

# Light and nutrient capture by common bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) in the Northern Highlands of Tanzania

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## Cover photo

Maize-bean intercrop at N2Africa demonstration trial H1 (Kyomo, Kilimanjaro Region, Tanzania)



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## Summary

Beans and maize are important food and cash crops for farmers in the Northern highlands of Tanzania, who often grow them together on one piece of land. The simultaneous growing of multiple crops in one field is often implemented to achieve efficient land use, to avoid risk and to improve soil fertility. Farmers commonly intercrop their maize and beans in alternating crops rows: one-by-one, or moja-moja in Swahili. The practice of planting maize and beans in a two-by-two (mbili-mbili in Swahili) alternating rows design has been introduced relatively recently. These cropping designs are referred to as moja and mbili cropping designs in this report. The mbili design had been introduced from Kenya and was proposed as a method to improve light availability for beans.

The aim of this thesis study was to further explore these two intercropping designs in Tanzania, in terms of resource capture. There was a special interest in light availability for beans, the proportions of nitrogen fixed by beans in the different cropping designs, and the differences between local and improved varieties of beans and maize. The study was part of the N2Africa project, which aims to show African smallholder farmers the potential benefits of nitrogen fixation by leguminous crops. One of the approaches of N2Africa is to install demonstration trials, on which various legume production technologies and varieties are shown to farmers. The Northern highlands of Tanzania are one of the focal areas of the project. During the second rainy season of 2016, the moja and mbili intercropping designs with maize and beans were included for the first time on six demonstration trials in the Kilimanjaro Region. Each of those trials consisted of six plots: (1) sole-cropped local bean, (2) sole-cropped improved bean, (3) sole-cropped improved maize, (4) moja-intercropped local beans and local maize, (5) mbili-intercropped local beans and local maize, (6) mbili-intercropped improved beans and improved beans.

The setup of the demonstration was not ideal for comparing sole and intercropped designs, because there were no plots available with sole-cropped local maize or with a moja-intercropped improved varieties. The moja design that was available (with local maize and bean varieties), represented the common intercropping practice in the region: without the use of fertilizers and insecticides. Those inputs were used on all other plots, however. The moja design was almost an additive design compared to sole-cropped maize, but the spacing between maize rows was slightly larger in the moja-intercrop.

Relative yields of local maize were reduced by moja intercropping, compared to sole cropping. The increased total plant density sometimes compensated for the relatively poor performance. For the mbili intercrops, relative yields of the local and the improved bean and maize varieties were as expected based on the differences in plant densities of the intercrops and sole crops. LERs of the mbili designs were around 1 or slightly larger for most of the trials.

A larger fraction of photosynthetically active radiation (PAR) was available for beans (transmitted by the maize canopy) in the mbili intercrops than in the other designs.

Estimates of the proportion of N fixed ranged a lot. Estimates were largest for moja-intercropped local beans compared to the other designs, but too few data were available for statistical testing. On all plots except one, more nitrogen was fixed than was removed through harvesting of bean seed.

Grain and stover yields of the improved maize variety dropped faster with an increasing disease severity than for local maize. However, for similar diseases scores, yields of the improved variety were still often similar or larger compared to local maize.

It is expected that alterations in the row spacing in the mbili design may improve its land equivalent ratio. A next step would be to monitor the development of the component crops throughout the whole cropping season, but the setup of the demonstration trials would have to be altered.

# 1. Introduction

Land in the Northern highlands of Tanzania is increasingly pressured by a growing population (Misana et al., 2012). As a result of land scarcity, land is often over-cultivated and exploited (Campbell et al., 2003). Farmers indicate that soil fertility rapidly decreases and that food shortages easily occur (Campbell et al., 2003). In order to improve soil fertility and to increase crop production, many farmers intercrop their crops with legumes (Soini, 2005) because they fix atmospheric nitrogen and make it biologically available for subsequent crops (Giller, 2001). Additionally, intercropping maximizes land use, and economic risks are mitigated by producing two crops instead of one on the same area (Asfaw et al., 2012).

Annual crops that are commonly intercropped in the Tanzanian highlands are maize and beans (Soini, 2005). Common beans (*Phaseolus vulgaris* L.) are mainly grown for subsistence and sale, and they are an important source of protein and carbohydrates in the diet of most Tanzanians (Hillocks et al., 2006). After Kenya and Uganda, Tanzania produces the largest quantities of beans in Africa. In recent years, annual production of dry beans was close to 1,200,000 tonnes (FAO, 2014). Maize is another important staple crop of the rural poor in Tanzania, with a consumption of 73 kg per capita per year, constituting one-third of caloric intake (Minot, 2010). In the regions with bimodal rainfall, maize is produced in both rainy seasons. Maize harvesting in the unimodal regions occurs well before the maize of the second rainy season in other parts of the country is ready, resulting in a year-round domestic production (Barreiro-Hurle, 2012). Maize is grown by more than 80% of the Tanzanian farmers, and primarily consumed by the producing household and a small surplus is sold at the local markets as a source of cash (Minot, 2010). Maize surpluses beyond home consumption are mainly found in the Southern highlands (largely as a result of government support), whereas maize deficits sometimes occur in the Northern highlands (Barreiro-Hurle, 2012).

Regional bean yields have remained well below yield potential with an average yield of 0.6 t ha<sup>-1</sup> versus a potential of more than 2 t ha<sup>-1</sup> (Hillocks et al., 2006). This indicates that the cultivated crops are experiencing stress, which can have various causes ranging from water or nutrient shortages to damage by pests. Besides these external impacts, traditional planting practices may actually not be optimal for certain crop varieties, systematically resulting in yields below the potential. Spatial arrangements of crop rows in an intercropping design could influence the combined or individual performance of the component crops.

Farmers often intercrop beans and maize as alternating rows, locally referred to as 'moja moja' (in Swahili) intercropping (literally 'one one'). The Sustainable Agricultural Centre for Research Extension and Development in Africa (SACRED-Africa) introduced a two-by-two staggered row arrangement to allow for more light to reach the understorey beans (Woomer et al., 2004). It was called 'mbili mbili' (in Swahili), meaning 'two two', and it is also an acronym for 'Managing Beneficial Interactions in Legume Intercrops' (Mucheru-Muna et al., 2010). The one-by-one and two-by-two staggered row intercropping designs are hereafter referred to as *moja* and *mbili* intercropping.

A study by Mucheru-Muna (2009) revealed that mbili intercropping can result in a financial profit benefit as high as 40% compared with moja intercropping as a result of larger maize yields. Besides, the mbili system gave higher yields in a wide range of rainfall levels. It was emphasized, however, that modest nitrogen application was recommended to sustain yields. Woomer et al. (2004) found land equivalent ratios (LER) of 2.0 and 1.7 for mbili and moja intercropping, respectively. The LER for mbili intercropping was significantly larger than for moja intercropping. These values reveal that the level of production per area was larger for crops in an intercropped than in a sole-cropped design, and this was mainly attributed

to maize. Ndung'u et al. (2005) found bean yields of 1.23 kg ha<sup>-1</sup> in mbili designs, and of 1.04 kg ha<sup>-1</sup> in moja designs, whereas maize yields were not significantly different.

This thesis study focuses on an exploration of moja and mbili intercropping of beans and maize in terms of light and nutrient capture as part of the objectives of the N2Africa project. N2Africa is a research-and-development project showing African smallholder farmers the potential of leguminous crops, using air as a free, infinite source of nitrogen to be turned into sources of nutrition or cash. The project is led by Wageningen University and Research and collaborates with other development partners in various countries of Africa. Phase II of the N2Africa project is active in various African countries, the core including Tanzania, Uganda, Ethiopia, Ghana and Nigeria. The Northern highlands of Tanzania are one of the focus areas of N2Africa. N2Africa now aims to further study moja and mbili intercropping practices and to find out how to intensify productivity of bean and maize, as well as optimizing the nitrogen fixation potential of beans.

## 1.1 Sharing resources

Compared to sole cropping, intercropping can introduce additional sources of stress because crops start competing for light, space, nutrients and water. Interspecific competition (maize-bean) occurs on top of the already present intraspecific competition (maize-maize and bean-bean), and that may result in poorer performance of the component crops. However, if interspecific competition is less than intraspecific competition, there is no problem in intercropping. Crops may even facilitate each other, for example by increasing nutrient availability and acquisition (Brooker et al., 2015). Interactions can be quite complex and crops may compete for one factor but facilitate each other with regard to another factor.

Maize and common bean strongly differ in their shoot and root architectures, so they make use of light and soil nutrient resources to variable extents (Lynch & White, 1992). Planting a mixture of C4 and C3 species (often a tall/short combination) improves functional complementarity as a result of their differences in plant stature and mechanisms of photosynthesis. The light saturation level for photosynthesis is higher for C4 than for C3 crops (Trenbath & Francis, 1986). A tall C4 crop like maize can make use of the higher light intensity, while reducing the light intensity for an understorey C3 crop like beans. This can lead to an increase in radiation use efficiency (RUE) of the C3 species (Gou, 2017). A larger RUE does not automatically imply a larger yield, but the mean LER of C3/C4 intercrops is significantly larger than for C3/C3, according to a meta-analysis by Yu et al. (2016).

The exact cause of any benefit of nitrogen fixation for intercropped cereals has been debated. A facilitative effect of nitrogen fixation exists if fixed nitrogen is directly transferred to the cereal through root exudates. Proof on the transfer of nitrogen in legume intercrops remains limited. Evidence of significant transfer mainly originates from studies on long-term mixed grass-legume swards, although contradictions arise even in these crop systems (Giller et al., 1991). It appears to be more likely that benefits for the intercropped cereal are a result of 'nitrogen sparing'. Nitrogen fixation is stimulated by maize reducing soil N availability for beans. Therefore, beans make more use of atmospheric nitrogen and less of soil nitrogen, which remains available for the cereal (Giller, 2001; Hardarson & Atkins, 2003; Li et al., 2003).



## 1.2 Study objectives

The aim of this study is to understand yields of local and improved varieties of maize and beans in intercropped and sole-cropped plots, by investigating light and nutrient capture. Insight in the capture of resources by crops in common planting patterns is useful in the development of alternative planting recommendations that optimize light and nutrient use and yields of maize and beans.

In order to develop targeted farming practice recommendations that fit the needs of the regional farmers, it is important to supplement quantitative crop data with qualitative information obtained by interviewing farmers in the study area. Insight in their reasons for either intercropping (with legumes) or sole cropping, and comprehension of the yield constraints they most commonly encounter, places the agronomical field data into their socio-cultural context.

The following research questions are addressed in this study:

- Does intercropping increase productivity of maize and/or beans compared to sole cropping?
- Does intercropping increase the relative and absolute levels of nitrogen fixation by beans?
- Can differences in bean yields be explained by light availability in contrasting cropping designs?

Additionally, the local and improved varieties of maize and bean are compared in terms of disease resilience, nutrient uptake and chlorophyll contents.

## 2. Methodology

### 2.1 Study area

In Tanzania, the N2Africa project Phase II has established numerous demonstration trials in the districts Moshi Rural, Moshi Urban, Hai, Arumeru and Lushoto, since 2013 (Woomer et al., 2014). These trials consist of six plots, each exhibiting a specific legume variety or production technology. The trials are intended to create awareness among farmers of various approaches to legume production. Farmers are invited to come and have a look at the trials, and they receive information during extension days facilitated by N2Africa staff. Farmers may try technologies of their liking on their own land, and their feedback assures a co-development of production technologies with farmers and researchers.

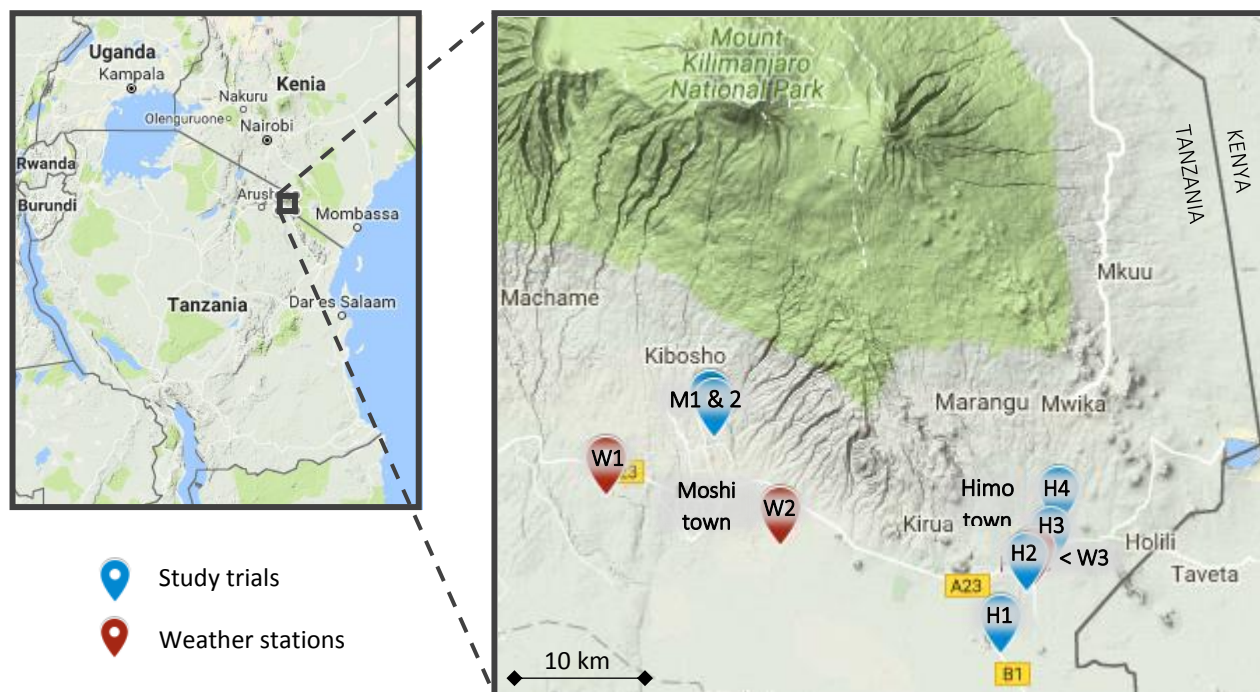
The study area covered six N2Africa demonstration trials in the Kilimanjaro region in Northern Tanzania. The demonstrated legumes were common bush beans (*Phaseolus vulgaris* L.) and the demonstrated technologies included intercropping of beans with maize (*Zea mays* L.), and the practices of row planting, the application of inorganic fertilizer and pesticides, regular weeding, specific row and plant spacing, and improved varieties. A detailed description of the setup of the trials is given in Section 2.1. This season (2016A) was the first time that the demonstration trials included not only beans but also maize, because intercropping is so common in the Kilimanjaro Region.

The study area is characterized by a volcanic soil type, rich in magnesium and calcium (Sarwatt & Mollel, 2006). The cropping season is bimodal, with a long rainy season (*Masika*) between March and June, and a short rainy season (*Vuli*) from the end of October to December. Field research took place during the long rainy season of 2016.

The region is divided into seven administrative districts, including Moshi Urban, Moshi Rural and Hai. Four of the study trials were situated in Moshi Rural, near Himo town, and hence coded H1-4. The other two trials were located in Moshi Urban district in the outskirts of Moshi town and coded M1 and M2. A map with the trial locations is shown in Figure 1 and the exact geographical location, altitude and the crops of the previous season are listed in Table 1. In the cropping season before this study took place (the long rainy season of 2015), and on the same pieces of land, all farmers produced maize and all but one farmer produced legumes. They had used various approaches of intercropping and sole cropping even within one field. They all used some nitrogen fertilizers in season 2015B but the quantities are unknown. Figures 2A and B show the accumulative rainfall and average mid-day temperatures in the study area. Soil characteristics of all trial locations are included in Table 2.

**Table 1.** Locations of the four trials near Himo town (H1-H4), and the two trials in Moshi town (M1, M2). Rates of applied fertilizer in season 2015 B, were unknown.

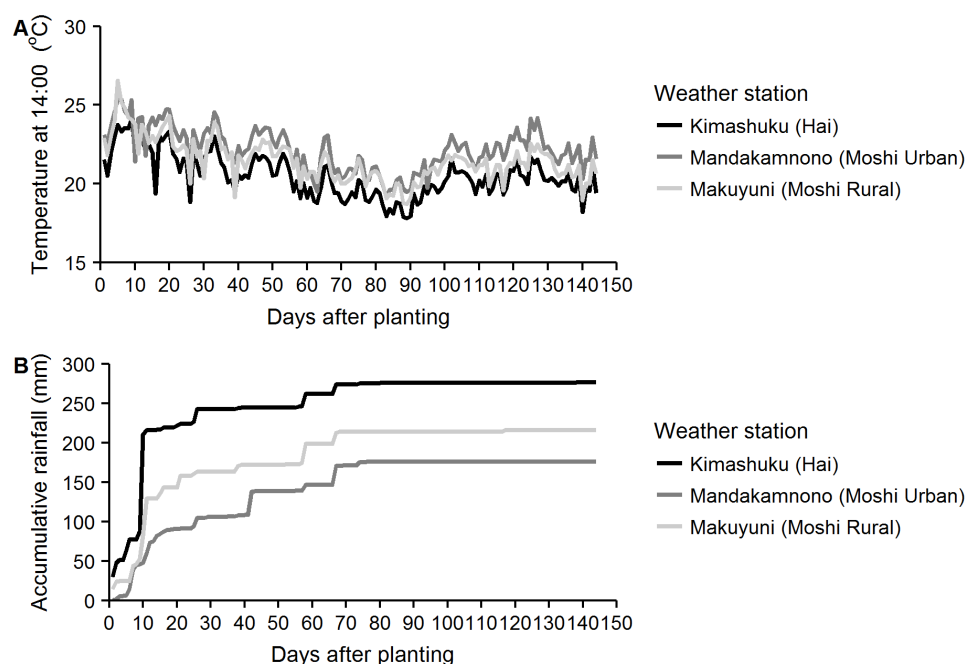
Trial	Latitude	Longitude	District	Elevation (masl)	Previous season (2015 B)
					<i>Crops</i> <span style="float:right"><i>Fertilizer</i></span>
H1	-3.454556	37.531533	Moshi Rural	727	Maize, sorghum, lablab <span style="float:right">UREA</span>
H2	-3.411917	37.554614	Moshi Rural	795	Maize, beans <span style="float:right">UREA</span>
H3	-3.393847	37.566583	Moshi Rural	833	Maize, beans <span style="float:right">NPK, UREA</span>
H4	-3.366175	37.571308	Moshi Rural	925	Maize, sunflower <span style="float:right">NPK, UREA</span>
M1	-3.305136	37.334925	Moshi Urban	996	Maize, beans <span style="float:right">UREA</span>
M2	-3.300719	37.333069	Moshi Urban	1014	Maize, beans <span style="float:right">DAP, UREA</span>



**Figure 1.** Study area. Blue markers indicate the six field trials (M1,M2, H1-4) in the Kilimanjaro Region, Tanzania. Red markers show the locations of weather stations (W1: Kimashuku village, W2: Mandakamnono village, W3: Makuyuni village). Map data © 2016 Google.

**Table 2.** Soil characteristics for each trial. Soil samples were taken in April 2016, prior to the installation of demonstration trials.

Trial	Texture	Clay (%)	Silt (%)	Sand (%)	pH	EC (mS/cm)	OC (%)	P (Bray) (ppm)	P (Olsen) (ppm)
H1	SCL	25	18	57	7.55	0.155	1.13	NA	46.40
H2	SCL	29	52	19	7.22	0.188	1.74	NA	119.20
H3	C	41	36	23	6.83	0.211	1.45	49.71	NA
H4	C	45	20	35	6.76	0.090	1.38	49.93	NA
M1	C	63	24	13	6.49	0.107	2.15	14.36	NA
M2	C	65	22	13	6.35	0.074	2.00	37.93	NA
Trial	N (%)	Ca (cmol+ kg <sup>-1</sup> )	Mg (cmol+ kg <sup>-1</sup> )	K (cmol+ kg <sup>-1</sup> )	Na (cmol+ kg <sup>-1</sup> )	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
H1	0.06	13.77	4.78	1.91	0.39	1.97	2.83	24.57	22.46
H2	0.09	17.87	4.86	2.01	0.56	2.99	2.59	26.35	57.54
H3	0.07	13.62	4.77	1.87	0.38	3.30	2.77	33.45	84.62
H4	0.06	12.38	4.87	0.37	0.46	4.09	0.92	38.32	101.54
M1	0.10	13.74	4.19	1.63	0.28	1.54	3.28	41.58	72.31
M2	0.08	12.45	4.27	1.00	0.42	1.28	2.79	42.22	88.62



**Figure 2.** (A) Mid-day temperatures and (B) accumulative rainfall in the study area. Weather data were obtained from KUKUA weather stations in the villages Kimashuku and Mandakamnono (W1 and 2 in Figure 1); 9.5 and 10.5 km to the trials in Moshi Urban District, respectively, and in Makuyuni village (W3); 0.5-6 km to the trials in Moshi Rural District. The planting date was April 15<sup>th</sup>, 2016.

