Soil fertility constraints for the production of common bean (*Phaseolus vulgaris* L.) in the Usambara Mountains of northern Tanzania

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Summary

A soil fertility study has been conducted in the West Usambara Mountains of Tanzania to assess the soil fertility constraints for the production of common bean (*Phaseolus vulgaris* L.). Common bean is a major grain legume in Tanzania, but smallholder farmers' yields are far below potential. The West Usambara Mountains is one of Tanzania's main bean production areas, facing the problems of low yield. The area is densely populated and the land has been cultivated intensively over the last decades causing a decline in soil fertility. Up-to-date information about the soil fertility status is needed to improve farmers' bean yields. The aim of this research is to describe the soil fertility constraints for the production of common bean. Experimental field trials with rhizobial inoculation (*Rhizobium tropici* CIAT899) and fertilization with P (26 kg P ha^{-1}) and K (25 kg K ha^{-1}) treatments were performed in a complete randomised block to investigate the effect of inoculation and P and K fertilization on nodulation and yield of common bean. These experiments demonstrate the soil fertility constraints for the production of common bean and how the production can potentially be improved. Soil samples were analysed for chemical and physical properties to describe the biophysical characteristics of the soil and analyse their relation to the treatment effect. Most probable number (MPN) counts were performed to determine the status of indigenous rhizobia populations in the soil and their relation to the response to inoculation. The field trials demonstrated a highly ($P<0.001$) significant response of nodulation to inoculation and P and K fertilization. Crop vigour and yield were also significantly ($P<0.001$) enhanced by P and K fertilization, but inoculation had no effect on crop vigour and yield. There was large variability in yield and yield response between fields, some poorly yielding fields had a lot of on-site variability due to environmental constraints. The chemical soil analysis demonstrated a deficiency in P and K while most other soil parameters were suitable for crop growth. The indigenous rhizobia populations in the area were present in large quantities ($1.2x10^2$ to $2.4x10^5$) which is likely to be the cause for the absence of a response to inoculation. However, nodulation and the impact of inoculation was probably also suppressed by environmental constraints. Management interviews with the field owners showed that farmers don not use any fertilizers or inoculum to beans, which is probably one of the main reasons for the nutrient deficiencies in the soil. Based on this research it can be established that the major soil fertility constraints for the production of common bean are deficiency of P and K in the soil; rhizobia populations are present and absence of compatible rhizobia nor N deficiency are major soil fertility constraints.

Chapter 1 Introduction

1.1 Problem definition

Agriculture is Tanzania's key sector: it is the main source of food for the nation (Muchena and Kiome, 1995), accounts for more than one quarter (27%) of the country's GDP and employs approximately three quarters of the working population (World Bank, 2014). Tanzania's most important grain legume is common bean (*Phaseolus vulgaris* L.). Common bean is a major staple in eastern Africa, where it is the second most important source of dietary protein after maize and the third most importance source of calories after maize and cassava (Hillocks et al., 2006). However, per capita bean consumption is declining as population increases outstrip production (Graham and Ranalli, 1997). Average bean yields in Tanzania are around 500 kg ha^{-1} whereas the attainable yield is 1500-3000 kg ha^{-1} under favourable field conditions (Hillocks et al., 2006).

The West Usambara Mountains in Northern Tanzania is one of Tanzania's main bean production areas. The region is an important agricultural area in Tanzania. It is densely populated and hosts a large community of smallholder farmers (Smithson et al., 1993) that are facing problems of low yield (Tenge, 2005).

Poor soil fertility is considered to be the major constraint for bean production across the region (Smithson et al., 1993). Over the last decades, the Usambara Mountains have been cultivated intensively due to an increased population, resulting in a decline in soil fertility and severe soil erosion (Mbaga-Semgalawe and Folmer, 2000). Many of the farming areas are located on these intensively cultivated and highly degraded areas, which are often located at steep slopes (Ndakidemi and Semoka, 2006). Field studies have demonstrated that especially the deficiency of P and K is the cause for growth and productivity problems observed in common bean (Smithson et al., 1993; Brodrick and Amijee, 1995; Amijee et al., 1998; Amijee and Giller, 1998; Giller et al., 1998; Ndakidemi and Semoka, 2006; Ndakidemi et al., 2006).

Degraded soils have a major impact on the livelihood of people, as a large part of the Tanzanians depend directly on agriculture. The major nutrients nitrogen (N), phosphorus (P) and potassium (K) are essential for growth and production of bean (Marschner, 2012). Beans are legumes that can fix atmospheric nitrogen (N_2) into the soil in symbiosis with soil rhizobia. But besides N, P and K are needed, and in nutrient deficient soils bean yield can be greatly improved with the application of moderate rates of

chemical fertilizers (Ndakidemi et al., 2006). Up-to-date information about the soil fertility status and major nutrient deficiencies is needed to improve farmers' yields and quality of life.

This research focuses on the soil fertility constraints to increase bean yields, such as soil nutrients and rhizobial bacteria of common bean in the West Usambara Mountains in Tanzania. Experimental field trials with rhizobial inoculation and P and K fertilization were performed to evaluate soil fertility constraints for the production of common bean. The outcomes of this study can contribute to improving soil fertility, and thus to productivity of common bean in this area.

1.2 Theoretical framework

1.2.1 Common bean

Common bean is the most important crop among the *Phaseolus* species. It is a crop with enormously variable morphology and growth patterns (Giller, 2001, pp 145-146). It is one of the principal food and cash legumes grown in the tropical world and most of the production takes place in developing countries (Pachico, 1989). Its high protein content (20-25%) supplements diets based on cereals, root and tuber crops and banana; a balanced diet can be obtained if cereals and legumes are consumed in the ratio 2:1 (Broughton et al., 2003).

In Tanzania, beans are the main grain legume crop, mostly produced by smallholder farmers for their own consumption. The main reasons for low crop yields obtained by most smallholder farmers are: poor seed quality, poor performance of the local landraces, mainly due to their susceptibility to pests and diseases, low soil fertility, drought and poor crop management, such as late weeding (Hillocks et al., 2006). Common bean is often intercropped with maize (Hillocks et al., 2006).

1.2.2 Soil fertility problems in the tropics

The major determinants of potential agricultural productivity are climate and soil characteristics. The tropics have the potential to be among the most productive cropping environments in the world, however the yields in tropical cropping systems are often very low (Giller, 2001). The low fertility of highly weathered and nutrient depleted soils is a major constraint to crop production in sub-Saharan Africa (Smaling et al, 1993; Okalebo et al, 2007). These soils are often derived from ancient parent rock that is poor in bases (Giller, 2001). Expanding populations have pushed agriculture onto more marginal, less fertile lands and have shortened the fallow periods available to restore soil fertility (Vlek, 1990). The amounts of nutrients exported from the field through crop harvest, and due to leaching and erosion is exceeding the inputs from organic manure and mineral fertilizers or from natural processes (i.e. from

atmospheric deposition or biological nitrogen fixation) (Smaling et al., 1993) causing an overall nutrient depletion of the soil.

In order to sustain food production in Africa the nutrients extracted from the soil need to be supplemented. Due to the rapidly expanding population, leaving the land fallow for a long period to restore soil fertility is often not an option anymore (Vlek, 1990). One way of restoring soil fertility is by applying inorganic fertilizers (Okalebo et al., 2007). Many studies have shown that the use of inorganic fertilizers can have very positive crop responses (Bationo, 2004). To achieve self-sufficiency in food production in sub-Saharan Africa, fertilizer consumption should be increased in many areas (Vlek, 1990).

1.2.3 Biological nitrogen fixation of common bean

All organisms require nitrogen (N) to live. After carbon, N is the element required in the largest quantity by plants (Marschner, 2012). The greatest proportion of N found on the earth is located in the earth as atmospheric nitrogen (N_2). Most organisms cannot utilize this source of N, but legume plants have the ability to make N_2 biologically available through biological nitrogen fixation (BNF). BNF is the process whereby a number of species of bacteria convert N_2 into ammonia (NH_3), which is biologically useful for plants (Unkovich et al., 2008; Giller, 2001). These bacteria, that are present in the soil, infect the roots of the plant and in symbiosis they form nodules in which BNF takes place.

Common bean is one of the many legume species able to fix N_2 through BNF and can nodulate with many different strains of root nodulating bacteria or rhizobia. Under favourable conditions it can nodulate and fix N_2 abundantly (Giller and Wilson, 1991). However, nodulation in the field is generally poor. The main constraint is not considered to be the absence of compatible rhizobia. Nodulation of common bean is probably limited by some environmental constraint or by other limiting nutrients than N or a combination of the two (Giller, 1990). Beans are mainly produced by smallholder farmers who grow the crop on low fertility soils with no or limited external input. Generally under these conditions effective nodulation with rhizobium is rarely observed and the amount of N_2 fixed is probably small (Amijee et al., 1990). Rates of BNF tend to be highest when plant-available mineral N in the soil is limiting but water and other nutrients are plentiful (Unkovich et al., 2008). In poor and degraded soils, there is thus a need for fertilizer application to enhance the BNF. When effective compatible rhizobia are absent, or only present in small numbers, the rhizobia bacteria can be added by inoculation. The crop yield response to inoculation depends on many local factors, but it has the potential to be an effective and cheap option to increase yield for resource poor farmers (Ndakidemi et al., 2006).

1.3 Literature review

A series of field experiments demonstrated P deficiency to be the main soil nutrient constraint to the nodulation of common bean in the West Usambara Mountains. In 1987 Smithson et al. (1993) diagnosed soil nutrient problems of common bean in the Lushoto area in the West Usambara Mountains. A strong inter-veinal chlorosis was observed that seem to be exacerbated by the addition of P fertilizer. Experimental trials with N, P and K in combination with soil and leaf analysis demonstrated that these symptoms were due to K deficiency. The trials also demonstrated a strong response in growth and yield to N, P and K additions.

Chemical soil analysis and estimation of indigenous rhizobial population in the soils in the Lushoto area by Amijee and Giller (1998) demonstrated that sufficient populations of soil rhizobia were naturally present in most soils in the region, but nodulation was constrained by deficiency of P. Field experiments with inoculation and P fertilizer application in combination with an estimation of indigenous rhizobia and soil chemical analysis by Amijee et al. (1998) found large populations (10^4 cells g^{-1} soil) and no yield response to inoculation. The study recommended fertilization with P to enhance effective nodulation. This was confirmed by the results presented by Giller et al. (1998), who showed a strong response in both nodulation and seed yields to the addition of P fertilizer in the region, while seed yield did not respond significantly to P fertilizer plus inoculation in comparison to P fertilizer alone. However, an overall analysis of all trials in northern Tanzania with common bean and inoculation combined showed a significant response to inoculation (Amijee and Giller, 1998). Similarly designed field experiments in the region with P fertilizer and rhizobia inoculation by Ndakidemi et al. (2006) demonstrated a strong increase of bean yield for both inoculation alone as well as for inoculation in combination with P fertilizer.

A soil fertility study in the West Usambara Mountains based on the chemical analysis of 30 soil samples by Ndakidemi and Semoka (2006) confirmed P deficiency as the major constraint. It also demonstrated N, K, magnesium (Mg) and calcium (Ca) deficiency at a large number of sites. Seed analysis indicated that molybdenum (Mo) and copper (Cu) are the most limiting micronutrients for BNF and growth of common bean (Brockrick and Amijee, 1995), though soil analysis found Cu (and other metallic micronutrients Iron (Fe) and Zink (Zn)) in adequate amounts in the soils (Ndakidemi and Semoka, 2006).

1.4 Objectives

In the West Usambara Mountains farmers' yields far below potential and can be improved with knowledge about the prevailing soil constraints to production. Previous research on the soil fertility constraints for the production on common bean in the area has demonstrated that P and K deficiency are the major nutrient deficiencies, and the production can potentially be improved by P and K fertilizer addition. The potential effect of inoculation is less clear, but it can also have an positive influence on yield.

However, there is no up-to-date information about the soil fertility constraints in this area. There are no detailed soil maps available and no experimental studies have been done in the last decade. Information about the current soil fertility status is needed to improve the farmers' bean yields: based of outcomes of this study recommendations on bean management (i.e. application of fertilizer and inoculum) can be established that can increase farmers' yields.

The main objective of this research is to describe the soil fertility constraints to the production of common bean in the Usambara Mountains in Tanzania. Within the scope of studying the soil fertility constraints in the Usambara Mountains the specific objectives were:

(1) To investigate the effect of common bean yield and nodulation to inoculation with rhizobia and fertilization with P and K.
(2) To describe biophysical characteristics of the soils and to analyse their impact on the response of yield of common bean to inoculation and fertilizers application.
(3) To determine the status of indigenous rhizobia populations and to analyse their impact on the response of nodulation of common bean to inoculation and fertilizers application.

N2Africa project

This research was conducted within the framework of N2Africa – a large scale, development and research project focused on "Putting N_2-fixation to work for smallholder farmers in Africa". One of the problems of common bean in Tanzania that N2Africa has encountered with is poor soil fertility. The project identified a need for more research on limiting nutrients and recommendations for fertilizer use (Ronner et al., 2012). This research will contribute to N2Africa by enhancing out understanding of soil fertility problems of common bean in Tanzania.

Chapter 2 Description of research area

2.1 West Usambara Mountains

The West Usambara Mountains are located in Northern Tanzania between latitude 4˚24'-5˚07' S and longitude 38˚10'-38˚42' E and cover an area of 1740 m^2 (Smithson et al, 1993). The altitudes range from 900 to 2300 m above mean sea level (Wickama and Mowo, 2001).

2.2.1 Climate

The average annual precipitation in the West Usambara Mountains is approximately 1000 mm falling in a bimodal pattern: a long rainy season (Masika) from March to May and a short rainy season (Vuli) from October to December (Vrieling et al., 2006; Ronner et al., 2012). Between January and February a dry spell occurs. The West Usambara Mountains have tropical mountain temperatures with high diurnal variation. There is cool period between May and August and a warm period between January and April; the average monthly temperatures vary from 18ºC in July to 23ºC in March (Vrieling et al., 2006).

In the long rainy season farmers plant in February/March and harvest in July/August. During the short rainy season planting is around November and harvest in January/February (Ronner et al., 2012). Due to the mountainous topography there is variation in rainfall amount, duration and distribution (Mbaga-Semgalawe, 1998), which affects agricultural production (Giller, 2001).

