Effect of cropping system design on severity of biotic stresses in common bean (*Phaseolus vulgaris*) and maize (*Zea mays*) in Northern Tanzania

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Abstract

Sustainable intensification of the agriculture in sub-Saharan Africa is essential to feed a growing population by increasing crop productivity and food security while preserving ecosystem services. The N_2Africa initiative is contributing to the onset of this by strengthening the implementation of nitrogen fixing leguminous species in smallholder farming systems. In the project country, Tanzania, common bean (*Phaseolus vulgaris*) constitute a staple food and principal source of dietary protein, however, the crop is subject to substantial yield losses caused by nutrient limitations and biotic stresses. As the latter has received less attention in N_2Africa, the object of this project is to identify occurring biotic stresses and the effect of cropping system or common bean cultivar (local, improved) on their severity. To address these a maize-common bean field trial at Kimashuku in the Northern Highland of Tanzania was studied throughout the long rain season, 2017. Data collection was done by in field measurements, visual scoring using standardized protocols and laboratory analysis.

At the site we scored the incidence and progression of damage done by 20 pests and 14 diseases while assessing crop light interception and development. Effect of rotation and intercropping was found to have a negligible effect on biotic stresses, with the large population and diversity observed a likely contributing factor plus plot size. Moreover, observations of substantial N, P, K and Mg deficiencies suggested that nutrient limitations were a major yield gap component at the site. Maize in a cropping system with the local common bean cultivar showed an overall higher performance with the rotation system scoring a land equivalent ratio of 1.64. However, a substantial yield gap of 64 to 86 % was found in the maize crop. On basis of our findings we recommend further studies on most important biotic stresses identified at the site and on near-future practices for smallholder farmers to control these.

Keywords: N_2Africa, Common bean (*Phaseolus vulgaris*), Maize (*Zea mays*), Cropping system, Biotic stress, Phenological dating, Light interception, Yield gap, Spottet stem borer (*Chilo partellus*), African bollworm (*Helicoverpa armigera*), Common rust (*Puccinia sorghi*), Eyespot (*Kabatiella zeae*), Alternaria leaf spot (*Alternaria alternate*), Angular leaf spot (*Isariopsis griseola*), Rhizoctonia root rot (*Rhizoctonia solani*)

Kimashuku field site in the Northern Highland of Tanzania at the beginning of the long rain season, end of March 2017 with view of Kilimanjaro in the horizon.

1. Introduction

The population of sub-Saharan Africa (SSA) is in exponential growth, and projected to double in the next 40 years, reaching an estimated 2 billion people (Cleland 2013; IBRD-IDA 2015). Dominated by smallholder farms the agricultural system of SSA has been incapable of meeting this subsequent food demand by means of an equivalent increase in productivity (Vanlauwe *et al*. 2014). Agricultural area has, therefore, on a continental level undertaken a substantial increase in past decades at direct expense of natural vegetation to increase the production (Brink and Eva 2009; Vanlauwe *et al*. 2015). A development that is furthermore escalated by a concurrent deterioration in soil fertility, caused by suboptimal management practices with nutrient deficiencies being a major yield gap component (Kihara *et al*. 2017). Current emphasis is, therefore, on a sustainable intensification of smallholder farms to i) narrow the gap between current and potential crop yields, ii) preserve ecosystem services and iii) increase the agricultural systems resilience to shocks and stresses (Vanlauwe *et al*. 2014). Introduction of novel practices is, nonetheless, impeded by smallholder farms limited finances, requiring low-risk options with short-tern return on their investments (Giller *et al*. 2013). A timely adaption of novel practices in this transition, therefore, require governmental intervention and other institutional initiatives such as the N_2Africa project (F. Baijukya 2014; E Ronner, Baijukya, and Giller 2012). The project approaches the topic from a soil fertility perspective by emphasizing an increased implementation of leguminous crops and biological nitrogen fixation in the smallholder farm system. In order to achieve this the project is conducting substantial research on development and selection of improved legume cultivars and *Rhizobium* inoculants (Giller *et al*. 2013). To ensure local adaptation the project is founded on delivery and dissemination of best available technology, aiming to reach more than 550.000 smallholder farmers in Africa by 2018 (Ampadu-boakye, Stadler, and Kanampiu 2017). This includes conducting small on-farm trials as an educative tool, making it possible to visualize the effect of novel practices without providing an expense or risk to the smallholder farmer.

In Tanzania, a project core country, common bean (*Phaseolus vulgaris* cv.) is the most widely cultivated grain legume often found intercropped alongside maize (*Zea mays*) (Hillocks *et al*. 2006; Esther Ronner and Giller 2013). This staple food crop constitutes a principal source of dietary protein in the rural household, complimenting a diet largely composed of maize, cassava root and plantain (Ugen *et al*. 2009). Yield of the common bean crop is, however, poor in the smallholder farming system with a yield average of 0.98 tons per hectare relative to a potential yield of 2.90 tons (FAO 2014; Schilt 2017). Consistent with this pattern is the yield of maize with an average of 1.63 tons per hectare relative to a potential yield of 8.90 tons (FAO 2014; YieldGap 2017). Contributing to these yield gap are severe nutrient limitation, inadequate agricultural practices, climatic factors and biotic stresses. The latter being a topic that has received less attention in the N_2Africa project although giving cause to substantial yield losses (Mwanauta, Mtei, and Ndakidemi 2015; Suleiman and Rosentrater 2015). In order to reduce the yield gap caused by biotic stresses in the Tanzanian smallholder farming system more knowledge is needed on the subject to advise farmers in means of controlling them.

The **objectives** of our project are, therefore, to i) Identify pests and diseases and their severity in a common bean – maize cropping system, ii) Assess the effect of intercropping and crop rotation as a control practice, iii) Study the relationship between selected plant physiological parameters and occurrence of biotic stresses and iv) Study the performance of a local and improved cultivar of common bean.

This will allow us to address the following **research questions**

- What are the most important biotic stresses in the common bean – maize cropping system?
- Does severity and/or incidence of pests and disease vary with cropping system? Intercropping?
- Does scoring of phenological stages, height and light interception provide relevant information to ascertain the influence of biotic stresses on crop from a holistic perspective?
- Does cultivar have a noticeable effect on resistance to biotic stresses and selected physiological parameters in common bean?

Methodologies for this project includes measurement of selected physiological parameters, in-field diagnosis and scoring of biotic stress at an ongoing field trial in the Northern Highland of Tanzania from sowing until harvest during the long rain season (March to June) 2017. Moreover, at terminus of the growth season, a laboratory analysis was included of collected samples to conduct a molecular diagnosis.

2. Materials and Methods

2.1 Kimashuku field trial site:

The field trial at Kimashuku is located in the Hai district belonging to the Kilimanjaro region of Northern Tanzania, with the latitude 03°18'03.74"S, and longitude 37°12'13.94"E, at an altitude of 1051 meter above sea level. Precipitation, humidity and temperature data were collected from a nearby Davis® Integrated Sensor Suite 6322C station "Mailia sita", located at latitude 03°19'36.91"S, and longitude 37°16'05.84"E operated by Kukua B.V. Weather service. The plots were irrigated by flooding with water from nearby stream twice a week from 2nd to 4th week after sowing until rain season started. Weeding of plots was done mechanically by manual labour when necessary, approximately, once a week until plants closed the rows. The labour was done by local workforce hired by a Ph.D student Eliakira Kisetu, responsible for the field site.

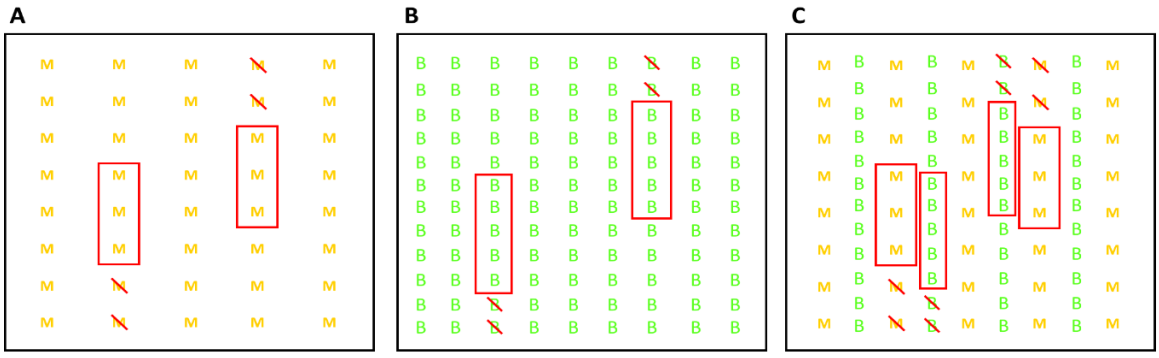
2.1.1 Plot design

In the field trial two cultivars of common bean (*Phaseolus vulgaris*) were deployed, a vine-growing local (Mkanamna) and a bushy-growing, improved (Lyamungu 90) developed by Selian Agricultural Research Institute in Arusha, Tanzania. Together with a local cultivar of maize (*Zea mays*) these were sown on the 6th of March 2017 in nine different cropping systems of monoculture, intercropping or rotation with a minimum of four replicates (Appendix, Table A1). The cropping systems were laid down in a randomized complete block design on the trial site in four rows each comprised of 11 plots (5 x 3.2 m). Distance between rows were 1 meter with no space between plots within the row except after plot number three, where a section in the size of a plot was left barrow to allow passage of water. Monoculture of maize was sown in five rows with an average within row distance of 30 cm and between row distance of 80 cm. Monoculture of common bean was sown in nine rows with an average within row distance of 10 cm and between row distance of 40 cm. In the intercropping plots five maize rows were sown and four rows of a common bean cultivar with similar within row distance as in monoculture and a between row distance of 40 cm. At sowing, triple superphosphate (TSP, 46% P_2O_5) fertilizer was applied per plant at a rate equivalent to 25 kg P ha^{-1} and 21 days after sowing (DAS) maize plants were applied urea (46% N) fertilizer by banding at a rate equivalent to 120 kg N ha^{-1}.

2.2 Dating of phenological stages:

The dating of plants phenological stages was conducted by sampling two times five plants per plot in a diagonal pattern and determining a mean value per plot at a given DAS (Figure 1, A-C). In the selection of plants the outer rows were discarded to avoid any boarder-effect on scores. In maize row two and four were assessed, discarding the initial two plants. In common bean row three and seven were assessed in monoculture, row two and four in intercropping plots. The dating of phenological stages was initiated 28 DAS and conducted approximately once a week, utilizing a pre-defined set of phenotypes for scoring the vegetative and reproductive stages for each crop given by literature.

Figure 1: Plant selection for assessments in plots with **A**) monoculture of maize (*Zea mays*), **B**) monoculture of common bean (*Phaeolus vulgaris*), and **C**) intercropping of maize and common bean



2.2.1 Dating phenological stages in maize

In the vegetative growth phase of maize, setting of leaf collar was scored as the number counted from below with the cotyledons as V1 and subsequent collars V2, V3,…, V_n (Dupont Pioneer 2016). Setting of tassels (R1) was equivalently dated and represented the transition from vegetative to reproductive growth followed by emergence of silks from young cobs as R2. Subsequent scoring of R3 to R6 stage in accordance to the utilized protocol by phenotypic observations were substituted with sampling of kernels for measurement of dry weight development. A single cob was collected per selected plot, with two replicates per cropping system (Table 2) per week after scoring of R2 stage until maturation. Collected cobs were dried by hanging from strings at 25-28°C in a ventilated room, and stored until weighing at terminus of the experiment.

2.2.2 Dating phenological stages in common bean

In the vegetative growth phase of common bean, number of nodes counted from below was scored when leaves extending from it were unrolled and leaf edges of each leaflet no longer touching (Lebaron 1974). Primary leaf node was noted as V1 with subsequent nodes as V2, V3,…,V_n. One blossom open at any node marked the transition to reproductive growth and was scored as R1. Pod setting was scored as R2 when pods at first blossom position was larger than ½ inch. Initial pod growth was scored as R3 when pod at first blossom position was larger than 1 inch, and when larger than 2 inch for the vine growing local cultivar and larger than 3 inch for the bushy growing improved cultivar as R4. Subsequent scoring of R5 to R9 stage in accordance to utilized protocol by phenotypic observations were substituted with measurements of seed dry weight development. Two times three pods were collected in the selection pattern previously described in two replicates per cropping system once a week from scoring of R3 stage until maturation (Figure 1). Collected pods were split and seeds dried at 25-28°C in a ventilated room, and stored until weighing at terminus of the experiment.

2.2.3 Measurement of plant height, plant number and yield

Six plants were selected in the pattern previously described and height was measured each week starting 36 DAS (Figure 1). Height of maize plants was measured from ground level to the point of top leaf separation at the highest collar or node of flag leaf when set. Height of common bean plants was measured from group level to the top node, in which trifoliate leaves were sufficiently unrolled so the two edges of each leaflet are no longer touching. Plants per plot were counted 32 DAS. Common bean crop was harvested at 97 DAS by uprooting the entire plant, discarding plants from boundary row and the initial two plants in each row, and set to dry for four days prior to threshing and weighing. Maize crop was harvested at 145 DAS, similarly discarding plants in boundary rows and kernels weighed per plot.

2.3 Crop light interception

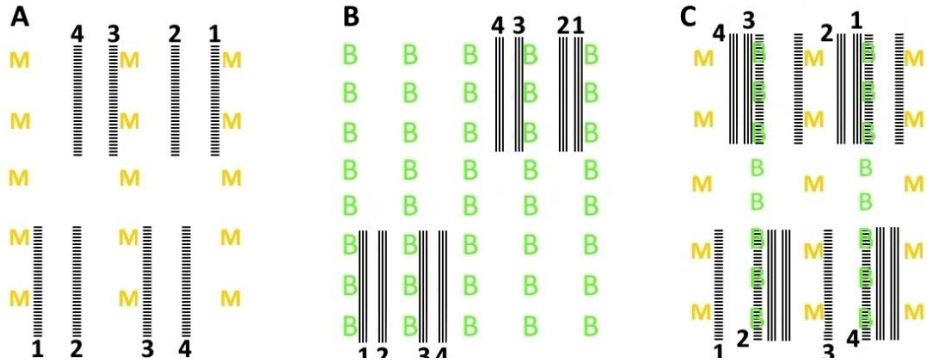
Measurements of light incidence within the plot was done to investigate light interception by the crops in the different cropping systems. Data was collected using an AccuPAR LP-80 ceptometer, Decagon Devices Inc™ instrument with an 86.5 cm probe containing eighty sensors along its length each measuring photosynthetically active radiation (PAR). In two plots per cropping system (Table 2), four measurements were done parallel to the row at ground level beneath and between plant rows respectively in both monoculture and intercropping plots of each crop (Figure 2 A-C). Simultaneous to the below canopy measurements, above canopy light incidence was

collected by an external spot sensor placed in the centre of each plot prior to measurements at a height of 3 meters to enable a calculation of fraction of PAR intercepted (F_{LI}). In order to subtract the light intercepted by maize crop when calculating F_{LI} of beans in intercropping plots, measurements were also done above bean plants at 30-50 cm height. Measurements were done in overcast conditions prior to row closing 43 DAS, then at 61, 79 and finally after bean harvest, nearing maturity of maize at 110 DAS.

$$F_{LI} = 1 - \frac{PAR_{Above\ canopy}}{PAR_{Below\ canopy}}$$

$$F_{LI,\ bean\ intercrop} = 1 - \frac{PAR_{Above\ canopy} * (1 - F_{LI\ \ maize\ intercrop})}{PAR_{Below\ bean\ canopy}}$$

Figure 2: Plant selection for assessment of light incidence in plots discarding the outer two rows of plants on the horizontal axis and on the vertical axis one row of plants in maize (*Zea mays*) and two rows in common bean (*Phaseolus vulgaris*). Measurement of light incidence at ground level beneath canopy (1,3) and between rows (2,4) in monoculture of **A**) maize and **B**) common bean. Measurement of light incidence at ground level beneath canopy (1,3), between rows (2,4 indicated in vertical stripes) and light incidence above (2,4 indicated in horizontal stripes) common bean plants at 30-50 cm in **C**) intercropping plots with maize. Stripes represent the assessed zone of 86.5 cm.



2.4 Scoring of disease, virus and pest severity and incidence:

Selection of plants for assessment of disease, viral and pest severity and incidence was conducted as previously described (Figure 1). On basis of observations in field all occurring biotic stress were noted and the most important scored for their severity according to literature protocols or scored for incidence if less important in regard to crop performance.

2.4.1 In maize

Severity was scored of the most important biotic stresses Eyespot, Grey leaf spot, Common rust and Stem borer foliage feeding. Common rust caused by the fungal pathogen *Puccinia sorghi* was scored for incidence 58 and 66 DAS. Severity was scored in accordance to a modified Cobb scale on basis of total leaf area affected by pustules on lowest leaf at 74, 77, 83, 86 and 91 DAS and leaf supporting upper ear at 79, 83, 86, 91 and 97 DAS (Peterson, Campbell, and Hannah 1948; Roelfs, Singh, and Saari 1992). Common rust disease is characterized by small, elongate, powdery dark brown pustules on both the adaxial and abaxial side of the leaf, distinguishing it from the circular, lighter cinnamon brown pustules of Southern rust predominantly confined to the adaxial side (CIMMYT 2004). Eyespot caused by the fungal pathogen *Kabatiella zeae* was scored for incidence at 58 and 66 DAS. Severity was scored on basis of total leaf area affected by the disease with visual grading support from a diagrammatic scale for Phaeosphaeria leaf spot disease on lowest leaf at 72, 77, 83, 86 and 91 DAS and leaf supporting upper ear at 79, 83, 86, 91 and 97 DAS (Sachs *et al*. 2011). Eyespot disease is characterized by small 1 to 4 mm, round, translucent lesions with tan coloured centre, black to purple margin and yellow halo (CIMMYT 2004). Grey leaf spot caused by the fungal pathogen *Cercospora zeae-maydis* and *C. sorghi* var. *maydis* was scored for severity in accordance to a 0 to 5 scale by Danson (2008) on basis of leaf area affected by the disease (Table 1). Lower leaf was scored 72, 77, 83, 86 and 91 DAS and leaf supporting upper ear at 79, 83, 86, 91 and 97 DAS. Grey leaf spot disease is characterized by regular, elongated brown-grey necrotic spots growing parallel to the veins (CIMMYT 2004).

