Assessing the rotational effects of maize (*Zea mays* L.) and soybean (*Glycine max (L.) Merr.*) through two field experiments for smallholder farms in Ethiopia

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Cover photo
Soybean-maize rotational cropping from the EIAR on-station field-trial plots in Bako, Oromia, Ethiopia.

Contents

List of tables

List of figures

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Abstract

Increasing land pressure and soil degradation, together with the limited availability of fertilizers, labour, equipment and technology, keeps production efficiency low for smallholder farmers. Maize (*Zea mays* L.) is the most farmed crop in Ethiopia, because it is seen as ideal food and cash crop, and is therefore cultivated throughout the country, in various agro-ecologies and socio-economic circumstances. Potentially, maize yield can be increased through the integration of legumes in the cropping system. However, the actual contribution of legumes to maize yield is highly situation specific. Indicating the importance of research done by the research-in-development group N2Africa, of which this study was part. N2Africa "aims to put nitrogen fixation to work for smallholder farmers in Africa", through improved integration of legumes into the smallholder farming systems. In Ethiopia N2Africa was focussed on a range of legumes, including soybean (*Glycine max (L.) Merr*). Possible management practices were tested on research stations throughout the country. For this study the field experiment of two of these research stations, Pawe and Bako, were selected, to test a number of management aspects. For maize, the effect of fertilization and previous soybean growth was tested. For soybean cultivation, fertilizer addition, rhizobium addition, and a rotational cropping sequence with maize cultivation was tested. All in all, this set up resulted in nine management treatments, for which yield data was collected by the research stations for three years (three cropping seasons, 2016 – 17 & 18). In addition to this, soil samples and sampling for the 15N abundance method was performed in 2018. This data was able to give an impression of the soil fertility and of the amount and percentage N fixed by soybean.

The aim of this study is to understand the effects of tested management aspects; rotational crop sequence and fertilization, on maize and soybean. For soybean the effect of re-inoculation is also tested. These three management aspects are assessed on four variables; grain yield, soil nutrient levels, nitrogen fixation and the possible farmer's profit. In order to develop an advice on sustainable intensification of maize and soybean cultivation for smallholder farmers in Ethiopia, these four variables are important. The results indicate variations per location and per year, making generalized advice more difficult. However, when combining results from the two locations and all four variables continuous maize with fertilizer inputs was deemed best. Two rotational treatments were seen as second and third best. A current common farmer practice was second best for both locations. For Pawe a fully fertilized rotation was third best, while for Bako this was a fully unfertilized rotation. Although, the management choice for both locations depends on the variable of interest.

1 Introduction

1.1 Smallholder farming in Ethiopia

Most farming systems in Ethiopia are mixed smallholder farms (often less than 2 ha (Vanlauwe et al., 2014)), accounting up to 96% of the total cultivated land (Cochrane & Bekele, 2018). Since land division and population growth have increased the pressure on the land, fallows have become scarce (Drechsel et al., 2001) and land degradation has increased (Holden & Shiferaw, 2004). Additional stress factors for smallholder farmers are the limited availability of fertilizers, labour, equipment and technology. In combination, this keeps production systems inefficient and yields low, (Giller et al., 2009). Additionally, 95% of farmers are farming in a rainfed system, increasing yield variability through climate dependence (Abate et al., 2015). With climate change rainfall is expected to become less reliable for Sub-Sahara Africa (SSA) in general (Bryan et al., 2009). This will increase the likelihood of poor yields and the vulnerability of the farmers.

Maize is the most farmed crop in Ethiopia, when looking at area coverage (16%) and production (26%)(Abdulkadir et al., 2017). Maize is seen as an ideal food and cash crop (Abera et al., 2013) and thus is cultivated throughout the country, in varied agro-ecologies and socio-economic circumstances (Abate et al., 2015). Even though maize is "one of the most productive crops in Ethiopia" (Abdulkadir et al., 2017), there is a gap in productivity between the yield from smallholder farmers (average yield 3.2 t ha^{-1}) and demonstration trials (average yield 5 – 6 t ha^{-1}). This lack in productivity limits the role maize can play in ensuring food security (Abera et al., 2013; Abdukadir et al., 2017). The two main constraints found are the limited use and availability of fertilizers and new varieties (Cheruiyot et al., 2001; Abera et al., 2013).

A current farmer practice to cope with the declining soil fertility, is to use a rotational cropping system. It differs per region which crops are used, although most often cereals (maize, teff, wheat or barley) are intercropped with legumes, such as faba bean, common bean, chickpea and soybean among others (Ronner & Giller, 2014; Atnaf et al., 2015). Legumes are important in the Ethiopian farming system, providing both food and feed, for farmers and livestock. The protein provided in grain legumes is an important addition of protein to local diets (Kamanga et al., 2010). In Ethiopia, legumes on average have become a higher value cash crop than cereals (Atnaf et al., 2015), and can increase food security (Holden & Shiferaw, 2004). Atnaf et al. (2015) indicates that there are yearly fluctuations in the productivity of different legumes. Overall soybean, chickpea, grass beans and faba bean have increased in productivity, and were the four highest legumes in productivity in 2012 (Atnaf et al., 2015). This being said, the most recognised trait of legumes is their ability to fix nitrogen, through a symbiosis with rhizobia in the root nodules. The actual amount of N that is fixed by the legume is difficult to assess, due to heterogeneity in farm management and soil properties (Zingore et al., 2008). These positive influences on soil fertility make legumes attractive for rotational cropping.

