. (2012). *Agriculture Analytical Report for the 2010 Census of population and housing*. MoFNP, Central Statistical Office. Retrieved from http://zambia.opendataforafrica.org

da Silva, D. A., Esteves, J. A. D. F., Messias, U. A., Teixeir, A., Goncalves, J. G. R., Chiorato, A. F., & Carbonell, S. A. M. (2014). Efficiency in the use of phosphorus by common bean genotypes. *Scientia Agricola, 71*(3). http://dx.doi.org/10.1590/S0103-9016201400030008

Doll, E. C., & Lucas, R. E. (1975). Testing soil for K, Ca and Mg. In L. M. Walsh, & J. D. Beaton (Eds.), *Soil testing and plant analysis* (pp. 133-152). Soil Analysis Society of America, Inc., Madison, Wisconsin, USA.

El-Katany, M. H. (2010). Enzyme production and nitrogen fixation by free, immobilized and co-immobilized inoculants of *Trichoderma harzianum* and *Azospirillum brasilense* and their possible role in growth promotion in tomato. *Food Technol. Biotechnol., 48*(2), 161-174.

Gupta, P. K. (2011). *A handbook of soil, fertilizer, and manure* (2nd ed.). Jodhpur, India: AGROBIOS.

Hamazakaza, P., Katungi, E., Reyes, B., Maredia, M., Muimui, K., & Ojara, M. (2014). *Assessing access and adoption of common bena improved varieties in Zambia*. Research Technical Report-CIAT & MSU.

Izquierdo, M. (1990). *Effects of N and P fertilizers on common bean (Phaseolus vulgaris L.) grown in a p-fixing Nicaraguan mollic andosol* (Master's thesis, Swedish University of Agricultural Sciences, Uppsala). Retrieved from http://www.vaxteko.nu/html/sll/slu/reports_diss_markvetenskap/RDV08/RDV08.HTM

Jayaraj, J., & Ramabadran, R. (1999). *Rhizobium Trichoderma* interaction *in vitro* and *in vivo*. *Indian Phytopathol, 52*, 190-192.

Kabahuma, M. K. (2013). *Enhancing biological nitrogen fixation in common bean (Phaseolus vulgaris L.)*. (Graduate Theses and Dissertations, Paper 13162, Iowa State University). Retrieved from http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=4169&context=etd

Kalra, Y. P., & Maynard, D. G. (1991). *Methods manual for forest soils and plant analysis* (p. 120).

Kouas, S., Labidi, N., Debez, A., & Abdelly, C. (2005). Effect of P on nodule formation and N fixation in bean. *Agron. Sustain. Dev, 25*, 389-393. http://dx.doi.org/10.1051/agro:2005034

Liu, W. C., Lund, L. J., & Page, A. L. (1989). Acidity produced by leguminous plants through symbiotic dinitrogen fixation. *J. Environ. Qual, 18*, 529-534. http://dx.doi.org/10.2134/jeq1989.00472425001800040025x

Mclean, E. O. (1982). Soil pH and lime requirement. In A. L. Page, R. H. Miller & D. R. Keeney (Eds.), *Methods of soil analysis, Part 2: Chemical and microbiological Properties* (2nd ed.). ASA, SSA, Madison, Wisconsin, USA.

Mcloughlin, J. J., & Dunican, L. K. (1985). Competition studies with *Rhizobium trijohi* in laboratory experiments. *Plant and Soil, 88*, 139-143. http://dx.doi.org/10.1007/BF02140673

Mohammadi, K., Shohrabi, Y., Gholamreza, H., Khalesro, S., & Majidi, M. (2012). Effective factors on biological nitrogen fixation. *African J. Agricult. Res., 7*(12), 1782-1788. http://dx.doi.org/10.5897/AJARX11.034

Montealegre, C., & Graham, P. H. (1996). Preference in the nodulation of *Phaseolus vulgaris* cv RAB 39.11. Effect of delayed nodulation or cell representation in the inoculant nodule occupancy by *Rhizobium tropici* UMR 1899. *Canadian Journal of Microbiology, 41*(42), 844-850. http://dx.doi.org/10.1139/m96-106

Muller, S. H., & Pereira, P. A. A. (1995). Nitrogen fixation of common bean (*Phaseolus vulgaris* L.) as affected by mineral nitrogen supply at different growth stages. *Plant and Soil, 177*(1), 55-61. http://dx.doi.org/10.1007/BF00010337

Mungai, N. W., & Karubiu, N. M. (2010). Effectiveness of rhizobia isolates from Njoro soils (Kenya) and commercial inoculants in nodulation of common beans (*Phaseolus vulgaris*). *Journal of Agriculture, Science and Technology, 12*(1), 47-59.