2.2.2 Geology and physical features

The Usambara Mountains are part of the Eastern Arc Mountains, which start in southern Kenya and progress through eastern Tanzania (Burgess et al., 2007). The Eastern Arc Mountains were formed during and after the Tertiary period. The West Usambara Mountains are an upraised horst of Precambrian metamorphic rocks, consisting of gneisses and granulite's (Pfeiffer, 1990; Mutakyahwa et al., 2003). The terrain is highly dissected with local slopes up to 90% (Vrieling et al., 2006). The West Usambara Mountains have several perennial and non-perennial rivers and streams flowing from the slopes through the valleys. Most of them originate from the sub-surface of forest reserves (Mbaga-Semgalawe, 1998).

The area has a lot of spatial variability in parent material and land use history. This spatial variability, in combination with the mountainous relief, results in spatial variability in soil type. This is visible in the small scale variation in soil colour, which ranges from orange to dark brown. Unfortunately there is no thorough soil classification of the West Usambara Mountains. The soils described in literature vary

widely; i.e. Humic Acrisols, Chromic Luvisols, Lixisols, Humic Nitisols, Ferrasols and Chromic Cambisols (Niemeyer, 1980; Mbaga-Semgalawe, 1998; Wickama and Mowo, 2001; Meliyo et al, 2002; Jones et al., 2003). The Soil and Terrain Database (SOTER) for South Africa classifies soils in the area as; 25% Humic Acrisols, 20% Ferric Lixisols, 15% Humic Nitisols, 15% Rhodic Ferrasols and 25% Eutric Leptosols (SOTERSAF, 2003). Acrisols and Lixisols are strong to slightly acid soils (respectively) with a low nutrient-holding capacity and clay-enriched subsoil, Ferrasols are also strongly weathered soils with a low nutrient-holding capacity, Nitisols are more developed soils with a better nutrient holding capacity and Leptosols are shallow soils over hard rock (Jones et al., 2003). The soils described are dominantly leached to very leached and shallow soils. However, a proper soil classification is needed to describe the soils in this area, as the soil descriptions provided in literature vary widely and do not take the large spatial variability in the area in account.

Erosion in the West Usambara Mountains mainly occurs in the form of sheet and rill erosion on fields that are annually cultivated. It is most severe at the onset of the long rainy season, when soil cover is poor due to land preparations and the rains are heavy (Vigiak et al., 2005). To sustain farming on Arcisols, erosion of the topsoil should be prevented, and adapted cropping systems with fertilization and careful management is required (Jones et al., 2003), but few soil and land cover measures have been adopted in the West Usambara Mountains (Vrieling et al., 2006).

2.2.3 Land use

The West Usambara Mountains are densely populated (200-400 inhabitants km^{-1}) because of its relatively cool climate and high rainfall (Tenge, 2005; Vigiak et al., 2005; Huijzenveld, 2008). The good climatic conditions have especially attracted farming communities and agriculture is the major economic activity in the area (Tenge, 2005).

The main food crops produced in the area are banana, maize, bean and potato and the main cash crops are tea, coffee and vegetables (Vigiak et al., 2005). Agricultural production is rained on steep slopes and irrigated in the valley bottom (Tenge et al., 2004; Tenge, 2005). Intercropping is a common farming practice (Vigiak et al., 2005). Besides production for local consumption, the area also produces food and cash crops for export to the surroundings and some major cities in Tanzania (Tenge, 2005). The area is famous for supplying vegetables (like tomatoes, cabbages and onions), fruits, tea, coffee, bananas and timber (Ndakidemi and Semoka, 2006).

The population in the West Usambara Mountains has increased steadily over the last decades, which has put a pressure on land and caused a decline in farm size (Tenge, 2005). Farm size in the West Usambara Mountains varies between 0.7 and 4.1 ha per household, fragmented in several small plots (Mbaga-Semgalawe and Folmer, 2000). Smallholder farmers are generally involved in mixed agricultural activities; they practice rain-fed cropping on the slopes, irrigated cropping in the valley bottom, livestock keeping and off-farm activities (Tenge et al., 2004). Livestock ownership is common in the area; 65% of the farmers own cattle, 40% own goats and 42% own sheep (Van den Brand, 2014). However, due to regulations to prevent erosion grazing of livestock is prohibited; farmers keep their livestock at their homestead and apply manure manually to their land. The use of fertilizers in the area is limited, especially the villages around Lushoto use very few fertilizers, where only 5% of the farmers used inorganic fertilizers and 9% used organic manure (Mowo et al., 2004). Most farmers are experiencing stress due to the decline in farm size and crop production and land degradation (Tenge, 2005) and have few resources to invest in fertilizers (Ndakidemi and Semoka, 2006).

2.2 Experiment field trial locations

The nine sites of the experimental field trial are located in the central western part of the West Usambara Mountains, all close to Lushoto village (Figure 1). The altitudes of the trial sites vary between 1218 and 1667 m above mean sea level (Table 1).

Table 1: Elevation, geographical position and slope of the nine field trial sites.

Field code	Field name	Elevation in m.a.s.l.	Latitude, S	Longitude, E	Position on slope	Slope gradient
F1	Mabughai	1667	04° 44.215’	038° 17.904’	foot slope	10-15%
F2	Jaegertal	1415	04° 47.111’	038° 17.808’	valley bottom	flat
F3	Lushoto	1444	04° 47.920’	038° 18.094’	slope	flat
F4	Kikurunge	1340	04° 50.795’	038° 21.290’	slope	25-30%
F5	Mschizii	1256	04° 48.854’	038° 20.538’	foot slope	15%
F6	Kwemsanga	1253	04° 49.509’	038° 20.902’	slope	20-25%
F7	Ngulwi	1423	04° 50.053’	038° 17.082’	slope	15%
F8	Mbuzii 1	1218	04° 52.256’	038° 20.256’	foot slope	10-15%
F9	Mbuzii 2	1286	04° 52.252’	038° 20.090’	slope	15-20%

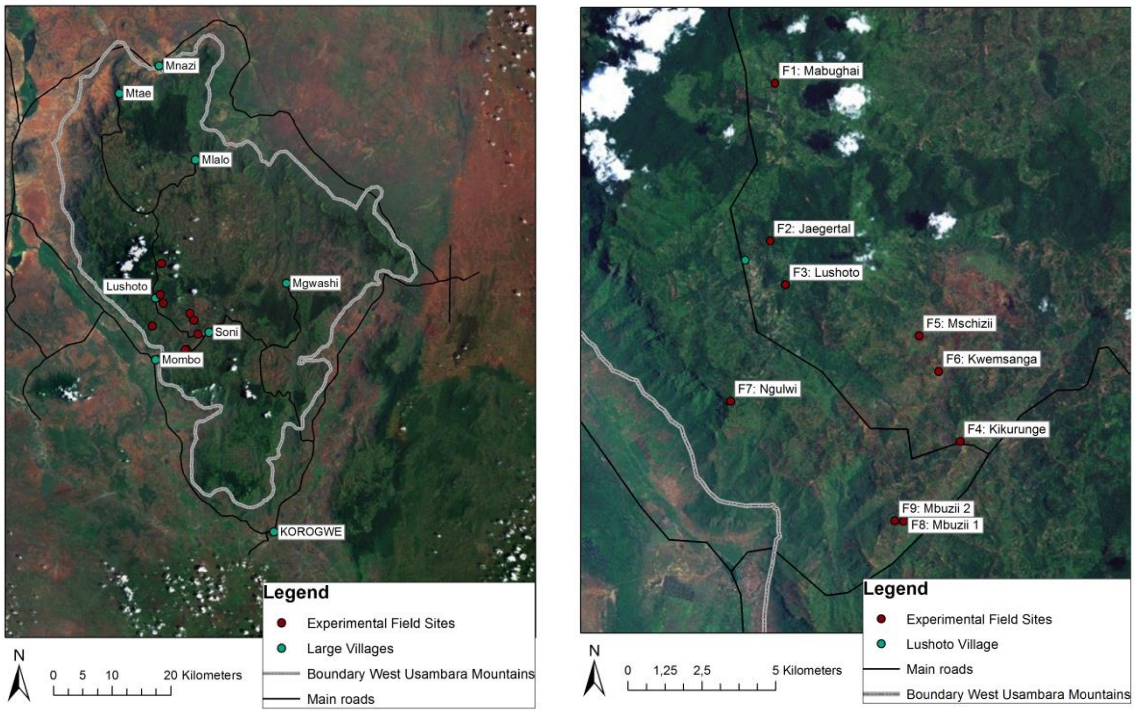

Figure 1 Overview of the Usambara Mountains with some of the large villages in green dots and the sites of the field trial in red dots (left) and an enlarged display of the field trial sites and their names (right).

Chapter 3 Materials and Methods

3.1 Field selection

One week before planting, the vicinity of district capital Lushoto was explored to select farmers' fields suited for the field trials. Nine fields were selected within a radius of 20 km of Lushoto, distributed over the area to capture the spatial variability. Attention was paid to select fields with minimal on-site variability like variation in soil fertility. Fields with a steep slope or several trees were not selected. It was attempted to have sites representative for the variety of fields used to produce common bean in this area: mostly low fertility smallholder farmers' fields on the slope, and some high fertility fields in the valley. Fields of different altitude, slope, position on slope, land-use and fertility were selected. Beforehand an estimation was made of the soil fertility; the expectation was that two fields were high, two were medium and five fields were low in fertility.

3.2 Experimental field trials

3.2.1 Set-up

The field trials were performed at the nine sites described in Section 2.2. The trials were conducted during the short rainy season of Northern Tanzania. All trials were planted between the 7th and 20th of November and harvested between the 27th of January and 12th of February. Bush bean variety Lyamungu 90 was used: a high yielding variety for mid altitude areas in Tanzania (Hillocks et al., 2006), bred by scientists from the Selian Agricultural Research Institute, Arusha, Tanzania.

3.2.2 Experimental trial design

The experiment had a randomised complete block design with a 2^3 factorial trial (three treatments, two levels). There were either two or three replicates at each site, depending on the field size (Appendix B).

Treatments

The 2^3 factorial treatments and levels were: inoculation with rhizobia (abbreviation: I) as *Rhizobium tropici* CIAT899 strain with a peat carrier supplied by LegumeTechnology UK, application of phosphorus (P) as 0 or 26 kg P ha^{-1} as Triple Super Phosphate (TSP) and application of potassium (K) as 0 or 25 kg K ha^{-1} as Muriate of Potash (MOP) (treatment numbers 1 and 3-9 in Appendix A).

The purpose of the I treatment was to study the potential influence of inoculation on BNF of common bean. The P and K treatments were included to study the major soil nutrient deficiencies and the potential effect of P and K fertilizer on common bean. Previous research has demonstrated that P and K were most deficient in this area (see Section 1.3). A factorial design was applied because it allows an assessment of interactions between I, P and K application. Based on previous research, interaction effects were expected, i.e. P and K fertilizer can enhance the effect of I (see Section 1.3). The fertilizer rates were based on recommendation by Smithson et al. (1993) for optimal fertilizer rates for farmers in the Lushoto area.

Two non-factorial treatments were added to the experiment: one extra control plot was included to decrease the uncertainty of the factorial design which depends on the control treatment (treatment number 2 in Appendix 1) and one treatment with P, K and N (25 kg N ha^{-1} as Calcium Ammonium Nitrate (CAN)) to compare the effect of N fertiliser with that of inoculation.

Lay-out

On each of the nine sites a plot with a total dimension between 350 m^2 and 550 m^2 was selected and divided into two or three replicates (blocks). The ten different treatment plots were randomly distributed in each block. Each plot was 3 by 2.5 meter, containing 5 rows of 3 m long with an intra-row spacing of 20 cm and a space of 50 cm between the rows. The two outer rows of the treatment plot were considered as border rows, leaving the three inner rows for observations and harvest. In these three rows, the outer plants were considered as border plants, six plants on the plot margin were uprooted for the nodulation measurement and the remaining inner plot of 2.2 by 1.5 m was used for the final harvest measurements. A distance of 50 cm was left between the different plots and a distance of 1 m between blocks. The lay-out of a trial site and subplots are shown in Appendix B.

3.2.3 Cultural practices

Sowing

Before sowing the rows were ploughed into a furrow. For the treatments with fertilizer applications, the fertilizer was distributed evenly in the furrow and covered with a small layer of soil. First the un-inoculated treatments were sown by applying three seeds at each spot with 20 cm spacing and covering the furrow with a layer of soil. The furrows of the treatments without fertilizer were also covered with a layer of soil before sowing to have the seeds sown at similar depths in all treatments. Subsequently, the remaining seeds were inoculated by dry inoculation. The rhizobial inoculum contained a polymer, so there

was no need for a carrier to stick the bacteria to the seeds. The seeds and inoculum were mixed in a plastic bag just before sowing and the inoculated seeds were sown as the uninoculated seeds.

Management

One to two weeks after emergence plants were thinned to two per stand by removing the weakest plant. The plant density after thinning was approximately 2 x 10^5 plants ha^{-1}. During the growing season the fields were visited several times to observe crop development. The fields were weeded once or twice if necessary. One field (Field 2: Jaegertal) was irrigated by the farmer several times during the season (Appendix C); all other fields were not irrigated.

3.2.4 Observations

Phenology

The following observations were made during the growing season: stand at emergence (number of plants in net plot); early deficiency signs (on scale from least healthy (most deficiency symptoms) to most healthy at a few weeks after emergence); crop vigour (on scale from poorest to best growth at flowering); plant height in cm (mean of ten plants per plot at the time of flowering); days to flowering (number of days to flowering of 50% of the plants); days to podding (number of days to podding of 50% plants); days to maturity (number of days to when 85% of plants has matured).

Nodulation

Effectiveness of nodulation was studied at 50% flowering. From each subplot, ten plants were uprooted with a spade from the area allocated for the nodulation measurement (Appendix B). If necessary, soil on the roots was removed in a bucket with water. From each individual plant the number of nodules and presence or absence of crown nodulation was noted. From each plant several nodules were randomly selected and cut open to assess the inner colour of the nodule (red, pink or brown = active; green, grey, white= inactive). Based on the number of active nodules each plant was assigned a nodulation effectiveness score ranging from 0 - 5 (0 = zero nodules; 1 = <5 nodules; 2 = 5-10 nodules; 3 = 11-20 nodules; 4 = 20-50 nodules; 5 = >50 nodules) according to the protocol of Bala et al. (2010). The nodulation score per plot was determined as the average score of the ten plants.

Weather data

Simple rain gauges were placed at each site (Anderson and Ingram, 1993). The rain gauges were emptied each field visit and the content was measured using a measuring cup (Appendix D).