Table 1: Five-step visual rating scale of Grey leaf spot disease (*Cercospora zeae-maydis* and *C. sorghi* var. *maydis*)severity in maize (*Zea mays*) after Danson *et al* (2008)

Severity score	Score phenotype
0	No symptoms
1	Very small necrotic lesions on leaves
2	Light necrosis covering less than 40% of the leaf area
3	Moderate necrosis on leaves, 40 to 60% of the leaf area
4	Severe necrosis on 60-80% of the leaf area
5	Very severe necrosis on more than 90% of the leaf area, or dead plants

Scoring of stem borer foliage feeding was conducted at 25, 29 and 34 DAS using a visual nine-step rating scale after Tefera *et al* (2011) (Table 2). Incidence of stem borer was conducted at 25 and 29 DAS with identification to specie level.

Table 2: Nine-step visual rating scale of stem borer leaf damage severity in maize (*Zea mays*) after Tefera *et al* (2011)

Severity score	Score phenotype
1	No visible leaf feeding damage
2	Few pin holes on older leaves
3	Several shot-holes injury on few leaves
4	Several shot hole injuries common on several leaves or small lesions
5	Elongated lesions larger than 2 cm long on a few leaves
6	Elongated lesions on several leaves
7	Several leaves with elongated lesions or tattering
8	Most leaves with elongated lesions or severe tattering
9	Plant dying as result of foliar damage

Incidence solely was scored for the less important biotic stresses. Physoderma brown spot caused by the fungal pathogen *Physoderma maydis* was scored for incidence at 93 DAS, diagnosed by circular dark brown spots on midribs, nodes and stem (CIMMYT 2004). Incidence of cob feeding damage was done at 93 DAS simultaneous to incidence scoring of aphid feeding damage to stem observed as brown patches under leaf sheaths.

2.4.2 In common bean

Severity was scored of foliage feeding at 29 and 34 DAS using a visual nine step rating scale after Schoonhoven & Pastor-Corrales (1987) on basis of % leaf area consumed by the pest (Table 3).

Table 3: Nine-step visual rating scale of foliage feeding in common bean (*Phaseolus vulgaris*) after Schoonhoven & Pastor-Corrales (1987).

Severity score	Score phenotype
1	No defoliation
2	Less than 5% of the leaf area consumed
3	Between 5 and 10% of the leaf area consumed
4	Between 10 and 15% of the leaf area consumed
5	Between 15 and 25% of the leaf area consumed
7	Between 25 and 50% of the leaf area consumed
9	More than 50% of the leaf area consumed

Owing to a number of co-occurring symptoms of disease, virus, pest damage and nutrient deficiencies in the crop incidence of biotic stress was conducted rather than scoring severity. Incidence was scored of Common bacterial blight, Alternaria leaf blight, Angular leaf spot, wilting and Bean common mosaic virus (BCMV). Common bacterial blight caused by the bacterial pathogen *Xanthomonas campestris* pv. *phaseoli* was scored at 74 and 77 DAS, diagnosed by symptoms of irregular necrotic lesions surrounded by a bright yellow margin on leaves (Hagedorn and Inglis 1986). Alternaria leaf blight cause by the fungal pathogen *Alternaria alternate* was scored at 83 DAS and diagnosed by symptoms of gray-brown oval lesions with concentric rings on leaves (Hagedorn and Inglis 1986). Angular leaf spot caused by the fungal pathogen *Isariopsis griseola* was scored at 83 DAS and diagnosed by symptoms of dark-brown to black, angular-shaped lesions on leaves and irregular shaped brown to reddish lesions on pods (Hagedorn and Inglis 1986). Total incidence of leaf blight and spot diseases was scored at 74 and

77 DAS. Total incidence of wilting was scored at 72, 74 and 77 DAS. Due to an inability to distinguish between the causative agents (*e.g.* bacterial wilt disease, root rot and nematodes) a broad positive score was deployed, spanning plants with at least one set of trifoliate leaves wilted to completely wilted plants. Root assessment was conducted at harvest by carefully digging up ten roots from selected plots at harvest 97 DAS (Table 2). Incidence of BCMV was done at 59 and 66 DAS, diagnosed by systematic symptoms of irregular shaped, light yellow and dark green mottling on leaves. Incidence of pod feeding was done at 59, 66, 72 and 77 DAS.

2.5 Laboratory diagnosis of disease

As symptoms of disease can be discrete or occur in an overlapping pattern it can be difficult to make an accurate in field diagnosis. A set of laboratory diagnostic tests was, therefore, conducted as a supplement to verify the initial diagnosis or identify the causative agent in case it was not possible in field. Sampling of plant material was done at 76 DAS, immediately put in labelled zip-lock bags and kept in a cold box prior to storage in a refrigerator at 5°C overnight (Table 4). On the following day, samples were sent by delivery service from Moshi to Dar es Salam in cold box, to be kept at -18°C at the IITA laboratory facility until laboratory tests could be conducted.

Table 4: Overview of sample collection of common bean (*Phaseolus vulgaris*) plant material for verification of in-field disease diagnosis or identification of causative agent behind observed symptoms in laboratory. Samples were collected 76 days after sowing in the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania.

No.	Plot	Tissue	In-field diagnosis		No.	Tissue	Plot	In-field diagnosis
B.5	406	Leaf	Healthy		B.17	Pod	309	Grey mold
B.7	303	Leaf	Alternaria leaf spot		B.18	Leaf	103	Overlap of several foliar diseases
B.8	103	Leaf	Alternaria leaf spot		B.22	Stem	207	Unidentified wilting disease
B.9	306	Leaf	Angular leaf spot		B.23	Stem	103	Unidentified wilting disease
B.11	307	Leaf	Anthracnose		B.26	Pod	307	Angular leaf spot
B.15	111	Leaf	Common bacterial blight		B.27	Pod	207	Unidentified
B.16	306	Leaf	Common bacterial blight					

Samples of dry bean roots were equivalently collected at harvest, 97 DAS from selected plots to identify the precise causative agent behind the observed root rot. Prior to analysis the sampled plant material was cut in < 5 mm pieces and grinded manually using a pre-sterilized mortar and pestle containing 1 mL of DNA extraction buffer (Cetyl trimethyl ammonium bromide, polyvinylpyrrolidone, β-mercaptanol, ethylenediaminetetraacetic acid). Hereafter the extracted DNA was purified from organic components by a chloroform-isoamyl alcohol (24:1) step with intermediary centrifugations. Total DNA concentration of the purified product was measured by a nanodrop instrument at 260 nm, after which they were diluted to a 25 ng/µL stock. Verification of fungal DNA in samples was done in a Polymerase Chain Reaction (PCR) protocol deploying universal ITS1-F or ITS3-F and ITS4-R primers with following thermocycling conditions; 5 min at 95°C, then 35 cycles (30 sec at 95°C, 30 sec 55°C, 1 min at 72°C), 5 min at 72°, respectively producing a amplicon of ≈ 800 or 300 base pair (bp). Diagnosis of diseases was, hereafter, conducted by deploying pathogen specific primers obtained from literature sources and manufactured by Integrated DNA Technologies, Inc. Belgium in a PCR protocol (Table 5). As the primer annealing temperature cited in the original papers consistently conflicted with the calculated annealing temperature, *i.e.* by subtracting 5 degrees from the lowest melting point of the primer set (T_m), some PCR protocols had to be run several times to identify optimal annealing temperatures. Standard thermocycling conditions was given by Promega© for GoTaq polymerase; 5 min at 95°C, then 35 cycles (30 sec at 95°C, 30 sec annealing temperature according to primer T_m, 1 min per 1 kb of amplicon at 72°C), 5 min at 72°C followed by 10°C soak. Each PCR test was done with a *Fusarium oxysporum* ssp. *cubanese* as fungal control to detect unspecific amplification of fungal DNA, however, it was not possible to include a positive control of the particular pathogens. Successful amplification of pathogen DNA was visualized by running PCR product on a 1.5% agarose gel containing a GelRed™ DNA dye. Observation of a clear single band in the expected kb size region was interpreted as a positive sample, and PCR product was sent to sequencing at Macrogen© USA. Output of separate forward and reverse sequence was aligned to generate a consensus sequence using BioEdit© Sequence Alignment Editor (v7.2.6.1) software to reduce likeliness of sequence errors. Consensus sequence was run through National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) database to verify product alignment with known sequences of the given pathogen, providing a strong diagnostic evidence in the absence of a positive control.

Table 5: Polymerase chain reaction thermocycling conditions and sequence of pathogen specific primers obtained from literature. Primer melting temperature was obtained from manufacturer of the primers Integrated DNA Technologies, Inc. BP = Base pair.

	Primer				Cycle					
Disease	Forward Sequence (5′ - 3′)	Reverse Sequence (5′ - 3′)	Melting temperature (°C)	Amplicon Size (bp)	Initial Denaturation	Cycles	Denaturation	Annealing	Extension	Final Extension
Sorghum downy mildew (Ladhalakshmi *et al*. 2009)	*SCAR-f1* TTG CAC AGC CAC TCT ATT	*SCAR-r1* AGT ATT TGG CAT CAA CTC	F: 51.3 R: 46.9	800	94°C 3 min	40	94°C 1 min	60°C 1 min	72°C 2 min	72°C 10 min
Common bacterial blight (Audy *et al*. 1994)	*X4c* GGC AAC ACC CGA TCC CTA AAC AGG-	*X4e* CGC CGG AAG CAC GAT CCT CGA AG	F: 61.9 R: 64.1	730	95°C 1 min	35	95°C 1 min	none	72°C 2 min	72°C 10 min
Alternaria leaf spot (Mmbaga, Shi, and Kim 2011)	*A1-f1* CCC ACC ACT AGG ACA AAC A	*A1-r1* GCT TAA TGG ATG CTA GAC CT	F: 54.3 R: 51.8	370	94°C 5 min	42	93°C 1 min	33°C 1 min	72°C 2 min	72°C 5 min
Angular leaf spot Andean group (Guzmán *et al*. 1999)	*Pa3093* CAA TCG CCG TAC ATG ACT AA	*Pa3185* CCG TTA CCT CTA TAT TCC CAA	F: 52.4 R: 51.2	390	94° 1 min	40	94°C 1 min	63°C 1 min	72°C 1 min	72°C 10 min
Angular leaf spot: Mesoamerican Group (Guzmán *et al*. 1999)	*Pm2981* CAA TCG CCG TTT ACG AAG AT	*Pm2982* CAA TCG CCG TCG ATC GAT GA	F: 53.2 R: 57.4	690	94° 1 min	30	94°C 1 min	62°C 1 min	72°C 1 min	72°C 10 min
Bacterial wilt (Tegli, Sereni, and Surico 2002)	*Cff*FOR2 GTT ATG ACT GAA CTT CAC TCC	*Cff*REV4 GAT GTT CCC GGT GTT CAG	F: 50.6 R: 53.2	306	94° 3 min	30	94°C 1 min	62°C 45 sec	72°C 30 sec	72°C 5 min
Fusarium wilt (Alves-santos *et al*. 2002)	*A280* TAT ACC GGA CGG GCG TAG TGA CGA TGG	*B310* CAG CCA TTC ATG GAT GAC ATA ACG AAT TTC	F: 64.6 R: 58.2	609	94° 5 min	40	94°C 1 min	65°C 1 min	72°C 2 min	72°C 10 min
Rhizoctonia root rot (Salazar, Julian, and Rubio 2000)	*Rhsp-1* AAC AAG GTT TCC GTA GGT G	*ITS4B* CAG GAG ACT TGT ACA CGG TCC AG	F: 52.4 R: 59.0	700	94° 2½ min	40	94°C 15 sec	59°C 30 sec	72°C 1½ min	72°C 10 min
Fusarium root rot (O'Donnell and Gray 1994)	*FspF* ACC CCC TAA CTC TTG TTA TAT CC	*FspR* GCG CAA TAC CCT GAG GCG	F: 53.5 R: 59.3	958	94° 5 min	35-40	94°C 35 sec	60°C 55 sec	72°C 2 min	72°C 10 min
Black root rot (Huang and Kang 2010)	*Tb1* TAT TCA TTG CTG AGT GGC	*Tb2* GGT TTT CCG GCA TGT TAT	F: 49.8 R: 50.4	330	94° 4 min	30	94°C 1 min	54°C 1 min	72°C 2 min	72°C 10 min

2.6 Statistical analysis

A descriptive statistical analysis was performed using R software version 3.4.2 (2017) to test for a significant effect ($p < 0.05$) of cropping arrangements and variety on crop height, development, dry matter weight, yield and severity of diseases and pest damage were analysed with an ANOVA on linear mixed models with the following random factors: DAS, block and plot. If statistical significance was found in the ANOVA test a Post-hoc least significance difference (LSD) multiple comparison analysis was conducted to identify the treatment varying from the monoculture treatment. The assumptions of homogeneity of variance and normal distribution of variance were checked with plots of residuals and Q-Q plots.

3. Results

3.1 Kimashuku field trial site:

Sowing of plants was done shortly after the first rainfalls on the site, and was due to a relapsing dry period kept irrigated by flooding until more frequent showers occurred, starting at 28 DAS (Figure 3B). Farmers managing the neighbouring fields missed this brief window of showers in early march and started sowing between 21 and 28 DAS (Figure 3.B). These fields consisted of maize intercropped with a local cultivar of common bean or sunflower (*Helianthus annuus*). The relative humidity in field remained high after rains stopped as a controlled network of streams continued to bring water from the foothills of Mt. Kilimanjaro to the area. From sowing the average temperature decreased steadily from a daily mean temperature above 30 degrees to around 22 degrees (Figure 3.A).

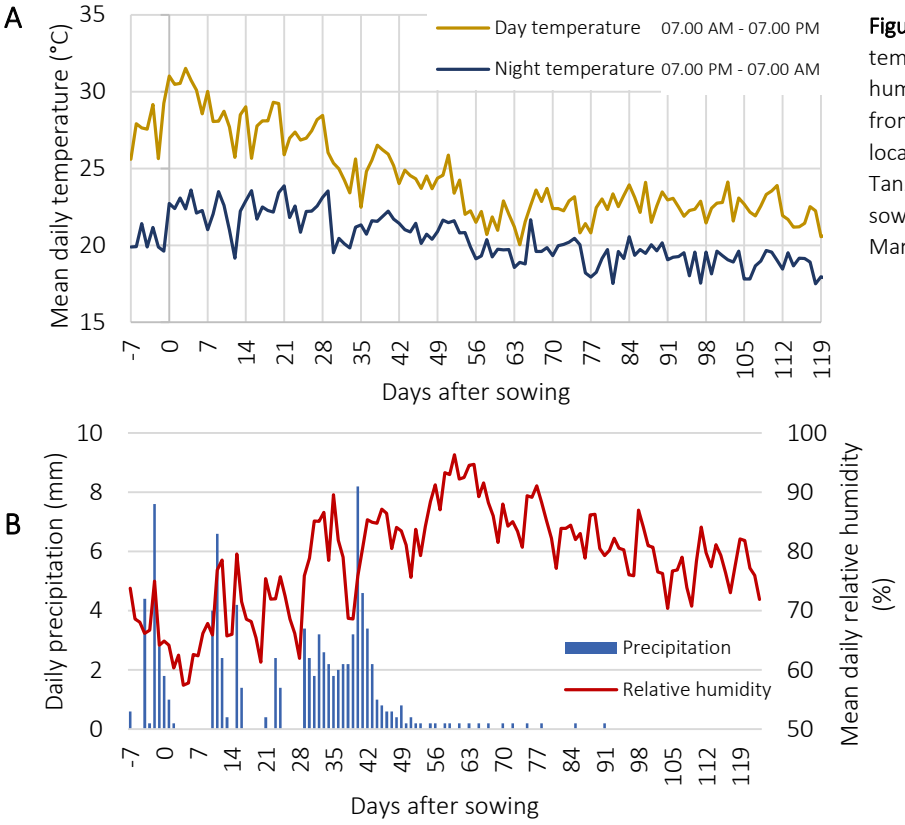


Figure 3: Mean day and night temperature (**A**), mean daily relative humidity and daily precipitation (**B**) from Maili sita weather station in located in the Northern Highland of Tanzania displayed as days after sowing of crops, with nul as the 6th of March 2017.

Amount of fertilizer disseminated on the site was inadequate to meet the crops demand, and particularly maize displayed severe deficiencies of nitrogen, potassium and magnesium. Symptoms of phosphorous was also observed in the maize crop, but was likely co-occuring with purpling caused by accumulation of anthocyanin pigments. The common bean crop only exhibited symptoms of magnesium deficiency (Figure 14.B).

3.2: Dating of phenological stages:

In order to study the effect of treatment on fundamental plant parameters, dating of vegetative and reproductive stages was done from 23 DAS until harvest of the crop (Appendix Table A3, Appendix Table A4).

3.2.1 Dating phenological stages in maize

The vegetative development of the maize crop progressed without any statistical significant differences relative to treatment, cropping systems or intercropped bean cultivar. Across treatments the maize

crop had set on average 11 to 14 nodes at 110 DAS (Figure 4.A). The maize crop was found to produce cobs (R_2 stage) in a brief time span around 72 DAS (Figure 4.B). For the following reproductive stages, it was decided to take kernel samples for measurement of dry weight progression rather than attempting to give a visual score of kernel development as traditionally done. As the collection of cobs in the plots, however, had direct influence on the plot yield at harvest, it was decided to minimize the collection of cobs, reducing the statistical strength of analysis on treatment level (Appendix Table A3.2). Average seed dry weight of all treatments at the measured time points show that kernel setting and cob growth occurs in the span from R_2 stage at 74 DAS until approximately 83 DAS, after which kernels start to fill in a linear pattern (Figure 4.C). At harvest, 145 DAS the average kernel weight across treatments was found to be 0.390 to 0.426 g. Neither cropping arrangement or common bean cultivar in the cropping system was found to have a significant effect on kernel size at harvest.

