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 total yield when inputs were combined. The % Nd fa, amount of N₂-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 Bugese due to the long maturity of the 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 high 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 N₂-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). These 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 Ni in the Muko site.

Keywords: 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 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 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, especially common beans and soybeans are the most promoted by policy makers and most researched by 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 (Rubigo 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 (Osunbode 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 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. Nutrient 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.

Increasing legume productivity depends on the success of the interaction between the legumes, rhizobium strains nodulating the legumes, 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 as important options to increase crop productivity (Zingore et al., 2008; Niyuhire et al., 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

Chapter 1
and soybean, and to the subsequent maize crop grown in rotation (Chapter 2);

1.4 Selection of the study sites

The study was carried out in farmers’ fields selected from the sites located in
Province. The study was carried out in farmers’ fields selected from the sites located in
Fig. 1.1 Ma p of Rwa n d a s h owi ng s i t e s wh er e t r i a l s we r e e s t a b l i s h e d ( l i g h t  g r e e n  c i r c l e s )

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 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 (DRI S) approaches. In this chapter, CND and DRI S 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 1
Chapter 2

Benefits of inoculation, P fertilizer and manure on yields of common bean and soybean also increase yield of subsequent maize.
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 legume strongly increased when other inputs were applied 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 N₂-fixed measured using the ¹⁵N-natural abundance method differed between the two legumes and varied between 15 and 198 kg N₂ ha⁻¹ for common bean and between 15 and 186 kg N₂ ha⁻¹ for soybean and differed enormously among treatments and AEZs. Application of inputs to the legume 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 stovever 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 disease, mobilization of poorly soluble P and increased mycorrhizal colonization of a 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., 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
Chapter 2

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 Gill, 2006).

Since indigenous rhizobia are not always sufficient in 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 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 response 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; Frank 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 agro-ecological 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 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 30°08'42" E at an altitude of 1435 m above sea level (masl), with a mean annual rainfall of 800 mm. Kanyozi 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 Kanyozi, 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 added as triple super phosphate. The experiments were laid out in a split plot design with P fertilizer as the main plot, inoculum as subplot, and barley as the reference crop. 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.
1.2

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 analyzed. In the legumes, biomass and nodulation were assessed at mid-podding. As a small sub-plot of 0.5 m² (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°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: 21-30 nodules; 5: > 30 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°C to constant weight. Grain yield is presented at 12.5% moisture content, stover (haulms + husks) at 0% moisture.
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 last maize plant of each row. Coobs were separated from stover and their fresh weights were determined. A sub-sample of stover and coobs was taken, and coobs were shelled. Coobs 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).

N2-fixation was measured using the 15N natural abundance method (Unkovich et al., 2008). After drying and grinding the shoot samples, 15N 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).

\%Ndfa = (δ^{15}N ref - δ^{15}N leg) / (δ^{15}N ref - B) × 100

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

Amount of N2-fixed = (%Ndfa × 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 N2-fixed – Total amount of N removed in grain

The total amount of N2-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 analyzed, 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.
Chapter 2

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 rotation effect in a split-split plot design using the Gen Stat 16th edition. The effect of different factors and their interactions were compared by computing the standard errors of difference (SED). Treatment means were compared using the least significant differences (LSD) at P ≤ 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 in Kamonyi, October 21 in Kayonza and October 23 in Bugesera. Common bean was harvested on January 23 in Kamonyi, January 28 in Bugesera and January 30 in Kayonza. Soybean was harvested a month later on March 4 in Bugesera, March 6 in Kayonza and March 7 in Kamonyi. Maize after common bean was sown on February 4 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
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 cmol c 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 Kamanzi or Kanyinza (0.9-1.0%). By contrast the largest P concentration was found in manure from Kamanzi. On average, 5 to 6% 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 Kamanzi and 50 kg N, 10 kg P and 35 kg K in Kanyinza.

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

<table>
<thead>
<tr>
<th>Soil parameter</th>
<th>Bugesera</th>
<th>Kamanzi</th>
<th>Kanyinza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH (H(_2)O)</td>
<td>6.2</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}))</td>
<td>1.8</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>C (g kg(^{-1}))</td>
<td>24.1</td>
<td>20.2</td>
<td>25.6</td>
</tr>
<tr>
<td>P (Olsen) (mg P kg(^{-1}))</td>
<td>15.7</td>
<td>10.2</td>
<td>18.1</td>
</tr>
<tr>
<td>Exchangeable K (cmol c kg(^{-1}))</td>
<td>0.4</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Exchangeable Ca (cmol c kg(^{-1}))</td>
<td>5.7</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Exchangeable Mg (cmol c kg(^{-1}))</td>
<td>1.8</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>ECEC (cmol c kg(^{-1}))</td>
<td>14.4</td>
<td>11.1</td>
<td>15.7</td>
</tr>
<tr>
<td>Sand (g kg(^{-1}))</td>
<td>380</td>
<td>490</td>
<td>360</td>
</tr>
<tr>
<td>Silt (g kg(^{-1}))</td>
<td>120</td>
<td>120</td>
<td>130</td>
</tr>
<tr>
<td>Clay (g kg(^{-1}))</td>
<td>500</td>
<td>400</td>
<td>510</td>
</tr>
</tbody>
</table>
### Table 2.1b Characteristics of the applied manure, averaged for each location.

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

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 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 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 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; * indicates significant difference.
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.

- R: without or with rhizobia (R) inoculation.

Grain yield (t ha$^{-1}$)

Biomass at mid-podding (t ha$^{-1}$)

between means;
Table 2.2 | Stove response of common bean, soybean to inoculation combined with fertilizer and three rates of manure at Bugesea, Kamonyi and Kayonza.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bugesea</th>
<th>Kamonyi</th>
<th>Kayonza</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stove (t ha⁻¹)</td>
<td>Stove (t ha⁻¹)</td>
<td>Stove (t ha⁻¹)</td>
</tr>
<tr>
<td>0 P</td>
<td>1.2</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>R+ 0 M</td>
<td>1.7</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>0 P+ R+ 0 M</td>
<td>1.5</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>0 P+ R+ 5 M</td>
<td>1.9</td>
<td>2.3</td>
<td>2.7</td>
</tr>
<tr>
<td>0 P+ R+ 10 M</td>
<td>1.9</td>
<td>2.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

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 (Fertilizer x Inoculum x Manure): 0.08, 0.18, 0.22, 0.15
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. Nodule score significantly differed (P < 0.001) among the three AEZs with Bamonyi 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 nodule of both legumes.

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

The amount of N₂-fixed was on average larger in soybean than common bean (Tables 2.3 and 2.4). For both legumes, the largest amount of N₂-fixed was observed at Bamonyi which received more and better distributed rainfall and had greater biomass production, and least at Kayonza for common bean and Bugeera for soybean. Inoculation combined with P fertilizer led to increased amount of N₂-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 N₂-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 Bamonyi for both legumes and the least uptake at Kayonza for common bean and Bugeera for soybean shoot N uptake. Both legumes had a greater shoot N and P uptake in treatments that
received full inputs and least in treatment. For both legumes and in all AE Zs, manure addition either alone or in combination with inoculation and fertilizer, strongly and consistently enhanced N and P uptake (Table 2.5). N and P uptake in treatments that received inoculation and 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 ha\(^{-1}\). Shoot N uptake in soybean increased as well with inoculation when combined with 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 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 ha\(^{-1}\). Similarly, inoculation and 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 N\(_2\) compared to more negative net N inputs observed in AE Zs 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 AE Z.
Fig. 2.4

No d u l e  m e a n  s c o r e  ( # )  r e s p o n s e :  ( a ,  c ,  e )  c o mmo n  b e a n ,  a n d  ( b ,  d ,  f )  s o y b e a n  t o  i n o c u l a t i o n ,  P  f e r t i l i z e r  a n d  t h r e e  

r a t e s  o f  ma n u r e  a t  ( a ,  b )  Bu g e s e r a ,  ( c ,  d )  K a m o n y i  a n d  ( e ,  f )  K a y o n z a .  E r r o r  b a r s  r e p r e s e n t  t h e  s t a n d a r d  e r ro r s  o f  d i f f e r e n c e  