## 2.2 Trial establishment and management

Each of the six trials consisted of six plots of 10x10 m with three different cropping designs: (1) sole cropping, (2) moja (one-by-one row) intercropping and (3) mbili (two-by-two rows) intercropping. The sole cropping and the mbili intercropping designs were executed with local and improved varieties of beans and maize. The six available combinations of cropping designs and varieties are shown in Table 3. The moja intercrop on trial H4 and the treatment with a mbili design and local varieties on trial H2 had not been installed as instructed and were excluded from the dataset. Some major management practices such as irrigation, weeding and the directions of plant rows, were different for some trials, as shown in Table 4.

**Table 3** Available treatments (combinations of a cropping design and a crop variety) in the study, with repetitions across six trials at different locations. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

Cropping design	Bean variety	Maize variety	Plots in the study (N)
Sole	Improved	-	6
	Local	-	6
	-	Improved	6
Moja	Local	& Local	5
Mbili	Improved	& Improved	6
	Local	& Local	5

**Table 4.** Crop management practices at the six trial locations. All plots were planted in rows.

Trial	Row direction	Irrigation	Weeding
H1	S-N	Yes	Thrice
H2	E-W	Yes	Twice
H3	E-W	No	Twice
H4	E-W	No	Twice
M1	WNN-ESS	No	Thrice
M2	NNW-SSE	No	Once

The demonstration trials were primarily intended to demonstrate technologies and to a lesser extent to set up a research trial. Therefore, only a limited number of practices was shown and not all possible combinations of cropping designs and varieties.

Moja-intercropped plots were intended to represent the common intercropping practice in the region, which also included that no inputs were used on these plots. The sole and mbili-intercropped plots were treated with inorganic fertilizer: DAP was applied in the furrow at a rate of 100 kg ha<sup>-1</sup> and there was top-dressing of urea only on maize at a rate of 65 kg ha<sup>-1</sup>. A non-systematic insecticide (Suracron 720 EC, active ingredient: Profenofros 720 g L<sup>-1</sup>) was also applied to the crops on sole and mbili-intercropped plots.

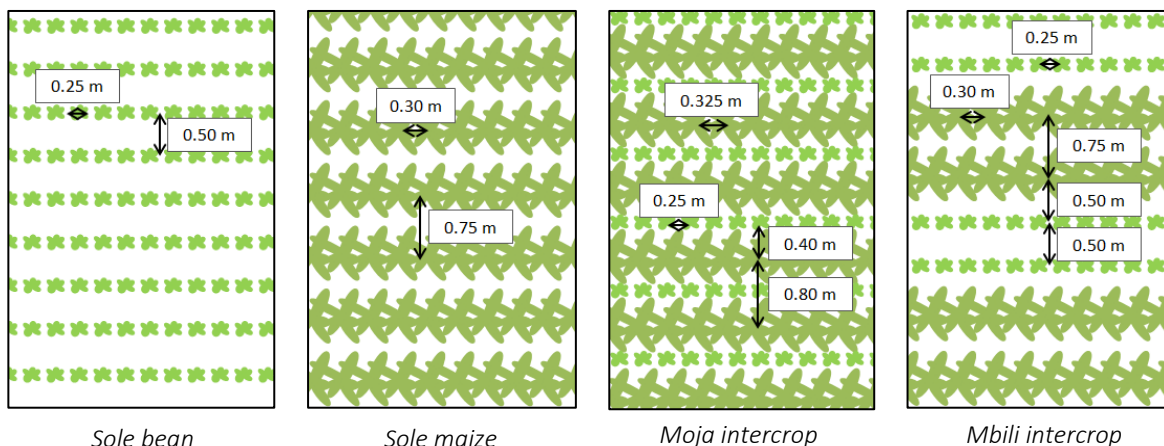
The local and improved bean varieties were Kariasii (also called Sura Mbaya) and Uyole Njano, respectively. Kariasii was locally produced and never certified. Uyole Njano was officially released by ARI-UYOLE Agricultural Research Institute (Mbeya, Tanzania) and has a yield potential of 2 t ha<sup>-1</sup> under optimal management. Uyole Njano was bred for tolerance to a range of diseases including leaf rust, common bacterial blight, halo blight, bean common mosaic virus, anthracnose and angular leaf spot (Kanyeka et al., 2007).

The local maize variety had no specific name. Farmers had been using it for generations, using saved seed from each last season. The improved maize variety was a certified hybrid variety, coded *DK 8031*, that was bred for tolerance to dry weather and to grey leaf spot, and for high yields (a potential of 5 to 8 t ha<sup>-1</sup>) even with low input farming (monsantoafrica.com, 2017).

Maize and beans were planted on the same day. Land preparation and weeding were performed manually using a hand hoe. Plant and row spacing details are shown in Table 5 and Figure 3. The timing of management practices and the measurements of this study can be found in Table 6.

**Table 5.** Plant spacing and density in the sole crops of maize and beans, and the moja and mbili intercrops: *Moja* ('one' in Swahili) intercropping: alternating crop rows. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows. *B* = bean, *M* = maize.

Design	Plants per hole	Row spacing (cm)	Plant spacing (cm)	Planting density (plants ha <sup>-1</sup> )
Sole cropping	B: 2	B-B: 50	B: 25	B: 160,000
	M: 1	M-M: 75	M: 30	M: 44,444
Moja intercropping	B: 2	B-M: 40	B: 25	B: 100,000
	M: 1		M: 32.5	M: 38,462
Mbili intercropping	B: 2	B-B: 50	B: 25	B: 66,494
	M: 1	B-M: 50	M: 30	M: 27,705
		M-M: 75		



**Figure 3.** Plot layout of in sole and intercropped bean (■) and maize (✕). Figure outlines do not represent plot boundaries.



**Table 6.** Timing of management practices at the six trials in this study. Management practices were the same for all plots within one trial. Data collection occurred on the day under “measurements” and during harvesting.

\* Maize on trial H3 was harvested by farmers before the agreed date and the yield was distributed among them. Maize yield of that trial is therefore unknown.

Trial	Planting	Days since planting			Pesticide application	Measurements	Harvesting	
		Weeding					Beans	Maize
	<i>Beans &amp; maize</i>	<i>1st</i>	<i>2nd</i>	<i>3rd</i>				
H1	22-4-2016	16	30	34	28	60	96	132
H2	19-4-2016	20	28		37	65	91	134
H3	20-4-2016	17	61		69	67	93	*
H4	15-4-2016	13	28		45	73	104	138
M1	17-4-2016	11	29	54	37	74	98	141
M2	19-4-2016	13			35	76	101	139
Average ± st. dev.		15±3	35±14	44±14	42±14	69±6	97±5	137±4

## 2.3 Data collection

### 2.3.1 Timing

At each trial, data collection occurred on three occasions: (1) just before physiological maturity, (2) at bean harvest and (3) at maize harvest. During the first visit, the following parameters were quantified: PAR interception, plant height, leaf SPAD and disease severity. During harvest, biomass samples were taken and seed and stover yield were measured.

As shown in Table 6, the crops were approximately 70 days old during the first measurements. At that moment, maize plants were in reproductive developmental stage R3 as defined by O’Keeffe (2015). During this stage, the size of the maize canopy remains stable until the crop starts to senesce (Tsubo & Walker, 2002). Beans were in pod filling stage (R7 or R8 stage), and some of the lower leaves were turning yellow.

### 2.3.2 Disease severity

In every plot - and for each of the available crops - the intensity of disease damage for each of 25 randomly chosen plants was scored on a scale of 0 to 10. This score was given for each plant and based on a visual interpretation of the proportion of plant tissue area showing symptoms of one of the four prevalent diseases: leaf rust (*Puccinia sorghi*) and grey leaf spot (*Cercospora zae-maydis*) for maize, and leaf rust (*Uromyces spp.*) and blight for beans (*Xanthomonas phaseoli* [common blight] and *Pseudomonas phaseolicola* [halo blight] were not distinguished). In some cases, there were small signs of other diseases like powdery mildew, but these were negligible compared to the predominance of the previously mentioned diseases. A score of 0 signified that the plant showed no visual signs of disease damage, a

score of 1 indicated that there was little damage (1-10% of plant tissue showed symptoms), a score of 2 meant that 11-20% of the plant was damaged, etc. A score of 10 would mean that the plant was dead as a result of disease damage. Separate scores were given for maize and beans, and for each of the four prevalent diseases, using the following formula:

$$\text{Disease severity score} = \frac{\text{sum of severity scores of sampled plants}}{N [= 25] * \text{maximum score [= 10]}} * 100$$

Essentially, the disease severity score is a rough impression of the percentage of leaf tissue in a plot that shows symptoms of a disease. However, disease severity in this report remains to be expressed as a score and not as a percentage because the proportion of leaf tissue that was affected by disease was not accurately measured; it is very likely that disease severity was overestimated (Bock et al., 2010). However, although the absolute levels of severity may be overestimations, the relative differences in disease severity are likely to be right and are therefore suitable to compare cropping designs and varieties.

From the separate disease scores, overall severity scores were calculated for beans and for maize by simply adding up the separate disease scores as follows:

$$\text{Overall severity score for beans} = \text{blight severity score} + \text{rust severity score}$$

$$\text{Overall severity score for maize} = \text{leaf spot severity score} + \text{rust severity score}$$

These overall severity scores per crop were used in all following linear modelling analyses, to account for the effects of disease when assessing the effects of other parameters.

Additionally, any visible signs of (micro) nutrient deficiencies and pest damage were noted for each plot.

### 2.3.3 PAR interception

Photosynthetically active radiation (PAR) is radiation in the 400-700 nm waveband, which is the portion of the radiation spectrum that is used by plants for photosynthesis. Biomass production is directly related to the amount of PAR intercepted by a crop canopy (Monteith and Moss, 1977). Total radiation available for plants is a combination of direct and diffuse light, and a sum of radiation transmitted by the canopy, scattered by leaves, and reflected by biomass and the soil. In this study, reflection from the soil was ignored for simplicity.

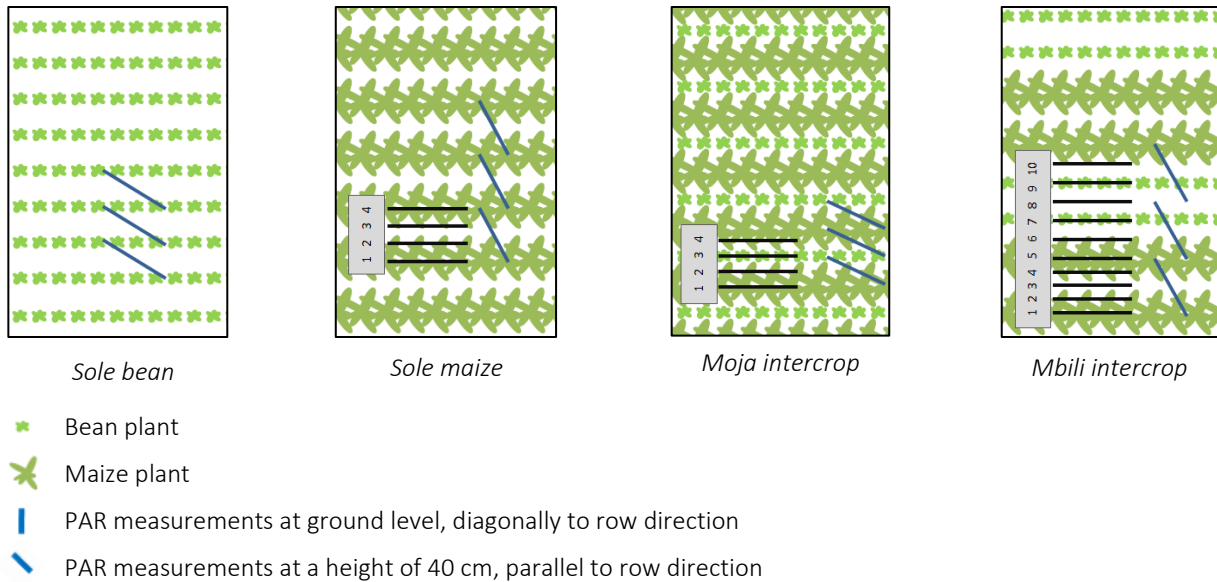
Because data collection took place during the rainy season, clear skies were rare. Therefore, PAR measurements were taken on overcast days with approximately only diffuse radiation, between 11:00 and 14:00. The fraction of PAR intercepted by the canopy was measured using the AccuPAR LP-80 (Decagon Devices Inc.). This tool consisted of a probe of 86.5 cm with eighty PAR sensors along its length, and an external spot sensor which was placed at a height of approximately 3 m, allowing for simultaneous PAR measurements above and below the canopy.

Figure 4 illustrates the following probe placements in the various cropping designs:

(a) To assess the proportion of PAR intercepted by the total canopy (for maize and/or beans), measurements were taken at ground level: the probe was held diagonally to cover the space between rows (the probe base and tip were placed at the crop shoots) and measurements were repeated in

different rows and at different positions within the same row, so that the average of measurements represented the fraction of light intercepted by the total canopy.

(b) To determine how much light was available (for bean plants) between rows of maize, measurements of PAR transmitted by maize were taken at 40 cm above the ground (top of most bean plants), placing the probe parallel to row direction and measuring at specific inter-row locations as shown in Figure 4. Sequences were repeated at different positions in the plot.



**Figure 4.** Probe positions of the AccuPAR LP-80 for PAR interception measurements in different cropping designs. The measurement sequences were repeated at different locations within the field.

### 2.3.4 Yield measurements

In each of the plots with beans, the number of pods per plant was counted for 30 random plants and the number of seeds per pod was determined for 30 random pods. Bean plants were uprooted and weighed. The plants were then threshed and the bean grains were weighed again. Fresh stover weight was calculated as the difference between the total weight of plants and the grain yield.

In each plot with maize, 30 random plants were cut at ground level and weighed. The cobs available in this sample were counted and separated from the stover. A sample of 9 cobs was weighed. These cobs were then threshed to determine kernel weight. Maize grain yields per plot were then calculated with use of the following formula:

$$\text{Fresh maize yield per plot} = (\text{kernel weight of sample} / 9) * \text{number of cobs per 30 plants} * (\text{total number of maize plants in plot} / 30)$$

The total number of plants in a plot were calculated based on the number of rows and the number of plants per row. Maize on trial H3 was harvested by farmers before biomass samples were taken. No yield data are available for maize on that trial.

For assessment of dry matter content and in preparation of material for nutrient analysis, samples of seed and stover were brought to the Tanzania Coffee Research Institute (TaCRI, Lyamungu-Moshi,

Tanzania). The biomass samples were weighed before and after drying in an oven at 70 °C until constant weight. Conversion factors between weights of fresh and dry material were used to calculate dry matter yields of bean and maize stover from the fresh stover weights that were assessed during harvesting.

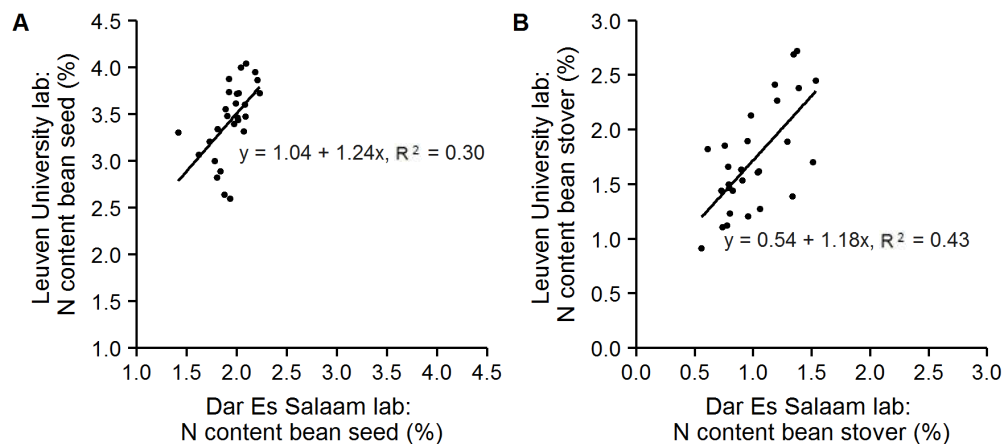
Partial land equivalent ratios (pLER) were calculated by dividing the yield of the intercrop by the yield of the sole crop (of that same variety, if possible). Because no sole crop of local maize was available, the pLER for the moja design was based on the yield of local moja-intercropped maize and the yield of sole-cropped improved maize.

Dry samples of grains and stover were ground with a simple electrical biomass grinder. Ground bean seeds and stover were sent to the IITA laboratory in Dar Es Salaam, Tanzania. for analysis of nutrient content and to the University of Leuven (KU Leuven, Belgium) for assessment of proportions of nitrogen isotopes, as explained in Section 2.3.5. Ground samples of maize biomass were also sent to Dar Es Salaam, but the results are not presented in this study because these samples were lost in the laboratory.

### 2.3.5 Soil and plant samples analyses for nutrient contents

Ashed bean biomass samples were analysed for the contents of N, Mg, K, Cu, Zn and Mn (with use of an Atomic Absorption Spectrophotometer), and P (with use of a UV-Vis Spectrophotometer). Standard samples were included to reveal any systematic errors of the method.

N contents of the same samples of beans were analysed in the IITA laboratory in Dar Es Salaam, Tanzania, and at the University of Leuven, Belgium. The analyses were compared to get an impression of the reliability of the results. Figure 5 reveals that the N contents measured in Leuven were consistently larger than those assessed in Dar Es Salaam. Besides, there was considerable spread of data around the trendlines. It is expected that the results from Leuven were more accurate than the results from Dar Es Salaam, because of the more advanced expertise and equipment available in Leuven. The comparison between laboratories suggested that results from Dar Es Salaam (also those presented in this thesis) were not entirely reliable and may be over or underestimates. It was not possible to compare results for nutrients other than N.



**Figure 5.** Comparison on N analyses of two laboratories: Leuven University and IITA, Dar Es Salaam. The labs received the same samples (same wrapping).

A soil nutrient analysis was executed for soils from all demonstration trials in the IITA laboratory in Dar Es Salaam, Tanzania, in October 2016. Soil samples had been taken by N2Africa staff prior to this study. Soils were analyzed for pH, organic carbon (Walkley-Black), total N (Kjeldahl), plant-available P (Bray and Olsen), exchangeable bases (Ca and Mg by atomic absorption spectrophotometry, K and Na by emission flame photometry), micronutrients (Cu, Zn, Mn, and Fe by DTPA extraction), and for the relative proportions of clay, sand and silt by hydrometer method. Results of this soil analysis were available on the level of whole demonstration trials, not on a plot level.

### 2.3.6 Nitrogen fixation

The relative and total amounts of nitrogen derived from the atmosphere (Ndfa) – fixed by beans – were determined with use of the  $^{15}\text{N}$  natural abundance method. This method is based on the N isotopic composition in beans and the weeds growing along with them (Ojiem et al., 2007).

The element nitrogen exists in a few variants – isotopes – differing in their number of neutrons. Two of these isotopes are stable:  $^{14}\text{N}$  (seven protons and seven neutrons, resulting in an atomic weight of 14) and  $^{15}\text{N}$  (with one extra neutron). The vast majority of naturally occurring nitrogen is constituted from the  $^{14}\text{N}$  isotope (almost 100%), whereas  $^{15}\text{N}$  is a lot less abundant (0.3663% of total atmospheric nitrogen). The  $^{15}\text{N}$  natural abundance method relies on differences in  $^{15}\text{N}:$  $^{14}\text{N}$  ratios in the atmosphere and in the soil (Shearer and Kohl, 1986). The abundance of  $^{15}\text{N}$  in a material is expressed as atom%  $^{15}\text{N}$ , which is the percentage of  $^{15}\text{N}$  atoms that are present in the total N pool of the material:

$$(1) \text{ atom}\% \text{ } ^{15}\text{N} = \frac{^{15}\text{N}}{^{15}\text{N} + ^{14}\text{N}} * 100$$

To show natural variation in  $^{15}\text{N}$  abundance, the atom%  $^{15}\text{N}$  of a material is often presented as the per mille deviation ( $\delta$ ) from atmospheric  $\text{N}_2$ , which has a constant abundance of 0.3663 atom%  $^{15}\text{N}$  (Mariotti, 1983):

$$(2) \delta^{15}\text{N} (\text{‰}) = \frac{\text{atom}\% \text{ } ^{15}\text{N}_{\text{sample}} - \text{atom}\% \text{ } ^{15}\text{N}_{\text{atmosphere}}}{\text{atom}\% \text{ } ^{15}\text{N}_{\text{atmosphere}}} * 1000$$

Legumes utilize two sources of nitrogen: the soil and the atmosphere. Therefore, the  $\delta^{15}\text{N}$  of legumes should be between those of the atmosphere and the soil. The  $\delta^{15}\text{N}$  of a non-legume represents solely the N isotopic composition of the soil. A comparison of the  $\delta^{15}\text{N}$  of a legume and a non-legume (reference plant) utilizing the same N pool, would reveal the proportion of nitrogen derived from the atmosphere (Ndfa) with use of the following formula (Shearer & Kohl, 1986):

$$(3) \text{ Ndfa} (\%) = \frac{\delta^{15}\text{N}_{\text{reference plant}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference plant}} - B} * 100$$

Plants can be used as reference plants if it can be assumed that they resemble the legume closely in terms of N uptake from the soil, if they utilize the same soil N pool as the legume, and if they do not nodulate. The  $B$  variable in formula (3) refers to the  $\delta^{15}\text{N}$  of the legume growing in a soil completely deprived of plant-available N, making the legume completely reliant on atmospheric  $\text{N}_2$ . The  $B$ -value is used to



account for isotopic fractionation of N that can occur during N<sub>2</sub>-fixation, and which can vary between species of legumes (Giller, 2001). The δ<sup>15</sup>N of a legume lacking soil N should resemble the δ<sup>15</sup>N of atmospheric N<sub>2</sub> (0‰). However, in practice, the δ<sup>15</sup>N of sampled plants is usually below zero because it is virtually impossible to collect complete root systems. Nodulated roots tend to be richer in <sup>15</sup>N compared to shoots (Unkovich et al., 1994).