Management interviews

The nine owners of the trial sites (mostly farmers) were interviewed by the researchers together with an extension officer. The interview was about household characteristics, previous management practices (for their farm in general and specifically for the experimental trial area) and perception of soil fertility. In addition, the owner was asked about his view on the conducted experiment and cooperation with the researchers.

3.2.5 Harvest

Before harvest, the actual harvest area was demarcated from the allocated harvest area (Appendix B) as the area of 2.2 by 1.5 m (3.3 m^2) where 66 plants were sown.

At harvest the number of mature plants inside the harvest area was recorded to determine the fraction of plants that reached maturity. This information is missing for the Mschizii field where plants were already randomly removed by the farmer. Because the harvested samples had to be at least 200 g of pods and 200 g of stems for each subplot, a different selection method was used for fields with little biomass:

- From the fields with very little biomass (Kikurunge, Kwemsanga, Ngulwi, Mbuzii 1 and Mbuzii 2) all plants inside the harvest area were harvested (harvest area of 3.3 m^2). For some of the subplots the harvest weight was so small that it was still less than 200 g
- From the fields with sufficient biomass (Mabughai, Jaegertal, and Lushoto) a selection of 20 plants was made systematically by harvesting the 20 plants at the same location from each subplot (harvest area of 1.0 m^2).

From all plants the aboveground biomass was removed. Because the remains of leaves on the plant varied between plots the leaves were removed from the stover and not taken in account. The pods were separated from the stems, and the number of pods and stems were recorded. Both samples were weighted to determine the fresh weight (FW). From the pods a subsample of 20 random pods was selected, weighted and separated into husks and seeds. The number of seeds was recorded and weighted to determine seed FW. The seeds subsample were dried in the oven at 55°C, and the husks subsample and stems were oven dried at 60°C, all for 24 hours. All the oven dried samples were weighted to determine dry weight (DW) seeds subsample, DW husks subsample and DW stems.

Calculations

All data were entered into Microsoft Excel (Excel) and the final yield components (i.e. number of pods m^{-2}, number seeds pod^{-1}, 100-seed weight and bean yield kg ha^{-1}) were calculated.

3.3 Soil sampling and analysis

3.3.1 Soil sampling

Soil samples were taken from each of the nine experimental field trial locations in October 2013, before the trials were planted. At each location 15-20 samples distributed over the whole area were taken of the 0-20 cm topsoil. These samples were mixed well and combined into one composite sample per site. From each composite sample a subsample of 300-400 g was selected, packed, labelled and stored in a fridge at 4ºC to be used for the MPN counts. The rest of the composite sample was air-dried. From each composite sample three air-dried subsamples (two for chemical analysis and one for the MPN counts) of 300-400 g was selected, packed, labelled and stored at a cool dry place.

3.3.2 Chemical and physical analysis

The air-dried subsamples for chemical analysis were sent to Mlingano analysis of chemical and physical soil properties; pH (H_20), % organic carbon (C) (Walkley-Black), total % N (Kjeldahl digestion), plant available P (Bray-I), cation exchange capacity (CEC) (extraction with ammonium acetate), cations (K, Ca, Mg and Na content) (atomic absorption spectrophotometry) and soil particle size (texture) (Bouyoucos).

Because the results from Mlingano had unreasonably high K values, replicates of the samples were send to Cropnuts for analysis of some chemical properties; pH (H_20), plant available P (Olsen), cation exchange capacity (CEC) (extraction with ammonium acetate) and cations (K, Ca, Mg and Na content) (atomic absorption spectrophotometry). The results from Cropnuts were used and presented in the report, because this laboratory is accredited by the Dutch Accreditation Authority (Cropnuts, 2014), except for organic carbon, total % N and soil particle size, which were only analysed at Mlingano (data from both laboratories can be found in Appendix E).

3.4 Most Probable Number counts

The Most Probable Number (MPN) count (Somesegaran and Hoben, 1985; Vincent, 1970; Woomer et al., 2011) was used to estimate the presence and size of the indigenous rhizobial population in the soils of the experimental field trial sites.

Design

Samples to be analysed for microbial activity are usually stored at 4ºC. In this case a normal kitchen fridge had to be used which fluctuated in temperature during storage. Besides these cooled samples, air-dried samples (Section 3.3.1) were used to reduce the uncertainty caused by the temperature fluctuations. For each of the nine soil samples six replicates were used: for replicate 1-4 the air-dried samples were used and for replicate 5 and 6 the samples stored in the fridge were used.

Sample preparation

The soil samples were prepared for dilution by removing all root or dirt material. A 10^{-5} dilution was prepared: 1 g of soil was added to 9 ml of distilled water and shaken with a vortex shaker for ten minutes (first dilution step). Then 1 ml of the first dilution step was added to 9 ml of water and shaken with a vortex shaker (second dilution step), with the use of a 1 ml aliquot. This step was subsequently repeated three more times to end up with a 10^{-5} dilution series.

Sterilization and pre-germination of seeds

The bush been seed Selian 94 was used as an indicator plant, because it has relatively small seeds and is easier to work with than Lyamungu 90. The seeds were surface sterilized with 95% alcohol (ethanol: C_2H_5OH) for 10 seconds and then rinsed with 2.5% sodium hypochlorite (NaClO) for ±4 minutes. They were rinsed with 6 changes of sterile distilled water, submerged with sterile water, left in the fridge for 4 h to imbibe and rinsed with two more changes of water. Autoclaved Petri dishes were prepared with autoclaved filter paper and moistened with sterile distilled water. The seeds were transferred to the Petri dishes, distributed evenly and left for 3 days to pre-germinate.

Implementation and measurement

In a laminar flow cabinet the 300 sterile growth pouches (Mega International, 2013) were opened, labelled and prepared in racks to keep them standing upright. Each growth pouches was filled with 20 ml of N-free Nutrient Solution (Broughton and Dillworth, 1971) using a pipette. From the Petri dishes with the pre-germinated seeds, the seeds with the highest viability were selected and gently placed in the paperwick though of the growth pouch (one seed per growth pouch). The pouches were inoculated with the soil dilution series with the use of 1 ml aliquots, starting with the highest dilution. Six growth pouches were not inoculated and served as a control.

The racks with growth pouches were placed in a clean, well-lit air-conditioned room. The plants were inspected frequently. When the growth pouches were getting dry 10 ml of N-free Nutrient Solution was added using a pipette. After 23 days and 26 days the presence or absence of nodules was recorded.

In some cases the observations showed negative results at low dilutions, while there were positive results at higher dilutions. This could be due to contamination, but this option was rejected because all controls were negative. It was assumed that the observed negative was false due to other constraints than the absence of rhizobia (like plant health) and adjusted according to Vincent (1970).

MPNES software

From the observed data the MPN was calculated with the use of MPNES software (Woomer et al., 1990). To check the MPNES method the MPN number from the MPNES software was compared with the MPN number from tables, which showed good correlations.

3.5 Data handling and analysis

All data were entered in Microsoft Excel. An overview Table of yield and soil parameters was created and saved as a CSV file. This file was entered into R (R Core Team, 2013) and field, replicate, treatment number, P application, K application, I application and N application were treated as factors (but only P and K as factorial), the yield parameters were treated as numeric variables.

Three separate Analysis of variance (ANOVA) were performed to detect statistically significant variation between different factors (treatments) on the yield variables;

1) The statistical significance of the effect of the factorial treatments. The three treatment effects analysed were P fertilization, K fertilization and inoculation as fixed effect (independent variable) (the treatment with N fertilization excluded) and the observations and yield parameters as response (dependent) variable. Because of the factorial design, all the treatments including P, K and I were selected and analysed. For example, to study the statistical significance of P fertilization, all treatments including P fertilization (so also P fertilization in combination with K fertilization or inoculation) were compared to all treatments without P fertilization (including other treatments). Also the interaction effects of all factors were analysed.

2) The statistical significance of the effect of inoculation and N fertilization in reference to only P and K fertilization has also been analysed with ANOVA. The tree treatments were: the P and K fertilization treatment (PK) as control treatment, the P and K fertilization in combination with inoculation treatment (PKI) and the P and K fertilization in combination with N fertilization (PKN).

The P-value and significance levels were obtained from the ANOVA analysis.

3) The statistical significance of the soil parameters on the treatment effect of P, K and I was analysed with a linear mixed model analysis (lme4 package) and ANOVA analysis. The soil parameters were treated as the fixed effect, the treatments as random effect and the yield parameters were treated as response. The P-value and significance level were obtained with an ANOVA analysis of the model with and without the tested variable.

Chapter 4 Results

4.1 Effect of inoculation and fertilizer application on common bean

4.1.1 Effect of treatments on nodulation

The nodulation of common bean was significantly enhanced by inoculation with rhizobia as well as fertilization with P and K (*P<0.001*) (Figure 2). The nodulation score increased from 1.1 to 1.9 by inoculum application without fertilization (Table 2). Fertilization with P and K increased the nodule score to 2.3 without inoculation, and 2.8 with inoculation.

Table 2 The effect of P, K and N fertilizer application and inoculation with rhizobia on the mean nodule score of all trials.

	-P		+P	
	-K	+K	-K	+K
-I	1.1	1.5	2.0	2.3
+I	1.9	1.9	2.1	2.8
+N	-	-	-	1.9

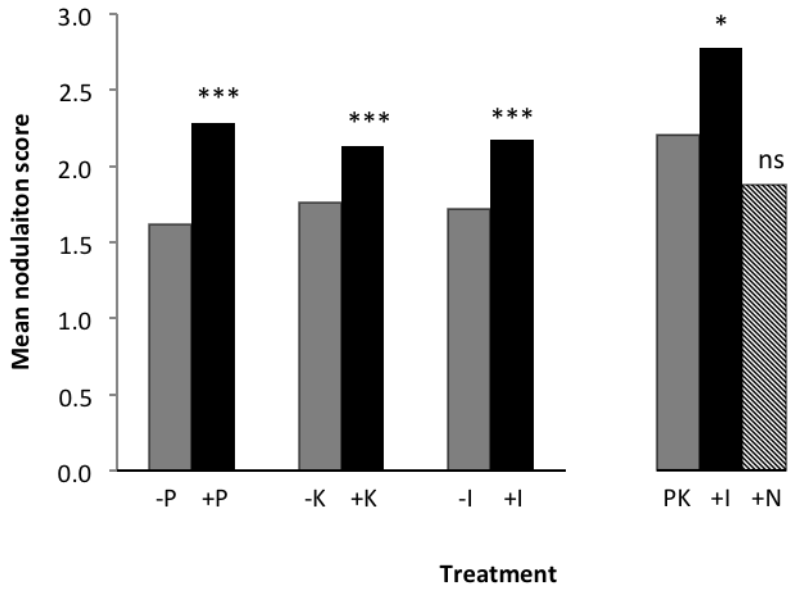

Figure 2 Left: mean nodulation score for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05. Right: mean nodulation score of the P and K fertilizer treatment (PK, grey) and P and K fertilizer in combination with inoculation (+I, black) or N fertilizer (+N, shaded). The treatment effect of I and N as the difference between PK and +I and +N. The statistical significance from the statistical significance of the analysis of variance (of +I and +N in reference to PK) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

In comparison to the P and K fertilization treatment, nodulation was significantly enhanced by the addition of rhizobia inoculation (*P<0.05*), with P and K alone having a mean nodulation score of 2.3 and with the addition of inoculation a nodulation score of 2.8 (Figure 2). The addition of N fertilization had a negative effect on nodulation, which was not significant and had a mean nodulation score of 1.9.

4.1.2 Effect of treatments on crop vigour

Several crop vigour parameters were significantly affected by the treatments. Crop vigour (scored on a scale from 1 to 5 by which 1=few and 5=abundant) was significantly increased from 2.0 to 3.5 with the addition of P and K fertilizer (*P<0.001*) (Table 3 and Figure 3). P fertilizer addition has a stronger effect on crop vigour than K fertilizer addition. Besides the individual effect of P and K fertilizer, the interaction between P and K was also significant (*P<0.05*) (Appendix F). The addition of inoculum increased crop vigour for some treatments but not for all (Table 3) and the overall effect of inoculation was not significant (Figure 4).

Table 3 The effect of P, K and N fertilizer application and inoculation with rhizobia on the mean crop vigour score of all trials.

	-P		+P	
	-K	+K	-K	+K
-I	2.0	2.4	3.1	3.5
+I	2.4	2.4	2.9	3.9
+N	-	-	-	4.5

Compared to the P and K fertilization treatment (3.5), inoculation did slightly increase crop vigour (3.9) but not significantly, while the addition of N fertilizer increased nodulation significantly (*P<0.001*) to a crop vigour score of 4.5 (Table 3 and Figure 3).

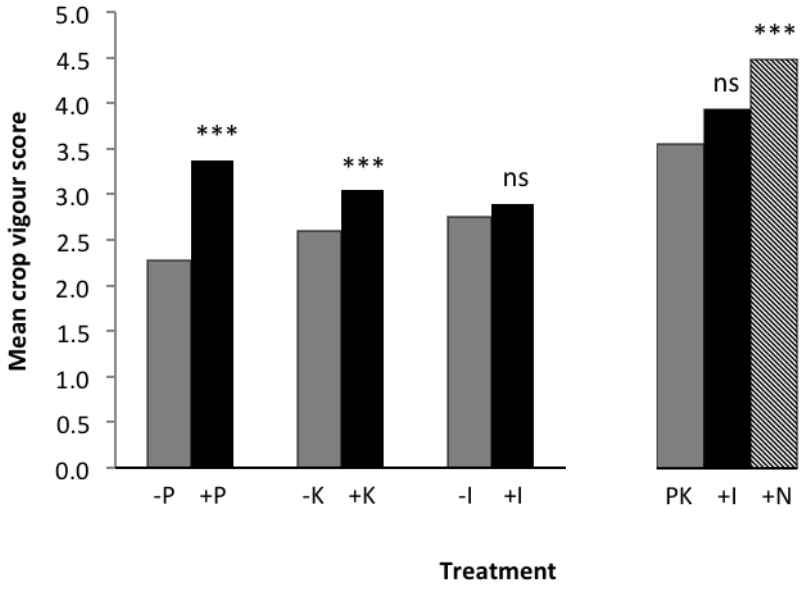

Figure 3 Left: mean <u>crop vigour</u> score for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05. Right: mean crop vigour score of the P and K fertilizer treatment (PK, grey) and P and K fertilizer in combination with inoculation (+I, black) or N fertilizer (+N, shaded). The treatment effect of I and N as the difference between PK and +I and +N. The statistical significance from the statistical significance of the analysis of variance (of +I and +N in reference to PK) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05

The deficiency signs scoring at early stage (on a scale from 1 to 5 by which 1=least healthy and 5=healthy) was decreased with the addition of P and K fertilizer, with a significantly higher mean early deficiency score of 3.8 and 3.3 for P and K fertilization treatment than the control (*P<0.001*) (Figure 4).