- / +  R:  wi t h o u t  o r  wi t h  r h i z o b i a  ( R)  i n o c u l a t i o n .  

between means;
Table 2.3: Common bean shoot $\delta^{15}N$, percentage of $N_2$ derived from atmosphere (Ndaf), and total $N$-fixed and net $N$ input as affected by treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Common bean shoot $\delta^{15}N$ (%)</th>
<th>$\delta^{15}N$ (%)</th>
<th>$\delta^{15}N$ (‰)</th>
<th>Common bean shoot $\delta^{15}N$ (%)</th>
<th>$\delta^{15}N$ (%)</th>
<th>$\delta^{15}N$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 M 15N</td>
<td>11.4 4.8 3.6</td>
<td>6.7 27 6.4</td>
<td>24 4.8 3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 M 15N</td>
<td>11.4 4.7 4.3</td>
<td>5.0 27 4.3</td>
<td>22 4.7 4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 M 30P</td>
<td>11.4 4.8 3.6</td>
<td>6.7 27 6.4</td>
<td>24 4.8 3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 M 30P</td>
<td>11.4 4.7 4.3</td>
<td>5.0 27 4.3</td>
<td>22 4.7 4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- $\delta^{15}N$: Nitrogen isotope ratio.
- Ndaf: Percentage of nitrogen fixed from the atmosphere.
- Net N input: The total amount of nitrogen fixed and net nitrogen input.
- R: without or with rhizobia (R) inoculation; trt: Treatment; ref: Reference.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soybean Shoot $\delta^{15}N$ (‰)</th>
<th>Soybean Shoot $\delta^{13}C$ (‰)</th>
<th>Soybean Shoot $\delta^{15}C$ (‰)</th>
<th>Soybean Shoot $\delta^{13}C$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SED (Fertilizer x Inoculum x Manure)</td>
<td>1.17</td>
<td>0.9</td>
<td>3.8</td>
<td>2.1</td>
</tr>
<tr>
<td>SED (Fertilizer x Inoculum)</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4 Soybean shoot $\delta^{15}N$ and $\delta^{13}C$ values and their ranges for different treatments.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bugeser AEZ</th>
<th>Kamonyi AEZ</th>
<th>Kyonza AEZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Common Bean</td>
<td>Soybean</td>
<td>Common Bean</td>
</tr>
<tr>
<td>0 P-R+ 0 M</td>
<td>8</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>0 P-R+ 10 M</td>
<td>12</td>
<td>119</td>
<td>21</td>
</tr>
<tr>
<td>30 P+R+ 0 M</td>
<td>11</td>
<td>77</td>
<td>7</td>
</tr>
<tr>
<td>30 P+R+ 10 M</td>
<td>29</td>
<td>200</td>
<td>23</td>
</tr>
<tr>
<td>Average</td>
<td>15</td>
<td>114</td>
<td>14</td>
</tr>
<tr>
<td>SED (Inoculum)</td>
<td>2.41</td>
<td>13.02</td>
<td>ns</td>
</tr>
<tr>
<td>SED (Fertilizer)</td>
<td>2.41</td>
<td>13.02</td>
<td>ns</td>
</tr>
<tr>
<td>SED (Manure)</td>
<td>2.41</td>
<td>13.02</td>
<td>ns</td>
</tr>
<tr>
<td>SED (AEZ)</td>
<td>2.96</td>
<td>15.94</td>
<td>6.35</td>
</tr>
</tbody>
</table>
3.5 Nutrient uptake and production in maize

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 in inoculation and fertilizer with or without manure addition. Similarly, inoculation with P fertilizer applied to soybean, 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\(^{-1}\) over maize uptake when grown after untreated soybean, and by 52 kg N ha\(^{-1}\) over maize yield when treatment of inoculants and P fertilizer with or without manure addition. 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 (see Chapter 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 realized as 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\(^{-1}\) 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\(^{-1}\) maize 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 inoculation was combined with P fertilizer 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 was combined with P fertilizer 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 straw yield response to inputs applied to the preceding legume 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*). N. B. Maize failed to yield any grain after soybean at Bugesera.

<table>
<thead>
<tr>
<th>Manure rates (t ha(^{-1}))</th>
<th>Grain yield (t ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bugesera</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manure rates (t ha(^{-1}))</th>
<th>Grain yield (t ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamonyi</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Treatment</td>
<td>Bugesera AEZ</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------</td>
</tr>
<tr>
<td></td>
<td>Treatment 1</td>
</tr>
<tr>
<td>Common bean - maize</td>
<td></td>
</tr>
<tr>
<td>Soybean - maize</td>
<td></td>
</tr>
<tr>
<td>Total P uptake</td>
<td>0 P - R+ 0 M</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total N uptake</td>
<td>0 P - R+ 0 M</td>
</tr>
<tr>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Average</td>
<td>45</td>
</tr>
</tbody>
</table>

Note: P = phosphorus, N = nitrogen, R = inoculum, M = manure. 
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.
Table 2.7 | Stover response of maize (t ha⁻¹) grown after common bean and soybean with and without inoculation, fertilizer and manure treatments

<table>
<thead>
<tr>
<th></th>
<th>Bugese AEZ</th>
<th>Kamonyi AEZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Common Bean</td>
<td>Maize</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>Maize</td>
</tr>
<tr>
<td>O P - R+ 0 M</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>O P - R+ 5 M</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>O P - R+ 10 M</td>
<td>4.8</td>
<td>3.5</td>
</tr>
<tr>
<td>0 P + R+ 0 M</td>
<td>6.0</td>
<td>4.2</td>
</tr>
<tr>
<td>0 P + R+ 5 M</td>
<td>6.2</td>
<td>7.1</td>
</tr>
<tr>
<td>0 P + R+ 10 M</td>
<td>5.8</td>
<td>7.3</td>
</tr>
<tr>
<td>30 P - R+ 0 M</td>
<td>4.5</td>
<td>3.5</td>
</tr>
<tr>
<td>30 P - R+ 5 M</td>
<td>5.1</td>
<td>4.1</td>
</tr>
<tr>
<td>30 P - R+ 10 M</td>
<td>3.9</td>
<td>6.0</td>
</tr>
<tr>
<td>30 P + R+ 0 M</td>
<td>3.7</td>
<td>5.9</td>
</tr>
<tr>
<td>30 P + R+ 5 M</td>
<td>6.2</td>
<td>5.3</td>
</tr>
<tr>
<td>30 P + R+ 10 M</td>
<td>7.7</td>
<td>8.0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>5.0</td>
<td>5.1</td>
</tr>
</tbody>
</table>

**SED** (Inoculum) 0.62 0.54
**SED** (Manure) 0.50 0.50
4. Discussion

4.1 Growth and yields of common bean and soybean

The better and well distributed rainfall at Kajonyi (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 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). 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., Mustone 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 Kajonyi which received the most rainfall, well distributed throughout 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 mechanisms behind are not well understood (Singh et al., 1997). The increase in nodulation observed with manure may be linked to its impact on availability of other nutrients, on moisture supply or on better soil structure creating a more favorable environment for nodulation.

The better nodulation at Kajonyi was translated into a larger %Nd fraction in both bean and soybean. There were no obvious or consistent effects of inoculation, P fertilizer or...
4.3 Residual effect of legumes on maize

The net N inputs from N2-fixation (calculated by subtracting the amount of N removed in grain from the amount of N2-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 highest yields tended to have the most negative net inputs this was not always the case. The values were more negative where %Nd-fa was smaller, especially at Bugese 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).