It was beyond the scope of this study to experimentally determine the B-value for the common beans and soil types in the trials. The %Ndfa estimates in the Results section are shown for a range of B-values, based on recommendations from literature (see Table 7).

In this study, biomass sampling for δ<sup>15</sup>N assessment occurred at harvest (97 days after planting, on average), when pods and shoots were dry. The weeds that were used as reference plants were at mid-flowering stage. In two out of the six trials, no reference plants were available because these trials had recently been weeded. In the other four trials, there were three species of suitable weeds that grew on at least two of the remaining trials. Pictures of the reference plants are included in Appendix I.

**Table 7.** B-values used for common beans in other studies implementing the natural abundance method for assessment of proportions of nitrogen fixed from the atmosphere.

B-value (‰)	Explanation	Recommended by:
0	Equal to atmospheric δ <sup>15</sup> N, assuming no isotopic fractionation	Unkovich et al., 2007
-0.63	This was the lowest δ <sup>15</sup> N value found in the collected legume samples. By using it as the B-value, the estimated %Ndfa for this specific sample would automatically be 100%.	Peoples et al. (1992, 2002)
-2.16	Mean value of experimentally determined B-values for common beans in the tropics	Unkovich et al. 2007 (Appendix 3)

The calculation for %Ndfa was based on whole plants. Whole plant δ<sup>15</sup>N was calculated as a weighted mean of the δ<sup>15</sup>N values found for seed and stover, using the following formula (Boddey et al., 1995):

$$(4) \delta^{15}N_{\text{whole plant}}(\text{‰}) = \frac{(\text{total seed } N * \delta^{15}N_{\text{seed}}) + (\text{total stover } N * \delta^{15}N_{\text{seed}})}{(\text{total seed } N + \text{total stover } N)} * 100$$

Total amounts of seed and stover N were calculated by multiplying the biomass N contents (%) of beans with plot-level grain and stover yields.

Total N and <sup>15</sup>N contents were determined separately for bean seed, bean stover, and the different weed species at the University of Leuven (Belgium). Analysis was performed with use of a Thermo Flash HT/EA Elemental Analyzer, coupled to a Thermo DeltaV Advantage IRMS through a Conflo IV interface; with a CO<sub>2</sub> trap installed inline and using certified standards (IAEA-N1 and IAEA-N2).

### 2.3.7 Leaf chlorophyll content

Incoming PAR is absorbed by chlorophyll pigments and used as energy for photosynthesis. Chlorophyll absorbs light in the wavelength regions of 400-500 nm (blue) and 600-700 nm (red). The hand-held SPAD-502Plus chlorophyll meter (*Konica Minolta*, 2016) was used to measure the light transmittance of these

wavelength regions through the maize canopy. The tool transforms the levels of absorbance to SPAD values on a scale of -9.9 to 199.9 which are proportional to leaf chlorophyll content.

Correlations between relative chlorophyll contents and rates of photosynthesis were revealed in studies by Ma et al. (1995) in soybean and Earl and Tollenaar (1997) in coffee, showing that SPAD readings can be used as quick indicators of leaf photosynthetic rates.

Because chlorophyll contains a lot of nitrogen, chlorophyll content can also be used as an indicator of nitrogen content of the crop as a basis for decisions in fertilization management (e.g. Blackmer & Schepers, 1995; Loh et al., 2002; Ruiz-Espinoza et al., 2010).

SPAD readings were performed on the youngest mature (trifoliolate) leaf of 25 randomly-picked plants of the available crop(s) in each plot. Per maize plant, six measurements per leaf (along its whole length, on both sides of the mid-vein) were averaged. For beans, three measurements (one on each leaflet of the trifoliolate) were averaged.

### 2.3.8 Survey

A short survey was conducted among farmers from villages in the proximity of the demonstration trials to learn about the socio-cultural context of intercropping practices. The interviews were held in collaboration with another research project involving surveys from KUKUA about weather variability and the provision of weather forecasts. The regional N2Africa field liaison officer informed farmers via extension workers and village chiefs about these studies on weather and intercropping. Farmers interested in collaboration gathered in Makuyuni and Mandakamnono village, Moshi Rural district. If the farmers grew legumes, the following questions were asked directly after the interview from KUKUA, via a Swahili translator:

- (1) General: name, age, village, phone number, highest level of education
- (2) Do you (sometimes) produce legumes? Why?
- (3) Which legumes do you produce? Do you produce a local or improved variety?
- (4) Do you sell (part of) your legume production? How much do you sell?
- (5) For legume production, do you practice sole cropping or intercropping? Why?
- (6) Which crop combinations do you use?
- (7) How do you arrange your intercrops? Why?
- (8) On the fields where you produce legumes, do you use crop rotations or do you plant the same crop(s) on the same piece of land every season? Why?
- (9) How satisfied are you with your legume yields in regular years?
- (10) What are common constraints for legume production?
- (11) How can legume production be improved, do you think?

A total of 44 farmers was interviewed and they were from the villages Kimashuku (14), Matala (7), Kirumeni (6), Mandakamnono (6), Makuyuni (5), Masaera (4) and Msufuni (2). Of these respondents, 23 were male and 21 were female. The highest education level of the respondents was primary school for three-quarters of the respondents, and secondary school for the others. Only one respondent had post-secondary education.

A bias exists in the pool of respondents, because they were not randomly selected and because only those prepared to take the effort of coming to meet were interviewed. All the farmers were familiar with the N2Africa project, although to different extents.

## 2.4. Statistical analysis

Statistical analysis of the data was conducted with *R* software version 3.3.2 (2016). It was impossible to test cropping design and variety as separate explanatory factors because there is no treatment with sole-cropped local maize, and no treatment with moja intercropping and improved varieties). Instead, all different combinations of cropping designs, varieties and use of inputs, were treated as separate treatments and analysed per crop. Table 8 shows the available treatments per crop.

**Table 8.** Cropping treatments: various combinations of (inter)cropping design and local or improved varieties of maize and beans.. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows. The inputs consisted of UREA, DAP and insecticides. The fertilizers were applied only in maize furrows and not on beans.

Treatment	Cropping design	Bean variety	Maize variety	Fertilizer inputs	Insecticides used	Plots in the study (N)
1	Sole bean	Improved	-	No	Yes	6
2	Sole bean	Local	-	No	Yes	6
3	Moja	Local	& Local	No	No	5
4	Mbili	Improved	& Improved	Yes	Yes	6
5	Mbili	Local	& Local	Yes	Yes	5

Treatment	Cropping design	Bean variety	Maize variety	Fertilizer inputs	Insecticides used	Plots in the study (N)
1	Sole maize	-	Improved	Yes	Yes	6
2	Moja	Local	& Local	No	No	5
3	Mbili	Improved	& Improved	Yes	Yes	6
4	Mbili	Local	& Local	Yes	Yes	5

Linear mixed models (LMM) were used to explore effects of treatments on factors like yields, nutrient contents and light interception for instance. These models were structured as follows:

$$\text{Dependent variable} \sim \text{combined disease severity score} + \text{treatment} + (\text{random factor: trial location})$$

The assumptions of homogeneity of variance and normal distribution of variances were checked with plots of residuals and Q-Q plots. These assumptions were in most cases somewhat violated. Analyses of variance (ANOVA) were conducted nonetheless, in order to be able to provide some interpretation of the data and get an impression of differences between cropping designs and varieties.

The bar graphs and tables in this report show predicted means resulting from such LMMs. Significant effects were tested with Tukey's HSD Post-hoc Test, comparing the means using a least significant difference (LSD) at P=0.05.

The trial setup sometimes made it difficult to statistically confirm whether any differences between cropping designs (e.g. in terms of yields and light interception) are the result of cropping designs or varieties or input uses. However, educated guesses can be made based on the degree to which particular designs differ.

In the analysis of grain and stover yields of beans and maize, yields of intercrops were compared to their expected yields. The expected yields of intercrops were based on the yields of sole crops of the same trial (and of the same variety, if possible), proportional to the difference in plant densities in the intercropped and sole-cropped plots. These proportions are listed in Table 9. There was no sole-cropped local maize available in the trials, so the expected yields for local maize intercrops had to be based on the available sole improved maize. Differences between actual and expected yields of intercrops were tested with a two-sided paired t-test.

Responses to survey questions were compared for male and female respondents, different education levels, and per village, with use of a chi-square test with a significance level at P=0.05.

**Table 9.** Proportional differences in the plant densities in sole and intercropped plots, to estimate yields of intercrops. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

Crop	Design	Planting density ( t ha <sup>-1</sup> )			Expected yield = X * sole yield
		<i>Intercrop</i>	/	<i>Sole</i>	
Bean	Moja	100,000	/	160,000	= 0.63
Bean	Mbili	66,494	/	160,000	= 0.42
Maize	Moja	38,462	/	44,444	= 0.87
Maize	Mbili	27,705	/	44,444	= 0.62

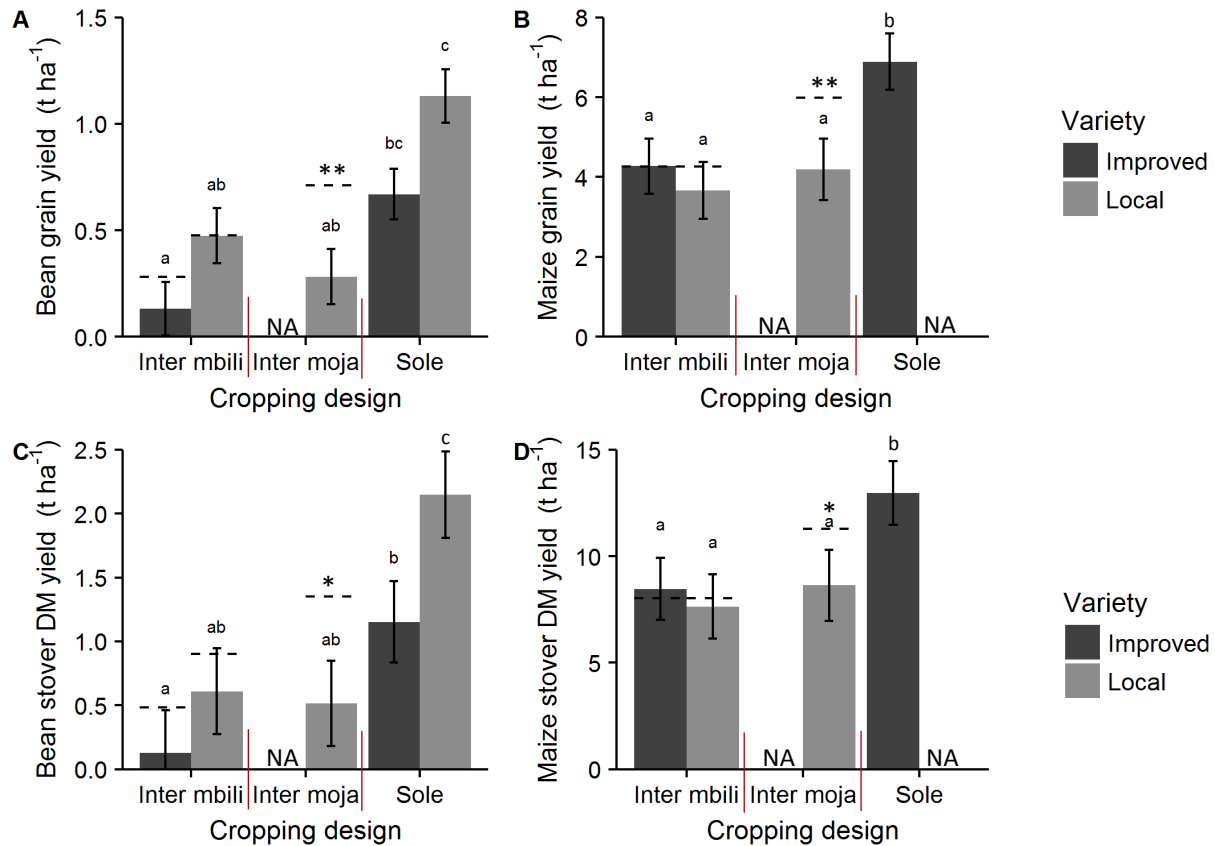
### 3. Results

#### 3.1 Grain and stover yields

Smaller yields were expected for intercrops than for the sole crops, proportional to the differences in plant densities. Actual grain and stover yields of beans and maize are shown in Figure 6, in which the dashed lines above the bars designate the expected yields. The expected yield for moja-intercropped local maize was based on the sole crop of improved maize because no sole crop of local maize was available.

Mean yields of mbili intercrops were at or slightly below the expected levels. Intercropped local beans and maize yielded less than expected ( $P < 0.01$  for grain yield, and  $P < 0.05$  for stover yield). Sole-cropped beans reached significantly larger plot-level yields than intercrops of that same variety ( $P < 0.05$ ). However, plot-level yields were similar for sole-cropped improved beans and intercropped local beans, and there were no significant differences between the yields of both varieties as a sole crop.

Maize stover yields were similar in all intercrops, regardless of the variety. Grain yields of improved maize were larger in the sole crop than in the intercropped designs ( $P < 0.01$ ).



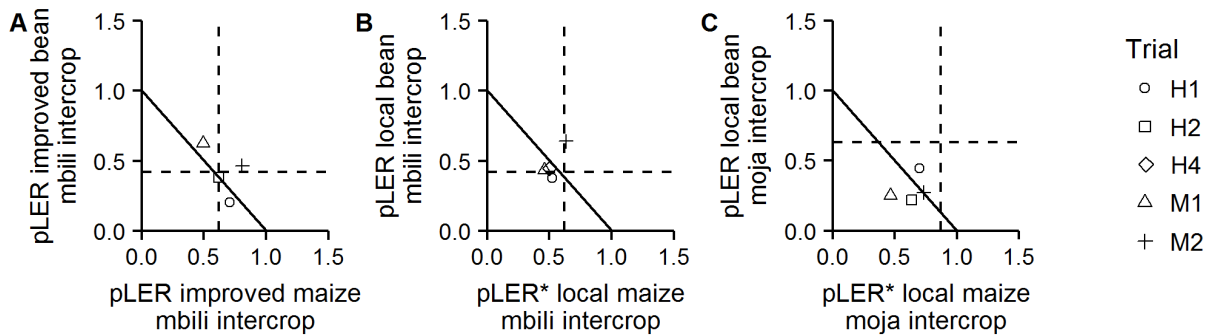
**Figure 6.** Grain and stover yields of grain and maize. Dashed lines above the bars signify the expected yields based on the different planting densities in the intercrop compared to the sole crop of the same variety (if possible). No yield data were available for sole local maize, so improved maize yields were used as a reference. Letters above the standard error bars signify significant differences (Tukey's HSD). Asterisks above the bars signify significant differences between actual (as measured in the field) and expected yields (two-sided paired T-tests) ('\*' for  $P < 0.05$ ; '\*\*' for  $P < 0.01$ ). *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.



Figure 7 shows the partial land equivalent ratios of beans and maize, based on grain yields. The pLER of local intercropped maize was calculated from the yield of intercropped local maize divided by the yield of sole-cropped improved maize, because no sole-cropped local maize was available.

The pLERs are sometimes larger than expected based on planting densities, but never for both crops at the same time. In the moja intercrop, the increased plant density reduced the yields of maize and beans. The reduced maize yields may be the result of competition by beans, or by comparing the yield of the local maize intercrop with the improved sole maize. Despite the relatively poor performance of the component crops, the LER was 1 or larger on trials H1 and M2, although lower than 1 on the other trials.

For mbili-intercropped maize, it differed across trials whether an LER close to 1 was mainly attributed to relatively large yields of maize or beans.



**Figure 7.** Partial land equivalent ratios (pLER) of beans versus the pLERs of maize, based on grain yields. Dashed lines signify the plant density of the intercrop compared to the sole crop. No data on maize yields were available for trial H3, no moja-intercropped plots were available on trial H4, and there was no yield on the plot with sole-cropped improved beans on trial H4. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows. \*The pLER values of local maize were calculated from the yield of intercropped local maize divided by the yield of sole-cropped improved maize, because no sole-cropped local maize was available.

Largest bean yields were measured on trial H1: 1.72 and 1.25 t ha<sup>-1</sup> for improved and local sole-cropped beans, respectively. The smallest bean yields were found on trial H4: bean yields in all of the plots were below 0.32 t ha<sup>-1</sup> and the sole-cropped improved beans even yielded absolutely nothing. Trial H4 had received less rainfall during the reproductive stages of development, according to the farmers who owned the land. Also for maize, the smallest yields were found on trial H4. Maize grain yields were largest on trial M1 and H1, with 7.7 and 7.8 t ha<sup>-1</sup> for sole-cropped improved maize. Bean and maize yields in intercrops were also slightly larger on trial H1 and trial M1, in comparison to the other trials.

The weight of 100 seeds and harvest indices of beans and maize, and the number of pods per plant and number of seeds per pod of beans (Table 10) were not significantly different per crop variety or cropping design.

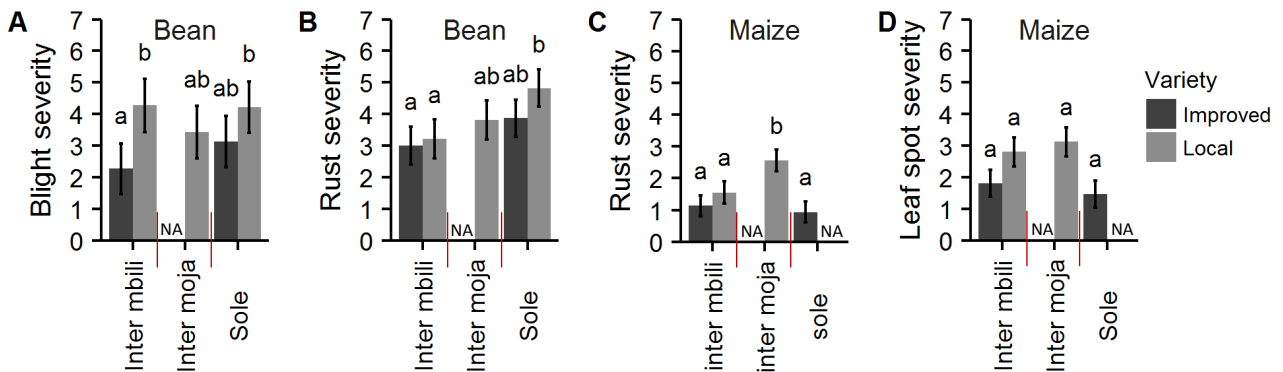
**Table 10.** Numbers of pods and seeds, and 100-seed weight of local and improved bean and maize varieties.

	Bean		Maize	
	Improved: Uyole Njano $\mu \pm SE$	Local: 'Kariasii' $\mu \pm SE$	Improved: DK 8031 $\mu \pm SE$	Local $\mu \pm SE$
Pods per plant	8.5 ± 0.9	9.0 ± 0.9		
Seeds per pod	2.7 ± 0.4	3.6 ± 0.2		
Weight of 100 seeds (g)	32.3 ± 1.8	27.3 ± 1.2	37.1 ± 2.6	37.4 ± 2.0
Harvest index (%)	46.0 ± 5.6	44.0 ± 4.8	32.5 ± 1.7	36.0 ± 1.9

### 3.2 Disease severity

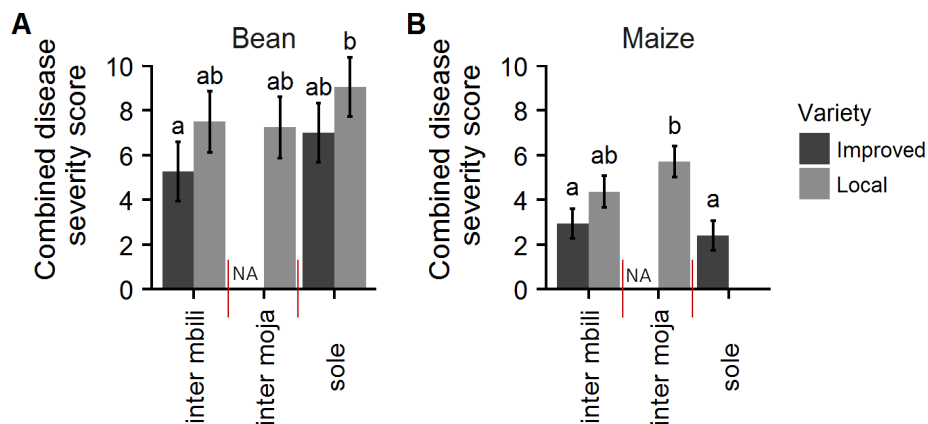
In beans, symptoms of blight (*Xanthomonas phaseoli* [common blight] and *Pseudomonas phaseolicola* [halo blight]) and leaf rust (*Uromyces spp.*) were visible on similar proportions of leaf tissue, compared to each other (Figure 8 A and B). Severity scores of improved and local varieties within the same cropping design were similar, with one exception for bean blight severity in mbili intercrops: severity scores were significantly smaller for the improved variety than for the local variety ( $P < 0.05$ ).