1.2 Rotational legume cropping

A current farmer practice is to plant legumes on the less fertile fields while maize (and other cash crops) are kept closer to the homestead on more fertile fields. This pattern is often also seen in a rotation, through maize being fertilized and the legumes unfertilized. As a result both N_2 fixation and legume yield is not maximized (Zingore et al., 2008). Other determining factors for legume growth and N_2 fixation are rainfall, rhizobial presence (depending on the amount and the species) (Rurangwa et al., 2018) and the variety that is used. Independent of the amount of N fixed, plant biomass left on (or in) the soil to decompose will become available in the soil (Lupwayi et al., 2011). Additionally, legumes also contribute to crop fertility with non-N effects, such as amended nutrient uptake (through mycorrhizae, Lupwayi et al. 2011)) and the breaking of pest and disease cycles (through the enhancement of microbial activity and diversity). Another positive non-N trait of legumes is the improvement of soil aggregates and thus soil structure (Lupwayi et al., 2011).

A review of research done on rotational effects in legume-cereal based systems in sub-Saharan Africa, showed that maize yield had an overall mean increase of 41% in comparison to continuous cereal cropping (Franke et al., 2018). However yield is not always increased by intercropping or rotational cropping (Tittonell & Giller, 2013). The large heterogeneity of SSA smallholder farmers makes accurate predictions on crop yield fluctuation difficult, making it key to analyse specific scenarios to identify sustainable improvements.

1.3 Soybean

Soybean has become increasingly popular and productive in Ethiopia in recent years, with especially a production potential in the South and West of Ethiopia (Bekabil, 2015). As a dietary addition, it rich in protein (40%), unsaturated fat (20%) and carbohydrates (29%), with valuable amino acids (Abebe et al., 2019). Soybean has not always been grown in Ethiopia and can be considered an exotic crop (Bekabil, 2015), therefore rhizobia addition is needed to stimulate nodulation (Giller, 2001).

Soybean maize rotations or intercrops can have a positive effect if integrated with soil and crop management. Research in Malawi found that on average the intercrop with soybean increases yield with 39% relative to continuous maize cropping. The proportion of N derived from the atmosphere (%Ndfa) depended on the variety of soybean. Local varieties fixed up to 65% of N (van Vugt et al., 2018). Ethiopian soils are often low in nutrients and soil organic matter (Getachew et al., 2017). Especially nitrogen and phosphorus are regarded as the most limiting factors (Debelle et al., 2002). Therefore, fertilizer additions may not only be necessary for maize growth but may also be necessary to stimulate soybean growth and N_2 fixation. A review of multiple SSA research indicates a strong advantage for a rotation with soybean, stating that the strongest residual effect of a legume on cereal was found with soybean and with groundnut (Franke et al., 2018). Research in Ethiopia also supports this, showing an increase in maize grain yield when intercropped with soybean (Kebebew et al., 2014; Abera et al., 2015), or grown in a rotation with other legumes or crops, e.g. haricot bean – niger seed – maize or finger millet – soybean - maize (Zerihun et al., 2013, Abebe et al., 2019).

1.4 N2Africa

This study is part of N2Africa, a large scale research based project, aiming to increase the use and yield of grain legumes in order to improve the food self-sufficiency and the diet of the smallholder farmers and the local community in SSA. This is done through linking theoretical knowledge with practical needs; selecting legume genotypes with the needed capacities, linking these with the 'best matching' rhizobia and finally tweaking the management until optimal for the region and farmer. In this way N2Africa has a best-fit approach. N2Africa is active in 11 African countries, working on adapting management systems, genotypes and stimulating rhizobia production and use.

Legumes have a great potential in Ethiopia, especially with the development of new soybean varieties. N2Africa has had a widespread influence on a multitude of levels; from farmers to private- and development organizations. Furthermore, events are organized focussing on farmer's education, for example through demonstration and adaptation trials, trainings and field days. Additionally, N2Africa works on a more national scale; in collaboration with the Ethiopian Institute of Agricultural Research (EIAR) and with Menagesha Biotech Industries (MBI). Through doing so N2Africa has clearly focussed on tackling problems at hand, such as the availability and access to improved seeds and rhizobia.

Among the many activities of N2Africa in Ethiopia research trials were conducted with a soybean-maize rotation on multiple research station (of the EIAR) for the duration of five years. This study is performed after three years of these research trials, and focusses on two (out of eight) of the research centres, Pawe and Bako. The two field experiments have the same set-up, and test certain management aspects for maize and soybean; crop sequence (continuous or rotational) and fertilization. For soybean, the effect of re-inoculation is also tested. These three management aspects are tested individually and in combination. For example, maize is cultivated with or without fertilization in both continuous and rotational cropping.

1.5 Research objective

The main objective of this study is to understand the effects of tested management aspects, rotational crop sequence and fertilization, on maize and soybean. For soybean, the effect of inoculation is also tested. These three management aspects are assessed on four variables; grain yield, soil nutrient levels, N_2 fixation and a cost-benefit analysis. In order to develop an advice on sustainable intensification of maize and soybean cultivation for smallholder farmers in Ethiopia, these four variables are important. With grain yield, soil nutrient levels and N_2 fixation shedding light on the agronomic affects. While the cost-benefit analysis gives an indication of cost, benefits, profits and the benefit cost ratio (BCR) per management treatment. The following four research questions are addressed in this study, each focussing on one of the four variables.

RQ1) How do crop sequence, fertilizer and rhizobia inputs affect the grain yield of maize and soybean?

The first hypothesis is that maize grain yield is expected to be higher when in a rotation with soybean, especially when both crops are fertilized. For soybean it is expected that yield is highest with continuous fertilizer and inoculant inputs, either in a continuous cropping sequence or in a rotational cropping sequence. Added rhizobia will improve the establishment of root nodules and thus aid soybean in higher growth and yield.

RQ2) What is the effect of crop sequence and fertilizer input on the soil nutrient content?