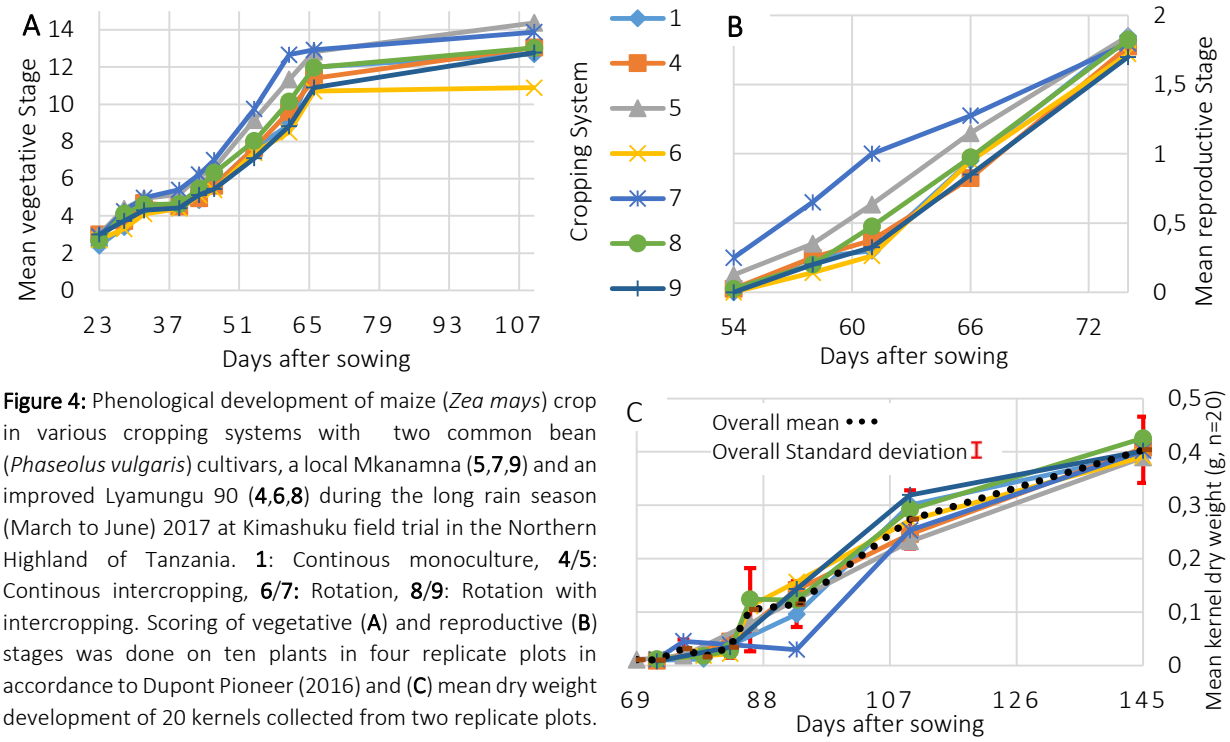


Figure 4: Phenological development of maize (*Zea mays*) crop in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) during the long rain season (March to June) 2017 at Kimashuku field trial in the Northern Highland of Tanzania. **1**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7**: Rotation, **8**/**9**: Rotation with intercropping. Scoring of vegetative (**A**) and reproductive (**B**) stages was done on ten plants in four replicate plots in accordance to Dupont Pioneer (2016) and (**C**) mean dry weight development of 20 kernels collected from two replicate plots.

3.2.2 Dating phenological stages in common bean

The vegetative development of both common bean cultivars progressed without any significant differences relative to cropping system. However, the vegetative development of the two cultivars was found to significantly differ ($p_{val,} << 0.05$) 69 DAS, with the improved cultivar setting on average 8 nodes relative to the local setting on average 12 (Figure 5.A). in consistence, the two cultivars were found to significantly differ at all three time points in scored reproductive development $p_{val,\ 43,\ 54,\ 59\ DAS}$,<< 0.05. The improved cultivar was observed to flower (R_1 stage) across treatments around 42 DAS relative to 47 DAS in the local cultivar (Figure 5.B).

Similar to maize, pods were collected weekly in the common bean crop to assess seed dry weight development, however, exerting a less destructing sampling effect. This assessment found that all plants of the improved cultivar had set pods (R_2 Stage) at 44 to 49 DAS while the local cultivar first at 52-53 DAS (Figure 5.B). Seed filling in the improved variety started around 55 DAS, while the local cultivar first showed increase seed dry weight at 61 DAS (Figure 5.C). Seed weight of the two common

bean cultivars was found to significantly differ at harvest 97 DAS (p_{val} << 0.05) with the improved cultivar had a mean seed weight of 0.33g, whereas the local cultivar produced a smaller seed with a mean weight of 0.15g (Figure 5.C).

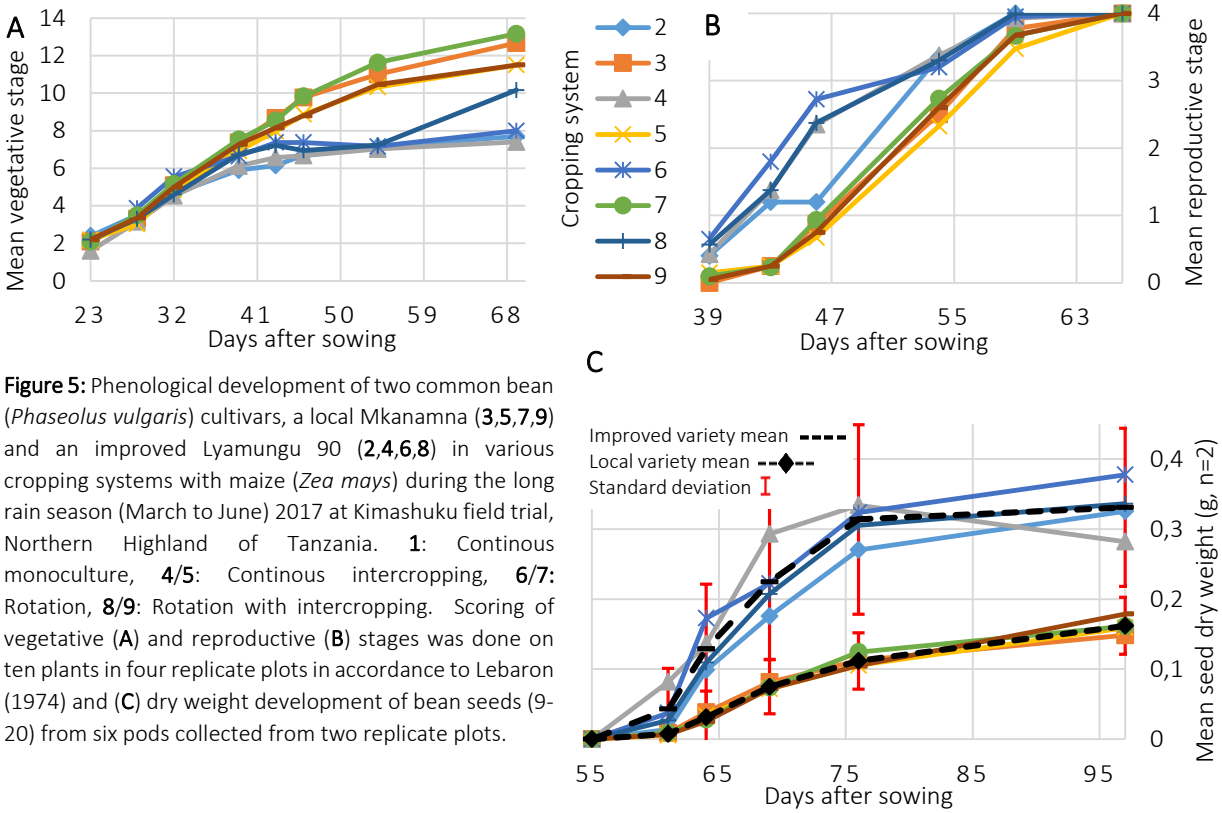


Figure 5: Phenological development of two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. **1**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation with intercropping. Scoring of vegetative (**A**) and reproductive (**B**) stages was done on ten plants in four replicate plots in accordance to Lebaron (1974) and (**C**) dry weight development of bean seeds (9-20) from six pods collected from two replicate plots.

3.2.4 Measurement of plant height, plant number and yield

Prior to senescence at 93 DAS the maize crop was found to measure a mean height of 126 to 170 centimetres across the treatments (Figure 7.A). Statistical analysis of height did not find any significant difference relative to cropping system nor variety of intercropped common bean cultivar in the cropping system. Plant count 32 DAS showed a relative even number of maize plants per plot, with an average across treatments from 63 to 77 plants (Appendix Table A2.1). The maize crop was harvested at 145 DAS, and across treatments produced an estimated average kernel yield from 1.28 to 3.23 tons per hectare (Figure 6). Cropping system was found to exert a significant effect on yield, with maize in rotation with intercrop of common bean producing the lowest mean yield of 1.67 tons per hectare (p_{val} << 0.05) relative to 2.7 to 2.99 tons per hectare in the other three cropping systems (figure 6). Cultivar of intercropped common bean in the cropping system had no significant effect on yield of maize.

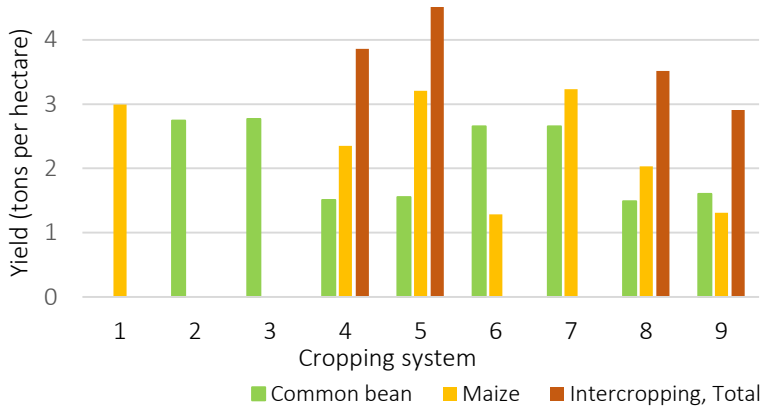
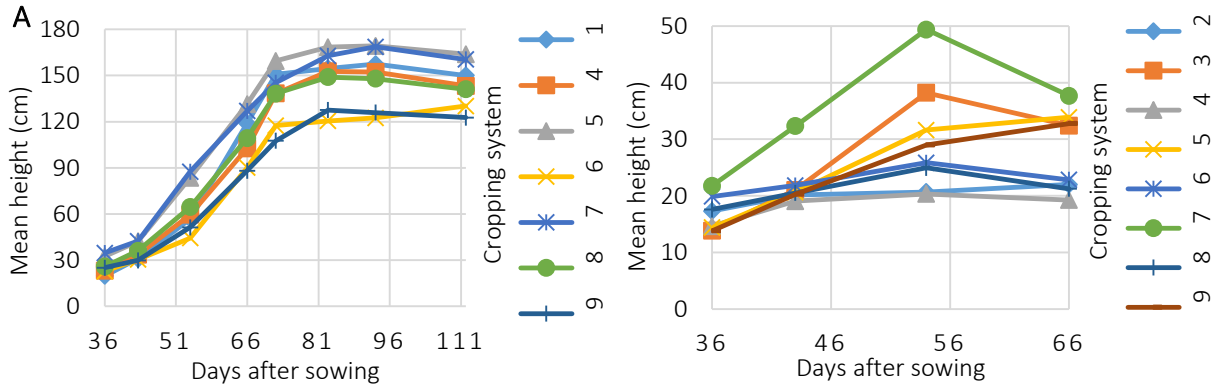


Figure 6: Yield of maize (*Zea mays*) and two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) in various cropping systems during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. **1**/**2**/**3**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation of maize with intercropping.

In the common bean crop, maximum height measured at 66 DAS was found to show a significant difference ($p_{val,}$<< 0.05) on cultivar level, with the improved cultivar having a mean height of 32.4 cm and the local cultivar that of 21.2 cm (Figure 7.B). In the continuous monoculture of the improved common bean cultivar cropping system substantial plot failure was observed with three out of four plots (Appendix Table A2.2). The crop was harvested at 97 DAS, and across treatments produced an estimated average seed yield from 1.49 to 2.77 tons per hectare (Figure 6). Cropping system was found to exert a significant effect on seed yield with intercropping systems producing on average 1.53-1.54 tons per hectare relative to 2.65-2.76 in monoculture ($p_{val,}$<< 0.05).

Figure 7: Height of crops cultivated in various cropping systems during the long rain season (March to June) at Kimashuku field trial, Northern Highland of Tanzania. **A**: Maize (*Zea mays*) measured from ground level to leaf separation at heights collar or flag leaf when set and **B:** Common bean (*Phaseolus vulgaris*), a local cultivar Mkanamna (**3**,**5**,**7**,**9**) and an improved cultivar Lyamungu 90 (**2**,**4**,**6**,**8**) measured from ground level to highest node. **1**: Continous monoculture of maize, **2**/**3**: Continous monoculture of common bean, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation with intercroppingMeasurements was done on six plants in four replicate plots.



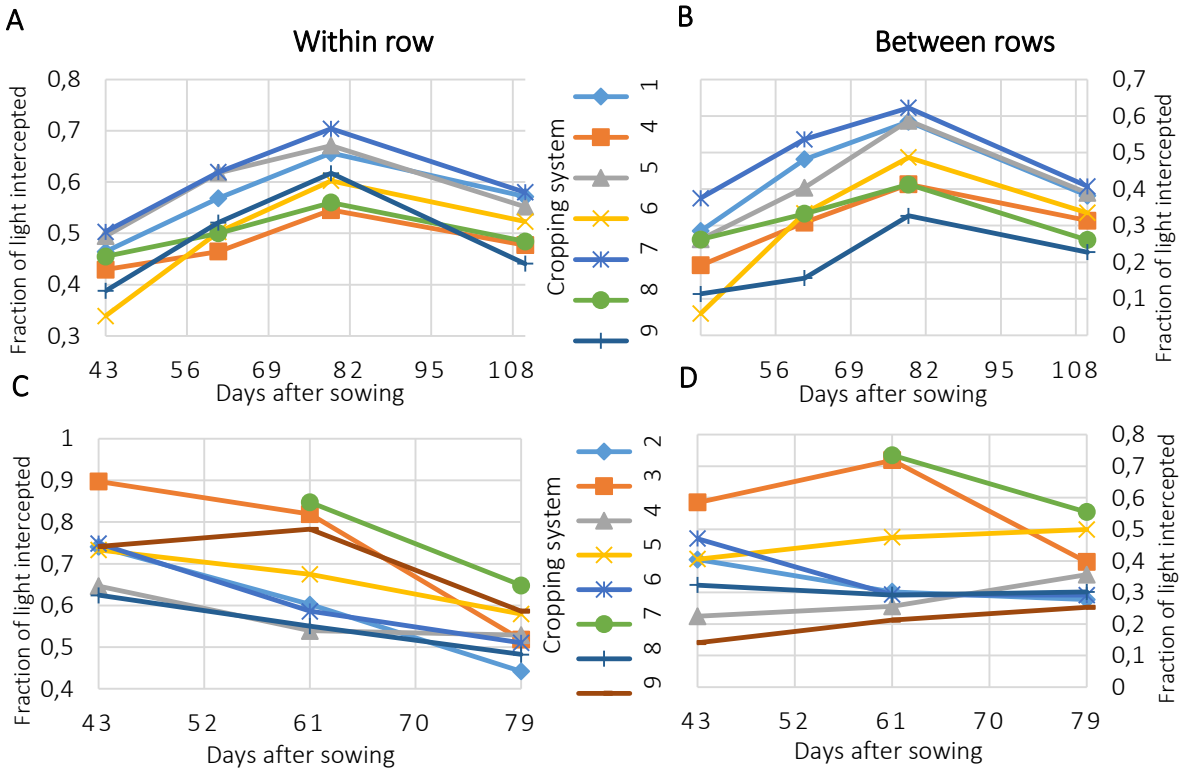
3.3 Crop light interception

Measurement of light interception was conducted to estimate the fraction of photosynthetically active radiation (PAR) that the crop intercepted and to study the effect of treatment on crop PAR interception. These measurements were conducted throughout the growth season, but statistical analysis was solely done on a single time point selected as the peak of canopy coverage prior to crop senescence.

The maize crop intercepted across treatments 56 to 70 % of PAR within the rows at 79 DAS, and 41 to 62 % between the rows (Figure 8 A-B). Cropping system was found to exert no significant effect on PAR intercepted in the maize crop. However, intercropping maize with the local common bean cultivar was found to significantly ($p_{val,}$ =0.040) enhance the interception of PAR within row by 9 % relative to that

of maize intercropped with the improved cultivar. The common bean crop intercepted across treatments 54 to 85 % of PAR within the rows at 61 DAS, and 21 to 73 % between rows (Figure 8 C-D). Cropping system nor cultivar was found to exert no significant effect on PAR intercepted in the common bean crop. The two common bean cultivars were, however, found to differ significantly in both within ($p_{val,}$ =0.001) and between row ($p_{val,}$ =0.034) PAR interception, with the local cultivar intercepting respectively 21 and 26 % more PAR.

Figure 8: Fraction of light intercepted in various cropping systems of maize (*Zea mays*) and two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) cultivated during the long rain season (March to June) 2017 at Kimashuku field trial site, in the Northern Highland of Tanzania. **1**: Continous monoculture of maize, **2**/**3**: Continous monoculture of common bean, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation with intercropping. Light incidence was measured using an 80 cm probe at ground level concurrent with measurement of above canopy light incidence using an external sensor at four sites in two replicate plots per cropping system. **A:** Within maize row, **B:** Between maize rows measured above bean crop at 30-50 cm height, **C:** Within bean row, **D:** Between bean rows.



3.4 Scoring of disease, virus and pest severity and incidence

As the field site at Kimashuku was not treated with pesticides during the long rain season (March to June) a large diversity and concentration was observed of both insect pests and diseases in the crops. To investigate if treatment, cropping system or common bean cultivar had an effect on the incidence and severity of biotic stresses these were scored accordingly throughout the growth season.

3.4.1 In maize

The first observation of disease in the maize crop was done 36 DAS, after a prolonged period of rain, with the relative humidity raising from 70 to 90 % (Figure 3.B). These were diagnosed as Common rust, Eyespot and Grey leaf spot, initially occurring solely on the lower leaves they progressed with differential severity up through the canopy (Figure 9 A-C). Later occurring diseases included Sorghum

downy mildew, Physoderma brown spot and the Maize strip virus, all at a low incidence level with little impact on the crop performance (Figure 9 D-F).