Gray leaf spot (*Cercospora zea-maydis*) was slightly more prevalent in maize than rust (*Puccinia sorghi*) as can be seen by comparing Figure 8 C and D. Only for moja-intercropped local maize, rust severity was slightly larger than for the other design and variety combinations ( $P < 0.05$ ).



**Figure 8.** Disease severity for blight and rust in beans, and rust and leaf spot in maize. The disease severity score is a rough indicator of the mean percentage of leaf tissue exhibiting disease symptoms. Letters above the standard error bars signify significant differences (Tukey's HSD). *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop

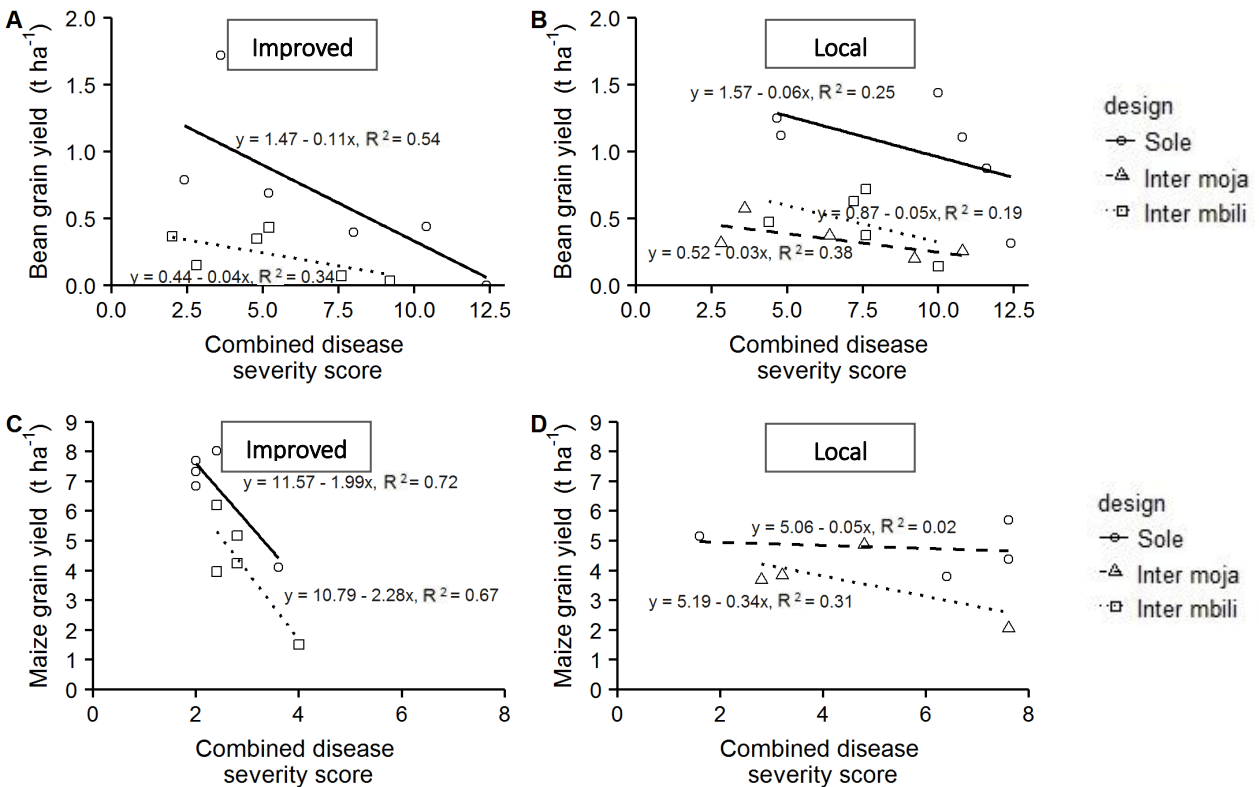
Combined disease severity scores were constituted of added-up severity scores of blight and rust for beans, and rust and leaf spot for maize. As shown in Figure 9, maize combined disease severity remained to be largest in the moja intercrops with the local maize. For beans, the combined disease severity was significantly smaller for improved beans in mbili intercrops than for local sole-cropped beans. Within the same variety, there were no differences in disease severity per cropping design.



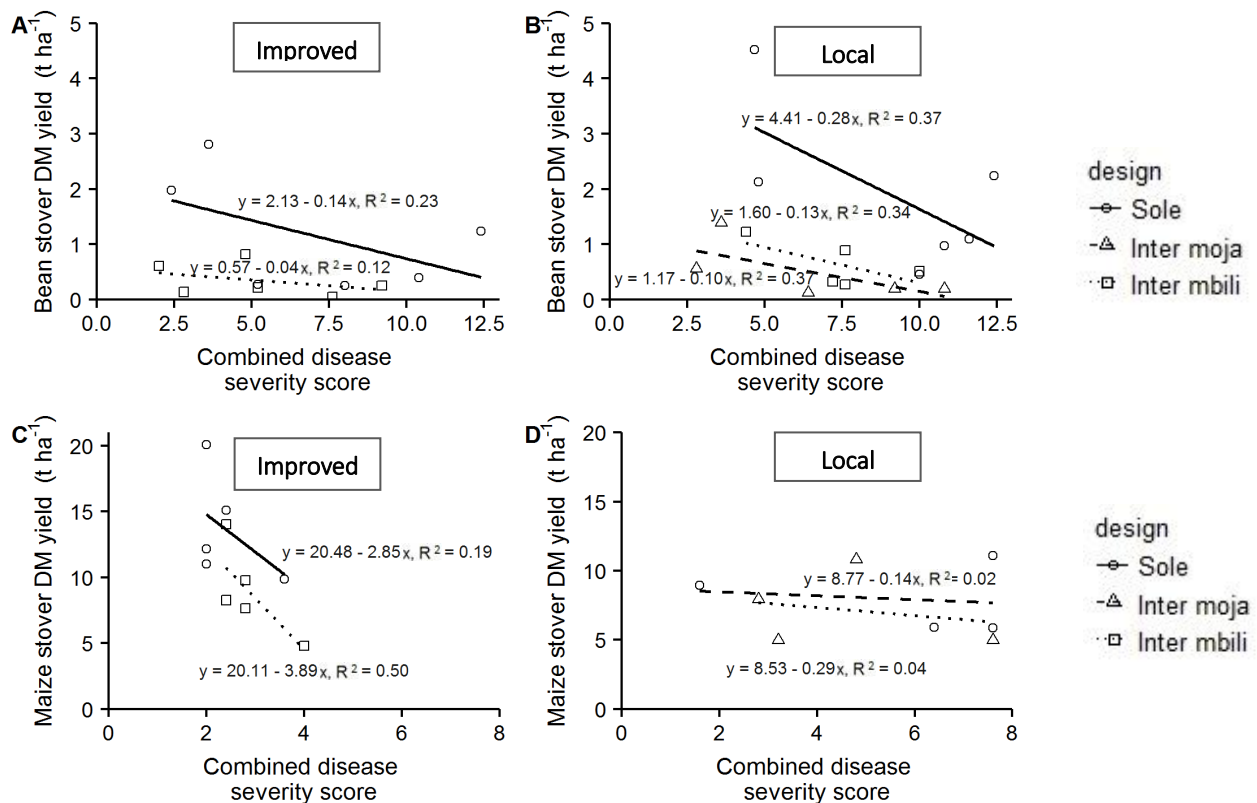
**Figure 9.** Combined disease severity score for bean (A) and maize (B) plots. Letters above the standard error bars signify significant differences (Tukey's HSD). *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

There was a negative correlation between severity scores and grain and stover yields, as depicted in Figure 10 and Figure 11. For improved beans and for both varieties of maize, there was an indication that grain yields were reduced by disease ( $R^2$  values ranged between 0.54 and 0.87; Figure 10 A, C and D). For local beans, the correlation between grain yield and disease severity was weaker (with  $R^2$  values ranging between 0.11 and 0.42; Figure 10 B). Similar trends were seen for stover yields and disease severity, as shown in Figure 11.

Grain and stover yields of improved maize drop faster with an increasing disease severity than for local maize: a shift in disease severity of one unit resulted in a drop in grain yields of almost 4 t ha<sup>-1</sup> for improved maize, and only 2 t ha<sup>-1</sup> for local maize (Figure 11 C and D). This suggests that the improved maize variety was more sensitive to disease. However, despite this the yields of improved maize remained larger than for local maize for similar disease scores. Especially when the disease damage was limited (a combined score below 3), larger yields were reached by the improved maize variety than by the local maize variety.



**Figure 10.** Bean and maize grain yields versus combined disease severity scores. Data were not corrected for trial location. Letters above the standard error bars signify significant differences (Tukey's HSD). *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crops rows.



**Figure 11.** Bean and maize stover yields versus combined disease severity scores. Data were not corrected for trial location. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

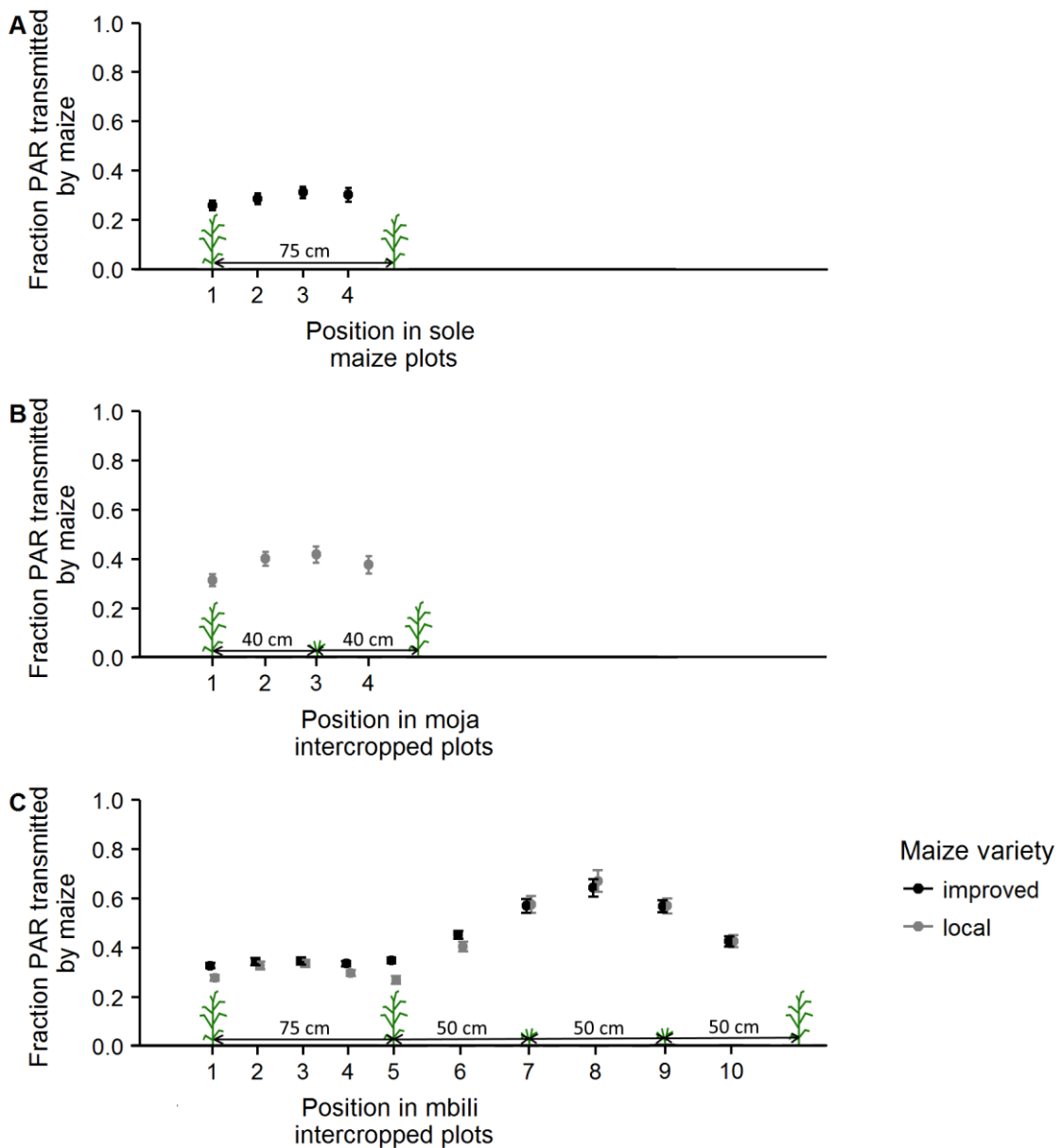
### 3.3 PAR interception

The fraction of PAR that was transmitted by the maize canopy was determined at specific positions across transects through plots at a height of 40 cm (height of bean plants), to find out how much PAR was available between rows of maize for (potential) use by beans. Soil coverage by the crop canopy was assumed to be at its maximum when these measurements were taken. Figure 12 provides an overview of the fractions of PAR transmitted throughout the canopy. Positions 1-4 in all cropping designs were similar in terms of probe placement of the PAR sensor relative to the rows of maize, although the absolute distances between those two rows of maize were slightly different: 75 cm in the sole and mbili designs, and 80 cm in the moja design. Because of that, fractions of light transmitted at positions 1-4 were expected to be similar, but there were some significant differences between the designs: less PAR was transmitted by sole-cropped improved maize than by mbili-intercropped improved maize on position 1 ( $P < 0.05$ ), and more light was intercepted on position 2 by sole-cropped improved maize than by moja-intercropped local maize ( $P < 0.05$ ). Fractions of PAR intercepted on positions 3 and 4 were the same in all cropping designs.

Within the maize rows and at the height of bean plants (40 cm), close to 75% of incoming PAR was intercepted by the maize plant parts above that height, regardless of maize variety. In the mbili designs, the maize canopy transmitted more light on the side with a neighbouring bean row than on the side with another row of maize. Compare for instance position 4 and 6 in the mbili designs. In position 4, leaves of two maize rows are overlapping, but on position 6 the coverage is attributed mainly to one row

of maize. The mbili design therefore resulted in the largest light availability per individual maize plant, but this had no influence on maize yields.

The measurement positions right above (potential) bean plants were also compared. These were positions 3 in all designs, and also position 7 and 9 in the mbili intercrops. As could be expected by the wider spacing of maize plants, more PAR was transmitted right above the beans in the mbili intercrops ( $P < 0.01$ , for positions 7 and 9) than in the other designs: fractions of PAR transmitted by maize above the beans were 57% in the mbili design, 42% in the moja design, and 31% in sole maize.



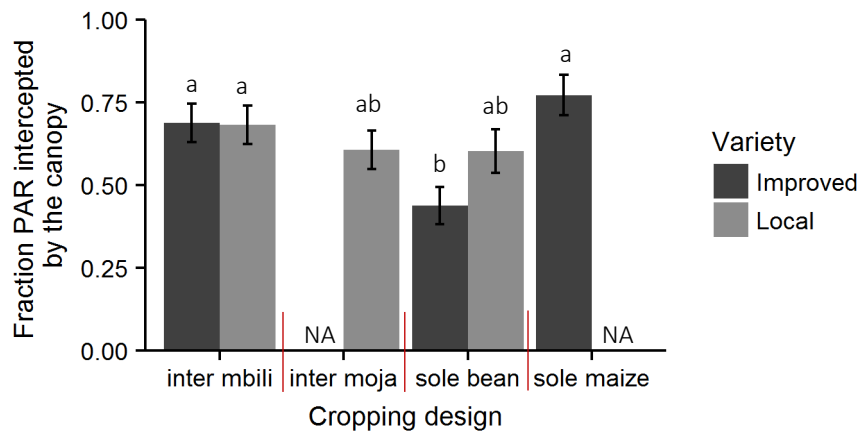
**Figure 12.** Fraction of PAR transmitted by the canopy at specific positions across transects through plots. The probe of the AccuPAR LP-80 was held parallel to row direction, at a height of 40 cm (top of bean plants). The positions of maize and bean plants are indicated in the plot (plant sizes are not accurate compared to inter-row spaces). *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.



There were no effects of row directions (see Table 4 in the Methods section) on fractions of PAR transmitted, likely because PAR measurements were performed in indirect sunlight.

When comparing light interception at ground level by total canopies (as shown in Figure 13), larger fractions of PAR were intercepted by sole-cropped improved maize and by the mbili designs than by sole-cropped improved beans. Less than 50% of available PAR was intercepted by sole-cropped improved beans.

PAR interception by the total canopy in the moja design was not larger than for sole maize, despite the addition of beans between the maize rows. This can be attributed to the observation that the local maize variety looked less vigorous than the improved variety. For beans it was the other way around: the local beans looked more vigorous than the improved beans. The mbili design was executed with improved and with local varieties of beans and maize, and the fractions of PAR intercepted were similar.



**Figure 13.** Fraction of PAR intercepted by the canopy. The probe of the AccuPAR LP-80 was held diagonally to row direction at the locations, at ground level. The positions of maize and bean plants are indicated in the plot. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

### 3.4 Biological nitrogen fixation

Proportions of total N and  $\delta^{15}\text{N}$  in beans per plot in each of the trials are shown in Table 11. Mean N contents of seed and stover were 3.4 and 1.7 %, respectively. The  $\delta^{15}\text{N}$ -enrichment of seed and stover were more similar to each other: 0.5 and 0.6 ‰, respectively. The  $\delta^{15}\text{N}$ -enrichment in seed and stover on trial M1 were significantly lower than in the other trials ( $P < 0.001$ ). Mean  $\delta^{15}\text{N}$  values were -2.2 ‰ for seed and -1.5 ‰ for stover on trial M1, whereas the mean  $\delta^{15}\text{N}$  values of the remaining trials were 2.1 ‰ for seed and 1.3 ‰ for stover. In terms of total N contents, the results were similar on trial M1 and the other trials. Table 11 also shows the N contents and  $\delta^{15}\text{N}$ -enrichments of standard samples which all came from the same batch of bean tissue and all should have the same nutrient composition. It can be seen in Table 11 that this is mostly the case, although for stover N contents a large difference (0.8 versus 1.6) was found. Those results are indicative of the error margin in the analysis.

Values of  $\delta^{15}\text{N}$  close to 0 suggest that the plant made relatively more use of atmospheric N pools than of soil N pools. Negative  $\delta^{15}\text{N}$  values indicate that the abundance of  $^{15}\text{N}$  in the biomass was lower than in the atmosphere.

Calculations on the proportion of N derived from the atmosphere (Ndfa) rely on the  $\delta^{15}\text{N}$  contents of shoots (including stover and seed) and of reference plants. Relative yields of grain and stover were used to estimate  $\delta^{15}\text{N}$  for whole shoots from the separate values for seed and stover, and the results are shown in Table 12. Paired with the beans, weeds were harvested on the same plots to use them as reference plants. Trials H3 and H4 had been weeded very recently and no suitable weeds were available. The  $\delta^{15}\text{N}$  of the different weed species are shown in Table 12. There were three different species of weeds that were deemed suitable as reference plants (pictures are included in Appendix I). The  $\delta^{15}\text{N}$ -enrichment of the weed species differed ( $P < 0.001$ ), but it was not known which of the weed species were most suitable to reflect soil  $\delta^{15}\text{N}$ -enrichment. Not all three of the weed species were available on each of the plots in the different trials. Sometimes, there was even only one species of weeds available. In almost all plots, the  $\delta^{15}\text{N}$  values of beans were consistently closer to zero than to the  $\delta^{15}\text{N}$  values of (paired) reference plants, indicating that beans were primarily fixing atmospheric nitrogen. The average of  $\delta^{15}\text{N}$ -enrichments of all weeds in a trial was assumed to reflect the soil in that trial and was used to calculate the proportion Ndfa.

Exceptionally low values for  $\delta^{15}\text{N}$  were found in beans and weeds in trial M1, indicating that there was something odd about the soil on that trial. Trial M1 was therefore not included in the further calculations of N fixation.

Figure 14 depicts the relative (14 A, B, C) and absolute (14 D, E, F) quantities of Ndfa. Because the choice of B-values (estimate of Ndfa if the atmosphere was the only source of N) highly influenced the outcome of calculations on N fixation, the results are presented for three different B-values. The tested B-values each led to estimates of nitrogen fixation up to 10% apart from each other. The proportions of Ndfa were similar in the intercropped designs compared to the sole crops. Moja-intercropped local beans sometimes fixed more nitrogen than beans in the sole-cropped and mbili designs. On all three trials included in this analysis, the proportion of Ndfa was largest in the moja intercrop.

