Enhancing biological nitrogen fixation and yield of soybean and common bean in smallholder farming systems of Rwanda

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Abstract

Legumes play a key role in soil fertility replenishment, yet the yields achieved are far below their potential due to poor management practices. The main objective of this thesis was to increase soybean and common bean productivity in the smallholder farming systems of Rwanda. Inputs of manure at different rates, mineral fertilizers and rhizobial inoculants were used.

Field trials evaluating the response of common bean and soybean to inoculation, P and manure (0, 5 and 10 t ha^{-1}) in three agro-ecological zones of Rwanda showed greater grain yield, biomass and stover yields when inputs were combined. The %Ndfa, amount of N_2-fixed, N and P uptake were larger in treated plots compared to control plots. Large variability in the data following inputs application was observed, but there was no clear relationship between the response to inputs and soil parameters. Inputs applied to the legumes lead to substantial increase in the yield of a subsequent maize crop. However, maize grown after soybean failed to yield in Bugesera due to the long maturity of the soybean variety used which resulted in late planting of the maize.

The role of manure on the survival of rhizobia in the soil was explored. The population of rhizobia in the soil was higher in plots that had received manure two seasons earlier compared with plots that had been inoculated or plots that had received P fertilizer only. The number of rhizobia in manured plots was still higher eighteen months from the first sampling. In the dry season rhizobial numbers decreased and increased again soon after during the rainy season.

The Northern Province of Rwanda is the best region for climbing bean. However, yields achieved are very poor. Trials evaluating the response of climbing bean to manure (0, 2 and 5 t ha^{-1}) and mineral fertilizers (N, P, K and their combination) were established in Kinoni and Muko villages with seven fields in each village. Results showed consistent yield increase when inputs were used together. Greater yields were achieved when manure was combined with NPK. In all cases larger responses were observed with the higher rate of manure. Similarly, inputs application increased the amount of N_2-fixed, N and P uptake.

Determination of limiting nutrients to climbing bean was performed using the Compositional Nutrient Diagnosis (CND) and the Diagnosis and Recommendation Integrated System (DRIS). The two approaches were useful in identifying nutrient limitations to climbing bean in Northern Rwanda. We observed deficiencies of Zn, N, K and P in Kinoni, and Zn, Mg, Ca, P and N in the Muko site.

Keywords: Agro-ecological zones; legumes; management practices; nutrient deficiency; smallholder farmers; survival

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Chapter 1

General Introduction

1.1 Grain legumes in cropping systems

Population increase in Rwanda has led to tremendous pressure on agricultural land. This has led to soil fertility depletion as farmers use little or no use of fertilizer. The soil fertility restoration through fallows is no longer possible (Rutunga et al., 1998). In light of ensuring food security, sustainable intensification of agricultural production is needed (Vanlauwe et al. 2014), and integrating legumes is a viable and key option to achieve this goal. Legumes establish symbiotic relationships with rhizobial bacteria and fix atmospheric nitrogen (Giller, 2001; Herridge et al., 2008). Legume residues by decomposing release nutrients to the soil or contribute to soil organic matter for long term sustainability (Vanlauwe et al., 2014).

In Rwanda, grain legumes are important food crops for both human consumption, animal feed and complement cereals in both production and consumption. Legumes grown in Rwanda include common beans, soybeans, groundnuts and peas. However, common beans and soybeans are the most promoted by policy makers and most researched by the National Agricultural Research Institutes and partners. Common beans are the staple food and constitute the main source of protein, and soybean demand is also increasing due to development of oil processing plants and animal feed units. Some grain legumes have a short growing period, thus providing food during the hunger period in the middle of the cropping season (Rubyogo et al., 2010), others (e.g. climbing bean) provide a continuous supply of green leaves and pods as well as dry grain throughout the growing season (Wortmann et al., 2001).

Growing legumes in rotation with cereals is further recognized as a cost-effective way by which farmers can maintain soil fertility. Legumes meet a large part of their N requirement through N2-fixation, thus sparing some of the soil N to the subsequent crops (Osunde et al., 2003). In addition to soil fertility improvement through N cycling, their rotation with cereals helps to control diseases and pests in cereals. Therefore, the integration of grain legumes in cropping systems increases crop yields and N efficiency. Legumes are also intercropped with cereals. This practice is intended to reduce risks during poor growing seasons. The risk avoidance is for example linked to the possibility of smallholder farmers to feed on leaves and green pods (e.g maize-common bean intercropping) early in the growing season (Woomer et al., 2004). Intercropping maize with legumes is also reported to give greater advantages under low soil fertility conditions (Kermah et al., 2017).

1.2 Management options for improving legume productivity

Legumes are important components of cropping systems because of their ability to fix atmospheric nitrogen, add substantial amounts of organic matter to the soil. Yet the yields realized in farmers' fields are usually very low. Nutrients deficiencies, soil acidity and moisture stress have been reported as environmental factors limiting legume productivity (Giller and Cadisch, 1995). This suggests the need for improved management practices to improve their productivity.

Increase in legume productivity depends on the success of the interaction between the legumes, rhizobium strain nodulating the legume, the management involved and their adaptation to a wide range of environments (Giller, 2013). Exploitation of the legume-rhizobium symbiosis in agricultural systems is crucial. However, rhizobium strains have to survive in soil in sufficient numbers to avoid repeated inoculation each season (Crozat et al., 1982). The survival of rhizobia in soil is also affected by many factors such as low soil pH, desiccation, nutrient deficiencies, extreme temperatures, cropping history and season (Hungria and Vegas, 2000; Giller, 2001). Improvement and exploitation of benefits associated with nitrogen fixation is a good alternative for smallholder farmers to replenish fertility of their soils. Application of organic manure improves the survival of rhizobia in soil by creating a favourable environment for their growth and multiplication (Zengeni et al., 2006).

Integrated soil fertility management (ISFM) is an option for increasing crop productivity through combining fertilizer use with other approaches to soil fertility management (Vanlauwe et al., 2010). For instance, application of P fertilizer to the legume in a legume-maize rotation cropping system yielded high grain and biomass of the legume, which in turn resulted in better performance of the subsequent maize crop (Vandamme et al., 2014). Manure contributes not only to the restoration of soil fertility in depleted fields, but also in improving the response of crops to other nutrients, and enhanced N and P uptake. Application of manure and mineral fertilizer are recognized as important options to increase crop productivity (Zingore et al., 2008; Niyuhire et al., 2017), and inoculation combined with fertilizers (organic and /or mineral) showed promising results in farmers fields (Ronner et al., 2016; Franke et al., 2019).

1.3 Objectives

The general objective was to increase common bean and soybean productivity in the smallholder farming systems of Rwanda. As the smallholder farmers are achieving low yields due to soil nutrients depletion, this thesis emphasises on the role of management in increasing the productivity of the two legumes. The specific objectives were to:

1) Assess the effects of management options on the productivity of common bean and soybean, and to the subsequent maize crop grown in rotation (Chapter 2);
2) Evaluate how manure and soil characteristics affect the survival of rhizobia in soils, and assess if there is need to inoculate in previously inoculated plots (Chapter 3);
3) Assess the effects of applied inputs to the productivity of climbing bean (Chapter 4);
4) Determine the limiting nutrients to climbing bean productivity using approaches to identify nutrient imbalances based on plant tissue analysis (Chapter 5).

1.4 Selection of the study sites

This study was conducted within the framework of N2Africa project: Putting nitrogen fixation to work for smallholder farmers in Africa (www.N2Africa.org). This project was working in 11 African countries including Rwanda. The project was focusing on main legumes grown in each country. In Rwanda, beans (both bush and climbing beans) and soybean were selected as they were the main legumes promoted in the country. Bush bean and soybean are grown in the low and medium altitudes of the country while climbing bean is mostly grown in the highlands of the Northern Province. The study was carried out in farmers’ fields selected from the sites located in the N2Africa project mandate area. These sites were located in the major bean and soybean growing area in Rwanda. The bush bean and soybean fields were selected in three contrasting agroecological zones (AEZs) of Rwanda (Chapter 2) to assess the effect of environment on their performance. In each AEZ, one district was selected and three fields were also selected from each district. The three districts were Bugesera, Kamonyi and Kayonza (Detailed information are presented in Chapter 2 Section 2.1). The trials of Chapter 3 were carried out in Bugesera and Kamonyi districts in the same treatments with Chapter 2 with slight modifications. The trials reported in Chapters 4 & 5 that focused on climbing bean were established in the Northern Province in Kinoni (Burera district) and Muko (Musanze district) sites, and seven fields per site were selected (Detailed information is presented in Chapter 4 Section 2.1). The figure below shows the study sites (light green circles) selected from Musanze and Burera districts for climbing bean, Bugesera, Kamonyi and Kayonza for bush bean and soybean.

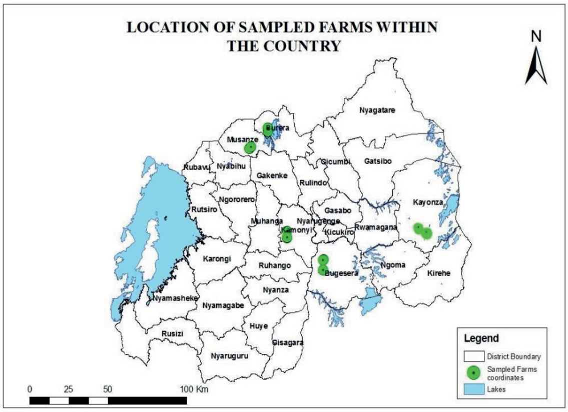

Fig. 1.1 Map of Rwanda showing sites where trials were established (light green circles)

1.5 Thesis outline

This thesis consists of six chapters: an introductory chapter, four research chapters and a chapter on discussion. Each of the research chapters represents one of the four objectives. Chapter 2 addresses Objective 1. It assesses how the use of inoculum combined with manure and P fertilizer enhances N2-fixation, yields, N and P uptake of common bean and soybean. It also evaluated how increased performance of the two legumes resulted in increased productivity of the subsequent maize crop grown in rotation. The fields and treatments involved in Chapter 2 were maintained with slight modifications to contain the trials of the Chapter 3. This chapter assesses the impact of manure on rhizobia survival in soil and on bean and soybean grain yields. It also assesses if there is a need to inoculate bean and soybean grown in previously inoculated soils. Chapter 4 assesses the role of manure and mineral fertilizer on climbing bean yields, N2-fixation, N and P uptake. The influence of soil characteristics on the response of climbing bean to inputs is discussed as well. Chapter 5 explores the nutrition status of climbing bean in the Northern Province of Rwanda using the compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) approaches. In this chapter, CND and DRIS norms were derived and compared. It also assessed the relationship between nutrient concentrations of foliar tissue and grain yield, and identified which nutrients are limiting in climbing bean in Northern Rwanda. Chapter 6 synthesizes the findings from the four chapters. It discusses the production of beans and soybeans in Rwanda, the impact of management options on the productivity of the two legumes in the smallholder farming systems, and the influence of seasonal variability on crop performance in monocropping systems. Crop diversification through intercropping is discussed as an option to mitigate climate shocks in drier regions. The chapter ends by providing a conclusion and recommendations for future studies.

Chapter 2

Benefits of inoculation, P fertilizer and manure on yields of common bean and soybean also increase yield of subsequent maize

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Abstract

Common bean and soybean yield poorly on smallholder farms in Rwanda. We evaluated the benefits of inoculation combined with P fertilizer and manure on yields of common bean and soybean in three agro-ecological zones (AEZs), and their residual effects on a subsequent maize crop. In the first season, the treatments included inoculum, three rates of manure, and two rates of P fertilizer, with nine replications (three per AEZ). Both legumes responded well to inoculation if applied together with manure and P fertilizer. Grain yields varied from 1.0 t ha^{-1} to 1.7 t ha^{-1} in unamended control plots to 4.8 t ha^{-1} for common bean and 3.8 t ha^{-1} for soybean in inoculated plots with both P and manure addition. The response of common bean and soybean to inputs varied greatly between AEZs. In the AEZ with low and erratic rainfall (Bugesera), yields of both legumes and maize were low and maize after soybean failed to yield any grain due to drought. In this regard, early maturing legume varieties are advised in regions of low rainfall. Responses of maize to an input applied to the legumes strongly increased when other inputs were applied together to the legume. This allowed greater maize yields which ranged from 0.8 t ha^{-1} in control plots to 6.5 t ha^{-1} in treatments previously inoculated with P and manure added for maize grown after common bean and from 1.9 t ha^{-1} in control plots to 5.3 t ha^{-1} for maize grown after soybean. The amount of N_2-fixed measured using the ^{15}N-natural abundance method differed between the two legumes and varied between 15 and 198 kg N_2 ha^{-1} for common bean and between 15 and 186 kg N_2 ha^{-1} for soybean and differed enormously among treatments and AEZs. Application of inputs to the legumes also resulted in enhanced N and P uptake of the subsequent maize. The use of inoculum combined with manure and P fertilizer is a good option for smallholder farmers growing common bean and soybean in rotation with maize. We observed strong effects of environment and call for care when targeting crops and technologies for sustainable crop production.

Keywords: Agro-ecological zone, inoculation, manure, P fertilizer, yield

1. Introduction

Legumes have an important role in improving soil health in sustainable agriculture (Vanlauwe et al., 2010). They have the ability, through symbiosis with rhizobia bacteria, to fix atmospheric nitrogen and yield well without mineral nitrogen fertilizer, improve soil fertility, and their rotation with cereals helps to control diseases and pests in cereals (Giller, 2001). However, the contribution of legumes to soil fertility is minimal if N2-fixation by the legume is constrained by an adverse environment (Giller and Cadisch, 1995). Integrated soil fertility management (ISFM) has gained much attention as a key option for boosting crop productivity through combining fertilizer use with other approaches to soil fertility management, adapted to local conditions (Vanlauwe et al., 2010). Various studies have shown the benefits of integrating ISFM in existing cropping systems. For instance, application of P fertilizer to the legume in a legume-maize rotation cropping system yielded high grain and biomass of the legume, which in turn resulted in better performance of the subsequent maize crop, thus reducing the need for external N fertilizer (Kihara et al., 2010, Vandamme et al., 2014). Targeting biological nitrogen fixation (BNF) technologies to agro-ecological niches within farming systems is of importance since the fertilizer is an expensive input which is hard to access for many smallholder farmers (Giller et al., 2013). If legume stover is not retained in the field, residual N is largely contributed by root and nodule senescence and fallen leaves (Ledgard and Giller, 1995). The benefits of legumes to the subsequent crops result not only from enhanced N availability following the legume crop but also from other rotational, non-N effects (Sanginga et al., 1999; Franke et al., 2018). These rotational effects include a reduction of pests and diseases, mobilization of poorly soluble P and increased mycorrhizal colonization of a subsequent cereal crop leading to enhanced P uptake (Bagayoko et al., 2000; Franke et al., 2018).

Population increase in Rwanda has led to small farm sizes, land fragmentation and soil fertility decline mainly as a result of intensive cropping with little or no nutrient inputs. The use of fallows to restore soil fertility is no longer possible (Rutunga et al., 1998). Common bean and soybean are the most widely cultivated legumes and promoted in the Rwandan Government’s Crop Intensification Programme (MINAGRI, 2009). The two legumes are grown for household consumption and for sale. Soybean cultivation is increasing due to its expanding market demand. Common bean is the main source of dietary protein: consumption was reported to be on average 38 kg of beans per person per year (CIAT, 2008). Yet, despite the high consumption of common bean and the expanding market demand of both beans and soybeans, yields achieved by smallholder farmers are poor: only 0.8–1.0 t bean ha^{-1} and 0.8–1.7 t soybean ha^{-1} (FAOSTAT, 2010).

Farmyard manure and mineral fertilizer are important options to increase crop productivity (Zingore et al., 2008). Manure contribute not only to the restoration of soil fertility in depleted fields, but also in improving the response of crops to other nutrients, and enhanced N and P uptake in the legume-cereal rotations. As manure supplies exchangeable bases and other micronutrients, this helps to alleviate deficiencies reducing legume nodulation and N2-fixation. Despite the 'One cow per poor family' initiative which was introduced by the national government to boost agricultural productivity, the use of cattle manure in Rwanda is constrained by on-farm availability (MINAGRI, 2009). As elsewhere in Africa, the use of mineral fertilizers in Rwanda is limited by high costs (Kelly et al., 2000) and poor distribution systems (Vanlauwe and Giller, 2006).

Since indigenous rhizobia are not always in sufficient numbers, effective enough or compatible with the specific legume crop to stimulate BNF and increase yields, inoculation of legumes with rhizobia is an important option for enhancing BNF in crop production systems (Giller, 2001). The effectiveness of BNF is affected by agro-ecological factors. For instance poor nodulation and poor plant vigor in beans grown in soil with low extractable P led to a poor BNF (Amijee and Giller, 1998). However, if P fertilizer was added to beans, consistent responses to inoculation in BNF and grain yield were achieved. Other environmental stresses, such as high temperatures and dry soil can affect the symbiosis between common bean rhizobia, leading to a lack of responses to inoculation (Hungria and Vargas, 2000).

Positive responses of cereal yields after the cultivation of legumes, relative to a cereal monoculture, have been reported frequently (Ojiem et al., 2014; Osunde et al., 2003; Franke et al., this volume). Yet we lack information on whether there are benefits of combined applications of inoculation with manure and/or P fertilizer application on the yields of grain legumes and whether these benefits are translated into increased yields of a subsequent cereal crop. We conducted a field study in three agroecological zones (AEZs) of Rwanda with the following objectives: (1) to assess the effect of inoculation, P fertilizer and manure addition on yield and yield components of common bean and soybean, (2) to evaluate the influence of environment on the response of the two legumes to inputs across the three AEZs, and (3) to evaluate how these treatments influence yield of a subsequent maize crop.

2. Materials and methods

2.1 Study sites

The study was carried out in farmers' fields in three contrasting AEZs of Rwanda. In each AEZ, one district was selected where trials were established. Bugesera district was selected from the Bugesera AEZ, located in the South-East of the country at 02°12'18" S and 300 08'42" E at an altitude of 1435 m above sea level (masl), with a mean annual rainfall of 800 mm. Kamonyi district from the Granitic ridge AEZ, in the central plateau of the country, at 2°00'25" S and 29°50'49" E, 1661 masl, 1200-1400 mm rain. Kayonza district from the Eastern plateau AEZ in the eastern part of the country, at 1°55'59" S and 30°31'13" E, 1601 masl, 1000-1200 mm rain.

2.2 Trial establishment

Three experimental fields per district were selected for each legume in the short rains (SR) 2014 and maize was planted in the same treatments after the two legumes in the long rains (LR) 2014. In Bugesera and Kayonza, each treatment block with common bean was next to the one with soybean and blocks were replicated on three different farms in the same village. In Kamonyi, all three common bean treatment blocks were placed next to each other on the same farm, and two soybean blocks were placed on one farm, and the third block on another farm.

Three treatment factors applied to the legumes were: 1) without or with inoculation with Rhizobium tropici CIAT 899 for common bean and Bradyrhizobium japonicum USDA 110 for soybean; 2) manure at three rates: 0, 5 and 10 t ha^{-1}; 3) P fertilizer at two rates: 0 and 30 kg P ha^{-1} added as triple super phosphate. The experiments were laid out in a split-split plot design with P fertilizer as the main plot, inoculum as sub-plot and manure as sub-sub-plot with a full set of treatments per block. Plot size was 5 m × 5 m. Next to each treatment block, a plot (5 m × 5 m) sown with maize served as a reference crop to assess BNF. The reference crop plots were fertilized with 5 t ha^{-1} of manure and weeds were controlled by hand. No P fertilizer was added to the reference crop.

The SR start in September and end in December, and the LR follow from March to June. Land was prepared with a hand hoe. Common bean variety RWR 2245 and soybean variety SB 24 were planted at a density of 50 cm × 10 cm for common bean and 40 cm × 10 cm for soybean with 1 m paths between main plots and sub plots to minimize cross-contamination. Manure applied to the experimental fields was provided by the participating farmers, and applied to her/his own field. In the LR 2014 season, maize variety ZM 607 was planted in all treatments at a density of 75 cm × 30 cm. No nutrients were added to the maize. No maize was planted at Kayonza as farmers mixed up the treatments during ploughing.

2.3 Measurements

2.3.1 Common bean and soybean

Prior to planting, soil and manure samples were collected from each experimental block for chemical analysis. Soil sampling (0–20 cm) at nine points in each field was done following a W shape. The nine samples were combined, air-dried and passed through a 2 mm sieve. Moreover, samples from the manure provided by the participating farmers were collected and chemically analysed. In the legumes, biomass and nodulation were assessed at mid-podding. A small sub-plot of 0.5 m^2 (leaving 0.5 m away from the plot border) was sampled. All plants were cut at ground level and fresh weight was determined. A sub sample was taken and weighed, sun dried, then oven dried at 65 ^{o}C to constant weight, and re-weighed for dry biomass yield determination. After cutting the biomass, the underground parts were gently uprooted, washed and nodule count was done by scoring 0-5 as follows: 0: No nodule; 1: < 5 nodules; 2: 5-10 nodules; 3: 11-20 nodules; 4: 21-50 nodules, and 5: >50 nodules. Final grain and stover yields were determined at crop maturity by harvesting all pods from the net plots excluding the outer plant lines of both sides of the plot, and determining total fresh weight. A sub-sample was taken, weighed and sun-dried for several days and then threshed by hand. Grains were cleaned by winnowing and subsequently weighed and the moisture content was determined using an electronic moisture meter. The haulms were harvested by cutting them at ground level. Total fresh weight of the haulms was taken. Representative sub-samples of haulms from each plot were taken, sun-dried, and then oven dried at 65 ^{o}C to constant weight. Grain yield is presented at 12.5 % moisture content, stover (haulms + husks) at 0 % moisture. After harvest, the residues remained in the field.

2.3.2 Maize

Maize grain and stover yield was measured at crop maturity. All maize plants within the harvest area were cut excluding one row at each side of the plot and the first and the last maize plant of each row. Cobs were separated from stover and their fresh weights were determined. A sub-sample of stover and cobs was taken, and cobs were shelled. Cobs and stover samples were sun-dried and oven-dried at 65 °C to constant weight and re-weighed. Maize grain yields are presented at 14 % moisture.

2.4 Plant analysis and measurements of nitrogen fixation

Common bean and soybean shoots, and maize stover and grain were ground and digested in hot H2SO4 and H2O2 (Parkinson and Allen, 1975). N and P concentrations in the digests were determined colorimetrically (Okalebo et al., 1993). N_2-fixation was measured using the ^{15}N natural abundance method (Unkovich et al., 2008). After drying and grinding the shoot samples, ^{15}N content was determined using a stable isotope mass spectrometer (Thermo Scientific, Delta V Advantage Isotope Ratio MS Coupled through Conflo IV to Thermo Scientific Flash HT/EA, KU Leuven). The proportion of N derived from atmosphere (%Ndfa) and amount of N_2-fixed for both legumes were calculated as follows (Unkovich et al., 2008):

$\%Ndfa = (\delta^{15}N\ ref - \delta^{15}N\ leg) / (\delta^{15}N\ ref - B) \times 100$

Where $\delta^{15}N$ ref and $\delta^{15}N$ leg are the ^{15}N natural abundance (‰) in the non-fixing reference crops (maize for this study) and the fixing species. The smallest values of $\delta^{15}N$ were used as the B-values and were -1.44 for common bean and -1.67 ‰ for soybean (Peoples et al., 2002).

Amount of N_2-fixed =(%Ndfa x Total N legume) / 100

Where Total N legume is the %N in the legume plant times the dry biomass yield of the

legume plant.

Net N input = Total amount of N_2-fixed – Total amount of N removed in grain

The total amount of N_2-fixed includes the N content in the below-ground parts, estimated at 30% of the amount of N2-fixed in the shoots (Unkovich et al., 2008). Since legume grains were not analysed, the N concentration in grain was estimated at 3.0% for common bean and 4.6% for soybean (Nijhof, 1987) and was multiplied with observed grain yield to obtain the total amount of N in grain.

Common bean and soybean P uptake was estimated as shoot P uptake determined at mid-podding, and maize N and P uptake was represented by the total amount of N and P in the aboveground parts (stover and grain) at harvest. Due to the large number of treatments and samples, nutrient concentrations and estimates of nitrogen fixation were made only in the 0 and 10 t ha^{-1} manure treatments.

2.6 Data analysis

Statistical analysis considered sites, fertilizer, inoculation and manure as fixed factors and replicates as random factors. Analysis of variance (ANOVA) was used to detect differences due to inputs and rotational effect in a split-split plot design using the GenStat 16th edition. The effect of different factors and their interactions were compared by computing the standard errors of difference (SED). Treatments means were compared using the least significant differences (LSD) at $P \leq 0.05$.

3. Results

3.1 Rainfall distribution and sowing dates

In all three AEZs, legume sowing was delayed by almost a month due to a late start of the rain season in 2013. Both legumes were sown on October 18 2013 in Kamonyi, October 21 in Kayonza and October 23 in Bugesera. Common bean was harvested on January 23 2014 in Kamonyi, January 28 in Bugesera and January 30 in Kayonza. Soybean was harvested a month later on March 4 2014 in Bugesera, March 6 in Kayonza and March 7 in Kamonyi. Maize after common bean was sown on February 4 2014 in Bugesera and February 5 in Kamonyi, while maize after soybean was sown on March 6 2014 in Bugesera and March 11 in Kamonyi. The maize variety took 141-146 days to mature, and the dry season in Bugesera started before maize sown after soybean was mature. Low rainfall, with dry spells in the middle of the season was observed during the LR. Bugesera received less and more poorly distributed rainfall (Fig. 2.1).

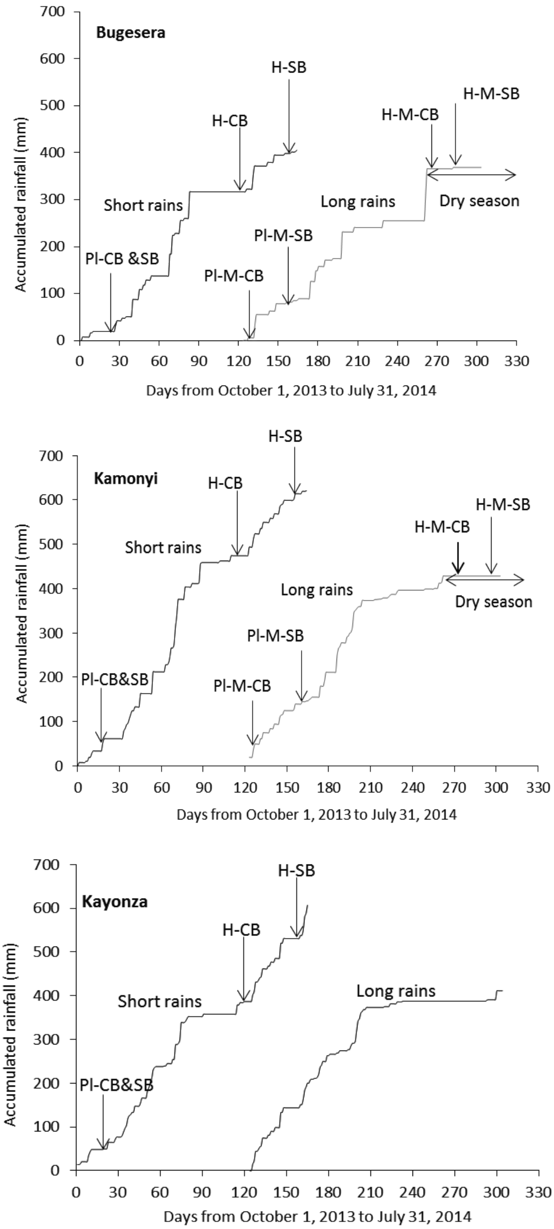

Fig. 2.1 Rainfall distribution, sowing and harvesting dates at Bugesera, Kamonyi and Kayonza. Key: Pl=Planting; H=Harvest; CB=Common bean; SB=Soybean; M=Maize; No maize was planted at Kayonza in the long rains.

3.2 Soil and manure characteristics

Soil and manure samples collected before trial establishment differed across the AEZs (Table 2.1a). Soil pH was slightly acid to near-neutral. Soil available P varied greatly among the samples taken within each AEZ and was below the critical value of 10 mg P kg^{-1} in 12 out of 18 experimental blocks. The soil organic carbon in the three AEZs was above the reported critical value of 1.5 % in all fields. Exchangeable cations were above the critical values of 0.2 for K and Mg, and 0.5 cmolc kg^{-1} for Ca, so availability of these elements was unlikely to limit crop growth. The nutrient content of the manure (Table 2.1b) varied among the AEZs. The N concentration in manure from Bugesera (1.8%) was double that in manure from Kamonyi or Kanyonza (0.9-1.0%). By contrast the largest P concentration was found in manure from Kamonyi. On average, 5 t of manure contained 90 kg N, 10 kg P and 70 kg K in Bugusera, 45 kg N, 25 kg P and 65 kg K in Kamonyi and 50 kg N, 10 kg P and 35 kg K in Kayonza.

Table 2.1a Soil characteristics of experimental sites, averaged across each location.

	Bugesera		Kamonyi		Kayonza	
Soil parameters	Average	Range	Average	Range	Average	Range
pH (H_2O)	6.2	5.0-7.5	6.2	5.5-6.5	6.2	5.4-6.6
Total N (g kg^{-1})	1.8	1.4-2.2	1.7	1.5-2.2	1.7	1.2-2.1
C (g kg^{-1})	24.1	16.3-31.4	20.2	16.4-24.8	25.6	18.6-32.5
P (Olsen) (mg P kg^{-1})	15.7	0.8-67.4	10.2	1.1-26.8	18.1	1.3-60.2
Exchangeable K (cmolc kg^{-1})	0.4	0.1-0.9	0.8	0.3-1.3	0.6	0.1-0.9
Exchangeable Ca (cmolc kg^{-1})	5.7	3.5-6.9	5.5	4.2-6.4	5.5	3.7-6.4
Exchangeable Mg (cmolc kg^{-1})	1.8	0.8-2.4	1.8	1.4-2.1	1.9	1.2-2.3
ECEC (cmolc kg^{-1})	14.4	5.8-20.0	11.1	8.4-17.9	15.7	6.1-25.0
Sand (g kg^{-1})	380	100-740	490	410-590	360	80-740
Silt (g kg^{-1})	120	70-200	120	50-180	130	90-180
Clay (g kg^{-1})	500	190-780	390	350-440	510	170-770

ECEC: Effective Cation Exchange Capacity

Table 2.1b Characteristics of the applied manure, averaged for each location.

	Bugesera		Kamonyi		Kayonza	
Soil parameters	Average	Range	Average	Range	Average	Range
pH (H2O)	8.7	8.3-9.3	8.2	7.7-9.0	8.5	7.5-9.6
C (%)	17.5	14.7-20.6	11.2	9.2-12.0	13.2	10.8-14.7
N (%)	1.8	1.6-2.1	0.9	1.0-1.4	1.0	0.7-1.6
P (%)	0.2	0.1-0.3	0.5	0.2-2.2	0.2	0.1-0.3
K (%)	1.4	0.7-2.5	1.3	0.7-1.5	0.7	0.3-1.3
Ca (%)	0.8	0.3-1.1	1.1	0.8-2.2	0.6	0.4-0.8
Mg (%)	0.4	0.2-0.5	0.9	0.3-1.4	0.3	0.1-0.4
S (%)	0.2	0.1-0.3	0.1	0.1-0.2	0.1	0.1-0.1
B (ppm)	43.4	22.0-48.4	40.9	20.3-52.0	43.6	18.5-57.4

3.3 Common bean and soybean yields

Grain and stover yield of common bean (Fig. 2.2, Table 2.2) and soybean (Fig. 2.3, Table 2.2) were greater in Kamonyi which received more and better distributed rainfall, though the differences were not significant. Small differences in biomass at mid-podding for both common bean ($P = 0.073$) and soybean ($P = 0.019$) were observed and biomass yield decreased with decreasing rainfall (Figs. 2.2 & 2.3).

Inputs of manure, inoculation and fertilizer significantly ($P < 0.001$) increased grain and stover yield, and biomass at mid-podding of both common bean and soybean, compared with unamended treatments across the three AEZs. Manure alone strongly increased the grain yield of common bean by 1.0 t ha^{-1}. The response to manure application increased with inoculation and P fertilizer application to 1.2 t ha^{-1}. Inoculation and P fertilizer increased the grain yield of common bean by 0.6 and 0.4 t ha^{-1} respectively. Although the overall effects of inoculation and P fertilizer were not significant, the combined treatment of inoculation and P together gave consistently the largest yield across all rates of manure at all three locations. The response of biomass at mid-podding to inoculation and P was strongest with the largest rate of manure. For instance in common bean, inoculation alone increased the biomass at mid-podding by 0.5 t ha^{-1}, and the response to inoculation due to manure and P fertilizer was increased to 1.7 t ha^{-1}. P fertilizer alone did not increase the biomass at mid-podding of common bean, but when added together with inoculation and manure gave an increase of 1.4 t ha^{-1}. The largest rate of manure increased the biomass at mid-podding of common bean by 1.4 t ha^{-1}, but together with inoculation and P fertilizer the response increased to 2.9 t ha-1. Similar trends were also observed for soybean (Fig. 2.3; Table 2.2). For both legumes, combined responses of all the three inputs together were greater than accumulated responses of single inputs for biomass at mid-podding. However, for grain and stover yields, synergistic effects of combined inputs were observed only for soybean and were not significant for common bean. For example in soybean, accumulated responses of single inputs was 4.9 and 1.4 t ha^{-1} against 6.2 and 1.6 t ha^{-1} achieved with combined responses of all inputs together for biomass at mid-podding and grain yield respectively.