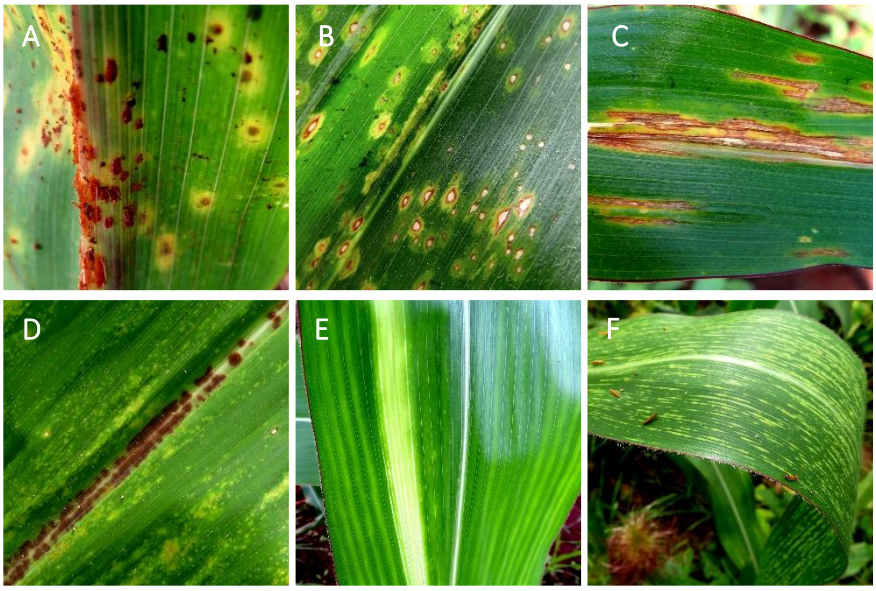


Figure 9: Foliar diseases and virus observed in maize (*Zea mays*) crop during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. **A:** Pustules of Common rust disease on abaxial site of lamina. **B:** Small circular lesions caused by Eyespot disease. **C:** Elongated, merging lesions caused by Grey leaf spot disease. **D:** Brown circular spots on mid rib and mottling of small chlorotic spots caused by Physoderma brown spot disease. **E:** Broad chlorotic band on leaf laminae caused by Sorghum downy mildew disease. **F:** Systematic striping caused by Maize stripe virus

At 97 DAS neither cropping system nor intercropped common bean cultivar was found to have a significant effect on the severity of Common rust, Eyespot or Grey leaf spot on lowest or upper ear supporting leaf. Common rust disease was the most important maize disease at the site, affecting across the treatments on average 10.3 to 19.10 % of the leaf area on lowest leaf at 91 DAS. Severity of the disease was found to be significantly higher ($p_{val} << 0.05$) on the lowest leaves with a mean score of 5.23 % leaf area relative to 1.59 % leaf area on the upper ear supporting leaf (Figure 10 C-D).

Following Common rust, Eyespot disease was the second most important disease in the maize crop with severity across treatments was found to be on average 2.6 to 5.0 % of leaf area affected at 91 DAS (Figure 10 A-B). In spite of a more even distribution throughout the canopy, severity of the disease was found to be significantly higher ($p_{val} << 0.05$) on the lowest leaves with a mean score of 2.28 % leaf area relative to 1.59 % leaf area on the upper ear supporting leaf.

Grey leaf spot disease was found to be of lesser importance on the site, with an average severity on the lower leaves 97 DAS of 1, translating to the phenotype “Very small necrotic lesions on leaves” given by the scoring scale after Danson *et al*. (2008) (Figure 10.E).

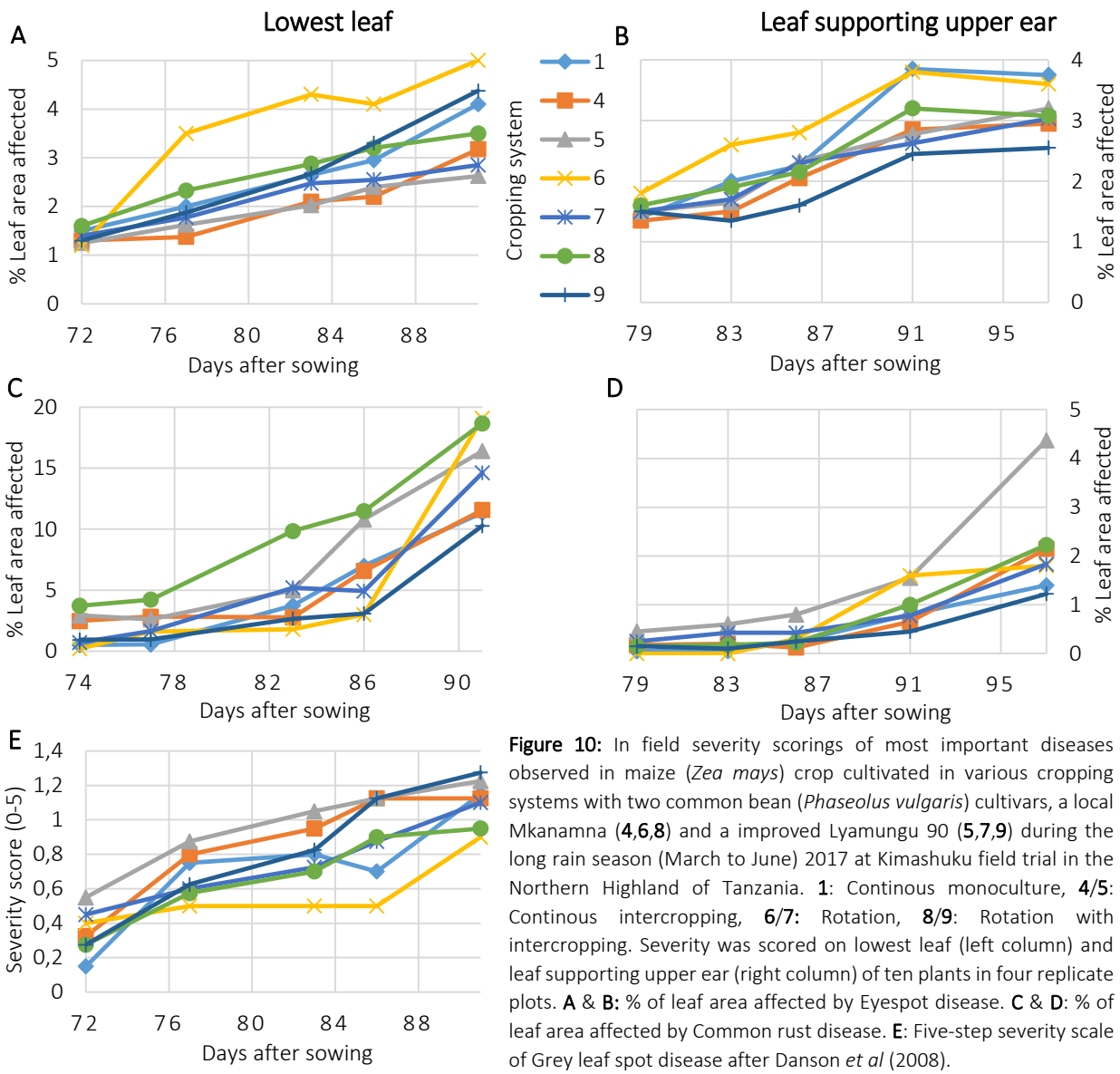


Figure 10: In field severity scorings of most important diseases observed in maize (*Zea mays*) crop cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**4**,**6**,**8**) and a improved Lyamungu 90 (**5**,**7**,**9**) during the long rain season (March to June) 2017 at Kimashuku field trial in the Northern Highland of Tanzania. **1**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7**: Rotation, **8**/**9**: Rotation with intercropping. Severity was scored on lowest leaf (left column) and leaf supporting upper ear (right column) of ten plants in four replicate plots. **A** & **B**: % of leaf area affected by Eyespot disease. **C** & **D**: % of leaf area affected by Common rust disease. **E**: Five-step severity scale of Grey leaf spot disease after Danson *et al* (2008).

The maize crop was equivalently infested with several pests including Stem borer species, African bollworm (*Helicoverpa armigera*), African stalk borer (*Busseola fusca*), Corn leaf aphids (*Rhopalosiphum maidis*) and an unidentified small blue beetle (Figure 11 A-F). Neither cropping system nor intercropped common bean cultivar was found to have any systematic significant effect on incidence or severity of pests in the maize crop.

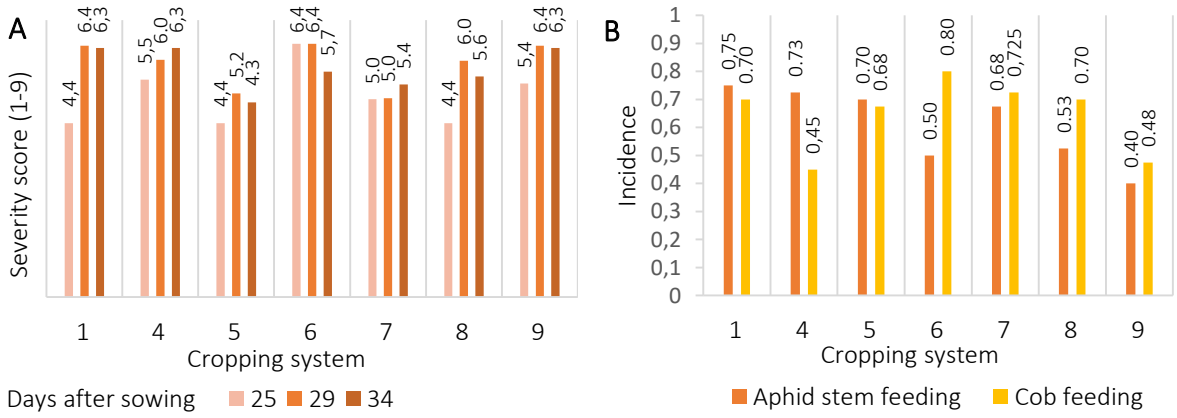
From first field assessment at 23 DAS it was evident that Stem borers was an important pest during the early stages of the maize crop. Incidence scoring at 29 DAS revealed that 80 to 100 % of plants at plot level were infested with at least one Stem borer specie. Majority of these were identified as Spottet (*Chilo partellus*) stem borer, whereas the African armyworm (*Spodoptera exempta*) was observed less frequently (Figure 11 A-B). Large infestation of African armyworm was, however, observed in neighbouring fields three weeks later. Collated foliage damage caused by Stem borers was scored in accordance to the 1-9 step scale given by Tefera *et al* (2011), and a mean severity score between 5 and 6 were found across treatments 29 and 34 DAS (Figure 12.A).

With setting of cobs in the maize crop around 54 DAS (Figure 4.B), a rapid infestation of African bollworm was observed at the site, feeding on tassels and subsequently cobs (Figure 11.C). Incidence of cob feeding was scored at 93 DAS, and found that 45 to 80 % of the plants across treatments exhibited minimum one cob showing feeding damage (Figure 12.B).

First observation of Corn leaf aphids was done 73 DAS, feeding on tassels and was subsequently found to cause brown patches under leaf sheaths on the stem (Figure 11.F). Incidence of aphid feeding damage was done 93 DAS, and revealed that 40 to 75 % of the plants across treatments were affected (Figure 12.B). Maize in rotation with common bean intercrop was found to show a significant (p_{val} = 0.034) effect, reducing mean incidence by 28.7 % relative to the continuous monoculture cropping system. The damage done my aphids at the site was, however, believe to be of minute importance.

Figure 11: Pests observed in maize (*Zea mays*) crop during the long rain season (March to June) at Kimashuku field trial, Northern Highland of Tanzania. **A:** Spottet stem borer (*Chilo partellus*). **B:** African armyworm (*Spodoptera exempta*). **C:** African bollworm (*Helicoverpa armigera*) feeding on tassels. **D:** African stalk borer (*Busseola fusca*) feeding on cob. **E:** Small dark blue beetle (unknown sp.) feeding on tassels. **F:** Corn leaf aphids (*Rhopalosiphum maidis*) in tassels.

Figure 12: In field scoring of **A:** stem borer feeding damage in accordance to a nine-step scale after Tefera *et al* (2011). **B:** incidence of cob feeding and aphid stem feeding in maize (*Zea mays*) 93 days after sowing. The crop was cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**4**,**6**,**8**) and an improved Lyamungu 90 (**5**,**7**,**9**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern highland of Tanzania. **1**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation with intercropping. Scoring was done on ten plants in four replicate plots.



3.4.2 In common bean

The first observation of disease in the common bean crop occurred 41 DAS, and during following weeks Alternaria leaf spot, Angular leaf spot, Anthracnose, Grey mold (*Botrytis cinera*), Common bacterial blight and Phytophthora leaf blight (*Phytophthora phaseoli*) disease were diagnosed in the field (Figure 13 A-F). Incidence of these diseases were scored, but due to the co-occurrence of several diseases and incapability to assure a consistently accurate separation of these, the data is not shown.

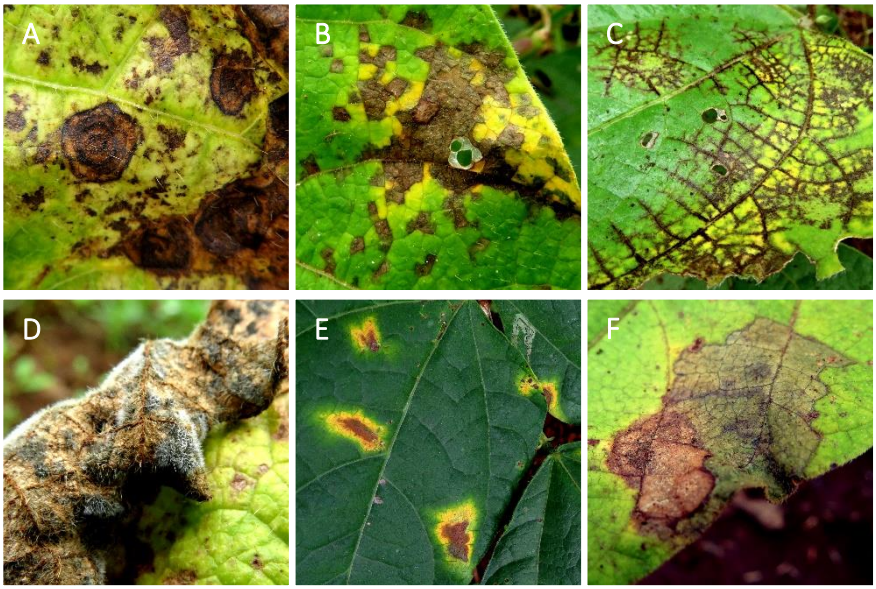


Figure 13: Diseases observed in common bean (*Phaseolus vulgaris*) crop during the long rain season (March to June) 2017 at Kimashuku field trial in the Northern Highland of Tanzania. **A:** Oval lesion with concentric rings caused by Alternaria leaf spot disease. **B:** Mosaic of Angular leaf spot disease lesions. **C:** Brick-red lesions on veins caused by Anthracnose disease. **D:** Necrotic tissue showing fungal hyphae caused by Grey mould disease. **E:** Irregular shaped lesions with bright yellow margin caused by Common bacterial blight disease. **F:** Water soaked lesion caused by Phytophthora leaf blight disease.

In addition to this assessment failure in field, the separation of viral symptoms from that of magnesium deficiency proved most difficult in field (Figure 14 A B). Severe infestation of whiteflies (*Bemisia* sp.) occurred at the site 25 DAS, and subsequent infestation of leaf hoppers (*Empoasca fabae*) observed at 36 DAS confirmed the presence of viral vectors (Figure 17.K)

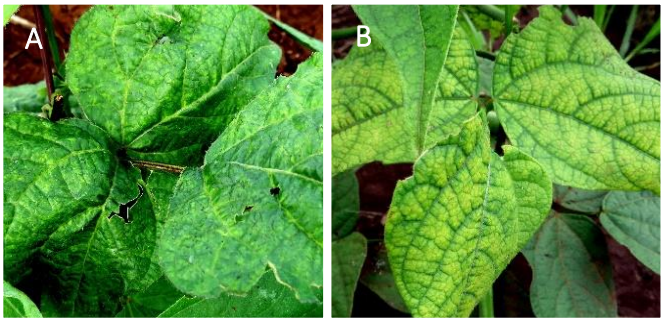


Figure 14: Virus observed in common bean (*Phaseolus vulgaris*) crop during the long rain season (March to June) at Kimashuku field trial, Northern highland of Tanzania. **A:** Systematic yellow-green mottling and malformation of leaf diagnosed as Bean common mosaic virus. **B:** Severe symptoms of magnesium deficiency, causing chlorosis of leaf with pronounced green veins.

In addition to the foliar diseases, assessment of roots at harvest 97 DAS revealed substantial damage to the root system (Figure 15). This was consistent with the observation of wilting plants throughout the growth season, and high failure rate previously mentioned of the improved cultivar in continuous monoculture. On basis of a red discolouration of root tissue found in all assessed plots, the causative agent was identified as Rhizoctonia root rot (*Rhizoctonia solani*) (Figure 3.4.24B). Observations of tunnelling, however, also indicated that feeding bean fly larvae (*Ophiomyia phaseoli*) were contributing to the root damage (Figure 16.C, Figure 17.L). The root assessment did, however, not reveal an immediate pattern between cropping systems or cultivars (Figure 15).