Too few data points remained to test for significant effects of treatments or bean varieties on nitrogen fixation. The results from trial H1 were considered to be most reliable because there were two of the same weeds species available in each of the plots.

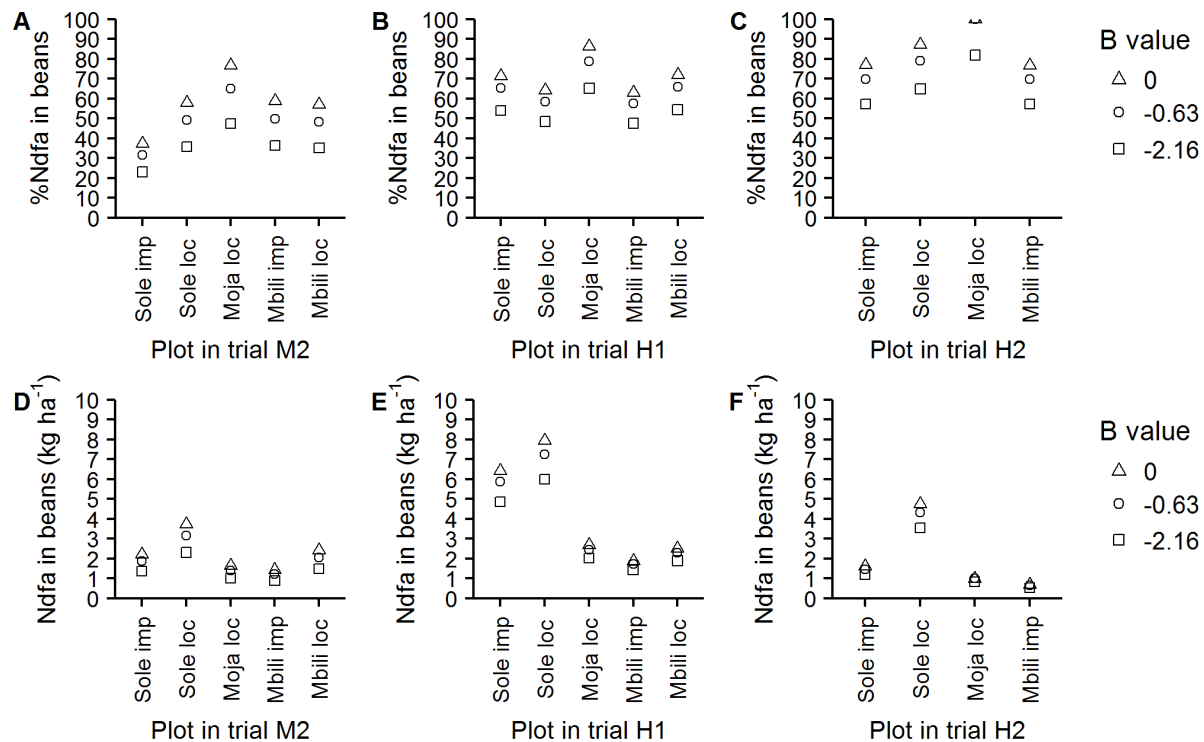
On trial M2, absolute amounts of seed N (mean: 2.50 kg ha<sup>-1</sup>, see section 3.5) were larger than the amounts of N fixed (mean: 1.43 kg ha<sup>-1</sup> at B = -2.16;  $P < 0.01$ ), revealing that in this trial, more N was removed by seed than was derived through fixation.

**Table 11.** Proportion of N and corresponding  $\delta^{15}\text{N}$ -enrichment of bean seed and stover. The six standard samples per tissue type were all the same batch of tissue material, and should have the same results of analysis. The standard errors of these standard samples provide an indication of the error margin of the analysis. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crops rows.

Trial	Design	Bean var.	N (%) seed		$\delta^{15}\text{N}$ (‰) seed		N (%) stover		$\delta^{15}\text{N}$ (‰) stover	
			$\mu \pm \text{SE}$	$\mu \pm \text{SE}$	$\mu \pm \text{SE}$	$\mu \pm \text{SE}$	$\mu \pm \text{SE}$	$\mu \pm \text{SE}$		
M1	Sole	Improved	3.7		-3.7		1.1		-0.8	
	Sole	Local	3.5		0.1		1.5		-1.3	
	Moja	Local	2.6	$3.6 \pm 0.2$	-4.0	$-2.2 \pm 0.8$	1.1	$1.4 \pm 0.1$	-2.6	$-1.5 \pm 0.5$
	Mbili	Improved	3.9		-2.5		1.4		-2.4	
	Mbili	Local	4.0		-1.1		1.9		-0.3	
M2	Sole	Local	3.5		1.8		1.2		1.0	
	Sole	Improved	3.5		2.6		1.6		1.8	
	Moja	Local	3.7	$3.7 \pm 0.2$	0.6	$1.5 \pm 0.3$	1.7	$1.6 \pm 0.1$	1.1	$1.5 \pm 0.2$
	Mbili	Improved	4.0		1.4		1.6		1.5	
	Mbili	Local	3.9		1.1		1.7		2.2	
H1	Sole	Improved	2.9		1.8		1.4		2.1	
	Sole	Local	3.3		2.2		1.8		2.5	
	Moja	Local	3.2	$3.3 \pm 0.2$	1.3	$1.8 \pm 0.2$	0.9	$1.6 \pm 0.2$	0.3	$1.9 \pm 0.4$
	Mbili	Improved	3.6		2.3		2.1		2.6	
	Mbili	Local	3.6		1.5		1.5		2.2	
H2	Sole	Improved	3.7		1.5		2.4		1.4	
	Sole	Local	3.4	$3.6 \pm 0.2$	0.3	$0.5 \pm 0.5$	2.3	$2.2 \pm 0.2$	1.5	$1.2 \pm 0.4$
	Moja	Local	3.4		-0.9		1.6		0.0	
	Mbili	Improved	3.9		1.2		2.4		1.9	
H3	Sole	Local	2.8		2.5		1.4		2.3	
	Sole	Improved	3.3		2.8		1.5		1.6	
	Moja	Local	3.0	$3.2 \pm 0.2$	-0.4	$0.9 \pm 0.7$	1.9	$1.8 \pm 0.3$	-1.0	$0.4 \pm 0.7$
	Mbili	Local	3.3		-0.9		1.2		-0.6	
	Mbili	Improved	3.6		0.5		2.7		-0.3	
H4	Sole	Improved	NA		NA		2.7		0.0	
	Sole	Local	2.6	$3.1 \pm 0.2$	0.3	$0.3 \pm 0.2$	1.3	$1.9 \pm 0.3$	0.6	$0.2 \pm 0.2$
	Mbili	Improved	3.7		0.7		2.4		0.0	
	Mbili	Local	3.1		-0.1		1.9		0.1	
Means of all trials			$3.4 \pm 0.1$		$0.5 \pm 0.3$		$1.7 \pm 0.1$		$0.6 \pm 0.3$	
Means of all trials except M1			$3.4 \pm 0.1$		$2.1 \pm 0.2$		$1.8 \pm 0.1$		$1.3 \pm 0.2$	
Standard sample			3.5		-0.6		0.8		-0.3	
Standard sample			3.5		-0.3		0.9		-1.2	
Standard sample			3.7	$3.7 \pm 0.1$	-0.5	$-0.3 \pm 0.2$	1.0	$1.0 \pm 0.1$	-1.0	$-0.8 \pm 0.2$
Standard sample			3.8		-0.4		0.8		-1.0	
Standard sample			4.2		0.5		1.2		-0.1	
Standard sample			3.6		-0.3		1.6		-1.0	

**Table 12.**  $\delta^{15}\text{N}$ -enrichment of whole bean shoots and for the available reference plants. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crops rows.

Trial	Design	Bean var.	$\delta^{15}\text{N}$ (‰) shoot		$\delta^{15}\text{N}$ (‰) reference plants			$\mu \pm \text{SE}$ (all ref. plants in trial)
			$\mu \pm \text{SE}$	A	B	C		
M1	Sole	Improved	-3.4		-3.3	0.5		
	Sole	Local	-0.1			4.3		
	Moja	Local	-3.8	-2.1 $\pm$ 0.3	-4.7		1.8	-2.3 $\pm$ 1.4
	Mbili	Improved	-2.5		-10.4		-3.7	
	Mbili	Local	-1.0		-3.3			
M2	Sole	Local	1.5		4.2		3.7	
	Sole	Improved	2.2		3.6		4.7	
	Moja	Local	0.8	1.5 $\pm$ 0.1	2.7			3.5 $\pm$ 0.3
	Mbili	Improved	1.4		1.7			
	Mbili	Local	1.5		2.9			
H1	Sole	Improved	1.9			7.4	6.4	
	Sole	Local	2.4			7.5	7.4	
	Moja	Local	0.9	1.9 $\pm$ 0.1	7.1		5.1	6.7 $\pm$ 0.4
	Mbili	Improved	2.4		6.8		8.0	
	Mbili	Local	1.9		6.5		4.3	
H2	Sole	Improved	1.5			8.2	6.0	
	Sole	Local	0.8			5.4	5.6	6.3 $\pm$ 0.9
	Moja	Local	-0.6	0.8 $\pm$ 0.2		9.6		
	Mbili	Improved	1.5				3.0	



**Figure 14.** Proportions Ndfa in beans (plot A-C) and the absolute quantities of Ndfa (plot D-F). The plot with mbili intercropping and local beans on trial H2 was not included in this thesis study because it was managed differently from all other plots in the study. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

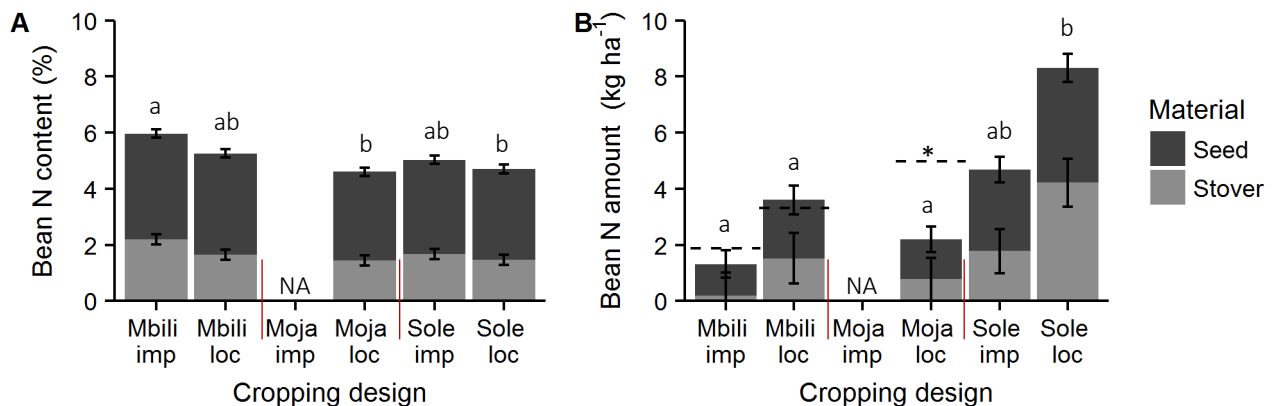
### 3.5 Bean nutrient contents

Data on tissue nutrient contents were only available for beans and are shown in Table 13. There was no correlation between tissue nutrient contents and bean yields. Nutrient contents did not differ between cropping designs and bean varieties, and were also not correlated to soil nutrient contents. Only plant Zn contents were correlated with soil Zn contents with an  $R^2$ -value of 0.53.

**Table 13.** Nutrient contents in seeds and stover of improved and local beans, as assessed in the IITA laboratory in Dar Es Salaam, Tanzania.

Nutrient content	Improved variety (Uyole Njano)		Local variety ('Kariasii')	
	Seed (N=11)	Stover (N=12)	Seed (N=16)	Stover (N=16)
	$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$	$\mu \pm SE$
N (%)	2.07 ± 0.03	1.31 ± 0.07	1.85 ± 0.04	0.91 ± 0.06
P (%)	0.21 ± 0.01	1.20 ± 0.02	0.35 ± 0.01	0.17 ± 0.02
Mg (%)	0.21 ± 0.01	0.39 ± 0.01	0.23 ± 0.01	0.38 ± 0.01
K (%)	1.32 ± 0.02	2.17 ± 0.09	1.47 ± 0.02	2.26 ± 0.07
Cu (ppm)	9.02 ± 0.54	8.57 ± 0.94	10.77 ± 1.06	8.08 ± 0.93
Zn (ppm)	29.98 ± 0.95	22.78 ± 2.38	26.33 ± 1.54	22.20 ± 1.74
Mn (ppm)	8.74 ± 1.30	70.90 ± 13.56	11.50 ± 1.58	82.00 ± 17.37

In almost all plots included in the study, some plants showed some visible signs of deficiencies of specific nutrients through discoloration of leaves and/or veins. Severity and incidence of visible disease deficiencies were not scored in detail and are often hard to diagnose accurately. The symptoms may not have been interpreted right. Mg and S deficiencies were most often observed, followed by Zn, N and P. Bean tissue was not analysed for S contents. However, S deficiencies were observed on some bean plants in almost all plots. There was no relation between nutrient contents and the occurrence of visible deficiency symptoms. A table with the nutrient contents of bean stover for each plot and the observed deficiencies is included in Appendix II.

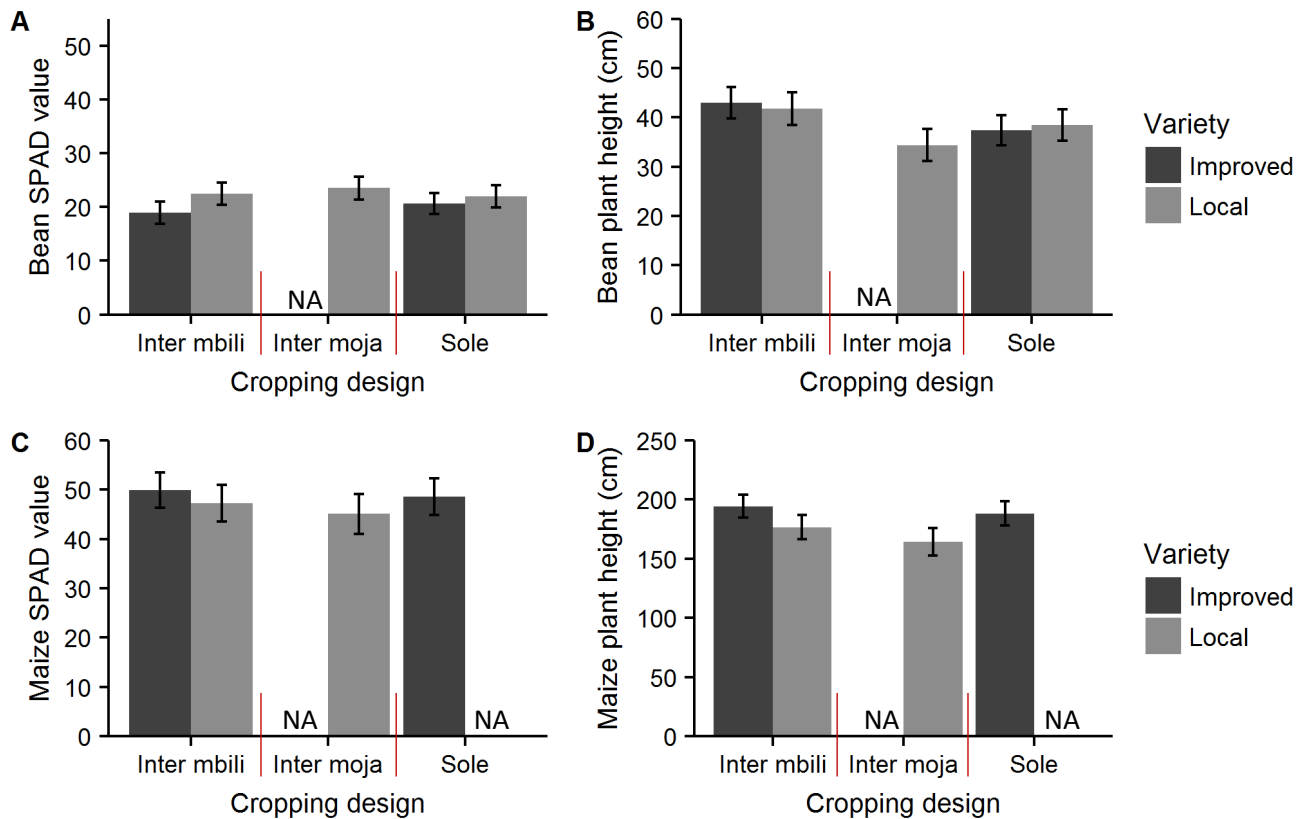


**Figure 15.** Relative and absolute nitrogen contents in the treatments. Letters above the standard error bars signify significant differences (Tukey's HSD) (valid for separate stover and seed N contents, as well as total N contents). Asterisks denote significant differences between actual and expected N quantities ('\*' for  $P < 0.05$ ). These N data originate from the University of Leuven. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

Tissue nitrogen contents had also been assessed at the University of Leuven. These data revealed that mbili-intercropped improved beans contained significantly larger proportions of nitrogen than the local varieties in the moja-intercropped and sole-cropped designs ( $P < 0.05$ ). In terms of absolute quantities of N, most N was accumulated by sole-cropped local beans (Figure 15). The differences in amounts of N across treatments reflected the differences in crop yields that were found for those treatments.

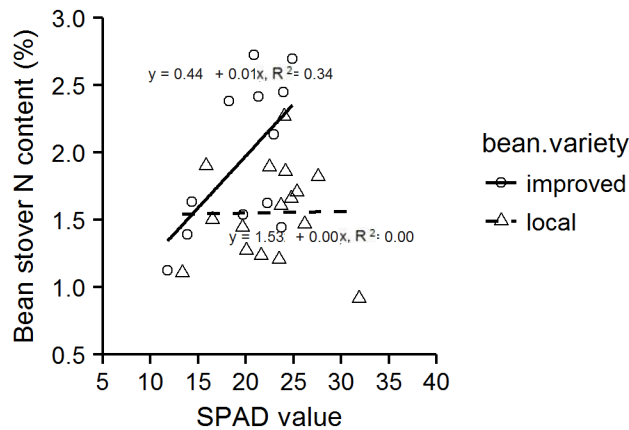
### 3.6 Leaf chlorophyll contents and plant height

For beans, mean SPAD values were 21 (SE=1). Mean SPAD values were more than twice as large for maize: 49 (SE=2). There were no differences in SPAD values between cropping designs and crop varieties. The mean heights of bean and maize plants were 39 cm (SE=1.4) and 183 cm (SE=6), respectively. There were no significant differences in plant height between the available treatments. Figure 16 depicts the mean SPAD values and heights of beans and maize.



**Figure 16.** SPAD values and height of bean and maize plants. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

SPAD values are used as indicators of the nitrogen content of plants. As shown in Figure 17, stover N contents indeed increased with proportional increases in SPAD values, but only in the improved bean.



**Figure 17.** Bean stover N content versus chlorophyll content (SPAD).

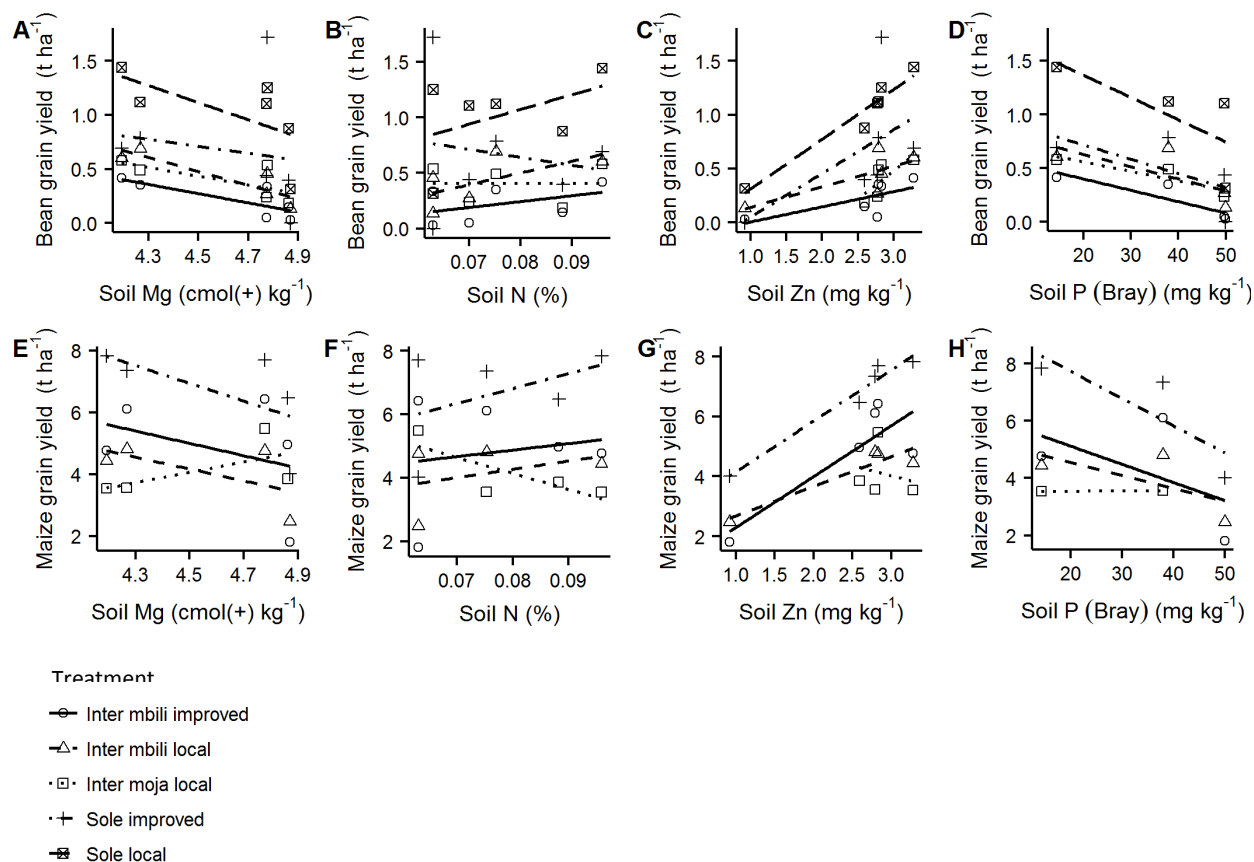
### 3.7 Soil nutrient contents

A correlation matrix of the soil properties (Appendix III) revealed many relationships between soil properties. Clay contents were negatively correlated with pH, organic carbon and Mg contents, and positively with Mn and the C:N ratios. Mg contents were positively correlated with Na and Cu. There were positive correlations between Zn and K contents, and negative correlations between Zn and Cu. Mn and Fe contents were strongly negatively correlated with pH and C:N ratios (but not with C and N contents separately), and positively with each other. Electrical conductivity was primarily correlated with K contents.