The rotation with continuous addition of fertilizer inputs is expected to increase the soil nutrients the most.

RQ3) What is the effect of crop sequence, fertilizer and rhizobia input on %N and amount N fixed by soybean?

The hypothesis is that %N fixed will be highest in continuous cropping with full fertilization and inoculation, with inoculation stimulating the %N fixed. The %N fixed is not expected to be limited by continuous inputs since soils are anticipated to be poor in soil fertility. The total amount of N-fixed is dependent on the amount of biomass. This is predicted to be highest in continuous cropping with full fertilization and inoculation.

RQ4) Overall, which cropping sequence and fertilizer treatment would be most profitable for farmers to implement?

In total question 1,2, and 3 build up to an agriculturally based advice for farmers as to which treatment to implement to gain the highest grain yield under sustainable conditions.
The most profitable treatment will most likely be a rotational cropping sequence with full fertilization. Fertilization will raise yields and profits, since soils are expected to be poor in soil fertility. The BCR is highest with low inputs and high outputs. Maize is higher yielding than soybean, so continuous unfertilized maize is expected to be highest in BCR.

2 Materials and Methods

The two, on-station long-term, field trials were started in 2016 in Pawe (Benishangul-Gumuz region) and Bako (Oromia region). The trials are planned to run for five years and this study was conducted after three years of the trials. The trials aim to assess the effect of rotational cropping and fertilization for maize and soybean, and the effect of re-inoculation for soybean. In order to do so yield data was collected by the research stations from 2016 onwards. Additionally, soil samples and N_2 fixation samples (from soybean and reference weeds) were taken in 2018.

2.1 Field experiments

Nine different treatments were made to test the three management aspects, either individually or in combination (Table 1). The fertilizer rate used for maize was 150 kg ha^{-1} of urea (supplying 69 kg ha^{-1} of N) and 100 kg ha^{-1} of NPS (supplying 19 kg ha^{-1} of N, 38 kg ha^{-1} P and 7 kg ha^{-1} S). Soybean received 50 kg ha^{-1} of NPS (supplying 8 kg ha^{-1} of N, 19 kg ha^{-1} P and 3.5 kg ha^{-1} S) and rhizobia inoculants (MAR-1495 from MBI). Continuous maize (CM) and soybean (CS) treatments with (+) and without (-) fertilization were the reference treatments to compare the rotational (R) treatments with. Rotational treatments either fully fertilized (RS+ M+), partially fertilized (RS-M+ or RS+M-) or without fertilization (RS-M-) were testing different management options for the soybean-maize rotation (N2Africa, 2016). All soybean treatments with fertilization receive both fertilizer and inoculants, unless further specified; CS+ 1xInoc and CS- 1xInoc. These two treatments both only receive inoculants in the first year of the trial, 2016. To test the effect of re-inoculation CS+ was compared to CS+ 1xInoc. CS- 1xInoc was not used in the inoculation comparison[1], and is referred to as CS- throughout this study (except for in Table 1).

Table 1 The nine treatments of the field trials indicated per year (M = maize; S= soybean; Inoc = rhizobia inoculation; + = with fertilization, - = without fertilization) (N2Africa, 2016).

#	Treatment	*2016*	*2017*	*2018*	*2019*	*2020*
T1	Continuous maize with fertilizer (CM+)	M +Urea +NPS	M +Urea +NPS	M +Urea +NPS	M +Urea +NPS	M +Urea +NPS
T2	Continuous maize without fertilizer (CM-)	M	M	M	M	M
T3	Continuous soybean with fertilizer (CS+)	S +NPS +Inoc	S +NPS +Inoc	S +NPS +Inoc	S +NPS +Inoc	S +NPS +Inoc
T4	Continuous soybean with fertilizer (but inoculation on 1st year only (CS+ 1xInoc)	S +NPS +Inoc	S +NPS	S +NPS	S +NPS	S +NPS
T5	Continuous soybean without fertilizer (but inoculation on 1st year only) (CS- 1xInoc)	S +Inoc	S	S	S	S
T6	Rotation with fertilization (RS+M+)	S +NPS +Inoc	M +Urea +NPS	S +NPS +Inoc	M +Urea +NPS	S +NPS +Inoc
T7	Rotation without maize fertilization (RS+M-)	S +NPS +Inoc	M	S +NPS +Inoc	M	S +NPS +Inoc
T8	Rotation without soybean fertilization (RS-M+)	S	M +Urea +NPS	S	M +Urea +NPS	S
T9	Rotation without fertilization (RS-M-)	S	M	S	M	S

[1] In order to be able to use CS- 1xInoc to test the effect of inoculation, a tenth treatment with continuous inoculation but no fertilization would be necessary.

Fields that had not undergone fertilization or inoculation in previous experiments for at least five years were chosen for the set-up for this field trial, in order to evade possible residual effects. The management was adjusted to prevent cross contamination, especially for the plots with inoculation. The spacing of the crops; seed rate, row spacing, plant spacing and number of seeds per hole, was prepared as indicated in Table 2. Table 2 also indicates the intended population of number of plants ha^{-1}.

Table 2 Seed rate, row spacing, plant spacing, number of seeds per hole and intended plant population (N2Africa, 2016).