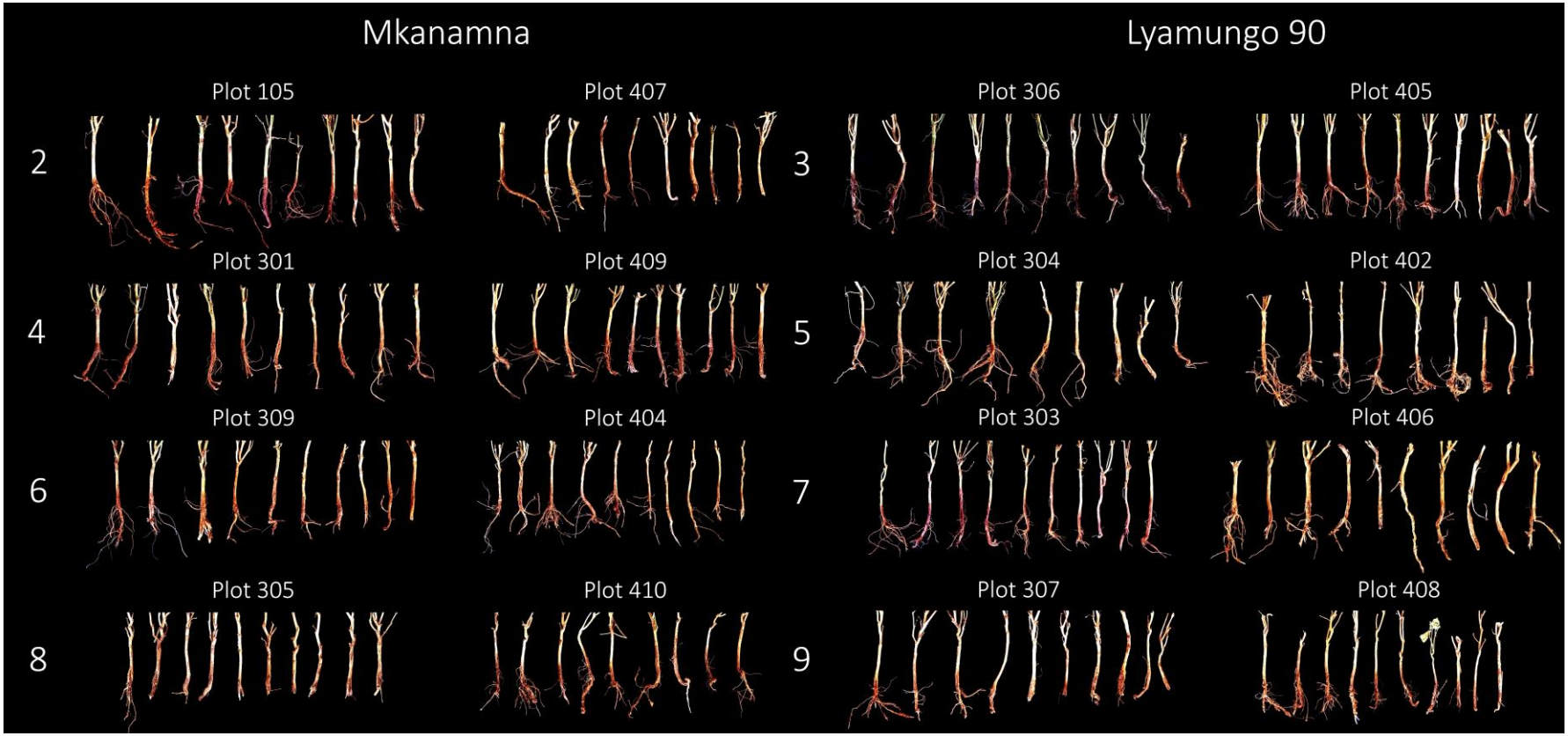


Figure 15: Root samples of two Common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (row **3**,**5**,**7**,**9**) and improved, Lyamungu 90 (row **2**,**4**,**6**,**8**) at harvest, cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) at Kimashuku field trial site, Northern highland of Tanzania. Figure shows ten rinsed roots collected from two selected plots per cropping system. Row **2** & **3**: Cultivated in continuous monoculture. Row **4** & **5:** Cultivated in continuous intercropping with maize. Row **6** & **7** Cultivated in rotation with maize. Row **8** & **9** Cultivated in rotation of maize followed by intercropping with maize.

Figure 16: Root damage observed in stunted common bean (*Phaseolus vulgaris*) plants cultivated during the long rain season (March to June) 2017 at Kimashuku field trial in the Northern Highland of Tanzania. **A:** Elongated, soft lesion on taproot diagnosed as Pythium root rot (*Pythium* sp.). **B:** Elongated red coloured lesion on taproot and stem diagnosed as Rhizoctonia root rot (*Rhizoctonia solani*). **C:** Small brown Bean fly (*Ophiomyia phaseoli*) pupa protruding from taproot

The common bean crop, moreover, accommodated a large population of insect pests with 12 identified species causing a considerable damage to foliage, inflorescence tissue and pods (Figure 17 A-L). All of the listed pests contributed to this damage, some in a brief time span synchronized with a specific developmental stage in the host, others continuous throughout the entire season.

Figure 17: Important pests observed in common bean (*Phaseolus vulgaris*) crop during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. **A:** African bollworm (*Helicoverpa armigera*). **B:** *Lepidopteran* sp. feeding on pod **C:** Chinese blister beetle (*Mylabris phalerata*) and **D:** False blister beetle (*Asclera ruficollis*) feeding on inflorescence tissue. **E:** Bean beetle (*Ootheca bennigseni* or *O. mutabilis*). **F:** African pod bug (*Clavigralla tomentosicollis*). **G:** Adult Southern green stink bug (*Nezara viridula*). **H:** Unidentified beetle mating. **I:** Unidentified beetle. **J:** Mealybug (*Pseudococcidae* sp.). **K:** Whitefly (*Bemisia* sp.) adult and eggs on abaxial leaf site. **L:** Emerging bean fly (*Ophiomyia phaseoli*) maggot.

Severity of foliage feeding done in accordance to a nine step scale by Schoonhoven and Pastor-Corrales (1987) based on leaf area consumed (Table 3). Mean scored generated from two scorings at 29 and 34 DAS found across the treatments an average severity score between 2.17 and 2.7, translating to the loss of 5 to 10 % of leaf area (Figure 19.A). Neither cropping system nor cultivar was found to exert a significant effect on foliage feeding severity.

Incidence of pod feeding was scored at 66, 72 and 77 DAS and revealed that 16 to 56 % of the plants across treatments exhibited minimum one pod showing feeding damage (Figure 19.B). The statistical analysis found a significant difference between the two cultivars (P_{val} << 0.05), with the improved cultivar having a mean incidence of 20 % relative to 54 % observed in the local cultivar. No assessment of post-harvest damage was done on seeds due to observation of a minute incidence level of pest emergence in seeds kept for dry weight measurement. Observed pests were identified as the bean weevil (*Acanthoscelides obtectus*), while an unknown larva specie was also observed more infrequent in the samples (Figure 18 A-B).

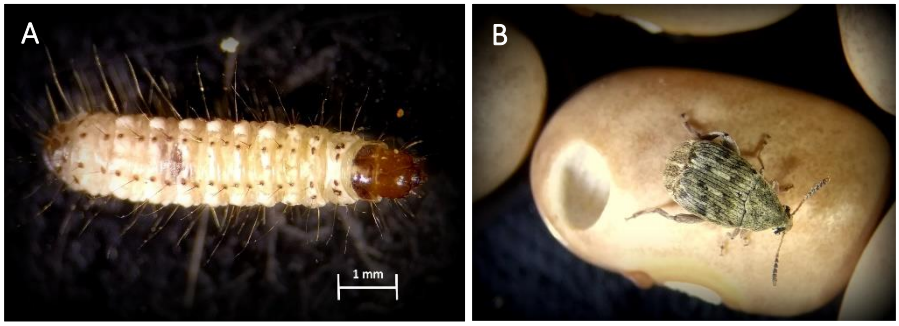


Figure 18: Emerged pests from Common bean (*Phaseolus vulgaris*) seeds harvested after the long rain season (March to June) 2017 at Kimashuku field trial site in the Northern Highland of Tanzania. **A:** unknown larvae. **B:** Bruchid beetle (*Acanthoscelides obtectus*)

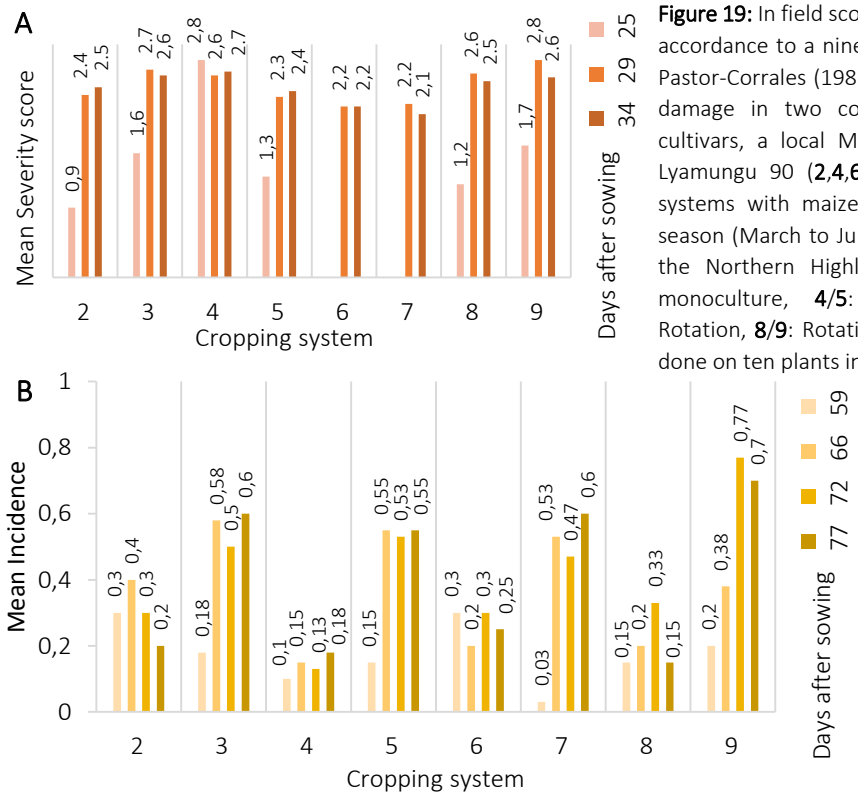


Figure 19: In field scoring of **A:** Foliage feeding damage in accordance to a nine-step scale after Schoonhoven and Pastor-Corrales (1987) and **B:** Incidence of pod feeding damage in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and improved Lyamungu 90 (**2**,**4**,**6**,**8**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial in the Northern Highland of Tanzania. **2**/**3**: Continous monoculture, **4**/**5**: Continous intercropping, **6**/**7:** Rotation, **8**/**9**: Rotation with intercropping. Scoring was done on ten plants in four replicate plots.

3.5 Laboratory diagnosis of disease

Inclusion of a laboratory diagnosis of collected plant samples had three purpose. Firstly, to verify the field diagnosis, secondly to identify causative agents of symptoms which could not be diagnosed in the field and thirdly to study the degree of overlapping diseases. The design of the laboratory diagnosis was to deploy pathogen specific primers found in literature in a PCR analysis, identify if single bonds could be located of expected base pair (bp) size, and if so these PCR products would be sent to sequencing. Issues with cited annealing temperature, however, resulted in the necessity of conducting several PCR runs at various temperatures to achieve a successful run without unspecific amplification. Prior to deployment of these specific primers, fungal DNA in the samples were verified by universal fungal primers (Figure 21.A).

The diseases observed in the maize crop were easily diagnosed, each showing distinct symptoms except that of Sorghum downy mildew. Sample M.6 and M.10 diagnosed with Sorghum downy mildew in the field did not produce positive bands in the PCR run deploying specific primers, putting question to the validity of the field diagnosis (Figure 9.E).

In the common bean crop, Grey mold, Phytophthora leaf blight and Anthracnose were found to be less important diseases and hence excluded from the diagnostic tests. The analysis focused instead on Common bacterial blight, Alternaria leaf spot, Angular leaf spot, Bacterial wilt, Fusarium wilt and root rot diseases. Sample B.8 and B.15 were found to produce clear single bonds in the expected region (730 bp) when deploying Common bacterial blight specific primers, using the protocol annealing temperature. Result of the latter sample were consistent with field diagnosis (Table 4, Figure 20.A). Sample B.7, 8, 9, 11, 15, 16, 18 and 27 produced a single band in the expected region (370 bp) when deploying Alternaria leaf spot specific primers at an adjusted annealing temperature of 46.8°C (Figure 20.B). For diagnosis of Angular leaf spot, it was not possible to identify a single set of primers in the literature search, instead two sets were used, capable of distinguishing between two pathogen groups, Andean and Mesoamerican. Sample B.9, 16 and 26 produced a single clear band in the run deploying the Andean primers at an adjusted annealing temperature of 46.2°C (Figure 20.C). This was consistent with the field diagnosis in sample B.9 and B.26, however, the band was found at around 700 bp instead of the expected size around 390 bp as cited by Guzmán *et al* (1999). Samples B.7, 8, 9, 15 and 16 produced a band at the expected region (690 bp) when deploying Mesoamerican primers at an adjusted annealing temperature of 53.2°C (Figure 20.D). Occurrence of additional bands in the PCR product, however, indicated an unspecific amplification. Sample B.19, 20 and 21 collected from plants showing symptoms of systematic wilting were tested for Bacterial wilt and Fusarium wilt disease (*Fusarium oxysporum* f.sp *phaseoli*) but did not produce single bands in the expected region (Data not shown).

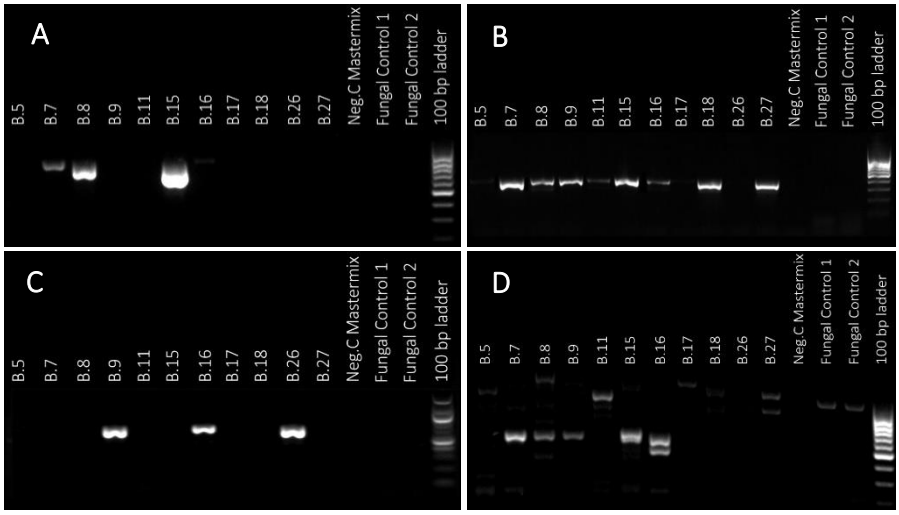


Figure 20: Polymerase chain reaction guided verification of in-field disease diagnosis in common bean (*Phaseolus vulgaris*) by deployment of specific primers visualised on 1.5% agarose gel electrophoresis. Plant material was collected during the long rain season (March to June) 2017 at Kimashuku field trial site in the Northern Highland of Tanzania. **A:** Common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) with primers after Audy *et al* (1994). **B:** Alternaria leaf spot (*Alternaria alternate*) with primers after Mmbaga *et al* (2011). Angular leaf spot (*Isariopsis griseola*), Andean group (**C**) and Mesoamerican group (**D**) with primers after Guzmán *et al* (1999). Fungal control 1&2: *Fusarium oxysporum* ssp*. cubanese*

Identification of the particular specie given cause to the observed root disease was a key objective in the laboratory diagnosis (Figure 15, Figure 16 A-B). Root samples of two selected plots per treatment was, therefore, subject to a PCR analysis deploying primers specific for Rhizoctonia root rot, Fusarium root rot and Black root rot (Figure 21. B-D). Specific primers for Pythium root rot (*Pythium* sp*)* was not identified in the literature search. Consistent with field diagnosis, all root samples were found to produce a band around the expected region (700 bp) in the run deploying Rhizoctonia root rot specific primers at an adjusted annealing temperature of 47.2°C (Figure 21.B). However, the fungal control containing DNA from leaf sample B.18, also produced a positive band, lowering the diagnostic strength of the observed bands. Several samples also produced a positive band in the expected region (958 bp) in the run deploying Fusarium root rot specific primers at an adjusted annealing temperature of 48.5°C (Figure 21.C). Lastly, none of the samples were found to produce bands in the expected region (300 bp) in the run deploying Black root rot specific primers (Figure 21.D).

In order to achieve sufficient degree of evidence to conclude if the samples contained the pathogen in question, PCR products containing single bands in the expected region were sent to sequencing (Table 6). Results of consensus sequences comparison with National Centre for Biotechnology Information Basic Local Alignment Search Tool database (NCIB BLAST) largely supported the infield diagnosis. Alternaria leaf blight and common bacterial blight were confirmed as present foliar diseases in the crop, while putative positive sample of Angular leaf spot, Andean group could not be confirmed as NCIB BLAST sequence database found no significant similarity to analysed products.

Our findings suggest that alternaria leaf spot was the most prevalent foliar disease in the common bean crops, however, occurring in an overlay with several other diseases at unknown severity as field diagnosis and scoring was not possible. The observed damage on roots giving cause to wilting and crop establishment failure in some plots was caused by Rhizoctonia root rot, with laboratory analysis supporting the field diagnosis (Figure 21.B). Fusarium root rot was, however, also confirmed in plot 307 and 408, cultivated with the local cultivar of common bean in rotation with intercropping.