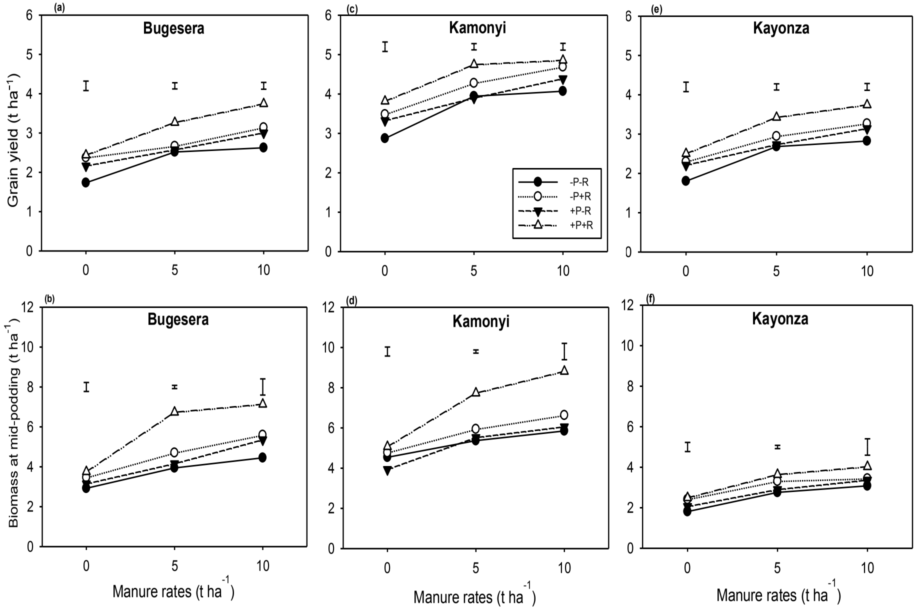

Fig. 2.2 (a, c, e) Grain and (b, d, f) biomass at mid-podding yield response of common bean to inoculation, P fertilizer and three rates of manure at (a, b) Bugesera, (c, d) Kamonyi and (e, f) Kayonza. Error bars represent the standard errors of difference between means; -/+ R: without or with rhizobia (R) inoculation.

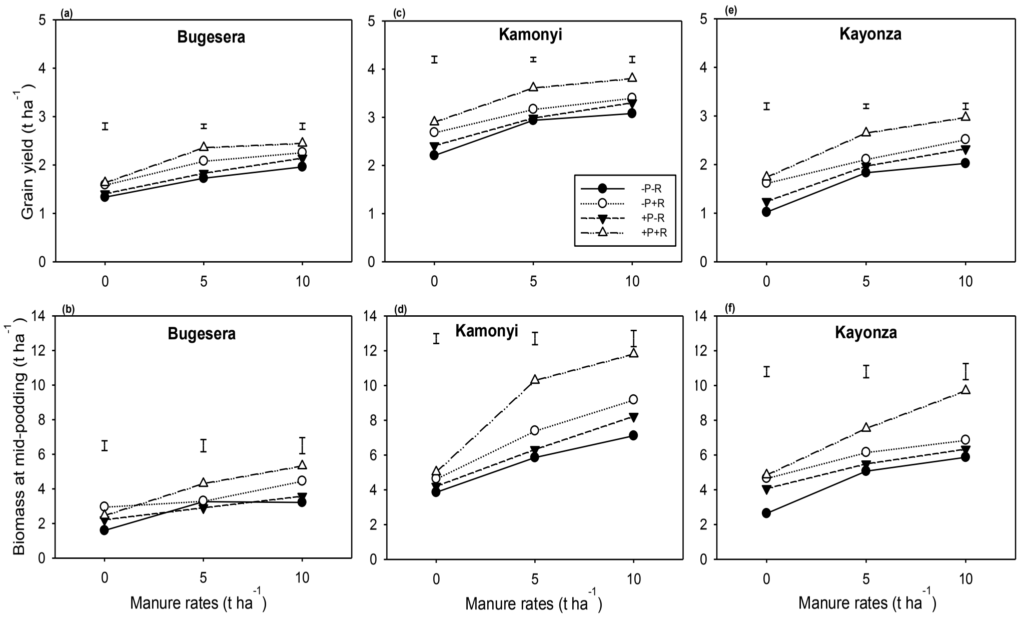

Fig. 2.3 (a, c, e) Grain and (b, d, f) biomass at mid-podding yield response of soybean to inoculation, P fertilizer and three rates of manure at (a, b) Bugesera, (c, d) Kamonyi and (e, f) Kayonza. Error bars represent the standard errors of difference between means; -/+ R: without or with rhizobia (R) inoculation.

Table 2.2 Stover response of common bean, soybean to inoculation combined with P fertilizer and three rates of manure at Bugesera, Kamonyi and Kayonza.

Treatments	Bugesera		Kamonyi		Kayonza	
	Common bean	Soybean	Common bean	Soybean	Common bean	Soybean
	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})
0P-R+0M	1.2	1.6	1.6	2.1	1.2	1.2
0P-R+5M	1.6	1.8	2.4	2.9	1.5	1.7
0P-R+10M	1.5	2.0	2.1	2.7	1.6	2.0
0P+R+0M	1.5	1.9	2.0	2.5	1.4	1.5
0P+R+5M	1.5	2.2	2.6	2.7	1.6	1.5
0P+R+10M	1.7	2.4	2.6	2.9	1.9	2.0
30P-R+0M	1.4	1.6	2.1	2.3	1.1	1.2
30P-R+5M	1.4	1.9	2.1	2.4	1.5	1.6
30P-R+10M	1.6	2.4	2.5	3.0	1.8	1.8
30P+R+0M	1.2	1.8	2.0	2.4	1.4	1.8
30P+R+5M	1.7	2.6	2.6	3.2	1.7	2.1
30P+R+10M	2.0	2.7	2.6	3.0	2.1	2.4
Average	1.5	2.1	2.3	2.7	1.6	1.7
SED (Inoculum)	0.04	0.13	0.12	0.10	0.07	0.05
SED (Manure)	0.06	0.11	0.08	0.11	0.11	0.08
SED (Fert x Inoc x Manure)	0.08		0.18	0.22		0.15
SED (Fertilizer)			0.02			

3.4 Nodulation, nitrogen fixation, N and P uptake and net N input

The number of nodules per plant in both common bean and soybean was assessed using nodule scores. Nodulation score significantly differed ($P < 0.001$) among the three AEZs with Kamonyi having the highest nodule score for both legumes. The nodule score of both common bean and soybean in the three AEZs increased with inoculation and increasing rate of manure (Fig. 2.4). There was no clear effect of P fertilizer on nodulation of both legumes.

Common bean generally fixed a smaller proportion of its nitrogen than soybean (Tables 2.3 and 2.4). The %Ndfa in common bean differed ($P = 0.003$) among the three AEZs and was on average largest in Kamonyi (53 %) and least in Bugesera (24 %). The %Ndfa in soybean was not affected by AEZ ($P = 0.317$). Surprisingly, inoculation had no significant effect on %Ndfa for either legume. Although 10 t ha^{-1} of manure often led to a smaller mean %Ndfa compared with unmanured treatments this difference was not significant.

The amount of N_2-fixed was on average larger in soybean than common bean (Tables 2.3 and 2.4). For both legumes, the largest amount of N_2-fixed was observed at Kamonyi which received more and better distributed rainfall and had greater biomass production, and least at Kayonza for common bean and Bugesera for soybean. Inoculation combined with P fertilizer led to increased amount of N_2-fixed by common bean and soybean, which was consistently more when combined with manure. Averaged over the three AEZs, inoculation combined with 30 kg P ha^{-1} increased the amount of N_2-fixed by common bean by 17 kg N ha^{-1} over the control and by 64 kg N ha^{-1} when manure was added at 10 t manure ha^{-1}. Similarly, inoculation combined with 30 kg P ha^{-1} increased the amount of N2-fixed by soybean by 16 kg N ha^{-1} without manure addition and by 57 kg N ha^{-1} when manure was added at 10 t manure ha^{-1}.

Shoot N and P uptake by common bean significantly differed ($P < 0.001$) among the three AEZs (Table 2.5). For soybean, significant difference ($P < 0.001$) between the three AEZs was observed in shoot N uptake but less strong differences ($P = 0.045$) in P uptake. A greater mean shoot N and P uptake was observed at Kamonyi for both legumes and the least uptake at Kayonza for common bean and Bugesera for soybean shoot N uptake. Both legumes had a greater shoot N and P uptake in treatments that received full inputs and least in unamended treatments. For both legumes and in all AEZs, manure addition either alone or in combination with inoculation and P fertilizer, strongly and consistently enhanced N and P uptake (Table 2.5). N and P uptake in treatments that received inoculation and P fertilizer were also small when no manure was added. For instance, inoculation combined with 30 kg P ha^{-1} increased mean shoot N uptake in common bean by 19 kg N ha^{-1} over the control without manure addition and by 115 kg N ha^{-1} when manure was added at 10 t ha^{-1}. Shoot N uptake in soybean increased as well with inoculation when combined with P fertilizer by 43 kg N ha^{-1} over the control without manure addition and by 193 kg N ha^{-1} with manure addition at 10 t ha^{-1}. Shoot P uptake in both legumes was less affected by inoculation when no manure was added. For example, inoculation combined with 30 kg P ha^{-1} increased shoot P uptake in common bean by 3 kg P ha^{-1} without manure and by 16 kg P ha^{-1} when manure was added at 10 t ha^{-1}. Similarly, inoculation and P fertilizer applied to soybean increased shoot P uptake by 4 kg P ha^{-1} without manure, and increased by 25 kg P ha^{-1} when manure was added (Table 2.5).

The net N input ranged widely from negative to positive for both common bean and soybean without any clear pattern or significant differences between treatments. A more positive net N input was observed in Kamonyi which received more and better distributed rainfall, where both legumes fixed a larger amount of N2 compared to more negative net N inputs observed in AEZs which experienced periods of dry spells (Tables 2.3 and 2.4). The net N input was strongly influenced by the amount of N_2-fixed, the total N in grain and AEZ.

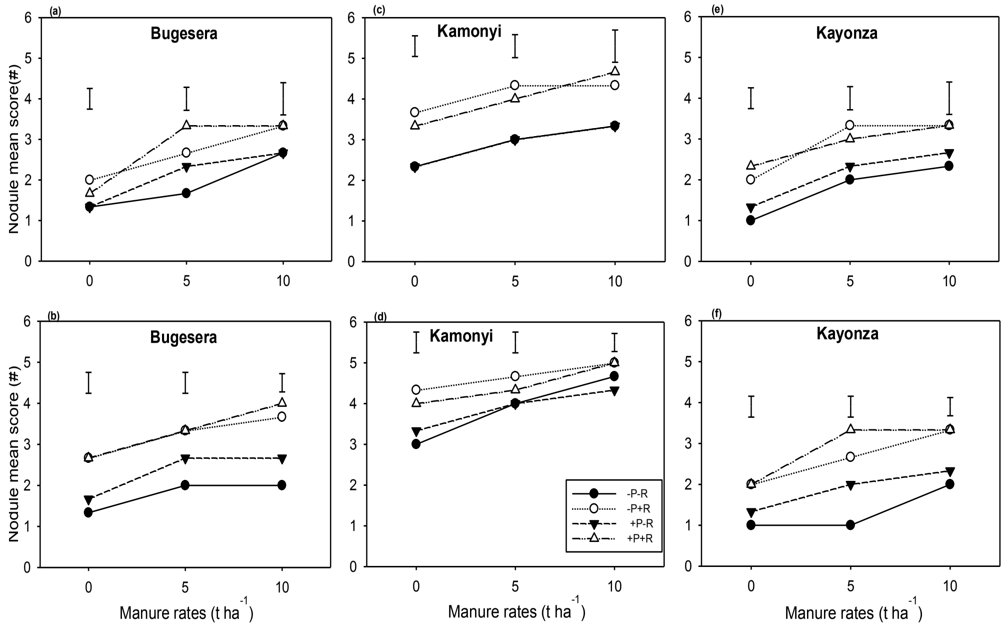

Fig. 2.4 Nodule mean score (#) response: (a, c, e) common bean, and (b, d, f) soybean to inoculation, P fertilizer and three rates of manure at (a, b) Bugesera, (c, d) Kamonyi and (e, f) Kayonza. Error bars represent the standard errors of difference between means; -/+ R: without or with rhizobia (R) inoculation.

Table 2.3 Common bean shoot $\delta^{15}N$, percentage of N_2 derived from atmosphere (Ndfa), amount of N_2-fixed and Net N input as affected by treatments

AEZs / Fertilizer (kg ha^{-1})	Inoculum	Manure (t ha^{-1})	$\delta^{15}N$ ref (‰)	shoot $\delta^{15}N$ (‰)	Range shoot $\delta^{15}N$ (‰)	%Ndfa	Total Amount N_2-fixed (kg ha^{-1})	Total N in grain (kg ha^{-1})	Net N input (kg ha^{-1})
Bugesera									
0P	-R	0M	7.1	4.7	4.3 - 5.0	27	22.2	52.0	-29.8
0P	-R	10M	7.1	5.8	5.6 - 6.1	14	17.1	78.7	-61.6
30P	+R	0M	7.1	4.8	3.4 - 6.8	27	26.5	73.1	-46.7
30P	+R	10M	7.1	4.8	3.6 - 6.7	27	64.9	112.3	-47.5
Average/AEZ				5.0		24	32.7	79.0	-46.4
Kamonyi									
0P	-R	0M	11.4	3.8	1.6 - 7.6	53	84.6	86.1	-1.6
0P	-R	10M	11.4	5.1	4.2 - 6.5	45	105.3	122.3	-17
30P	+R	0M	11.4	3.4	2.5 - 3.9	62	117.7	114.5	3.2
30P	+R	10M	11.4	4.1	3.5 - 5.2	55	197.7	145.6	52.0
Average/AEZ				4.1		53	126.3	117.1	9.2
Kayonza									
0P	-R	0M	8.4	4.1	-1.4-7.5	43	18.2	54.1	-35.9
0P	-R	10M	8.4	7.6	6.9 - 8.2	12	14.5	84.6	-70.1
30P	+R	0M	8.4	5.4	4.7 - 6.2	33	34	75.1	-41.1
30P	+R	10M	8.4	5.4	4.8 - 6.1	33	55.3	112.3	-57.0
Average/AEZ				5.6		30	30.5	81.5	-51.0
SED (Fertilizer x Inoculum x Manure)				0.89		9.27	13.5	5	16.66
SED (AEZ x Manure x Inoculum)				1.09		11.35	16.54	6.12	20.41
SED (AEZ x Fertilizer x Inoculum)				1.09		11.35	16.54	6.12	20.41
SED (AEZ x Fertilizer x Inoculum x Manure)				1.54		16.06	23.39	8.66	28.86

-/+ R: without or with rhizobia (R) inoculation; trt: Treatment; ref: Reference

Table 2.4 Soybean shoot $\delta^{15}N$, percentage of N_2 derived from atmosphere (%Ndfa), amount of N_2-fixed and Net N input as affected by treatments

AEZs / Fertilizer (kg ha^{-1})	Inoculum	Manure (t ha^{-1})	$\delta^{15}N$ reference (‰)	Shoot $\delta^{15}N$ (‰)	Range Shoot $\delta^{15}N$ (‰)	%Ndfa	Total amount N_2-fixed (kg ha^{-1})	Total N in grain (kg ha^{-1})	Net N input (kg ha^{-1})
Bugesera									
0P	-R	0M	8.0	3.2	-0.2 - 5.4	46	25.0	61.4	-36.4
0P	-R	10M	8.0	5.2	4.0 - 6.7	28	14.9	90.2	-75.3
30P	+R	0M	8.0	4.2	3.0 - 6.0	39	28.4	75.1	-46.7
30P	+R	10M	8.0	5.3	4.1 - 7.5	28	50.4	112.6	-62.3
Average/AEZ				4.5		35	29.7	84.8	-55.2
Kamonyi									
0P	-R	0M	8.4	3.7	0.3 - 6.7	44	71.0	101.5	-30.6
0P	-R	10M	8.4	2.1	1.2 - 3.8	62	184.3	141.6	42.8
30P	+R	0M	8.4	2.8	0.1 - 5.4	54	117.8	133.5	-15.6
30P	+R	10M	8.4	3.8	0.0 - 6.1	43	186.0	175.0	11.0
Average/AEZ				3.1		51	139.8	137.9	1.9
Kayonza									
0P	-R	0M	8.0	0.9	-1.7 - 5.1	69	58.5	47.0	11.5
0P	-R	10M	8.0	5.8	5.0 - 6.3	21	50.1	93.1	-43.0
30P	+R	0M	8.0	4.8	2.4 - 6.3	32	56.3	80.1	-23.8
30P	+R	10M	8.0	5.5	3.9 - 6.6	24	90.8	136.6	-45.8
Average/AEZ				4.3		37	63.9	89.2	-25.3
SED (Fertilizer x Inoculum x Manure)				0.95		12.76	19.52	5.05	19.86
SED (AEZ x Manure x Inoculum)				1.17		15.62	23.9	6.19	24.32
SED (AEZ x Fertilizer x Inoculum)				1.17		15.62	23.9	6.19	24.32
SED (AEZ x Fertilizer x Inoculum x Manure)				1.65		22.1	33.81	8.75	34.4

Table 2.5 Shoot N and P uptake (kg ha^{-1}) of common bean and soybean as influenced by treatments

Treatments	Bugesera AEZ				Kamonyi AEZ				Kayonza AEZ			
	Common bean		Soybean		Common bean		Soybean		Common bean		Soybean	
	Shoot P uptake	Shoot N uptake	Shoot P uptake	Shoot N uptake	Shoot P uptake	Shoot N uptake	Shoot P uptake	Shoot N uptake	Shoot P uptake	Shoot N uptake	Shoot P uptake	Shoot N uptake
0P-R+0M	8	59	4	36	23	121	14	109	4	51	6	70
0P-R+10M	12	119	21	45	28	157	26	213	9	89	11	169
30P+R+0M	11	77	7	54	27	134	19	147	6	77	10	144
30P+R+10M	29	200	23	144	44	254	53	338	10	122	24	312
Average	15	114	14	70	31	167	28	202	7	85	13	174
SED (Inoculum)	2.41	13.02	ns	17.27	2.41	13.02	ns	17.27	2.41	13.02	ns	17.27
SED (Fertilizer)	2.41	13.02	ns	17.27	2.41	13.02	ns	17.27	2.41	13.02	ns	17.27
SED (Manure)	2.41	13.02	5.19	17.27	2.41	13.02	5.19	17.27	2.41	13.02	5.19	17.27
SED (AEZ)	2.96	15.94	6.35	21.15	2.96	15.94	6.35	21.15	2.96	15.94	6.35	21.15

3.5 Nutrient uptake and production in maize

Maize accumulated more N and P when grown after common bean and soybean at Kamonyi than at Bugesera ($P < 0.001$; Table 2.6). The preceding legume also influenced N and P uptake by maize, with more N uptake achieved when maize was grown after common bean and more P uptake achieved when maize was grown after Soybean. In all cases, greater uptake was achieved in treatments that previously had received full inputs compared with previously unamended plots or plots that had received inoculation and P fertilizer without manure addition. Inoculation combined with 30 kg P ha^{-1} applied to common bean, on average, increased N uptake of the subsequent maize by 27 kg N ha^{-1} over maize uptake after unamended common bean and by 64 kg N ha^{-1} when manure was added at 10 t ha^{-1}. Similarly, inoculation with P fertilizer applied to soybean, on average, increased N uptake of the subsequent maize by 16 kg N ha^{-1} over maize uptake when grown after untreated soybean, and by 52 kg N ha^{-1} when manure was added at 10 t ha^{-1}. Maize P uptake grown after both common bean and soybean was increased by inputs applied to the two legumes and followed similar trend as N uptake (Table 2.6).

Greater maize grain and stover yields after common bean were observed at Kamonyi (Fig. 2.5; Table 2.7). The late planting of maize due to the longer duration of the soybean crop at Bugesera, meant that the season ended before maize had yielded any grain. At Kamonyi, greater maize yield was achieved in treatments that previously received manure relative to unamended treatments or treatments with inoculation combined with P fertilizer. Whether maize was grown after common bean or soybean, synergistic effects of combined application of inputs applied to the two legumes were seen in increased yield of the maize. For instance, accumulated responses to the application of single inputs applied to the legume were 2.8 and 1.7 t ha^{-1} for maize grain yield grown after common bean and soybean respectively, whereas the combined effects of all inputs applied together were 3.0 and 3.4 t ha^{-1} for maize grain yield grown after common bean and soybean respectively. Similarly, when inoculation had been applied to the previous common bean, mean maize grain yield increased by 1.3 t ha^{-1} over maize yield after untreated common bean, and this response increased to 1.6 t ha^{-1} when manure and P fertilizer were added to inoculation. When manure had been applied to common bean, the maize grain yield increased by 0.7 t ha^{-1} over maize after unamended common bean, and the response increased by 1.3 t ha^{-1} when inoculation and 30 kg P ha^{-1} were added with manure in the legume phase. For maize grown after soybean, there was strong residual effect from inputs applied to soybean. For example in the case of maize grain yield grown after soybean, inoculation response to application of manure and P fertilizer increased from 0.8 to 2.4 t ha^{-1}, and manure response to application of P fertilizer and inoculation increased from 0.2 to 2.5 t ha^{-1} (Fig. 2.5). Maize stover yield response to inputs applied to the preceding legumes followed similar pattern as grain yield (Table 2.7).

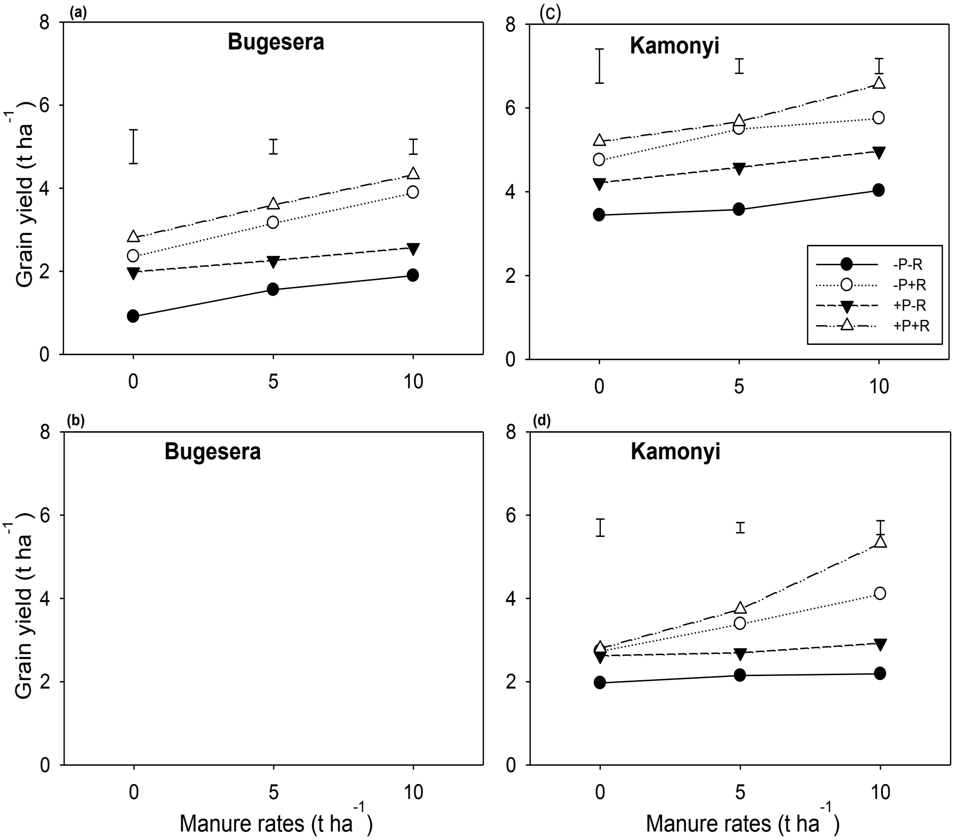

Fig. 2.5 Maize grain yield after: (a, c) common bean and (b, d) soybean receiving different treatments at (a, b) Bugesera and (c, d) Kamonyi. Error bars represent the standard errors of difference between means; -/+ R: without or with rhizobia (R) inoculation. N.B. Maize failed to yield any grain after soybean at Bugesera.

Table 2.6 Total N and P uptake (kg ha^{-1}) of maize grown after common bean and soybean with and without inoculation, P fertilizer and manure

Treatments	Bugesera AEZ				Kamonyi AEZ			
	Common bean-Maize		Soybean-Maize		Common bean-Maize		Soybean-Maize	
	Total P uptake	Total N uptake	P uptake	N uptake	Total P uptake	Total N uptake	Total P uptake	Total N uptake
0P-R+0M	2	33	4	24	16	98	18	69
0P-R+10M	3	68	5	24	23	119	35	68
30P+R+0M	2	75	8	47	21	111	30	78
30P+R+10M	10	116	9	64	21	144	55	134
Average	4	73	7	40	20	118	35	87
SED (Inoculum)	n.s	n.s	n.s	n.s	n.s	n.s	6.04	4.62
SED (Fertilizer)	n.s	n.s	n.s	n.s	n.s	n.s	6.04	4.62
SED (Manure)	n.s	15.58	n.s	n.s	n.s	15.58	6.04	4.62
SED (AEZ)	3.34	15.58	n.s	n.s	3.34	15.58	6.04	4.62
SED (Inoculum x manure)	n.s	n.s	n.s	n.s	n.s	n.s	n.s	6.54

Common bean-Maize: Maize grown after common bean; Soybean-Maize: Maize grown after soybean; ns: not significant at $p < 0.05$; 0M/10M: 0 and 10 t ha^{-1} of manure; P and N uptake by maize after soybean at Bugesera was calculated from stover only since maize did not yield any grain

Table 2.7 Stover response of maize (t ha^{-1}) grown after common bean and soybean with and without inoculation, P fertilizer and manure

Treatments	Bugesera AEZ		Kamonyi AEZ	
	Common bean-Maize	Soybean-Maize	Common bean-Maize	Soybean-Maize
	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})	Stover (t ha^{-1})
0P-R+0M	2.8	3.6	6.0	1.7
0P-R+5M	3.5	2.9	6.6	1.8
0P-R+10M	4.8	3.5	6.2	1.9
0P+R+0M	6.0	4.2	6.4	2.3
0P+R+5M	6.2	7.1	7.6	2.9
0P+R+10M	5.8	7.3	7.3	3.5
30P-R+0M	4.5	3.5	6.4	2.2
30P-R+5M	5.1	4.1	7.0	2.3
30P-R+10M	3.9	6.0	8.1	2.5
30P+R+0M	3.7	5.9	5.8	2.4
30P+R+5M	6.2	5.3	7.6	3.2
30P+R+10M	7.7	8.0	7.6	4.6
Average	5.0	5.1	6.9	2.6
SED (Inoculum)	0.62	0.54		
SED (Manure)		0.59		0.50

4. Discussion

4.1 Growth and yields of common bean and soybean

The better and well distributed rainfall at Kamonyi (Fig. 2.1b) resulted in greater yields of stover and grain compared with Bugesera and Kayonza (Figs. 2.2 and 2.3; Table 2.2). Despite the differences in overall productivity, the response of both legumes to the various treatments was remarkably consistent across the locations. Manure strongly increased the yield of both legumes. Inoculation and P fertilizer resulted in greater yield of both common bean and soybean in all three AEZs, and these treatment effects increased with increasing rate of manure applied. This suggests that manure contributed nutrients other than N and P or had other beneficial effects on growth of legumes (Zingore et al., 2008). The individual effects from inoculation, P fertilizer or manure on increasing biomass at podding of common bean were clear, and effects were stronger when all inputs were combined suggesting synergy. Similar synergistic effects when different sources of nutrients are used together have been reported elsewhere (e.g. Mustonen et al., 2013).

There was also evidence for synergistic effects of all inputs on soybean stover and grain yields but not for common bean where the effects of the inputs appeared to be additive.

4.2 Nodulation, N2-fixation, N and P uptake and net N input by the legumes in response to inputs

Greater nodulation was observed in soybean than common bean, and the largest nodule scores were seen in Kamonyi which received the most rainfall, well distributed through the season (Fig. 2.4). Inoculation significantly increased the nodule score of both legumes. Application of P fertilizer alone did not significantly enhance the nodule score of common bean but did for soybean. These differences in response to P fertilizer between the two legumes could be due to differences in P demand although the mechanisms behind are not well understood (Singh et al., 1997). The increase in nodulation observed with manure may be linked to its impacts on availability of other nutrients, on moisture supply or on better soil structure creating a more favourable environment for nodulation.

The better nodulation at Kamonyi was translated into a larger %Ndfa in both bean and soybean. There were no obvious or consistent effects of inoculation, P fertilizer or manure on %Ndfa. Similarly, Vandamme et al. (2014) observed that increasing P rates did not result in increased N_2-fixation in different soybean genotypes in Kenya. The N available from manure is often depleted in 15N compared with the atmosphere (Inácio et al., 2015) which could overestimations of %Ndfa in the control treatments although we cannot assess how important this effect might have been. The smaller observed at Bugesera in both legumes could be due to water limitation resulting from dry spells and less total rainfall at this location.

The largest amounts of N_2-fixed were achieved in both legumes when inoculation, P fertilizer and manure were applied together, reflecting the impacts on growth and yield at all three sites. The higher rainfall recorded at Kamonyi also resulted in a larger amount of N_2-fixed than at Bugesera and Kayonza. Ojiem et al. (2007) also observed enhanced legume growth, biomass production and larger N_2-fixation in agroecological zones with higher rainfall in Western Kenya.

The net N input from N_2-fixation (calculated by subtracting the amount of N removed in grain from the amount of N_2-fixed in each treatment) was negative in most cases for both common bean and soybean, with no consistent differences among treatments (Tables 2.2 and 2.3). Although treatments with the largest yields tended to have the most negative net inputs this was not always the case. The values were more negative where %Ndfa was smaller, especially at Bugesera and Kayonza the two sites which received least rainfall. Such results are not uncommon: there are many reports of negative net N inputs for grain legumes (e.g. Giller et al., 1994; Osunde et al., 2003). Net N inputs of grain legumes may be limited due to stresses such as drought that result in poor growth of the legume (Vanlauwe and Giller, 2006).

Greater shoot N and P uptake was observed at Kamonyi with higher rainfall and in treatments that received combined inoculation with manure and P fertilizer (Table 2.5). The greater N and P uptake in treatments that received manure relative to treatments with P fertilizer and inoculation, suggests that N and P supplied by manure also were readily available for plant uptake and positively contributed to plant growth.

4.3 Residual effect of legumes on maize

Maize grain and stover yields matched closely the pattern of biomass production and yield of the previous common bean and soybean treatment (Fig. 2.5; Table 2.7). The greatest residual effects of the legumes and largest maize yields were observed at Kamonyi with highest rainfall; matching observations of Ojiem et al. (2014) in western Kenya. Despite the fact that soybean fixed more N than common bean, its long duration led to the delay in sowing of the subsequent maize crop at Bugesera, resulting in complete failure to yield of the maize crop. These suggests the need for early maturing soybean varieties in Bugesera, or that farmers may be better to use common bean in rotation with maize. Inoculation along with manure and P fertilizer application to common bean and soybean significantly enhanced the yields of the subsequent maize crop. Application of inputs to the legumes also resulted in enhanced N and P uptake of the subsequent maize than that of maize after unamended legumes (Table 2.6). Whilst the residual benefits of P fertilizer and manure could be due to residual P availability (Wolf et al., 1987) and slow mineralization of the manure applied to the legumes, the increase in maize yield due to rhizobial inoculation can only be due to the improved growth and N_2-fixation of the previous legume. Our results support the suggestions that inputs are best targeted to legumes in rotation with cereals to maximise legume production and N_2-fixation as well as residual benefits to cereals (Giller, 2002).

We observed strong yield increases and N uptake of maize after common bean and soybean amended with inputs despite the negative net N input observed in some treatments. The increase of maize yield despite negative net N input also could be a result of another rotational effect rather than simply the carry-over of N from the soybean residue (Sanginga et al., 2001). Among these rotational effects include improvement of soil physical properties allowing better exploration of the soil by maize after the legumes (Sanginga et al., 2001), control of diseases and pests in cereals (Giller, 2001), high mycorrhizal infection and reduced incidence of nematode damage (Bagayoko et al., 2000). Nevertheless, the fact that legume inoculation also had strong residual benefits in production of the subsequent maize crop leads us to concur with Kermah et al. (2018) that the net N input from legumes is a poor indicator of effects of legumes on soil fertility.

4.4 Relevance of the study for Rwandan smallholder farmers

Cultivation of legumes as sole crops is promoted through the government-led Crop Intensification Program (CIP) in Rwanda, with the recommendation that legumes are grown in rotation with cereals. Smallholder farmers largely grow legumes with little or no fertiliser. Most mineral or organic fertiliser is targeted to cash crops (e.g. tomato, vegetables, Irish potato) that have a ready market. Both common bean and soybean are important sources of income for farmers and our results show that if inputs are targeted to enhance legume production, greater yields are achieved both from the legume itself and the subsequent cereal crop. Although we compared manure applications of 5 or 10 t ha^{-1}, the current recommendation in Rwanda is 10 t ha^{-1} for food crops (MINAGRI, 2010).