Legumes	*Seed rate (kg/ha)*	*Row spacing (cm)*	*Plant spacing (cm)*	*# of seeds/hole*	*Intended population (#plants ha^{-1})*
Soybean	80	60	5	1	333,333
Maize	25	75	30	1	44,444

2.2 Study area

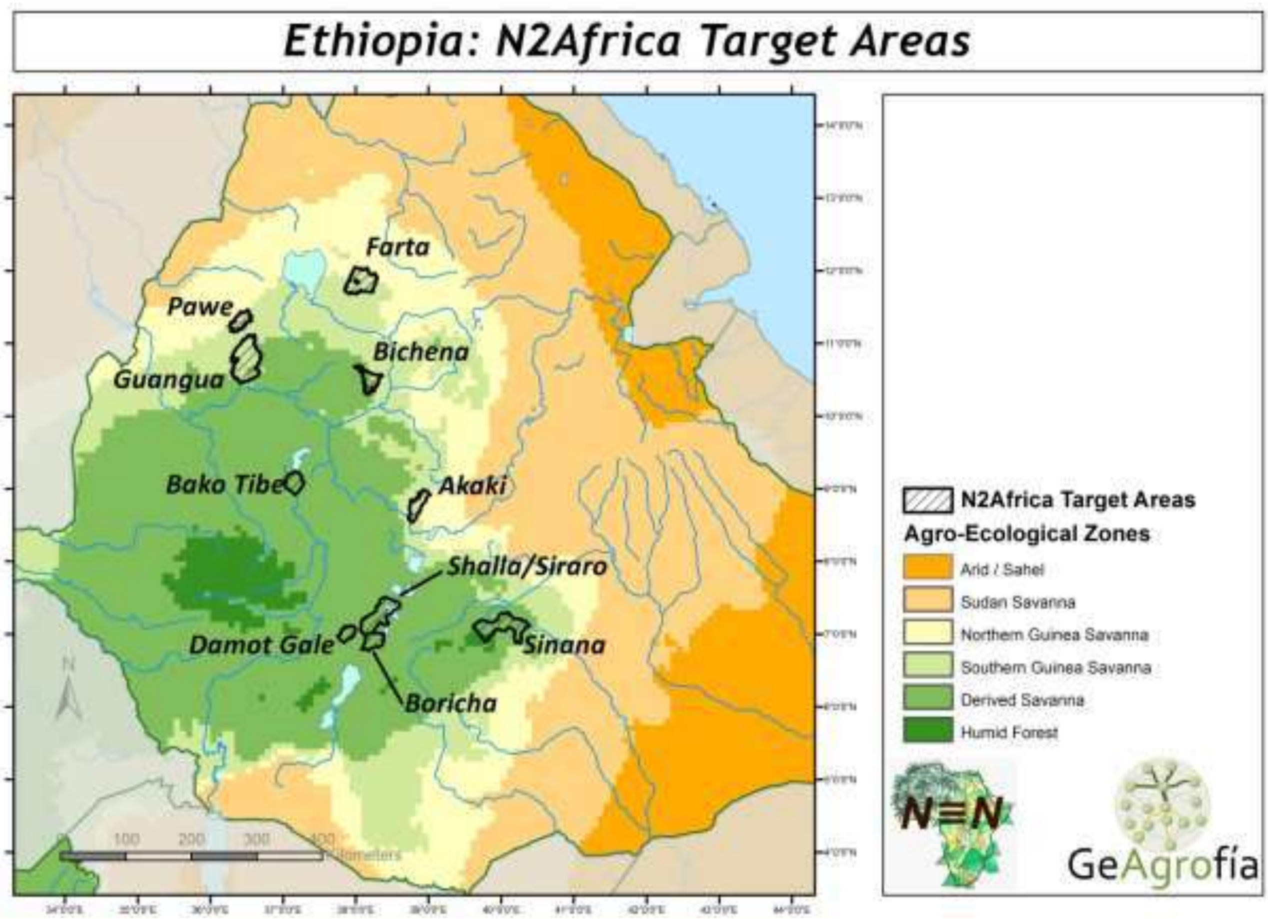


Figure 1 The study area of Pawe and Bako (Tibe) indicated, among the other N2Africa target areas for Ethiopia. The Agro-Ecological Zones are indicated in the legend (Ronner & Giller, 2014).

Pawe is located in the Benishangul-Gumuz region (Ethiopia & Eritrea RCPVs, 2018). The agro-ecological zone (AEZ) is classified as mid-altitude sub-humid (Mosisa et al., 2011) and hot to warm, with annual minimum and maximum temperatures of 16.3°C and 32.6° C. Pawe has two rainy seasons with an average annual rainfall of 1321 mm (Climate Data, 2018). The research centre itself is located at an elevation of 1120 m at the latitudes and longitudes of 11°19’N, 36°24’E (Figure 1). Figure 2 is a soil map of Ethiopia, for Pawe it predominantly indicates vertilsols, nitisols and leptosols (Haile & Moog, 2016).

Bako (or Bako Tibe) is part of the Oromia region. This region is characterized by flat plains and mountains which are crossed by a number of rivers both in the wet and dry season. The AEZ is classified as mid-altitude with two rainy season and an average annual rainfall of 1550 mm. It has hot humid weather, with a minimum temperature of 13.2°C and maximum of 28°C (IQQO, 2018). The research centre is located at an elevation of 1650 m at the latitude and longitude of 9°6'N, 37°9'E (Figure 1) (N2Africa, 2016). The soil type in Bako is dominated by nitosols. As indicated in Figure 2, vertisols, luvisols and andosols are also present (Haile & Moog, 2016).