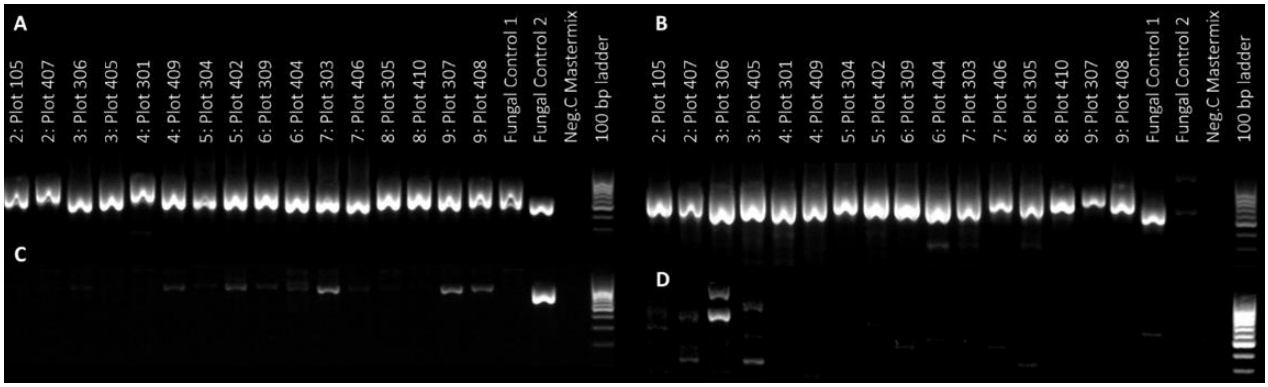


Figure 21: Polymerase chain reaction guided identification of root rot disease in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**2**,**4**,**6**,**8**) and an improved Lyamungu 90 (**3**,**5**,**7**,**9**) by deployment of specific primers visualised on 1.5% agarose gel electrophoresis. Root material was collected from two replicate plots per cropping system 97 days after sowing at Kimashuku field trial in Northern Highland of Tanzania. Cropping systems comprise of continuous monoculture (**2**,**3**), continuous intercropping with maize (*Zea mays*) (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). **A:** Verification of fungal DNA in samples by deployment of ITS1-ITS4 primers. **B:** Rhizoctonia root rot (*Rhizoctonia solani*) with primers after Salazar *et al* (2000). **C:** Fusarium root rot (*Fusarium oxysporum* f.sp *phaseoli*) with primers after O'Donnell and Gray (1994) and **D:** Black root rot (*Thielaviopsis basicola*) with primers after Huang and Kang (2010). **Fungal control 1**: B.18 leaf sample exhibiting several fungal

Table 6: Overview of products from a Polymerase Chain Reaction guided disease diagnosis deploying pathogen specific primers, the field diagnose in Common bean (*Phaseolus vulgaris*) crop at Kimashuku field trial, Northern Highland of Tanzania, and the subsequent result of consensus sequence comparison when run through National Centre for Biotechnology Information Basic Local Alignment Search Tool database.

Disease	Causative agent / Primer specificity	Sample	Field diagnosis	Consensus Sequence	
				Size (bp)	Identity
Common bacterial blight	*Xanthomonas campestris* pv. *phaseoli*	B.8	Alternaria leaf spot	821	*Xanthomonas* sp.
		B.15	Common bacterial blight	819	*Xanthomonas* sp.
Alternaria leaf spot	*Alternaria alternata*	B.7	Alternaria leaf spot	286	*Alternaria alternata*
		B.8	Alternaria leaf spot	253	*Alternaria alternata*
		B.9	Angular leaf spot	282	*Alternaria alternata*
		B.15	Common bacterial blight	337	*Alternaria alternata*
		B.18	Overlap of several foliar diseases	376	*Alternaria alternate*
		B.27	*Unidentified*	375	*Alternaria alternata*
Angular leaf spot (Andean)	*Isariopsis griseola*	B.9	Angular leaf spot	660	No significant similarity found
		B.16	Common bacterial blight	665	No significant similarity found
		B.26	Angular leaf spot	644	No significant similarity found
Angular leaf spot (Mesomerican)		B.9	Angular leaf spot	877	No significant similarity found
		Plot			
Rhizoctonia root rot	*Rhizoctonia solani*	407	*Unidentified root rot*	873	Poor sequence quality
		405	*Unidentified root rot*	868	*Rhizoctonia solani*
		409	*Unidentified root rot*	814	*Rhizoctonia solani*
		402	*Unidentified root rot*	815	*Rhizoctonia solani*
		404	*Unidentified root rot*	888	*Rhizoctonia solani*
		406	*Unidentified root rot*	853	*Rhizoctonia solani*
		410	*Unidentified root rot*	808	*Rhizoctonia solani*
		408	*Unidentified root rot*	769	*Rhizoctonia solani*
Fusarium root rot	*Fusarium solani* f.sp *phaseoli*	307	*Unidentified root rot*	854	*Fusarium* sp.
		408	*Unidentified root rot*	936	*Fusarium* sp.

Discussion

In this section main findings of the research at the Kimashuku site will be discussed using available literature on the field to mine relevant information in the pursuit to answer research questions presented in the introduction. Structure of this section will be largely like previous sections, starting with discussing the field site, then plant physiological parameters and lastly observed biotic stresses, severity of their occurrence and possible effect observed of cropping system and cultivar.

Establishment of the crop went accordingly, and the decision to sown after initial showers turned out to be correct, putting the crop three full weeks ahead of later sown neighbouring fields. In spite of a relapsing dry period at the Kimashuku site, the local stream swelled by rains falling at the foothills of Mt. Kilimanjaro. This water resource was diverged by an intricate network of smaller channels to the field for flood irrigation during periods of insufficient precipitation. In practice this was done by blockage of selected streams using sandbags, directing the water unto fields prepared with square patches (2-3 m^2) surrounded by a soil ridge (10 cm) to avoid runoff and ensure water infiltration. Distribution of this water resource is organized by a local authority, and as majority of smallholder farms were solely rain fed it suggested a lack of power, *i.e.* financial means to secure it in case of insufficient precipitation (Tittonell and Giller 2013). Stakes are, therefore, high when settling on a sowing date, depending on a prolonged period of rain to ensure a successful crop, explaining the delayed sowing on neighbouring fields. Water constitute a yield limiting factor for most smallholder farms in the Northern Highland in dry periods during the rainy season (Rockstrom 2000). Is it to be expected that these events will occur more frequent in near-future as a consequence of global warming, with a projected increase in spatial and temporal variation in rainfall events (Adhikari, Nejadhashemi, and Woznicki 2015; Hartmann *et al*. 2013). On site, inhomogeneous weeding and flood irrigation is likely to have caused some variation in crop performance, weakening the statistical analysis of assessed parameters.

Detailed information on climatic parameters obtained from the local weather station showed a high relative humidity in the interval 68 to 95 %, and mean day temperature from 20 to 27 degrees from 30 DAS and throughout the growth season (Figure 3 A-B). These environmental conditions are recognised to favour the development of most pathogens often dependent on prolonged periods of leaf wetness to infect host plant, and certain temperature to proliferate (Agrios 2005). Accompanied by a highly monotonous cropping system on neighbouring fields, largely consisting of maize intercropped with the local common bean cultivar cultivated, the conditions for severe epidemics were present at the site.

The Northern Highland of Tanzania constitute one of East Africas most fertile areas. Recent survey of the volcanic soil by Funkawa *et al*. (2012) found that the soil was characterized by a very fine texture (36.2% sand, 28.7% silt, 35.1% clay), rich on organic matter (43.3 g kg^{-1}), nitrogen (3.4 g kg^{-1}) and with an availability of phosphorus pentoxide at 0.43 g kg^{-1}. pH of the volcanic soil was found to be slightly acidic with a score of 5.9 at which favours the availability of most plant macro and micronutrients except magnesium and to a less degree phosphorus (Kihara *et al*. 2017). In spite of a rich soil and dissemination of triple phosphate fertilizer and urea banding in maize during the establishment phase, symptoms of severe nutrient deficiencies were observed throughout the field. Maize displayed particularly severe nitrogen deficiencies while limitations in magnesium and potassium were less pronounced. Common bean displayed magnesium deficiency solely, however, likeness to viral symptoms made it difficult to separate the stresses and estimate the extent (Figure 14 A-B). Observed deficits is not likely not caused by innate soil deficiencies on site, but rather the product of a negative nutrient balance throughout its cultivated history. This is consistent with a study by Baijukya *et al*. (2005), who found a negative nutrient

balance of 15 to 7 kg nitrogen, 2 to 1 kg phosphorus and 14 to 4 kg potassium per hectare per year in a maize based cropping system in North-West Tanzania.
The N_2Africa project emphasizes integration of leguminous crops alongside application of phosphorus fertilizer as an effective measure, affordable on short term for the smallholder farmers (Giller *et al*. 2013). In some soils, denoted as non-responsive the addition of macronutrients (N, P, K) solely is not sufficient, as deficiencies in secondary nutrients and micronutrients are equivalently limiting crop productivity (Kihara *et al*. 2017). Hence, crops cultivated on these soils will also not respond accordingly to introduction of nitrogen fixing leguminous species as a singular intervention. In a meta-analysis by Kihara *et al*. (2017) on data from 40 individual studies in SSA, they discovered that application of sulphur and micronutrients increased maize yield by 0.84 tons per hectare. At the Kimashuku site, application of potassium and magnesium fertilizer in particular would be beneficial for crop productivity.

Our study on the effect of cropping system on occurrence and severity of biotic stress at Kimashuku was done from a holistic perspective with inclusion of selected plant physiological parameters. These included measurement of yield, height, phenological development and light interception. In the follow subsection, findings on the effect of treatment (*i.e.* common bean cultivar x cropping system combination), cropping system (*i.e.* Cont. monoculture, Cont. intercropping, rotation and rotation with intercropping) and cultivar will be discussed for the maize and common bean crop respectively.
In maize, continuous intercropping and rotation cropping system with the local common bean cultivar was found to produce the highest yield of respectively 3.21 and 3.23 tons per hectare in the fifth growth season on site (Figure 6). Relative to continuous monoculture these cropping systems resulted in a yield increase of 220 to 240 kg per hectare. These treatments were, equivalently found to exhibit the highest mean crop height of 169 cm (Figure 7.A). In addition to this the continuous intercropping system with the local cultivar yielded 1.56 tons per hectare, resulting in a land equivalent ratio (LER) of 1.64.

$$Land\ equivalent\ ratio = \frac{Y_{Maize,\ intercrop}}{Y_{Maize,\ monoculture}} + \frac{Y_{bean,\ intercrop}}{Y_{Maize,\ monoculture}}$$ (Mead and Willey 1980)

High partial LER value of maize (>1) suggest that nutrient competition with the intercrop is negligible in this treatment combination relative to the profits of nitrogen fixation. This LER ratio is consistent with findings in a recent study by Kermah *et al*. (2017) on maize intercropped with soya bean, groundnut and cowpea. In the study they concluded that productivity of intercropping systems was enhanced on less fertile soils, allowing a more even competition between the crops than on fertile soils where maize tended to outcompete the legume specie. Use of intercropping practice is, therefore, an important measure in the sustainable intensification of SSA agriculture, and increase land productivity.
The maize crop, however, displayed a high variation in yield across treatments, with the lowest at 1.28 tons per hectare. In accordance to cited water-limited yield potential, *i.e.* maximum yield in a rain fed production system, of 8.9 tons per hectare this translates to a yield gap of 63.7 to 85.7 % (YieldGap 2017; Tittonell and Giller 2013). In our study we found that the cropping system of maize in rotation with intercrop to yield a significantly lower output of kernels relative to monoculture. As this cropping system was expected to rank among the highest yielding, being the most diverse, we expect this result to be linked to an underlying factor on site rather than the cropping system. From the south to north on our site ran a gradient of crop vigour from high to low likely caused by difference in water availability. By stochastic event, most of the replicate plots of this cropping system was placed in the northern sector, resulting in this outlying result in spite of a randomized complete block design.

In the dating of phenological stages throughout the growth season we found that the maize crop to a large extent developed uniformly across treatments. Majority of leaf collars in the crop was set 66 DAS, and transition from vegetative to reproductive growth started at 54 DAS with setting of tassels (Figure 4 A-B). Replacement of the traditional phenological scoring of kernel development with dry weight measurement showed that majority of kernel growth occurred from 83 to 110 DAS (Figure 4.C).
Interception of PAR is highly correlated to crop productivity and was hence included as a key parameter in the holistic approach to assess crop performance (Monteith and Moss 1977). At the peak of canopy coverage, the maize crop intercepted between 56 to 70 % of PAR within the rows and 41 to 62 % between rows (Figure 8 A-B). We found that inclusion of the local common bean cultivar in the cropping system led to a significantly higher (9 %) interception of PAR within the maize row. These systems, moreover, displayed a slightly tighter row closing capacity.
In the common bean crop, intercropping treatments were found to yield 1.53 to 1.54 tons per hectare, translating to a partial LER of > 0.56 (*i.e.* 56 %), with bean in monoculture yielding on average 2.74 tons per hectare (Figure 6) (Mead and Willey 1980). This significant yield reduction is directly linked to the light competition with intercropped maize, reducing intercepted PAR by 63 % relative to monoculture treatment (Data not shown) (Figure 8 C-D). An increased nutrient utilization as a result of higher LER rations, *i.e.* an increased productivity per area, however, requires an equivalent increase in nutrient return to no fuel a more negative nutrient balance (Kimani *et al*. 2013). Yield found in the monoculture plots was very close to the cited water-limited yield potential, with a yield gap of 5 % (Schilt 2017)**.** The two common bean cultivars were found to significantly differ in both maximum height, seed DW, vegetative and reproductive growth. The local Mkanamna cultivar was characterized by a delayed development, smaller seed size and a more vigorous vine growth, intercepting a larger fraction of PAR and growing taller. None of the cropping systems stood out in performance on selected plant physiological parameters.

In the following subsection our findings on biotic stresses in the field will be discussed in relation to plant physiological parameters and cropping system. The severity of biotic stresses was done in accordance to literature by a single individual, and diagrammatic scales were used when possible to reduce the variability linked to visual scorings (Nutter and Schultz 1995). This approach requires no investment in instruments and methods described in this report can direly applied by N_2Africa research personal in the field after a brief study. Doing so, this will contribute to an increased awareness of the identification of biotic stresses, occurrence of these and their impact on crop productivity.
At the Kimashuku site a large number of pest were observed in the crop with 6 species identified in maize and 14 species in common bean during the growth season (Figure 11, Figure 17, Figure 18). Damage done by these pests was found to be particularly severe in the early stages of the crop. A likely factor contributing to this observation is the delayed establishment of crops on neighbouring plots, attracting flying pests from a substantial radius, seeking out a host plant. In the maize crop these were predominantly Spottet stem borer (*Chilo partellus*) and to a lesser degree African armyworm (*Spodoptera exempta*) with 80 to 100 % of assessed plants infested with minimum one pest. These gave cause to substantial feeding damage and reduced canopy area by folding several tips together prior to pupating. On the used nine step visual scale by Tefera *et al*. 2011, cultivation of maize in a cropping system with the local common bean cultivar was found to reduce mean severity by half a score (nonsignificant, $P_{val} = 0.0139$) relative to the improved cultivar. While this difference in score is relatively small, an increased nutritional status of the plant has been found to reduce the yield losses caused by

the Spottet stem borer (Mgoo *et al*. 2006). This would imply a higher uptake of biologically fixated nitrogen in the maize plants in these cropping systems. Improving soil fertility is, therefore, a long-term control practice where deployment of parasitoid wasps has been a large research topic in recent years, showing promising results in SSA (Kipkoech *et al*. 2010). Intercropping has been found in other cropping systems to reduce infestation and abundance of stem borers, however, we did not find any noticeable effect on severity nor incidence score (Degri, Mailafiya, and Mshelia 2014; Maluleke, Addo-Bediako, and Ayisi 2005). In these studies, they proposed that host-finding of female stem borer was interfered by the non-host intercrop, reducing ovipositional success. It is like that at the population densities that we observed at the Kimashuku site, that this reduction was negligible. Moreover, the small plot size might cloud the effect of intercropping.

Foliage feeding was equivalently an issue in the early stages of the common bean crop with various pests, *e.g*. African bollworm (*Helicoverpa armigera*) Bean beetle (*Ootheca bennigseni, O. mutabilis*) and *Lepidopteran* sp. identified. Four weeks after sowing these were found to cause a mean reduction of leaf area by five to ten %, after which foliage feeding subsided as leaves started to reach maturity.

Our scoring did not reveal a significant difference in neither cropping system or between common bean cultivars in relation to foliage feeding. Particularly the absent effect of intercropping was contrasting our expectations. As leaves matured on the crops, the pest population shifted and started to feed particularly on the inflorescence tissue, *i.e.* tassels and cobs in maize, flowers and pods in common bean. African bollworm (*Helicoverpa armigera*) and to a lesser extent African stalk borer (*Busseola fusca*) was found in the maize crop with 45 to 80 % of plants across treatment exhibiting minimum one cob showing feeding damage 93 DAS (Figure 12.B). The feeding damage was, mostly confined to the cob tip and likely had a minute effect on the total yield. However, it is not known if the feeding can interfere with proper kernel setting. Pest feeding damage have, nonetheless, been found to constitute a key entry site for ear rots capable of causing extensive yield losses (Blandino *et al*. 2008). Ear rots are infamous producers of mycotoxins and a serious food safety issue in SSA, capable of causing severe health disorders (Mukanga *et al*. 2010).

Pod feeding in the common bean also gave cause to substantial damage from pod setting around 39 DAS until drying around 77 DAS (Figure 19.B). Assessment of incidence revealed a significant difference in the two cultivars with Local cultivar had on average 54 % of plants with minimum one feeding wound on pods relative to 20 % in the improved cultivar. This observation indicates a feeding preference either caused by a repellent effect in the improved cultivar, or synchronization of pests with pod setting in the local cultivar at the site (Jayasinghe, Premachandra, and Neilson 2015).

Post-harvest damage in particularly bean seeds have been cited as an important component in the biotic yield gap. However, due to low emergence levers observed in collected seeds this assessment was not done (Umbeyeyi and Rukazambuda 2016; Ebinu *et al*. 2016) (Figure 18 A-B).

Disease at the Kimashuku site started to occur around five weeks after sowing, concurrent with a prolonged period of rain fall and increase in relative humidity. To study their impact and progression on the crop, we decided to score severity on lowest leaf, and successively ear-supporting leaf as it is has been shown to play an important role in kernel filling (Sanchez-Bragado *et al*. 2014). Common rust disease (*Puccinia sorghi*) was found to score the highest severity on lowest leaves, causing a loss of 10 to 19 % of leaf area across treatments 91 DAS (Figure 10 C-D). In SSA agriculture, Common rust is a wide-spread disease giving cause to substantial yield losses of 35 % (Vivek *et al*. 2010; Dey *et al*. 2012). Eyespot disease (*Kabatiella zeae*) was found to occur evenly throughout the canopy at a lower severity,

causing a loss of 2.6 to 5 % of leaf area 97 DAS (Figure 10 A-B). In spite of being a relative important disease on the site little literature is available on Eyespot disease, evidently occurring as a minor or upcoming disease in SSA agriculture.