The question remains as to whether these are feasible rates of manure for smallholders? To answer this question, we need to consider livestock numbers, livestock ownership and farmers' resources. In recent years, the government of Rwanda has promoted ownership of improved dairy cows and zero grazing through the "One Cow per Poor Family" programme. This programme targets poor and vulnerable farmers with no cattle and less than 0.75 ha of land, on condition that the first offspring is passed on to another farmer (MINAGRI, 2009). This programme is expected to improve livelihoods through consumption and sales of milk and increased crop productivity through use of manure. A remarkable increase in the size of the national herd has been observed rising to 1.33 million cattle in 2013 from 755,000 in 2000, with the projects of 1.67 million in 2017 and 1.92 million by 2020 (MINAGRI, 2013).

There are 2.41 million households in rural Rwanda of which 32% own cattle (NISR, 2014); so 771,200 households own 1,33 million cattle, giving an average 1.7 cattle hh-1. It has been shown that a local Ankole weighing 300 kg, reared in a zero-grazing system can produce 6 t of manure per year (MINAGRI, 1990). Assuming each head cattle produces 6 t of manure annually each household could produce on average 10.2 t of manure per year. Landholdings in Rwanda are generally very small but vary from one region to another. At national level more than 60 % of households have less than 0.7 ha. Some districts are densely-populated such as Kamonyi which has a population density of 519 inhabitants/km^2. By contrast, Bugesera district has 280 inhabitants / km^2 and Kayonza is the least populated with 178 inhabitants/km^2 (NISR, 2014). In all three districts average farm size is reported to be 0.5-1 ha per household. The above calculations suggest it is feasible for farmers who own at least one head of cattle to apply manure at rates of 5 to 10 t ha^{-1} per year.

Bucagu et al. (2014) working in Isimbi (Southern province) and Kageyo (Northern Province) of Rwanda showed that farmers with 0.5 to 2 ha of lands and one or two head of cattle were able to invest organic manure in food crop production. In northern Rwanda, Franke et al. (2019) found that poor farmers were cultivating on poorly fertile soils and achieved poor yields of climbing beans, while wealthier farmers could invest in organic and inorganic fertiliser and achieve greater yields. Analysis in southwest Rwanda suggests that limited fodder availability at village scale may limit the expansion of the One Cow per Poor Family programme to all households (Klapwijk et al., 2014). Although some farmers bought manure for growing tomatoes and vegetables in our study sites, there was no fixed price. In Kamonyi, a pit of approximately 5 t manure was sold for 20,000 Rwandan Francs (€24), whereas in Bugesera it was only 10,000 Rwandan Francs (€12) and Kayonza between 15,000-20,000 Rwandan Francs. It is unlikely that the poorer farmers would be able to invest in purchasing manure for crop production.

5. Conclusion

The use of inoculum combined with manure and P fertilizer on common bean and soybean showed great potential to enhance not only the yields of the legume but also production of the subsequent maize crop. In the drier agroecology of Bugesera, maize failed to yield any grain when grown after soybean due to the delayed planting due to the long duration of soybean compared with common bean. Early maturing soybean varieties are required in such regions of low and erratic rainfall in case of soybean-maize rotations. Our results also show strong influence of the agroecological environment and call for careful strategies when targeting technologies and crops for sustainable intensification of crop production.

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Chapter 3

The impact of manure on the survival of rhizobia in the soil and on bean and soybean yield in two districts of Rwanda

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Abstract

The survival of rhizobia in soils is crucial for grain legume production in smallholder cropping systems and is affected by soil and environmental factors. We evaluated the effect of manure on rhizobial survival in soil and the need to re-inoculate bean and soybean in previously inoculated farmers' fields in Bugesera and Kamonyi districts in Rwanda. The background rhizobial population was estimated at the start of the trial and at later stages using the most probable number (MPN) plant infection method and indicated a small initial population size in ten out of twelve fields. In the first season, bean and soybean were grown without and with inoculum, manure at three rates: 0, 5 and 10 t ha^{-1} and P fertilizer at two rates: 0 and 30 kg P ha^{-1} added as triple super phosphate in split-split plot design. In the following season, all plots were cropped with maize with a uniform management. In the third season, covered in this study, bean and soybean were grown with the experimental units of the first season, split into two halves with one half uninoculated and the other half inoculated. Inoculation and manure addition in Season 1 increased the population size of rhizobia and yields of both bean and soybean in season 3. Grain yields in Season 3 varied from 1.4 t ha^{-1} to 1.6 t ha^{-1} in uninoculated, unmanured plots to 3.8 t ha^{-1} for bean and 2.8 t ha^{-1} for soybean in inoculated plot receiving 10 t manure ha^{-1}. Re-inoculation in Season 3 did not significantly increase the yields of bean and soybean in plots that had been inoculated in Season 1 across the manure rates. Cropping history had a strong effect on rhizobial population size. Small numbers 1-2 cells g^{-1} soil were obtained with soybean since soybean had not been cropped in those fields before, and the numbers increased to tenfold four months after planting soybean. Rainfall and manure strongly influenced the survival of rhizobia in the soil. The largest population of 10^4 cells g^{-1} soil was recorded during the rainy season in March and April in plots that had received manure, while the smallest population size of less than 10^1 cells g^{-1} soil was obtained during the dry season in July in uninoculated and unmanured plots. The use of manure can help smallholder farmers to grow legumes without repeated inoculation and achieve greater yields.

Keywords: cropping history, manure, re-inoculation, rhizobia dynamics

1 Introduction

Legumes are important crops in agriculture, partly because most of them establish symbiotic relationships with rhizobial bacteria (Herridge et al., 2008). Rhizobia colonise nodules on the plant roots (Giller, 2001) and fix atmospheric nitrogen. Through this symbiosis with rhizobia, legumes play an important role in nitrogen cycling and in improving agricultural productivity. The success in the legume-rhizobium interaction in case legumes are grown without inoculation depends on the abundance of effective indigenous rhizobia in the soil (Thies et al., 1991b). Three situations have been identified where it is necessary to introduce rhizobia to ensure effective nodulation and biological nitrogen fixation: (1) in the absence of compatible rhizobia; (2) when there is a small population of compatible rhizobia resulting in slow nodulation; and/or (3) ineffective or less effective indigenous rhizobia than the selected inoculants for a particular legume host (Giller, 2001). In these situations, inoculation of legumes with rhizobia is important for enhancing biological nitrogen fixation (BNF) in crop production systems.

Rhizobia can survive in the soil as free-living bacteria in the absence of host crops in numbers sufficient for effective nitrogen fixation by a legume crop, and form a large proportion of nodules (Elkins et al., 1976; Crozat et al., 1982; Zengeni et al., 2006). The factors affecting the survival of rhizobia in soil have been examined extensively. Among them there are low soil pH and aluminium toxicity, desiccation, nutrient deficiencies, salinity/alkalinity, extreme temperatures and other toxicities (Mahler and Wollum II,1982; Zahran, 1999; Hungria and Vegas, 2000; Giller, 2001). Moreover, the survival of rhizobia in soil is correlated with soil clay content, percentage carbon and moisture availability (Zengeni et al., 2006) and may be affected by the cropping history and season (Andrade et al., 2002). The application of organic manure improves the survival of rhizobia in soil by creating a favourable environment for their growth and multiplication. Organic manure improves the soil carbon, increases the water-holding capacity, neutralizes acidic conditions, and supplies exchangeable bases and other micronutrients (Nzuma et al., 1998; Zengeni et al., 2006; Zingore et al., 2008).

The question how long subsequent legume crops can benefit from previous inoculation and how the longevity of rhizobia in the soil can be extended is especially relevant in smallholder systems where inoculants are often unavailable. Nitrogen fixation often plays a key role in crop production in smallholder systems, as a source of N complementing N fertilizer. Apart from their role in nitrogen fixation and soil fertility restoration, legumes in Rwanda play a key role in crop production. Bean is the main source of dietary protein and is considered as the ‘meat’ for many Rwandans country side. Consumption was reported to be on average 38 kg of beans per person per year (CIAT, 2008). Soybean cultivation is becoming important due to its expanding market demand, and has been included among the priority crops in the Crop Intensification Program (CIP). Since 2012, soybean is used for edible oil processing and animal feeds after establishment of a public-private partnership business venture (Mount Meru Soyco Limited) in Kayonza district which has a capacity of 200 t per day. In Rwanda, legume residues also are used as feeds for livestock or as mulch in banana and coffee plantations and help to suppress weeds, conserve soil moisture and recycle nutrients to the soil. Most smallholder farmers in Rwanda have access to small amounts of manure as a result of the "One Cow per Poor Family" programme (MINAGRI, 2013; Bucagu et al*.,* 2014), but the use of inorganic fertiliser is still low despite the government subsidy policy (MINAGRI, 2014).

To address questions on the need to inoculate legumes growing in previously inoculated soil and the factors that influence rhizobial survival in the soil, we conducted field trials in different sites in Rwanda aimed to: 1) assess the effect of manure on rhizobial survival in the soil and on grain yield of bean and soybean, 2) evaluate if there is a need to re-inoculate bean and soybean after inoculation two seasons earlier.

2. Materials and methods

2.1 Study site and trials establishment

The study was carried out in farmers' fields in two contrasting agroecological zones (AEZ) in Rwanda: Bugesera AEZ and the Granitic ridge AEZ. Bugesera district was selected within the Bugesera AEZ, located in the South-East of the country at 02°12'18" S and 30^0 08'42" E at an elevation of 1435 m above sea level (masl) and a mean annual rainfall of 800 mm. Kamonyi district was selected within the Granitic ridge AEZ, in the central plateau of the country, at 2°00'25" S and 29°50'49" E, 1661 masl, 1200-1400 mm mean annual rainfall. In each AEZ, three fields for bean and three for soybean were selected. The selection was based on fields with no history of rhizobia inoculation, with comparable fertility status and not located in areas potential for water-logging. Rainfall data for each site were obtained from the nearest Rwanda Meteorology stations.

In Season 1 (short rains 2014, Sep 2013 – Jan 2014, referred here as Season 1) fields were cropped with beans and soybeans with the following treatments: 1) without or with inoculation with *Rhizobium tropici* CIAT 899 for bean and *Bradyrhizobium japonicum* USDA 110 for soybean; 2) manure at three rates: 0, 5 and 10 t ha^{-1}; 3) P fertilizer at two rates: 0 and 30 kg P ha^{-1} added as triple super phosphate. The manure used was collected from the participating farmers and was analysed for chemical properties (Chapter 2). On average the pH of the manure was 8.7 and 8.2 for Bugesera and Kamonyi respectively. The N concentration in manure was 1.8% in Bugesera and 0.9% in Kamonyi. P concentration in manure was 0.2% in Bugesera and 0.5% in Kamonyi. The K concentration in manure was 1.4 % in Bugesera and 1.3 % in Kamonyi. Organic carbon was 17.5 % in Bugesera and 11.2 % in Kamonyi.

The experiments were laid out in a split-split plot design with P fertilizer as the main plot, inoculum as sub-plot and manure as sub-sub-plots with a full set of treatments per block. Sub-sub-plot size was 5.0 m × 5.0 m. Bean variety RWR 2245 and soybean variety SB 24 were planted at a density of 50 cm × 10 cm for bean and 40 cm × 10 cm for soybean with 1 m paths between sub plots to minimize cross-contamination of inoculants. Results from Season 1 (yields, soil and manure characteristics) are presented in Chapter 2.

After legume harvest, a maize crop was planted in the long rains 2014 (March 2014 – July 2014, referred here as Season 2) in all treatments. Maize variety ZM 607 was planted at a density of 75 cm × 30 cm. No nutrients were added to the maize, while weeds were regularly controlled by hand weeding. After maize harvest, a re-inoculation trial was established (short rains 2015, from Sep 2014 – Jan 2015, referred to as Season 3). The experimental lay-out of Season 1 was retained. However, none of the treatments in Season 1 were applied in Season 3. Instead, each sub-sub-plot was split into two halves with one half uninoculated and the other half inoculated. Inoculant used is produced by the microbiology department of the Rwanda Agriculture Board (RAB). It consisted of *rhizobium tropici* CIAT 899 for bean and *bradyrhizobium japonicum* USDA 110 for soybean. Inoculant was applied at a rate of 80 g per 10 kg seeds. Seeds were placed into a large bucket, then moistened with clean cold water and mixed until all seeds were evenly coated with water. Then immediately inoculant was added onto the moistened seeds and mixed thoroughly until all seeds were covered by inoculant. This was done in the field under a shade to avoid direct sun to the inoculant and the seeds were planted immediately.

Half a sub-sub-plot was 5.0 m × 2.0 m with a 1 m path between the halves to minimize rhizobial contamination. In the following season (long rains 2015, from March 2015 to July 2015, referred to as Season 4), farmers were allowed to plant maize in all fields as in Season 2. The last sampling for this study (soil for MPN) was done in April 2015 during Season 4.

2.2 Assessing rhizobial population dynamics

Before Season 1 (September 2013), soil samples were collected in all fields for chemical analysis and determination of the initial population background of rhizobia. The rhizobia population in the different sub-sub plots was monitored through soil sampling and the most probable number method (Vincent, 1970) was used to estimate the rhizobia population. Sampling was done in January, March, July 2014 and April 2015 (Fig. 3.1). For all the sampling times, soil samples were collected in treatments that had received P fertiliser and without or with inoculation across the manure rates in Season 1. The bean variety RWR 2245 and the soybean variety SB24 were used as the trap host.

2.3 Field measurements

Above-ground biomass production and nodulation in bean and soybean in Season 3 were assessed at mid-podding by harvesting an area of 0.5 m^2 for bean and 0.4 m^2 for soybean. All plants were cut at ground level and fresh weight was determined. A sub sample was taken and weighed, sun dried, then oven dried at 65 ^oC to constant weight, and re-weighed for dry biomass yield determination. After cutting the above-ground biomass, the underground parts of all plants in the biomass harvest area were gently uprooted, washed and nodule count was done by scoring 0-5 as follows: 0: No nodule; 1: < 5 nodules; 2: 5-10 nodules; 3: 11-20 nodules; 4: 21-50 nodules, and 5: >50 nodules. Final grain yield was determined at crop maturity by harvesting all pods from the net plots (8 m^2) excluding the outer plant lines of both sides of the plot, and determining total fresh weight. A sub-sample was taken, weighed and sun-dried for several days and then threshed by hand. Grains were cleaned by winnowing and subsequently weighed and the moisture content was determined using an electronic moisture meter. Grain yield is presented at 12.5 % moisture content, biomass yield at 0% moisture.

2.4 Data analysis

Statistical analysis was performed using the GenStat 16th edition. Data were analysed with a linear mixed model with site × fertiliser × inoculum × manure as fixed factors and replication as random factor. Inoculation in Season 1 and Season 3 were taken as levels of the inoculum factor. Rhizobial population data were log-transformed before analysis. A repeated measurement analysis was performed to assess rhizobial population dynamics over time, taking replicates as subjects and months from first sampling (sampling time) as time points. Sampling time × inoculum × manure × site were considered as fixed factors. Since sampling time was not equally spaced, antedependence model order 1 was chosen for correlation within subjects (replicates) across sampling time. The factor inoculum for rhizobial dynamics assessment refers to inoculation in Season 1. Treatment means were compared using the standard errors of difference between means (SED) at $p < 0.05$.

3. Results

3.1 Rainfall distribution and soil sampling for rhizobia count

In both Bugesera and Kamonyi, the amount of rainfall received at the different soil sampling times differed (Fig 3.1). The initial soil sampling in September 2013 (S0) provided the initial population background of rhizobia before the start of the experiment, January 2014 (S1) was the period of the short dry season when legumes of Season 1 were harvested, March 2014 (S2) and April 2015 (S4) in Season 2 and Season 4 respectively when maize was grown were period of rainy season, and July 2014 (S3) in a dry period without cropping (off-season).

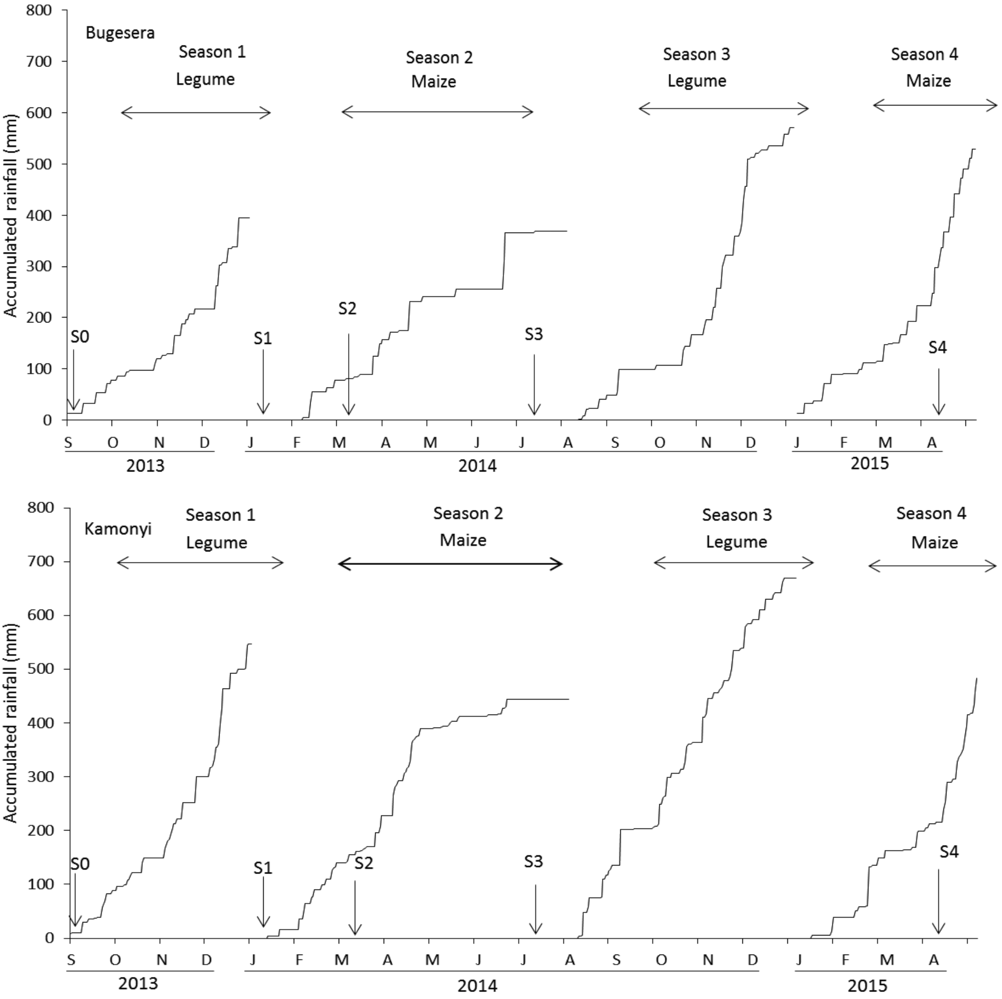

Fig. 3.1. Rainfall distribution in Bugesera and Kamonyi indicating time of soil sampling for rhizobia count (S0, S1, S2, S3 & S4). S0: September 2013, S1: January 2014; S2: March 2014; S3: July 2014 and S4: April, 2015.

3.2 Field soil characteristics and rhizobial background of the trial sites

In the study sites, farmers generally grow legumes in rotation with maize. However, few farmers do rotate with sorghum or tubers. In this study, rotations were between bean and maize except two fields which beans were grown in rotations with sorghum and cassava. No soybean had been grown in those fields. The fields also had no history of rhizobia inoculation neither for bean nor for soybean. The initial rhizobial population background was low in ten out of twelve fields. The absence of soybean in these cropping systems may explain the low initial rhizobial population background with soybean as the trap host compared to bean (Table 3.1). Soil pH in the experimental fields was slightly acid to near-neutral. The soil organic carbon in the two AEZs was above the reported critical value of 1.5 % in all fields and clay content was above 20 %. On average, 5 t of manure contained 90 kg N, 10 kg P and 70 kg K in Bugesera, 45 kg N, 25 kg P and 65 kg K in Kamonyi (Chapter 2).

Table 3.1 Characteristics of the trial sites and the initial rhizobial background population in Bugesera and Kamonyi AEZs

AEZ / Field	Previous crops	Test legume	pH (H_2O)	C (g kg^{-1})	Clay (g kg^{-1})	Sand (g kg^{-1})	Rhizobial cells g^{-1} soil (x10^{3})
Bugesera							
1	Bean-Sorghum	Bean	6.2	28.4	746	160	3.2
2	Bean-Maize	Bean	6.2	29.5	782	98	0.142
3	Bean-Maize	Bean	6.2	16.8	242	560	0.018
4	Bean-Maize	Soybean	5.0	16.3	191	739	0.002
5	Bean-Maize	Soybean	6.4	31.4	754	100	0.002
6	Bean-Maize	Soybean	7.5	22.1	294	601	0.001
Mean per AEZ			6.2	24.1	502	376	
Kamonyi							
1	Bean-Cassava	Bean	6.4	21.7	353	594	3.2
2	Bean-Maize	Bean	6.1	16.4	384	433	0.026
3	Bean-Maize	Bean	6.3	24.8	444	500	0.1
4	Bean-Maize	Soybean	5.5	19.4	422	408	0.002
5	Bean-Maize	Soybean	6.5	18.7	370	483	0.002
6	Bean-Maize	Soybean	6.5	19.9	352	544	0.001
Mean per AEZ			6.2	20.2	388	494	

3.3 Effects of cropping history, sampling time and manure on the survival of rhizobia

Initial rhizobial background recorded before establishment of the trials (September 2013) was small at both sites and there was no significant differences between the two sites. In both sites, initial rhizobial background with soybean as trap host was very small (Table 3.1). Four months from first sampling, a tenfold increase in rhizobial population was observed in the control treatments in soybean fields. The survival of rhizobia in the two study sites during the different sampling time was better in Bugesera ($P = 0.03$) in bean fields and Kamonyi ($P<0.001$) in soybean fields. However, there was no evidence of the cause for these differences found in our study, and it is difficult to elucidate exactly to what extent soil properties could have influenced rhizobia survival in the two sites.

The time of soil sampling significantly ($P<0.001$) influenced the population of rhizobia in soil for both bean and soybean at both sites. The population of rhizobia generally increased in the rainy seasons, and decreased during the dry season (Fig. 3.2). Inoculation and manure application increased ($P<0.001$) rhizobia populations up to 10^3 cells g^{-1} soil in January (four months after first sampling), and increased further up to 10^4 cells g^{-1} soil during the rainy season of March (six months after first sampling). The rhizobia numbers then decreased in all treatments in the dry season of July (ten months after first sampling) to populations less than 10^1 cells g^{-1} soil. Rhizobia numbers at eighteen months after first sampling during the rainy season of April were again higher ranging from 10^1 to 10^4 cells g^{-1} soil (Fig. 3.2). Manure application in Season 1 consistently increased rhizobia populations throughout the trial, with greater populations at the high manure application rate. At both sites, the population of rhizobia in the manured treatments were generally higher in inoculated than in uninoculated treatments. Inoculation alone also increased the population of rhizobia, but the numbers decreased during the dry season. This stresses the role of manure in the survival of rhizobia in soil by creating favourable conditions for their growth and multiplication.

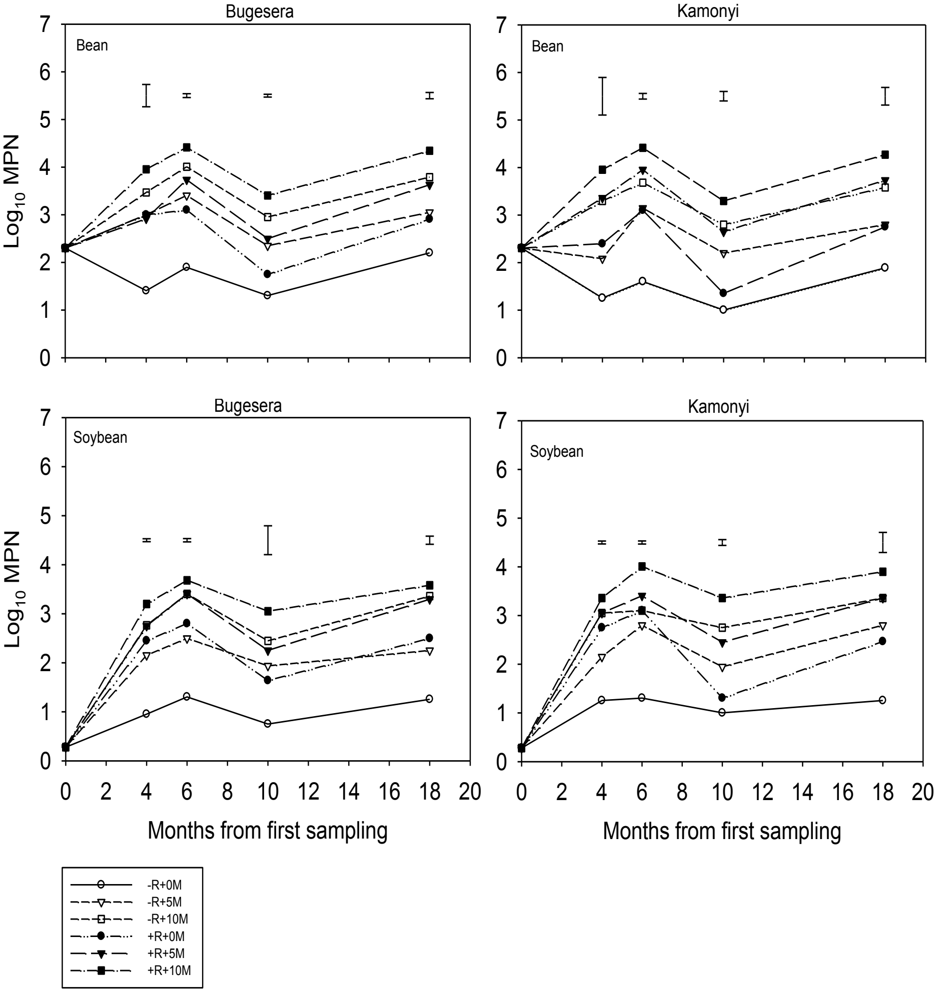

Fig. 3.2 Temporal dynamics of rhizobial populations with bean and soybean as trap hosts in Bugesera and Kamonyi in manured plots with or without inoculation. Rhizobia count was done in September, 2013, January, March and July 2014, and April 2015. Error bars represent standard errors of difference between means.

3.4 Nodulation score as affected by manure and inoculation

The nodule score of bean at both sites and soybean in Kamonyi was not influenced by inoculation in Season 1 but did increase *(P*<0.001) nodulation of soybean in Bugesera. Re-inoculation in Season 3 increased the nodule score of bean at both sites and soybean in Bugesera when no manure had been added in Season 1. There was an interaction (*P* = 0.002) between inoculation either in Season 1 or in Season 3 and manure application on the nodule score of bean at both sites but was not the case for soybean. There was no effect of inoculation in Season 1 or Season 3 on the nodule score of soybean in Kamonyi (Fig. 3.3). A maximum nodulation score of soybean in Kamonyi was observed irrespective of manure applications and inoculation. This may be due to the better survival of rhizobia nodulating soybean after introduction of soybean at this site. Manure added in Season 1 consistently increased ($P<0.001$) the nodule score of bean in Bugesera and Kamonyi in both uninoculated and inoculated either in season 1 or season 3. There was no difference between 5 t and 10 t manure ha^{-1} in increasing nodulation.

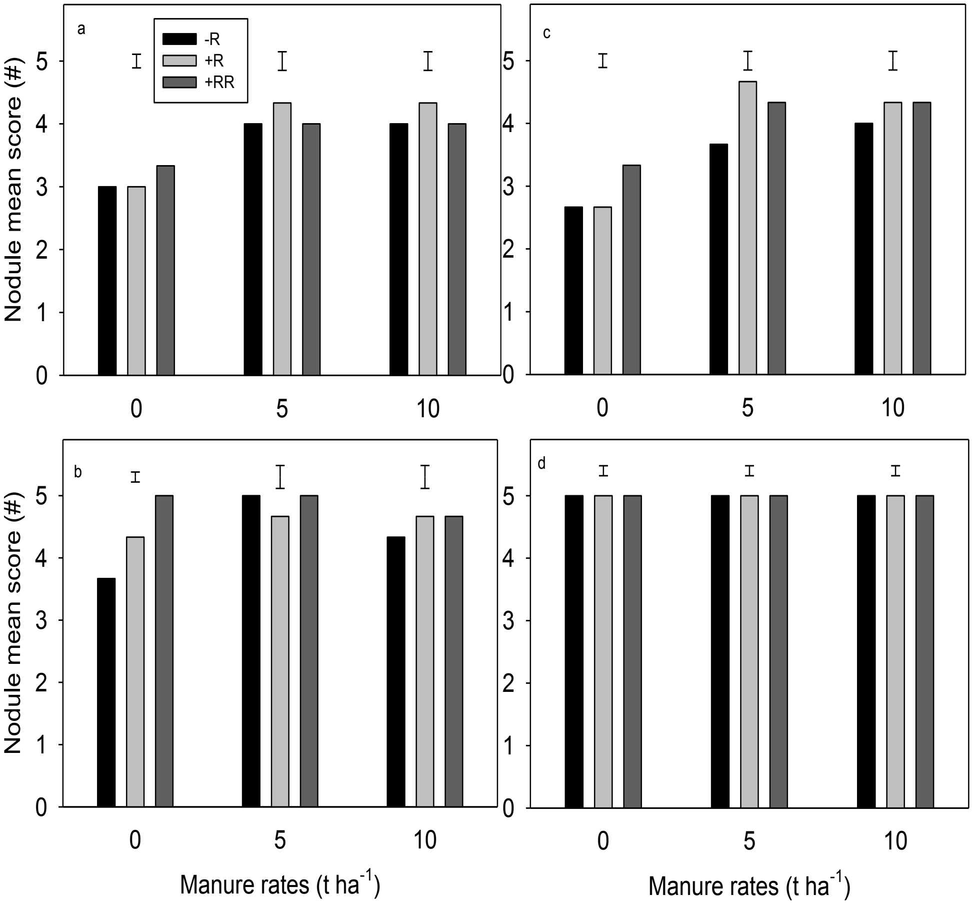

Fig. 3.3 Nodule score (#) response: (a, c) bean, and (b, d) soybean to inoculation and three rates of manure at (a, b) Bugesera, and (c, d) Kamonyi in season 3. Error bars represent the standard errors of difference between means; -R: without inoculation; +R: Inoculated in season 1; +RR: Re-inoculated in season 3.

3.5 Grain yield response of bean and soybean to manure application

The manure applied in Season 1 increased grain yield of both bean and soybean in Season 3 at both study sites ($P<0.001$) (Tables 3.2 & 3.3), though the increase was slightly greater in Kamonyi than in Bugesera. Inoculation in Season 1 significantly ($P<0.001$) increased yield of both bean and soybean in Bugesera and Kamonyi. No significant effect from P applied in Season 1 was observed in Season 3 in both legumes and sites. In Season 3, inoculation significantly ($P<0.001$) increased the yields of both bean and soybean, but there were no significant differences in yields observed between plots re-inoculated in Season 3 and those that had been inoculated in Season 1 across the manure rates though slightly greater yields were most achieved in re-inoculated plots. In both sites, yields of uninoculated plots also increased with increasing manure rates (*P*<0.001). For both bean and soybean there was an interaction inoculum–manure (*P*<0.001) at both sites, and manure–fertiliser for bean in Bugesera (*P*<0.001) and Kamonyi (*P*=0.019). No interaction inoculum–fertilizer was observed for both legumes at both sites. Small yields were consistently achieved in uninoculated and unmanured plots and responses to inoculation were less pronounced in manured plots. This could indicate that manure inputs are beneficial to plant growth in cases where inoculants are unavailable. The response to inoculation was variable for both bean and soybean at both sites. However, for both bean and soybean there was a significant (*P*<0.001) response to inoculation in unmanured plots (Fig. 3.4).