The crop height was significantly enhanced by P fertilization (*P<0.001*) and K fertilization (*P<0.05*) (Figure 5). Inoculation resulted in slightly higher plants but had no significant impact.

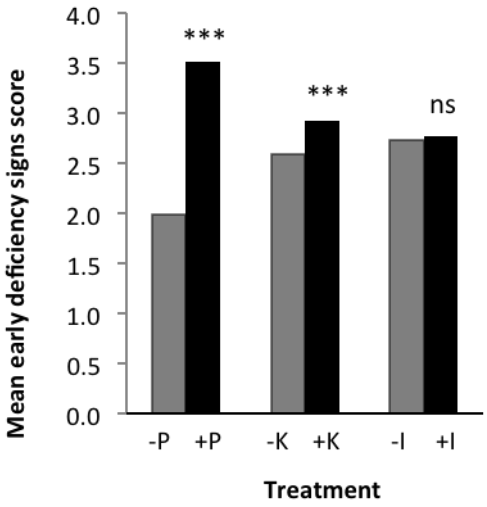

Figure 4 Mean <u>early deficiency signs</u> score for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

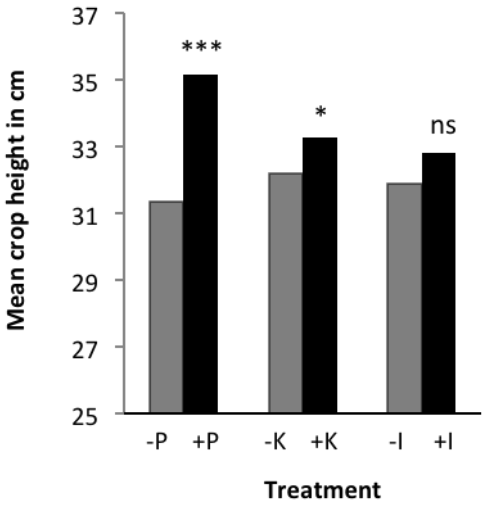

Figure 5 Mean <u>crop height</u> in cm for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

4.1.3 Effect of treatments on yield

The treatment effect on yield was significant for the addition of P and K fertilizer (*P<0.001*), but not for inoculation (Figure 6). There were no significant interaction effects between different treatments (Appendix F). Without any treatment the mean bean yield was 452 kg ha^{-1}, the overall response of bean yield to P and K fertilizer was strong with a mean yield of 686 kg ha^{-1} with P fertilizer addition and 650 kg ha^{-1} with K fertilizer addition (Figure 6). The overall response to inoculation was not significant (Figure 6). Inoculation only increased bean yield slightly in combination with P and K fertilizer (Table 4).

Table 4 The effect of P, K and N fertilizer application and inoculation with rhizobia on the mean bean yield of all trials.

	-P		+P	
	-K	+K	-K	+K
-I	452	511	658	712
+I	447	480	514	755
+N	-	-	-	791

In comparison with the treatment with combined P and K fertilizer application without I (712 kg ha^{-1}), yields were slightly increased by the addition of inoculation (755 kg ha^{-1}) or N fertilizer (791 kg ha^{-1}) but not significantly (Table 4 and Figure 6).

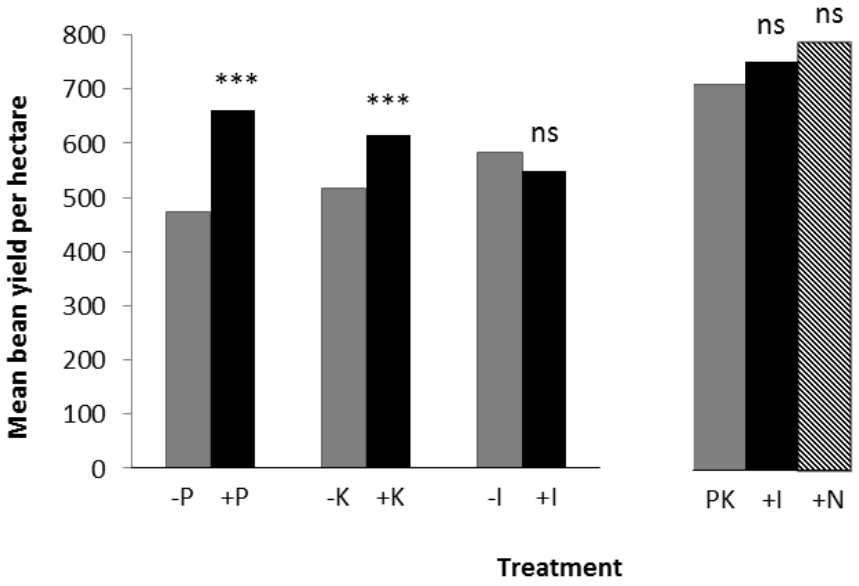

Figure 6 Left: mean <u>bean yield</u> per hectare for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05. Right: mean bean yield per hectare of the P and K fertilizer treatment (PK, grey) and P and K fertilizer in combination with inoculation (+I, black) or N fertilizer (+N, shaded). The treatment effect of I and N as the difference between PK and +I and +N. The statistical significance from the statistical significance of the analysis of variance (of +I and +N in reference to PK) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05

4.1.4 Effects of treatments on stem and seed weight

The dry weight of the plant stems increased significantly with the addition of P (*P<0.001*) and K (*P<0.01*) fertilizer (Figure 7). The fertilizer effect is not as strongly reflected in the increase of the weight of 100 seeds, which only increased significantly (*P<0.05*) with the application of P fertilizers (Figure 8).

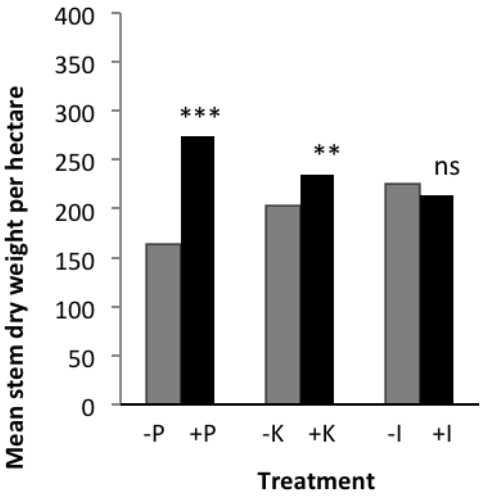

Figure 7 Mean stem dry weight in kg per hectare for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

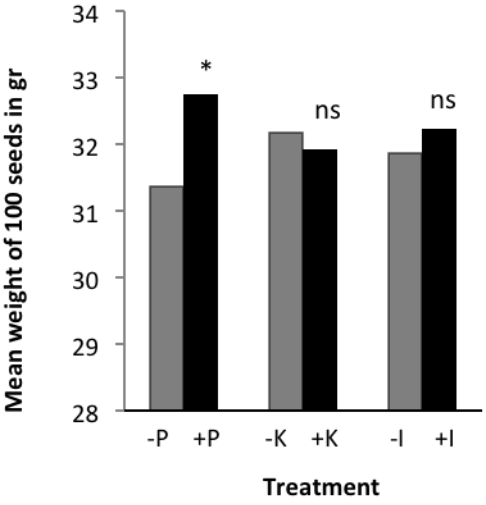

Figure 8 Mean weight of 100 seeds in gram for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

4.1.5 Effect of treatments on yield for individual fields

The variability in yield over the different fields was large, the average yield per field ranged from 78 kg ha^{-1} to 2037 kg ha^{-1} (Table 5). The highest yielding field was the irrigated field (Field 2 Jaegertal). In general, the fields with the highest yield in the control treatment also had the highest absolute and relative response in yield to the application of fertilizers. The nodulation and yield response to the treatments per field can be found in Appendix H.

Table 5 Bean yield in kg per ha for the fields individually; field average of all treatments, averge of the control treatments, average of all treatments with both P an K fertilizer and the percentual increase of the yield obtained with both P and K fertilizer in reference to the control (not taking the treatment with N in account).

	Bean yield in kg per ha			Percentage increase[a]
Fields	Field average	Control average	P and K treatments average	
#1 Mabughai	871	522	1345	258%
#2 Jaegertal	2037	1630	2490	264%
#3 Lushoto	609	389	965	123%
#4 Kikurunge	138	115	204	178%
#5 Mschizii	NA			
#6 Kwemsanga	266	176	234	133%
#7 Ngulwi	269	227	380	168%
#8 Mbuzii 1	161	205	138	68%
#9 Mbuzii 2	78	97	128	131%

[a] Percentage increase of the average of treatments with P and K fertilizer application in reference to the average control treatments.

There was also a lot of variability in the yield response to the different treatments (Table 6). Site variation significantly affected the response to P (*P<0.001*) (Appendix F). The response to P ranged from -15% to +89% between sites (Table 6).

The average response to inoculation was negative, though insignificant (the response is calculated as percentage increase of the average yield of all treatments with I compared to the average yield of all the treatments without I, which also include the fertilizer treatments).

Table 6 Yield response to the different inputs. Field average of bean yield for treatment with the input compared to average bean yield without that certain input.

	Percentage yield response		
Fields	P	K	I
#1 Mabughai	9%	78%	-25%
#2 Jaegertal	54%	15%	-3%
#3 Lushoto	55%	83%	-6%
#4 Kikurunge	23%	71%	2%
#5 Mschizii			
#6 Kwemsanga	-2%	2%	13%
#7 Ngulwi	74%	23%	-6%
#8 Mbuzii 1	-15%	-16%	-12%
#9 Mbuzii 2	89%	76%	-10%
Average	36%	30%	-8%

4.1.6 Impact of treatments on normal yielding fields

Field 1, 2 and 3 were the only fields with a yield above 500 kg hectare^{-1} (Table 5) ans who had much less field variability than the other fields (Table 6). These fields are considered to be more representative for bean yields in Tanzania (Table 5 and Section 1.1) and the potential effect of treatments on bean yield. The treatment effect of P and K fertilizer addition on yield was much stronger for these fields, with an average yield increase from 847 kg hectare^{-1} in the control to 1627 kg hectare^{-1} with the addition of P and K fertilizer (Table 7). The effect of P or K fertilizer addition is highly significant, but the effect of inoculation or N fertilizer is not (Figure 9).

Table 7 The effect of P, K and N fertilizer application and inoculation with rhizobia on the mean bean yield of Field 1, 2 and 3.

	-P		+P	
	-K	+K	-K	+K
-I	847	1043	1391	1483
+I	841	958	994	1627
+N				1688

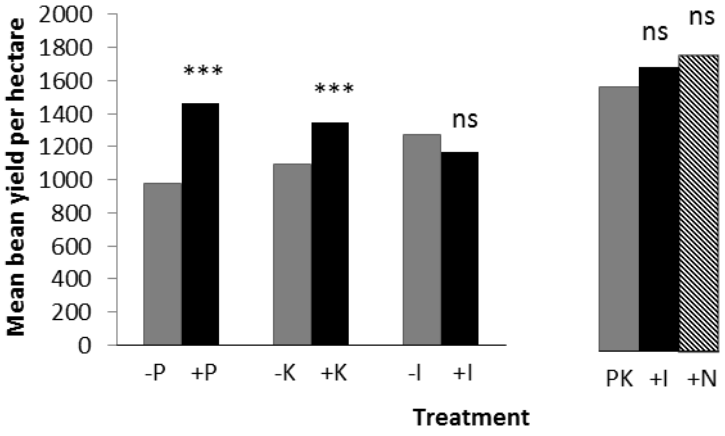

Figure 9 Left: mean bean yield per hectare of Field 1, 2 and 3 for all factorial treatments with (+, black) and without (-, grey) P fertilizer (P), K fertilizer (K) and inoculation (I). The treatment effect of P, K and I as the difference between – and +. The statistical significance from the analysis of variance (of treatment effect: + treatment in reference to – treatment) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05.

Right: mean bean yield per hectare of Field 1, 2 and 3 of the P and K fertilizer treatment (PK, grey) and P and K fertilizer in combination with inoculation (+I, black) or N fertilizer (+N, shaded). The treatment effect of I and N as the difference between PK and +I and +N. The statistical significance from the statistical significance of the analysis of variance (of +I and +N in reference to PK) as: ***: P < 0.001, **: 0.001 ≤ P < 0.01, *:0.01 < P < 0.05, ns: not significant, P≥0.05

4.2 Relation between biophysical characteristics and treatment effect

4.2.1 Biochemical characteristics of the study area

The trial soils predominantly had a clay texture (Table 9). The soil available P value ranged between 1.5 and 10.8 and was below the critical value of 15 mg kg^{-1} (Landon, 1991) (Table 8). The K concentration in the soil ranged between 0.1 and 0.3 $cmol_{(+)}$ K kg^{-1} and was below the critical value of 2.0 (Anderson, 1973) for most sites. The pH was suitable for crop growth (5.2-6.9). The soils in the valley (Jaegertal and Mschizii) that were expected to be more fertile do not stand out as more fertile from the chemical analysis. The fields very close to each other (Mbuzii 1 and Mbuzii 2) have corresponding chemical soil properties.

Table 8 Soil chemical properties of the experimental fields.