In our study we found no effect of cropping system on occurrence of diseases in the maize crop. However, our scorings revealed that the diseases occurred with a significantly higher severity on the moister lower leaves relative to the upper ear supporting leaf. As severity of biotic stresses across the cropping systems was relatively uniform we conclude that it was not the primary factor contributing to the observed yield reduction in the maize rotation and intercropping system.

Grey leaf disease (*Cercospora zeae-maydis*, *C. sorghi* var. *maydis*) has been cited as one of the most severe diseases in SSA agriculture, capable of causing substantial yield losses (Nega, Lemessa, and Berecha 2016; Ward and Nowell 1998). However, at Kimashuku site Grey leaf spot was found to cause negligible damage relative to above mentioned disease (Figure 10.E).

In common bean our data from scoring of severity and incidence was decided to not be included in the report as the accuracy of in-field assessment could not be guaranteed due to overlap of diseases and likeness of their symptoms. Recognition of these and the ability to separate these in the given circumstances require a certain amount of training (Nutter and Schultz 1995). Nonetheless, when occurring in isolation, Alternaria leaf spot (*Alternaria alternata*), Angular leaf spot (*Isariopsis griseola*), Common bacterial blight (*Xanthomonas campestris* cv. *phaseoli*) and Anthracnose (*Colletotrichum lindemuthianum*) and Bean mosaic virus were identified in the field (Figure 17). Throughout the field, symptoms of wilting and high failure rate in monoculture treatment of the improved cultivar suggested a soil-borne biotic stress, impairing root functioning. Consistent with this was the observation of a lower rate in establishing a healthy crop in the improved cultivar, with a mean of 140 plants per plot relative to 182 in the local cultivar. Assessment of roots led to the observation that most of the compromised plants had a distinct red discolouration of the cortex, suggesting Rhizoctonia root rot (*Rhizoctonia solani*) (Figure 15, Figure 16). The screening, however, also revealed to a less degree, tunnelling in the tap root caused by feeding bean fly (*Ophiomyia phaseoli*) maggots. As the field diagnosis of particularly the foliar diseases in common bean was problematic, a laboratory diagnosis was included in the study. These tests confirmed the diagnosis of Common bacterial blight, Angular leaf spot and Alternaria leaf spot (Table 6). The latter, was found on several leaves not diagnosed with Alternaria leaf spot suggesting that this was the most prevalent foliar disease in the common bean crop. While the causative agent has been thoroughly studies in other agricultural important crops, literature on its occurrence and potential to control it in common bean systems is scarce (Thomma 2003). Angular leaf spot disease, however, is known to cause devastating yield losses in SSA, with a recent study by Mongi *et al*. (2017) showing a loss of up to 60 % in the Southern Highland of Tanzania. Our findings suggest that Angular leaf spot was a disease of minor importance on the site.

Analysis of the root samples revealed that Rhizoctonia root rot was present in all sampled plots without discriminating between cropping systems, indicative of a large pathogen population giving cause to substantial root damage (Naseri and Mousavi 2015). In addition to this, Fusarium root rot (*Fusarium solani* f.sp *phaseoli*) was confirmed in two plots (307, 408), cultivated with maize in a rotation with intercrop of the local common bean cultivar. This severe infestation of root rots is likely the cause to the non-existent effect of cropping system on the site. Rotation and cropping system is a key control practice, cited in several studies to reduce the pool of soil-borne pathogens in the soil compartment (Larkin and Honeycutt 2006; Abdel-Monaim and Abo-Elyousr 2012). At this infestation level it is,

however, likely that the rotation needs to be extended with more than one growth season between common bean crops to produce an effect on the soil borne diseases.

Knowledge on the biotic stresses occurring in SSA is insufficient to advise farmers on effective control practices other than emphasizing good agricultural practices. At present farmers who can afford it, therefore, dependent on use of pesticides to combat biotic stresses, and often applying it in an inefficient manner without protection gear (Macharia 2015).

5. Conclusion & further recommendations

This study produced a rare insight in the occurrence and progression of biotic stresses in relation to crop developmental stage and light interception in maize and common bean. Throughout the long rain season at Kimashuku site in the Northern Highland of Tanzania we identified 20 pests and 14 diseases in the crop. In maize, important biotic stresses included Spottet stem borer, African bollworm, Common rust and Eyespot, while in common bean various foliage and pod feeding pests, Alternaria leaf spot and Rhizoctonia root rot. Effect of cropping system was found to be largely negligible on the incidence and severity of these with the large population and diversity of biotic stresses a likely contributing factor to this. Observations of severe symptoms of N, P, K, and Mg nutrient deficiencies, moreover, led us to draw the conclusion that nutrient limitation, *i.e.* abiotic stress was a major yield gap component on the site. Inclusion of the local common bean cultivar in a cropping system with maize was found to increase yield, height, interception of PAR, crop development and lower score in severity or incidence of most of the assessed biotic stresses. The overall best performing treatment combination was maize in rotation with the local cultivar, producing the highest land equivalent ratio of 1.64. Yield gap of maize relative to water-limited yield potential across treatments was 63.7 to 85.7 %, while 5 % in monoculture treatment of common bean.
Inclusion of the selected plant physiological parameters provided an insight in crop functioning but limited information on the individual biotic stresses, clouded by a number of other abiotic and biotic stresses.

Our study proposes the following, **further recommendations**

- Increase the awareness of biotic stresses in N_2Africa project countries by training field extension officers in identification and assessment of their severity in field
- Investigate near-future control methods for the most important biotic stresses
- Inclusion of measurements on plant nutritional status (N, P, K, Mg, S)
- Increase the plot size to amplify the effect of cropping system on occurrence of biotic stresses

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Appendices

Appendix 1: Plot design and cropping history at Kimashuku field trial site

Table A1: Cropping systems of maize (*Zea mays*) (**M**) and two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**BL**) and improved Lyamungu 90 (**BI**) at Kimashuku field trial, Northern Highland of Tanzania. Per cropping system two plots were selected for additional assessments (*).

				Growth Seasons				
				2015		2016		2017
		Treatment	Plot	1	2	3	4	5
Cropping system(s)	1	Continuous monoculture of maize	101*	M	M	M	M	M
			204*	M	M	M	M	M
			310	M	M	M	M	M
			411	M	M	M	M	M
	2	Continuous monoculture of improved common bean	105*	BI	BI	BI	BI	BI
			208	BI	BI	BI	BI	BI
			311	BI	BI	BI	BI	BI
			407*	BI	BI	BI	BI	BI
	3	Continuous monoculture of local common bean	111	BL	BL	BL	BL	BL
			206	BL	BL	BL	BL	BL
			306*	BL	BL	BL	BL	BL
			405*	BL	BL	BL	BL	BL
	4	Continuous intercropping of maize and improved common bean	109	M+BI	M+BI	M+BI	M+BI	M+BI
			207	M+BI	M+BI	M+BI	M+BI	M+BI
			301*	M+BI	M+BI	M+BI	M+BI	M+BI
			409*	M+BI	M+BI	M+BI	M+BI	M+BI
	5	Continuous intercropping of maize and local common bean	110	M+BL	M+BL	M+BL	M+BL	M+BL
			205	M+BL	M+BL	M+BL	M+BL	M+BL
			304*	M+BL	M+BL	M+BL	M+BL	M+BL
			402*	M+BL	M+BL	M+BL	M+BL	M+BL
	6	Rotation of maize with improved common bean	102	BI	M	BI	M	BI
			108	M	BI	M	BI	M
			201	BI	M	BI	M	BI
			211	M	BI	M	BI	M
			308*	M	BI	M	BI	M
			309*	BI	M	BI	M	BI
			403*	M	BI	M	BI	M
			404*	BI	M	BI	M	BI
	7	Rotation, maize and local common bean	103	BL	M	BL	M	BL
			107	M	BL	M	BL	M
			203	BL	M	BL	M	BL
			210	M	BL	M	BL	M
			302*	M	BL	M	BL	M
			303*	BL	M	BL	M	BL
			401*	M	BL	M	BL	M
			406*	BL	M	BL	M	BL
	8	Rotation, maize and intercropping maize with improved common bean	104	M+BI	M	M+BI	M	M+BI
			202	M+BI	M	M+BI	M	M+BI
			305*	M+BI	M	M+BI	M	M+BI
			410*	M+BI	M	M+BI	M	M+BI
	9	Rotation, maize and intercropping maize with local common bean	106	M+BL	M	M+BL	M	M+BL
			209	M+BL	M	M+BL	M	M+BL
			307*	M+BL	M	M+BL	M	M+BL
			408*	M+BL	M	M+BL	M	M+BL

Appendix 2: Crop height and yield

Figure A2.1: Mean plants per plot and height development of maize (*Zea mays*) cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**1**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). SD = Standard deviation. DAS = Days after sowing.

		n	Mean plants per plot	Height Mean of six samples, cm								Plot mean yield ± SD Tons per hectare
				36	43	54	66	72	83	93	112	
DAS												
Cropping System	1	2	77 ± 9	19.4 ± 6.1	30.9 ± 7.9	56.8 ± 18.9	118.9 ± 33.8	150.8 ± 22.8	154.6 ± 25.3	157.4 ± 23.5	150.1 ± 23.2	3.00 ± 0.76
	4	4	63 ± 10	23.9 ± 5.9	33.5 ± 9.7	59.5 ± 22.1	102.7 ± 39.3	138.4 ± 29.0	152.8 ± 25.3	152.3 ± 25.2	143.3 ± 24.3	2.35 ± 1.72
	5	4	72 ± 4	31.3 ± 8.7	42.3 ± 14.9	83.6 ± 23.2	131.4 ± 37.9	159.5 ± 27.3	168.4 ± 21.2	169.3 ± 21.2	163.9 ± 23.0	3.21 ± 1.11
	6	1	68 ± 0	22.8 ± 4.5	30.4 ± 8.0	44.3 ± 11.4	90.2 ± 26.7	117.5 ± 15.0	120.5 ± 19.8	122.5 ± 18.5	130.2 ± 14.2	1.28 ± 0.00
	7	4	67 ± 10	34.6 ± 10.1	42.4 ± 14.7	87.5 ± 26.0	126.9 ± 38.8	145.4 ± 32.7	162.9 ± 25.1	168.7 ± 21.6	160.4 ± 23.4	3.23 ± 0.88
	8	4	63 ± 10	26.7 ± 8.6	36.2 ± 15.2	64.5 ± 25.2	109.4 ± 37.3	138.3 ± 32.1	149.0 ± 25.0	148.0 ± 26.6	141.1 ± 29.8	2.03 ± 1.40
	9	4	64 ± 8	24.2 ± 7.2	29.9 ± 9.6	51.3 ± 16.1	88.1 ± 33.0	107.6 ± 29.9	127.6 ± 20.6	125.9 ± 22.7	122.7 ± 27.6	1.31 ± 0.24

Table A2.2: Mean plants per plot and height development of two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**2**,**3**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). SD = Standard deviation. DAS = Days after sowing.

		n	Mean plants per plot	Height ± SD Mean of six samples, cm				Plot mean yield ± SD Tons per hectare
				36	43	54	66	97
DAS								
Cropping System	2	1	221 ± 54	17.3 ± 4.9	20.2 ± 3.3	20.7 ± 4.2	22 ± 2.0	2.74 ± 0.00
	3	4	263 ± 38	13.9 ± 4.0	21.0 ± 7.3	38.2 ± 10.5	32.4 ± 8.5	2.77 ± 0.05
	4	4	87 ± 11	14.7 ± 5.7	19.1 ± 6.7	20.3 ± 5.2	19.3 ± 4.6	1.51 ± 0.05
	5	4	100 ± 15	14.4 ± 5.6	20.6 ± 7.7	31.6 ± 11.7	33.9 ± 9.0	1.56 ± 0.15
	6	4	215 ± 35	19.9 ± 5.3	21.8 ± 5.2	25.9 ± 11.0	22.8 ± 4.9	2.65 ± 0.13
	7	3	261 ± 128	21.8 ± 6.1	32.3 ± 12.8	49.4 ± 9.0	37.7 ± 7.9	2.65 ± 1.08
	8	4	75 ± 17	17.6 ± 5.4	20.5 ± 5.1	25.0 ± 8.8	21.3 ± 4.8	1.49 ± 0.12
	9	4	125 ± 28	13.8 ± 7.0	20.3 ± 5.2	29 ± 7.8	32.8 ± 7.4	1.60 ± 0.05

Appendix 3: Developmental parameters of maize

Table A3.1: Mean score of phenological stages in maize (*Zea mays*) cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**1**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Scoring method after Dupont Pioneer (2016), with mean generated from 10 scorings per n (plot). SD = Standard deviation. DAS = Days after sowing.

			Vegetative stage, Mean ± SD									
	DAS	n	23	28	32	39	43	46	54	61	66	110
Cropping system	1	2	2.4 ± 0.7	3.4 ± 0.5	4.4 ± 0.9	4.6 ± 0.9	4.9 ± 0.9	5.7 ± 1.0	7.7 ± 1.4	8.9 ± 1.8	12.0 ± 1.8	12.7 ± 1.5
	4	4	3.0 ± 0.0	3.7 ± 0.6	4.7 ± 0.8	4.5 ± 1.0	5.0 ± 1.1	5.6 ± 1.3	7.7 ± 2.1	9.6 ± 2.6	11.4 ± 3.0	13.1 ± 1.1
	5	4	3.0 ± 0.7	4.4 ± 0.7	5.0 ± 0.8	5.2 ± 0.9	6.2 ± 1.1	6.6 ± 1.3	9.2 ± 2.0	11.3 ± 2.9	12.8 ± 1.9	14.3 ± 0.9
	6	1	2.7 ± 0.5	3.3 ± 0.5	4.1 ± 0.3	4.4 ± 0.8	5.1 ± 1.0	5.4 ± 1.0	7.4 ± 1.7	8.5 ± 2.0	10.7 ± 1.5	12.0 ± 0.9
	7	4	2.9 ± 0.3	4.3 ± 0.6	5.0 ± 0.8	5.4 ± 1.0	6.2 ± 1.2	7.0 ± 1.3	9.8 ± 1.7	12.7 ± 2.3	12.9 ± 1.7	13.9 ± 1.1
	8	4	2.7 ± 0.7	4.2 ± 0.6	4.6 ± 0.9	4.7 ± 1.0	5.5 ± 1.2	6.4 ± 1.4	8.0 ± 2.0	10.2 ± 2.4	12.0 ± 2.1	13.0 ± 1.1
	9	4	3.0 ± 0.5	3.7 ± 0.6	4.3 ± 0.9	4.4 ± 0.9	5.1 ± 1.3	5.5 ± 1.4	7.1 ± 1.7	8.8 ± 2.1	10.9 ± 2.1	12.8 ± 1.9

			Reproductive stage, Mean ± SD				
	DAS		54	58	61	66	74
Cropping system	1	2	0.0 ± 0.0	0.3 ± 0.4	0.3 ± 0.5	1.0 ± 0.7	1.9 ± 0.4
	4	4	0.0 ± 0.2	0.3 ± 0.5	0.4 ± 0.5	0.8 ± 0.6	1.8 ± 0.4
	5	4	0.1 ± 0.3	0.4 ± 0.5	0.6 ± 0.6	1.2 ± 0.6	1.9 ± 0.4
	6	1	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.4	1.0 ± 0.5	1.9 ± 0.6
	7	4	0.3 ± 0.4	0.7 ± 0.6	1.0 ± 0.6	1.3 ± 0.7	1.8 ± 0.4
	8	4	0.0 ± 0.2	0.2 ± 0.4	0.5 ± 0.5	1.0 ± 0.6	1.8 ± 0.4
	9	4	0.0 ± 0.0	0.2 ± 0.4	0.3 ± 0.5	0.9 ± 0.6	1.7 ± 0.4

Table A3.2: Mean kernel dry weight development in maize (*Zea mays*) cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**1**), continuous intercropping (**4**,**5**), rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Number in superscript represent sample n with mean generated from individual weight of 20 kernels per n (plot). SD = Standard deviation. DAS = Days after sowing.

		Mean kernel weight (g)								
	DAS	69	72	76	79	83	86	93	110	145
Cropping system	1		0.010^{2}					0.096^{2}	0.301^{1}	0.396^{2}
	4		0.009^{2}					0.141^{2}	0.246^{1}	0.407^{4}
	5	0.011^{2}		0.019^{2}			0.079^{2}		0.232^{1}	0.390^{4}
	6		0.012^{2}		0.018^{1}	0.022^{1}	0.111^{1}	0.157^{1}	0.272^{1}	0.391^{4}
	7		0.011^{2}	0.045^{1}		0.039^{2}		0.030^{1}	0.253^{2}	0.402^{4}
	8		0.012^{1}		0.018^{1}	0.028^{1}	0.124^{1}	0.123^{1}	0.292^{1}	0.426^{4}
	9		0.008^{1}			0.034^{2}		0.143^{2}	0.319^{1}	0.402^{4}
Mean		0.011	0.010	0.032	0.016	0.034	0.105	0.115	0.274	0.404
SD		0.002	0.003	0.017	0.005	0.020	0.078	0.043	0.055	0.062

Appendix 4: Developmental parameters of common bean

Table A4.1: Mean score of phenological stage in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**2**,**3**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Scoring method after Lebaron (1974), with each mean generated from 10 scorings per n (plot). SD = Standard deviation. DAS = Days after sowing.