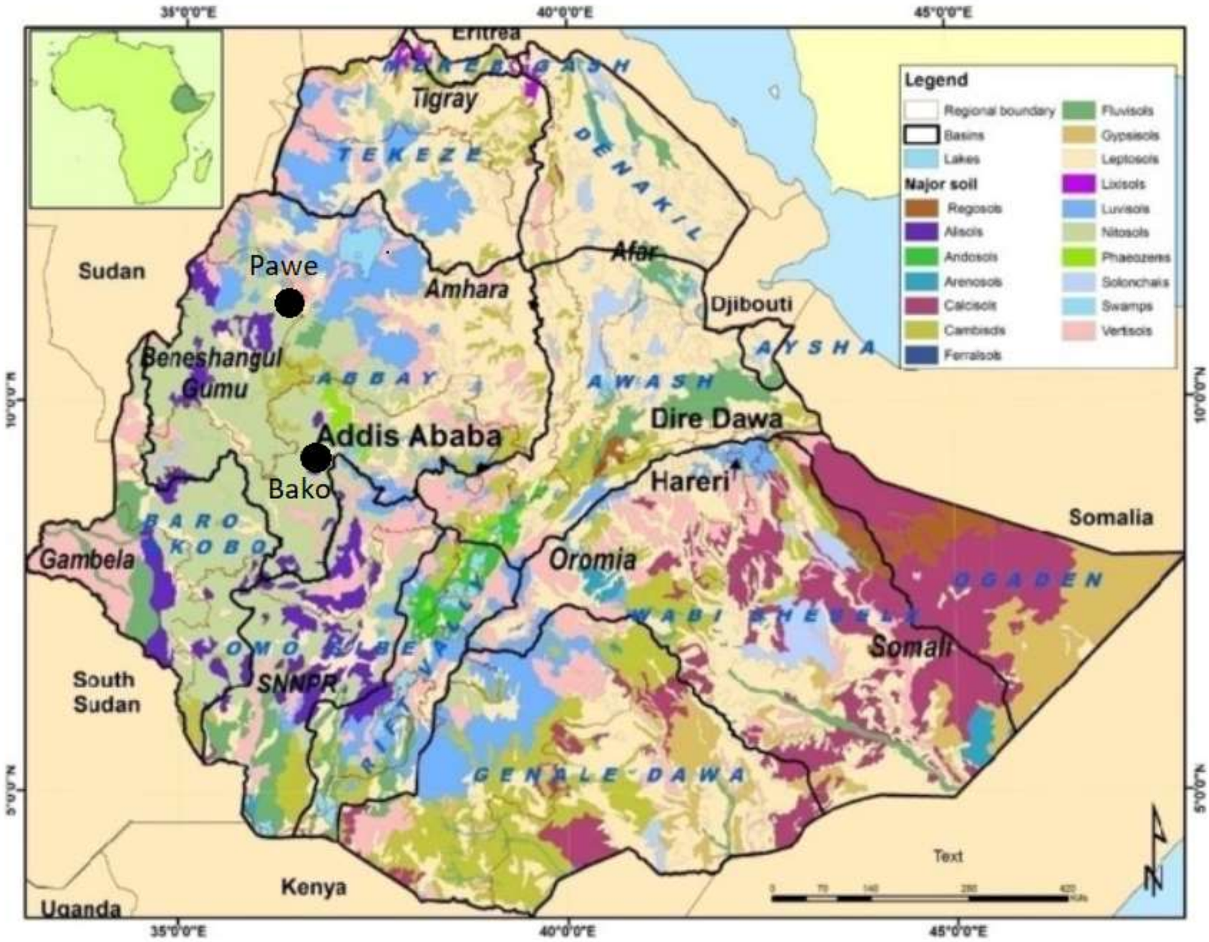


Figure 2 Soil map of Ethiopia with major soil types indicated. The black lines are indications for basins, regional boundaries are white (Haile & Moog, 2016).

2.3 Field set up

Both locations had the same set-up for the field trial. However, since each research station was in charge of their own field trial, small differences between the locations are present over the years.

2.3.1 Pawe

The field experiment followed a randomized complete block design (RCBD) with four replicates of each plot (36 plots in total). The field chosen for this experiment was on site and appeared to be level. The maize variety cultivated (in all three years) was BH-540. This is an intermediate maturing hybrid maize variety with high yield, and a moderate adaption for drought and disease stress (Eyasu et al., 2018). In 2016 and 2017 the soybean variety used was Belessa-95, while in 2018 the variety was TGx-13-3-

2644. The choice of soybean variety was made per year. Each year the highest yielding variety for the region was selected by the research station. Other aspects that were deemed important from TGx-13-3-2644 were large seed size and a high market demand. The rhizobium strain used was always MAR-1495.

Inputs were added to the different treatments at sowing. Further management of the plots within this experiment were ploughing and ridging. The plots were ploughed manually twice a year; at planting and after harvest. Especially the plots treated with inoculum were prepared carefully as not to inoculate any other plots (N2Africa, 2016). In Pawe this was done through keeping a ridge at the edge of every plot, as to stop the soil from spreading to other plots. Especially in the rainy season this is important and difficult. The exact field set-up is given in Figure 3 with plot (P) and treatment indications, as well as the measurements of the plots and the overall field. Full treatment descriptions are given in Table 2.