Greater shoot N and P uptake was observed at Kamonyi with higher rainfall and in treatments that received combined inoculation with manure and fertilizer (Table 2.5). The greater N and P uptake in treatments that received manure relative to treatments with fertilizer and inoculation, suggests that N and P supplied by manure were readily available for plant uptake and positively contributed to plant growth.
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 Bugeera, resulting in complete failure to yield of the maize crop. These suggest the need for early maturing soybean varieties in Bugeera, 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 an ended legume (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 N2-fixation of the previous legume. Our results support the suggestion that inputs are best targeted to legumes in rotation with cereals to maximize legume production and N2-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 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 Kermahe 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 fertilizer. Most mineral or organic fertilizer is targeted to cash crops (e.g. tomatoes, 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...
The current recommendation in Rwanda is 10 t/ha for food crops (MINAGRI, 2010). The question remains as to whether these are feasible rates 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. An 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 771,228 households own 1,33 million cattle, giving an average 1.7 cattle/hh. It has been shown that a local Ankole weighing 300 kg, reared in a zero-grazing system can produce 6 to 12 t of manure per year (MINAGRI, 1990). Assuming each head cattle produces 6 to 12 t of manure annually each household could produce on average 10.2 t of manure per year. Land holdings 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 Kamoyny which has a population density of 519 inhabitants/km2. By contrast, Bugese district has 280 inhabitants/km2 and Kayonza is the least populated with 178 inhabitants/km2 (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 per year.

Buca et al. (2014) working in Isimbri (Southern province) and Kagayo (Northern Province) of Rwanda showed that farmers with 0.5 to 2 ha of land and one or two heads of cattle were able to invest organic manure in food crop production. In northern Rwanda, Frank 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 fertilizer and achieved greater yields. Analysis in southwestern Rwanda suggests 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 in our study sites, there was no fixed price. In Kamoyny, a pit of

Chapter 2
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
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 beans and soybeans 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, beans and soybeans were grown without and with inoculum, manure at three rates: 0, 5 and 10 t ha\(^{-1}\), and fertilizer at two rates: 0 and 30 kg P ha\(^{-1}\) added as triple superphosphate in split-split plot design. In the following season, all plots were cropped with maize with uniform management. In the third season, covered in this study, beans and soybeans were grown with the experimental units of the first season, split into 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 beans and soybeans 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 beans and 2.8 t ha\(^{-1}\) for soybeans in inoculated plots receiving 10 t manure ha\(^{-1}\). Re-inoculation in Season 3 did not significantly increase the yields of beans and soybeans 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 soybeans since soybeans had not been cropped in those fields before, and the numbers increased to ten fold four months after planting soybeans. Rainfall and manure strongly influenced the survival of rhizobia in the soil. The largest population of 104 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 101 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 colonize nodules on plant roots (Giller, 2001) and fix atmospheric nitrogen. Through this symbiosis with rhizobia, legumes play an important role in nitrogen cycling and 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 (Thiessen et al., 1991). 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 less effective indigenous rhizobia than the selected inoculant 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 numbers sufficient for effective nitrogen fixation by a legume crop, and form large proportions of nodules (Elkins et al., 1976; Crozet et al., 1982; Zengeni et al., 1998). The factors affecting rhizobium survival in soil have been examined extensively. Among them are low soil pH and aluminum toxicity, desiccation, nutrient toxicities (Mahler and Wollum II, 1982; Zahran, 1999; Hungria and Vegas, 2000; Giller, 2001). Moreover, moisture availability (Zengeni et al., 2006) and may be affected by the crop. The longevity of rhizobia in the soil is related to soil clay content, percentage carbon and history and season (Andrade et al., 2002). The application of organic manure improves rhizobia survival in soil by creating a favorable environment for their growth and multiplication. Organic manure improves soil carbon, increases water-holding capacity, neutralizes acidic conditions, and supplies exchangeable bases and other micronutrients (Zengeni et al., 1998; Zengeni et al., 2006; Zingore et al., 2008). The question how 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 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
4 2

Chapter 3

2. Materials and methods
2.1 Study site and trials establishment
The study was carried out in farmers’ fields in two contrasting agroecological zones 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°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 Rwandan Meteorology stations.

In Season 1 (short rains 2014, Sep 2013–Jan 2014, referred here as Season 1) fields were cropped with beans and soybean 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 superphosphate. The manure used was collected from participating farmers and was analysed for chemical...
On average the pH of the manure was 8.7 and 8.2 for Bugesera and Kamanzi respectively. The N concentration in manure was 1.8% in Bugesera and 0.9% in Kamanzi. P concentration in manure was 0.2% in Bugesera and 0.5% in Kamanzi. The K concentration in manure was 1.4% in Bugesera and 1.3% in Kamanzi. Organic carbon was 17.5% in Bugesera and 11.2% in Kamanzi.

The experiments were laid out in a split-split plot design with P fertilizer as the main plot, inoculum as subplot and manure as sub-subplots with a full set of treatments per block. Sub-subplot size was 5.0 m × 5.0 m. Bean variety RW2245 and soybean variety SB24 were planted at a density of 50 cm × 10 cm for bean and 40 cm × 10 cm for soybean with 1 m paths between subplots to minimize 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 ZM607 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 layout of Season 1 was retained. However, none of the treatments in Season 1 were applied in Season 3. Instead, each sub-subplot 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 in 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-subplot 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 rhizobial population in the different sub-subplots was monitored through soil chemical analysis.
s ampling 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 fertilizer and without or with inoculation across the manure rates in Season 1. The bean variety RW R2245 and the soybean variety S B24 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 m² for bean and 0.4 m² 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 0-50 nodules, and >50 nodules. Final grain yield was determined at crop maturity by harvesting all pods from the net plots (8 m²) 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 × fertilizer × 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. Rhizobia population data were log-transformed before analysis. A repeated measurement analysis was performed to assess rhizobia 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, an antedependence model order 1 was chosen for correlation within subjects (replicates). The factor inoculum for rhizobia 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 Kamanji, 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 with no 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, S4: April 2015.

3.2 Field soil characteristics and rhizobial background of the trial sites
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 low at both sites and there was no significant difference between the two sites. In both sites, initial rhizobial population with soybean as trap host was very small (Table 3.1). Four months from first sampling, a ten fold increase in rhizobial population was observed in the control treatments in soybean fields. The survival of rhizobia in the two study sites during different sampling times was better in Bugesera (\(P = 0.03\)) in bean fields and in 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 during 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.
3.4 Nodulation score as affected by manure and inoculation

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, January, March and July 2014, and April 2015. Error bars represent standard errors of differences between means.

3.4 Nodulation score as affected by manure and inoculation
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 Kanyinya (Fig. 3.3). A maximum nodule score of soybean in Kanyinya 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 Bugese and Kanyinya in both uninoculated and inoculated either in season 1 or season 3. There was no difference between 5 and 10 t manure ha$^{-1}$ in increasing nodule.
Fig. 3.3

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) (Table 3.2 & 3.3), though the increase was (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 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 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–fertilizer 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

<table>
<thead>
<tr>
<th></th>
<th>Bugesera</th>
<th>Kamonyi</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>Ferilizer + / -</td>
<td>Rb</td>
<td>Rb</td>
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<td>Inoculum /</td>
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<td>Manure</td>
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<td>(tha⁻¹)</td>
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<tr>
<td>Mean</td>
<td>2.3</td>
<td>2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Note: No inoculation in season 3; ns: not significant at p = 0.05

Chapter 3
Fig. 3.4. Response to inoculation (t ha$^{-1}$) in Season 3 for (a, c) beans and (b, d) soybean grain yield response to inoculation in Season 3 for (b, d) beans and (a, c) soybean. Error bars represent standard error of means of individual treatments.
4. Discussion

4.1 Influence of cropping history on rhizobial survival

Rhizobia in soils without a history of inoculation (e.g. Giller, 2001; Andrade et al., 2002; Zengeni et al., 2006). The low initial size of rhizobia in the soil (Bottomley, 1992; Andrade, 2002). The low initial size of rhizobia in soil (Karanja et al., 1995; Mendes, 1998). After infection of soybean fields. Although there was no evidence of the ca
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 September, and the short rains start and continue to December, with a short dry season in January and February during harvesting of legumes. The larger rhizobia numbers observed during the rainy season and sharp decline during the dry season confirms observations of Zenge 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; Zenge 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 Zenge 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 Buge se and Kamo nyi 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 favorable environment for rhizobia growth and survival. Manure is also known for its limiting 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 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
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 small holder 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 and 10 t 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 small holder farmers.
Acknowledgements

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Chapter 4

The response of climbing bean to fertilizer and organic manure in the Northern Province of Rwanda
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 NP K. Large variability in yield between fields was observed. Application of fertilizer together with manure increased the grain yield from 1.5 t/ha in Kinoni, and from 2.6 t/ha in Muko. Fertilizer and/or manure increased the yield from 0.8 t/ha in Kinoni and from 1.5 t/ha in Muko. Application of 30 kg P/ha and 5 t manure/ha led to increased N and P uptake (from 49 to 106 kg N/ha and from 6.1 to 12.4 kg P/ha in Kinoni and from 46 to 128 kg N/ha and from 5.3 to 17.9 kg P/ha 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 NP K application was observed in plots where soil available P and soil exchangeable K were relatively low. Our results show the benefits of using manure along with mineral fertilizers for increased climbing bean yields and nutrient uptake in smallholder farming systems.