			Vegetative stage, Mean ± SD							
	DAS	n	23	28	32	39	43	46	54	69
Cropping system	2	1	2.4 ± 0.5	3.5 ± 1.0	4.7 ± 1.2	5.9 ± 1.3	6.2 ± 1.2	6.7 ± 1.3	7.2 ± 0.9	7.7 ± 0.9
	3	4	2.1 ± 0.6	3.3 ± 0.9	5.1 ± 1.1	7.4 ± 1.7	8.7 ± 1.8	9.8 ± 2.1	11.0 ± 2.3	12.7 ± 1.9
	4	4	1.6 ± 0.5	3.2 ± 0.7	4.5 ±1.1	6.2 ± 1.3	6.6 ± 1.2	6.7 ± 1.2	7.0 ± 1.1	7.4 ± 1.0
	5	4	2.2 ± 0.6	3.0 ± 1.0	4.9 ± 1.4	6.9 ± 1.9	8.0 ± 1.7	8.9 ± 1.7	10.3 ± 1.8	11.5 ± 1.9
	6	4	-	3.9 ± 0.7	5.6 ±1.1	6.7 ± 1.3	7.4 ± 1.1	7.4 ± 1.0	7.2 ± 1.0	8.0 ± 0.9
	7	3	2.1 ± 0.7	3.5 ± 1.1	5.2 ± 1.6	7.5 ± 1.9	8.5 ± 1.9	9.8 ± 2.0	11.6 ± 1.4	13.2 ± 1.7
	8	4	2.2 ± 0.8	3.3 ± 0.9	4.6 ± 1.1	6.7 ± 1.1	7.2 ± 1.1	7.0 ± 1.1	7.2 ± 1.0	10.2 ± 0.9
	9	4	2.2 ± 0.4	3.4 ± 0.7	5.0 ± 1.2	7.3 ± 1.6	8.2 ± 1.1	8.2 ± 1.2	10.5 ± 2.1	11.5 ± 2.0

			Reproductive Stage, Mean ± SD					
	DAS	n	39	43	46	54	59	66
Cropping system	2	1	0.4 ± 0.5	1.2 ± 0.8	1.2 ± 1.0	3.4 ± 0.8	4.0 ± 0.0	4.0 ± 0.0
	3	4	0.0 ± 0.0	0.3 ± 0.4	0.9 ± 0.8	2.5 ± 1.1	3.8 ± 0.6	4.0 ± 0.0
	4	4	0.4 ± 0.5	1.4 ± 0.8	2.4 ± 1.0	3.4 ± 1.2	3.9 ± 0.3	4.0 ± 0.0
	5	4	0.2 ± 0.4	0.3 ± 0.5	0.7 ± 0.8	2.3 ± 1.2	3.5 ± 0.8	4.0 ± 0.0
	6	4	0.7 ± 0.5	1.8 ± 0.5	2.7 ± 0.6	3.2 ± 0.0	4.0 ± 0.2	4.0 ± 0.0
	7	3	0.1 ± 0.3	0.2 ± 0.4	0.9 ± 0.9	2.7 ± 1.0	3.7 ± 0.8	4.0 ± 0.0
	8	4	0.6 ± 0.5	0.1 ± 0.7	2.4 ± 0.9	3.3 ± 1.1	4.0 ± 0.0	4.0 ± 0.0
	9	4	0.1 ± 0.2	0.3 ± 0.4	0.8 ± 1.0	2.6 ± 1.1	3.7 ± 0.7	4.0 ± 0.0

Table A4.2: Mean seed dry weight development in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**3**,**5**,**7**,**9**) and an improved Lyamungu 90 (**2**,**4**,**6**,**8**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**2**,**3**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Mean generated individual weighing of seeds (< 20) from six pods per n (plot). SD = Standard deviation. DAS = Days after sowing.

		n	Seed dry weight, Mean ± SD					
	DAS		55	61	64	69	76	97
Cropping system	2	1	0.000 ± 0.00	0.014 ± 0.03	0.099 ± 0.09	0.176 ± 0.08	0.270 ± 0.11	0.325 ± 0.15
	3	2	0.000 ± 0.00	0.009 ± 0.01	0.039 ± 0.07	0.081 ± 0.04	0.112 ± 0.04	0.148 ± 0.03
	4	2	0.000 ± 0.00	0.082 ± 0.08	0.136 ± 0.09	0.293 ± 0.10	0.334 ± 0.10	0.282 ± 0.08
	5	2	0.000 ± 0.00	0.006 ± 0.00	0.034 ± 0.02	0.072 ± 0.03	0.105 ± 0.04	0.159 ± 0.04
	6	2	0.000± 0.00	0.004 ± 0.05	0.173 ± 0.11	0.222 ± 0.12	0.323 ± 0.15	0.378 ± 0.11
	7	2	0.000± 0.00	0.009 ± 0.01	0.028 ± 0.02	0.074 ± 0.03	0.124 ± 0.04	0.161 ± 0.04
	8	2	0.000 ± 0.00	0.027 ± 0.03	0.109 ± 0.06	0.207 ± 0.11	0.305 ± 0.15	0.336 ± 0.11
	9	2	0.000 ± 0.00	0.007 ± 0.01	0.025 ± 0.02	0.072 ± 0.04	0.105 ± 0.04	0.179 ± 0.04
Mean Improved			0.000 ± 0.00	0.043 ± 0.09	0.129 ± 0.09	0.225 ± 0.11	0.314 ± 0.14	0.331 ± 0.11
Mean Local			0.000 ± 0.00	0.008 ± 0.01	0.031 ± 0.04	0.075 ± 0.04	0.112 ± 0.04	0.162 ± 0.04

Appendix 5: Scoring of biotic stresses in maize crop

Table A5: Mean severity and incidence of occurring biotic stresses in maize (*Zea mays*) cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**4**,**8**,**9**) and an improved Lyamungu 90 (**5**,**7**,**9**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**1**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). **A:** Common rust disease, severity scoring method after Danson *et al* (2008). **B:** Eyespot disease, severity scoring method after Sachs *et al* (2011). **C:** Grey leaf spot disease, severity scoring method after Danson *et al* (2008). **D:** Stem borer foliage feeding damage, severity scoring after Tefera *et al* (2011). Means are generated from 10 scorings per n (plot). SD = Standard deviation. DAS = Days after sowing.

A			Mean ± SD, % area affected Lowest leaf					Mean ± SD, % area affected Upper ear leaf				
	DAS	n	72	77	83	86	91	79	83	86	91	97
Cropping system	**1**	2	0.5 ± 0.5	0.6 ± 0.5	3.8 ± 7.1	7.0 ± 14.0	11.4 ± 21.7	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.4	0.8 ± 1.1	1.4 ± 1.6
	4	4	2.5 ± 8.5	2.8 ± 7.0	2.8 ± 6.0	6.6 ± 13.0	11.6 ± 16.5	0.2 ± 0.4	0.2 ± 0.4	0.1 ± 0.3	0.7 ± 1.1	2.2 ± 2.2
	5	4	2.9 ± 7.9	2.6 ± 8.0	5.0 ± 9.5	10.8 ± 15.1	16.4 ± 18.9	0.5 ± 0.9	0.6 ± 1.6	0.8 ± 0.18	1.6 ± 3.3	4.4 ± 9.6
	6	1	0.2 ± 0.4	1.6 ± 3.4	1.8 ± 3.3	3.0 ± 6.1	19.1 **± 30.5**	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	1.6 ± 1.8	1.8 ± 1.7
	7	4	0.7 ± 1.1	1.7 ± 3.5	5.2 ± 6.9	4.9 ± 5.7	14.6 **± 19.8**	0.3 ± 0.8	0.4 ± 0.9	0.4 ± 0.9	0.8 ± 1.1	1.8 ± 1.8
	8	4	3.7 ± 9.8	4.2 ± 11.5	9.9 ± 19.5	11.5 ± 22.6	18.7 **± 25.6**	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4	1.0 ± 1.2	2.2 ± 2.2
	9	4	0.9 ± 2.1	1.0 ± 2.4	2.7 ± 6.2	3.1 ± 6.1	10.3 **± 17.0**	0.2 ± 0.4	0.1 ± 0.3	0.3 ± 0.4	0.5 ± 0.9	1.2 ± 1.3

B			Mean ± SD, % area affected Lowest leaf					Mean ± SD, % area affected Upper ear leaf				
	DAS	n	72	77	83	86	91	79	83	86	91	97
Cropping system	**1**	2	1.5 ± 0.9	2.0 ± 1.7	2.7 ± 2.2	3.0 ± 2.3	4.1 ± 2.5	1.4 ± 1.0	2.0 ± 1.2	2.3 ± 2.1	3.9 ± 1.7	3.8 ± 1.7
	4	4	1.3 ± 0.8	1.4 ± 1.1	2.1 ± 1.2	2.2 ± 1.4	3.2 ± 2.6	1.4 ± 0.8	1.5 ± 1.0	2.1 ± 1.2	2.9 ± 1.1	3.0 ± 1.1
	5	4	1.3 ± 0.7	1.6 ± 1.8	2.0 ± 1.8	2.4 ± 2.7	2.6 ± 1.8	1.5 ± 0.9	1.7 ± 1.1	2.3 ± 1.7	2.8 ± 1.6	3.2 ± 2.2
	6	1	1.2 ± 0.6	3.5 ± 3.0	4.3 ± 2.6	4.1 ± 2.6	5.0 ± 2.9	1.8 ± 1.0	2.6 ± 0.8	2.8 ± 1.1	3.8 ± 1.0	3.6 ± 1.0
	7	4	1.4 ± 0.8	1.8 ± 1.9	2.5 ± 3.0	2.6 ± 2.9	2.9 ± 2.4	1.5 ± 0.9	1.7 ± 1.2	2.3 ± 2.3	2.6 ± 1.5	3.0 ± 1.6
	8	4	1.6 ± 1.1	2.3 ± 2.1	2.9 ± 2.5	3.2 ± 2.9	3.5 ± 1.8	1.6 ± 1.0	1.9 ± 1.0	2.2 ± 1.2	3.2 ± 1.0	3.1 ± 1.4
	9	4	1.3 ± 0.8	1.9 ± 2.0	2.7 ± 1.7	3.3 ± 3.8	4.4 ± 3.3	1.5 ± 0.9	1.4 ± 0.8	1.6 ± 0.9	2.5 ± 0.9	2.6 ± 1.1

C			Mean ± SD, 0-5 Severity score Lowest leaf					D	Mean ± SD, 1-9 Severity score			Mean ± SD, Pest incidence (0-1)
	DAS	n	72	77	83	86	91		25	29	34	29
Cropping system	**1**	2	0.15 ± 0.37	0.75 ± 0.71	0.80 ± 0.70	0.70 ± 0.80	1.15 ± 1.04		4.4 ± 1.7	6.4 ± 1.3	6.3 ± 1.4	0.90 ± 0.31
	4	4	0.33 ± 0.53	0.80 ± 0.93	0.95 ± 0.90	1.13 ± 0.97	1.13 ± 0.97		5.5 ± 1.4	6.0 ± 1.3	6.3 ± 1.4	1.00 ± 0.00
	5	4	0.55 ± 0.60	0.88 ± 0.85	1.05 ± 0.81	1.13 ± 0.88	1.13 ± 0.83		4.0 ± 2.3	5.2 ± 1.4	4.9 ± 1.5	0.95 ± 0.22
	6	1	0.40 ± 0.52	0.50 ± 0.71	0.50 ± 0.71	0.50 ± 0.71	0.90 ± 0.74		6.4 ± 1.4	6.4 ± 1.0	5.7 ± 1.1	1.00 ± 0.00
	7	4	0.45 ± 0.64	0.60 ± 0.71	0.73 ± 0.72	0.88 ± 0.76	1.10 ± 0.84		5.0 ± 1.1	5.0 ± 1.0	5.4 ± 1.5	0.85 ± 0.36
	8	4	0.28 ± 0.51	0.58 ± 0.71	0.70 ± 0.76	0.90 ± 0.84	0.95 ± 0.81		4.4 ± 1.1	6.0 ± 1.1	5.6 ± 1.2	0.95 ± 0.22
	9	4	0.28 ± 0.45	0.63 ± 0.78	0.83 ± 0.84	1.13 ± 0.91	1.28 ± 0.85		5.4 ± 1.2	6.4 ± 1.2	6.3 ± 1.1	1.00 ± 0.00

Appendix 6: Scoring of biotic stresses in common bean crop

Table A6: Mean severity and incidence of occurring biotic stresses in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**2**,**4**,**8**,**9**) and an improved Lyamungu 90 (**3**,**5**,**7**,**9**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**2**,**3**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Foliage feeding, scoring method after Schoonhoven and Pastor-Corrales (1987). Means are generated from 10 scorings per n (plot). SD = Standard deviation. DAS = Days after sowing.

			Foliage feeding Mean ± SD, 1-9 Severity score			Pod feeding Mean ± SD, Incidence (0-1)			
	DAS	n	25	29	34	59	66	72	77
Cropping system	**2**	1	0.9 ± 1.2	2.4 ± 0.8	2.5 ± 1.2	0.30 ± 0.48	0.40 ± 0.52	0.30 ± 0.48	0.20 ± 0.42
	3	4	1.6 ± 1.4	2.7 ± 1.5	2.6 ± 1.3	0.18 ± 0.65	0.58 ± 0.63	0.50 ± 0.65	0.60 ± 0.62
	4	4	2.8 ± 1.7	2.6 ± 1.1	2.7 ± 0.8	0.10 ± 0.39	0.15 ± 0.42	0.13 ± 0.43	0.18 ± 0.44
	5	4	1.3 ± 1.2	2.3 ± 1.5	2.4 ± 1.3	0.15 ± 0.64	0.55 ± 0.64	0.53 ± 0.66	0.55 ± 0.64
	6	4	-	2.2 ± 0.9	2.2 ± 0.9	0.30 ± 0.49	0.20 ± 0.45	0.30 ± 0.46	0.25 ± 0.47
	7	3	-	2.2 ± 0.8	2.1 ± 0.8	0.03 ± 0.59	0.53 ± 0.65	0.47 ± 0.66	0.60 ± 0.63
	8	4	1.2 ± 1.3	2.6 ± 1.2	2.5 ± 0.9	0.15 ± 0.42	0.20 ± 0.45	0.33 ± 0.47	0.15 ± 0.42
	9	4	1.7 ± 2.3	2.8 ± 1.7	2.6 ± 0.8	0.20 ± 0.65	0.38 ± 0.66	0.77 ± 0.63	0.70 ± 0.58

Appendix 7: Crop light interception

Figure A6.1: Fraction of light intercepted (F_{LI}) in maize (*Zea mays*) cultivated in various cropping systems with two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**1**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**). Light incidence was measured at ground level within row, between row and above intercropped beans (**5**,**6**,**8**,**9**) at 30-50 cm height using an 86.5 cm probe at four sites in two replicate plots per cropping system. SD = Standard deviation. DAS = Days after sowing.

		Mean fraction of light intercepted															
		Within row								Between rows							
DAS		43		61		79		110		43		61		79		110	
		F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD
Cropping system	**1**	0.46	0.09	0.57	0.06	0.66	0.06	0.57	0.06	0.28	0.06	0.48	0.10	0.58	0.08	0.38	0.09
	4	0.43	0.07	0.47	0.15	0.55	0.09	0.48	0.11	0.19	0.07	0.31	0.10	0.41	0.13	0.32	0.12
	5	0.49	0.13	0.62	0.13	0.67	0.04	0.55	0.05	0.26	0.14	0.41	0.17	0.59	0.06	0.39	0.07
	6	0.34	0.09	0.50	0.03	0.60	0.06	0.52	0.03	0.06	0.19	0.33	0.07	0.49	0.15	0.33	0.07
	7	0.50	0.13	0.62	0.08	0.70	0.05	0.58	0.08	0.38	0.12	0.54	0.11	0.62	0.03	0.41	0.08
	8	0.45	0.06	0.50	0.09	0.56	0.11	0.48	0.09	0.26	0.08	0.33	0.12	0.41	0.11	0.26	0.11
	9	0.39	0.10	0.52	0.09	0.62	0.10	0.44	0.06	0.11	0.07	0.16	0.06	0.33	0.11	0.23	0.05

Figure A6.2: Fraction of light intercepted (F_{LI}) in two common bean (*Phaseolus vulgaris*) cultivars, a local Mkanamna (**5**,**7**,**9**) and an improved Lyamungu 90 (**4**,**6**,**8**) cultivated in various cropping systems with maize (*Zea mays*) during the long rain season (March to June) 2017 at Kimashuku field trial, Northern Highland of Tanzania. Systems comprise of continuous monoculture (**2**,**3**), continuous intercropping (**4**,**5**) rotation (**6**,**7**), and rotation with intercropping (**8**,**9**)**.** Light incidence was measured at ground level within row and between row plus above intercropped beans (**5**,**6**,**8**,**9**) at 30-50 cm height, parallel to rows using an 86.5 cm probe at four sites in two replicate plots per cropping system. **A:** F_{LI} estimated using the above canopy photosynthetically active radiation (PAR) measured at 3 meter. **B:** F_{LI} estimated after subtraction of light intercepted by maize crop by using above bean crop PAR measurement in intercropping plots. SD = Standard deviation. DAS = Days after sowing.

A		Mean fraction of total above light intercepted											
		Within row						Between rows					
DAS		43		61		79		43		61		79	
		F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD
Cropping system	2	0.74	0.10	0.60	0.15	0.44	0.10	0.40	0.15	0.30	0.08	0.28	0.08
	3	0.90	0.07	0.82	0.11	0.52	0.15	0.59	0.29	0.72	0.18	0.40	0.20
	4	0.71	0.14	0.64	0.10	0.66	0.05	0.35	0.12	0.42	0.15	0.54	0.11
	5	0.79	0.04	0.76	0.12	0.74	0.02	0.53	0.10	0.62	0.10	0.68	0.02
	6	0.75	0.18	0.59	0.11	0.61	0.09	0.47	0.16	0.30	0.07	0.29	0.12
	7	-	-	0.85	0.08	0.65	0.11	-	-	0.73	0.17	0.55	0.17
	8	0.70	0.07	0.66	0.12	0.63	0.14	0.43	0.15	0.45	0.17	0.50	0.14
	9	0.77	0.09	0.81	0.06	0.69	0.07	0.32	0.13	0.44	0.10	0.53	0.10

B		Mean fraction of available light intercepted											
		Within row						Between rows					
DAS		43		61		79		43		61		79	
		F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD	F_{LI}	SD
Cropping system	4	0.65	0.19	0.54	0.10	0.53	0.05	0.22	0.14	0.26	0.14	0.36	0.14
	5	0.73	0.07	0.68	0.14	0.58	0.04	0.41	0.15	0.47	0.11	0.50	0.04
	8	0.63	0.09	0.55	0.14	0.48	0.16	0.32	0.17	0.29	0.19	0.30	0.17
	9	0.74	0.10	0.78	0.07	0.59	0.12	0.14	0.17	0.22	0.13	0.25	0.16