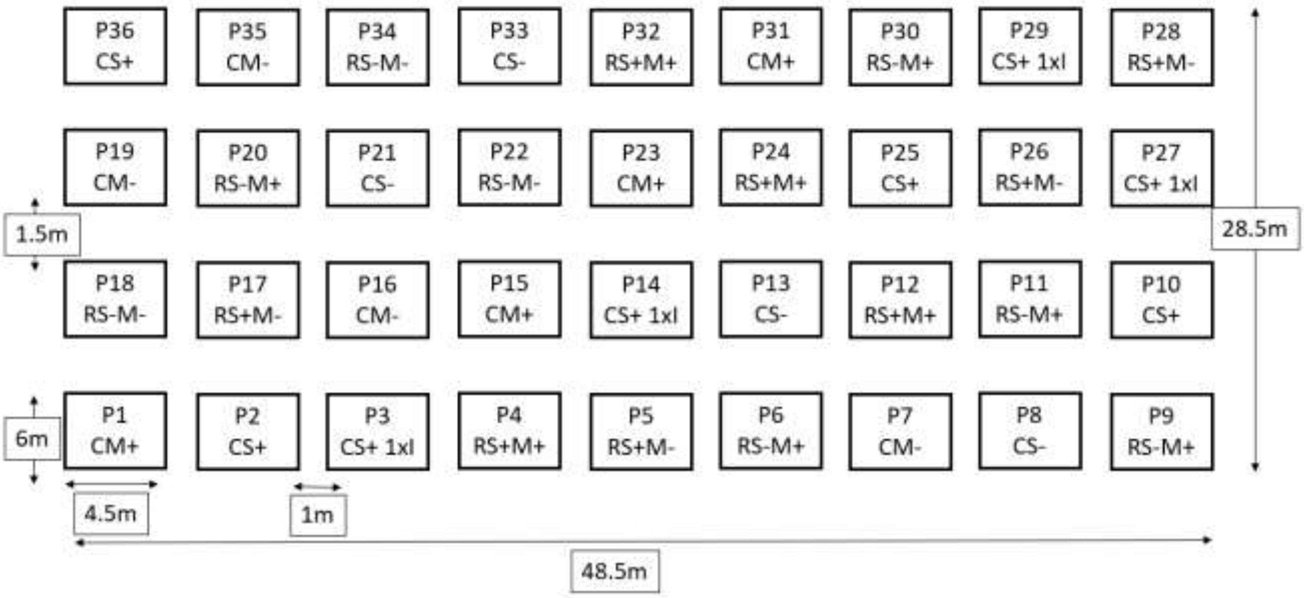


Figure 3 Field trial set-up for Pawe on-station trial, with plot (P) and treatment indications (C = continuous, R = rotational, M = maize, S = soybean, + = with fertilization, - = without fertilization, 1x I = inoculation only in 2016).

2.3.2 Bako

In all years (2016, 2017 and 2018) Belessa-95 was used as the soybean variety. Belessa-95 was selected since it is high yielding, and had a high market demand in Bako. The rhizobium strain used was always MAR-1495. Before establishment of the experiment in 2016 the field was ploughed by a tractor, disked and harrowed. During the experiment the plots are hand ploughed twice a year at sowing and harvest. The inputs of the treatments are added at sowing and ridges are kept to prevent contamination. Maize variety used: BH-660. BH-660 is a late maturing hybrid more suitable for a high rain fall region (Aman et al., 2016). The variety was selected on the basis of high yield and climate suitability.

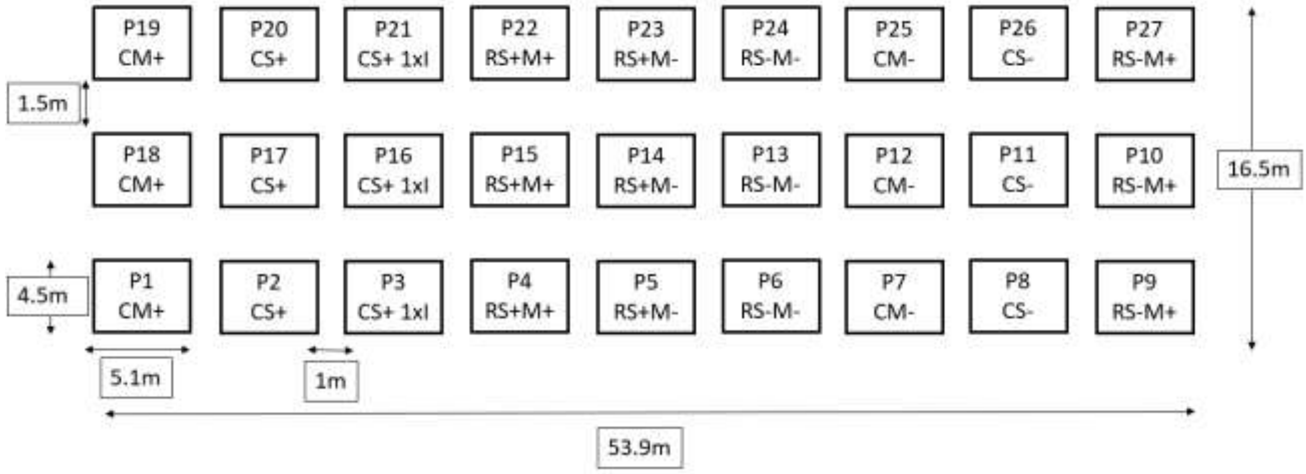


Figure 4 Field trial set-up for Bako on-station trial, with plot (P) and treatment indications (C = continuous, R = rotational, M = maize, S = soybean, + = with fertilization, - = without fertilization, 1x I = inoculation only in 2016).

The field experiment in Bako has three replicates side by side, following the very slight slope of the field. In figure 4 the exact field set-up is given (of the 27 plots in total), with plot (P) and treatment indicated, as well as the size of the plots and of the field. Full treatment descriptions are given in Table 2.

2.4. Crop measurements

The yearly crop measurements taken, were: stand count per plot, plant height, number of pods per plant, fresh- and dry biomass, seed moisture percentage, 100 seed weight, grain-, husk- and haulm yield. The following plant measurements were measured from five randomly selected plants from the middle rows; plant height, number of pods per plant and biomass sampling. For these measurements, sampling excluding the borders to eliminate border effect. At Pawe these plants were removed from a diagonal destructive row, while in Bako they were randomly sampled from the plot. The other measurements were assessed per plot.