						Exchangeable bases				
	pH	Org C	Total N	Av. P	CEC	Ca	Mg	K	Na	EC
Fields		%C	%N	mg kg^{-1}	cmol kg^{-1}					mS cm^{-1}
#1 Mabughai	5.3	2.6	0.3	6.1	17.6	3.9	0.8	0.1	0.2	1.1
#2 Jaegertal	5.6	2.4	0.3	1.5	21.7	5.6	1.4	0.3	0.2	2.7
#3 Lushoto	5.2	2.5	0.3	10.8	12.5	2.4	0.7	0.2	0.2	1.2
#4 Kikurunge	6.9	2.3	0.2	2.5	24.2	8.0	3.1	0.1	0.3	0.3
#5 Mschizii	6.6	2.0	0.1	5.7	15.7	5.0	1.8	0.3	0.3	0.7
#6 Kwemsanga	6.4	1.4	0.1	2.3	16.8	5.4	1.6	0.2	0.2	0.2
#7 Ngulwi	6.0	2.5	0.2	2.5	16.7	5.2	1.3	0.2	0.2	0.7
#8 Mbuzii 1	6.1	2.0	0.2	2.7	21.5	6.3	2.2	0.2	0.3	0.8
#9 Mbuzii 2	6.1	2.4	0.2	1.5	18.7	5.4	2.0	0.3	0.3	1.2

Table 9 Physical properties of the soil samples. Particle size classifications in µm as percentage by weight of the whole sample and texture class according to USDA 1951 standards (Landon, 1991)

	%Clay	% Fine Silt	% Coarse Silt	% Sand	Texture class	
Fields	<2 µm	2-20 µm	20-50 µm	50-2000 µm	Abbreviation	Class name
#1 Mabughai	24	14	4	58	SCL	Sandy clay loam
#2 Jaegertal	36	20	4	40	CL	Clay loam
#3 Lushoto	28	14	6	52	SCL	Sandy clay loam
#4 Kikurunge	44	10	4	42	C	Clay
#5 Mschizii	46	12	4	38	C	Clay
#6 Kwemsanga	42	8	4	46	SC	Sandy clay
#7 Ngulwi	40	12	6	42	CL/C	Clay (loam)
#8 Mbuzii 1	32	10	8	50	SCL	Sandy clay loam
#9 Mbuzii 2	50	8	4	38	C	Clay

4.2.2 Relation between biophysical characteristics and fertilizer effect

There is no linear relation between the soil concentrations of P or K and the response in yield to fertilization with P and K (Appendix I). Most soils are deficient in K and all soils are deficient in P concentration in the soil (Figure 10 and 11).

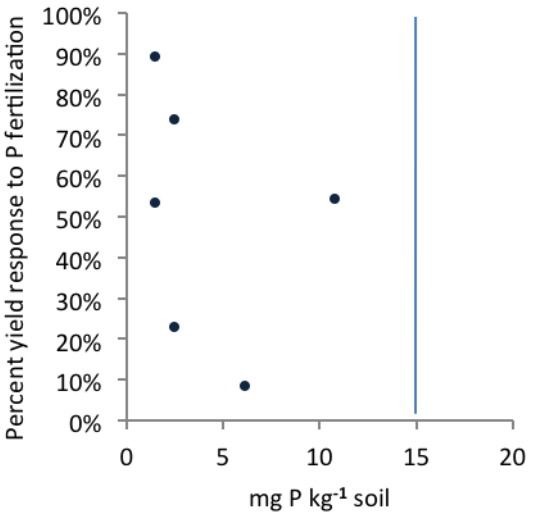

Figure 10 Percentage yield response to P fertilization against the soil available P concentration of the trial sites. Percentage yield response as actual yield response to P fertilization divided by absolute yield of treatments without P fertilization. In blue the critical value of soil P (Landon, 1991).

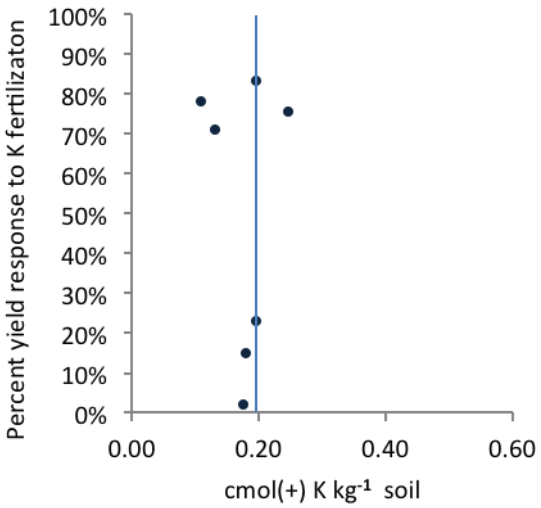

Figure 11 Percentage yield response to K fertilization against the soil available K concentration of the trial sites. Percentage yield response as actual yield response to K fertilization divided by absolute yield of treatments without K fertilization. In blue the critical value of soil K (Anderson, 1973).

4.3 Indigenous rhizobia population

4.3.1 Indigenous rhizobia population

The indigenous rhizobia populations were present in large quantities in the soils of the experimental fields (Table 10). The number of cell rhizobia gram soil^{-1} varies from 1.18x10^{2} to >2.40x10^{5}.

Table 10 Estimation of the populations of rhizobia capable of nodulation common bean in the soils of the experimental fields.

Fields	Number of rhizobia Cells gram soil^{-1}
#1 Mabughai	1.04x10^{3}
#2 Jaegertal	1.75x10^{4}
#3 Lushoto	1.18x10^{2}
#4 Kikurunge	1.60x10^{5}
#5 Mschizii	5.00x10^{4}
#6 Kwemsanga	>2.40x10^{5}
#7 Ngulwi	2.38x10^{4}
#8 Mbuzii 1	2.13x10^{4}
#9 Mbuzii 2	3.25x10^{4}

4.3.2 Relation between indigenous rhizobia population and nodulation

Field variation also had a significant effect on nodulation (*P<0.001*), while the variation per block did not. There was no significant response between the rhizobia population in the soil and nodulation (Appendix J). The effect of inoculation on nodulation was not related to the rhizobia population in the soil (Figure 12).

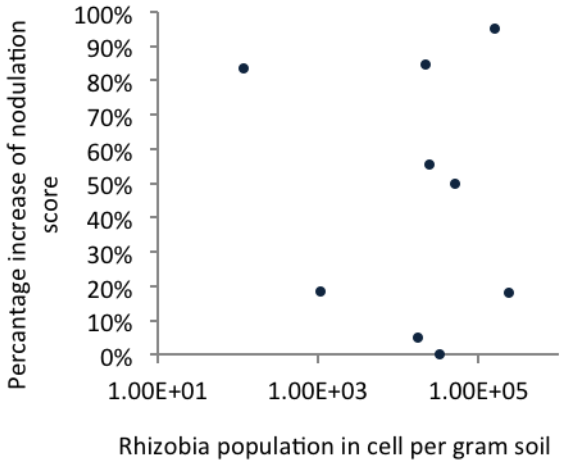

Figure 12 Rhizobia population in cells per gram soil of the nine experimental fields against the percentage increase of nodulation of the inoculation treatment.

4.4 Management interviews

Most of the owners of the trial sites had farming as their main occupation, with either primary or secondary school as their highest education obtained (Table 11). Maize and beans were the main crops, though not all farmers produced beans. Most farmers also produced other crops like vegetables and potatoes.

Table 11 Socio-economic characteristics of the owners of the trial sites.

Fields[a]	Main occupation	Gender	Age	Education, highest level	Household size[b]	Main crops produced on entire farm[c]
#1 Mabughai	Headmaster school	male	43	diploma education	4	M, P
#2 Jaegertal	Farmer	male	45	secondary	8	V, P, M
#3 Lushoto	Head resource center	male	NA	NA	NA	NA
#4 Kikurunge	Agricultural teacher	female	NA	NA	NA	M, B
#5 Mschizii	Farmer	male	53	secondary	10	V, C, T
#6 Kwemsanga	Farmer	male	32	primary	6	V, M ,B , C
#7 Ngulwi	Farmer	female	38	secondary	6	M, B, P, T , C
#8 Mbuzii 1	Farmer	male	60	secondary	5	M, B, T
#9 Mbuzii 2	Farmer	male	49	primary	6	B

[a] Not all socio-economic characteristics of the Lushoto and Kikurunge field owners were considered as they were not farmers

[b] Number of people of the household, including the farmer

[c] Crops abbreviated as: M=maize, B=beans, P=(sweet)potatoes, V=cash crop vegetables (like cabbage, lettuce, broccoli), T=tomatoes, C=cassava

Most of the trial site owners kept cattle as livestock, and used their manure as inorganic fertilizer (Table 12). It was also common for the farmers to purchase manure in buckets from other farmers. Manure was mainly applied to maize and vegetables, and never to beans. Most of the farmers also applied inorganic fertilizers to their crops, but only to cash crop vegetables and maize. The most widely applied type of fertilizer was DAP. None of the trial owners were familiar with inoculums nor had they ever applied it. The bean seeds used were always local varieties from their own stock or market.

Table 12 Livestock keeping and use of organic and inorganic fertilizers

Fields	Livestock		Use of manure			Use of inorganic fertilizer		Use of inoculum
	Cows	Sheep/ goats	From own cattle	Pur-chased	Crops[a]	If yes: Type	Crops[a]	
#1 Mabughai	0	0	no	yes	M, P	DAP	?	no
#2 Jaegertal	4	0	yes	yes	V, M	DAP	V	no
#3 Lushoto	7	0	yes	No	NA	DAP	V	no
#4 Kikurunge	0	0	no	yes	M	DAP	M	no
#5 Mschizii	1	3	?	yes	V, M	NPK	V, M	no
#6 Kwemsanga	4	7	yes	yes	V, M	Urea	V	no
#7 Ngulwi	2	0	yes	no	M	NA	NA	no
#8 Mbuzii 1	3	0	yes	no	M	NA	NA	no
#9 Mbuzii 2	3	0	yes	no	M	NA	NA	no

[a] Crops abbreviated as: M=maize, B=beans, P=(sweet)potatoes, V=cash crop vegetables (like cabbage, lettuce, broccoli), T=tomatoes, C=cassava

Most of the sites were previously used to produced maize and beans, and in some cases potatoes, tomato or vegetables (Table 13). Because of unsatisfying yields it was common for some owners to leave the site fallow in the Vuli (short rainy) season. Several owners were facing growth problems due to aphids and root rot, and had observed a yellowing of the bean leaves.

Table 13 Field history: previous crops produced on the site, owner's perception of soil fertility and crop problems observed by the owner.

Fields	Previous crops		Owners' perception of soil fertility	Crop problems observed by owner	
	Vuli 2012[a]	Masika 2013[a]		Diseases or insects	Growth problems
#1 Mabughai	M, B, P	M, B, P	not fertile	NA	Stunted growth
#2 Jaegertal	M	P	very fertile	aphids	NA
#3 Lushoto	V	V	poor/ moderate	NA	NA
#4 Kikurunge	fallow	M, B	poor/moderate	aphids	NA
#5 Mschizii	B	M, T	not fertile	aphids, root rot	yellowing leaves
#6 Kwemsanga	fallow	M	not fertile	aphids	yellowing leaves
#7 Ngulwi	M, B	fallow	fertile	aphids, root rot	yellowing leaves
#8 Mbuzii 1	fallow	M	good	NA	NA
#9 Mbuzii 2	fallow	B	not fertile	root rot	yellowing leaves

[a] Crops abbreviated as: M=maize, B=beans, P=(sweet)potatoes, V=cash crop vegetables (like cabbage, lettuce, broccoli), T=tomatoes, C=cassava. Vuli is the short rainy season and Masika the long rainy season.

Chapter 5 Discussion

5.1 Field trials for nutrient deficiency analysis

5.1.1 Phosphorus and potassium deficiency

Nodulation

The addition of P and K fertilizer had a positive effect on nodulation. Even without inoculation the average nodulation score increased from 1.1 to 1.5, 2.0 and 2.3 for P, K and P+K fertilization respectively (Section 4.1.1). These results indicate that nodulation is suppressed by nutrient deficiencies of the plant, rather than absence of indigenous rhizobia in the soil (other constraint than nutrient deficiencies will be discussed in Section5.3.2). This is in line with the results of Amijee and Giller (1998) and Amijee et al. (1998) and Giller et al. (1998) who observed a strong response to inoculation only in combination with P fertilizer and recommended P fertilization to enhance effective nodulation.

Crop vigour and yield

The addition of P and K fertilizer had an overall highly significant positive effect on crop vigour and yield, demonstrating a deficiency of P and K in the soil (Section 4.1.2 and 4.1.3). These results are in accordance with previous research in this area (Section 1.3). Smithson et al (1993) diagnosed P and K deficiency to be the major constraint of common bean growth and yield. Several other field studies with P fertilizer confirmed P deficiency to be the major constraint (Amijee et al., 1998; Amijee and Giller, 1998; and Giller et al., 1998; Ndakidemi et al, 2006)

5.1.2 Nitrogen deficiency

Nodulation

In most fields plants nodulated without inoculation, but inoculation enhanced nodulation (Section 4.1.1). Active nodulation indicates that the plant is able to fix nitrogen, and does not have a (substantial) N deficiency. The addition N fertilization supressed nodulation, but not to such an extent that nodulation was absent.

Crop vigour and yield

Inoculation did not significantly influence crop vigour nor yield, even though nodulation was increased by inoculation (Section 4.1.2 and 4.1.3). One determinant of the success of inoculation is whether the soil is deficient in N (in the case of the absence of rhizobia population)(Thies, 1991b). The weak response to inoculation could therefore indicate that there is no N deficiency in the soil. There was also no significant yield response to N fertilization, which indicates that the either there is no N deficiency in the soil, or N fertilization does not provide more N than BNF does. However, other factors than N deficiency play a major role in the effect of inoculation on yield, which are described in Section 5.3.

The addition of N fertilizer had a significant positive effect on crop vigour, while inoculation did not (Section 4.1.2). This can be explained by the plants' need for nitrogen before BNF can be effective. At an early stage, the plant needs N to establish crop vigour which it needs to start nodulating and perform BNF (Marschner, 2012). Crop vigour was significantly enhanced with the addition of N fertilizer, but not with inoculation (Figure 3). This addition of N could have favoured plant growth in an early stage when the plant was not yet able to perform BNF. Later, when the plant has invested in nodules and can perform BNF, the influence of N fertilization is compensated by the N provided by BNF, which can explain why there was no significant effect of N fertilization on bean yield (Figure 6).

5.1.3 Field variability

Experimental field trials with fertilizers are the most reliable way of determining nutrient availability for crops in soils (Marschner, 2012). However, besides the treatment factors there are always other factors influencing the growth and yield of the crop.

These factors can either cause within-site variability (when there is variation in the presence and severity of this factor per field treatment plot) and/or limit the treatment response and yield in general (when it limits nutrient uptake or yield in general). The presence of these factors is demonstrated by the large variability in treatment response within fields (Appendix H), and the large variability between fields (Section 4.1.5). For the purpose of the experimental trials, within-site variability should be avoided as much as possible. The causes variability between treatments and sites make it more complex to analyse the trial results. However, variability between fields is common and it was also aimed to represent different farmers' field conditions.

The treatment response to P and K fertilizer was strongest on fields that were relatively high yielding in the control. Several fields had an overall low yield (even with fertilization) and a lot of within-site

variability. There overall low yield and variability in the treatment response indicates that major constraint to nodulation, crop vigour, and yield were not N, P and K deficiencies in the soil but another overriding constraint. This does not mean that the soil is not deficient in nutrients, but other factors that limit plant growth were overriding.