Keywords: Phaseolus vulgaris; nitrogen fixation; 15N natural abundance; ...
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 land holdings 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 achieving this goal. Legumes are important crops both for supply of food and fodder and for soil improvement. Legumes fix atmospheric N2 through symbiosis with rhizobia and contribute N to the soil for use by other crops (Frank 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., 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 in 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 Muynæza, 1995). Climbing beans are reported to be less constrained by diseases and much more productive than bush beans (Wortmann et al., 2001), as well as being less prone to root rots (Sperling and Muynæza, 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 Muynæza, 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 below-ground crop residues (Niyyhère 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 losses from the steep slopes observed in the Eastern African highlands (Wortmann et al., 2001). However, availability of stacking material, the increased labor requirements for stacking 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 started in the early 1970s at the former ‘Institut des Sciences Agronomiques du Rwanda’ (ISAR), now Rwanda Agriculture Board (RAB) (Sperrling and Muyaneza, 1995). However, bush bean was predominant, and climbing beans were found only in the northwest of the country (Sperrling 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 (Frank and de Wolf, 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 (Frank and de Wolf, 2011).

Many studies have shown the need for balanced fertilization in soils that have been cropped continuously (Smithson 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 productivity of climbing bean (Frank et al., 2019). There is limited information on the integrated use of farmyard manure and mineral fertilizer 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 fertilizer (both alone and in combination) and manure on yields of climbing bean, (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 sides 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 Bure 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°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 (H2O), 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.
2.2 Trial establishment

The trials were established in the long rainy season of 2014. Climbing bean variety RW V1129 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 + NP K. 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 NP K 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 m² and a net area for biomass sampling of 1.75 m². 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 N₂-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.
2.3 Yield assessment

Above ground biomass was determined at late pod-filling stage. A subplot of 1.75 m² (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 subsample 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 subsample 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 hauMs were harvested by cutting at ground level and weighed. Representative subsamples of hauMs 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 N₂-fixation

Dry climbing bean biomass was ground and digested in hot H₂SO₄ and H₂O₂ (Parkinson and Allen, 1975). N and P concentrations in the digests were determined by colorimetric methods (Okalebo et al., 1993). The proportion of plant N from N₂-fixation was measured using the 15N natural abundance method (Unkovich et al., 2008). After drying and grinding the samples, 15N 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, KUL Leuven).

The δ¹⁵N value and the proportion of N derived from atmosphere (%Ndₐ) were calculated. The %Ndₐ (Equation 1) and amount of N2-fixed (Equation 2) were calculated as follows (Unkovich et al., 2008):

\[
%\text{Nd}_\text{a} = \frac{\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{leg}}}{\delta^{15}\text{N}_{\text{ref}} - B} \times 100
\]

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

Amount of N fixed [kg ha⁻¹] = (%Ndₐ × Total N legume [kg ha⁻¹]) / 100

where %Ndₐ is the percentage of N from N₂-fixation; Total N legume is the product of shoot N and P uptake was determined at late pod-filling.

2.5 Data analysis

Statistical analysis was performed using GenStat (version 16, VSNI 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 N₂-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 Kinon, 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\(\text{ha}^{-1}\) of manure contained 60 kg N\(\text{ha}^{-1}\), 15 kg P\(\text{ha}^{-1}\) and 55 kg K\(\text{ha}^{-1}\) in Kinon and 65 kg N\(\text{ha}^{-1}\), 15 kg P\(\text{ha}^{-1}\) and 70 kg K\(\text{ha}^{-1}\) in Muko.

<table>
<thead>
<tr>
<th>Table 4.1 Characteristics of the soil from the Kinon (low potential) and Muko (high potential) sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kinon</strong> (low potential)</td>
</tr>
<tr>
<td><strong>Soil parameter</strong></td>
</tr>
<tr>
<td>pH (H(_2)O)</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}))</td>
</tr>
<tr>
<td>Organic C (g kg(^{-1}))</td>
</tr>
<tr>
<td>Available P (Olsen) (mg P kg(^{-1}))</td>
</tr>
<tr>
<td>Exchangeable K (cmol c kg(^{-1}))</td>
</tr>
<tr>
<td>Exchangeable Ca (cmol c kg(^{-1}))</td>
</tr>
<tr>
<td>Exchangeable Mg (cmol c kg(^{-1}))</td>
</tr>
<tr>
<td>ECEC (cmol c kg(^{-1}))</td>
</tr>
<tr>
<td>Sand (g kg(^{-1}))</td>
</tr>
<tr>
<td>Silt (g kg(^{-1}))</td>
</tr>
<tr>
<td>Clay (g kg(^{-1}))</td>
</tr>
</tbody>
</table>

ECEC: Effective Cation Exchange Capacity
Table 4.2: Characteristics of the applied manure at the Kinoi (n = 7) and Muko (n = 7) sites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kinoi</th>
<th>Range</th>
<th>Muko</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H₂O)</td>
<td>8.5</td>
<td>7.7-9.0</td>
<td>8.7</td>
<td>8.2-9.6</td>
</tr>
<tr>
<td>C (%)</td>
<td>15.5</td>
<td>12.0-19.3</td>
<td>18.4</td>
<td>12.3-29.1</td>
</tr>
<tr>
<td>N (%)</td>
<td>1.2</td>
<td>0.7-1.6</td>
<td>1.3</td>
<td>0.6-1.9</td>
</tr>
<tr>
<td>C: N</td>
<td>14.0</td>
<td>7.5-27.6</td>
<td>14.9</td>
<td>9.7-23.4</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.3</td>
<td>0.1-0.5</td>
<td>0.3</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>K (%)</td>
<td>1.1</td>
<td>0.5-2.0</td>
<td>1.4</td>
<td>0.7-4.0</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1.1</td>
<td>0.6-1.6</td>
<td>1.1</td>
<td>0.7-1.4</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.6</td>
<td>0.4-0.7</td>
<td>0.6</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.1</td>
<td>0.1-0.2</td>
<td>0.1</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>B (ppm)</td>
<td>37.2</td>
<td>22.7-46.0</td>
<td>31.6</td>
<td>25.2-44.7</td>
</tr>
</tbody>
</table>