Table 3.2 Bean and soybean grain yield (t ha^{-1}) as affected by manure and inoculation

		Common bean					Soybean				
		Bugesera		Kamonyi		Mean	Bugesera		Kamonyi		Mean
Fertilizer +/- Inoculum	Manure (t ha^{-1})	-R^a	+R^b	-R^a	+R^b		-R^a	+R^b	-R^a	+R^b	
-P-R	0	1.6	2.3	1.7	2.0	1.9	1.5	1.7	1.4	1.8	1.6
	5	2.5	2.7	2.6	2.8	2.7	2.1	2.2	2.0	2.2	2.1
	10	2.9	2.7	2.8	2.7	2.8	2.3	2.2	2.1	2.2	2.2
Mean		2.3	2.6	2.4	2.5	2.4	2.0	2.0	1.8	2.1	2.0
-P+R	0	2.2	2.4	1.9	2.2	2.2	1.7	1.8	1.6	1.8	1.7
	5	3.0	2.9	3.3	3.1	3.1	2.4	2.4	2.5	2.4	2.4
	10	3.2	3.4	3.7	3.2	3.4	2.6	2.5	2.7	2.4	2.6
Mean		2.8	2.9	3.0	2.8	2.9	2.2	2.2	2.3	2.2	2.2
+P-R	0	2.1	2.4	1.9	2.3	2.2	1.6	1.9	1.6	1.9	1.8
	5	2.6	2.6	2.5	2.7	2.6	2.1	2.1	2.0	2.1	2.1
	10	2.7	2.8	2.6	2.9	2.8	2.2	2.3	2.1	2.3	2.2
Mean		2.5	2.6	2.3	2.6	2.5	2.0	2.1	1.9	2.1	2.0
+P+R	0	2.4	2.5	2.1	2.4	2.4	1.9	2.0	1.8	2.0	1.9
	5	2.8	3.1	2.9	3.5	3.1	2.4	2.5	2.3	2.3	2.4
	10	3.3	3.5	3.0	3.8	3.4	2.7	2.6	2.8	2.8	2.7
Mean		2.8	3.0	2.7	3.2	2.9	2.3	2.4	2.3	2.4	2.3
SED interaction		0.08	0.08	0.14	0.14		ns	ns	ns	ns	

-/+ R: without / with rhizobia (R) in Season 1, ^{a}: No inoculation in season 3; ^{b}: With inoculation in season 3; ns: not significant at $p < 0.05$

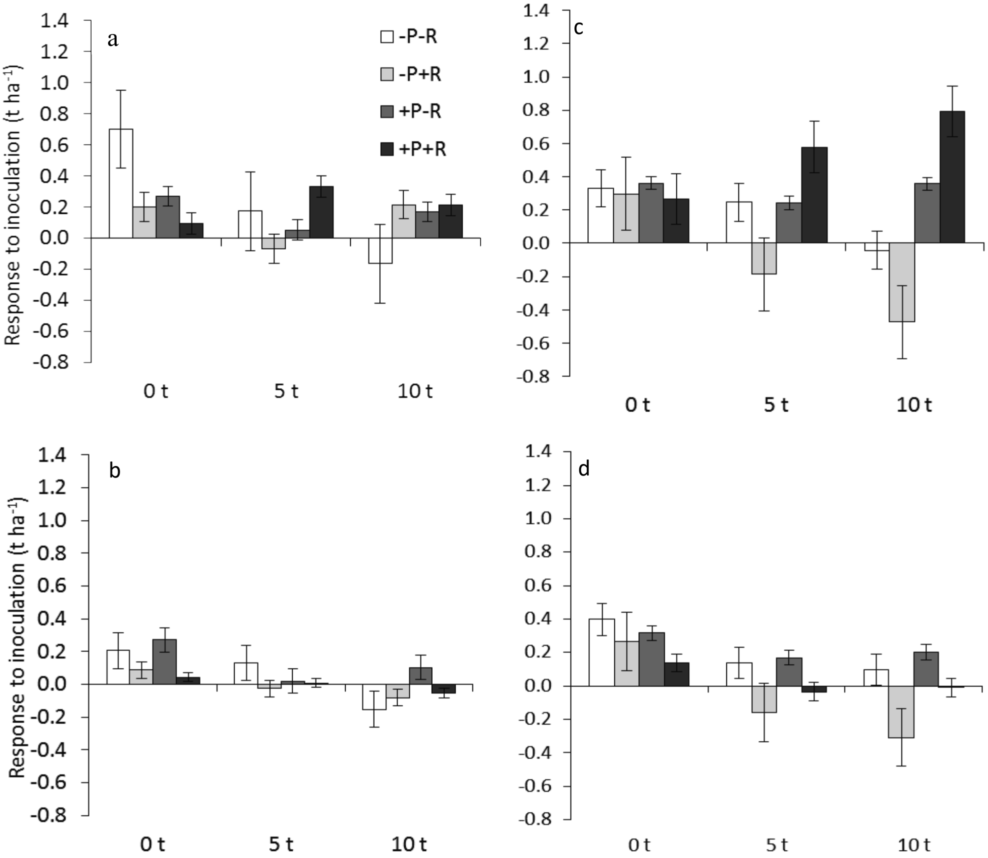

Fig. 3.4. (a, c) Bean and (b, d) soybean grain yield response to inoculation in Season 3 in (a, b) Bugesera and (c, d) Kamonyi across three rates of manure (t ha^{-1}). Error bars represent standard error of means of individual treatment.

4. Discussion

4.1 Influence of cropping history on rhizobial survival

The population size of rhizobia recorded before establishment of the trials (September 2013) was small at both sites and there was no significant differences between the two sites, except fields where bean had been grown before (Table 3.1). Low background rhizobial populations is not uncommon: there are reports of low population size of rhizobia in soils without a history of inoculation (e.g. Giller , 2001; Andrade et al., 2002; Zengeni et al., 2006). The population size of rhizobia in the soil depends on many factors such as field history, soil characteristics and the presence of a host plant. The presence of a compatible host legume has been reported to strongly influence the size of rhizobia in the soil (Bottomley, 1992; Andrade, 2002). The low initial population background observed may be explained by the history of experimental fields which had not been inoculated before and sampling was done after a non-legume crop. The very low initial population background observed with soybean as a host supports findings by Mahler and Wollum, 1982 who observed smaller rhizobial population of soybean before planting and gradual increase during the growing season. However, there is a controversial debate on the extent to which cropping history may affect the size of rhizobial population in the soil. Marshall et al. (1993) showed that the number of *R. leguminosarum* bv. *Viciae* did not depend on how recently the crop was grown, while many others reported cropping history to have a critical influence on the size of rhizobia in soil (Karanja et al., 1995; Mendes and Bottomley, 1998).

After introduction of legumes (October, 2013), the population size increased as revealed by the MPN count. The stronger increase of rhizobia population after planting soybean (Fig. 3.2) even in the uninoculated and unmanured plots emphasizes the important role of cropping history in the survival of rhizobia in the soil. This increase of rhizobia population in control plots upon introduction of a host legume may indicate that there was less influence of soil properties on rhizobial numbers in the two sites rather a lack of a compatible host crop. The survival of rhizobia in the two study sites during the different sampling time was better in Bugesera in bean fields and Kamonyi in soybean fields. Although there was no evidence of the cause for these differences found in our study, this may be due to the differences in optimum conditions for survival of rhizobia infecting bean and those infecting soybean at the two sites. It is difficult to elucidate exactly to what extent soil properties should have influenced rhizobia survival in the two sites. Nevertheless, the high organic carbon at both sites may have supported the survival of rhizobia especially in Kamonyi which had slightly sandy soil compared to Bugesera. The soils from the two sites had nutrients content mostly above reported critical values. Both sites had mostly a pH above 5 and organic carbon above 1 % with clay content above 20 % though on average Bugesera site had higher soil organic carbon and clay content compared with Kamonyi site.

4.2 Temporal dynamics of rhizobial populations and their response to *manure*

The population size of rhizobia decreased following the decrease in rainfall, with higher numbers in March and April during the rainy season and less in July which was the dry season. The month of July is a dry period as the dry season starts from June to mi-September, and the short rains start and continue to December, with a short dry season in January and February during harvesting of legumes. Then the long rains follow from March to June. The larger rhizobia numbers observed during the rainy season and sharp decline during the dry season confirms observations of Zengeni et al. (2006) in sandy soils at two smallholder sites in Zimbabwe. Similarly, Andrade et al. (2002) observed strong effects of season on bean nodulating rhizobia population in Brazilian soil. The higher rainfall during the rainy season in March and April increased soil moisture content, which in turn influenced positively the population size of rhizobia. Many researchers reported soil moisture to be among the most limiting factors for rhizobial survival in soil (e.g: Mahler and Wollum, 1980; Hungria and Vargas, 2000; Zengeni et al., 2006). The different MPNs revealed that rhizobia survive in soils (Fig. 2), and the population size of rhizobia was not affected by the length of the period from first sampling. The largest rhizobia numbers were recorded eighteen months from the first sampling. Similarly Zengeni et al. (2006) observed higher population numbers even at three years after inoculation.

Application of manure significantly improved the survival of rhizobia in soil at all of the sampling time (Fig. 2). The population size of rhizobia in both Bugesera and Kamonyi increased with increasing manure rate, with very small numbers in unmanured treatments across the sampling time. Increased survival of rhizobia observed in manured plots may be linked to its impact on availability of many nutrients, on moisture retention or on better soil structure creating a more favourable environment for rhizobia growth and survival. Manure is also known for its liming effect (Nzuma et al., 1998), supply of exchangeable bases and other micronutrients (Zingore et al., 2008). All these characteristics of manure may contribute to the better survival of rhizobia in manured treatments compared with unmanured treatments. The survival of rhizobia in soil for longer period of time when soil conditions are optimum has been reported elsewhere. For instance, *Phaseolus*-nodulating rhizobial isolates were detected six years after lime application in Brazilian soils (Andrade et al. 2002). Rhizobia also survive in soil in the absence of a plant host by using available organic material as sources of carbon and energy (O’Hara, 2001). The manure applied in

Season 1 served as a source of organic carbon and other essential nutrients to the rhizobia thus enabling them to survive better up to Season 3 after a rotation with a cereal crop.

4.3 Effect of manure on nodulation score, yield and response to re-inoculation

Re-inoculation of the plots inoculated in season 1 increased the nodule score of bean when no manure was added. Increased nodulation observed in the manured treatments may be partly due to its direct impact on availability of nutrient for crop growth as well as by creating more favourable conditions for nodulation and survival of rhizobia. Increased organic matter in soil enhances growth and multiplication of rhizobia with time, and allow nodulation. Manure applied in Season 1 increased grain yield of bean and soybean in Season 3 at both sites. This could be linked to the better growth of the two legumes as manure supplied many nutrients, improved soil structure and moisture retention allowing exploration of a large proportion of soil. The uninoculated and unmanured plots consistently had the smallest grain yield. The responses to re-inoculation were variable. Consistent responses were observed in unmanured plots, while some negative responses were seen in the manured plots (Fig. 4). This emphasizes the great role of manure in improving plant growth and yield in smallholder farming systems.

5. Conclusion

Our results clearly demonstrate the important role of manure as a key in enhancing rhizobial survival in soil, stimulating response to inoculation and increased legume grain yield. The greater survival of rhizobia was observed at both 5 t and 10 t manure ha^{-1}, and least in unmanured plots. Strong effects of rainfall on the population size of rhizobia were observed, with rhizobia numbers decreasing with decreasing rainfall. Inoculation increased grain yield of both bean and soybean but greater yields were achieved when manure was added. Our results did not show significant effect of re-inoculation of the previously inoculated plots across manure rates, rather significant and consistent responses were observed in unmanured plots. Cropping history also had an effect on the initial population background of rhizobia in soil. This was indicated by very small numbers when the legume has not been cropped before and strong increase once the legume was introduced in the field. Soil properties may have had effect on the survival of rhizobia in the two study sites, but it was not proved in this study. The greater yields achieved in Season 3 coupled with consistent rhizobial survival in manured plots are good indicators that farmers can grow bean and soybean without repeated inoculation if manure is used in the first season. This can assist smallholder farmers in making decisions as to how best to use the limited amounts of manure and rhizobial inoculants they are able to source.

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Chapter 4

The response of climbing bean to fertilizer and organic manure in the Northern Province of Rwanda

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Rurangwa, E., Vanlauwe, B., Giller, K.E., (under review), The response of climbing bean to fertilizer and organic manure in the Northern Province of Rwanda. Nutrient Cycling.

Abstract

Climbing beans play a central role in food security of rural households in the densely populated highlands of East and Central Africa. Soil fertility degradation and the lack of nutrient inputs are major limitations to yield of beans and other crops. We conducted field trials in Northern Rwanda in Kinoni and Muko villages to evaluate the effect of mineral N, P and K fertilizers (both alone and in combination) and manure on nitrogen fixation, grain yields of climbing bean in smallholder farmers' fields. The trials were laid down in a randomized complete block design (RCBD) with seven replicate blocks in each village. Manure and fertilizer application led to greater yields in all fields and the largest yields were achieved when manure was combined with NPK. Large variability in yield between fields was observed. Application of fertilizer together with manure increased the grain yield from 1.5 t ha^{-1} to 3.9 t ha^{-1} in Kinoni, and from 2.6 t ha^{-1} to 5.4 t ha^{-1} in Muko. Fertilizer and / or manure increased stover yield from 0.8 t ha^{-1} to 2.3 t ha^{-1} in Kinoni and from 1.5 t ha^{-1} to 3.4 t ha^{-1} in Muko. Application of 30 kg P ha^{-1} and 5 t manure ha^{-1} led to increased N and P uptake (from 49 to 106 kg N ha^{-1} and from 6.1 to 12.4 kg P ha^{-1} in Kinoni and from 46 to 128 kg N ha^{-1} and from 5.3 to 17.9 kg P ha^{-1} in Muko). There was no clear relationship between soil fertility characteristics and the response of climbing bean to applied inputs at Muko site. However, at Kinoni site, limited response to manure and NPK application was observed in plots where soil available P and soil exchangeable K were relatively low. Our results show the benefits of using manure alongside with mineral fertilizers for increased climbing bean yields and nutrient uptake in smallholder farming systems.

Keywords: Phaseolus vulgaris; nitrogen fixation; ^{15}N-natural abundance; nutrients uptake.

1. Introduction

In densely populated areas of sub-Saharan Africa, nutrient availability is a major limitation to crop growth, since soil fertility regeneration through fallowing land is no longer possible. There is an urgent need to improve agricultural productivity as landholdings have reduced in area due to population growth. To feed the rapidly growing population, sustainable intensification of agricultural production is needed (Vanlauwe et al., 2014), and integrating legumes is key to achieve this goal. Legumes are important crops both for supply of food and fodder and for soil improvement. Legumes fix atmospheric N_2 through symbiosis with rhizobia and contribute N to the soil for use by other crops (Franke et al., 2018). The use of legumes in rotation may lead to a reduction in fertilizer-N use, reduced pest and weed occurrence, and improved soil quality (Unkovich et al., 1997; Giller, 2001).

Despite the low yields achieved by farmers, common bean (*Phaseolus vulgaris* L.) remains a major crop in Eastern and Southern Africa (Wortmann et al., 2001). Bush bean varieties have been under cultivation since the introduction of common bean in the 16th century, while climbing bean varieties were only found in a few locations, and on small plots. The introduction of improved climbing bean varieties in Rwanda resulted in their rapid spread, also into neighboring countries (Sperling and Muyaneza, 1995). Climbing beans are reported to be less constrained by diseases and much more productive than bush bean (Wortmann et al., 2001), as well as being less prone to root rots (Sperling and Muyaneza, 1995). Climbing beans can produce grain yields nearly three-fold the yield of bush beans and grow vertically, making climbing bean a crop with great potential in densely-populated areas (Musoni et al., 2014).

The indeterminate growth of climbing beans allows them to provide a continuous supply of green leaves and pods as well as dry grain throughout the growing season (Sperling and Muyaneza, 1995; Wortmann et al., 2001). Strong residual effects have been reported when maize is grown after climbing bean, which are attributed to the large amounts of above-and belowground crop residues (Niyuhire et al., 2017), changes in microbial activities (Turco et al., 1990) and access to more P (Bainville et al. 2005). Climbing beans also provide valuable residues for livestock feed. Improved soil cover from climbing beans helps in suppressing weed growth as well as reduces water and soil loss from the steep slopes observed in the Eastern African highlands (Wortmann et al., 2001). However, availability of staking material, the increased labour requirements for staking and the longer growing period are identified by farmers as disadvantages of climbing bean (Sperling et al., 1992).

In Rwanda, beans are known as the “meat” of the Rwandan countryside. Research on improved climbing bean varieties in Rwanda started in the early 1970s at the former ‘Institut des Sciences Agronomiques du Rwanda’ (ISAR), now Rwanda Agriculture Board (RAB) (Sperling and Muyaneza, 1995). However, bush bean was predominant, and climbing beans were found only in the northwest of the country (Sperling and Muyaneza, 1995). Recently, the bean programme in Rwanda released a number of high yielding varieties, with high Fe and Zn contents that are mostly grown in the cool and humid northern highlands of the country (Musoni et al., 2014). New varieties of climbing beans suitable for production in the low-elevation zones have also been released (Musoni et al., 2014). The Northern Province of Rwanda has a very high population density estimated at 528 persons km^{-2} (NIS 2012). This has resulted in small landholdings which are continuously cropped resulting in small yields. In this region, up to 95% of the households grow climbing beans (Franke and deWolf, 2011; NIS, 2010). Smallholder farmers largely grow legumes with little or no fertilizer, as most mineral or organic fertilizer is targeted to cash crops (e.g. tomato, vegetables, Irish potato) that have a ready market. Only 12-21 % of the farmers in Northern Province of Rwanda use mineral fertilizer in climbing bean fields (Franke and deWolf 2011).

Many studies have shown the need for balanced fertilization in soils that have been cropped continuously (Smithson et al., 1993; Zingore et al., 2008a, 2008b). Integrated soil fertility management (ISFM) has been adopted as a framework for boosting crop productivity (Vanlauwe et al., 2010). One of the best options of addressing soil fertility declines and increasing fertilizer use efficiency is the combined application of organic and mineral fertilizers. A previous study in Northern Province of Rwanda showed that the inherent soil fertility characteristics affected the impact of applied nutrient inputs on the productivity of climbing bean (Franke et al., 2019). There is limited information on the integrated use of farmyard manure and mineral fertilizers on the performance of climbing bean in Rwanda.

The specific objectives of this study were to: (1) evaluate the effect of mineral N, P and K fertilizers (both alone and in combination) and manure on yields of climbing bean in two sites (seven fields in each) in Northern Rwanda; (2) assess the effect of mineral P fertilizer and manure on nitrogen fixation, N and P uptake, and (3) explore the influence of soil fertility characteristics on the response of climbing bean to applied nutrients.

2. Materials and methods

2.1 Study sites, soil sampling and field selection

The experiments were conducted in farmers' fields in Kinoni and Muko villages in the northern province of Rwanda. The objective was to select locations (villages) with low and high potential for cultivation of climbing beans based on soil fertility status and elevation. The colour of the soil, soil drainage, soil depth and crop performance over previous years were the selection criteria used by the farmers from each village. In addition to the soil fertility information, the size of the fields was also among the criteria as many farmers could not get the desired field size. In each village, fields with comparable fertility status, size and not located in areas potential for water-logging were selected. In each village, seven fields (blocks) were selected. The soils in Muko and Kinoni are classified as Andosols in the World Reference Base for Soil Resources (FAO, International Soil Reference and Information Centre and International Society of Soil Science).

The information received from farmers of both villages on climbing bean performance over years coupled with their differences in elevation, allowed to classify fields in Muko as high potential and those in Kinoni as low potential. The fields in Muko were located in the volcanic plains of Musanze district, with soils having dark colour, deep and better water drainage, good history for crop performance (according to farmers), and less prone to erosion. The fields in Kinoni were located on steep slopes on the side of the hills which expose them to erosion that may affect soil fertility over time and plant performance (Rugazura et al. 2015). Kinoni village is located in Burera district at 1°28'26.3" S and 29°50'4.8" E at an elevation of 2182 m above sea level (masl), with a mean annual rainfall of 1500 mm and a mean annual temperature of 21 ^{0}C. Muko village is located in Musanze district at 1°30'27.5" S and 29° 36'23.8" E at an elevation of 1850 m above sea level (masl), with a mean annual rainfall of 1400 mm and a mean annual temperature of 17.8 ^{0}C. In addition to the farmers' classification, soils were sampled from each field (block) at a depth of 0-20 cm from nine points following a W pattern. The nine samples were thoroughly mixed and a composite sample was taken, air-dried and passed through a 2 mm sieve, then taken to the laboratory at Crop Nutrition Laboratory Services in Nairobi, Kenya and analysed for pH (H_2O), total N, available P (Olsen), Organic C, effective cation exchange capacity (ECEC), exchangeable cations (K, Ca, Mg, Na) and texture using standard methods (Okalebo et al., 2002). Rainfall distribution and sowing dates are shown in figure 4.1.

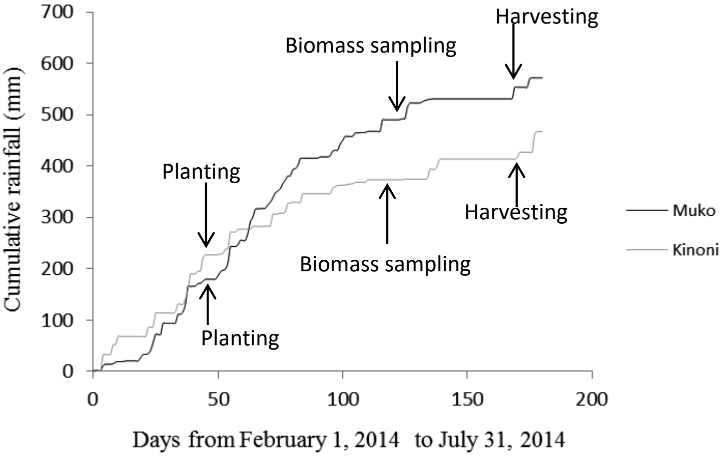

Fig. 1 Rainfall distribution during the experimental period at the Kinoni and Muko sites.

2.2 Trial establishment

The trials were established in the long rainy season of 2014. Climbing bean variety RWV 1129 was planted at 50 cm inter-row and 20 cm intra-row spacing, with three seeds per planting hole and thinned to two at first weeding. Treatments included manure (obtained from the participating farmers, and applied to her / his own field) at three rates: 0 t ha^{-1}, 2 t ha^{-1} and 5 t ha^{-1} of dry weight, mineral fertilizer treatments: None, +N, +P, +K and +NPK. P was applied in form of TSP at a rate of 30 kg P ha^{-1}; K was applied as KCl at 30 kg K ha^{-1}, N was applied as urea, split 50-50 at sowing and at first weeding (3 weeks after planting) and applied at a rate of 60 kg N ha^{-1} and NPK as a combination of N (60 kg N ha^{-1} as urea applied as in the sole N treatment), P (30 kg P ha^{-1} as TSP) and K (30 kg K ha^{-1} as KCl). With the rates of 2 t ha^{-1} and 5 t ha^{-1} of manure, each plot received 3.6 and 9.0 kg of manure respectively and was applied in the planting rows at sowing. Plot size was 4.5 m × 4 m with final harvest area of 8.75 m^2 and a net area for biomass sampling of 1.75 m^2. Each field was treated as a single complete replicate (block). Adjacent to each replicate (block), a plot (4.5 m × 4 m) was sown with maize to serve as reference plant for measurement of N_2-fixation. The reference plant was planted at the same time as climbing bean. Weeds were regularly controlled using a hand hoe by the farmers when needed. Figure 4.2 shows the relationship between N applied through manure and its C:N ratio, indicating the variation in manure quality used in the trials.

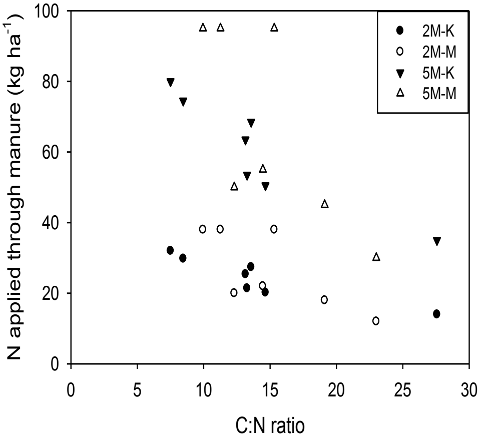

Fig. 4.2 Relationship between C: N ratio and N applied through manure (kg ha^{-1}) at Kinoni and Muko sites. 2M-K, 2M-M, 5M-K and 5M-M represent 2 and 5 t of manure ha^{-1} at Kinoni (K) and Muko (M) sites

2.3 Yield assessment

Above ground biomass was determined at late pod-filling stage. A sub plot of 1.75 m^2 (leaving 0.5 m away from the plot border) was sampled, all plants were cut at ground level and fresh weight was determined in the field. A sub-sample was taken and weighed, sun-dried, and then oven dried at 65 °C to constant weight to determine the dry matter contents. Final grain and stover yields were determined at crop maturity. All pods were harvested in the net plots excluding the outer rows of both sides of the plot, and the total fresh weight was determined. A sub-sample was taken, weighed and sun-dried for several days and then threshed by hand. Grains were cleaned by winnowing and subsequently weighed and the moisture content was determined using an electronic moisture meter. The grain yield was calculated at 12.5 % moisture. The haulms were harvested by cutting at ground level and weighed. Representative sub-samples of haulms from each plot were taken sun-dried, then oven dried at 65 °C to constant weight, and the dry weight was recorded. Stover yield and biomass yield at late pod-filling were calculated at 0 % moisture.

2.4 Assessment of N and P uptake and N_2-fixation

Dry climbing bean biomass was ground and digested in hot H_2SO_4 and H_2O_2 (Parkinson and Allen, 1975). N and P concentrations in the digests were determined by colorimetric methods (Okalebo et al., 1993). The proportion plant N from N_2-fixation was measured using the ^{15}N natural abundance method (Unkovich et al. 2008). After drying and grinding the samples, ^{15}N content was determined using a stable isotope mass spectrometer (Thermo Scientific, Delta V Advantage Isotope Ratio MS Coupled through Conflo IV to Thermo Scientific Flash HT/EA, KU Leuven). The $\delta^{15}N$ value and the proportion of N derived from atmosphere (%Ndfa) were calculated. The %Ndfa (Equation 1) and amount of N_2-fixed (Equation 2) were calculated as follows (Unkovich et al., 2008):

$$\%\text{Ndfa} = (\delta^{15}\text{N ref} - \delta^{15}\text{N leg}) / (\delta^{15}\text{N ref} - B) \times 100 \qquad \text{(Eq. 1)}$$

where $\delta^{15}N$ ref and $\delta^{15}N$ leg are the ^{15}N natural abundance (‰) in the non-fixing reference crops (maize) and the fixing species (climbing bean) respectively. The smallest values of $\delta^{15}N$ for climbing bean was used as the *B*-value (Peoples et al. 2002) and in this case was -1.7 ‰.

$$\text{Amount of N fixed [kg ha}^{-1}] = (\%\text{Ndfa} \times \text{Total N legume [kg ha}^{-1}])/100 \qquad \text{(Eq. 2)}$$

where %Ndfa is the percentage of N from N_2-fixation; Total N legume is the product of the %N in the legume plant and the dry biomass yield of the legume plant divided by 100. The total amount of N fixed was calculated to include the N content in the below ground parts, estimated at 30% of the amount of N_2-fixed in the shoots (Unkovich et al., 2008). Shoot N and P uptake was determined at late pod-filling.

2.5 Data analysis

Statistical analysis was performed using GenStat (version 16, VSN International Ltd). A mixed effects linear model was used for data analysis with sites × fertilizer × manure as fixed factors. Fields (blocks) were nested under sites and included in the model as random factors to account for their effects on grain, biomass and stover yields. Furthermore, N and P uptake and N_2-fixation were analyzed at fertilizer × manure level since there were no significant differences when site was included as fixed factor. Treatment means were compared using the standard error of differences between means (SED) at $P \leq 0.05$ significance level.

3. Results

3.1 Soil and manure characteristics

Soil and manure characteristics differed within each site and between the sites though overall differences between the two sites were not significant (Tables 4.1 & 4.2). Soil pH was slightly acid to near-neutral. Mean soil available P was above the critical value of 10 mg P kg^{-1}, but P availability varied within and across sites. In Kinoni, 4 out of 7 fields had available P concentrations far below the critical value. In Muko, soils had larger concentrations of available P with only 2 out of 7 fields with available P below the critical value. The soil organic carbon in the two sites was above the reported critical value of 1.5 % in all fields. Exchangeable cations were above the critical values of 0.2 for Mg in all fields and K was sufficient (>0.2 cmol$_c$ kg^{-1}) in 9 out of 14 fields, and exchangeable Ca was sufficient (> 0.5 cmol$_c$ kg^{-1}) in all fields. The manure varied in nutrient content (Table 4.2). On average, 5 t ha^{-1} of manure contained 60 kg N ha^{-1}, 15 kg P ha^{-1} and 55 kg K ha^{-1} in Kinoni and 65 kg N ha^{-1}, 15 kg P ha^{-1} and 70 kg K ha^{-1} in Muko.

Table 4.1 Characteristics of the soil from the Kinoni (*n*=7) and Muko (*n*=7) sites

	Kinoni (low potential)		Muko (high potential)		
Soil parameters	Mean	Range	Mean	Range	P-value
pH (H_2O)	6.5	6.3-6.7	6.5	6.4-6.8	ns
Total N (g kg^{-1})	2.4	1.8-3.5	3.1	2.2-4.0	ns
Organic C (g kg^{-1})	26.0	16.0-38.0	34.0	19.0-48.0	ns
Available P (Olsen) (mg P kg^{-1})	14.0	2.3-45.9	32.0	3.0-74.1	ns
Exchangeable K (cmol$_c$ kg^{-1})	0.2	0.1-0.6	0.4	0.1-0.7	ns
Exchangeable Ca (cmol$_c$ kg^{-1})	6.4	5.2-7.1	6.7	6.0-7.3	ns
Exchangeable Mg (cmol$_c$ kg^{-1})	2.1	1.3-2.9	1.6	0.8-2.7	ns
ECEC (cmol$_c$ kg^{-1})	15.4	10.1-20.8	14.0	9.0-18.3	ns
Sand (g kg^{-1})	376	140-688	605	269-887	0.075
Silt (g kg^{-1})	278	96-433	203	56-353	ns
Clay (g kg^{-1})	346	144-764	192	57-358	ns

ECEC: Effective Cation Exchange Capacity

Table4.2 Characteristics of the applied manure at the Kinoni (*n*=7) and Muko (*n*=7) sites

	Kinoni		Muko	
Parameters	Mean	Range	Mean	Range
pH (H_2O)	8.5	7.7-9.0	8.7	8.2-9.6
C (%)	15.5	12.0-19.3	18.4	12.3-29.1
N (%)	1.2	0.7-1.6	1.3	0.6-1.9
C:N	14.0	7.5-27.6	14.9	9.7-23.4
P (%)	0.3	0.1-0.5	0.3	0.1-0.5
K (%)	1.1	0.5-2.0	1.4	0.7-4.0
Ca (%)	1.1	0.6-1.6	1.1	0.7-1.4
Mg (%)	0.6	0.4-0.7	0.6	0.4-1.0
S (%)	0.1	0.1-0.2	0.1	0.1-0.3
B (ppm)	37.2	22.7-46.0	31.6	25.2-44.7

3.2 Yields and responses to inputs

Application of fertilizer and / or manure significantly ($P<0.001$) increased the grain yield (Fig 4.3a, b) and stover yields (Fig. 4.3c, d) at both sites. However, there was large variability in yield among fields, with grain yield ranging from 0.5 t ha^{-1} to 6.0 t ha^{-1}. Yields in control plots ranged from 0.5 t ha^{-1} to 3.2 t ha^{-1}, while yields with NPK and / or manure ranged from 1.3 t ha^{-1} to 6.0 t ha^{-1} (Fig. 4.4) On average grain yield was 2.8 t ha^{-1} and 4.1 t ha^{-1} for Kinoni and Muko respectively. Average stover yield was 1.6 t ha^{-1} and 2.4 t ha^{-1} for Kinoni and Muko respectively. Application of manure alone increased the grain yield from 1.5 t to 2.8 t ha^{-1} in Kinoni (Fig. 4.3a), and from 2.6 t ha^{-1} to 4.4 t ha^{-1} in Muko (Fig. 4.3b). In all fields, yields increased with NPK and / or manure addition. Fields in Kinoni village gave significantly ($P<0.001$) smaller grain yield than in Muko. Application of fertilizer alone increased the grain yield from 1.5 t ha^{-1} to 2.4 t ha^{-1} in Kinoni, and from 2.6 t ha^{-1} to 3.7 t ha^{-1} in Muko. Application of fertilizer together with manure increased the grain yield from 1.5 t ha^{-1} to 3.9 t ha^{-1} in Kinoni, and from 2.6 t ha^{-1} to 5.4 t ha^{-1} in Muko. There was large variability in stover yield among fields, ranging from 0.4 t ha^{-1} to 4.0 t ha^{-1}. Stover yield in control plots ranged from 0.4 t ha^{-1} to 2.0 t ha^{-1}, while stover yield with NPK and / or manure ranged from 1.2 t ha^{-1} to 4.0 t ha^{-1}. Stover yield varied greatly and the increase due to inputs application did not follow the same pattern as grain and biomass yields. On average fertilizer and / or manure increased stover yield from 0.8 t ha^{-1} to 2.3 t ha^{-1} in Kinoni and from 1.5 t ha^{-1} to 3.4 t ha^{-1} in Muko. Biomass yield significantly ($P<0.001$) increased with addition of fertilizer and / or manure at both sites (Fig. 4.3e, f). Large variability in biomass yield and response to applied inputs was also observed which followed the same pattern as grain yield at both sites.

In general, manure application led to a substantial increase in the grain yield with response of fertilizer to manure application ranging from 1.0-1.7 t ha^{-1} (Fig. 4.5). Responses to inputs were smaller in Kinoni than in Muko. On average, greater response of fertilizer to manure addition was achieved when manure was used together with N or NPK fertilizers and was least with P alone though there were no significant differences among treatments at the Kinoni site. Responses to NPK were improved by manure addition at both sites (Fig. 4.5). There was also a weak relationship between N applied through manure and climbing bean grain yield response to manure application (data not shown).