The following field were very likely to be constrained by overriding factors: #4 Kikurunge, #6 Kwemsanga, #7 Ngulwi, #8 Mbuzii 1 and #9 Mbuzii 2. It was in the current set-up not possible to determine the severity of these constraints nor which factors were most influential for each field. However, the most probable overriding factors, based on field observations, are discussed below.

1) *Root rot*: at several fields (Mschizii, Mbuzii 1 and Mbuzii 2) severe root rot was observed. Root rot does not only suppress growth in general but also limits for nutrient uptake and thereby the treatment response, and the possibility for nodulation. The root rot observed had a spatial variability and probably caused within-site variability to plant growth and yield treatment response.
2) *Insects:* at most fields insects like stem maggots, leaf hoppers were observed, which are common insects in Africa (Graham and Ranalli, 1997). The severity of insects ranged from low to severe. It is very likely that insects have caused within-site variability.
3) Management history: Management history can cause within-site variability, for instance when farmers use fertilizers (manure, ash etc.) it is often not spread homogenously over the field. It is also related to the overall treatment effect and yield per field, as it is a main determinant for soil fertility (Section 5.4).
4) Drought: Another factor influencing crop growth was drought, but instead of causing within-site variability it generally limits the treatment effect for the whole field. Drought limits the plant's ability to take up nutrients from the soil (Marschner, 2012). There were several periods of drought during the growth season (Appendix D) which has probably limited nutrient uptake as well as crop growth and yield. The only irrigated field (F2 Jaegertal) had a much higher yield (average of 2437 kg ha^{-1}) than all the other fields (averages ranging from 78 to 871 kg ha^{-1}) (Table 5). Besides the higher yield it also showed the strongest response to fertilizer application (Table 5). The precipitation measurements with our rain gauges are not detailed enough to analyse the impact of precipitation on yield, but it is clear that (of non-irrigated fields) the lowest yielding fields were also the fields which received the least precipitation (Table 5 and Appendix D). It is expected that drought played a major role and has constrained plant growth and yield as well as the response to the treatments.

5.2 Soil analysis for nutrient deficiency analysis

The soil chemical analysis was performed by two laboratories Cropnuts and Mlingano (Section 3.3.2). The data from the two laboratories conflicted for several parameters (Appendix E), which cannot be explained. Of the parameters that were analysed in both laboratories, only the results from Cropnuts were presented in the report because this laboratory is accredited by the Dutch Accreditation Authority (Cropnuts, 2014). However, there were still problems with the available P values from the Cropnuts laboratory, which ranged between 16.3 - 55.2 and contradicting with the trial results, previous literature and the available P values from Mlingano (Appendix E). Therefore Cropnuts checked and elaborated their Olsen P method and found that their activated charcoal was contaminated with P causing their available P results to be too high. The available P values were reanalysed and those values were in accordance with other results and used in this report.

5.2.1 Phosphorus and potassium deficiency

Phosphorus

The available P(Olsen) ranged between 1.6 and 10.9 mg P kg^{-1} with average of 4.0 mg P kg^{-1}, which is all below the critical value of 15 mg P kg^{-1} (Landon, 1991) needed for crop growth. The available P in the soil is low, which provides a good indication of soil P deficiency (Bolland and Gilkes, 1992). These results are in accordance with the strong response to P fertilizers in the experimental field trials (Section 4.1.2) and P deficiencies found in the soil in previous research in the region (Section 1.3) with available P values ranging from 1.6 - 9.6 (Smithson et al., 1993), 1.68 - 6.3 (with Bray-I) (Ndakidemi et al., 2006) and 0.2 - 6.6 (Amijee and Giller, 1998).

Potassium

The K values found with chemical analysis range from 0.1 – 0.3 (Table 8) which is below the critical value of 0.2 (Anderson, 1973) for most fields, indicating K deficiency in the soil. These low K values are lower but in accordance with previous research with values of 0.2 - 0.8 (Smithson et al, 1993), 0.1 - 0.6 (Ndakidemi and Semoka, 2006), 0.1 - 0.9 (Amijee and Giller, 1998) and 0.3 - 1.8 (Mowo et al. 2006). The low K values found with chemical soil analysis correspond with the strong response to K fertilizer in the trials (Section 4.1). All concentrations in the soil were deficient and almost all field respond to K fertilization.

The soil chemical analysis of P and K is in accordance with the trial response, as both methods point out P and K deficiency in the soil. However, the soil data is not indicative for the relative response of the crop to nutrient applications, because we are dealing with multiple interacting constraints. Soil test are especially useful when they are correlated to trial treatment responses, as yield is a function of many variables besides the nutrient availability in the soil (Sanchez, 1976). This is clearly reflected in the absence in a relation between soil chemical properties and the yield response; the response to P or K fertilizer does not increase when available P and K values in the soil decreases (Figure 9 and 10). There was a large variation in severity and impact of environmental constraints on yield between fields (Section 5.1.3) which were overriding P and K deficiency. This variation has caused a variation in treatment response rather than the available P and K in the soil, and is the cause for the absence of a relation between trial yield response and soil chemical properties.

5.2.2 Overall fertility

No singe soil parameters (besides available P and K concentration in the soil) were related to the yield response in the trials, as these parameters are only one of the many hundred variables that influence crop growth (Sanchez, 1976). However, an estimate of soil fertility based in the soil chemical analysis will be discussed.

Besides deficiency of P and K the soils were reasonably fertile and suitable for crop growth. A low pH can constrain the nodulation, growth and yield of common bean (Buerkert et al., 1990; Fageria et al., 2008) but the soils are not acid and have a pH within the range of 5.5-6.3 which is suitable for crop growth (Landon, 1991). The organic carbon content ranges between 1.4 and 2.6 %C, the critical value for organic carbon is considered to be 2 %C (Kemper and Koch, 1966; Greenland et al., 1975). The total N concentration in the soils ranged between 0.1-0.3 %N, which can be rated as low to medium (Landon, 1991). Most of the sites have reasonable C content, only one of the fields had a carbon content below the critical value (F6 Kwemsanga). Common bean is sensitive to salinity (Doorenbos en Kassam, 1979). The EC values above the optimal range of 0-0.7 (Landon, 1991) indicate that the soil might be too saline. However, the fields with the highest EC values are the highest yielding fields, indicating that other factors than EC determined yield. Concentrations of Ca, Mg and Na and were reasonable for crop growth (Landon, 1991). The CEC values in the soil ranged between 12 and 24 cmol kg^{-1}, which is not as low as would be expected for weathered soils. It indicates that the soil ability to retain nutrients is relatively reasonable (Sanschez and Logan, 1992).

5.3 Biological nitrogen fixation

5.3.1 Rhizobia population

Rhizobia populations present in the soils ranged between $1.18x10^2$ to $>2.40x10^5$ cells g $soil^{-1}$, seven of the nine fields had a population larger than 10^4 cells g $soil^{-1}$. This is larger than the populations found by Amijee and Giller (1998), who found populations up to 10^4 cells g $soil^{-1}$, and even some soils without any rhizobia bacteria present. Common bean is a major crop in the area which has been cultivated on many of the study sites in the past (Section 4.3), so the presence of rhizobia in the soil is likely.

5.3.2 BNF determinants

The amount if N fixed biologically depends on many factors. With the knowledge of the indigenous rhizobia population in the soil the main determinants of BNF are known and their role in this study area is discussed below.

The main determinants of BNF of common bean are:

1) Presence of indigenous rhizobia population: The absence or presence of compatible rhizobia populations in the soil is one of the determinants of the success of inoculation. The probability of enhancing yield with inoculation decreases with increasing numbers of indigenous rhizobia. When rhizobia populations are above 10 cells g $soil^{-1}$ a yield effect is not likely (Thies et al., 1991a). The rhizobia populations found are of such quantities (Section 4.3.1) that a response of inoculation is not expected and this was also observed as the inoculation effect on yield was not significant (Section 4.1.3). Nodulation increased by inoculation (Figure 2), which could indicate that the inoculum strains applied were of a better quality than the indigenous strains. However, the difference between the inoculum and indigenous strains was not large enough to effect yield (Section 4.1.3).

2) N deficiency in the soil: Another determinant is N availability (in the soil or by fertilizer application) for crop growth. With sufficient N available the plant will not invest in nodules (Marschner, 2012). The trials demonstrated no effect of N fertilization on yield. This is likely to be an effect of the indigenous rhizobia population in the soil causing the plant to be able to fix N (Section 5.1.2) (Thies et al., 1991b).

3) Other nutrient deficiencies. Nodulation was significantly enhanced with P and K fertilizer addition, indicating that P and K deficiency limited BNF (Section 5.1.1). By adding P and K fertilization plant health and thus its ability to fix N is increased. P is known to play an important role in BNF, and in the case of P deficiency P addition increases nodulation and yield (Marschner, 2012), which was demonstrated by the trials (Section 4.1.1).

4) Environmental constraints: High soil temperatures can limit nodulation (Giller, 2001). However, the soil temperatures were not so high that this has been a constraint. Drought is also a very harmful constraint to BNF (Kantar et al., 2010), but it is impossible to assess to what extent it has had an influence. The combination of drought, diseases, insects and other environmental constraints were very likely to have suppressed BNF by reducing the plant health to such a stage that it will not invest in nodulation. Fields with poor plant health (Section 5.1.4) were in general the poorly yielding fields, indicating that nodulation is closely linked to plant health (Appendix H). At these fields there was no active nodulation, not even for the treatments with inoculation and fertilization, which indicates that environmental constraints have limited BNF at these fields.

The MPN counts demonstrated that rhizobio populations are present in the soil. A response to inoculation is in that case less likely, but still possible, especially with a high quality inoculum. The results of this research did not show a positive significant effect to inoculation. However, it is very likely that the environment constraints suppressed nodulation, and therefore the impact of inoculation. More research is needed to investigate the potential impact of inoculation on yield.

5.4 Relation with management

Soil fertility is closely linked to land management. The field owners indicated to use some inorganic fertilizers but not much, and if applied it was to vegetables or maize. This is accordance with previous research by Mowo et al. (2006) who attributes this to the high prices of fertilizer, absence of suppliers and information on how to use fertilizers. The interviews with the field owners (Section 4.) demonstrated that the field owners never apply inoculum, fertilizer or manure on beans, which is one the main causes for the deficiencies of P and K. If they do use chemical fertilizer, it is mostly DAP, which lacks K and causes the lack for K at the fields.

Most of the low yielding trials were fields that the owners do not use for cultivation in the short rainy season and leave fallow, because of unsatisfying yields. Those fields (Kikurunge, Kwemsanga, Mbuzii 1 and Mbuzii 2, Table 13) were also fields that were very poorly yielding with average yields ranging from 78-266 kg ha^{-1}, which is indeed not rewarding for farmers. This does not explain why there is a low yield, but does point out that farmers are aware of the drought in the short rainy season (Section 5.1.3).

Most of the problems observed during the trials were also observed by the field owners. Also many farmers indicated they had observed a yellowing of the leaves, which is interpreted as ‘Usambara Mottle’,

an indicator for K deficiency (Smithson et al., 1993). This is in accordance with the strong response to K fertilizer demonstrated by the trials.

5.5 Spatial variability Usambara Mountains

Position on slope

The only field that was located in the valley bottom (Jaegertal) is the field with the highest yield, but this is mostly due to the irrigation it received (5.1.3). There seems to be a relation between slope gradient and yield (Table 1 and Table 5), with the most healthy fields located on lower slopes or flat areas. There is no relation in exchangeable K or available P in the soil and position on slope, altitude or slope gradient (Table 1 and Table 8), in contrast to Mowo et al. (2006) who found a variation in exchangeable K and available P along the slopes around Lushoto. This indicates that the exchangeable K and available P value in the soils of the trial sites is stronger related to factors like management and soil type than variation on the slope.

Precipitation

The precipitation measured with rain gauges showed a decrease in the spatial variation of precipitation to the south (Figure 1 and Appendix D). These measurement are not robust enough to derive a spatial pattern in precipitation. However, this pattern is reflected by the low yielding fields (and also the fields that farmers tend to leave fallow in the short rainy season due to drought) that are located in the southern part of the research area.

Nutrient deficiencies

Fertilizer trials scattered throughout an area can be used to determine the general nutrient deficiencies and average fertilizer requirements of a region (Sanchez, 1976). The soil nutrient deficiencies depend on the local characteristics. The spatial variability in soils and environment in the Usambara Mountains (Section 2.2) complicates the extrapolation of the trial result to a larger area. However, the overall positive response to P and K fertilization was very strong and only one of the nine sites did not show a positive response to the application of P and K fertilizer. Keeping local variability in mind, the results indicated that the soils in the area are generally deficient in P and K for the production of common bean.

5.6 Recommendations for N2Africa

N2Africa will be working in the Usambara Mountains in the near future with the purpose of improving legume yields of smallholder farmers. Based on the finding of our research it is recommended they focus their activities on the availability and information on use of fertilizers. The trial results and soil analysis have demonstrated that the soils in the area are deficient in P and K. Inorganic and organic fertilizers are needed by the farmers to improve their yield. The use of inoculum might increase yields slightly but it is not a major constraint, as indigenous rhizobia populations are present in the study area. However, the impact of inoculation should be studied more extensively, as nodulation was probably suppressed by drought or poor plant health. Besides the application of fertilizers, the quality of seed and availability of quality seeds should be assessed.

The use of fertilizers have a high potential to increase farmers yields and has the addition of P and K fertilizer has the potential to double farmers yields in this area (Section 4.1.6). N2Africa should focus on the availability and affordability (for example providing inorganic fertilizers in very small amounts) of the fertilizers, as the famers in this area are resource poor, and are not very likely to invest in expensive fertilizers. Besides this, it is very important to increase the knowledge about soil fertility constraints and the how use of organic and inorganic fertilizers can tackle these soil fertility constraints. Farmers should be made aware that the application of fertilizers does not only have a high potential for maize and vegetables, but also for beans.

However, it should be kept in mind that there were many factors other than soil fertility constraints limiting the production of common bean on the other fields. Five out of nine sites had very poorly yields due to constraints like drought, insects and diseases. On these fields, the introduction of fertilizers, inoculum or quality seed will not be rewarding due to these other overriding factors. Future research should focus on these other limiting factors in order to achieve rewarding yields in this area.

When establishing trials to study or demonstrate nutrient deficiencies in the future it is recommended to establish these in the long rainy season, as precipitation is not enough for crop growth in the short rainy season in some regions. It should also be taken in account that the study area covers a small part of the West Usambara Mountains and local circumstances at other areas in the Usambara Mountains should be assessed.