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 yield (Fig. 4.3c, d) at both sites. However, there was large variability 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 NP K 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 Kinoi and Muko, respectively. Average stover yield was 1.6 t ha⁻¹ and 2.4 t ha⁻¹ for Kinoi and Muko, respectively. Application of manure alone increased the grain yield from 1.5 t to 2.8 t ha⁻¹ in Kinoi (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 NP K and/or manure addition. Fields in Kinoi 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 Kinoi, 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 Kinoi, 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 NP K 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
Input application did not follow the same pattern as grain and biomass yields. On average, fertilizer and/or manure increased yield from 0.8 t/ha to 2.3 t/ha in Kinon and from 1.5 t/ha to 3.4 t/ha 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 (Fig. 4.5). Responses to inputs were smaller in Kinon than in Muko. On average, greater response of fertilizer to manure addition was achieved when manure was used together with N or NP K fertilizers and was least with P alone though there were no significant differences among treatments at the Kinon site. Responses to NP K 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, %Nd f a and amount of N2-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. 4.3af: The effects of manure rates (t ha⁻¹) on grain yield (a,b), stover yield (c,d), and biomass at late pod-filling (e,f) at Kinoni and Muko. Error bars represent the standard error of differences between treatment means.
Fig. 4. Climbing bean yield in control against (a) yield with manure (kg ha\(^{-1}\)), (b) yield with NP K (kg ha\(^{-1}\)), (c) yield with manure (kg ha\(^{-1}\)) against yield with NP K + manure (kg ha\(^{-1}\)), and (d) yield with NP K (kg ha\(^{-1}\)) against yield with NP K + manure (kg ha\(^{-1}\)). 2M-K, 2M-M, 5M-K, and 5M-M represent 2 and 5 tons 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 NP K fertilizer at Kinoni and Muko sites. Encircled data points have been excluded from the regression analysis.
Fig. 5: 

(a) Relationship between (a) Response to NPK (kg ha\(^{-1}\)) 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. 2 M-K, 2 M-M, 5 M-K, and 5 M-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 input application and was on average smaller in Kinon 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 Kinon 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 Kinon 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 Kinon 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 Kinon 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 nutrient 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 Kinon. 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 N₂-fixed in Muko as more biomass could have been produced after biomass sampling.

The %Nd f a was on average lower in Muko (high potential) compared to Kinon (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 %Nd f a but was positively observed with the amount of N₂-fixed. Positive relationships were also observed between the %Nd f a and the amount of N₂-fixed and between the amount of N₂-fixed and biomass N (data not presented). The amount of N₂-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. 4.7 a, b). The %Nd f a 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 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 circled data values have been excluded from the regression analysis.
Table 4.3

<table>
<thead>
<tr>
<th>Site / Fertilizer</th>
<th>Shoot δ(^{15})N (%)</th>
<th>Shoot δ(^{15})N Range (%)</th>
<th>%Nd fa</th>
<th>Total N in shoot (kg ha(^{-1}))</th>
<th>Total Amount N(^2)-fixed (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinoni 0 P</td>
<td>0.2 - 1.7</td>
<td>4.4</td>
<td>48.5</td>
<td>32.7</td>
<td>32.7</td>
</tr>
<tr>
<td>5</td>
<td>0.0 - 0.7</td>
<td>3.8</td>
<td>89.8</td>
<td>52.1</td>
<td>52.1</td>
</tr>
<tr>
<td>30 P</td>
<td>2.2 - 1.3</td>
<td>3.0</td>
<td>63.5</td>
<td>37.0</td>
<td>37.0</td>
</tr>
<tr>
<td>30 P</td>
<td>2.6 - 1.0</td>
<td>5.9</td>
<td>106.3</td>
<td>59.1</td>
<td>59.1</td>
</tr>
<tr>
<td>Mean / Site</td>
<td>2.3</td>
<td>4.3</td>
<td>77.2</td>
<td>45.2</td>
<td>45.2</td>
</tr>
<tr>
<td>SED (Fertilizer)</td>
<td>ns</td>
<td>ns</td>
<td>3.7</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>SED (Manure)</td>
<td>ns</td>
<td>ns</td>
<td>4.4</td>
<td>5.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

SED (Fertilizer)  ns  ns  3.7  ns
SED (Manure)      ns  ns  4.4  5.2

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 NP K application were observed in plots where soil available P and exchangeable K were <10 mg kg\(^{-1}\) and <0.2 cmol\(^{+}\) 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 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 stove 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 Mukovu 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., 1990). Management options including use of manure, stacking density and height have been identified as key factors influencing climbing bean productivity (Reckling, 2011; Musoni et al., 2014; Franke et al., 2016). 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 (Niyingirwa et al., 2017). Manure is reported to increase the crop yield and responses to applied fertilizers (Rurangwa et al., 2016). People et al. (2009) reported that plant biomass has been reported to be the best predictor of nitrogen fixation (Salvagatti et al., 2009) mainly because most factors affecting nitrogen fixation also affect leaf photosynthesis and biomass production (Peoples et al., 2009).

4.2 N and P uptake and N2-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 Ojieme 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 N2-fixation (Fig. 4.7d) which was positively correlated with the biomass productivity (Fig. 4.7b). Plant biomass was positively correlated with the biomass productivity (Fig. 4.7b) which was positively correlated with the biomass productivity (Fig. 4.7b).
The Nd varied greatly between fields ranging from 1.12 to 99.7% with a mean of 39%. Observed variability in Nd is not uncommon in smallholder farming systems, and has been reported extensively. Giller, (2001) reviewing different papers reported high variation in Nd (0-73%) and amount of N2-fixed (2-125 kg N ha⁻¹). Ojieme et al. (2007) working in Western Kenya also observed variability in Nd ranging between 7-90%. People et al. (2009) reported Nd ranging between 10-151%; and Reckling, (2011) observed a variation between 13-66%.

Using maize as a reference crop has been reported to underestimate the Nd (Ojieme et al., 2007). This may explain the small Nd 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 Nd observed, environmental and management effects are reported among others to affect the Nd (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 the amount of N2-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 (Wormann, 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 beans with indeterminate growth. For this reason, there is a need for biomass sampling at different growth stages for more accurate determination of N2-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, Frank 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, Frank 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 yield responses in plots that received manure in combination with NP K fertilizer. Increased yields and responses when inputs are used together have been reported extensively (Ronne et al., 2019).
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 cmolc 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 fixed. Many researchers have reported large variability of the δ¹⁵N when maize was used as a reference crop (Ojiem et al. 2007; Chikowo et al. 2004). In our study the δ¹⁵N 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/cm³, and by computing (1000 × 0.2 × 1.3 × g N/kg soil), 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 some general validity (Janssens et al., 1990), N varies from 94-1052 kg N/ha and is higher than 20% of N uptake from the soil. With these assumptions, the δ¹⁵N 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 %Nd f, Nd fs and total N harvested in research, Reckling, (2011), used δ¹⁵N of 9 N₂-fixing weeds and observed % Nd f ranging from 11.37 to 77.97% with an average of 49%, Nd fs ranging from 31 to 410 kg N ha⁻¹ and a total N harvested ranging from 95 to 557 kg N ha⁻¹. Ojem et al. (2007) observed variations in δ¹⁵N among sites in Western Kenya for both maize and broad-leaved weeds, and maize had lower δ¹⁵N values compared to broad-leaved weeds. On average, maize δ¹⁵N was 3.20 ‰ and 5.89 ‰ for broad-leaved weeds which was closer to the 8‰ δ¹⁵N of total soil N. These authors concluded that g N₂-fixation are underestimated. The average 5.4 ‰ which is not far from that reported above for broad-leaved weeds and the corresponding δ¹⁵N of maize used as the reference crop is 1.68 to 6.31‰ compared with 0.44 to 4.10‰ (Reckling, 2011) and -0.70 to 3.74 (Ojem et al., 2007). With these findings, it remains uncertain which specific factor governs the %Nd f in the small holder farming systems characterized by heterogeneous fields.

5. Conclusion

Application of fertilizer inputs led to greater yields in all fields of the study sites. The fertilizers proved to be beneficial in increasing...
Mineral fertilizer in increasing climbing bean yield. The use of manure and fertilizer strongly increased the uptake of Na and P at both sites. Targeting the best time for biomass sampling in climbing bean for N2-fixation estimation seems to be difficult, and multiple biomass sampling at different growth stages are advised for accurate N2-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 %Nd fa observed in this study, it remains unclear which specific factors govern the %Nd fa in the smallholder farming systems. The use of a range of non-N2-fixing leguminous crops and different methods is recommended.

Acknowledgement

We thank the Bill & Melinda Gates Foundation for funding through a grant to Wageningen University to support the project N2 Africa: Putting Nitrogen Fixation to Work for Smallholder Farmers in Africa (www.N2Africa.org). We are also grateful to the participating farmers for providing the land and their enthusiastic collaboration with the trials.
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
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 DRI S 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 (DRI S). 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. DRI S 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, 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 DRI S and CND indices, indicating the usefulness of both approaches in diagnosing nutrient deficiencies in climbing bean. Despite the application of manure and NP K fertilizers, N and P indices remained negative at both sites and K at Muko 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 fertilizers inputs are major limitations to crop production.