There were interactions site × manure × fertilizer for grain yield ($P = 0.019$), biomass ($P = 0.02$) and stover ($P = 0.003$), but not for N and P uptake, %Ndfa and amount of N_2-fixed. The variability in yields and response to inputs observed between sites may be linked to the inherent soil fertility and differences in past management practices though it was not assessed in this study.

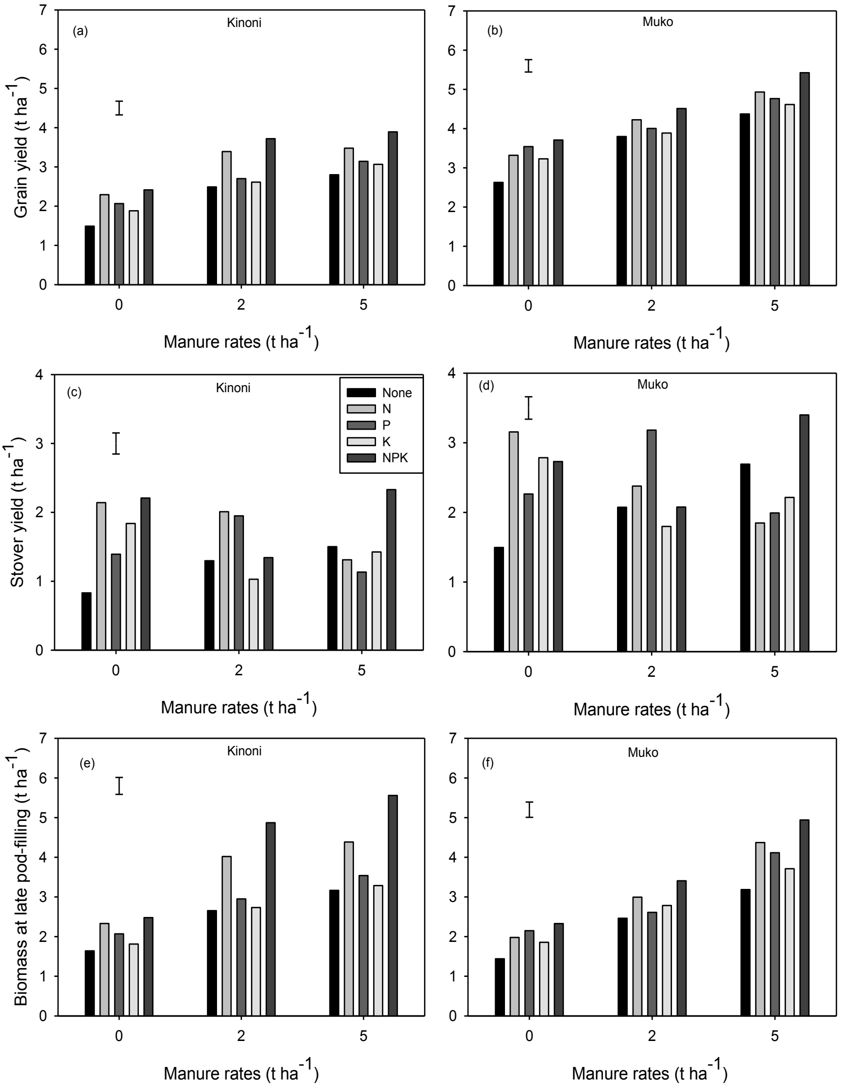

Fig. 4.3 Climbing bean grain (a, b), stover yields (c, d) and biomass at late pod-filling (e, f) as affected by inputs at Kinoni and Muko. None: Control (no fertilizer added); Error bars represent the standard error of differences between treatment means.

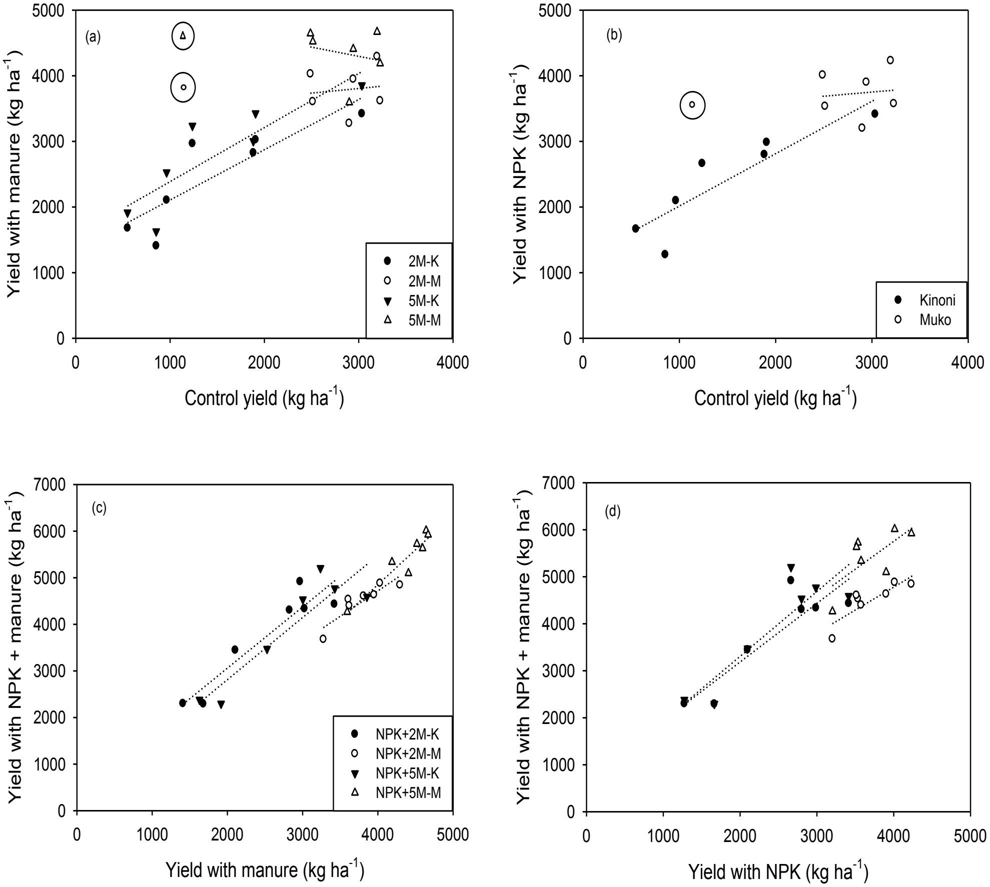

Fig.4 Climbing bean grain yield in control against (a) yield with manure (kg ha^{-1}), (b) yield with NPK (kg ha^{-1}), (c) yield with manure (kg ha^{-1}) against yield with NPK + manure (kg ha^{-1}) and (d) yield with NPK (kg ha^{-1}) against yield with NPK + manure (kg ha^{-1}). 2M-K, 2M-M, 5M-K and 5M-M represent 2 and 5 t of manure ha^{-1} at Kinoni (K) and Muko (M) sites. The dashed lines represent linear regression lines for (a, c, d) the manure rates and/or NPK and (b) NPK fertilizer at Kinoni and Muko sites. Encircled data points have been excluded from the regression analysis.

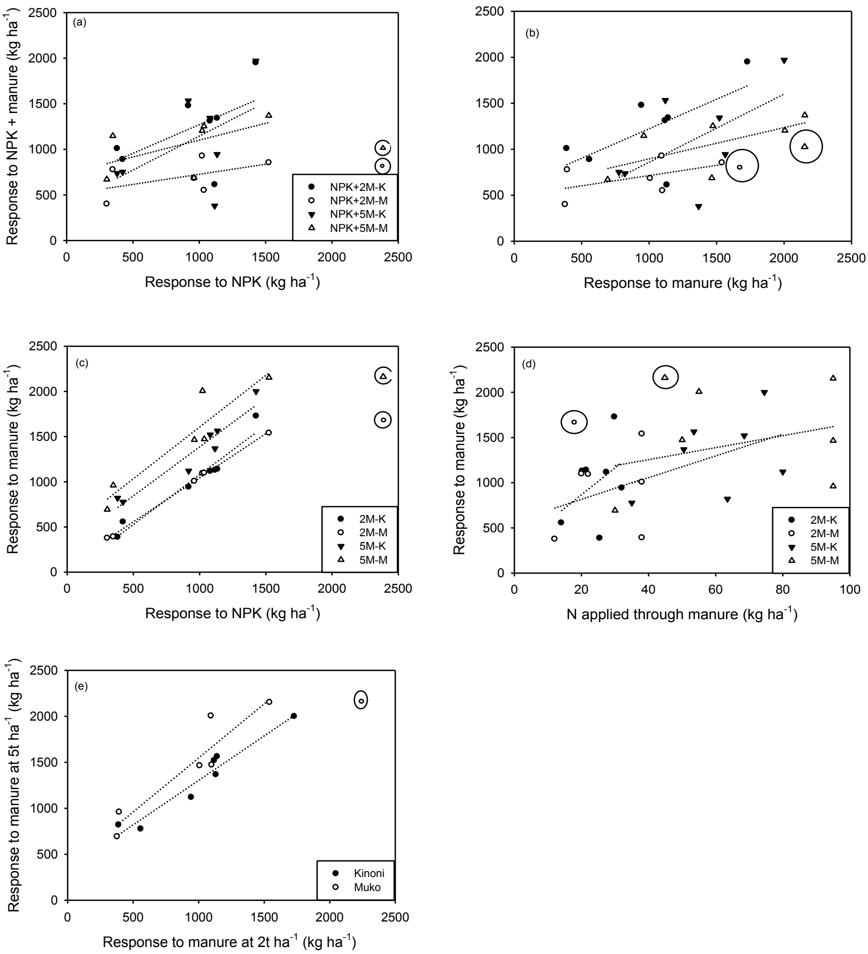

Fig. 5 Relationship between (a) Response to NPK (-control) against response to NPK and manure (-manure), (b) response to manure (-control) against response to NPK and manure (-manure), (c) response to NPK (-control) against response to manure (-control), (d) N applied through manure against response to manure (-control), and (e) response to manure at 2 t ha^{-1} against response to manure at 5 t ha^{-1} at Kinoni and Muko sites. 2M-K, 2M-M, 5M-K and 5M-M represent 2 and 5 t of manure ha^{-1} at Kinoni (K) and Muko (M) sites. The dashed lines represent the linear regression for (a, b) NPK and 2 and 5 t manure ha^{-1} at Kinoni and Muko sites, and (c, d, e) 2 and 5 t manure ha^{-1} at Kinoni and Muko sites. Encircled data points have been excluded from the regression analysis.

3.3 N and P uptake and nitrogen fixation

Shoot N and P uptake was improved by inputs application and was on average smaller in Kinoni than in Muko. In both sites, greater uptake was achieved in plots that received both fertilizer and manure, followed by plots with manure, then plots with P fertilizer alone and was least in plots that had not received any amendment. Variability in shoot N and P uptake was observed, with shoot N uptake ranging from 15.1 to 176.4 kg N ha^{-1} in kinoni and from 15.8 to 181.1 kg N ha^{-1} in Muko. Shoot P uptake also varied and ranged from 1.9 to 25.8 kg P ha^{-1} in Kinoni and from 1.9 to 25.4 kg P ha^{-1} in Muko. Application of inputs increased shoot N and P uptake in all fields in both sites. On average, 30 kg P ha^{-1} and 5 t manure ha^{-1} applied together increased N uptake from 48.5 to 106.3 kg N ha^{-1} in Kinoni and from 45.9 to 128.3 kg N ha^{-1} in Muko. Application of 30 kg P ha^{-1} and 5 t manure ha^{-1} also increased P uptake from 6.1 to 12.4 kg P ha^{-1} in Kinoni and from 5.3 to 17.9 kg P ha^{-1} in Muko (Fig. 4.6). Increased shoot N and P uptake in treated plots than in control plots may be a result of many nutrients supply including N and P, greater root system development leading to exploitation of a big volume of soil. Although N and P uptake increased with inputs application, there was no relationship between N applied through manure and shoot N uptake and weak relationship between P applied through manure and shoot P uptake (data not shown).

Greater grain yields were observed in Muko at small amount of biomass (data not presented), and may indicate more plant growth after biomass harvest (which was done before leaves started to senesce) in Muko than in Kinoni. This high grain yield in Muko at small biomass yield may also indicate a continuous biomass production, N and P accumulation after biomass harvest at this site. This may also have led to under-estimation of amount of N_2-fixed in Muko as more biomass could have been produced after biomass sampling.

The %Ndfa was on average lower in Muko (high potential) compared to Kinoni (low potential), but there was no significant difference between treatments (Table 4.3). No relationship was observed between biomass at late pod-filling and the %Ndfa but was positively observed with the amount of N_2-fixed. Positive relationships were also observed between the %Ndfa and the amount of N_2-fixed and between the amount of N_2-fixed and biomass N (data not presented). The amount of N_2-fixed was increased by application of fertilizer and manure, and was positively influenced by the biomass N (data not presented). Similar positive relationships were also observed for grain yield and N and P uptake (Fig.4.7a, b). The %Ndfa slightly correlated with shoot N uptake but no relationships were observed with N, P and K applied through manure (data not shown).

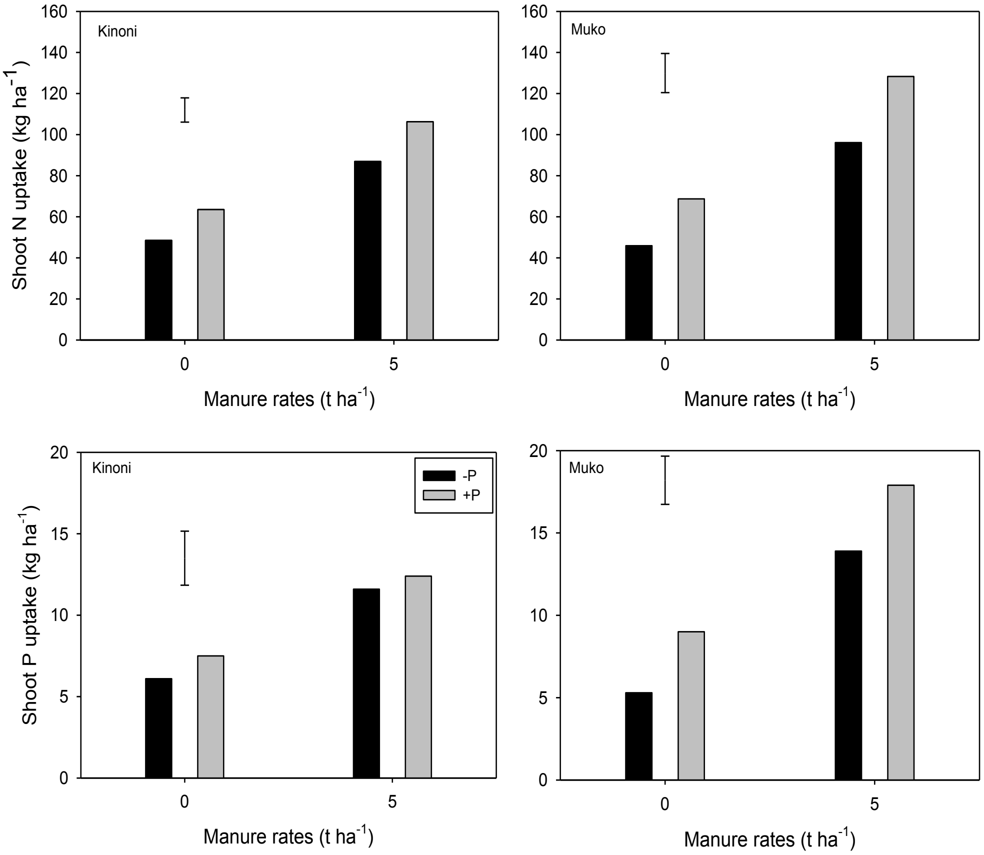

Fig. 6 Shoot N and P uptake at late pod-filling as affected by treatments at Kinoni and Muko. Error bars represent the standard error of differences between treatment means.

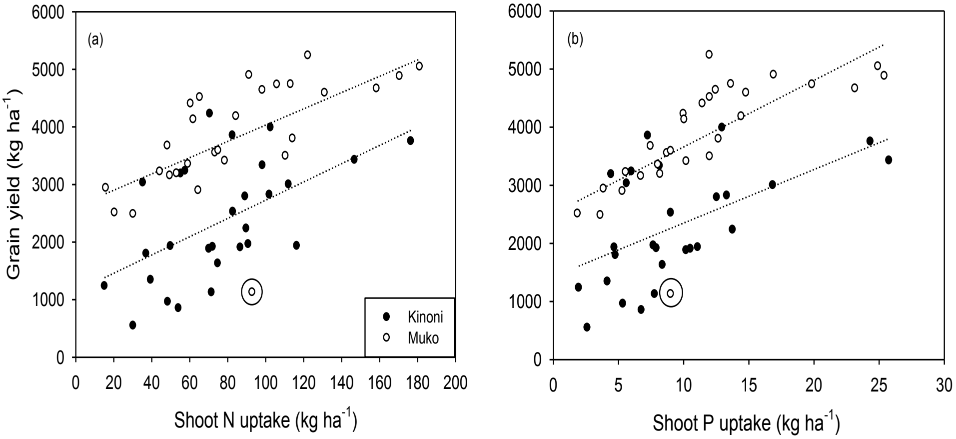

Fig. 7 Climbing bean grain yield (kg ha^{-1}) as affected by (a) shoot N uptake and (b) shoot P uptake at late pod-filling. The dashed lines represent linear relationships for Kinoni and Muko sites. The encircled data values have been excluded from the regression analysis.

Table 4.3 Climbing bean shoot $\delta^{15}N$, $\delta^{15}N$ reference crop, %Ndfa, Total N in shoot and amount of N_2-fixed as affected by treatments

Sites / Fertilizer (kg ha^{-1})	Manure (t ha^{-1})	shoot $\delta^{15}N$ leg (‰)	Range shoot $\delta^{15}N$ (‰)	%Ndfa	Total N in shoot (kg ha^{-1})	Total Amount N_2-fixed (kg ha^{-1})
Kinoni						
0P	0	2.0	-1.7-4.4	49	48.5	32.7
0P	5	2.3	0.7-3.8	42	89.8	52.1
30P	0	2.2	1.3-3.0	42	63.5	37.0
30P	5	2.6	1.0-5.9	37	106.3	59.1
Mean/Site		2.3		43	77	45.2
SED (Fertilizer)		*ns*		*ns*	*3.7*	*ns*
SED (Manure)		*ns*		*ns*	*4.4*	*5.2*
Muko						
0P	0	3.3	0.9-6.3	32	45.9	18.6
0P	5	3.4	1.8-5.2	30	96.1	42.2
30P	0	3.1	0.9-4.2	33	68.7	32.9
30P	5	2.2	0.4-4.1	46	128.3	79.0
Mean/Site		3		35	84.7	43.2
SED (Fertilizer)		*ns*		*ns*	*3.7*	*ns*
SED (Manure)		*ns*		*ns*	*7.8*	*8.5*

Reference plant (Maize) values: Kinoni: 5.2(3.0-7.1) and Muko: 5.6 (4.2-7.6)

Ndfa: percentage of N_2 derived from atmosphere; leg: legume crop; ref: reference maize crop

3.4 Soil fertility characteristics and response to applied inputs

Responses to inputs were observed in both sites ($P<0.001$), yet there were no clear relationships with soil parameters. In Kinoni, weak responses to manure and NPK application were observed in plots where soil available P and exchangeable K were < 10 mg kg^{-1} and < 0.2 cmol$_c$ kg^{-1} respectively (data not presented). However, this was not the case in Muko, suggesting that P and K were not limiting at this site. In neither of the sites was a clear relationship observed between soil N and response to inputs, suggesting that N was not limiting either. There were also no clear relationships between response to P and soil available P, response to N and soil N and response to K and exchangeable K (data not shown) at both sites. In general, larger responses were observed in Muko though there was no clear relationship with soil parameters.

4. Discussion

4.1 Yields response to inputs and fertilizer response to manure application

Grain, biomass and stover yields increased with manure and fertilizer inputs at both sites. Applied individually, manure led to a greater yield increase than mineral fertilizer at both Kinoni and Muko villages. Strong increase of crop yields following manure application has been reported elsewhere. Zingore et al. (2008b) observed a substantial soybean yield increase as a result of manure application compared with yields achieved with application of single super phosphate (SSP). The strong effects of manure in increasing crop yields are attributed to its multiple functions such as supply of many nutrients including micronutrients, increasing soil organic matter contents as well as improving soil conditions needed for crop performance (Zingore et al., 2008a, b; De Ridder and Van Keulen, 1990). Management options including use of manure, staking density and height have been identified as key factors influencing climbing bean productivity (Reckling, 2011; Musoni et al., 2014; Franke et al., 2019) in Rwanda, and yield variability was strongly related to resource endowment (Franke et al., 2019). In our experiments, farmers used similar stake quality and management was the same during the study period. The differences in yields observed could be linked to differences in past management practices among the participating farmers. Increased yield of climbing bean when organic and inorganic fertilizers are combined has been reported elsewhere (Niyuhire et al., 2017). Manure is reported to increase the crop yield and responses to applied fertilizers (Rurangwa et al., 2018), and past manure application was identified as the most factor affecting yields and yields response to applied fertilizers (Njoroge et al., 2019). Surprisingly we found no clear relationships between measured soil characteristics and climbing bean yields.

4.2 N and P uptake and N_2-fixation

The total N and P contents in the shoots were small in non-amended plots, and significantly increased with application of fertilizer and manure. On average shoot N uptake increased from 48.5 to 128 kg N ha^{-1} which is in the range of shoot N uptake reported by Ojiem et al. (2007) on various legume crops ranging from 10-486 kg N ha^{-1}. Increased N and P uptake may be a result of increased plant growth hence increased plant demand. Shoot N uptake increased with increasing N_2-fixed (Fig 4.7d) which was positively correlated with the biomass productivity (Fig. 4.7b). Plant biomass has been reported to be the best predictor of nitrogen fixation (Salvagiotti et al., 2008; Peoples et al., 2009), mainly because most of the factors affecting nitrogen fixation also affect leaf photosynthesis and biomass production (Peoples et al., 2009).

The %Ndfa varied greatly between fields ranging from 1.12 to 99.7 % with a mean of 39 %. Observed variability in %Ndfa is not uncommon in smallholder faming systems, and has been reported extensively. Giller, (2001) reviewing different papers reported high variation in %Ndfa (0-73%) and amount of N_2-fixed (2-125 kg N ha^{-1}), Ojiem et al. (2007) working in Western Kenya also observed variability in %Ndfa ranging between 7-90%. Peoples et al. (2009) reported %Ndfa ranging between 10-51%; and Reckling, (2011) observed a variation between 13-66%. Using maize as a reference crop has been reported to underestimate the %Ndfa (Ojiem et al., 2007). This may inpart explain the small %Ndfa observed in this study compared with the findings by Reckling (2011) in the same region. Although we could not explain the reason for the large variability in the %Ndfa observed, environmental and management effects are reported among others to affect the %Ndfa (Graham, 1981 and Bliss, 1993). The greater grain yield achieved in Muko despite the relatively small amount of biomass may indicate more plant growth after biomass harvest in Muko than in Kinoni. Increased plant growth after biomass harvest may have led to an underestimation of amount of N_2-fixed at this site as more biomass could have been produced after biomass sampling. Earlier studies showed that more of the plant N is acquired from atmosphere in the post-flowering stage compared with the earlier stages of growth (Wortmann, 2001). However, in this study biomass was sampled at late pod-filling stage, and this shows difficulties in knowing the best time for biomass sampling in climbing bean with indeterminate growth. For this reason, there is a need for biomass sampling at different growth stages for more accurate determination of N_2-fixation.

4.3 Soil fertility characteristics and response to applied inputs

Strong responses to applied inputs were observed which varied within and between the two study sites. The soils of both study sites had a wide range of nutrient contents ranging from very small to very high. However, there was no clear relationship between soil parameters and responses to applied inputs. Similarly, Franke et al. (2019) working in Northern Rwanda observed a poor correlation between soil characteristics and response to inputs. Large variability in responses was observed within and across sites, with poor yields even in some fields with good soil characteristics. This explains the lack of correlation between soil parameters and yields of climbing bean. In line with the results of this study, Franke et al. (2019) observed that organic manure application rate was positively associated with climbing bean yield. They found that greater yields were achieved in plots that received more organic manure. In this study, we also observed greater yields and yields responses in plots that received manure in combination with NPK fertilizer. Increased yields and responses when inputs are used together have been reported extensively (Ronner et al., 2016; Rurangwa et al., 2018). In Kinoni, some weak responses to manure and NPK application were observed in plots where soil available P (< 10 mg kg^{-1}) and exchangeable K (< 0.2 $cmol_c$ kg^{-1}) were limiting. However, this was not consistent as it was not seen in Muko, and it remains difficult to explain. Comparable results were reported by Franke et al. (2019) who found responses to DAP fertiliser in soil where available P was more limiting and with no relationship between soil N and response to DAP.

Although not assessed in this study, the large variability in yields observed may be linked to past management and inherent soil fertility. Variability in yields and responses to applied inputs in smallholder farmers is not uncommon. Ronner et al. (2016) working on soybean in Northern Nigeria found large variability in soybean yields and response to P +/ or inoculation. In our study, the largest response to applied inputs was observed at Muko where the control yields were greatest, matching observations of Ronner et al. (2016) in Norther Nigeria. Similarly, Njoroge et al. (2019) working in Western Kenya observed variability in maize yield and yield responses to applied fertilizers, and reported past manure application as the main factor affecting responses to applied inputs. We did not investigate the history of our field trials, but the variability in yield observed maybe linked to the differences in past management practices among the participating farmers. In the study sites many farmers rotate climbing beans with other crops such as potatoes, tomatoes, vegetables and fertilize them with manure and or fertilizer (Franke et al., 2019). The same source states that the amount of manure that farmers apply depends on their wealth category, and the number of livestock they own.

4.4 Reflections on the measurements of nitrogen fixation using maize as a reference crop

The use of maize as a reference crop may lead to underestimation or overestimation of the %Ndfa and its corresponding amount of N_2-fixed. Many researchers have reported large variability of the $\delta^{15}N$ when maize was used as a reference crop (Ojiem et al., 2007; Chikowo et al., 2004). In our study the $\delta^{15}N$ of the maize reference crop varied from 3.0 to 7.6 (‰) and led to large variability of the %Ndfa which ranged from 1.12 to 99.7 % with a mean of 39 %. The total N harvested (total N in the shoot + total N in grain) varied widely, ranging from 52 to 382 kg N ha^{-1} with an average of 204 kg N ha^{-1}. The total amount of N derived from nitrogen fixation (calculated based on the %Ndfa and total N harvested) varied from 4 to 253 kg N ha^{-1} with an average of 79 kg N ha^{-1}. These results lead to very low to very high N derived from the soil (Ndfs) ranging from 0.4 to 378 kg N ha^{-1} with an average of 125 kg N ha^{-1}.

Considering the total soil N in the top soil layer (20 cm) plus N from manure applied in one ha, using a soil bulk density of 1.3 g / cm^3, and by computing (10000 × 0.2 × 1.3 × g N / kg soil)/1000; where 1.3 is the soil bulk density in g / cm^3, and g N / kg is the measured N content in 1 kg of soil, and then all converted into kg N / ha, the total N in the top soil layer ranges from 4680-10400 kg N / ha. Assuming that 2% of the total soil N is mineralized in one season, an approximation which appears to have some general validity (Janssen et al., 1990), N supply from the soil obtained would vary from 94-1052 kg N / ha and is higher than 20% of N up taken from the soil. With these assumptions, though there was large variability in observed %Ndfa, the results obtained with the $\delta^{15}N$ estimates that suggest the climbing beans are getting 0.4 to 378 kg N / ha from the soil appear to be feasible.

Similar variability in %Ndfa, Ndfs and total N harvested was observed when using broad-leaved weeds as reference plants in the same region for climbing beans. In his research, Reckling, (2011), used $\delta^{15}N$ of 9 non N_2-fixing weeds and observed % Ndfa ranging from 11.37 to 77.97% with an average of 49%, Ndfs ranging from 31 to 410 kg N ha^{-1} and a total N harvested ranging from 95 to 557 kg N ha^{-1} with an average of 182 kg N ha^{-1}.

Ojem et al. (2007) observed variations in $\delta^{15}N$ among sites in Western Kenya for both maize and broad-leaved weeds, and maize had lower $\delta^{15}N$ values compared to broad-leaved weeds. On average, maize $\delta^{15}N$ was 3.20 ‰ and 5.89 ‰ for broad-leaved weeds which was closer to the 8‰ $\delta^{15}N$ of total soil N. These authors concluded that when maize is used as reference crop the %Ndfa and the corresponding N_2-fixation are underestimated. The $\delta^{15}N$ of maize used as the reference crop in our study was on average 5.4‰ which is not far from that reported above for broad-leaved weeds in Kenya. The greater variation and small %Ndfa observed in our study may partly be a result of the observed $\delta^{15}N$ of the legume (climbing bean) which was low to very high as well (-1.68 to 6.31‰) compared with -0.44 to 4.10‰ (Reckling, 2011) and -0.70 to 3.74 (Ojiem et al., 2007). With these findings, it remains uncertain which specific factors govern the %Ndfa in the smallholder farming systems characterized by heterogeneous fields.

5. Conclusion

Application of fertilizer inputs led to greater yields in all fields of the study sites. The use of manure alongside with mineral fertilizers proved to be beneficial in increasing climbing bean yields. Greater yields were achieved when manure was used together with NPK fertilizer. Applied individually, manure seemed to be more beneficial than mineral fertilizer in increasing climbing bean yield. The use of manure and fertilizer strongly increased the uptake of N and P at both sites. Targeting of the best time for biomass sampling in climbing bean for N_2-fixation estimation seems to be difficult, and multiple biomass sampling at different growth stages are advised for accurate N_2-fixation determination. Variability in yields and response to inputs coupled with the lack of correlation between soil characteristics and response to inputs call for a deeper understanding of the field's history/ past management before fertilizer application. Looking at the large variability in %Ndfa observed in this study, it remains unclear which specific factors govern the %Ndfa in the smallholder farming systems. The use of a range of non N_2-fixing leguminous crops and different methods is recommended.

Acknowledgement

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Chapter 5

The use of the compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) for evaluating the nutrition status of climbing bean in Northern Province of Rwanda

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This Chapter will be published in a modified version as:

Rurangwa, E., Vanlauwe, B., Nziguheba, G., Giller, K.E., The use of the compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) for evaluating the nutrition status of climbing bean in Northern Province of Rwanda

Abstract

Soil fertility decline is a major factor limiting crop production in the smallholder farming systems of Rwanda. The use of fertilizers either organic or inorganic remains low and smallholder farmers achieve poor yields. The low use of fertilizers coupled with the current blanket fertilizer recommendations call for site-specific fertilizer targeting deficient nutrients. This study aimed to: (1) derive and compare CND and DRIS norms for climbing bean, (2) assess the relationship between nutrient concentrations of foliar tissue and yield, and (3) identify which nutrients are limiting in climbing bean in Northern Rwanda, using the Compositional Nutrient Diagnosis (CND) and the Diagnosis and Recommendation Integrated System (DRIS). Climbing bean leaf samples were collected from 56 plots in Northern Rwanda and were analyzed for N, P, K, Ca, Mg, Cu, Mn and Zn. DRIS and CND approaches were applied to rank nutrients according to their degree of limitation to climbing bean. Results suggested that Zn was the most limiting nutrient at both sites. In Kinoni Zn was the most limiting, followed by N, K and P; while in Muko Zn was the most limiting, followed by Mg, Ca, P and N. The results also showed strong relationship between DRIS and CND indices, indicating the usefulness of both approaches in diagnosing nutrient deficiencies in climbing bean. Despite the application of manure and NPK fertilizer, N and P indices remained negative at both sites and K at Kinoni may indicate low efficiency of applied fertilizers. Climbing bean was not able to take up in sufficient quantities even some nutrients that were highly available in soil, suggesting an interaction issue that hinders their uptake.

Keywords: Deficiencies, foliar nutrient concentration, norms, indices, nutrient imbalance

1. Introduction

Population increase in Rwanda has led to high pressure on land resulting in intensive cultivation and depletion of soil nutrients. The use of chemical fertilizers remains very limited despite the Government initiative of Crop Intensification Program (CIP), in which subsidized fertilizers are provided to farmers for use in selected priority crops (FAO, 2019). Decreasing soil fertility coupled with little or no use of fertilizer inputs are major limitations to crop production.

The Northern Province of Rwanda is a major climbing bean growing area due to the favourable climatic conditions (Sperling and Muyaneza, 1995; Musoni et al., 2014). However, few farmers in this area apply fertilizer to climbing bean and they achieve only small yields commonly below 1 t ha^{-1}, way below the potential rainfed yields of 5 t ha^{-1} (Franke and de Wolf, 2011; Musoni et al., 2014; Franke et al., 2019). Depletion of soil nutrients leads to nutrient deficiencies or imbalances in agricultural systems, leading to decreases in yield and nutritional quality of the harvested products (Nziguheba et al., 2009). Recent studies in Northern Province of Rwanda showed that only farmers in the wealthier categories were able to retain crop residues in their farms and also invest in manure or fertilizer (Reckling, 2011; Franke et al., 2019). There is a need for understanding the primary deficient nutrients in the crop as opposed to the commonly-used blanket fertilizer recommendations to be able to provide balanced nutrition.