Musandu, A. A. O., & Joshua, O. O. (2001). Response of common bean to *Rhizobium* inoculation and fertilizers. *The Journal of Food and Technology in Africa, 6*(4). http://dx.doi.org/10.4314/jftaa.v6i4.19303

Muthamia, J., Kimani, P. M., Chemining'wa, G., & Esilaba, A. O. (2015). Effects of *Rhizobium*, *Azospirillum*, and *Rhizobium Azospirillum* coinoculation inoculants on climbing beans growth and development in low and high phosphorus sand media. *Proceedings of the 5th Post graduates PhD. Seminar on communication of research findings at the University of Nairobi, Faculty of Agriculture*. Retrieved from http://agriculture.uonbi.ac.ke/sites/default/files/cavs/agriculture/larmat/Proceedings%20of%20the%205th%20PhD%20seminar%20HELD%20on%2018th%20SEPTEMBER%202015.pdf

Mweetwa, A. (2011). Potential for increased yield and market opportunities for beans. *SanBio, 2*(2).

Mweetwa, A. M., Eckhardt, E. A., Stott, D. E., Chilombo, G., Armstrong, A. P., Schulze, D., & Nakatsu, C. H. (2014). Isolation and characterization of Chilembwe and Sinda rock phosphate solubilizing soil microorganisms. *African Journal of Microbiology Research, 8*(34), 3191-3203. http://dx.doi.org/10.5897/AJMR2014.6923

Mweetwa, A. M., Mulenga, M., Mulilo, X., Ngulube, M., Banda, J. S. K., Kapulu, N., & Ngandu, S. H. (2014). Response of cowpea soya beans and groundnuts to non-indigenous legume inoculants. *Sustainable Agriculture Research, 3*(4), 84-95. http://dx.doi.org/10.5539/sar.v3n4p84

Nakitto, A. M., Muyona, J. H., & Nakimbungwe, D. (2015). Effects of combined processing methods on the nutritional quality of beans. *Food Science & Nutrition, 3*(3), 233-241. http://dx.doi.org/10.1002/fsn3.209

Nyau, V. (2014). Nutraceuical perspectives and utilization of common beans (*Phaseolus vulgaris* L.): A review. *African Journal of Food, Agriculture Nutrition Development, 14*(7).

Otieno, P. E., Muthomi, J. W., Cheminig'wa, G. N., & Nderitu, J. H. (2007). Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected grain legumes. *African Crop Science Conference Proceedings, 8*, 305-312.

Panda, S. C. (2011). *Soil management and organic farming*. Jodhpur, India: AGROBIOS.

Pereira, J. L., Queiroz, R. M. L., Charneau, S., Felix, C. R., Ricart, C. A., Lopes da Silva, F., … Noronha, E. F. (2014). Analysis of Phaseolus vulgaris response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani.* http://dx.doi.org/10.1371/journal.pone.0098234

Rodino, A. P., Santalla, M., De Ron, A. A. M., & Drevon, J. J. (2005). Variability in symbiotic nitrogen fixation among white landraces of common bean from the Iberian peninsula. *Symbiosis, 40*, 69-78.

Rubio, M. B., Quijada, N. M., Pérez, E., Domínguez, S., Monte, E., & Hermosa, R. (2014). Identifying beneficial qualities of *Trichoderma parareesei* for plants. *Appl. Environ. Microbiol., 80*(6), 1864-1873. http://dx.doi.org/10.1128/AEM.03375-13

Samavat, S., Samavat, S., Mafakheri, S., & Shakour, J. (2012). Promoting common bean growth and nitrogen fixation by the co-inoculation of *Rhizobium* and *Pseudomonas fluorescen*s isolates. *Bulgarian Journal of Agricultural Science, 18*(3), 387-395.

Samolski, I., Rincón, A. M., Pinzón, L. M., Viterbo, A., & Monte, E. (2012). The qid74 gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiology, 158*, 129-138. http://dx.doi.org/10.1099/mic.0.053140-0

Sharma, A., Jadeja, K. B., Kataria, G. K., Ananika, M., & Kumar, D. J. (2014). Plant growth promoting effect of *Trichoderma* on groundnut. *AGRES-An International e-Journal, 3*(4), 324-352.

Singleton, P. W., & Tavares, J. W. (1986) Inoculation response of legumes in relation to the number and effectiveness of indigenous Rhizobium populations. *Appl Environ Microbiol., 51*(5), 1013-1018.

Stajkovic, O., Delic, D., Josic, D., Kuzmanovic, D., Rasulic, N., Knezevic-Vukcevic, J. (2011). Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth-promoting bacteria. *Romanian Biotechnological Letters, 16*(1), 5919-5926.

Tatarani, G., Dichio, B., & Xiloyannis, C. (2012). Soil fungi-plant interaction. *Advances in selected plant physiology aspects*. Retrieved from http://cdn.intechopen.com/pdfs-wm/35827.pdf

Thomas, G. W. (1982). Exchangeable Cations. In A. L. Page, R. H. Miller & D. R. Keeney (Eds.), *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties* (2nd ed., pp. 159-165). ASA, SSA, Madison, Wisconsin.

Yadegari, M., & Rahmani, H. A. (2010). Evaluation of bean (*Phaselous vulgaris*) seeds inoculation with *Rhizobium phaseoli* and plant growth promoting Rhizobacteria (PGPR) on yield and yield components. *African Journal of Agricultural Research, 5*(9). http://dx.doi.org/10.3923/pjbs.2008.1935.1939