Lowest yields of maize and beans were found on trial H4. Effects of drought were visible, but on that trial some nutrients may have been limiting as well. Soil Zn, K, N, Ca and EC were relatively low compared to the other trials. Soil Mg, P and Fe contents on trial H4 were relatively large however.

As explained in Section 3.5, nutrient deficiencies were visible primarily for Mg and S, and to a lesser extent for Zn, N and P. Figure 18 shows the relationships between bean and maize grain yields and those soil properties. Table 14 provides an overview of the strengths of any relationships that were found as the corresponding  $R^2$ -values for beans and maize. Appendix III contains graphs and a table of  $R^2$ -values for the other measured soil properties. The low yields on trial H4 have a strong effect on the slope of the relations between bean yields and soil properties.





**Figure 18.** Relationships between grain yields of beans (A-D) and maize (E-H) and soil properties. The  $R^2$ -values associated with these relationships are shown in Table 14. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

**Table 14** Strength of correlations between soil characteristics and grain yields of beans, and maize expressed as  $R^2$ -values. The plus and minus symbols between parenthesis indicate whether the correlation is positive or negative, for those relations with an  $R^2$ -value > 0.40. *Moja*: alternating crop rows. *Mbili*: two-by-two alternating crop rows.

Design	Bean variety	Mg	N	Zn	P-Bray
Sole	Improved	0.02	0.02	0.28	0.31
Sole	Local	0.34	0.18	<b>0.93 (+)</b>	<b>0.42 (-)</b>
Mbili	Improved	<b>0.57 (-)</b>	0.15	<b>0.43 (+)</b>	<b>0.71 (-)</b>
Mbili	Local	<b>0.74 (-)</b>	0.32	<b>0.51 (+)</b>	<b>0.42 (-)</b>
Moja	Local	<b>0.43 (-)</b>	0.00	<b>0.48 (+)</b>	<b>0.67 (-)</b>

Design	Maize variety	Mg	N	Zn	P-Bray
Sole	Improved	0.31	0.15	<b>0.96 (+)</b>	0.00
Mbili	Improved	0.10	0.02	<b>0.66 (+)</b>	0.04
Mbili	Local	0.27	0.09	<b>0.82 (+)</b>	0.00
Moja	Local	0.29	<b>0.52 (-)</b>	0.03	<b>0.62 (+)</b>

### 3.8 Farmers' survey

A group of 44 legume-producing farmers (23 male, 21 female) from different households were interviewed to get insight into their considerations with regard to legume production. In explaining their reasons for producing legumes, the nutritional value of legumes was mentioned by 82% of the respondents, (good) market value by 44% and soil fertility benefits by 34%. The most popular legumes were bush beans, which were grown in 2016 by all respondents except one. Climbing bean varieties were grown by 32% of the respondents. Farmers more often grew improved bean varieties than local bean varieties. A handful of farmers grew legumes like groundnut, cowpea, pigeon pea and leguminous trees. Of all respondents, 57% used all their legume produce for home consumption, 36% sold a small proportion, three farmers sold more and no-one sold everything.

Intercropping was a common practice in legume production: it was implemented by 89% of the respondents. Legumes were sole-cropped by 37%. Some farmers had two legume fields and used both practices. The respondents were asked to explain why they chose for a certain practice, and they could mention multiple reasons. If farmers chose intercropping, they did so to make optimal use of space (65%, and primarily because of land scarcity) and to reduce risk (40%). Other reasons that were mentioned included tradition (10%) reducing pressure of pests (13%) and weeds (10%). Farmers who sole-cropped their legumes explained that they did so to achieve larger yields (88%), and to avoid competition for nutrients (19%) and light (13%).

When farmers intercropped, 97% of them combined common beans and maize. 18% of the farmers also added sunflower to that combination. Three farmers used the same combinations, but with pigeon pea instead of beans. Alternating rows (moja intercropping) were the most popular cropping design (implemented by 80% of the farmers), primarily because of tradition (38%), because it was easy (31%), to secure light availability for the crops (31%) or for appropriate nutrient availability (22%). A few farmers explained that they chose moja intercropping because it would lead to good yields and good soil fertility.

Only three farmers applied mbili intercropping, and they did so because of tradition and to increase yields. Two farmers randomly distributed the crops that they grew together, because it was easy during planting, and as a result of tradition.

Legumes were grown on the same plot each year by 64% of the respondents, because limited land was available (96%), because that specific plot was suitable for the legumes, or because of tradition (21%). Farmers who rotated their legumes (39%), did so because of tradition (62%), for soil quality benefits (59%), or to avoid diseases (41%). Several farmers explained that their practice depended on the season and on which plots could be irrigated.

Most farmers were not satisfied with their legume yields (36%). Many farmers were somewhat satisfied (34%), 27% was satisfied, and 5% was very satisfied with their legume yields.

The respondents were asked what they perceived as the most important constraints to legume production. Pest insects were mentioned most often (75% of the respondents), followed by irregular rain (59%), legume diseases (34%), drought (32%), poor availability of improved seed (34%), poor seed quality (32%) and low soil fertility (25%). Other reasons that were mentioned included knowledge deficits, counterfeit inputs, and too much rainfall.

Half of the farmers explained how they thought legume production could be improved. Farmers made remarks about the importance of improved seed. They wanted them to be more easily available (cheaper) and they should give high yields and be drought-resistant. Many respondents said that they wanted more frequent or elaborate extension services with regard to farming practices and

entrepreneurship. Problems of counterfeit fertilizers had to be tackled, access to capital needed to be easier, and better irrigation could be beneficial.

There were no differences in the answers of groups based on education level, gender, and village.

## 4. Discussion

### 4.1 Yields

#### 4.1.1. Relative yields

It is common to present yield results of intercrops in terms of a land equivalent ratio (LER), which is the relative sole-cropped area to intercropped area that is needed to provide equal yields with the different practices. LERs could not be calculated for all trials, because no moja-intercropped plot was available on trial H4 and no maize yield data were available from trial H3. On the remaining trials, the LERs of the intercropped designs were close to 1, showing that those intercropping designs were equally productive compared to sole cropping on the same land area. The productivity per plant was decreased in the moja design compared to sole crops, but this was (partly) compensated for by an increased total plant density compared to sole-cropped maize.

In the moja-intercrop, yields were lower than expected for beans ( $P < 0.01$ ) and for maize ( $P < 0.05$ ). Bean performance was likely reduced by the increased competition for soil nutrients and water, and the increased shading by maize. With regard to the reduced maize yields, it needs to be taken into account that expected yields for intercropped local maize were based on sole-cropped improved maize because no sole crop of local maize was available. The local maize variety may have performed differently as a sole crop than the improved maize variety, so the pLER of local maize can be an over- or underestimation. Moja-intercrops were only available for the local varieties.

It was not only the intercropping practice that was different for the moja-intercropped plots compared to the other treatments, because the moja-intercropped plots also received no fertilizer and pesticides. Besides, they were planted less carefully than the other designs. Because they were intended to represent farmers' practices, planting was executed by the farmers who owned the land, with less instruction from N2Africa staff than on the other plots. Although average inter- and intra-row spacing was the same, spacing was more variable and that resulted in some gaps in different parts of the plots. Rows were also less straight than in the other designs. These aspects may have contributed to the relatively poor yields.

In the mbili designs, beans and maize yielded as expected based on the planting density differences across designs. If yield is used as an indicator, maize did not benefit from replacing a neighbouring maize row on one side with beans, despite increased light availability and presumably less competition for soil nutrients and water.

Other studies on moja and mbili intercropping found that crop yields were improved by intercropping and especially by mbili intercropping with simultaneous planting (Mucheru-Muna et al., 2010; Ndung'u et al., 2005; Woomer et al., 2004). In the moja intercrops, those studies maintained the same inter-row spacing of maize in the moja and the sole-cropped design (75 cm), and beans were simply planted in the middle between those rows (additive design). Increasing crop densities beyond replacement, usually leads to larger LER (Yu et al., 2016). In the trials of this thesis study, row spacing in the sole crop was 75 cm, and 80 cm in the moja design. With regard to mbili intercropping, the above-mentioned studies planted twin rows of maize 50 cm apart, next to a strip 100 cm wide for two rows of beans. The N2Africa trials maintained a distance of 75 cm within paired maize rows, and a distance of 150 cm between pairs of maize rows, which is a lot wider. Distances between rows of maize and beans were always similar to the inter-row distances of sole-cropped beans. This was done to avoid that beans would

be negatively affected by the increased nutrient requirements from maize in comparison to neighbouring beans and to allow for more light to reach the beans. However, bean yields in the mbili design were not larger than in the moja design.

Other studies on maize-bean intercrops found that relatively low maize densities at 37,000 plants ha<sup>-1</sup> optimize maize yield, sole or intercropped (Mutungamiri et al., 2001), and that intercropped beans require maize densities even lower than that (Molatudi & Mariga, 2012). On the demonstration trials, maize densities were 38,462 plants ha<sup>-1</sup> in the moja intercrop, 27,705 plants ha<sup>-1</sup> in the mbili intercrop and 44,444 in the sole crop. The impression was that plant densities were actually a little bit lower than intended at planting. Germination levels were not assessed, however. Planting densities were assumed to be similar on all trials because planting was coordinated by N2Africa staff. Effects on yield may not have been only due to competition between maize and beans but also to poor germination. Sole bean plots in trial H3 showed many gaps where there were grass weeds. Possibly some beans did not emerge or the seedlings were pulled out during weeding, or the bean plants were already outcompeted by grasses just before weeding.

There were no differences in the number of pods per plant, number of seeds per pod and the weight of 100 seeds for the different treatments. Ndung'u et al. (2005) did find effects of cropping design on the number of pods per plant, with largest numbers in sole crops, then in mbili crops, and smallest number of pods in the moja systems. Different varieties were used however, which had a characteristic of producing more pods per plant (an average of 23 as opposed to 9 in this thesis study).

#### 4.1.2. Absolute yields and constraints

Grain yields of sole-cropped beans were 0.7 ( $\pm$  0.1) t ha<sup>-1</sup> for the improved and 1.1 ( $\pm$  0.1) t ha<sup>-1</sup> for the local variety, but these yields were not significantly different from each other. Local bean yields were at the national average and the improved bean yields have reached less than half of their potential at 3 t ha<sup>-1</sup> (Shenkalwa et al., 2013). Mean grain yields for sole-cropped improved maize were 6.5 t ha<sup>-1</sup>, which were within the range of potential yields determined for this maize variety DK 8031 (monsantoafrica.com, 2017). Maize yields - whether sole or intercropped and regardless of the variety – were much larger than the average yields across the country which have been fluctuating around 1 to 3 t ha<sup>-1</sup> between 2000 and 2010 (Barreiro-Hurle, 2012). This may be attributed to the frequent monitoring of the trials by N2Africa staff and timely weeding. Besides, fertilizers and pesticides were applied on most plots, whereas the majority of Tanzanian farmers rarely use these inputs. On average, Tanzanian farmers use only 8 kg ha<sup>-1</sup> of fertilizers, and only 0.7% of maize farmers uses improved maize varieties together with fertilizers (Boniphace et al., 2015). The moja-intercropped plots had not received any fertilizer or pesticides, however, and maize yields on these plots (3.8  $\pm$  0.7 t ha<sup>-1</sup>) were still larger than the national average. This suggests that the relatively high yields cannot only be attributed to good management but likely also to soil and weather conditions that were favourable to maize.

Lowest bean and maize yields were found on trial H4, which was slightly drier than the other trials according to the farmers who owned the land, and there were no options for supplemental irrigation. Soil factors may also have reduced the yields, because soil contents of Zn, K, N and Ca were relatively low on trial H4 compared to the other trials. Some stress as a result of drought was evident in the crop phenotypes in trial H4. Drought tends to be more influential than other abiotic stresses on bean performance and overall productivity (Darkwa et al., 2016). Beans were in a slightly later stage of pod filling and a larger proportion of the leaves were turning yellow, compared to the beans in the other trials.

Adequate amounts of rainfall are essential for beans during the stages of flowering and pod setting (Darkwa et al., 2016; Katungi et al., 2009). Szilagyi (2003) compared bean yields in a regular and a dry year ('dry' meant only one-third of the rainfall of the regular year) in Romania, and found that drought strongly reduced the numbers of pods per plant (by 60%), the number of seeds per pod (by 26%), and ultimately the bean yields (by 80%), and similar results were found in Ethiopia (Darkwa et al., 2016). On dry trial H4, the mean number of seeds per pod was only 1.1 (as opposed to 3.6 for the other trials), but the mean number of pods per plant was the same as for the other trials. This finding indicates that drought stress occurred after pod formation and at the onset of pod filling.

Under dry conditions, intercropping could have been advantageous for beans because of the shade provided by maize (Woomer et al., 2004), but the relative difference of bean yields as sole and intercrop was not larger on trial H4 than in the other trials (see Appendix IV).

Harvest indices (HI) of local and improved maize were around 34 %, whereas the HI of mature maize can reach 50 % (Tollenaar et al., 2006). The HI of beans was around 45%, which is right in the middle of the range of bean HIs described by Giller (2001), which is 21-64%.

Maize yields were calculated from a sample of only nine cobs per plot. A larger sample could provide a more accurate estimate of the maize yield.

## 4.2 Diseases

A study on cereal-legume intercropping by Hauggaard-Nielsen et al. (2008) revealed clear reductions of disease severity in the tested cereals and legumes in intercrops compared to sole crops. No such effects of cropping design were found in this thesis study when comparing intercrops and sole-crops with the same variety.

Improved bean variety Uyole Njano was bred for tolerance to a range of diseases (Kanyeka et al., 2007) but was still affected by leaf rust and blight. Both diseases affected similar proportions of leaf tissue. For maize, predominant diseases were leaf rust and leaf spot, despite being bred for tolerance to leaf spot (monsantofafrica.com, 2017). Although a look at the disease symptoms in the field led to the impression that diseases would be no strong constraint, there were negative correlations between bean and maize yields and disease severity. Grain and stover yields of improved maize dropped twice as much as the yields of the local variety, showing that the improved maize variety DK 8031 was more sensitive to disease than the local variety. Despite this, yields of improved maize were in most cases similar or larger compared to the local variety, for the same disease scores. When the disease score was small (around 2), yields of the improved variety were a lot larger than those of the local variety. This implies that if disease severity can be kept to a minimum – e.g. with use of fungicides – the improved variety is the best option in terms of yield returns. No fungicides were applied on the trials included in this study.

Because disease severity was assessed when the crops were already in their reproductive stages, it is unknown during which stage of development the disease started to infect the crops. Effects of bean rust are most severe when infection occurs during or just prior to flowering (Hagedorn & Inglis, 1986). Effects of maize rust on yields are more severe when infection occurs early in the cropping season (Kloppers & Tweer, 2009) but symptoms are usually first found when maize starts tasseling (CYMMIT, 2004). Early onset of gray leaf spot in maize stimulates earlier leaf senescence, less grain fill, and a reduction in photosynthetic leaf area which results in the reallocation of carbohydrates from stalks to ears and effectively making the stalk weaker (Jackson et al., 2008). As soon as a plant is affected by one disease, it may be more easily affected by another as a result of reduced resilience/resistance.

Having several very severely infected plants may differently affect yields compared to having many slightly infected plants. Severity scores of individual diseases were based on the same plants, but were not necessarily noted down in the same order. It was not possible to check whether the diseases occurred in most cases on the same plant or not. The impression from being in the field was that the different diseases occurred together on the same plant in about half of the cases.

It is likely that this combined disease overestimated actual disease severity because the scores of disease severity were not very precise as a result of inexperience of the author of this thesis. A score of 1 was given when 1 to 10% of leaf tissue showed symptoms. Addition of component scores leads to the impression that more than 10% of leaf tissue was affected whereas in practice it may have been still less than 10%. Disease severity scores were always assessed and discussed by two researchers, to ensure that relative differences in disease severity between plots were accurately represented, even if absolute levels of disease severity were not correct and likely overestimated.

### 4.3 PAR interception

The rather straightforward result of the different intercropping practices was that more light was available (i.e. more light was transmitted by maize) right above the bean plants in the mbili design than in the moja design. In the mbili designs, a bean row was located between a maize row and another bean row, which provided no shading. The fraction of light transmitted in the mbili design by maize right above a (potential) row of beans, was a factor 1.4 larger than in the moja design and a factor 1.8 larger compared to sole maize. Woomer et al. (2004) also found an increase in light availability for beans with a factor 1.5 in the mbili designs compared to moja designs, despite a smaller width of the strip with two bean rows compared to this study (100 cm compared to 150 cm, in the mbili design). So, more space available for beans in the mbili design in this study compared to that of Woomer et al. (2004) did not result in even more light availability for beans. This may be a result of measuring PAR interception on overcast days. On sunny days, the sunlight casts hard shadows, and the differences in PAR are large for sunny and shaded positions. On overcast days, the indirect sunlight that remains is a result of diffuse reflection and the light comes in not only from above but from all sides, reflecting from neighbouring leaves. Weather conditions did not allow for doing measurements solely on sunny days.

Although the improved maize variety appeared slightly taller, there were no significant differences in the height of maize plants for the two varieties. There were small differences in the fractions of PAR intercepted on positions between two rows of maize, so these were likely due to the differences in row spacing (75 cm in the sole and mbili designs, and 80 cm in the moja design). What may have slightly influenced the results is that there were some more gaps in the moja design as a result of poor planting practice, as explained before.

Hadi and Hashem (2006) studied the tolerance of common beans to different levels of shade (0, 20, 40 and 55% of incoming light) in Iran, and found that beans compensated for shading by increasing their leaf area and by extending the duration of grain filling. In that study, the numbers of grains per pod and per plant were decreased by shading, but total yields were not. The number of grains per pod and per plant, and seed weight, were the same for all treatments in this study.

Crop dry matter output as a fraction of the intercepted radiation has been termed radiation use efficiency (RUE,  $\text{g MJ}^{-1}$ ) (Monteith & Moss, 1977). More specifically, it is the slope of the relationship between dry matter accumulation throughout the growing season and the cumulative radiation

intercepted (Sinclair & Muchow, 1993). Practical limitations made it impossible to monitor biomass accumulation and PAR interception throughout complete crop development, so RUE could not be assessed. Tsubo and Walker (2002) measured light interception levels throughout the development of maize-bean intercrops, and found that the RUE of beans was larger in intercrops (as was the yield), and similar for maize in intercrops and sole crops. Yield advantages in intercrops can be the result of greater radiation interception or greater radiation use efficiency (Gou, 2017). Intercrops did not result in yield advantages in this study, despite larger light availability for beans in the mbili design, and the RUE remains unknown.

There were no significant differences in the fractions of PAR intercepted by the total canopies, except for the total canopies of sole improved maize and sole improved beans. At ground level, sole improved maize intercepted 77% of available PAR, and sole improved beans intercepted only 44% on average. The results show that intercropping did not lead to larger total fractions of light intercepted compared to sole cropping. The moja design was almost an additive intercropping design compared to sole maize, but the fraction of PAR intercepted by the canopy was not larger than for sole maize. This can be explained by the differences in leaf area of the local and the improved maize variety: the local maize variety in the moja intercrop looked less vigorous than the improved maize variety.