These measurements were taken at different growth stages of the crops throughout the season. The stand count was taken at harvest and estimated per plot through counting each axis of the plot. Plant height was measured at harvest maturity from ground level to the tip of the plant. Fresh biomass was measured at mid-flowering. The dry biomass was weighed after oven drying at 120 ^{o}C for 48 hours. For the seed moisture percentage the difference in seed weight was measured before and after the seeds are sun-dried for a week and winnowed. The measured seed moisture percentage was used for calculating dry seed yield per hectare from harvested plot area. The standard moisture content used for soybean is 10% and 12.5% for maize. 100 seed weight was measured after drying, for maize seed, 500 seed weight was used to obtain a better average. Husk- and haulm yield (soybean only) were also measured after harvest. Threshing was done by hand.

At neither of the research stations pests or diseases were persistent enough to need scoring.

2.5 N_2 fixation

For the N_2 fixation measurements the 15N abundance method was used. The 15N abundance method has the advantage that it can be used anywhere since it needs no special interventions in terms of addition of isotopes. This also made it the most suited for this research. The basis for the 15N abundance method is that soils are slightly enriched with ^{15}N relative to the atmosphere. ^{14}N is lighter than the ^{15}N isotope, causing ^{14}N to react faster, especially in gaseous losses from the soil (Giller, 2001). The reference plant is necessary to compare the amount of ^{15}N, due to this the method is very dependent on the reference plant, which should be selected with care (Peoples et al., 2002). Broad-leafed weed species function well as reference plants, their rooting systems are often similar to that of the legumes. It is necessary that the weeds have the same treatment as soybean (Unkovich et al., 2008), and preferred that the reference plants have the same growth period. The magnitude of this effect is dependent on uniformity of the soil profile (and thus connected to rooting depth) and on the uniformity of the $\delta^{15}N$ values of the reference plant over time. If both are reasonably stable the effect of the growing period of the reference plant is likely to be small (Giller, 2001).

The samples of soybean and reference weeds were sampled from each plot. Soybean plants were not sampled from the border rows of the plot, to avoid border effects. Weeds were sampled from all over the plot, depending on the abundance, species and size of the weeds. Larger weeds were selected to try and obtain older plants which should be closer to the growth period of soybean. Care was also taken to sample weeds of multiple species (indicated in Appendix I), creating a mixed and bulked sample, in order to limit a species effect (Goh, 2007). These samples were collected at mid-podding in 2018, only.

Fresh weight of the samples was taken, and after oven drying for 72 hours at 70 °C the dry weight was measured. After which the samples were ground to 1 mm and ground again with a ball mill. The analysis of %C, %N, $\delta^{15}N$ and $\delta^{13}C$ was performed by KU Leuven (University of Leuven, Belgium). To calculate the %Ndfa the Formula 1 was used (Unkovich et al., 2008).

$$\%Ndfa = \frac{(\delta 15\text{Nreference plant} - \delta 15\text{Nfixing plant})}{(\delta 15\text{Nreference plant} - B)} \qquad \textit{(Formula 1)}$$

With 'B' being the $\delta^{15}N$ of a legume when grown with atmospheric N_2 as the sole source of N. The B value depends on the variety and on the inoculant used. For this study it was deemed most accurate to choose the lowest value from the dataset itself. For this calculation the lowest value from Pawe and Bako have been used respectively.

2.6 Soil measurements

After harvest in 2018 soil samples were taken. Per plot a composite sample was made from five randomly sampled points with a cylindrical auger at a depth of 0-20, using the zigzag method. These samples were air dried for a week, ground (with mortar and pestle) and sieved with a 2mm steel sieve. After which the sample size was reduced to 100 g through spreading the full sample on a sheet, to avoid selection of particle size. From this a representative subsample was taken.

Soil samples for two treatments (with three replicates from each location) were sent for chemical and physical analysis to Yara Analytical Services (Pocklington, UK). Samples analysed were CM+ and CM-, these were expected to be most different in soil values. The analysis included; total N (Kjeldahl), P (Olsen method, Na (sodium or calcium sulphate), CEC (leached with 1 M ammonium acetate followed by 10% potassium chloride) pH (water), amount of organic matter (Dumas Combustion), K, Ca and Mg (1M Ammonium nitrate). The physical analysis included the soil texture classification of %Sand, %Silt, %Clay (laser diffraction). Soil parameters were given in PPM (mg kg^{-1}), when necessary these values were converted to mmol kg^{-1} using Formula 2.

$$PPM \rightarrow \frac{mmol}{kg} = \frac{\left(\frac{PPM}{1000}\right)}{Molar\ mass} * 1000$$ *(Formula 2)*

2.6.1 N and P balances

Next to measuring soil fertility, another way of analysing the effect of the management treatments on soil nutrients is through an input – output balance. For N and P a restricted balance was made, based on data available. The main restriction was that only above ground values for biomass yield, grain yield and fertilizer were taken into account, thus excluding any influence from root biomass (%root N and %root P). The biomass yield (kg ha^{-1}) and grain yield (kg ha^{-1}) were converted into kg N ha^{-1}, and kg P ha^{-1} with the N and P contents (%) given in Table 3 (Nijhof, 1987). The first assumption is that grain yield (kg N ha^{-1}, and kg P ha^{-1}) was the only offtake from the plots.

Table 3 Average %N content and %P content (Nijhof, 1987).