The Northern Province of Rwanda is a major climbing bean growing area due to the favorable climatic conditions (Franke et al., 2011). 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 yield of 5 t ha\(^{-1}\) (Frank and de Wolf, 2011; Musoni et al., 2014). 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 (Nzigouheba et al., 2009). Recent studies in the Northern Province of Rwanda showed that only farmers in the wealthier categories were able to retain crop residues in their farms. There is a need for understanding the primary deficient nutrients as opposed to the commonly-used blanket fertilizer recommendations 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 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 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 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 distribution 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 Engine (DARE) is a system to diagnose and recommend fertilizer treatment for climbing beans.

The DARE system follows a decision-making process that includes data collection and storage in a database, diagnostic phases, and recommendation phases. The diagnostic phases consist of Symptom Evaluation, Plant Nutritional Diagnosis, and Management Options. The recommendation phase includes two parts: Fertilizer Recommendations and Final Recommendations. The DARE system is implemented in a computer program that can be used by farmers to diagnose their climbing bean crops and recommend appropriate fertilizer treatments. The DARE system is designed to be user-friendly and accessible to farmers without specialized knowledge in plant nutrition or disease management.
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 Worthington 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 (Khiairi et al., 2001a). However, other studies on tomato (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, 1988; Darra et al., 1992), but some researchers have suggested that norms calculated from local data may improve DRIS diagnosis. 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

2.2 Leaf sampling and analysis

Chapter 5
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, Worth 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. Malvolti (1989) recommended that the reference population should be obtained with 80% maximum yield observations. Worman 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 beans. 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 nutrient 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 Vx for nutrient x, are computed from the nutrient x and the geometric mean of all nutrients and Rd (filling value between 100% and the sum of d nutrient proportions); where d
Chapter 5

...nutrients under consideration (Parent and Dafir, 1992). The CND indices and nutrient imbalance indices (CND<sup>r</sup>) were computed for both low and high-yield subpopulations (Khiairi et al., 2001a). The calculation of CND norms was based on methods outlined by Khiairi et al. (2001b). Plant tissue forms a d-simplex (S<sub>d</sub>) made of d nutrients and a filling value (R<sub>d</sub>) defined as (Parent and Dafir, 1992):

\[
S_d = \frac{1}{d+1}
\]

The filling value computed as:

\[
R_d = 100 - (N + P + K + ...)\]

The geometric mean (G) computed as:

\[
G = \sqrt[d]{N \times P \times K \times \cdots R_d}
\]

Where 100 is the dry matter concentration (%); N, P, K, ... are nutrient proportions (%); and R<sub>d</sub> the filling value computed as:

\[
R_d = 100 - (N + P + K + ...).
\]

\[
G = (N \times P \times K \times \cdots R_d)^{\frac{1}{d}}
\]

The nutrient imbalance index of a diagnosed specimen, which is its CND<sup>r2</sup>, was computed as:

\[
r^2 = I^2_N + I^2_P + I^2_K + \cdots I^2_{R_d}
\]

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 DRI S, norms consist of average and standard deviation of dual ratio between nutrients obtained from a high-yield subpopulation. The coefficient 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 (NI I) (the sum of absolute values of indices) were computed.
Values of separate nutrient indices were computed for both low and high-yielding subpopulations (Waltworth and Sumner, 1987), based on ratios of each nutrient relative to all other nutrients using the equations provided by Waltworth and Sumner (1988). If we consider hypothetical nutrients A through N, then:

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

$$B_{\text{index}} = f\left(\frac{B}{C}\right) + f\left(\frac{B}{D}\right) + f\left(\frac{B}{E}\right) + \ldots + f\left(\frac{B}{N}\right)$$

$$N_{\text{index}} = f\left(\frac{N}{C}\right) + f\left(\frac{N}{D}\right) + f\left(\frac{N}{E}\right) + \ldots + f\left(\frac{N}{M}\right)$$

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 $CV_i$ is the coefficient of variation associated with that norm.

DRI S 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 deficiency that nutrient is relative to others (Nziguih 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:

$$\text{NII} = |I_N| + |I_P| + |I_K| + |I_Ca| + |I_Mg| + |I_Cu| + |I_Mn| + |I_Zn|$$

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

### 2.4 Statistical analysis

Statistical analysis was performed using GenStat (version 16, VSNI 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 (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 5.40% 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. 56 out of 56 plots had leaf P concentrations below the critical deficiency concentration of 0.4%. For K, 9 plots from Kinon 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 out of 56 plots, of which 11 plots were from Kinon 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 Kinon had leaf Zn concentrations below the critical deficiency concentration of 35 mg kg\(^{-1}\), and only 6 plots from Muko had leaf Zn concentrations 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 (Figure 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; Table 2). For P, 36
Concentrations (Fig. 5.1a). The relationship between leaf P concentration and grain yield showed similar patterns 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 Kinon showing a C-shaped curve with larger grain yield at small leaf K concentrations, while in the Muko plots there was a broader 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.
Fig. 5.1 Relationship between climbing bean 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). Leaf nutrient concentrations differed significantly (p ≤ 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 ≤ 0.05) and lower Ca, Mg and Mn (p ≤ 0.05) compared with the low-yielding subpopulation. Mean concentration for P % was slightly higher in the high-yielding subpopulation and leaf concentration in Cu mg kg⁻¹ was slightly higher in the low-yielding 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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Low-yielding subpopulation</th>
<th>High-yield subpopulation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>2.92 ± 0.71</td>
<td>3.50 ± 0.58</td>
<td>0.01</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.33 ± 0.14</td>
<td>0.39 ± 0.08</td>
<td>n.s</td>
</tr>
<tr>
<td>K (%)</td>
<td>1.24 ± 1.09</td>
<td>2.04 ± 1.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>3.54 ± 1.07</td>
<td>2.75 ± 0.97</td>
<td>0.02</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.79 ± 0.47</td>
<td>0.42 ± 0.29</td>
<td>0.01</td>
</tr>
<tr>
<td>Cu (mg kg(^{-1}))</td>
<td>4.8 ± 1.43</td>
<td>4.33 ± 1.35</td>
<td>n.s</td>
</tr>
<tr>
<td>Mn (mg kg(^{-1}))</td>
<td>112.01 ± 43.71</td>
<td>68.93 ± 18.90</td>
<td>0.001</td>
</tr>
<tr>
<td>Zn (mg kg(^{-1}))</td>
<td>19.75 ± 8.27</td>
<td>31.09 ± 6.30</td>
<td>0.001</td>
</tr>
</tbody>
</table>
### Table 5.2: Average grain yields, nutrient concentrations in leaf tissue and DRI S indices of climbing bean at Kinoi and Muko sites

<table>
<thead>
<tr>
<th>Leaf nutrient concentration</th>
<th>DRI S Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites / Fertilizer</td>
<td>Yield (kg ha⁻¹)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Kinoi</strong></td>
<td></td>
</tr>
<tr>
<td>Manure</td>
<td>1490</td>
</tr>
<tr>
<td>+ NP K</td>
<td>2800</td>
</tr>
<tr>
<td>+ NP K</td>
<td>2415</td>
</tr>
<tr>
<td>+ NP K</td>
<td>3893</td>
</tr>
<tr>
<td>+ NP K</td>
<td>3710</td>
</tr>
<tr>
<td>+ NP K</td>
<td>3710</td>
</tr>
<tr>
<td>+ NP K</td>
<td>3710</td>
</tr>
<tr>
<td>+ NP K</td>
<td>3710</td>
</tr>
</tbody>
</table>

| **Muko**                   |                 |   |   |   |    |    |    |    |    |
| Manure                      | 2628            | 3.73 | 0.40 | 2.65 | 2.51 | 0.31 | 4.98 | 131.09 | 19.31 |
| + NP K                      | 4373            | 3.65 | 0.39 | 2.32 | 2.48 | 0.31 | 4.01 | 70.27   | 29.04 |
| + NP K                      | 3710            | 2.74 | 0.37 | 0.26 | 4.37 | 1.03 | 4.37 | 96.71   | 29.04 |
| + NP K                      | 3710            | 2.74 | 0.37 | 0.26 | 4.37 | 1.03 | 4.37 | 96.71   | 29.04 |
| + NP K                      | 3710            | 2.74 | 0.37 | 0.26 | 4.37 | 1.03 | 4.37 | 96.71   | 29.04 |

| SED fertilizer             | 146.8           |  |   |   |    |    |    |    |    |
| SED manure                 | 146.8           |  |   |   |    |    |    |    |    |
| 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 ≤ 0.05.