Nutrient concentrations of foliar tissue are useful indicators of the nutritional status of the plants (Wortmann et al., 1992). The nutritional state of plants influences the dry biomass production, and deficiency of essential nutrients prevents the maximum potential productivity (Serra et al., 2012). The leaf tissue is an important part of the plant where the physiological; activity happens, and shows easily the nutritional disturbance. With this knowledge, it is necessary to specify the nutrient limitations for better targeting of fertilizer recommendations to address existing nutrient deficiencies for increased crop production. The nutritional diagnosis approach is a complementary tool to soil analysis for diagnosis of nutrient deficiencies.

Visual diagnosis of plant nutritional deficiency has been commonly used but it is of limited utility as deficiency symptoms show up in the plant only when the deficiency is acute (Marshner, 1995). Several methods have been derived to investigate the dynamics of the leaf tissue composition and translocation into the plant, but most of them considered each nutrient independently (Wortmann, 1992). Foliar nutrient levels are much affected by plant age as well as interactions affecting nutrient uptake and distribution (Wortmann, 1992). Analysis and interpretation of foliar tissue composition results is complicated due to its dynamic nature. The Diagnosis and Recommendation

Integrated System (DRIS) (Beaufils, 1973) and the Compositional Nutrient Diagnosis (CND) (Parent and Dafir, 1992) are two methods commonly used to diagnose nutrient imbalances in crops, as both methods take nutrient interactions into consideration.

DRIS is based on dual ratio functions while CND is based on row-centred log ratios where each nutrient is adjusted to the geometric mean of all nutrients and to a filling value (Rd). DRIS norms for dry bean (*Phaseolus vulgaris* L.) were estimated from a broad-based data set by Wortmann et al. (1992). Previous studies reported DRIS method to be inferior to CND in diagnosing imbalances as it assumes additivity of dual ratios, and does not directly take into account higher order interactions (Parent and Dafir, 1992). DRIS is also reported to be less sensitive for early detection of N stress in sweet corn compared with CND (Khiari et al., 2001a). However, other studies on tomatoes (Parent et al., 1993) and potatoes (Parent et al., 1994a), DRIS and CND provided similar results.

Norms for DRIS have been obtained by several authors (Sumner, 1981; Beverly, 1993), but some researchers have suggested that norms calculated from a local data base may improve DRIS diagnosis (Dara et al., 1992). It has also been shown that DRIS norms for a crop differ between regions (Walworth and Sumner, 1987), and yet we lack CND norms specific to beans. The specific objectives of this study were: (1) derive and compare CND and DRIS norms for climbing bean, (2) assess the relationship between nutrient concentrations of foliar tissue and yield, and (3) identify which nutrients are limiting in climbing bean in Northern Rwanda.

2. Materials and methods

2.1 Study site, Soil sampling and fields selection

The experiments were conducted in farmers' fields in Kinoni and Muko villages in the northern province of Rwanda. The objective was to select locations (villages) with low and high potential for cultivation of climbing beans based on soil fertility status and elevation. In each village, seven fields (blocks) were selected to make a total of 14 fields in both villages. Soil samples were taken from each field (block) at a depth of 0-20 cm from nine points following a W pattern and composite was made (The details on soil sampling, analysis, fields selection and establishment are presented in Chapter 4).

2.2 Leaf sampling and analysis

Leaf tissue analysis was done on 56 samples from experimental fields. The 56 samples were collected in treatments with no inputs, with full NPK, with NPK + manure, and with manure only selected from a set of 210 plots. In this chapter, samples were collected in plots that received manure at 0 t ha^{-1} and 5 t ha^{-1} of dry weight, and NPK was applied as a combination of N (60 kg N ha^{-1} as urea applied as in the sole N treatment), P (30 kg P ha^{-1} as TSP) and K (30 kg K ha^{-1} as KCl). Plot size was 4.5 m x 4 m, and each field was treated as a single complete replicate (block).

Leaf samples were collected from the uppermost fully expanded leaf of the main stem 8-12 weeks after planting (Wortmann et al., 1992). Recently matured leaves were picked as they best reflect the general nutrient status of the whole plant during the period of most intensive growth. From each sampled treatment plot and for all replications (blocks), at least 20 leaves were collected on healthy plants and oven dried at 65^0C to constant weight. Sample analyses included the determination of macronutrients (N, P, and K) and micronutrients (Ca, Mg, Cu, Mn and Zn). ICP-OES (inductively coupled plasma optical emission spectrometry), ICP-OES, Varian 720 ES, KU Leuven, BE analysis was used to determine nutrient content in digest after digestion of collected dry samples with nitric acid.

2.3 Analytical approach for the compositional nutrient diagnosis (CND) and the diagnosis and recommendation integrated system (DRIS)

Grain yield data from experimental fields was used to differentiate between low-yield and high-yield subpopulations. The high-yield subpopulation data sets were used to develop the norms. Researchers have proposed several ways to select the reference population (high-yield subpopulation). For instance, Walworth and Sumner (1987) suggested that the reference limit to separate two populations should be arbitrarily selected, as each population is supposed to present the normal distribution. Letzsch and Sumner (1984) recommended that the reference population should contain at least 10% of the overall database observations. Malavolta (1989) recommended that the reference population should be obtained with 80 % maximum yield observations. Wortmann et al. (1992) estimated norms for beans using 306 cases including 48 samples from Rwanda, and set a minimum yield at 1100 kg ha^{-1} while estimating norms. However, this yield cut-off seems too low for climbing bean. Using their yield cut-off value in our data set, only 3 samples remained in the low-yield subpopulation. In this study, the yield cut-off between the low and high-yield subpopulations was based on the optimum climbing bean yield that can be achieved if there are no nutrients limitations (4000 kg ha^{-1}). Based on that, 19 out of 56 samples were referred to as high-yield subpopulation, and it contains the 10 % of the total data set.

The CND norms are the means and standard deviations of row-centred log ratios in the high-yield subpopulation. Row-centred log ratios, denoted as V_x for nutrient x, are computed from the nutrient x and the geometric mean of all nutrients and R_d (filling value between 100% and the sum of *d* nutrient proportions); where *d* is the number of nutrients under consideration (Parent and Dafir, 1992). The CND indices and nutrient imbalance indices (CND r^2) were computed for both low and high-yield subpopulations (Khiari et al., 2001a).

The calculation of CND norms was based on methods outlined by Khiari et al. (2001b). Plant tissue composition forms a d-dimensional nutrient arrangement; i.e. simplex (S^d) made of d +1 nutrient proportions including d nutrients and a filling value (R_d) defined as (Parent and Dafir, 1992):

$$S^d = [(N, P, K,\cdots\cdots, R_d): N > 0, P > 0, K > 0,\cdots Rd > 0, N + P + K +\cdots+ R_d =100]$$

Where 100 is the dry matter concentration (%); N, P, K,. ...are nutrient proportions (%); and R_d is the filling value computed as:

$R_d = 100 - (N + P + K +....)$.

A geometric mean (G) computed as:

$G = (N \times P \times K \times\cdots\times R_d)^{1/d+1}$ is used to divide nutrient proportions to derive row-centred log ratios as follows:

$V_N = \ln |N / G|, V_P = \ln |P / G|, VK = \ln |K / G|, \cdots, V_{Rd} = \ln |R_d / G|$

and

$V_N + V_P + V_K +\cdots, + V_{Rd} = 0$

Where V_x is the CND row-centred log ratio expression for nutrient X.

From the high bunch weight subpopulation, CND norms, which are the means and standard deviations of row-centred log ratios, denoted as V^*_N, V^*_P, V^*_K, ... V^*_{Rd} and SD^*_N, SD^*_P, SD^*_K, ... SD^*_{Rd}, respectively, were then calculated. The CND indices, denoted as I_N, I_P, I_K, ... I_{Rd} were then calculated from the row-centred log ratios as follows:

$I_N = (V_N - V^*_N) / SD^*_N; I_P = (V_P - V^*_P) / SD^*_P, I_K = (V_K - V^*_K) / SD^*_K, ... I_{Rd} = (V_{Rd} - V^*_{Rd}) / SD^*_{Rd}$

The nutrient imbalance index of a diagnosed specimen, which is its CNDr^2, was computed as: $r^2 = I^2_N + I^2_P + I^2_K +\cdots+ I^2_{Rd}$

As a result of the indices, the values closer to zero indicate the higher probability to obtain a high yield, whereas the negative or positive values indicate the imbalance at both directions, deficiency or surplus, respectively.

For DRIS, norms consist of average and standard deviation of dual ratio between nutrients obtained from a high-yield subpopulation. The coefficients of variation (CVs) of the same data are used as a measure of the relative spread of the yield response curve. The indices represent the mean of all the function values involving the particular nutrient. The function values were added (+) to determine the indices for the nutrient in numerator and were subtracted (+) if the required nutrient index is in the denominator. The indices and nutrient imbalance indices (NII) (the sum of absolute values of separate nutrient indices) were computed for both low and high-yielding subpopulations (Walworth and Sumner, 1987), based on ratios of each nutrient relative to all other nutrients using the equations provided by Walworth and Sumner (1988). If we consider hypothetical nutrients A through N, then:

$$\text{A index} = \frac{f\left(\frac{A}{B}\right) + f\left(\frac{A}{C}\right) + f\left(\frac{A}{D}\right) + \cdots + f\left(\frac{A}{N}\right)}{z}$$

$$\text{B index} = \frac{-f\left(\frac{A}{B}\right) + f\left(\frac{B}{C}\right) + f\left(\frac{B}{D}\right) + \cdots + f\left(\frac{B}{N}\right)}{z}$$

$$\text{N index} = \frac{-f\left(\frac{A}{N}\right) - f\left(\frac{B}{N}\right) - f\left(\frac{C}{N}\right) - \cdots - f\left(\frac{M}{N}\right)}{z}$$

$$f\left(\frac{A}{B}\right) = \left(\frac{A/B}{a/b} - 1\right)\frac{1{,}000}{CV} \text{ if A/B} \geq \text{a/b or}$$

$$f\left(\frac{A}{B}\right) = \left(1 - \frac{a/b}{A/B}\right)\frac{1{,}000}{CV} \text{ if A/B} < \text{a/b}$$

Where A/B denotes the value of the ratio of the two elements in the tissue being diagnosed, z is the number of nutrients under consideration, a/b is the optimum value or norm for that ratio, and CV is the coefficient of variation associated with that norm. DRIS indices can range from negative to positive depending on whether a nutrient is deficient or excessive relative to other nutrients considered. The more negative an index is the more imbalance or deficient that nutrient is relative to others (Nziguheba et al., 2009). In this study the indices for N, P, K, Ca, Mg, Cu, Mn and Zn were calculated. For any sample, the nutrient indices sum to zero. The measure of the total nutritional imbalance, the nutrient imbalance index (NII), was calculated using the absolute values of the indices generated for the sample as shown in the examples below:

$$\text{NII} = |\text{IN}| + |\text{IP}| + |\text{IK}| + |\text{ICa}| + |\text{IMg}| + |\text{ICu}| + |\text{IMn}| + |\text{IZn}|$$

The greater the sum, the more the imbalance among nutrients (Snyder and Kretschmer, 1987). The relationships between CND and both DRIS indices were explored using regressions.

2.4 Statistical analysis

Statistical analysis was performed using GenStat (version 16, VSN International Ltd). To assess the effects of inputs on climbing bean yield and leaf nutrient concentrations, a mixed effects linear model was used for data analysis considering sites × fertilizer × manure as fixed factors. Fields (blocks) were nested under sites and included in the model as random factors. Treatment means were compared using the standard error of differences between means (SED) at $P \leq 0.05$ significance level.

3. Results

3.1 Leaf nutrient concentrations

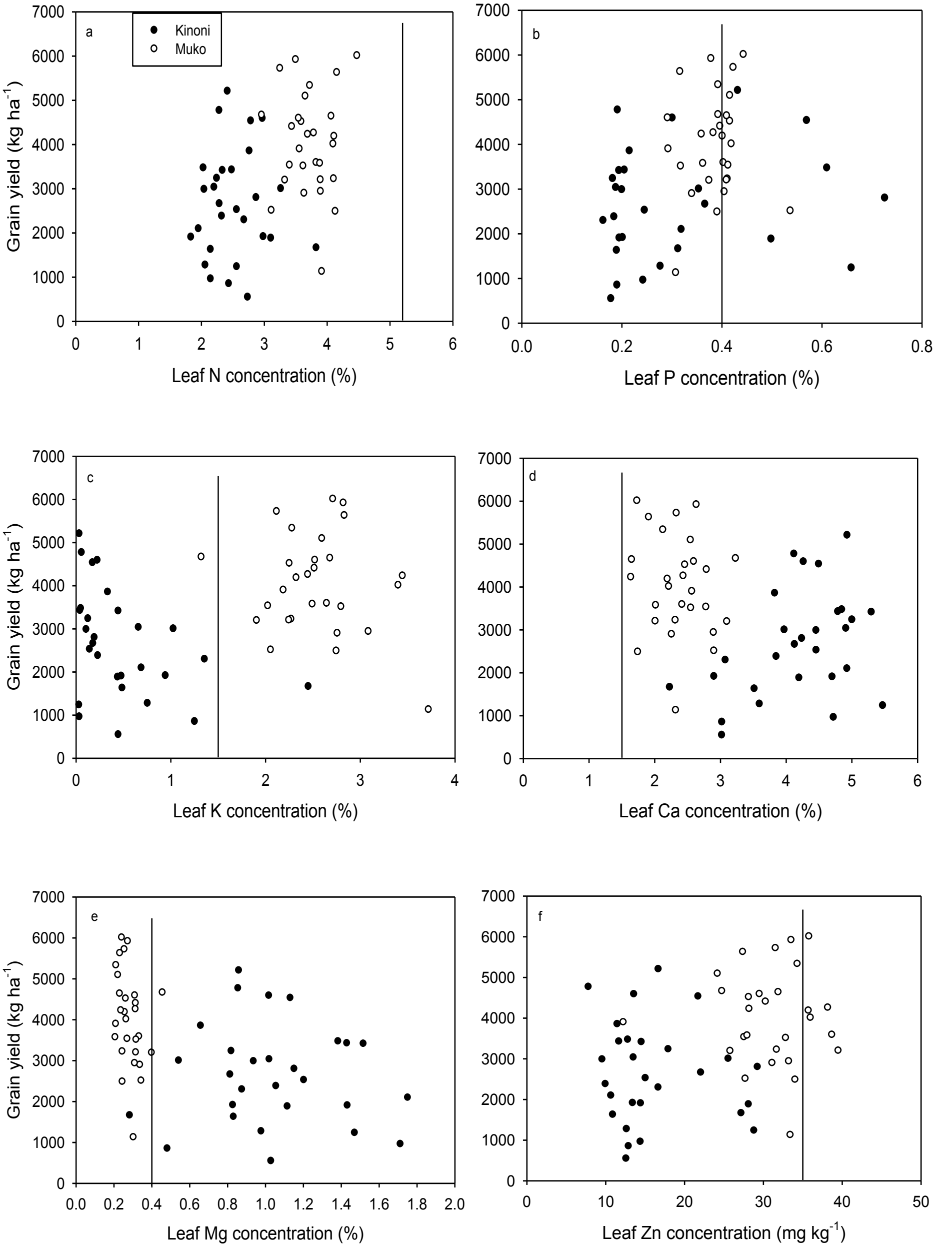
The measured leaf nutrient concentrations for N, P, K, Ca, Mg, Cu, Mn and Zn obtained were highly variable. For instance leaf N concentration ranged from 1.83 to 4.48 % with a mean of 3.13%, leaf P concentration ranged from 0.16 to 0.73% with a mean of 0.35%, leaf K concentration ranged from 0.03 to 3.72% with a mean of 1.51%, leaf Ca concentration ranged from 1.64 to 5.4%) with a mean of 3.27%, leaf Mg concentration ranged from 0.21 to 1.75% with a mean of 0.66%, leaf Cu concentration ranged from 2.19 to 7.82 mg kg^{-1} with a mean of 4.64 mg kg^{-1}, leaf Mn concentration ranged from 33.20 to 211.96 mg kg^{-1} with a mean of 97.39 mg kg^{-1}, and leaf Zn concentration ranged from 7.82 to 39.51 mg kg^{-1} with a mean of 23.60 mg kg^{-1}. Leaf N concentrations obtained at both sites were below the critical deficiency concentrations of 5.2% reported by Reuter and Robinson (1997; Table 2). For P, 36 plots out of 56 had leaf P concentrations below the critical deficiency concentration of 0.4%. For K, 9 plots from Kinoni had values below the reported critical deficiency concentration of 1.5%. Leaf Ca concentrations from all the plots were above the critical deficiency concentration of 1.5%. For Mg, the leaf Mg concentrations were below the critical deficiency concentration of 0.4% in 27 out of 56 plots, of which 26 plots were from the Muko site. Leaf Cu concentrations were below the critical deficiency concentration of 5.0 mg kg^{-1} in 30 plots out of 56 plots, of which 11 plots were from Kinoni and 19 plots from Muko sites. For Mn, only 3 plots from the Muko site had leaf Mn concentrations below the critical deficiency concentration of 50 mg kg^{-1}. All the plots in Kinoni had leaf Zn concentration below the critical deficiency concentration of 35 mg kg^{-1}, and only 6 plots from Muko had leaf Zn concentration above the critical deficiency.

Statistical analysis showed significant differences ($p < 0.001$) in leaf nutrient concentrations between the two sites for all the nutrients except P. However, no significant differences were observed for fertilizer and manure treatments in increasing the leaf nutrient concentrations of the measured parameters across sites. There were no interactions between treatments.

3.2 Relationship between grain yield and leaf nutrient concentrations

Climbing bean leaf nutrient concentrations for N, P, K, Ca, Mg, Cu, Mn and Zn were plotted against the grain yield (Fig. 5.1) to assess the relationships between plant growth and nutrient concentrations of foliar tissue. Leaf N concentrations in all plots were below the reported critical deficiency concentrations of 5.2% (Reuter and Robinson, 1997). A positive relationship between grain yield and leaf nutrient concentrations was observed. Climbing bean grain yield increased with increasing leaf

N concentrations (Fig. 5.1a). The relationship between leaf P concentration and grain yield showed similar pattern as leaf N (Fig. 5.1b). The relationship between leaf K concentration and grain yield showed separate trends between the two sites, with plots in Kinoni showing a C-shaped curve with larger grain yield at small leaf K concentrations, while in the Muko plots there was a more broad nutrient range (Fig.5.1c). Negative relationships were observed between grain yield and leaf Ca concentration (Fig. 5.1d), leaf Mg concentration (Fig.5.1e), leaf Zn concentration (Fig. 5.1f), leaf Mn concentration (Fig. 5.1h) and the sites clearly separated from each other. Grain yield also increased with increasing Cu, Mn and Zn leaf concentrations then decreased.


a
Kinoni
Muko
Grain yield (kg ha^{-1})
Leaf N concentration (%)
b
Leaf P concentration (%)
c
Leaf K concentration (%)
d
Leaf Ca concentration (%)
e
Leaf Mg concentration (%)
f
Leaf Zn concentration (mg kg^{-1})
0
1000
2000
3000
4000
5000
6000
7000

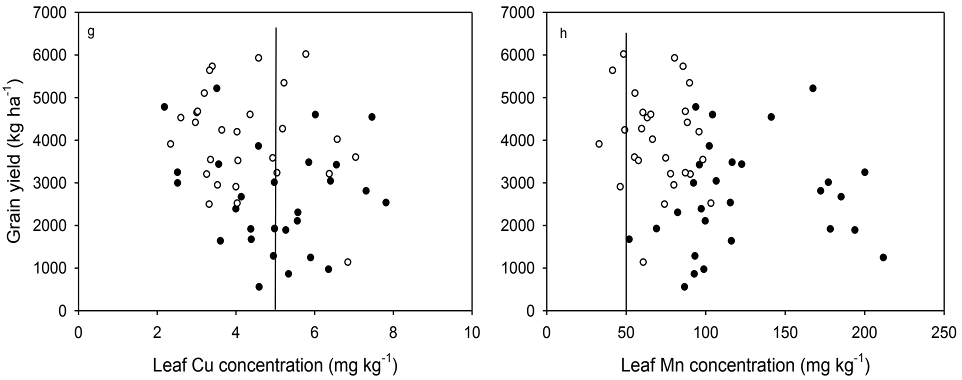


Fig. 5.1 Relationship between climbing bean leaf nutrient concentrations and grain yield (kg ha^{-1}) at Kinoni and Muko sites. Vertical lines represent the critical nutrient concentration level for bean production, below which deficiencies can be assumed according to Reuter and Robinson (1997).

3.3 Grain yield, leaf nutrient concentrations and DRIS indices

Grain yield differed between the two sites and increased ($p < 0.001$) with inputs application (Table 5.2). The leaf nutrient concentrations differed significantly ($p \leq 0.05$) between the two subpopulations except for P and Cu (Table 5.1). The high-yielding subpopulation had significantly higher N, K and Zn ($p \leq 0.05$) and lower Ca, Mg and Mn ($p \leq 0.05$) compared with the low-yielding subpopulation. The mean concentration for P% was slightly higher in high-yielding subpopulation and leaf concentration in Cu mg kg^{-1} was slightly higher in low-yield subpopulation but with no significant differences between the two subpopulations. Leaf nutrient concentrations also differed significantly between the two sites ($p < 0.001$). Application of NPK and manure did not consistently increase leaf nutrient concentrations at both sites. DRIS indices also differed between the two sites ($p < 0.001$), and varied with inputs application (Table 5.2).

Table 5.1 Comparison of leaf nutrient concentrations between low (n=37) and high (n=19)-yielding populations

	Low-yielding subpopulation		High-yielding subpopulation		
Nutrients	Mean	SD	Mean	SD	Pvalue
N (%)	2.92	0.71	3.50	0.58	0.01
P (%)	0.33	0.14	0.39	0.08	n.s
K (%)	1.24	1.09	2.04	1.08	0.04
Ca (%)	3.54	1.07	2.75	0.97	0.02
Mg (%)	0.79	0.47	0.42	0.29	0.01
Cu (mg kg^{-1})	4.80	1.43	4.33	1.35	n.s
Mn (mg kg^{-1})	112.01	43.71	68.93	18.90	0.001
Zn (mg kg^{-1})	19.75	8.27	31.09	6.30	0.001

Table 5.2 Average grain yields, nutrient concentrations in leaf tissue and DRIS indices of climbing bean at Kinoni and Muko sites

			Leaf nutrient concentration								DRIS Indices							
			(%)					($mg\ kg^{-1}$)										
Sites / Fertilizer ($kg\ ha^{-1}$)	Manure ($kg\ ha^{-1}$)	Yield ($kg\ ha^{-1}$)	N	P	K	Ca	Mg	Cu	Mn	Zn	N	P	K	Ca	Mg	Cu	Mn	Zn
Kinoni																		
-NPK	0	1490	2.43	0.30	0.48	4.29	1.18	5.72	104.98	13.17	-7.20	-6.00	-12.80	5.80	20.30	19.27	24.10	-46.33
-NPK	5	2800	2.64	0.23	0.44	4.07	0.90	4.58	117.76	19.92	-0.80	-8.80	-13.60	7.30	16.00	5.85	28.98	-28.12
+ NPK	0	2415	2.48	0.34	0.69	4.13	1.06	4.79	105.82	17.36	-8.40	-2.70	-11.50	4.90	17.70	7.10	21.38	-32.72
+ NPK	5	3893	2.50	0.35	0.30	4.23	1.03	4.32	96.71	28.91	-5.20	-4.10	-14.60	5.90	18.10	-2.98	15.80	-9.08
SEDfertilizer		146.8	ns	ns	ns	ns	ns	n.s	n.s	2.48	ns	1.7	ns	ns	ns	4.02	n.s	5.39
SEDmanure		146.8	ns	ns	ns	ns	ns	n.s	n.s	2.48	1.7	ns	ns	ns	ns	4.02	n.s	5.39
Muko																		
-NPK	0	2628	3.73	0.40	2.65	2.51	0.31	4.98	131.09	19.31	0.50	-1.10	4.40	-1.20	-2.60	4.330	28.37	-24.53
-NPK	5	4373	3.65	0.39	2.32	2.48	0.31	4.01	70.27	29.04	0.80	0.10	2.00	-0.70	-2.20	-1.480	0.77	-0.46
+NPK	0	3710	3.74	0.37	2.66	2.25	0.26	4.37	79.9	29.31	2.10	-1.10	6.10	-1.70	-5.40	0.600	6.28	-2.84
+NPK	5	5425	3.79	0.39	2.54	2.25	0.25	4.35	72.62	31.76	1.90	1.50	4.70	-1.70	-6.40	-0.480	1.75	1.66
SEDfertilizer		182.3	ns	ns	ns	ns	ns	n.s	10.82	3.05	ns	ns	ns	ns	0.90	n.s	4.25	4.51
SEDmanure		182.3	ns	ns	ns	ns	ns	n.s	10.82	n.s	ns	ns	ns	ns	ns	n.s	4.25	4.51
Adequate ranges[a]			5.2-5.4	0.4-0.6	1.5-3.5	1.5-2.5	0.4-0.8	5.0-15	50-400	35-100								

ns: not significant at $p \leq 0.05$. [a]: Adequate ranges for bean leaf nutrient concentrations (Reuter and Robinson, 1997)

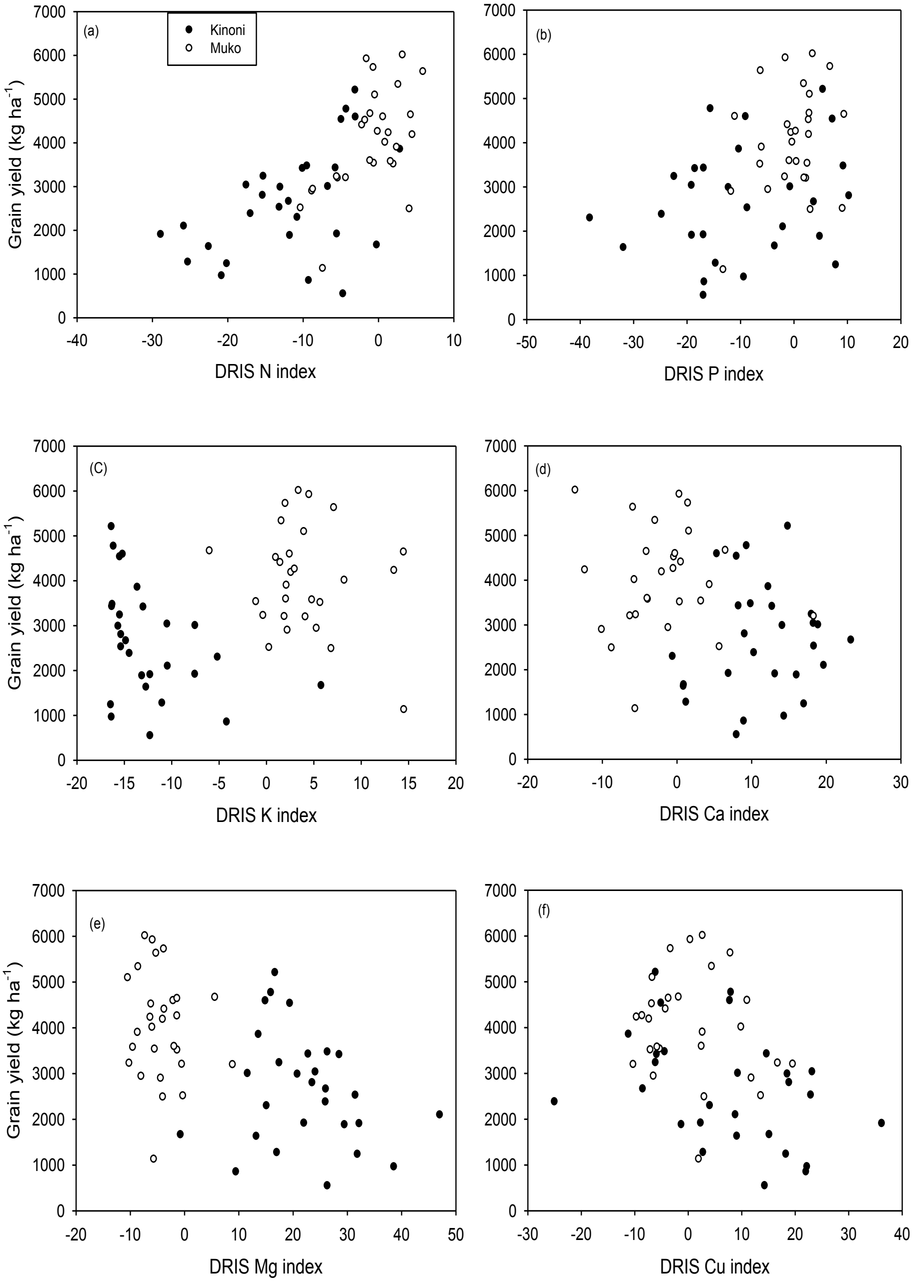
The means and CVs of the selected nutrient ratios, for high-yielding subpopulations (DRIS norms) are presented in Table 3. The variances of the nutrient ratios of the low and high-yielding subpopulations differed significantly ($p \leq 0.05$) except for N/P, K/P, Zn/P, Ca/Cu, Mn/Ca and Mn/Mg. These norms were used to calculate DRIS nutrient indices and Nutrient Imbalance Indices.

Looking at the DRIS indices and obtained grain yields, a positive relationship was found between N-index and grain yield (Fig. 5.2a). A similar but weaker relationship was found for the P-index (Fig. 5.2b). Negative relationships were found for K, Mg, Cu and Mn indices, where grain yield decreased with increasing index values (Fig. 5.2c, e, f, g). Those nutrients except K were not applied as fertilizers in the trials. For Ca and Zn, no clear relationship with grain yield was found. However, at Muko higher grain yield was observed at higher Zn index (Fig. 5.2h).

Table 5.3 Means and coefficient of variation-CV (%) of nutrient ratios, for low and high-yielding populations

	Low-yielding subpopulation		High yielding subpopulation[a]		
Ratios	Mean	CV (%)	Mean	CV (%)	Pvalue
N/P	10.05	30.53	9.36	21.02	ns
K/N	0.37	73.98	0.55	50.86	0.031
N/Ca	0.98	55.21	1.47	41.93	0.003
Mg/N	0.32	74.81	0.14	89.67	0.003
Cu/N	1.76	43.43	1.27	34.77	0.014
Mn/N	0.002	50.27	0.004	29.93	0.001
Zn/N	0.0007	46.53	0.0009	24.84	0.019
K/P	3.91	79.95	5.33	54.97	ns
P/Ca	0.10	52.34	0.16	34.25	0.001
P/Mg	0.68	79.41	1.25	42.17	0.001
Cu/P	0.0017	50.52	0.0012	48.15	0.024
Mn/P	0.042	64.22	0.019	38.68	0.001
Zn/P	0.007	55.65	0.008	32.60	n.s
K/Ca	0.48	103.76	0.94	62.73	0.005
K/Mg	3.70	115.47	7.82	59.43	0.002
K/Cu	2860.58	94.82	5252.42	63.10	0.006
K/Mn	126.23	102.43	314.80	64.88	0.001
K/Zn	690.29	95.39	709.41	68.22	n.s
Ca/Mg	5.78	42.66	7.73	25.16	0.005
Ca/Cu	8096.58	43.75	6843.06	40.66	n.s
Mn/Ca	0.003	49.74	0.003	42.01	n.s
Ca/Zn	2205.94	54.53	923.53	38.56	0.001
Mg/Cu	1738.05	61.25	1016.35	66.87	0.011
Mn/Mg	0.021	72.86	0.023	49.30	n.s
Mg/Zn	496.92	74.95	139.08	66.71	0.001
Cu/Mn	0.05	43.34	0.07	39.35	0.006
Cu/Zn	0.29	45.40	0.14	20.63	0.001
Mn/Zn	6.71	46.39	2.28	28.69	0.001

[a] The means and CV(%) of ratios for high yielding subpopulation are the DRIS norms


(a)
● Kinoni
○ Muko
Grain yield (kg ha^{-1})
7000
6000
5000
4000
3000
2000
1000
0
-40
-30
-20
-10
0
10
DRIS N index
(b)
-50
-40
-30
-20
-10
0
10
20
DRIS P index
(C)
-20
-15
-10
-5
0
5
10
15
20
DRIS K index
(d)
-20
-10
0
10
20
30
DRIS Ca index
(e)
-20
-10
0
10
20
30
40
50
DRIS Mg index
(f)
-30
-20
-10
0
10
20
30
40
DRIS Cu index

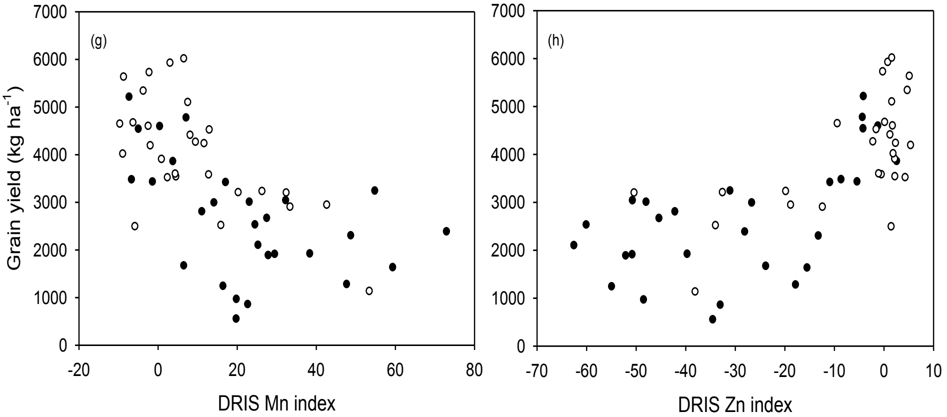


Fig. 5.2 Climbing bean grain yield as a function of calculated DRIS indices for the nutrients N, P, K, Ca, Mg, Cu, Mn and Zn.