Chapter 6 Conclusion

The objective of this research was to describe the soil fertility constraints to the production of common bean in the Usambara Mountains. Based on this research it can be established that major soil fertility constraints of common bean are P and K deficiency.

- The highly significant response in crop vigour, biomass and yield to P and K fertilization have established P and K to be deficient in the soil. This was confirmed by the soil chemical properties.
- The selected soil chemical properties demonstrated no other soil parameters to be a major constraint to crop growth besides P and K deficiency.
- Most probable number counts showed that rhizobia populations were present in sufficient quantities in the soil. This is in line with the lack in response to N fertilization and inoculation. Based on this research the absence of rhizobia populations in the soil nor N deficiency seems a major soil fertility constraint. However, it is very likely that the impact of inoculation was suppressed by environmental constraints, and more research is needed to conlude about the potential impact if inoculation.
- The nodulation assessment indicated P and K deficiency to be limiting nodulation and BNF.

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Appendix A : Treatments

Table A1: Factors and levels of the factorial trial.

Factors	Treatment (levels)		Unit of train or fertilizer
	-	+	
inoculation	No	Yes	*Rhizobium* strain CIAT-899
P	0	26	kg ha^{-1} P as triple superphosphate (TSP)
K	0	25	kg ha^{-1} K as muriate of potash (MOP)
N	0	25	kg ha^{-1} N as calcium ammonium nitrate (CAN)

Table A2: Treatment structure (factorial in italic)

	Treatment number	Treatments:
	1	control 1
Factorial	*2*	*control 2*
PKI	*3*	*K*
T2-T9	*4*	*P*
	5	*Inoc*
	6	*P + K*
	7	*K + inoc*
	8	*P + inoc*
	9	*P + K + inoc*
	10	P + K + N

Appendix B: Field trial lay-out

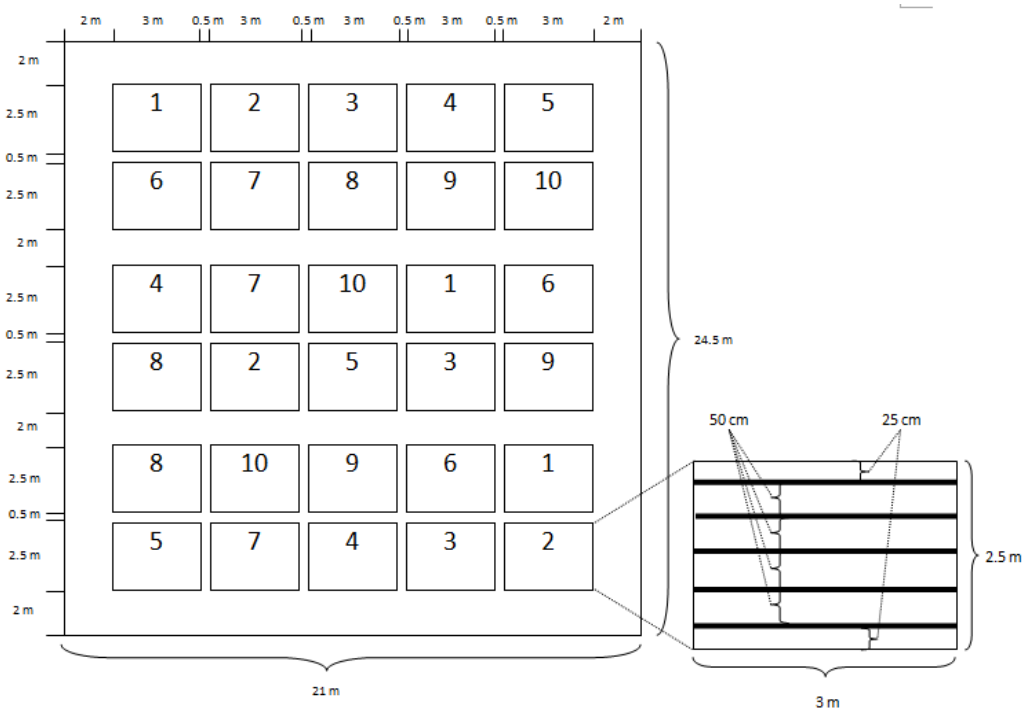

Figure A1 Schematic display of trial lay-out in case of three replicates.

Table A3 Field size per replica and in case of two or three replicates

	Total parcel		Net dimension gross plots (exl borders)	
	Parcel	dimension	Parcel	dimensions
One plot	9.5 x 21 m	199.5 m^2	5 x 15 m	75 m^2
+ one rep	17 x 21 m	357 m^2	10 x 15 m	150 m^2
+ two rep	24.5 x 21 m	514.5 m^2	15 x 15 m	225 m^2

Table A4: Number of replicates per field

Field	Number of replicates
#1 Mabughai	3
#2 Jaegertal	3
#3 Lushoto	2
#4 Kikurunge	3
#5 Mshizii	2
#6 Kwemsanga	3
#7 Ngulwi	3
#8 Mbuzii 1	3
#9 Mbuzii 2	2

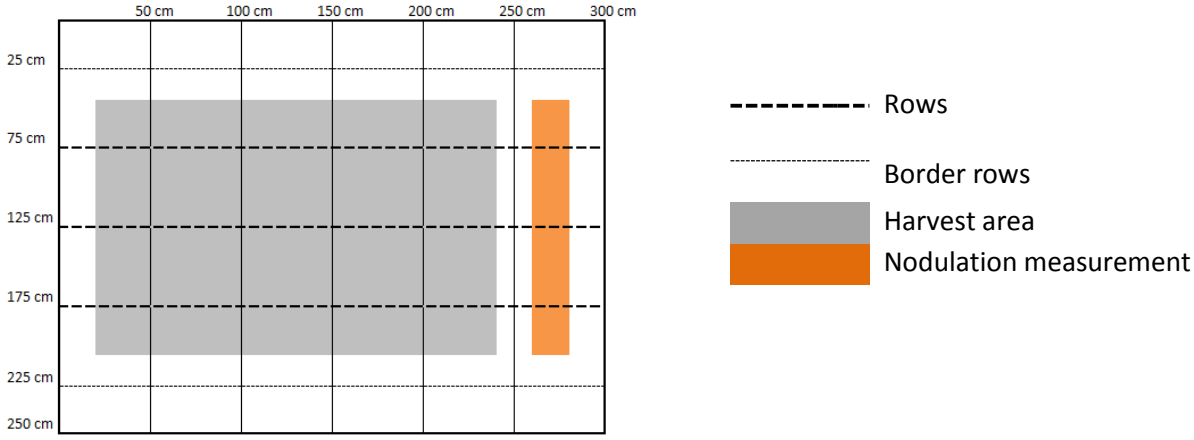

Figure A2 Schematic display of one treatment plot:

Appendix C: sowing and management dates

From Nov 2013 to Feb 2014

Table A5 Plant phenological dates

Fields	Planting	Emerge	50% Flowering	50% Podding	Maturity	Harvest
#1 Mabughai	8-11	16-11	26-12	2-1		12-2
#2 Jaegertal	7-11	17-11	23-12	30-12	15-1	11-2
#3 Lushoto	12-11	25-11	4-1	11-1		11-2
#4 Kikurunge	11-11	2-12	7-1	14-1		10-2
#5 Mshizii	15-11	30-11	28-12	4-1	13-1	9-2
#6 Kwemsanga	9-11	30-11	24-12	31-12	11-1	Rep. 1 27-1, Rep. 2+3 9-2
#7 Ngulwi	13-11	25-11	25-12	1-1	11-1	28-1
#8 Mbuzii 1	19-11	28-11	29-12	5-1	13-1	29-1
#9 Mbuzii 2	19-11	28-11	29-12	5-1	13-1	29-1

Table A6 Date of management practices

Fields	Thinning	Weeding	Irrigation
#1 Mabughai	28-11	07-12	-
#2 Jaegertal	28-11	09-12	10-11; 15-12; 29-12
#3 Lushoto	30-11	19-12	-
#4 Kikurunge	05-12	20-12	-
#5 Mshizii	03-12	20-12	-
#6 Kwemsanga	03-12	05-12	-
#7 Ngulwi	29-11		-
#8 Mbuzii 1	02-12	09-12	-
#9 Mbuzii 2	02-12	09-12	-

Table A7 Dates of data recording

Fields	Stand at emerge	Early deficiency signs	Crop vigour
#1 Mabughai	28-11	4-12	26-12
#2 Jaegertal	28-11	6-12	19-12
#3 Lushoto	30-11	19-12	4-1
#4 Kikurunge	5-12	20-12	14-1
#5 Mshizii	3-12	18-12	4-1
#6 Kwemsanga	3-12	18-12	24-12
#7 Ngulwi	29-11	18-12	25-12
#8 Mbuzii 1	2-11	20-12	5-1
#9 Mbuzii 2	2-11	20-12	5-1

Appendix D: precipitation estimation

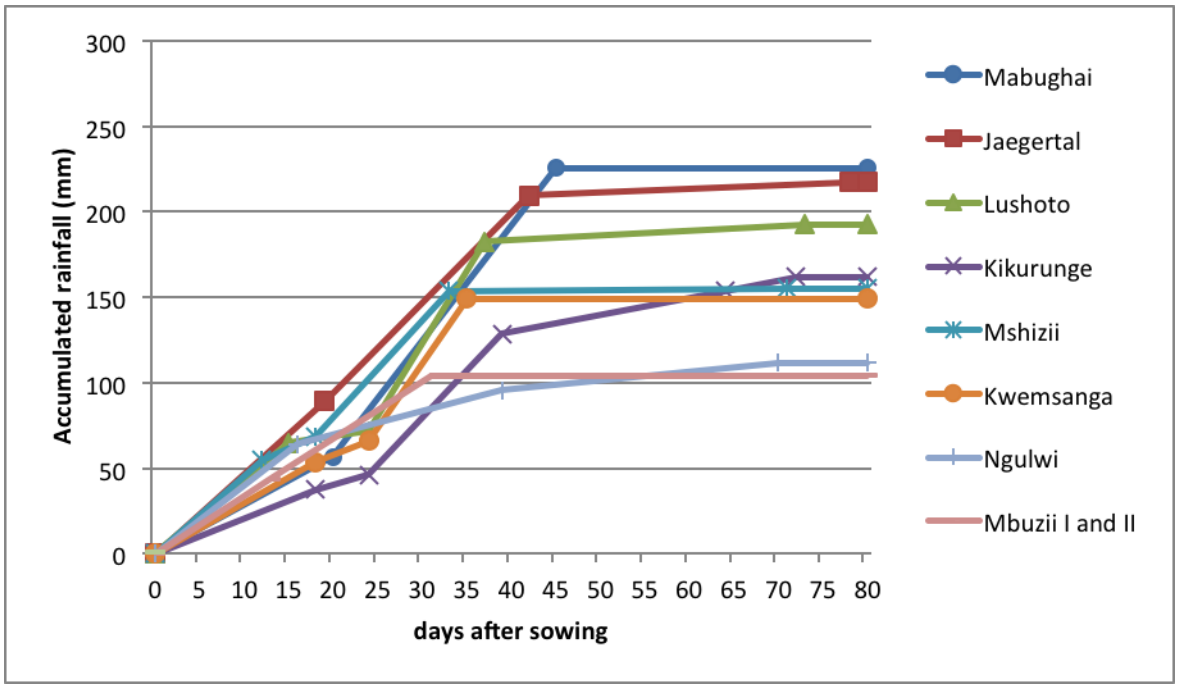

Figure A3 Cumulative precipitation per field in days after sowing

Appendix E: Soil data from both laboratories

Table A8 Chemical properties of the soil from both the Cropnuts (Cr) and Mlingano (Ml) laboratory.

	Chemical analysis															
Parameter	pH		Available P.		K		Ca		Mg		Na		EC(S)		CEC	
Laboratory	Cr	*Ml*	Cr	*Ml*	Cr	*Ml*	Cr	*M*	Cr	*M*	Cr	*Ml*	Cr	*Ml*	Cr	*Ml*
Method	*H20*		Olsen-P	*Br-I*	AAS		AAS		AAS		AAS				ammonium acetate extraction	
Unit	pH		mg kg^{-1}		me $100g^{-1}$		me $100g^{-1}$		me $100g^{-1}$		me $100g^{-1}$		mS cm^{-1}		me $100g^{-1}$	
F1 Mabughai	5.3	*5.4*	6.1	*3.9*	0.1	*0.3*	3.9	*2.1*	0.8	*0.5*	0.2	*0.3*	1.1	0.1	17.6	*9.2*
F2 Jaegertal	5.6	*5.5*	1.5	*3.2*	0.2	*0.5*	5.6	*4.4*	1.4	*1.6*	0.2	*0.3*	2.7	0.2	21.7	*9.0*
F3 Lushoto	5.2	*5.3*	10.8	*1.9*	0.2	*0.5*	2.4	*3.7*	0.7	*0.7*	0.2	*0.2*	1.2	0.1	12.5	*7.2*
F4 Kikurunge	6.9	*6.3*	2.5	*2.9*	0.1	*0.3*	8.0	*5.8*	3.1	*1.1*	0.3	*0.3*	0.7	0.1	24.2	*11.6*
F5 Mschizii	6.6	*6.3*	5.7	*3.9*	0.2	*0.4*	5.0	*3.3*	1.8	*2.0*	0.3	*0.4*	0.7	0.1	15.7	*10.6*
F6 Kwemsanga	6.4	*6.3*	2.3	*3.8*	0.2	*0.4*	5.4	*3.6*	1.6	*1.7*	0.2	*0.2*	0.7	0.1	16.8	*9.8*
F7 Ngulwi	6.0	*6.2*	2.5	*2.9*	0.2	*0.3*	5.2	*3.3*	1.3	*1.5*	0.2	*0.1*	0.7	0.1	16.7	*8.2*
F8 Mbuzii 1	6.1	*6.0*	2.7	*2.5*	0.2	*0.4*	6.3	*4.9*	2.2	*2.1*	0.3	*0.3*	0.8	0.1	21.5	*10.2*
F9 Mbuzii 2	6.1	*6.1*	1.5	*3.7*	0.2	*0.5*	5.4	*3.6*	2.0	*2.1*	0.3	*0.1*	1.2	0.1	18.7	*9.8*
Average	6.0	*5.9*	4.0	*3.2*	0.2	*0.4*	5.2	*3.9*	1.7	*1.5*	0.3	*0.2*	1.1	*0.1*	18.4	*9.5*