*Adapted and translated from the original text.*
The means and CVs of the selected nutrient ratios, for high-yielding populations (DRI S norms) are presented in Table 3. The variances of the nutrient ratios of the low- and high-yielding subpopulations differed significantly (p ≤ 0.05) except for N/P, K/P, Zn/P, Ca/Cu, Mn/Ca and Mn/Mg. These norms were used to calculate DRI S nutrient indices and Nutrient Imbalance Indices. Looking at the DRI S 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. However, at Muko, higher grain yield was observed at higher Zn index (Fig. 5.2h).
<table>
<thead>
<tr>
<th>Ratio</th>
<th>Low-yielding population</th>
<th>High-yielding population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>CV (%)</td>
<td>Mean</td>
</tr>
<tr>
<td>N/P</td>
<td>10.0</td>
<td>5.9</td>
</tr>
<tr>
<td>K/N</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>N/Ca</td>
<td>0.98</td>
<td>5.5</td>
</tr>
<tr>
<td>Mg/N</td>
<td>0.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Cu/N</td>
<td>1.76</td>
<td>4.3</td>
</tr>
<tr>
<td>Mn/N</td>
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</tr>
<tr>
<td>Zn/N</td>
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<tr>
<td>K/P</td>
<td>3.91</td>
<td>7.9</td>
</tr>
<tr>
<td>P/Ca</td>
<td>0.1</td>
<td>0</td>
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<tr>
<td>P/Mg</td>
<td>0.68</td>
<td>7.9</td>
</tr>
<tr>
<td>Cu/P</td>
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</tr>
<tr>
<td>Mn/P</td>
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<td>6.4</td>
</tr>
<tr>
<td>Zn/P</td>
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<tr>
<td>K/Ca</td>
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<tr>
<td>K/Mg</td>
<td>3.7</td>
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</tr>
<tr>
<td>K/Cu</td>
<td>2860.58</td>
<td>94.8</td>
</tr>
<tr>
<td>K/Mn</td>
<td>126.23</td>
<td>102.43</td>
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<tr>
<td>K/Zn</td>
<td>6.909</td>
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<tr>
<td>Ca/Mg</td>
<td>5.78</td>
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<td>Ca/Cu</td>
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<tr>
<td>Mn/Ca</td>
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<td>Ca/Zn</td>
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<td>Mg/Cu</td>
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<td>Mn/Mg</td>
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<td>Mg/Zn</td>
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<tr>
<td>Cu/Mn</td>
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<td>43.3</td>
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<tr>
<td>Cu/Zn</td>
<td>0.29</td>
<td>45.4</td>
</tr>
<tr>
<td>Mn/Zn</td>
<td>6.71</td>
<td>46.4</td>
</tr>
</tbody>
</table>

*Table 5.3* The means and coefficient of variation (CV%) of nutrient ratios for low and high-yielding populations.
3.4 CND norms

The CND norms, which are the means and standard deviations of VN, VP, VK, VCa, VMg, VCu, VMn and VZn, for the high-yielding subpopulation, are presented in Table 5.4. The variances of the low and high-yielding subpopulations differed significantly (p ≤ 0.05) for VN, VP, VCa, VMg, VMn and VZn. The variances for other nutrients did not differ significantly (p ≤ 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 r² 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. CND r² was 36.01 and 10.46 for Kinoni and Muko respectively.
Table 5.4  

<table>
<thead>
<tr>
<th></th>
<th>Low-yielding population</th>
<th>High-yielding population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Row-centred log-ratios</strong></td>
<td><strong>Mean</strong></td>
<td><strong>S.D.</strong></td>
</tr>
<tr>
<td><strong>VNa</strong> 2.59</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td><strong>VP</strong> 0.34</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td><strong>VK</strong> 1.09</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td><strong>VCa</strong> 2.76</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td><strong>VMg</strong> 1.10</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td><strong>VCu</strong> 6.14</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td><strong>VMn</strong> 3.02</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td><strong>VZn</strong> 4.77</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td><strong>VRd</strong> 6.06</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

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 Kinon 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 5.4 c, d). The positive indices indicate that these nutrients are sufficient while the negative indices are deficient in the leaf tissue.

At Kinon the order of nutrient limitation is as follows: 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, and Zn. The continuous lines are regression lines for CND and DRIS indices at Kinoni (R² = 0.85) and Muko (R² = 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 assume that the distribution of observations at low yield is skewed (Walworth and Sumner, 1987, Wairegi and Van Asten, 2011).

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 (Kinon) 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, Nziguhebza et al.
1.04 observed negative 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 in addition may have contributed to the negative indices observed regardless of manure and NP K fertilizer applied in the trials. This supports observations by Nzihenhe 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 small-holder 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.

4.2 CND and DRIS Norms

In this study, DRIS and CND gave similar results matching observations by Parent et al. (1993) working on tomato es and Parent et al. (1994a) working on potato es. Although the order of nutrient limitations 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 (Khairi et al., 2001b) and on banana (Wairigi and van Assten, 2011)). Worman et al. (1992) estimated norms for dry beans collected from various countries and reported N to be adequate and K to be the most limiting, and reported that the order of nutrient 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 beans in Northern Rwanda. We observed...
d e f i c i e n c i e s  o f  Z n ,  N,  K a n d  P  i n  K i n o n i ,  a n d  Z n ,  Mg ,  Ca ,  P  a n d  N i n  M u k o  d e s p i t e 
the addition of manure and NP K fertilizers in the experimental trials. Both DRI S 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 
DRI S 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 DRI S and CND approaches were small.

Considering the capabilities of both DRI S 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 N2 Africa: 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 DRI S 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, soybeans, and maize if inputs are used. Chapter 2 assessed how the use of inoculum combined with manure and fertilizer on common beans and soybeans 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 (Thuisman 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 (Thuisman 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
Rhizobia generally increased in the rainy season, and decreased during the dry season. Application of manure also resulted in the survival of rhizobia in soil. The population was 2.8 t ha\(^{-1}\) in previously inoculated plot and was 3.0 t ha\(^{-1}\) when re-inoculated in Season 3.

For instance, in Bugesera common bean yield significantly increased the yields of bean and soybean in plots that had been inoculated months after first sampling to populations less than 101 cells g\(^{-1}\) soil. Rhizobia numbers then decreased in all treatments in the dry season of July (ten months after first sampling) to populations less than 101 cells g\(^{-1}\) soil. We observed that the choice of the legumes to use in rotations is a key determinant in legume-cereal rotation sequence in the smallholder farming systems. Chapter 3 evaluated the effects of 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\(^{-1}\).
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 1.3 t 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 NP K alone increased climbing bean yield by 1.0 t ha⁻¹, and when NP K was combined with 2 t manure ha⁻¹, the yield increased by 2.1 t ha⁻¹ and 2.6 t ha⁻¹ when NP K 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 (%Nd f a) by the legumes, yet it remains unclear which specific factors govern the %Nd f a in the small holder 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 (DRI S). 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, followed by N, K and P; while in Muko Zn was the most limiting, followed by Mg, Ca, P.