3. 4 CND norms

The CND norms, which are the means and standard deviations of V_N, V_P, V_K, V_{Ca}, V_{Mg}, V_{Cu}, V_{Mn} and V_{Zn}, for the high-yielding subpopulation, are presented in Table 5.4. The variances of the low and high-yielding subpopulations differed significantly ($p \leq 0.05$) for V_N, V_P, V_{Ca} V_{Mg}, V_{Mn} and V_{Zn}. The variances for other nutrients did not differ significantly ($p \leq 0.05$) between the two subpopulations. These norms were used to estimate nutrient indices for N, P, K, Ca, Mg, Cu, Mn, Zn and Rd and CND r^2 values. In Kinoni, obtained indices of -1.75, -1.04, -1.11 and -2.10 for N, P, K and Zn respectively suggest that those nutrients were deficient, while Ca (1.35), Mg (1.78), Cu (0.73) and Mn (1.90) were sufficient. In Muko, N (0.14), K (0.49) and Mn (0.62) were sufficient while P (-0.19), Ca (-0.41), Mg (-0.46), Cu (-0.13) and Zn (-1.07) were deficient. CNDr^2 was 36.01 and 10.46 for Kinoni and Muko respectively.

Table 5.4 Means and standard deviation (SD) of row-centred log-ratios for low and high-yielding populations

	Low-yielding subpopulation		High-yielding subpopulation[a]		
Row-centred log- ratios	Mean	SD	Mean	SD	Pvalue
V_N	2.59	0.22	2.76	0.14	0.003
V_P	0.34	0.39	0.55	0.23	0.040
V_K	1.09	1.30	1.75	1.30	ns
V_{Ca}	2.76	0.40	2.48	0.40	0.017
V_{Mg}	1.10	0.75	0.47	0.62	0.003
V_{cu}	-6.14	0.31	-6.27	0.28	n.s
V_{Mn}	-3.02	0.34	-3.50	0.25	0.001
V_{Zn}	-4.77	0.41	-4.28	0.21	0.001
V_{Rd}	6.06	0.13	6.03	0.14	ns

[a]The means and SD for high- yielding subpopulation are the Compositional Nutrient Diagnosis (CND) norms for d = 8 nutrients

3.5 DRIS and CND indices

The DRIS and CND indices obtained were both positive and negative. A strong relationship between DRIS and CND indices was observed (Fig. 5.3). For both DRIS and CND, the index values for Ca, Mg, Cu and Mn were positive at Kinoni site while N, P, K and Zn were negative at this site (Fig. 5.4a, b). At Muko site, K and Mn were positive for both DRIS and CND, N was positive for CND while P, Ca, Mg, Cu and Zn were negative for both DRIS and CND (Fig 5.4c, d). The positive indices indicate that these nutrients are sufficient while the negative indices are deficient in the leaf tissue. At Kinoni the order of nutrients limitation is as follow: Zn < N < K <P, while at Muko Zn < Mg < Ca < P <N. At both sites the results suggest that Zn was the most limiting.

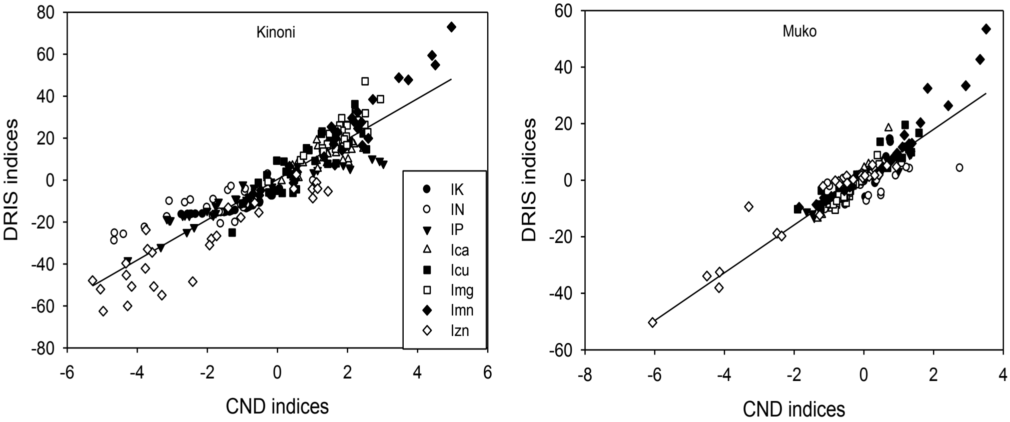


Fig. 5.3 Relationship between CND and DRIS nutrient indices for N, P, K, Ca, Mg, Cu, Mn and Zn. The continuous lines are regression lines for CND and DRIS indices at Kinoni (R^2 = 0.85) and Muko (R^2 = 0.84).

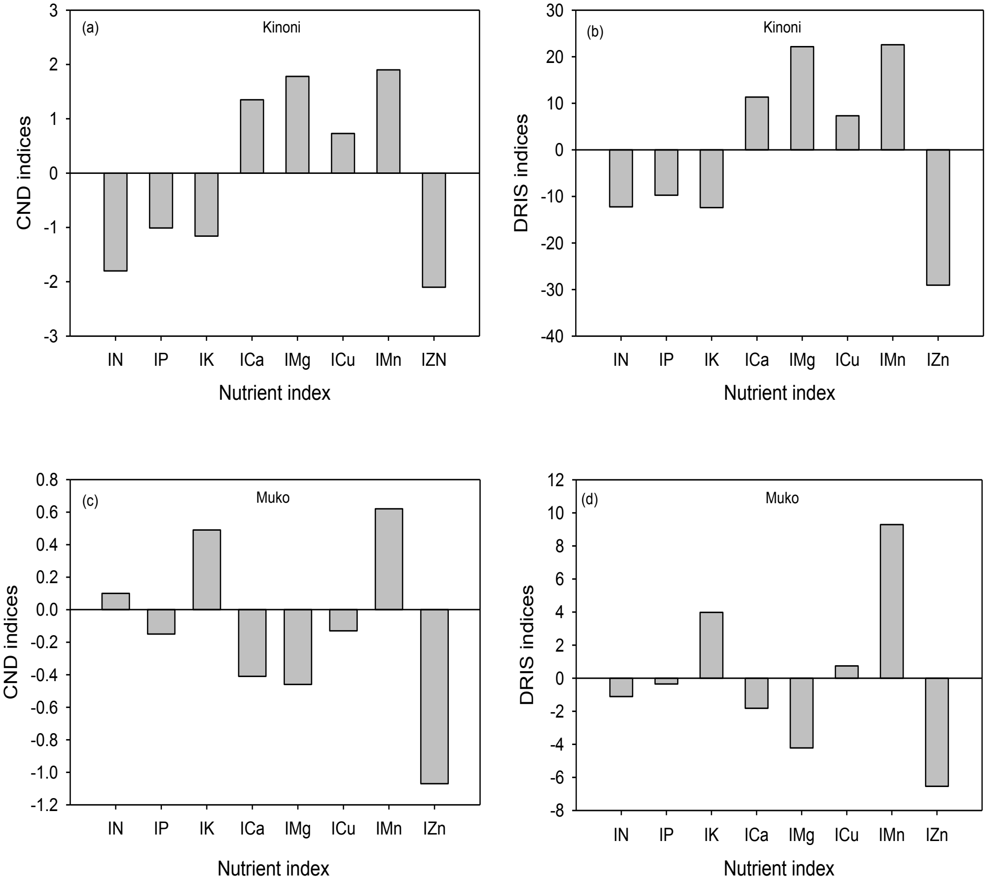


Fig. 5.4 CND (a, c) and DRIS (b, d) nutrient index values for leaf tissue at Kinoni (a, b) and Muko (c, d) sites.

4. Discussions

4.1 Relationships between Nutrient Concentrations and grain yield

The results from this study showed significantly higher N, K and Zn in high-yield subpopulation compared with low-yield subpopulation (Table 5.1). The leaf concentration in P also was higher in high yield subpopulation though there was no significant difference. Other nutrients were higher in low-yield subpopulation compared with high-yield subpopulation. The higher leaf N concentration in the high yield subpopulation compared with the low yield subpopulation has been reported elsewhere (Walworth and Sumner, 1987; Wairegi and Van Asten, 2011). The significantly higher Ca and Mg ($P < 0.05$) in the low-yield subpopulation compared with the high-yield subpopulation may be due to a negative interaction between N, P, K, Ca and Mg in the plant. These differences in nutrient concentrations, between the two subpopulations, justify the use of the high-yield subpopulation to derive norms instead of using all the observations. Using the high yield subpopulation to derive norms assumes that the distribution of observations at low yields is skewed (Walworth and Sumner, 1987, Wairegi and Van Asten, 2011).

Before establishing the trials, soil samples were collected and analyzed. The results are presented in Chapter 4 Section 3.1. The results showed very low to very high nutrients at both sites. Only Ca and Mg were sufficient. Responses to inputs were observed in both sites ($P<0.001$), however, there were no clear relationships between response to inputs and soil parameters. In Kinoni, some weak responses to manure and NPK application were observed in plots where soil available P and exchangeable K were limiting. However, this was not the case in Muko, suggesting that P and K were not limiting at this site. In neither of the sites was a clear relationship observed between soil N and response to inputs, suggesting that N was not limiting either. However, the DRIS and CND indices showed that N and P were limiting at both sites and K was limiting in Kinoni, while Ca and Mg were limiting in Muko. The soil results confirm the need for leaf nutrient analysis to compliment soil chemical analysis in determining which nutrients are limiting.

Addition of manure and NPK fertilizers did not consistently alleviate N, P and K deficiency. Slight increases in leaf nutrient concentrations in plots that received manure and NPK fertilizer were observed though there was no significant difference except for Zn (Kinoni) and Mn (Muko). The lack of relationship between soil parameters and response to inputs in addition to the negative indices for N, P and K may indicate low efficiency of applied fertilizer, and that climbing bean was not able to take up in sufficient quantities even nutrients that were highly available in soil, suggesting an interaction issue that hinders their uptake. Similarly, Nziguheba et al.

(2009) observed negative N indices in treatments that received N in Shika site in Western Kenya, and concluded that it may be an indication that N was becoming imbalanced relative to other nutrients. In another experiment, they reported that when the rate of P application was increased to 40 kg P ha^{-1}, the trend was reversed, P indices becoming positive in most of the fields, whereas more than 50% of the fields had negative P indices when the rate was reduced to 20 kg ha^{-1}. Repeated cultivation of the tiny fields in Northern Rwanda with little inputs addition may have contributed to the negative indices observed regardless of manure and NPK fertilizer applied in the trials. This supports observations by Nziguheba et al. (2009) working on maize in West African Savanna in a long term experiment under 20 years of continuous cultivation. In the context of smallholder farmers growing climbing beans in Northern Rwanda, further increase of the application rates may not be economically feasible. Soil fertility replenishment may target the use of micronutrients in addition to N, P and K, and development of site-specific fertilizer recommendations instead of blanket recommendations targeting deficient nutrients.

In addition to the soil N taken by the plants, nitrogen fixation by the legumes contributes to the leaf N concentration. Deficiencies in essential plant nutrients are able to affect nodule formation and the total amount of N_2-fixed (Giller, 2001). Improving nitrogen fixation in climbing bean may also contribute to the residual N and minimize additional mineral N fertilizer.

4.2 CND and DRIS Norms

In this study, DRIS and CND gave similar results matching observations by Parent et al. (1993) working on tomatoes and Parent et al. (1994a) working on potatoes. Although the order of nutrients limitation slightly differed between DRIS and CND, both approaches identified Zn as the most deficient and P as the least deficient. Close relationship between CND and DRIS indices (Fig. 5.3) suggests that differences between both approaches are minimal. Similar observations have been reported by other researchers (e.g. on carrot (Parent et al., 1994), sweet corn (Khiari et al., 2001b) and on banana (Wairegi and van Asten, 2011)). Wortmann et al. (1992) estimated norms for dry beans collected from various countries and reported N, P to be adequate and K to be the most limiting, and reported that the order of nutrients requirements varies less due to the differences in the plant age. They recommended estimation of norms for beans for specific conditions.

5. Conclusion

DRIS and CND indices were closely related, and both approaches were useful in identifying nutrient limitations to climbing bean in Northern Rwanda. We observed deficiencies of Zn, N, K and P in Kinoni, and Zn, Mg, Ca, P and N in Muko despite the addition of manure and NPK fertilizers in the experimental trials. Both DRIS and CND indices suggested that Zn was the most limiting at both sites. Improvement of soil fertility in Northern Rwanda may target the use of micronutrients in addition to N, P and K, and development of site-specific fertilizer recommendations instead of blanket recommendations may help farmers to increase their farm productivity. Both DRIS and CND norms derived in this study proved to be reliable for diagnosing nutrient imbalances in climbing bean in Northern Rwanda, and overall differences between the DRIS and CND approaches were small.

Considering the capabilities of both DRIS and CND in detecting limiting nutrients in climbing bean, it is necessary to conduct similar trials in a wide range of environment and include micronutrients in addition to N, P and K. Experiments to test the identified nutrients indicated to be limiting for climbing bean production are necessary as well to confirm whether they are truly limiting in the field.

Acknowledgement

We thank the Bill & Melinda Gates Foundation for funding through a grant to Wageningen University to support the project N2Africa: Putting Nitrogen Fixation to Work for Smallholder Farmers in Africa (www.N2Africa.org). Jiska van Vliet is acknowledged for the good discussions on the application of DRIS and CND. We are also grateful to the participating farmers for providing the land and their enthusiastic collaboration with the trials.

Chapter 6

General discussion

6.1 General findings

The main findings of this thesis are of great relevance to the smallholder farmers of Rwanda. They clearly demonstrate that it is feasible to double or even triple the yields of beans, soybean and maize if inputs are used. Chapter 2 assessed how the use of inoculum combined with manure and P fertilizer on common bean and soybean enhances not only the yields of the legume but also the productivity of the subsequent maize crop. For instance, inoculation alone increased the grain yield by 0.6 and 0.4 t ha^{-1} for common bean and soybean respectively compared with unamended plots. This effect of inoculation on common bean is greater than the 0.3 t ha^{-1} reported in Ethiopia and 0.4 t ha^{-1} reported in Tanzania (Thuijsman et al., 2018). On average, addition of P fertilizer increased the grain yield by 0.4 and 0.2 t ha^{-1} for common bean and soybean respectively compared with the control. Increased yield due to P addition observed for common bean is similar to the one reported in Ethiopia (Thuijsman et al., 2018). When P was combined with inoculation, common bean grain yield increased by 0.6 t ha^{-1} which is similar to the increase reported in Ethiopia. Inoculation combined with P fertilizer increased soybean grain yield by 0.6 t ha^{-1} which shows a good response of P to inoculation since P alone increased the soybean yield by 0.2 t ha^{-1}. When P fertilizer was combined with manure at 5 and 10 t ha^{-1}, common bean yield increased by 0.9 and 1.4 t ha^{-1} respectively. Addition of manure alone significantly increased the yield of both legumes. For example, manure added at 5 and 10 t ha^{-1} increased the grain yield of common bean by 0.9 and 1 t ha^{-1} respectively, while soybean yield increased by 0.6 and 0.8 t ha^{-1} respectively. Inoculation combined with manure at 5 and 10 t ha^{-1} increased the grain yield of common bean by 1.2 and 1.6 t ha^{-1} respectively, and soybean yield increased by 0.9 and 0.8 t ha^{-1} with 5 and 10 t ha^{-1} respectively. Inoculation combined with P and manure at 5 and 10 t ha^{-1} increased the grain yield of common bean by 1.7 and 2.0 t ha^{-1} respectively, while soybean yield increased by 1.4 and 1.6 t ha^{-1} respectively. The results also show that there were no significant differences between the two rates of manure in increasing the yield of both legumes.

Application of inputs to the legumes also resulted in enhanced grain yield, N and P uptake of the subsequent maize than that of maize after unamended legumes. For instance, maize yields ranged from 0.8 t ha^{-1} in control plots to 6.5 t ha^{-1} in treatments that previously received P with inoculation and manure added for maize grown after common bean and from 1.9 t ha^{-1} in control plots to 5.3 t ha^{-1} for maize grown after soybean. When inoculation had been applied to the previous common bean, average maize grain yield increased by 1.3 t ha^{-1} over maize yield after untreated common bean, and this response increased to 1.6 t ha^{-1} when manure and P fertilizer were added to inoculation. For maize grown after soybean, there was strong residual effect from inputs applied to soybean. In the case of maize grain yield grown after soybean, inoculation response to application of manure and P fertilizer increased from 0.8 to 2.4 t ha^{-1}, and manure response to application of P fertilizer and inoculation increased from 0.2 to 2.5 t ha^{-1}. We observed that the choice of the legumes to use in rotations is a key determinant in legume-cereal rotation sequence in the smallholder faming systems relying on rain fed agriculture. In the drier agro-ecology of Bugesera, maize failed to yield when grown after soybean due to the long duration of soybean that delayed the planting of maize compared with common bean. Chapter 3 evaluated the effects of manure on rhizobial survival in soil and the need to re-inoculate bean and soybean in previously inoculated fields in Bugesera and Kamonyi Districs. The trials were established in Season 3 after maize (Season 2) and common bean and soybean (Season1) in the same fields with slight modifications (Details are in Section 2.1 of the Chapter 3). Grain yields in Season 3 varied from 1.4 t ha^{-1} to 1.6 t ha^{-1} in uninoculated, unmanured plots to 3.8 t ha^{-1} for bean and 2.8 t ha^{-1} for soybean in plots that had been inoculated receiving 10 t manure ha^{-1} in season 1. Re-inoculation in Season 3 did not significantly increase the yields of bean and soybean in plots that had been inoculated in Season 1 across the manure rates. For instance, in Bugesera yield of common bean was 2.8 t ha^{-1} in previously inoculated plot and was 3.0 t ha^{-1} when re-inoculated in season 3. For soybean yield of the previously inoculated (Season 1) was 2.3 t ha^{-1} and 2.4 t ha^{-1} after re-inoculation in Season 3, and in Kamonyi also soybean yield was 2.3 t ha^{-1} in plot inoculated in Season 1, and 2.4 t ha^{-1} when re-inoculated in Season 3. Application of manure also resulted in the survival of rhizobia in soil. The population of rhizobia generally increased in the rainy seasons, and decreased during the dry season. Inoculation and manure application increased rhizobia populations up to 10^3 cells g^{-1} soil in January (four months after first sampling), and increased further up to 10^4 cells g^{-1} soil during the rainy season of March (six months after first sampling). The rhizobia numbers then decreased in all treatments in the dry season of July (ten months after first sampling) to populations less than 10^1 cells g^{-1} soil. Rhizobia numbers at eighteen months after first sampling during the rainy season of April were again higher ranging from 10^1 to 10^4 cells g^{-1} soil in treatments that had received manure in Season 1. In Chapter 4, we assessed the role of manure and mineral fertilizer on climbing bean yields, N_2-fixation, N and P uptake. We observed large variability in yield among fields, with grain yield ranging from 0.5 t ha^{-1} to 6.0 t ha^{-1}. Averaged across the sites, manure addition at 2 t and 5 t h^{-1} increased climbing bean yield by 1.1 and 1.5 t ha^{-1} respectively, which is greater than 0.9 and 1.0 t ha^{-1} reported in Chapter 2 for bush bean at 5 and 10 t manure ha^{-1}. Application of P fertilizer alone increased climbing bean grain yield by 0.7 t ha^{-1} and is greater than 0.4 t ha^{-1} reported in Chapter 2 for bush bean. When P was combined with 2 t manure ha^{-1} climbing bean yield increased by 1.3t ha^{-1}, while P combined with 5 t manure ha^{-1} climbing bean yield increased by 1.9 t ha^{-1} and it is greater than 0.9 and 1.4 t ha^{-1} obtained for bush bean when P was combined with 5 and 10 t ha^{-1} respectively. Application of NPK alone increased climbing bean yield by 1.0 t ha^{-1}, and when NPK was combined with 2t manure ha^{-1}, the yield increased by 2.1 t ha^{-1} and 2.6 t ha^{-1} when NPK was combined with 5 t manure ha^{-1} and this is greater than the observed increase for bush bean when all inputs are combined. These findings suggest that climbing bean is more responsive to inputs than bush bean. I also observed large variability in the proportion of nitrogen fixed (%Ndfa) by the legumes, yet it remains unclear which specific factors govern the %Ndfa in the smallholder farming systems. Chapter 5 explored the nutrition status of climbing bean in the Northern Province of Rwanda using the compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS). The indices obtained from the two approaches were closely related, and both approaches were useful in identifying potential nutrient limitations to climbing bean in Northern Rwanda. Results suggested that Zn was the most limiting nutrient at both sites. In Kinoni Zn was the most limiting, followed by N, K and P; while in Muko Zn was the most limiting, followed by Mg, Ca, P and N.

6.2 Bean and Soybean production in Rwanda

Legumes are important crops and establish symbiotic relationships with rhizobial bacteria and fix atmospheric nitrogen (Giller, 2001; Herridge et al., 2008), thus contributing to N cycling and improving agricultural productivity. Legumes are multipurpose crops and are consumed either directly as food or in various processed forms or as feed in many farming systems. Legume crops are often grown as rotation crops or intercropped with cereals because of their role in nitrogen fixation. Common bean and soybean are the most widely cultivated legumes and promoted in the Rwandan Government's Crop Intensification Program that was established in 2008 (MINAGRI, 2009). Beans are the primary source of dietary proteins in Rwanda as they supply 28% of national dietary proteins compared to 11% from animal sources (MINAGRI, 2010). Soybean represents also an important source of proteins and its market demand is increasing although it grows slowly compared with beans. Currently there are two bigger companies: Mount Meru SOYCO Ltd and African Improved Foods (AIF). Mount Meru SOYCO Ltd opened in Rwanda in March 2014 and requires a supply of 45,000 MT of soybean annually for edible oil and soy cake production. Africa Improved Foods (AIF) that produce infant fortified foodstuffs consume about

17,000 MT per year is operational since December 2016. The demand for soybean grains by the two companies alone is at 62,000 MT / year, which is almost double the total national annual production. There are also other small soybean processing plants (e.g. COCOF, SOSOMA).

In Rwandan farming systems, where soils are cultivated for a long time with little or no use of fertilizers, beans and soybeans become the major source of N through biological nitrogen fixation and thus contribute to soil fertility. Soybean is grown across the country but the most suitable lands are located in the Eastern, Southern and Western Provinces. Common bean is the main source of dietary protein and its consumption was reported to be on average 38 kg of beans per person per year (CIAT, 2008), and it is known as the "meat" of the Rwandan countryside. In Rwanda both bush and climbing beans are grown, with bush beans dominating in the low and mid-altitude while climbing beans are mostly found in the highlands of the country. However, climbing bean varieties adapted to low and mid-altitude have been developed and adopted as well. Climbing bean is a suitable crop for intensification in more densely populated areas due to its indeterminate growth habit which allows greater production per unit area. The indeterminate growth of climbing beans allows them to provide a continuous supply of green leaves and pods throughout the growing season as well as well as dry grain once pods mature (Sperling and Muyaneza, 1995; Wortmann et al., 2001). Figs 6.1 & 6.2 below show soybean and bean production area (ha), and production amount (t) from 1994 to 2017. Soybean production area is increasing, but grows more slowly compared with beans.

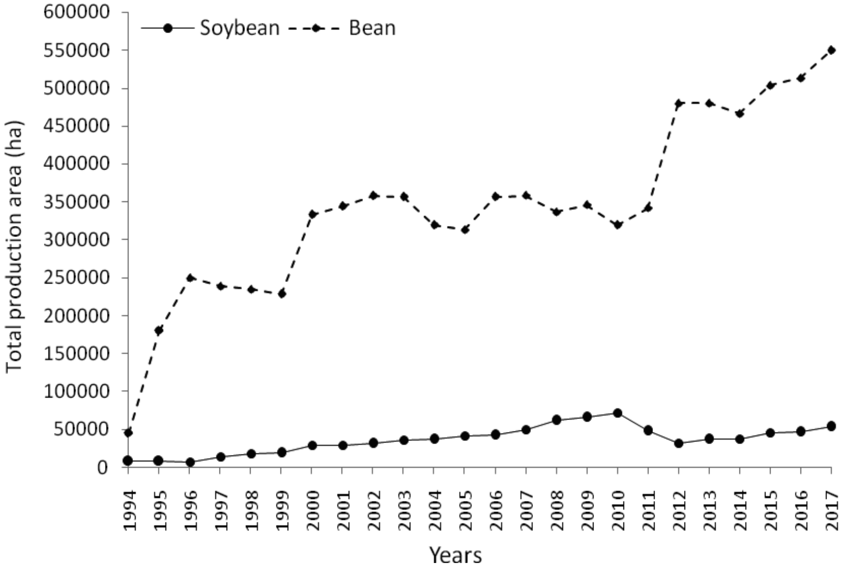


Fig. 6.1 Trend of soybean and bean production area (ha) in Rwanda from 1994 to 2017 (FAOstat, 2019)

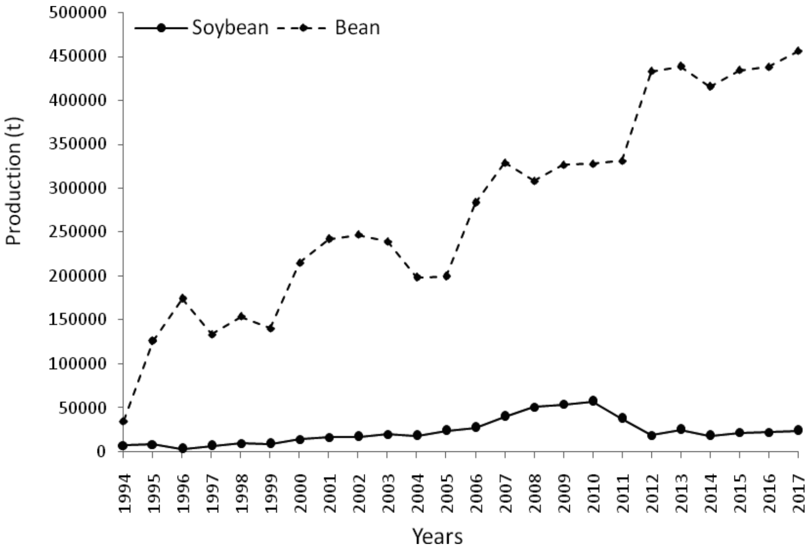


Fig. 6.2 Trend of soybean and bean production (t) in Rwanda from 1994 to 2017 (FAOstat, 2019)

Although soybeans and beans are important legumes in Rwanda for both human consumption and soil fertility improvement, their productivity in the smallholder farming systems remains poor. Integrated soil fertility management (ISFM) through combining fertilizer use with other approaches to soil fertility management is a key option (Vanlauwe et al., 2010). The use of farmyard manure and mineral fertilizer is recognized as an important management practice in increasing crop productivity (Zingore et al., 2008). Beside its role in soil fertility restoration, manure improves the response of crops to other nutrients, and enhances N and P uptake in the legume-cereal rotations (Chapters 2 and 4). It also improves the survival of rhizobia in soil by creating a favourable environment for their growth and multiplication (Chapter 3). Manure is reported to improve the soil carbon, increases the water-holding capacity, neutralizes acidic conditions, and supplies exchangeable bases and other micronutrients (Nzuma et al., 1998; Zengeni et al., 2006; Zingore et al., 2008). However, manure of low quality generally causes immobilization of N in the first season, but can release large amounts of N in subsequent seasons (Nyamangara et al., 2009). This may justify the greater effects of manure after three seasons when common bean and soybean were grown after maize (Chapter 3). Chapter 5 showed the deficient nutrients in climbing bean that could lead to yield limitation.

Inputs used in this study consisted of inoculum, cattle manure at 2, 5 or 10 t ha^{-1}, N at a rate of 60 kg N ha^{-1}, P at 30 kg P ha^{-1}, K at 30 kg K ha^{-1} and NPK as a combination of N, P and K. The 10 t manure ha^{-1} treatment is in line with the application rates recommended by the Ministry of Agriculture for food crops (MINAGRI, 2010). The question arises as to which farmers can afford to use 2, 5 or 10 t manure ha^{-1}, or have such large amounts of manure available, is discussed in Chapter 2 Section 4.4 and Section 6.3.1 of this general discussion.

6. 3 Cropping in the smallholder farming systems of Rwanda

6.3.1 Production technologies and farmers typology

In Rwanda, population increase has led to shortage in land availability, land fragmentation and soil fertility decline (Rutunga et al., 1998). Through the government-led Crop Intensification Program (CIP), intensification is promoted as a strategy to increase crop productivity and farmers' incomes (MINAGRI, 2009). In this program, land use consolidation was implemented since 2008 with the basic idea that farmers consolidate their land parcels and cultivate one selected priority crop while keeping ownership of their lands intact (MINAGRI, 2009). Expectations from this policy are expansion of the land area under cultivation of priority crops, increased crop yields, and improved food security among farm households. Before introduction of the land consolidation programme, farmers were cultivating on scattered small fields which could be several km apart. Participating farmers receive subsidized inorganic fertilizers and improved seeds for priority crops selected for a given region. In consolidated lands, identified priority crops are grown in monoculture systems where cereals, mostly maize is cultivated in rotation with legumes. Maize is either rotated with common beans or soybeans. The two legumes are grown for household consumption and for sale. Yet, despite the high consumption of common bean and the expanding market demand of both beans and soybeans, yields achieved by smallholder farmers are poor: only 0.8–1.0 t bean ha^{-1} and 0.8–1.7 t soybean ha^{-1} (FAOSTAT, 2010).

In Chapter 2 of this thesis, I reported strong effects of fertilizer inputs on common bean and soybean yields and residual benefits to the subsequent maize in rotation. The results clearly showed that when inputs are applied to the legumes, corresponding benefits are translated into increased yields of a cereal in rotation. Chapter 4 also stresses the benefits of combined use of inorganic and organic fertilizers on nutrient uptake by climbing bean, N_2-fixation and yields. Assumptions made in Chapter 2 Section 4.4 on cattle manure availability support the findings of Bucagu et al. (2014) who showed that farmers with 0.5 to 2 ha of lands and one or two head of cattle were able to invest organic manure in food crop production. Based on the number of households that own cattle (771,200 households), number of cattle they have (1.33 million cattle) as reported in Chapter 2 Section 4.4, there are on average 1.7 cattle hh^{-1}. The quantity of manure that can be obtained per cattle per year based on the reported quantity of manure (kg DM) per Tropical Livestock Unit (TLU) produced per day of 2.3 kg DM TLU^{-1} d^{-1} (Castellanos-Navarrete et al.,2015) equals 939 kg. So, if each house hold owns 1.7 cattle, the quantity of manure that can be produced is 1427 kg. This suggests that farmers who have 0.2 ha of land and one to two cattle (Bucagu et al., 2014; Klapwijk et al., 2014), can only apply 1 to 2 t manure ha^{-1}. The rates of 5 and 10 t ha^{-1} can be applied by farmers in the wealthier category who have many cattle. The farmers with limited quantity of manure can be advised to apply manure in the planting rows. The land-constrained farmer category (poorest) that has difficulties in getting fodder even to keep one cattle, can hardly invest in manure for crop production as manure is needed in large quantities. A partial budget analysis, taking into account the cost of inputs and yields is performed. Labour cost is not included.

Net benefit = Total revenue (TR) − Total cost (TC)

Total revenue is estimated as the product of grain yield (t ha^{-1}) and grain price (US$ kg^{-1}). Seeds prices are obtained from RAB as prices of certified seeds. Soybean seeds and fertilizers are subsidized and the costs have been recently published (July 1, 2019) by the Ministry of Agriculture and Animal Resources. Beans are not subsidized. Grain prices are the current local market prices at Huye district. TC is the sum of the costs of inputs (seeds, fertilisers and insecticides). Details of unit costs for seeds, fertilizers and insecticides and grain prices are presented in Table 6.1 below.