#### 4.4 Nitrogen fixation

Only three trials (M2, H1 and H2) were suitable for determination of nitrogen fixation levels because reference weeds were available. The  $\delta^{15}\text{N}$ -enrichment of shoots were comparable or on the low side compared to values for *Phaseolus vulgaris* in literature (Giller, 2001; Unkovich et al., 2008; Warinner et al., 2013). Exceptionally low  $\delta^{15}\text{N}$  values were found on trial M1 despite having total N contents of seed and stover similar to the other trials. Negative values often result from the uptake of mineral N, which contains relatively less  $^{15}\text{N}$  than soil organic N (Mariotti et al. 1981, as cited by Kahmen et al., 2008). However, the soil mineral N contents were low (see Table 2) and no fertilizer was applied to beans. The very low  $\delta^{15}\text{N}$  values found on trial M1 suggest that there was something odd about that soil.

Plant sampling for  $\delta^{15}\text{N}$  assessment was done during bean harvest (97 days after planting, on average), when pods and shoots were dry. In other assessments of N fixation with use of the  $^{15}\text{N}$  natural abundance method, legume samples were often collected at the stages of pod formation or mid-flowering, when the legumes are presumably at peak biomass production and before commencement of plant senescence (e.g. Mortimer et al., 2008; Mucheru-Muna et al., 2010; Unkovich et al., 1994). Unkovich et al. (1994) studied  $\delta^{15}\text{N}$  levels (B-values) throughout the development of field pea and lupin, grown in a medium lacking plant-available N and fully dependent on N fixation. For both legumes – and especially for peas –  $\delta^{15}\text{N}$ -enrichment decreased with time (indicating an increase in  $\text{N}_2$  fixation). In peas,  $\delta^{15}\text{N}$  decreased by 0.8‰ between 55 and 120 days after planting. Nodules start to senesce after flowering (Araujo et al., 2000). The  $\delta^{15}\text{N}$  levels found in this thesis study were therefore likely somewhat lower than if they had been determined before plant senescence. The reference weeds were in their flowering stage when they were sampled.

Estimates of the percentage of N fixed differed highly across trials and for different B-values. In all three included trials, largest proportions of N fixed were estimated for moja-intercropped beans, but it was not possible to statistically test differences in Ndfa for the different cropping designs.



Weed species A was available only on trial M2, species B was sampled on trials H1 and H2, and species C was sampled on trials M2, H1 and H2. The quality of the sampled weed species in their use as reference plants is unknown. To avoid making use of various weeds that do not grow everywhere, it could be useful to plant non-nodulating beans next to the plots. They would have been more similar to the nodulating beans in terms of nutrient uptake, and more suitable as reference plants.

Mucheru-Muna et al. (2009) also tested nitrogen fixation in moja and mbili intercrops. They used maize as a reference plant, but did not specify any  $\delta^{15}\text{N}$  levels for maize and for beans. They found that nitrogen fixation was not influenced by intercropping. Average levels of fixation were 60% Ndfa in sole and intercrops, whereas common beans fix on average 40% of total N (Hardarson & Atkins, 2003). High levels of nitrogen fixation suggest that some soil nitrogen may have been spared for maize.

It is only on trial M2 that nitrogen removal through seeds exceeded nitrogen fixation, resulting in a nitrogen loss (assuming that bean residues remained on the plots). It is not known how much nitrogen was removed from the system by maize.

## 4.5 Nitrogen levels and SPAD values

Results from lab analysis at the University of Leuven revealed slight differences in absolute and relative nitrogen contents of beans among the various treatments. Relative nitrogen contents were larger for mbili-intercropped improved beans than for moja- and sole-cropped local beans. Differences were small however, so the absolute amounts of nitrogen accumulated by the beans simply reflected the yields that were found for those treatments: N quantities in moja-intercropped local beans were lower than expected, as were their yields. Differences in absolute amounts accumulated by improved and local beans within the same cropping designs, were not significant.

SPAD values have been used as indicators for nitrogen contents of leaf tissue. Although a trend line through data on bean stover N content versus SPAD values showed a perfect relation ( $y=0.44 + 0.01x$ ), the  $R^2$  was only 34% as a result of scattered data (Figure 16). Local bean N content could not at all be predicted by their SPAD values. SPAD values were also not explained by PAR availability. Although average SPAD values of maize and beans were normal (Darkwa et al., 2016; Earl & Tollenaar, 1997), there may have been some extra variability as a result of dust and pollen that could have influenced the relationships with other factors. Darkwa et al. (2016) found that grain yields of beans were explained by leaf chlorophyll content, but no such effects were found in this thesis.

## 4.6 Nutrient contents of soil and bean tissue

Soil samples had been taken in April 2016, right before planting maize and beans. Prior to installation of the demonstration trials, the land had been managed and cropped as the local farmers who owned the land saw it to be suitable.

Ndakidemi & Semoka (2006) tested soil fertility in the Western Usambara Mountains in Northern Tanzania, and compiled from literature the critical levels of soil nutrients for most crops. Table 15 shows the ranges of soil nutrient contents found on the demonstration trials, next to a list of the critical levels of those nutrients. In almost all cases, sufficient nutrients were available for good growth of beans and maize. Soil nutrient contents were always well below toxic levels (Brodrick et al., 1995). P-Bray and OC

levels were on the low side on only one trial each. Soil N contents were low on all trials, but UREA and DAP were applied in the maize furrows after soil sampling.

Of all trials, the lowest contents of Zn, K, N and Ca were found on trial H4, where yields were already reduced by limited rainfall. Drought has been found to increase sensitivity of cereals to soil Zn deficiencies (Bagci et al., 2007). Soil Cu contents were highest on trial H4 and closest to – yet still below – the critical level for maize and beans.

**Table 15.** Soils in the trials compared to critical nutrient levels. \*As cited in Ndakidemi & Semoka, 2006.

Soil characteristic or nutrient	Range found on demonstration trials	Critical level for most crops	Reference
pH	6.35 – 7.55	6 – 7.5 (beans)	Shenkalwa et al. (2013)
EC (mS cm <sup>-1</sup> )	0.074 – 0.211	12 cmol/kg	NNS (1990) *
OC (%)	1.13 – 2.15	2	Greenland et al. (1975) *
P-Bray (mg kg <sup>-1</sup> ) (four trials)	14.36 – 49.93	15	FMANR (1990) *
P-Olsen (mg kg <sup>-1</sup> ) (two trials)	46.40 – 119.20		
N (%)	0.06 – 0.10	0.2	NNS (1990) *
Ca (cmol+ kg <sup>-1</sup> )	12.38 – 17.87	5	Marx et al. (1996) *
Mg (cmol+ kg <sup>-1</sup> )	4.19 – 4.87	2	Schwartz and Coralles (1989) *
K (cmol+ kg <sup>-1</sup> )	0.37 – 2.01	0.5 (beans)	Anderson (1973) *
Cu (ppm)	1.28 – 4.09	9 (beans) and 5 (maize)	Schulte et al. (1999)
Zn (ppm)	0.92 – 3.28	0.6	Silanpää (1982) *
Mn (ppm)	24.57 – 42.22	5.0	Silanpää (1982) *
Fe (ppm)	22.46 – 101.54	0.3 – 10	Lindsay and Cox (1985) *

According to literature, bean production and nitrogen fixation are mainly constrained by soils that are too acidic or too alkaline. Acidic soils primarily limit the availability of P, which is essential for crop growth and root nodulation, and alkaline soils often have deficiencies of B, Fe, Mn and Zn, which are important for the formation and functionality of nodules (Giller, 2001). Especially the availability of P and B can have strong impacts on nitrogen fixation (Graham & Vance, 2003; Hardarson & Atkins, 2003; Yamagishi & Yamamoto, 1994). Soil B contents were not assessed, but the neutral soil pH at the trial sites was favourable for its sufficient availability.

Although *Phaseolus* beans can be grown on wide range of soil types, loamy soils (medium texture) are most favourable (Shenkalwa et al., 2013). Soil textures in all trials were clayey except for two (H1 and H2) which were a sandy clay loam.

Data on tissue nutrient contents were only available for beans, and they were not correlated to soil nutrient contents except for Zn ( $R^2=0.53$ ). The use of discoloration symptoms of nutrient deficiencies as an indicator of tissue nutrient contents for plots was not reliable because those contents were not lower in those plots where symptoms were found, than in plots where no symptoms were observed. However, plant sampling for nutrient analysis occurred on a random basis and those sampled plants did not always show deficiencies. Symptoms may be a good indicator of nutrient contents at plant-level, and were likely a result of very localized differences in soil nutrient availability.

Soil and bean tissue analyses had been performed in the IITA laboratory in Dar Es Salaam, Tanzania. N contents in bean tissue were also determined at the University of Leuven in Belgium. As shown in Figure 5 and as explained in the Methods sections, a comparison of the results of the different labs revealed that the laboratory of Dar Es Salaam had overestimated biomass N contents. It is unknown

whether any systematic errors occurred in the analyses for other nutrients, but the comparison of laboratories suggests that the analyses from Dar Es Salaam may not have been completely accurate.

## **4.7 Farmers' considerations regarding legume intercropping**

Farmers were interviewed to learn about common legume production practices and about reasons for either or not applying intercropping in a certain way. The large majority of the respondents intercropped their legumes, and almost all of those farmers used a combination of beans and maize. The farmers who sole-cropped their legumes did so primarily to get larger yields.

Moja intercropping was most common, and only three out of 44 farmers applied mbili intercropping. For both intercropping practices, the main reasons were tradition and ease. It was not clear whether farmers had consciously chosen moja intercropping over mbili intercropping because of practical reasons, or whether farmers were not familiar with the mbili design. Details on row and plant spacing in the intercropping designs were not explained.

Land scarcity was one of the main drivers to apply intercropping and in the decision to use legumes in rotation or to plant them at the exact same plot each season. Farmers wanted to reduce risk and produce multiple crops on small pieces of land. Pest insects and irregular rain were most often mentioned as the most important constraints to legume production.

When farmers produced beans, they most often used improved rather than local varieties. Farmers also explained that there was a large demand for new improved bean varieties that were more resistant to drought and diseases, and that the accessibility and price of improved varieties were a problem.

A demand for more (frequent / diverse) extension services was also expressed. N2Africa's efforts were appreciated.

The farmers were interviewed rather fast, so as not to take much of their time and to limit their explanations to the most important factors. Reasons may have been mentioned by more farmers if they had been given more time to answer.

## **4.8 Improving practices and further research**

### **4.8.1. Composition of the demonstration trials**

The main purpose of the demonstration trials installed by N2Africa that were used in this study was to show farmers various cropping practices, different maize and bean varieties, and to show the common farmers' practice of moja intercropping as a reference. Farmers are invited to come and see the trials and to receive information from N2Africa staff.

The setup of the trials made it difficult to interpret whether any observed differences in for instance yield are a result of differences in total plant densities, row or plant spacing, any interactions between maize and beans, crop variety, or the use of fertilizer and/or insecticides. Of course it is not possible to vary all these factors one by one because it would require too many plots, but the treatments

could be made at least a little more comparable in the future. It could be considered to use the same plant spacing and row spacing between (twin) rows in all plots, and to apply fertilizers and insecticides on all or on none of the plots. It would mean that the moja-intercrop no longer represented the farmers' practice, but it could be assumed that farmers are aware of their common practices and that it is not strictly necessary to include it in the demonstration trial. Inclusion of a plot with moja-intercropped improved maize and a plot with sole-cropped local maize would make it possible to calculate pLER based on yields of the intercrop and sole crop of the same maize variety. If land area is limiting, it could be considered to plant a local and an improved variety on the halves of a single plot. Total plot area per sole-cropped variety would become very small, but there would at least be an impression of the sole-cropped yields of both varieties.

Signs in front of each plot could make it easier for visitors of the demonstration trial to understand the differences in cropping practices on the various plots. Despite the consistent use by N2Africa of the terms 'improved' and 'local' to describe varieties, I would suggest to avoid continuous referral to a new variety on a trial as 'improved' because it could lead to wrong assumptions on its performance (e.g. yield or disease sensitivity) that might not actually be valid under farmers' management. It could also be presented as an 'alternative' variety.

#### **4.8.2. Improving the intercropping design**

The near-additive moja design with rows of beans added between the rows of maize (row spacing was slightly different on in the sole and the moja design), resulted in a reduced bean yield compared to local sole-cropped beans. This suggests that the beans suffered from competition by maize. Wider row spacing of maize may reduce that competition, but it also decreases the total plant density. It is unknown which of those effects most strongly influence the total yield.

As for the mbili intercrop, it could be interesting to slightly reduce the spacing between twin rows of maize to test if they would yield differently. Cob set may become lower but this can be compensated by an increased plant density. The twin rows of beans could also be placed a bit closer together, so that they are a bit further away from the maize rows and receive more PAR and presumably less competition by maize for soil nutrients and water. Woomer et al. (2004), Mucheru-Muna et al. (2009) and Ndung'u et al. (2005) all used a mbili design with 0.5 m between the twin maize rows, and a strip of a width of 1 m for the two rows of beans. In those studies, LERs of the mbili designs were larger than of the moja designs. A slightly wider strip for the bean rows (e.g. 1.25) could also be tested, to improve light availability.

#### **4.8.3. Competition throughout time**

Different bean varieties can vary in their abilities to develop successfully under shaded conditions in particular growth stages. Davis and Garcia (1983) found larger bean and maize yields when beans were planted between rows of maize that were reaching physiological maturity (relay intercropping), than in maize-bean intercropping systems with simultaneous planting, in Colombia. Relay intercropping avoided effects of bean competition on maize. Davis and Garcia (1983) speculated that beans are able to develop successfully under shaded conditions during the early growth stages (relay intercropping), but that shading becomes more stressful if it starts from the flowering stage onwards (when maize has grown such

that it starts covering the beans, after simultaneous planting). On N2Africa's demonstration trials, maize and beans were planted on the same day, so it could be interesting to try relay intercropping in following seasons, and study crop development throughout the whole growing season.

Woolley et al. (1993) also defined the growth stages of beans until early flowering as the critical period for weed control. The demonstration trials were weeded one up to three times before flowering, so it is assumed that competition with weeds was not a problem. Competition with maize for soil nutrients may have been there during this critical period for beans. Intercrops do not only compete for light and nutrients but also for water. Rajcan and Swanton (2001) describe the stage of pollination as especially critical for water stress in maize.

Monitoring crop growth and weather conditions throughout a whole season would give more insight into actual competition between maize and beans for light, nutrients and water. Ideally this would be studied for the duration of several seasons. It could be tested whether soil fertility benefits become apparent as a result of producing beans.

## 5. Concluding remarks

The simultaneous growing of multiple crops is often done to maximize land use, to overcome cropping risks if one crop fails in the season, to make use of available light and to explore different soil nutrient niches. Competition of component crops occurs throughout the cropping season, but the research setup was limited to only determining crop and canopy variables when the crops close to physiological maturity and at harvest: the end result of crop competition. Data were collected at the end of only a single season.

Not all cropping designs were available for local as well as improved crop varieties. The treatments (design/variety/management combinations) being as they were, the following conclusions could be drawn:

- Relative yields of local maize were reduced by moja intercropping, compared to sole cropping. The increased total plant density sometimes compensated for the relatively poor performance.
- For the mbili intercrops, relative yields of the local and the improved bean and maize varieties were as expected based on the differences in plant densities of the intercrops and sole crops. LERs of the mbili designs could be calculated for four out of the six trials, and they were around 1 or slightly larger.
- More PAR was available for beans in the mbili intercrops than in the other designs.
- Estimates of the proportion of N fixed ranged a lot. Estimates were largest for moja-intercropped local beans compared to the other designs, but significant differences between treatments could not be determined.
- On all plots except one, more nitrogen was fixed than was removed through harvesting of bean seed.
- The improved maize variety DK 8031 was more sensitive to leaf spot and rust than the local variety. Grain and stover yields of the improved maize variety dropped faster with an increasing disease severity than for local maize. However, for similar diseases scores, yields of the improved variety were still often similar or larger compared to local maize.
- SPAD values cannot always be used as a reliable indicator of nitrogen contents.
- Mbili intercropping is rarely used (3 out of 44 interviewed farmers).
- According to farmers, main constraints to legume production are pests, irregular rainfall, diseases, and the poor availability and quality of improved seeds. Improvements to legume production can mainly be made through improved varieties and extension services.
- There were no differences between treatments with regard to SPAD, nutrient contents (except nitrogen, with a slightly larger N proportion for improved mbili beans compared to local moja and sole beans) and plant height.

Next steps in research on this topic could be the monitoring of crop competition throughout one or more whole cropping seasons, slightly denser plant spacing in the mbili cropping design, and a more balanced setup of the demonstration trials to facilitate comparisons between treatments.

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# Appendix

## Appendix I – Reference weeds

Figure 1 shows pictures of the weeds that were used as reference plants for assessment of the proportion of N derived from N fixation, using the  $\delta^{15}\text{N}$  natural abundance method.



Figure 1. Pictures of the three species of weeds that were used as reference plants for assessment of nitrogen fixation with use of the  $\delta^{15}\text{N}$  natural abundance method.

## Appendix II – Tissue nutrient content & visible nutrient deficiencies

**Table 1.** Nutrient contents of common bean stover per plot. Cells in the table are colored if any visible signs of a deficiency for that nutrient were observed in that plot. In all cases, only very few bean plants showed signs of deficiencies (discoloration of leaves and/or veins) and it is possible that the symptoms were not always interpreted right. Sulphur (S) deficiencies were observed, but bean tissue was not analyzed for S contents. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.

Trial	Design	Bean variety	N (%)	P (%)	Mg (%)	K (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Other deficiencies
M1	Sole	Improved	0.77	0.09	0.35	1.68	6.61	21.35	166.03	S
	Sole	Local	0.79	0.09	0.37	2.00	5.79	13.89	255.77	S
	Moja	Local	0.73	0.09	0.35	1.91	8.26	14.33	213.46	S
	Mbili	Improved	1.33	0.16	0.36	2.24	8.68	20.47	167.31	S
	Mbili	Local	1.29	0.21	0.36	2.33	7.02	30.26	173.72	
M2	Sole	Local	0.95	0.10	0.29	2.04	6.61	19.15	78.21	
	Sole	Improved	1.04	0.13	0.30	2.04	7.44	17.69	51.92	S
	Moja	Local	1.51	0.13	0.32	2.29	9.50	16.81	95.51	S
	Mbili	Improved	0.90	0.13	0.32	1.97	5.37	12.72	76.92	S
	Mbili	Local	0.78	0.09	0.30	2.13	5.37	17.98	58.33	S
H1	Sole	Improved	0.82	0.19	0.41	2.18	7.44	17.54	59.62	S
	Sole	Local	0.61	0.14	0.39	2.83	6.61	20.18	44.23	S
	Moja	Local	0.56	0.06	0.37	2.72	3.72	15.50	26.32	
	Mbili	Improved	0.98	0.18	0.42	2.42	4.55	14.91	60.53	S
	Mbili	Local	0.79	0.15	0.41	2.29	7.02	14.77	55.26	S
H2	Sole	Improved	1.18	0.22	0.41	2.23	8.68	28.65	45.26	
	Sole	Local	1.20	0.31	0.41	2.20	9.92	34.06	52.11	S
	Moja	Local	1.04	0.23	0.38	2.60	5.79	20.47	48.95	S
	Mbili	Improved	1.53	0.25	0.42	2.40	9.09	20.47	70.53	
H3	Sole	Local	0.73	0.24	0.42	2.15	7.44	25.73	44.21	
	Sole	Improved	0.90	0.20	0.42	2.45	6.20	16.23	30.00	S
	Moja	Local	0.76	0.17	0.43	2.41	7.02	20.91	54.74	
	Mbili	Local	0.80	0.23	0.42	2.33	5.79	27.78	50.00	S
	Mbili	Improved	1.37	0.28	0.44	2.70	11.57	30.56	52.63	S
H4	Sole	Improved	1.34	0.30	0.44	1.78	10.74	33.92	40.53	
	Sole	Local	1.06	0.27	0.47	2.04	16.12	33.04	31.58	S
	Mbili	Improved	1.39	0.28	0.43	1.95	16.53	38.89	29.47	
	Mbili	Local	0.95	0.23	0.45	1.94	17.36	30.41	29.47	

**Table 2.** Visible signs of nutrient deficiencies in plots with maize. In all cases, only very few bean plants showed signs of deficiencies (discoloration of leaves and/or veins) and it is possible that the symptoms were not always interpreted right. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.