Appendix F: Statistical results ANOVA in R

Table A9 Statistical significance from the ANOVA analysis Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

	perc.plants.emergence			perc.plants.maturity			early.deficiency.score			crop.vigour.score			nodulation.score		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
F	5.0609	2.2E-16	***	4.9022	6.6E-16	***	10.889	0.02733	*	5.569	0.23609		275.847	2.2E-16	***
block	2.6524	1.4E-10	***	0.5697	0.43217		16.426	0.03892	*	28.204	3.6E-05	***	10.259	0.11984	
P	0.0276	0.3069		0.1546	0.0506	.	136.889	2.2E-16	***	72.593	2.2E-16	***	31.178	1.8E-13	***
K	0.5979	4.7E-06	***	0.0038	0.75774		7.5	0.00058	***	11.837	5.4E-06	***	9.759	9.8E-06	***
I	0.0082	0.57699		0.0104	0.609		0.32	0.46802		1.14	0.14294		11.591	1.7E-06	***
P:K	0.0163	0.43227		0.2263	0.01846	*	0.145	0.62427		2.731	0.02419	*	0.792	0.19211	
P:I	0.0421	0.2069		0.0001	0.95265		1.41	0.12872		0.31	0.44395		0.714	0.2152	
K:I	0.0019	0.78786		0.0886	0.13766		0.167	0.59995		0.032	0.80495		0.002	0.94161	
P:K:I	0.0111	0.51591		0.0405	0.31432		2.689	0.03669	*	3.2	0.01485	*	2.222	0.02983	*
F:P	0.3664	0.09325	.	0.7221	0.01558	*	33.926	1.1E-07	***	34.764	5.2E-09	***	10.284	0.00698	**
F:K	0.769	0.00069	***	0.2451	0.52206		12.621	0.011	*	9.197	0.03221	*	6.77	0.0757	.
F:I	0.2729	0.24787		0.2556	0.49281		3.434	0.68094		4.117	0.45428		8.572	0.02289	*
F:P:K	0.3893	0.07212	.	0.1584	0.77853		6.954	0.1856		8.513	0.04806	*	8.59	0.02262	*
F:P:I	1.4138	2.3E-07	***	0.2337	0.55468		0.496	0.99908		1.477	0.94358		2.375	0.73894	
F:K:I	0.4288	0.04585	*	0.1173	0.88641		4.641	0.46896		2.216	0.83393		3.63	0.45047	
F:P:K:I	0.3795	0.08059	.	0.348	0.27921		4.539	0.48578		5.272	0.27252		4.556	0.28301	
Residuals	3.3788			4.7177			77.824			67.713			59.407		

Table A10 Statistical significance from the ANOVA analysis Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

	crop.height			grain.yield.ha			grain.yield.plant			dw.stem.ha			dw.stem.plant		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
F	9052.8	2.2E-16	***	24686.7	2.2E-16	***	148.252	2.2E-16	***	3554.8	2.2E-16	***	17.3547	2.2E-16	***
block	719.4	9.3E-05	***	1440.7	7.8E-09	***	7.571	1E-09	***	534	2.7E-10	***	2.8835	2.1E-12	***
P	991.7	3.2E-11	***	543.8	1.2E-07	***	2.149	9.2E-07	***	500.2	2.2E-16	***	2.3421	2.2E-16	***
K	68.2	0.04908	*	201.8	0.00081	***	1.029	0.0005	***	39.5	0.00823	**	0.2119	0.00367	**
I	6.1	0.5534		5.1	0.58621		0.007	0.77569		5.1	0.3351		0.0278	0.28547	
P:K	32.1	0.17478		36.8	0.14474		0.291	0.05948	.	4	0.39545		0.0787	0.07365	.
P:I	12.5	0.39487		10.9	0.42584		0.084	0.30901		3.1	0.45201		0.0275	0.28847	
K:I	2.5	0.70245		2.4	0.70873		0.053	0.41842		0	0.9377		0.0016	0.79985	
P:K:I	10.2	0.4421		24	0.23848		0.086	0.30431		17.9	0.07275	.	0.0877	0.05911	.
F:P	523.8	6.8E-05	***	567.1	0.0001	***	3.433	9.1E-06	***	387.2	6.9E-10	***	2.1269	1E-11	***
F:K	157.3	0.11385		232.3	0.06853	.	1.09	0.10678		111.3	0.00765	**	0.4582	0.02071	*
F:I	56.4	0.65575		85.6	0.65785		0.517	0.6013		28.8	0.62789		0.1573	0.59239	
F:P:K	146.4	0.14075		237	0.06286	.	1.138	0.09007	.	68.8	0.09389	.	0.2767	0.19015	
F:P:I	1.9	0.99978		137.9	0.3345		0.863	0.23065		27.4	0.65932		0.1517	0.61787	
F:K:I	38.3	0.8138		200.9	0.11994		1.143	0.08845	.	74.6	0.06782	.	0.4046	0.04125	*
F:P:K:I	47	0.73845		250	0.0495	*	1.451	0.02788	*	50	0.25346		0.2093	0.38007	
Residuals	1472.2			1996.4			10.153			650.4			3.0966		

Table A11 Statistical significance from the ANOVA analysis Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

	100.seed.weight			nr.pods.m2			nr.pods.plant			nr.seed.pod		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
F	12874.7	2E-16	***	343917	2.2E-16	***	1035.86	2.2E-16	***	120.794	2.2E-16	***
block	443.4	0.323		19323	5.2E-11	***	57.65	1.8E-12	***	2.626	0.00613	**
P	120.9	0.03223	*	8878	2.2E-10	***	19.7	2.6E-09	***	0.102	0.24844	
K	0.7	0.86715		2118	0.00094	***	5.8	0.00069	***	1.228	9.7E-05	***
I	6.6	0.61389		12	0.7986		0.01	0.87124		0.031	0.52292	
P:K	15.1	0.44589		318	0.19091		1.85	0.05131	.	0.003	0.8408	
P:I	1.9	0.78482		16	0.7698		0.19	0.52849		0.154	0.15623	
K:I	57.6	0.13743		53	0.59328		0.63	0.2523		0.011	0.70616	
P:K:I	47.9	0.17554		216	0.28028		0.34	0.39799		0.005	0.79527	
F:P	263.5	0.26108		14058	1.5E-10	***	41.11	2E-11	***	1.021	0.10851	
F:K	243.8	0.31528		3009	0.02867	*	7.3	0.06449	.	1.605	0.01019	*
F:I	166.7	0.59662		1611	0.28075		5.17	0.22496		0.385	0.74618	
F:P:K	113.3	0.81726		2586	0.0596	.	6.21	0.1246		1.443	0.02012	*
F:P:I	123.6	0.77722		1226	0.4696		3.89	0.4273		0.976	0.12813	
F:K:I	412.4	0.05165	.	2865	0.03689	*	9.52	0.01542	*	0.588	0.46281	
F:P:K:I	494	0.01943	*	1452	0.35017		4.78	0.27726		2.572	0.00016	***
Residuals	3249.3			21516			60.31			9.546		

Appendix G: Significance of I and N in relation to PK only

Table A12 Statistical significance from the ANOVA analysis of I and N in relation to PK treatment Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

	perc.plants.emergence			perc.plants.maturity			early.deficiency.score			crop.vigour.score			nodulation.score		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
PKI	0.0105	0.6876		0.02	0.5711		4	0.03335	*	0.111	0.701495		9	0.04133	*
PKN	0.0044	0.795		0.0007	0.9131		16.333	4.03E-05	***	10.083	0.000479	***	1.333	0.42632	

	crop.height			grain.yield.ha			grain.yield.plant			dw.stem.ha			dw.stem.plant		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
PKI	75.3	0.4257		1.2	0.9392		0.011	0.9212		16.44	0.5543		0.1253	0.4459	
PKN	225.8	0.1706	***	14.9	0.7916		0.162	0.7099		58.56	0.2661		0.4397	0.1555	

	100.seed.weight			nr.pods.m2			nr.pods.plant			nr.seed.pod		
	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.	sum sq.	p-value	sign.
PKI	0.165	0.6434		3	0.9743		0	1		0.214	0.6088	
PKN	1.042	0.2465		206	0.7955		1.41	0.6877		0.006	0.9325	

Appendix H:

Table A13 Response in yield to the fertilizer effect in kg per ha for the field individually

Treatment	F1	F2	F3	F4	F6	F7	F8	F9
C	522	1630	389	115	176	227	205	97
K	938	1575	618	144	294	181	200	4
P	885	2738	551	61	341	281	131	32
I	606	1349	568	174	349	184	123	11
PK	1477	2256	718	179	245	371	141	112
KI	605	1844	426	93	349	162	122	97
PI	598	2132	252	65	276	285	192	81
PKI	1107	2684	1091	225	168	404	148	106
PKN	1451	2530	1084	209	288	365	126	165

Table A14 Response in nodualtion score to the fertilizer effect for the field individually

Treatment	F1	F2	F3	F4	F6	F7	F8	F9
C	2	3	1	0	0	0	1	0
K	3	4	1	0	1	1	0	0
P	3	4	2	1	1	2	1	0
I	3	4	3	0	1	2	2	0
PK	4	4	2	1	1	2	1	0
KI	3	3	2	1	1	2	1	0
PI	3	4	3	1	0	2	1	0
PKI	4	4	4	2	2	4	2	0
PKN	3	3	3	0	1	3	0	0

Appendix I: Soil parameters on yield response

Table A15 Mixed model results of the effect of soil parameters on the yield effect to the P, K and I treatments

Fixed effect/ indep. variable		response/dependent variable											
tested variable:	control variable:	grain.yield.ha			dw.stem.ha			crop.vigour.score			nodulation.score		
soil parameter	treatment	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.
soil.ph	P	4.1189	0.04241	*	4.6679	0.03073	*	1.2057	0.2722		3.7811	0.05184	.
	K	4.1229	0.04231	*	4.6663	0.03076	*	1.2057	0.2722		3.7824	0.05179	.
	I	4.1313	0.0421	*	4.6652	0.03078	*	1.2057	0.2722		3.782	0.05181	.
soil.org.c	P	0.6043	0.4369		0.7128	0.3985		0.4441	0.5051		0	0.9971	
	K	0.6057	0.4364		0.7095	0.3996		0.4441	0.5051		0	0.997	
	I	0.6082	0.4355		0.7074	0.4003		0.4441	0.5051		0	0.997	
soil.total.n	P	5.7034	0.01693	*	6.1567	0.01309	*	1.0633	0.3025		2.6356	0.1045	
	K	5.7072	0.0169	*	6.1406	0.01321	*	1.0633	0.3025		2.6356	0.1045	
	I	5.7141	0.01683	*	6.13	0.01329	*	1.0633	0.3025		2.6356	0.1045	
soil.av.p	P	0.6123	0.4339		0.78	0.3775		0.0877	0.7671		0.2034	0.652	
	K	0.613	0.4336		0.78	0.3786		0.0877	0.7671		0.2033	0.652	
	I	0.6143	0.4332		0.77	0.3793		0.0877	0.7671		0.2033	0.652	
soil.cec	P	0.0042	0.9484		0.0005	0.9822		1.7964	0.1801		0.07	0.7913	
	K	0.0041	0.9488		0.0006	0.9801		1.7964	0.1801		0.0701	0.7911	
	I	0.004	0.9496		0.0007	0.9788		1.7964	0.1801		0.0701	0.7912	
soil.ca	P	0.9084	0.3406		1.0026	0.3167		2.4078	0.1207		0.9826	0.3216	
	K	0.9089	0.3404		0.9989	0.3176		2.4078	0.1207		0.9833	0.3214	
	I	0.9099	0.3401		0.9965	0.3181		2.4078	0.1207		0.9831	0.3214	

Table 16 Mixed model results of the effect of soil parameters on the yield effect to the P, K and I treatments

Fixed effect/ indep. variable		response/dependent variable											
tested variable:	control variable:	grain.yield.ha			dw.stem.ha			crop.vigour.score			nodulation.score		
soil parameter	treatment	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.
soil.mg	P	2.6186	0.1056		2.1339	0.1441		1.4596	0.227		4.0086	0.04527	*
	K	2.6183	0.1056		2.1268	0.1447		1.4596	0.227		4.0102	0.04523	*
	I	2.6169	0.1057		2.1222	0.1452		1.4596	0.227		4.0097	0.04524	*
soil.k	P	0.2297	0.6317		0.0103	0.9192		0.0056	0.9405		3.0843	0.07905	.
	K	0.2269	0.6338		0.01	0.9202		0.0056	0.9405		3.0822	0.07915	.
	I	0.221	0.6383		0.0099	0.9209		0.0056	0.9405		3.0828	0.07913	.
soil.na	P	1.9679	0.1607		0.7525	0.3857		0.2812	0.5959		4.9588	0.02596	*
	K	1.9606	0.1615		0.7468	0.3875		0.2812	0.5959		4.9559	0.026	*
	I	1.9446	0.1632		0.7431	0.3887		0.2812	0.5959		4.9567	0.02599	*
soil.ec	P	10.137	0.00145	**	15.123	1.01E-04	***	0.0304	0.8616		3.1511	0.07588	.
	K	10.172	0.00143	**	15.099	1.02E-04	***	0.0304	0.8616		3.1529	0.07579	.
	I	10.245	0.00137	**	15.083	1.03E-04	***	0.0304	0.8616		3.1524	0.07581	.

Appendix J: Treatment response to rhizobia population

Table 17 Mixed model results of the effect of soil rhizobia population on the yield effect to the P, K and I treatments

Fixed effect/ indep. variable		response/dependent variable											
tested variable:	control variable:	grain.yield.ha			dw.stem.ha			crop.vigour.score			nodulation.score		
soil parameter	treatment	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.	Chi-sq.	P-value	Sign.
soil.rhizobia.pop	P	1.037	0.3085		1.6382	0.2006		0.6127	0.4338		9.00E-04	0.9764	
	K	1.041	0.3076		1.6446	0.1997		0.6127	0.4338		9.00E-04	0.9765	
	I	1.0497	0.3056		1.6487	0.1991		0.6127	0.4338		9.00E-04	0.9765	

Appendix K: Crop vigour photos

Jaegertal 19/12/13

R1T1 - Control

R1T2 - Control

R1T3 - K

R1T4 - P

R1T5 - Inoc.

R1T6 - K+P

R1T7 - K+Inoc.

R1T8 - P+Inoc.

R1T9 - K+P+Inoc.

R1T10 - P+K+N

Ngulwi 25/12/13

R1T1 - Control

R1T2 - Control

R1T3 - K

R1T4 - P

R1T5 - Inoc.

R1T6 - K+P

R1T7 - K+Inoc.

R1T8 - P+Inoc.

R1T9 - K+P+Inoc.

R1T10 - P+K+N