Table 6.1 Input and grain prices used in estimating total cost (TC) and total revenue (TR) based on average yield from bush bean, soybean and climbing bean experimental trials

Input costs (US$ ha^{-1})	
Soybean Seeds	28.0
Bush bean seeds	46.2
Climbing bean seeds	52.7
Urea	0.5
NPK	0.7
TSP	0.6
DAP	0.6
Manure (2 t ha^{-1})	22.0
Manure (5 t ha^{-1})	54.9
Manure (10 t ha^{-1})	109.9
Insecticide	17.6
Inoculants	3.3
Grain prices (US$ kg^{-1})	
Soybean	0.5
Bush bean	0.5
Climbing bean	0.5

Rwf: Rwandan francs; 1 US$=910 Rwf Checked online on 26th August, 2019

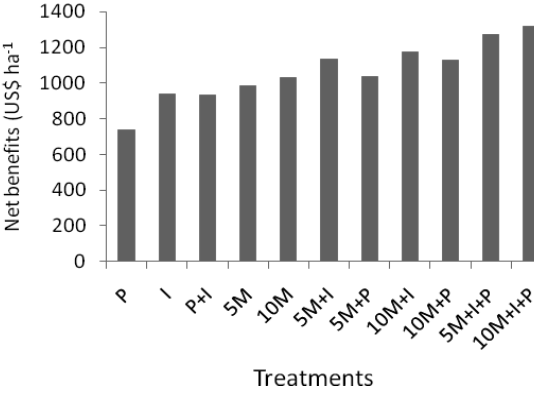


Fig. 6.3 Net benefits of soybean from a partial budgeting analysis as affected by different treatments. The yield data used are averages from Bugesera, Kamonyi and Kayonza (Chapter 2). I: stands for inoculation; 2 M and 5 M stand for 2 and 5 t of manure respectively.

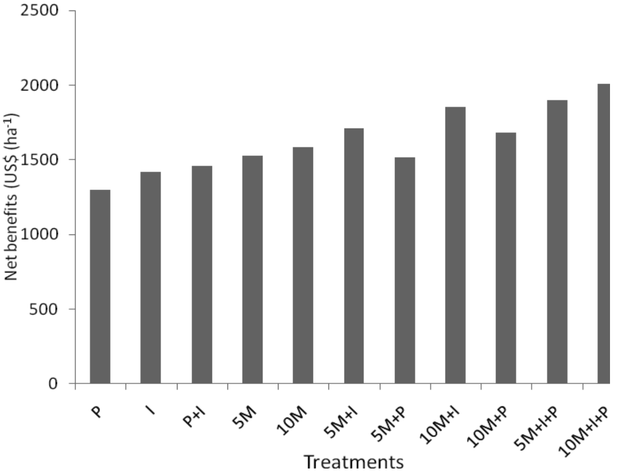


Fig. 6.4 Net benefits of bush bean from a partial budgeting analysis as affected by different treatments. The yield data used are averages from Bugesera, Kamonyi and Kayonza (Chapter 2). I: stands for inoculation; 2 M and 5 M stand for 2 and 5 t of manure respectively.

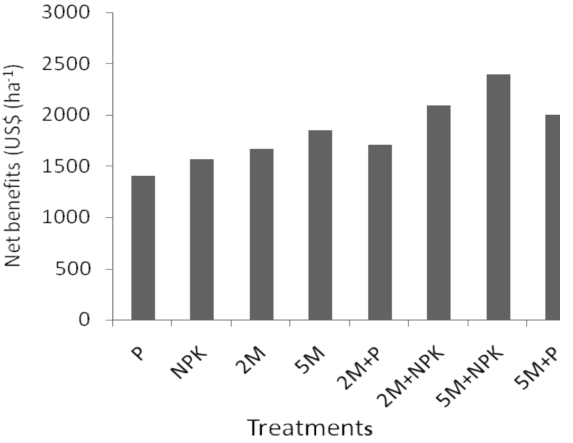


Fig. 6.5 Net benefits of climbing bean from a partial budgeting analysis as affected by different treatments. The yield data used are averages from Kinoni and Muko (Chapter 4). 2 M and 5 M stand for 2 and 5 t of manure respectively.

The above figures show net benefits from growing soybean (Fig. 6.3), bush bean (Fig. 6.4) and climbing bean (Fig. 6.5). From these figures, it is clear that larger net benefits are obtained when inputs of fertilizers are combined due to greater grain yields. Net benefit from inoculation is comparable to that of 5 and 10 t manure ha^{-1} despite the greater yields achieved with the two rates of manure. This is due to the fact that inoculants are cheaper compared with manure. This is very interesting for the resource-constrained farmers (poorest) since they are not able to invest in manure for crop production. The net benefit with 2 t manure ha^{-1} for climbing bean (Fig 6.5) is comparable to that of 5 t, it may be recommended for farmers with one or two cattle who do not have access to higher quantity of manure, and use them in planting rows. In Chapter 2 we reported consistent effects of combined use of manure and inoculation on legumes and to the subsequent maize in rotation, and the results of the Chapter 3 showed strong effects of manure on rhizobial survival in soil for up to three seasons without re-inoculation. These results suggest that manure combined with inoculation may be recommended over mineral fertilizer taking into account that the crop in rotation may be grown without fertilization. This combination of manure and inoculation may be recommended to farmers who are able to invest in manure (those with cattle). Although the high rates of manure resulted in greater yields, observed yields with 5 t ha^{-1} combined with inoculation (Chapter 2) and 2 t ha^{-1} for climbing bean (Chapter 4) indicates that farmers with 0.2 ha of lands that are able to keep one or two cattle, may go for 2 t of manure ha^{-1} combined with inoculation and apply it to the planting rows as it was done in this study.

The poor farmers as defined in the nationwide Ubudehe household typology (Ansoms, 2008) are land constrained. The findings of this study also match with results published in a report by Hengsdijk et al. (2014) in which a large data set from the N2Africa project (www.N2Africa.org) with baseline information of more than 3000 farm households from eight countries in humid and semi-arid SSA including Rwanda was analysed. In this report, three scenarios were made for which food self-sufficiency and land gap calculations were done to assess to what extent food self-sufficiency and economic returns of households are affected by production technologies: i) actual yields of maize and soybean (Baseline Scenario); ii) a doubling of actual maize yields and fertilization of soybean with P fertilizers resulting in higher soybean yields than in the Baseline Scenario (Scenario 2); iii) 80% of simulated water limited maize yields combined with attainable soybean yields using a combination of P fertilizers and inoculants (Scenario 3). Below (Figs. 6.2, 3 & 4), I presented some results from Rwanda (Scenarios 1 & 3) to show how farmers in the land-constrained category are food insecure. What is interesting in this report that matches with our findings

(Chapters 2, 3 & 4) is that when combined inputs are used, yields increased 4 to 5 times from scenario 1 to scenario 3. Greater yields result in less land required for achieving food self-sufficiency of households, and thus more surplus land as well as higher gross returns. From the report, there was a strong impact due to inputs use (Scenario 3) compared with base line scenario. In Scenario 3 most of households are able to achieve food self-sufficiency, except for approximately 10-20% of the households that remained dependent on food obtained off-farm. For instance in Musambira (Kamonyi) farmers that are food self sufficiency increased from 11% to 83%, Musenenyi (Bugesera) from 44% to 82%, Rukara (Kayonza) from 20% to 84%, Burera from 41% to 82% and Gakenke from 43% to 82% for baseline scenario to scenario 3 respectively. Burera and Gakenke are basically known for maize and climbing bean. Our results when inputs are used together match the results of this report that Scenario 3 can be considered as an upper limit of what farmers may be able to achieve in terms of food self-sufficiency with proper management.

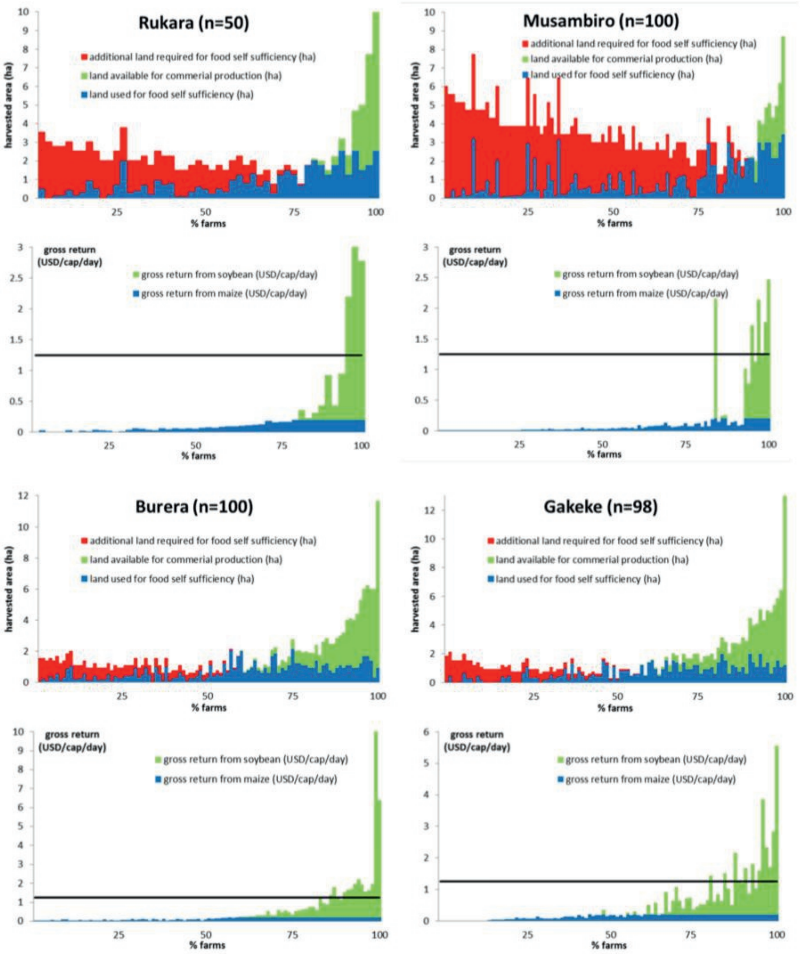


Fig. 6.2 Food self-sufficiency and land gap analysis in the Baseline Scenario based on two cropping seasons for four action sites in Rwanda. For each action site on top the food-self-sufficiency analysis based on current maize yields. For each site at the bottom the associated gross returns from growing soybean (current yields) on surplus land (green area) and the self-consumed maize valued using prevailing market prices (blue area). The black horizontal line indicates the poverty benchmark of 1.25 USD cap^{-1} day^{-1} (Hengsdijk et al., 2014).

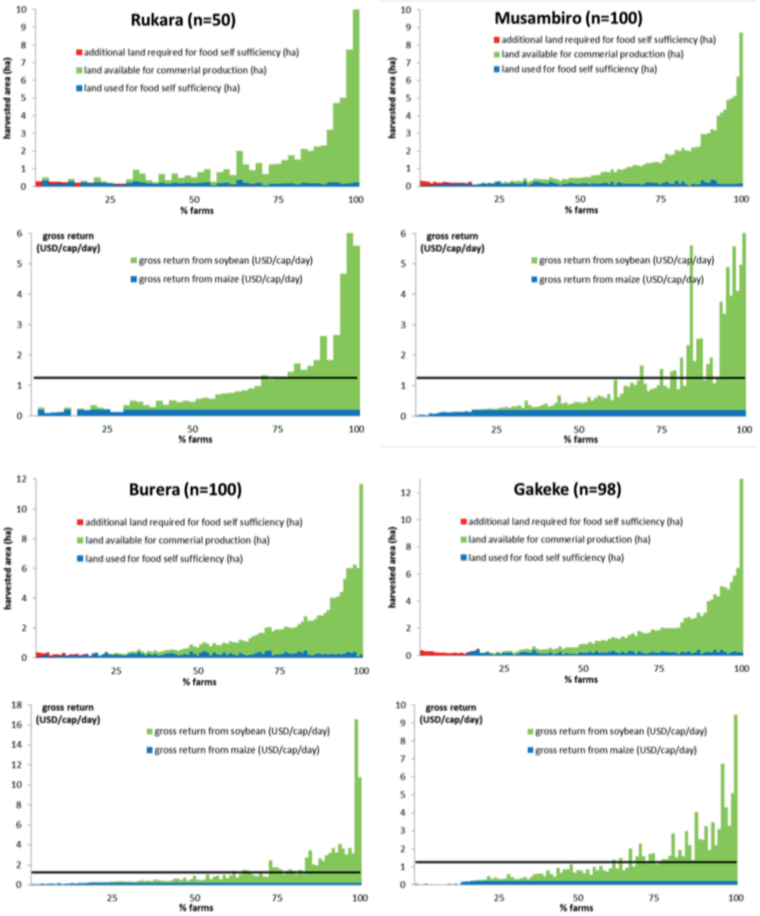


Fig. 6.3 Food self-sufficiency and land gap analysis in scenario 3 based on two cropping seasons for four action sites in Rwanda. For each action site on top the food-self-sufficiency analysis based 80% water-limited maize yields. For each site at the bottom the associated gross returns from growing soybean (P fertilizer + inoculants) on surplus land (green area) and the self-consumed maize valued using prevailing market prices (blue area). The black horizontal line indicates the poverty benchmark of 1.25 USD cap^{-1} day^{-1} (Hengsdijk et al., 2014).

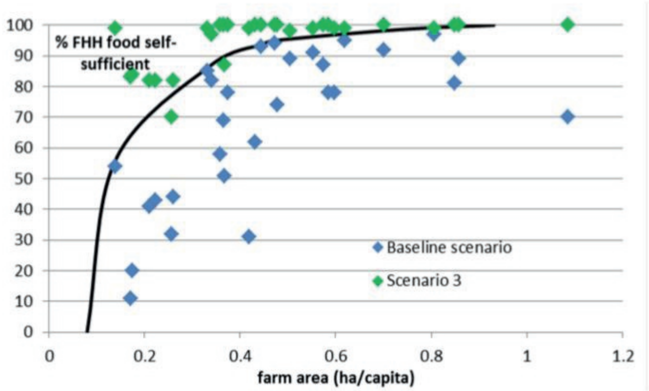


Fig. 6.4 Relationship between area of land holding (farm area) per capita and the percentage of households achieving food self-sufficiency in the Baseline Scenario and Scenario 3 for the 29 N2Africa action sites. The line indicates the upper boundary for the percentage of household that is able to reach food self-sufficiency at a given available land holding per capita (Hengsdijk et al., 2014).

6.3.2 Seasonal variability and its impact on the current production systems

Rwanda has two rainy seasons per year, with the long rain season starting from February to June while the short rain season runs from September to December followed by a short dry season from January to February. Rainfall distribution varies geographically and dry spells occur regularly and can result in severe yield declines or crop failure since agriculture is mainly rain fed. This dependency of agriculture on rainfall leads to strong climate shocks and unpredictable agricultural production from one season to another. The Eastern Province that has more arable lands, drought is a major constraint particularly in Bugesera and Umutara agro-ecological zones. In the conclusion of the Chapter 2, I highlighted the strong effects of rainfall and maturity period of crops used in rotations. It was observed that in the drier agroecology of Bugesera, maize failed to yield any grain when grown after soybean due to the delayed planting of maize since the soybean variety used took longer to mature compared with common bean. In these situations early maturing soybean varieties maybe recommended in soybean-maize rotations.

Legume-cereal rotations have many advantages. Legumes get part of their N requirement through N_2-fixation, thus sparing some of the soil N to the subsequent crops in addition to the residual N from nodule senescence and fallen leaves (Osunde et al., 2003). There are also other rotational effects such as reduction of pests and diseases, mobilization of poorly soluble P and increased mycorrhizal colonization of a subsequent cereal crop leading to enhanced P uptake, improved soil moisture management, effective weed control, better exploitation of moisture and nutrients at different soil depth by differences in rooting pattern of rotating crops (Bagayoko et al., 2000; Franke et al., 2019).

Despite the above benefits from legume-cereal rotations, promoting monoculture in the smallholder farming systems that are fully dependent on rainfall for crop production may have strong impacts on their livelihood. This is more pronounced in the poorest farmer's category that is land-constrained and also not able to invest in fertilizers (both inorganic and organic) to produce surplus food.

6.3.3 Crop diversification as an option to mitigate climate shocks in smallholder farming systems

Monoculture is dominant in Rwanda since the introduction of the crop intensification program in 2008. Maize is either rotated with beans or soybeans. Variability in rainfall distribution has been the main cause of crop failure. Crop diversification through intercropping is reported as an alternative to crop rotation in drier regions. Intercropping system is intended to reduce risks during poor growing seasons and could mitigate risk of crop failure (Willey, 1990) in the sense that if the main crop (cereal) fails to produce yield due to erratic distribution of rainfall within a season, the added grain legume provides food for the farm household (Rusinamhodzi et al., 2012). Enhanced productivity of intercrops in low fertility soils may be of great importance for the poorer farmers who are cultivating on poor soils and achieve poor yields. Increased yields and productivity of intercrops relative to sole crops result from complementary use of resources for growth by the intercrop components (Willey, 1979; Rao and Singh, 1990; Willey, 1990).

The success of an intercropping system is reported to be dependent on many factors including intercropped species, their spatial arrangements, densities per species, and management practices including fertilizer application, timely weeding (Giller, 2001). For instance, intercropping cereals with climbing bean has not been successful and did not show promise as a better legume for intercropping with cereals mainly due to its growth habit. The large climbing bean yield loss when intercropped with maize is attributed to greater overlap with the increasingly dominant maize (Clark and Francis, 1985). Nevertheless, some promising results when climbing bean was intercropped with banana have been reported (Ronner et al., 2019), depending on the management (pruning) of the banana plants to reduce competition for light. Climbing bean is a major grain legume in Rwanda especially in the highlands of the Northern Province. It is grown in rotation either with maize, sorghum or Irish potatoes.

6.4 Concluding remarks and recommendations for future research

The present study focused on understanding the role of management options in increasing nitrogen fixation and productivity of common bean and soybean in the smallholder farming systems of Rwanda. Large variability in the data and to some extent lack of consistent relationships between response to inputs and soil characteristics were observed. The fact that the study was conducted in farmer's fields with diverse management background may partly explain the variability observed. The management used in this study resulted in increased yields, N and P uptake (Chapters 2 and 4) and increased rhizobial survival in soil (Chapter 3). A study in Northern Province of Rwanda showed that the inherent soil fertility characteristics affected the impact of applied nutrient inputs on the productivity of climbing bean (Franke et al., 2019). However, I did not find any clear relationship between soil fertility characteristics and the response of climbing bean to applied nutrients. In the same sites, a large variability in the %Ndfa was observed and it remained unclear which specific factors govern the %Ndfa in the smallholder farming systems. The inputs used in this study strongly increased the productivity of common bean and soybean. It may be important to investigate intercropping of bean or soybean-maize especially in the drier agroecological zone of Bugesera and in similar environments of the country that are facing a challenge of rainfall variability. Experiments to test the identified nutrients indicated to be limiting for climbing bean production are necessary as well to confirm whether they are truly limiting in the field.

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Summary

Agriculture in Rwanda is dominated by smallholder farming systems which are land constrained, with little or no use of inputs. Inclusion of legumes in such systems is recognised as a potential pathway for sustainable intensification. Legumes establish symbiotic relationships with rhizobial bacteria and fix atmospheric nitrogen, thus contributing to N cycling and improving agricultural productivity. However, nutrients deficiencies, soil acidity and moisture stress have been reported as environmental factors limiting legume productivity. This suggests the need for improved management practices to improve their productivity. Soybean and common bean are two major legumes cultivated in Rwanda. However, their productivity remains poor mainly as a result of low soil fertility and little or no use of fertilizers.

The general objective of this thesis was to increase soybean and common bean productivity in the smallholder farming systems of Rwanda. In this regard, we evaluated the benefits of inoculation combined with P fertilizer and manure on yields of common bean and soybean in three agro-ecological zones (AEZs), and their residual effects on a subsequent maize crop (Chapter 2). This activity was run in three consecutive seasons. In the first season, the two legumes were tested in the three contrasting agro-ecological zones to assess the effects of environment on their performance. The treatments included inoculum (with and without), three rates of manure (0, 5, 10 t ha^{-1}), and two rates of P fertilizer (0 and 30 kg P ha^{-1}), with three replications per AEZ. Both legumes responded well to inoculation if applied together with manure and P fertilizer. The response of the two legumes to inputs varied between AEZs, with better yields in the AEZ with higher rainfall. In the second season, a trial with maize was established in the same treatments of the two legumes with a uniform management to assess the residual effects on maize productivity. We observed greater yields, N and P uptake in treatments that previously had received full inputs compared with previously unamended plots or plots that had received inoculation and P fertilizer without manure addition. However, maize grown after soybean in Bugesera failed to yield due to the long maturity period of the soybean variety used that delayed the planting of maize. In addition to the longevity of the soybean variety used, high variability in rainfall distribution observed in Bugesera throughout the study period also contributed to the smaller yields observed in this site.

To understand the effect of manure on rhizobial survival in soil and the need to re-inoculate bean and soybean in previously inoculated farmers' fields (Chapter 3), bean and soybean were grown with the experimental units of the first season (Chapter 2), split into two halves with one

half uninoculated and the other half inoculated. Trials were established in Bugesera and Kamonyi, but Kayonza was omitted as farmers mixed up the treatments before planting. These trials also evaluated if there is a need to inoculate in previously inoculated soils. We observed strong effect of the manure applied in Season 1 on grain yield of both bean and soybean in Season 3 at both study sites. For instance, average grain yield of common bean in uninoculated, unmanured plots was 1.6 and 1.5 t ha^{-1} for Bugesera and Kamonyi respectively, and was 2.3 and 2.4 t ha^{-1} in previously manured plots for Bugesera and Kamonyi respectively. Soybean yield also increased by 0.8 and 0.7 t ha^{-1} in previously manured plots compared with untreated plots. Inoculation in Season 1 also increased yield of both bean and soybean in Season 3 in both sites. The yield of common bean increased by 0.6 and 0.2 t ha^{-1} when grown in plots that had been inoculated in Season 1 in Bugesera and Kamonyi respectively. No significant effect from P applied in Season 1 was observed in Season 3 in both legumes and sites. There were also no significant differences in yields observed between plots re-inoculated in Season 3 and those that had been inoculated in Season 1 across the manure rates though slightly greater yields were achieved in re-inoculated plots. The response to inoculation was variable for both bean and soybean at both sites. However, for both legumes there was a significant response to inoculation in unmanured plots. Before establishing the trials in Season 1, initial rhizobial background recorded was very small in both sites. This was mainly due to the fact that the fields of the trials had no history of inoculation. However, a sharp increase in rhizobial population up to tenfold was observed four months from first sampling in the control treatments in soybean fields. Manure applied in Season 1 consistently increased rhizobia populations throughout the trial, with greater populations at the high manure application rate. Although the number of rhizobial population decreased in dry season in all treatments, a higher number of rhizobial population of 10^4 cells g^{-1} soil at eighteen months after first sampling was recorded during the rainy season in previously manured plots. At both sites, the population of rhizobia in the manured treatments were generally higher in inoculated than in uninoculated treatments. The survival of rhizobia in Bugesera was better in bean fields while it was better in Kamonyi for soybean. However, there was no evidence of the cause for these differences found in our study, and it remained unclear to what extent soil properties could have influenced rhizobia survival in the two sites.

In Chapter 4, we evaluated the effect of mineral N, P and K fertilizers (both alone and in combination) and manure on yields of climbing bean in Kinoni and Muko villages in Northern Rwanda. We also assessed the effect of mineral P fertilizer and manure on nitrogen fixation, N and P uptake, and explored the influence of soil fertility characteristics on the response of climbing bean to applied nutrients. The results showed strong effect of fertilizer and / or manure in increasing the grain yield, biomass and stover yields at both sites. However, there was large variability in yield among fields. In all fields, yields increased with NPK and / or manure addition, and fields in Kinoni village gave significantly smaller grain yield than in Muko. On average, greater response of fertilizer to manure addition was achieved when manure was used together with N or NPK fertilizers and was least with P. There was no clear relationship between response to inputs and soil characteristics. The variability in yields and response to inputs observed between sites may be linked to the inherent soil fertility and differences in past management practices between participating farmers. Application of inputs increased shoot N and P uptake in all fields in both sites. Inputs application to climbing bean improved shoot N and P uptake in both sites. Greater uptake was achieved in plots that received both fertilizer and manure, followed by plots with manure, then plots with P fertilizer alone and was least in plots that had not received any amendment. The %Ndfa varied greatly between fields ranging from very small to very high, and was on average lower in Muko compared to Kinoni. Positive relationships were observed between the %Ndfa and the amount of N_2-fixed and between the amount of N_2-fixed and biomass N. The amount of N_2-fixed was increased by application of fertilizer and manure, and was positively influenced by the biomass N. Similar positive relationships were also observed for grain yield and N and P uptake.

Looking at the limited resources of many farmers growing beans, especially in the densely populated high lands of the Northern Province of the country, Chapter 5 was initiated to understand and identify the limiting nutrients to climbing bean production. Two approaches (Compositional Nutrient Diagnosis (CND) and the Diagnosis and Recommendation Integrated System (DRIS) were performed to assess the relationship between nutrient concentrations of foliar tissue and yield, and also to identify nutrients that are limiting in climbing bean in Northern Rwanda. The results indicated high variability in the measured leaf nutrient concentrations for N, P, K, Ca, Mg, Cu, Mn and Zn obtained. Leaf N (%) concentrations obtained at both sites were below the critical deficiency concentrations, and leaf Ca was above the critical deficiency concentrations in all plots. A positive relationship between grain yield and leaf nutrient concentrations was observed for N, P and K but negative relationships were observed between grain yield and leaf Ca concentration, leaf Mg concentration, leaf Zn concentration and leaf Mn concentration. DRIS and CND indices were closely related, and both approaches were useful in identifying nutrient limitations to climbing bean in Northern Rwanda. We observed deficiencies of Zn, N, K and P in Kinoni, and Zn, Mg, Ca, P and N in Muko. Both DRIS and CND norms derived in this study proved to be reliable for diagnosing nutrient imbalances in climbing bean in Northern Rwanda, and overall differences between the DRIS and CND approaches were small.

The inputs used in this study proved to be crucial in increasing the productivity of soybean and beans. Greater yields in all the trials were achieved at higher rates or when inputs are combined. Considering land and cattle ownership in Rwanda, we observed that higher rates of manure for instance may be recommended for farmers in the wealthier category, while the resource-constrained farmers may go for rhizobial inoculants. The farmers with one to two cattle can use both inoculants and manure at lower rates (1-2 t ha^{-1}). Other options such as intercropping legumes-cereal maybe evaluated in farming systems in Rwanda especially in drier agro-ecological zones.

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List of publications

Peer reviewed publications

1. **Rurangwa, E**., Vanlauwe, B., Giller,. K.E., 2018. Benefits of inoculation, P fertilizer and manure on yields of common bean and soybean also increase yield of subsequent maize. Agric. Ecosyst. Env. 261, 219-229.
2. Mukamuhirwa, F., **Rurangwa, E**., 2018. Evaluation for High Iron and Zinc Content among Selected Climbing Bean Genotypes in Rwanda. Adv Crop Sci Tech 6: 344. doi:10.4172/2329-8863.1000344.
3. Nsengiyumva, A., Mbabazi, M.P., **Rurangwa, E**., Shukla, J. and Ntaganira, E., 2017. Effect of biofortified beans adoption on socio economic welfare of farmers in Eastern Rwanda. IJSTR 6, 10. ISSN 2277-8616.
4. Nsengiyumva, A., Byamushana, C.K., **Rurangwa, E**., 2017. Evaluation of the response of two soybean varieties to rhizobia inoculation for improved biological nitrogen fixation. IJSTR. 6, 09. ISSN 2277-8616.
5. Rutikanga, A., Sivirihauma, C., Ocimati, W., Night, G., Murekezi, C., Ndungo, V., Mugiraneza, T., **Rurangwa, E**., Blomme, G., 2016. Breaking the cycle of Xanthomonas campestris pv. Musacerum in infected fields through the cultivation of annual crops and disease control in adjacent fields. J. Phytopathol. 164, 659-670.
6. Jefwa, J., Vanlauwe, B., Coyne, D., van Asten, P., Gaidashova, S., **Rurangwa, E.**, Mwashasha, M. and Elsen, A., 2010. Benefits and Potential Use of Arbuscular Mycorrhizal Fungi (AMF) in Banana and Plantain (*Musa* spp.) Systems in Africa. Proceedings of International Conference of Banana and Plantain in Africa. Eds. Dubois, T et al. 2010. Acta Hort. 879, 479-486. ISHS 2010.

Non-peer reviewed publications

1. **Rurangwa, E.,** Jefwa, J., Vanlauwe, B., Gaidashova, S. and Blomme, G., 2012. Indigenous arbuscular mycorrhizal fungi improve field establishment of tissue cultured AAA-EA and AAB bananas in Rwanda. Integrated Soil Fertility Management in Africa: From microbes to Markets. Safari Park Hotel, Nairobi, Kenya. 22-26 October 2012.
2. Waweru, B., Gaidashova, S., **Rurangwa, E.** and Jefwa, J., 2012. Effect of inoculation time on growth of two banana genotypes inoculated with commercially produced

arbuscular mycorrhizal fungi. Integrated Soil Fertility Management in Africa: From microbes to Markets. Safari Park Hotel, Nairobi, Kenya. 22-26 October 2012.

3. **Rurangwa, E.**, Jefwa, J., Vanlauwe, B. and Turoop, L., 2011. Indigenous Arbuscular Mycorhizal Fungi and growth of Tissue Cultured Banana Plantlets under Nursery Conditions in Rwanda**.** Poster presented in CIALCA Conference on Challenges and opportunities for Agricultural Intensification of the Humid Highland Systems of Sub-Saharan Africa. Kigali, Rwanda. 24-27 October 2011.
4. Gaidashova, S., Night, G., **Rurangwa, E.,** Gahakwa, D., Mukandinda, A., Uwimpuhwe, B., Karemera, F., Mugiraneza, T., Kajuga, J., Murekezi, C., Tinzaara, W., Karamura, E. and Jogo, W., 2011. Adoption of technologies disseminated for the control of banana xanthomonas wilt in Rwanda**.** CIALCA Conference on Challenges and opportunities for Agricultural Intensification of the Humid Highland Systems of Sub-Saharan Africa. Kigali, Rwanda. 24-27 October 2011.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Effects of environment, management practices, legume and rhizobium genotypes on nitrogen fixation and yield of common bean and soybean in the farming systems of East and Central Africa (2011-2012)

Writing of project proposal (4.5 ECTS)

- Enhancing biological nitrogen fixation and yield of soybean and common bean in smallholder farming systems of Rwanda

Post-graduate courses (5.4 ECTS)

- Sense PhD; Sense (2012)
- Introduction to R for statistical analysis; PE&RC (2015)
- Grasping sustainability; Sense (2017)
- N2Africa project writeshop; Wageningen, the Netherlands (2013)

Laboratory training and working visits (4.5 ECTS)

- Training on soil sampling and handling for rhizobia population count and nodulation scoring; Rwanda Agriculture Board, Microbiology Laboratory (2012)
- Basic training on inoculum production and legume inoculation process; Rwanda Agriculture Board, Microbiology Laboratory (2012)

Invited review of (unpublished) journal manuscript (1 ECTS)

- Experimental Agriculture: constraints and opportunities for crop intensification including medicinal plants in eastern and northern Rwanda (2016)

Deficiency, Refresh, Brush-up courses (7.5 ECTS)

- Systems analysis, simulation and systems management; PPS (2011)
- Basic statistics; PE&RC (2015)

Competence strengthening / skills courses (3.9 ECTS)

- Techniques for writing and presenting a scientific paper; WGS (2011)
- Scientific publishing; WGS (2015)
- Data management planning; WGS (2015)
- Project and time management; WGS (2015)
- Information literacy including endnote introduction; WGS (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC First years weekend (2011)
- Meeting with Bill Gates (2011)
- PE&RC Day (2014)
- PE&RC Last years weekend (2014)
- PhD Carousel (2017)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Rwanda agriculture board seminars; Rwanda (2012-2018)
- Rwanda agriculture board microbiology laboratory team discussions; Rwanda (2012-2018)
- N2Africa project closing phase I and launching phase II; Nairobi, Kenya (2013)
- Seminar on nitrogen fixation: from microbes to plant; Wageningen, the Netherlands (2015)
- N2Africa project annual planning meeting; Zimbabwe (2016)
- N2Africa project annual planning meeting; Rwanda (2018)

International symposia, workshops and conferences (5 ECTS)

- Integrated soil fertility management in Africa: from microbes to markets, Nairobi, Kenya (2012)
- Pan-African Grain Legume Research Conference and World Cowpea Research Conference; Livingstone, Zambia (2016)

About the author

Edouard Rurangwa was born on 4 August 1978 in Cankuzo, Burundi. He grew up in Mushiha where he attended primary education before going for high school at collège Kigamba. In 1994 his parents returned to Rwanda and Edouard continued his high school at Ecole des Sciences de Byimmana before joining the University of Rwanda in 1999. He graduated in Agricultural Science (option: Soil and Environmental management) in 2005. In October 2006, he was employed as a junior researcher in banana programme by the former Institut des Sciences Agronomiques du Rwanda (Currently Rwanda Agriculture and Animal Resources Development Board or RAB for short). He was involved in many research and extension activities across Rwanda. In August 2007, Edouard obtained a scholarship funded by TSBF-CIALCA project to pursue an MSc programme at Jomo Kenyatta University of Agriculture and Technology. He was enrolled in Agricultural Science in Horticulture department. His research focused on looking at the influence of arbuscular mycorrhizal fungi on nursery inoculated tissue cultured banana and their initial field performance. After his MSc, Edouard was promoted as an Associate Research Fellow in banana program and continued to serve RAB until he started his PhD programme at Wageningen University in October 2011 with support of the N2Africa project. From August 2017 onwards, Edouard is leading the soybean programme at RAB, in charge of research and technology transfer activities.

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