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

Rwandan Government’s Crop Intensification Program that was established in 2008 (MINAGRI, 2009). 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.
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 Rwanda 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 beans are 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 leaves and pods throughout the growing season as well as dry grain once pods mature (Wormann et al., 2001). Figures 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 (FAOs tat, 2019)
Fig. 6.2 | Trend of soybean and bean production (t) in Rwanda from 1994 to 2017 (FAOs t a t, 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 practice in increasing crop productivity (Zingore et al., 2008). Besides 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 (Chapter 2 and 4). It also improves the survival of rhizobia in soil by creating a favorable environment for their growth and multiplication (Chapter 3).

Manure is reported to improve soil carbon, increase water-holding capacity, neutralizes acidic conditions, supplies exchangeable bases and other micronutrients (Zingore et al., 2008).

However, low-quality manure generally causes immobilization of N in the first season, but can release large amounts of N in subsequent seasons (Nyangara 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.
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 (Rutungay 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 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 organic fertilizers and improved seeds for selected priority crops 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 organic and/or synthetic fertilizers on nutrient uptake by climbing bean, N₂ 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 heads of cattle were able to invest organic manure in food crop production. Based on the number of ears, Chapter 2 Section 4.4 supports the findings of Bucagu et al. (2014) who showed that farmers with 0.5 to 2 ha of lands and one or two heads of cattle were able to invest organic manure in food crop production.
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 household 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 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. Apart a 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 Huyn district. TC is the sum of the costs of inputs (seeds, fertilizers and insecticides). Details of unit costs for seeds, fertilizers and insecticides and grain prices are presented in Table 6.1 below.
Table 6.1  

<table>
<thead>
<tr>
<th>Input costs (US$ ha⁻¹)</th>
<th>Soybean Seeds</th>
<th>Bush bean seeds</th>
<th>Climbing bean seeds</th>
<th>Urea</th>
<th>NPK</th>
<th>TSP</th>
<th>DAP</th>
<th>Manure (2 t ha⁻¹)</th>
<th>Manure (5 t ha⁻¹)</th>
<th>Manure (10 t ha⁻¹)</th>
<th>Insecticide</th>
<th>Inoculant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28.0</td>
<td>46.2</td>
<td>52.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>22.0</td>
<td>54.9</td>
<td>109.9</td>
<td>17.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Grain prices (US$ kg⁻¹)  

<table>
<thead>
<tr>
<th>Soybean</th>
<th>Bush bean</th>
<th>Climbing bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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

Fig. 6.3 Net benefits  

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 partial budgeting analysis as affected by different treatments. The yield data used are averages from Busesera, Kamonyi and Kayonza (Chapter 3).

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 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 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 to 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 who do not have access to high 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) indicate that farmers with 0.2 ha of land 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 was done in this study.

The poor farmers as defined in the nationwide Ubudehe household typology (Ansooms, 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 N2 Africa 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 analyzed. 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 ing of actual maize yields and fertilization of soybean with P fertilizers resulting in higher soybean yields than 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 (Scenario 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 yields of maize and soybean (Baseline Scenario); ii) a double increment of maize and soybean in the Baseline Scenario (Scenario 2); iii) 80% of simulated water limited maize yields

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Chapter 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 baseline 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 food self-sufficiency increased from 11% to 83%, Musenenyi (Bugesera) from 44% to 82%, Rukara (Kayonza) from 20% to 84%, Burerwa from 41% to 82% and Gakenke from 43% to 82% for baseline scenario to scenario 3 respectively. Burerwa 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 (Hendrix 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 site at the 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 (Heinse 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 N2 Africa 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 (Hengdijk 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 rainy season starting from February to June while the short rainy 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 rainfed. This dependence 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 took longer to mature compared with common bean. In these situations early maturing soybean varieties may be recommended in soybean-maize rotations.

Legume-cereal rotations have many advantages. Legumes get part of their N requirement through N2-fixation, thus sparing some of the soil N to the subsequent crops in addition to the residual N from nodules senescence and fallen leaves (Osunde et al., 2003). There are also other rotational effects such as reduction of pests and...
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6.3.3 Crop diversification as an option to mitigate climate shocks in smallholder farming systems

Crop diversification through intercropping is reported as an alternative to crop rotation in drier regions. Intercropping systems are intended to reduce risks during poor growing seasons and the distribution of rainfall within a season, the enhanced productivity of intercrops in low fertility soils may be of great importance for the poorer farmers who are cultivating on poor soils and achieving 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, 1990; Willey, 1990). The success of an intercropping system is reported to be dependent on many factors including intercropped species, their spatial arrangement, densities per species, and stance, intercropping cereals with climbing beans 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 major grain legume in Rwanda especially in the highlands of the Northern Province. It

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 unable to invest in fertilizers (both inorganic and organic) to produce surplus food.

2000; Franke et al., 2019).
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 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 showed that the inherent soil fertility characteristics affected the impact of applied nutrient inputs on productivity of climbing bean (Frank 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 %Nd fa was observed and it remained unclear which specific factors govern the %Nd fa 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 dryer agroecological zone of Bugesea 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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References
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 recognized 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, nutrient 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) (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 uniform management to assess their residual effects on maize productivity. We observed greater yields, N and P uptake in treatments that previously 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 farmers’ fields (Chapter 3), bean and soybean were grown with the experimental units of the first season (Chapter 2), split into halves with one
half uninoculated and the other inoculated. Trials were established in Bugesera and Kmonyi, but Kayoza 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 Kmonyi respectively, and was 2.3 and 2.4 t ha⁻¹ in previously manured plots for Bugesera and Kmonyi 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 Kmonyi 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 rhizobial 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 10⁴ cells g⁻¹ soil 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 Kmonyi 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 rhizobial survival in the two sites.

In Chapter 4, we evaluated the effect of mineral N, P and K fertilizer (both alone and in combination) and manure on yields of climbing bean in Kibutu and Mukobwe 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 starch yield at both sites. However, there was large variability in yields among fields. In all fields, yields increased with NPK and/or manure addition, and fields in Kino 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. Variability in yields and response to inputs observed between sites may be linked to the inherent 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. Input 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 %Nd fava varied greatly between fields ranging from very small to very high, and was on average lower in Muko compared to Kino. Positive relationships were observed between the %Nd fava and the amount of N2-fixed, and between the amount of N2-fixed and biomass N. The amount of N2-fixed was increased by application of fertilizer and manure, and was positively influenced by biomass N. Similar positive relationships were also observed for grain yield and N and P uptake.

Looking at the limited resources of many farmers, especially in the densely populated highlands of the Northern Province of the country, Chapter 5 was initiated to understand and identify the limiting nutrients to climbing bean production. Two approaches (Composite Nutrient Diagnosis (CND) and the Diagnosis and Recommendation Integrated System (DRISS) 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. DRISS 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 Kino, and Zn, Mg, Ca, P and Ni in Muko. Both DRISS and CND norms derived in this study proved to be reliable for diagnosing...
Nutrient imbalances in climbing beans in Northern Rwanda, and overall differences between the DRI S 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 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) . Other options such as intercropping legumes-cereal may be evaluated in farming systems in Rwanda, especially in drier agro-ecological zones.
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List of publications

Peer reviewed publications


Non-peer reviewed publications


PE&RC Training and Education Statement

Review of literature (6 ECTS)
- Effects of environment, management practices, legume and rhizobium genotype on nitrogen fixation and yield of common bean and soybean in 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; 
- 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; 
- 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)
- 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)
- 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 year weekend (2011)
- Meeting with Bill Gates (2011)
- PE&RC Day (2014)
- PE&RC Last year weekend (2014)
- PhD Carousel (2017)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)
- Rwanda agriculture board seminars; Rwanda (2012)
- 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)
- 2nd 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 Musha 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 École des Sciences de Byimanana 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 (Current: 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-CA project to pursue an MS 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 MS, Edouard was promoted as an Associate Research Fellow 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 onward, Edouard is leading the soybean programme at RAB, in charge of research and technology transfer activities.
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After the defence, there will be a reception.

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

On Monday 9 December 2019 at 11:00 hours in the Aula of Wageningen University, Generaal Foulkesweg 1a, Wageningen

Invitation

You are cordially invited to attend the defence of my PhD thesis.