Trial	Design	Maize variety	N	P	Mg	K	Cu	Zn	Mn	S
M1	Sole	Improved		■	■					■
	Moja	Local	■	■	■					■
	Mbili	Improved		■	■					
	Mbili	Local	■	■	■			■		
M2	Sole	Improved		■	■					
	Moja	Local	■	■	■					■
	Mbili	Improved		■	■					■
	Mbili	Local		■	■					■
H1	Sole	Improved	■	■	■	■				■
	Moja	Local	■	■	■	■				■
	Mbili	Improved		■	■	■				■
	Mbili	Local		■	■	■				■
H2	Sole	Improved		■	■					
	Moja	Local	■	■	■	■	■			■
	Mbili	Improved	■	■	■	■				■
H3	Moja	Local	■	■	■	■				
	Sole	Improved		■	■					
	Moja	Local	■	■	■	■				■
	Mbili	Improved		■	■					■
H4	Mbili	Improved	■	■	■			■		■
	Sole	Improved		■	■					
	Mbili	Local	■	■	■			■		

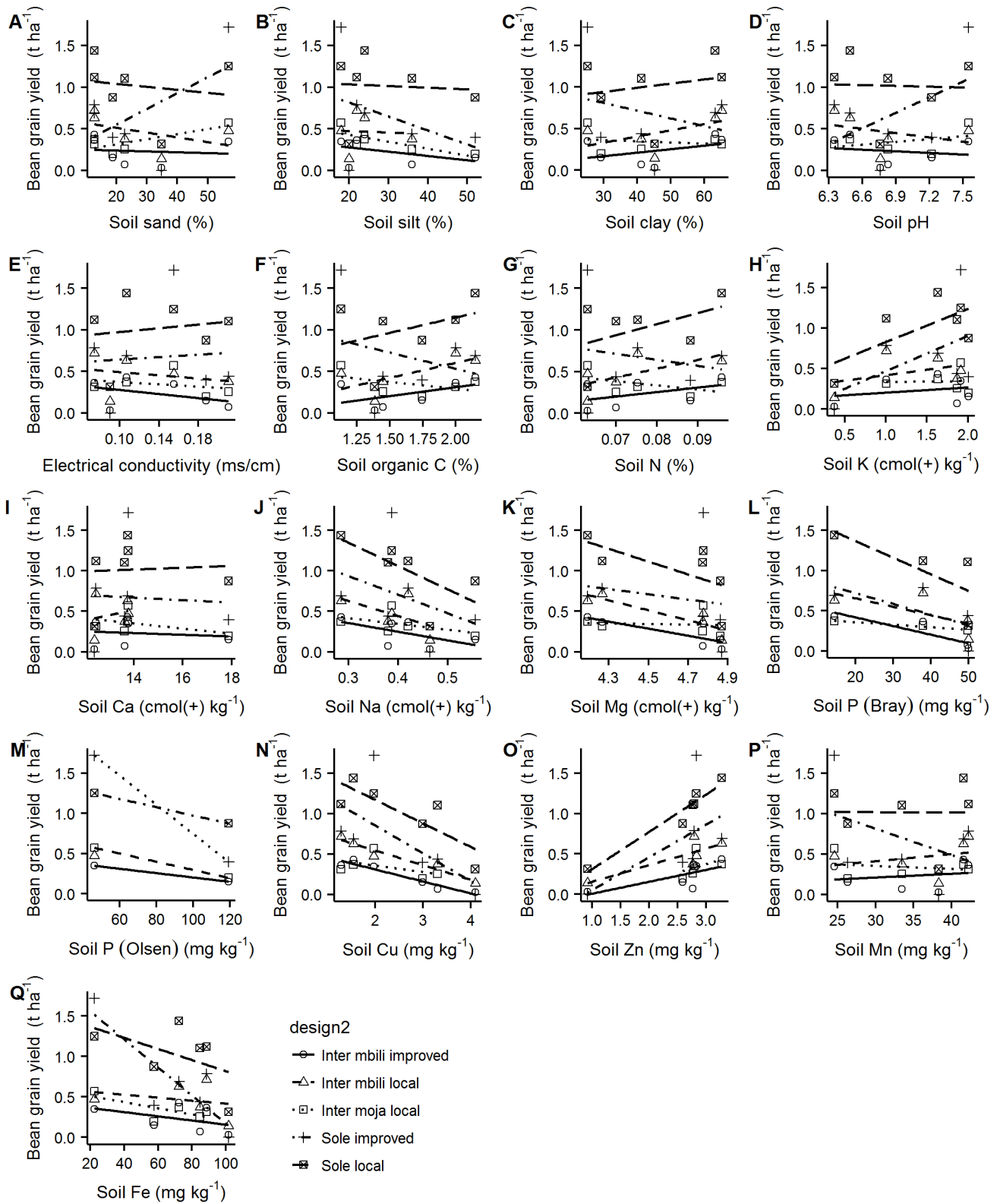
  

■	Signs of deficiency observed in some maize plants
■	Signs of deficiency observed in some bean plants
■	Signs of deficiency observed in maize and bean plants

## Appendix III – Soil nutrient contents

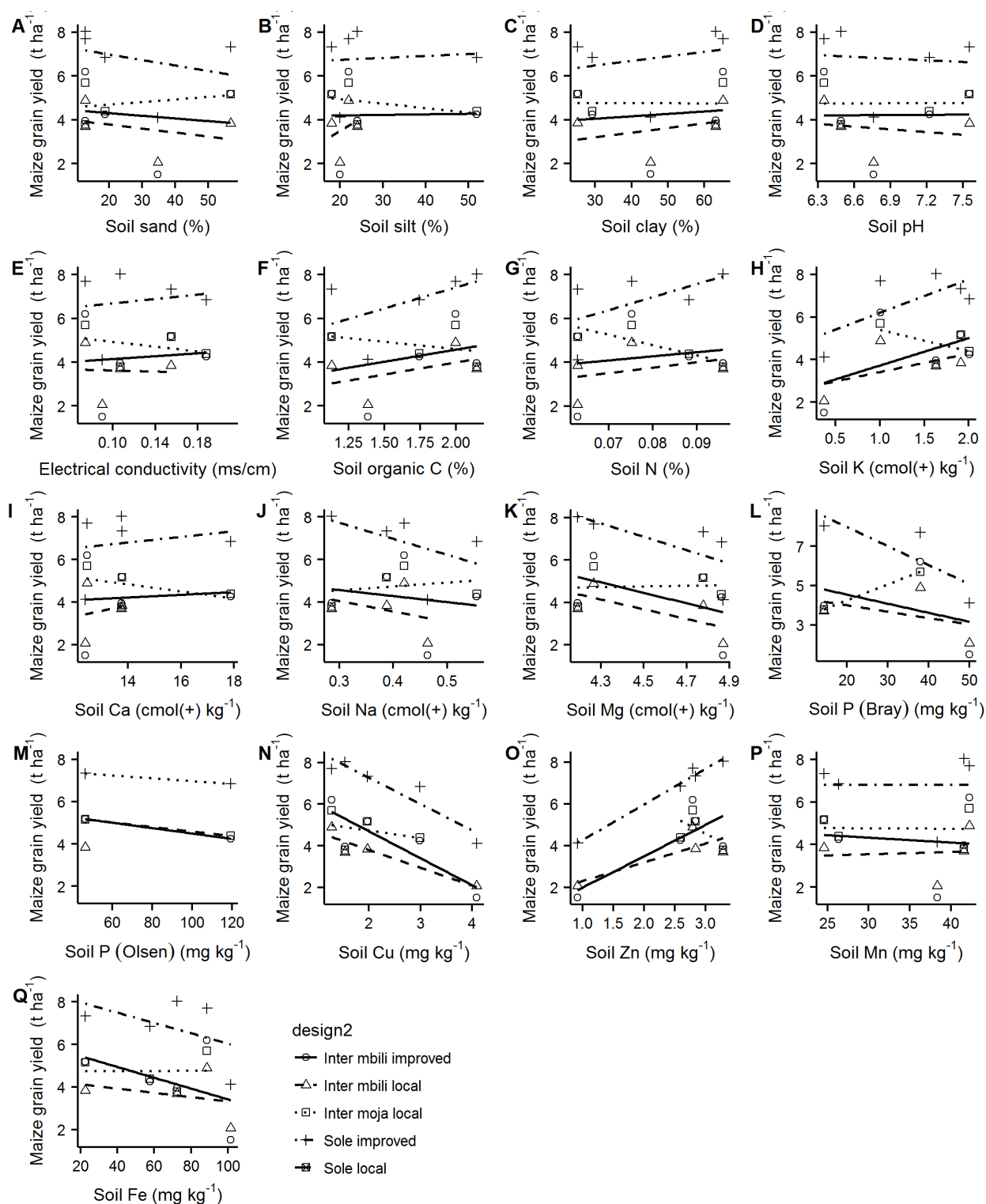
**Table 3.** Correlation matrix of soil properties. Soil samples had been taken on six trials (H1, H2, H3, H4, M1, M2) in April 2016. \*P-Bray contents were only available for trials M1, M2, H3 and H4.

	pH	EC	OC	Clay	Silt	Sand	N	Ca	Mg	K	Na	Cu	Zn	Mn	Fe	C:N
pH	1.00	0.43	-0.67	-0.95	0.11	0.72	-0.41	0.39	0.60	0.43	0.45	-0.10	0.09	-0.97	-0.81	-0.97
EC	0.43	1.00	-0.21	-0.56	0.69	-0.08	-0.01	0.53	0.39	0.76	0.27	0.05	0.44	-0.63	-0.29	-0.54
OC	-0.67	-0.21	1.00	0.72	0.31	-0.87	0.95	0.30	-0.76	0.14	-0.27	-0.39	0.41	0.56	0.27	0.64
Clay	-0.95	-0.56	0.72	1.00	-0.29	-0.63	0.49	-0.43	-0.80	-0.35	-0.64	-0.19	0.08	0.96	0.60	0.90
Silt	0.11	0.69	0.31	-0.29	1.00	-0.56	0.47	0.87	0.28	0.49	0.61	0.20	0.21	-0.35	0.03	-0.10
Sand	0.72	-0.08	-0.87	-0.63	-0.56	1.00	-0.80	-0.34	0.47	-0.10	0.06	0.00	-0.24	-0.54	-0.54	-0.70
N	-0.41	-0.01	0.95	0.49	0.47	-0.80	1.00	0.54	-0.68	0.39	-0.15	-0.51	0.57	0.29	0.01	0.39
Ca	0.39	0.53	0.30	-0.43	0.87	-0.34	0.54	1.00	0.19	0.61	0.61	-0.12	0.34	-0.56	-0.36	-0.35
Mg	0.60	0.39	-0.76	-0.80	0.28	0.47	-0.68	0.19	1.00	-0.15	0.80	0.74	-0.59	-0.61	-0.01	-0.49
K	0.43	0.76	0.14	-0.35	0.49	-0.10	0.39	0.61	-0.15	1.00	-0.09	-0.60	0.88	-0.58	-0.68	-0.57
Na	0.45	0.27	-0.27	-0.64	0.61	0.06	-0.15	0.61	0.80	-0.09	1.00	0.62	-0.50	-0.53	0.02	-0.29
Cu	-0.10	0.05	-0.39	-0.19	0.20	0.00	-0.51	-0.12	0.74	-0.60	0.62	1.00	-0.84	0.07	0.66	0.22
Zn	0.09	0.44	0.41	0.08	0.21	-0.24	0.57	0.34	-0.59	0.88	-0.50	-0.84	1.00	-0.19	-0.56	-0.26
Mn	-0.97	-0.63	0.56	0.96	-0.35	-0.54	0.29	-0.56	-0.61	-0.58	-0.53	0.07	-0.19	1.00	0.76	0.96
Fe	-0.81	-0.29	0.27	0.60	0.03	-0.54	0.01	-0.36	-0.01	-0.68	0.02	0.66	-0.56	0.76	1.00	0.86
C:N	-0.97	-0.54	0.64	0.90	-0.10	-0.70	0.39	-0.35	-0.49	-0.57	-0.29	0.22	-0.26	0.96	0.86	1.00
P*	0.64	0.33	-0.90	-0.77	0.26	0.72	-0.98	-0.48	0.89	-0.36	0.85	0.75	-0.65	-0.67	0.82	-0.20



**Figure 2.** Relationship between bean grain yield and various soil characteristics. Data were not corrected for trial location. NB: relationships exist between the depicted soil properties, as shown in Table 4 of the Appendix. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.





**Figure 3.** Relationship between maize grain yield and various soil characteristics. Data were not corrected for trial location. NB: relationships exist between the depicted soil properties, as shown in Table 4 of the Appendix. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.



**Table 4.** Strength of correlations between soil characteristics and grain yields of **beans**, expressed as R<sup>2</sup>-values. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.

Design	Bean variety	Clay	Silt	Sand	pH	EC	OC	P -Bray	N
Sole	Improved	0.06	0.12	0.26	0.20	0.00	0.06	0.31	0.02
Sole	Local	0.04	0.00	0.02	0.00	0.02	0.11	0.42	0.18
Mbili	Improved	0.14	0.13	0.01	0.02	0.13	0.21	0.71	0.15
Mbili	Local	0.24	0.00	0.17	0.09	0.04	0.41	0.36	0.33
Moja	Local	0.03	0.58	0.56	0.12	0.06	0.17	0.93	0.17
Design	Bean variety	Ca	Mg	K	Na	Cu	Zn	Mn	Fe
Sole	Improved	0.00	0.02	0.20	0.11	0.37	0.28	0.15	0.66
Sole	Local	0.00	0.34	0.40	0.39	0.62	0.93	0.00	0.21
Mbili	Improved	0.01	0.58	0.05	0.28	0.91	0.45	0.03	0.15
Mbili	Local	0.03	0.71	0.12	0.24	0.92	0.63	0.07	0.05
Moja	Local	0.16	0.01	0.00	0.19	0.20	0.11	0.03	0.42

**Table 5.** Strength of correlations between soil characteristics and grain yields **maize**, expressed as R<sup>2</sup>-values. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.

Design	Maize variety	Clay	Silt	Sand	pH	EC	OC	P -Bray	N
Sole	Improved	0.05	0.00	0.07	0.00	0.02	0.21	NA	0.26
Mbili	Improved	0.01	0.00	0.01	0.00	0.01	0.05	NA	0.02
Mbili	Local	0.08	0.05	0.08	0.02	0.00	0.15	NA	0.07
Moja	Local	0.00	0.10	0.06	0.00	0.07	0.09	NA	0.57
Design	Maize variety	Ca	Mg	K	Na	Cu	Zn	Mn	Fe
Sole	Improved	0.03	0.39	0.41	0.18	0.84	0.98	0.00	0.18
Mbili	Improved	0.00	0.18	0.21	0.02	0.68	0.54	0.01	0.16
Mbili	Local	0.02	0.37	0.19	0.07	0.84	0.56	0.00	0.06
Moja	Local	0.15	0.00	0.21	0.04	0.07	0.20	0.00	0.00

## Appendix IV – Yields per trial

**Table 6.** Grain yields of beans and maize on each plot on six different trial locations. *Moja*: alternating rows of maize and beans. *Mbili*: two-by-two alternating rows of maize and beans.

Trial	Design	Bean			Maize			
		Variety	Grain yield (t ha <sup>-1</sup> )	Intercrop yield / sole crop yield (same variety)	Variety	Grain yield (t ha <sup>-1</sup> )	Intercrop yield / sole crop yield (same variety)	
M1	Sole	Improved	0.690		-	-		
	Sole	Local	1.440		-	-		
	Moja	Local	0.580	0.40	Local	3.547		
	Mbili	Improved	0.415	0.60	Improved	4.767	0.61	
	Mbili	Local	0.605	0.42	Local	4.437		
	Sole	-	-		Improved	7.833		
M2	Sole B	Local	1.120		-	-		
	Sole B	Improved	0.785		-	-		
	Moja	Local	0.490	0.44	Local	3.556		
	Mbili	Improved	0.351	0.45	Improved	6.105	0.83	
	Mbili	Local	0.690	0.62	Local	4.805		
	Sole	-	-		Improved	7.350		
H1	Sole	Improved	1.720		-	-		
	Sole	Local	1.250		-	-		
	Moja	Local	0.535	0.43	Local	5.478		
	Mbili	Improved	0.335	0.20	Improved	6.422		
	Mbili	Local	0.455	0.36	Local	4.760	0.83	
	Sole	-	-		Improved	7.700		
H2	Sole	Improved	0.395		-	-		
	Sole	Local	0.875		-	-		
	Moja	Local	0.185	0.21	Local	3.856		
	Mbili	Improved	0.145	0.37	Improved	4.960	0.77	
	Sole	-	-		Improved	6.471		
H3	Sole	Local	1.105		-	-		
	Sole	Improved	0.439		-	-		
	Moja	Local	0.239	0.54	Local	No yield data		
	Mbili	Local	0.270	0.24	Local	No yield data		
	Mbili	Improved	0.050	0.11	Improved	No yield data		
	Sole	-	-		Improved	No yield data		
H4	Sole	Improved	0		-	-		
	Sole	Local	0.315		-	-		
	Mbili	Improved	0.030	div/0	Improved	1.815		
	Mbili	Local	0.135	0.43	Local	2.475	0.45	
	Sole	-	-		Improved	4.014		
			Plant density intercrop / plant density solecrop					
			Moja	0.63			Moja	0.87
			Mbili	0.42			Mbili	0.62

## Appendix V – Survey form

The survey questions were asked by a Swahili translator, and filled in in the following form:

### Introduction

N2Africa is a project that aims to improve the production of legumes, because they fix nitrogen from the atmosphere at make it available in the soil, thereby improving soil fertility. Besides, legumes like beans or peas are very nutritious and contain many proteins. In order to develop improved production technologies, we first need to learn from you about traditional practices and the constraints you experience. This is why I would like to ask you a few questions about legume production.

*[General remark: Try to avoid mentioning the answering options to the farmer. Just fill in what he/she mentions. After his/her answer, do ask whether that was it or whether he/she has more to say.]*

<b>Date:</b>		<b>Location:</b>	
<b>Name of person filling in the form:</b>			
<i>[Fill in about the respondent:]</i>			
<b>Name:</b>			
<b>Age:</b>			
<b>Sex:</b>			
<b>Village:</b>			
<b>Highest level of education:</b>		<i>[Indicate the number of years this education was attended]</i>	
Primary			
Secondary			
Post-secondary			
University			
Other:			
<b>1a. Do you (sometimes) produce legumes?</b>			
Yes		No	
<b>1b. Why?</b> <i>[Multiple options possible]</i>		<b>1b. Why not?</b> <i>[Multiple options possible]</i>	
Soil fertility benefits		Low market value	
Nutritional value		Simply no interest in legume production	
Tradition		Other:	
Good market price			
Other:			
<b>End of questionnaire if no legume production</b>			
<b>2a. Which legumes do you produce?</b> <i>[Multiple options possible]</i>		<b>2b. Do you use a local or improved variety?</b> <i>[Encircle what is applicable for the crops in the left column]</i>	
Bush bean		Local / improved	
(Semi) climbing bean		Local / improved	
Mung bean / green gram		Local / improved	
Pigeon pea		Local / improved	
Cowpea		Local / improved	

Groundnut		Local / improved
Leguminous trees		Species:
Other:		Local / improved
<b>3. Do you sell (part of) your legume production? If yes, how much do you sell?</b>		
No, all is for home consumption		
Yes, a little		
Yes, about half		
Yes, most		
Yes, all		
<b>4a. For legume production, do you practice sole cropping or intercropping? <i>[Both options possible!]</i></b>		
Intercropping		Sole cropping
<b>4b. Why? <i>[Multiple options possible]</i></b>		<b>4b. Why? <i>[Multiple options possible]</i></b>
Increase production of legume / intercrop / both		Higher yield
Increase soil cover		Avoid competition for light
Reduce weed pressure		Avoid competition for nutrients
Reduce pest pressure		Tradition
Optimal use of space		Other:
Reduce production risk (hope that at least one crop performs well)		
Tradition		
Other:		
<b>5a. Which crop combinations do you use? <i>[Should involve a before-mentioned legume!]</i></b>		<i>[If more than one combination is mentioned:]</i> <b>5b. Which combination performs best? <i>[Mark that crop combination in the left column]</i></b>
Bean – Maize		
Bean – Maize – Sunflower		
Pigeon pea – Maize		
Pigeon pea – Maize – Sunflower		
Other:		
Other:		
Other:		
<b>6a. How do you arrange your intercrops? <i>[Multiple options possible]</i></b>		<b>6b. Why like this?</b>
One-by-one alternating rows (I-I-I-I-)		Easy practice / low labour intensity
		Tradition
		Light availability / reduce competition for light
		Nutrient availability / reduce competition for nutrients
		Other:

Two-by-two alternating rows (II--II--II--)	Easy practice / low labour intensity	
	Tradition	
	Light availability / reduce competition for light	
	Nutrient availability / reduce competition for nutrients	
	Other:	
Randomly distributed	Easy practice / low labour intensity	
	Tradition	
	Light availability / reduce competition for light	
	Nutrient availability / reduce competition for nutrients	
	Other:	
Other:	Easy practice / low labour intensity	
	Tradition	
	Light availability / reduce competition for light	
	Nutrient availability / reduce competition for nutrients	
	Other:	
<b>7a. On the fields where you produce legumes, do you use crop rotations or do you plant the same crop(s) on the same piece of land every season? [Multiple options possible, if the farmer does not use the same method all the time]</b>		
Crop rotation	<b>7b. Why?</b>	
	Avoid diseases	
	Tradition	
	To improve soil quality	
	Other:	
Same crops every season	Easy planning	
	Tradition	
	No problems with diseases	
	Limited land available	

		Other:	
<b>8. How satisfied are you with your legume yields in regular years?</b>			
Not satisfied			
Not very satisfied			
Satisfied			
Very satisfied			
<b>9. What are common constraints for legume production? <i>[Multiple options possible]</i></b>			
Pests (insects)		No fertilizers	
Pests (animals)		Flooding	
Disease		Too much rain	
Weeds		Irregular rain	
Low soil fertility		Drought	
Availability of improved seed		Seed quality	
Other:			
<b>10. How can legume production be improved, do you think?</b>			