	N content (%)	*P content (%)*
Maize grain	1.55	0.48
Maize biomass	0.9	0.22
Soybean grain	6.1	0.68
Soybean biomass	1.05	0.3

Then, for both N and for P two separate calculations were made, including and excluding crop residues as input in the next year. Crop residues were not removed from the plot and thus not true additions. Thus, in the calculations 'without crop residues' the biomass (in kg N ha^{-1}, and kg P ha^{-1} from the previous year) is not taken into account. The assumption was, that the only input was fertilizer in kg ha^{-1} (for N and P). A second calculation was made to illustrate the benefits of actively incorporating the crop residues remaining in the field in the next season, 'with crop residues'. Since, it is often the case that crop residues are taken off or eaten by livestock after harvest (McGuire, 2007). When crop residues were incorporated, the biomass yield (kg N ha^{-1}, and kg P ha^{-1}) of the previous year[2] together with fertilizer inputs (kg ha^{-1} for N and P) constitute the assumed plot inputs. Another assumed plot input for the N balances, is the amount of N fixed by the soybean. Data for the amount of N fixed by soybean was collected in 2018. With the assumption that the variation in amount of N fixed between the years is of minimal influence, the data from 2018 was used as input for each year, per corresponding treatment.

$$Balance\ 2016 = Input\ 2016 - Output\ 2016$$ *(Formula 3)*

$$Balance\ 2017 = Balance\ 2016 + (Input\ 2017 - Output\ 2017)$$ *(Formula 4)*

$$Balance\ 2018 = Balance\ 2017 + (Input\ 2018 - Output\ 2018)$$ *(Formula 5)*

The balances were made in such a manner that the balance of the previous year has a knock on effect on the balance of the current year. The calculations made, are given in Formula 3, 4 and 5 (input N balances = fertilizer + amount N fixed (+ crop residues), input P balances = fertilizer (+crop residues),

[2] Biomass yield (kg N ha^{-1}, and kg P ha^{-1}) from 2016 was included in N and P input in 2017, and biomass yield (kg N ha^{-1}, and kg P ha^{-1}) from 2017 formed part of the N and P inputs in 2018.

output = grain yield). Balances were relative, or at least there were no starting values. Calculations were only done for Pawe, biomass data for 2017 from Bako was missing. Therefore, the crop residues could not be calculated for all necessary years. For the full dataset see Appendix II.

2.7 Cost-benefit analysis

The cost-benefit analysis was based on economic values from 2017[3]. Seed and produce prices fluctuate annually, seasonally and regionally. Generally best prices for produce will be obtained in (larger) cities with a seasonal high in June to August (WFP Ethiopia, 2018). Regional and temporal fluctuations in market prices can be very influential for the farmers' profit.

This analysis was done with values from 2017 from Bako, regional and temporal fluctuations within Ethiopia and the trial period were not included. The labour cost was not included in this analysis since the treatments were deemed to be fairly equal in labour. Generally, labour was expected to only have a minor effect on profitability (Ronner et al., 2016). Prices for soybean and maize seeds and sale from Bako, are given in Table 3.

Table 4 Seed and produce prices for maize and soybean in Ethiopia[3]

	Maize	*Soybean*
Seed (Birr kg^{-1})	18.8	16.0
Sale of grain (Birr kg^{-1})	6.0	7.5

As cost, the cost of seeds and of fertilizers, when added, were included (cost = seed price (+fertilizers)). The benefits were defined as the grain yield times the price for the sale of grain (Table 3)(benefit = grail yield * grain price). And profit was defined as the benefits minus the costs (profit = benefit – cost). Benefit cost ratio is calculated through dividing the benefits by the cost (BCR = benefit/cost). Cumulative costs, benefits and profits are added to indicate the overall worth of the treatments, and to be able to compare all three years (although different crops are grown and soybean is grown twice in the rotational treatments). An average BCR is also made with the cumulative cost and benefit, again as comparison for the three years. Seed costs together with the seed rate were converted to prices per hectare (25 kg ha^{-1} * 18.8 Birr kg^{-1} = 470 Birr ha^{-1} for maize and 80 kg ha^{-1} * 16.0 Birr kg^{-1} = 1280 Birr ha^{-1} for soybean). For fertilizer urea and NPS are used; urea costs 11.5 Birr kg^{-1}, and NPS costs 14 Birr kg^{-1}. 150 kg urea and 100 kg NPS is added to maize ha^{-1}, and 50 kg NPS is added to soybean ha^{-1}. Which makes the costs of maize fertilization 3,125 Birr ha^{-1}, and soybean 700 Birr ha^{-1}. All prices were converted to USD, with a conversion rate taken from December 2018. This was the time of the last harvest, taken into account in this study, therefore a logical time for the sale of maize and soybean grain. 1 Birr was worth 0.03390 USD at this time. Table 5 gives a price indication in Birr ha^{-1} and USD ha^{-1} for maize with and without fertilization and soybean with and without fertilization.

Table 5 Price indication for the purchase of maize and soybean either with or without fertilizer additions in Birr ha^{-1} and USD ha $^{-1}$.

	Birr ha^{-1}	*USD ha^{-1}*
Maize with fertilizer (M+)	3595	122
Maize without fertilizer (M-)	470	16
Soybean with fertilizer (S+)	1980	67
Soybean without fertilizer (S-)	1280	43

[3] Values were collected by N2Africa in 2017 for Bako.

2.8 Data analysis

For the statistical analysis of this study *R* software version 3.4.4 (2018) was used with RStudio version 1.2.1335 (2009-2019). To identify the factors of influence within the treatments, each treatment was split into multiple factorial variables. These factors were: rotation, maize fertilizer, soybean fertilizer, crop and inoculant. Another factor used was a combination of maize fertilizer, soybean fertilizer and crop (CropFert), coded as M+, M-, S+ and S-. In the random linear mixed models (REML) that were made additional factors were added for location and year, as well as a random factor (the plot replicates, indicating a block factor). After which an analysis of variance (ANOVA) was performed. A Tukey post hoc test analyzed the specific differences between the factorial variables (and thus the treatments), at p = 0.05.

Grain yield of both maize and soybean for all three years is given in the full dataset in Appendix III, Table 1. From this, the grain yield of soybean for all three years was analyzed, to highlight general trends per location, per crop and per year. (e.g. Grain yield ~ Rotation * Fertilization * Year + (1|Replicates)). Since only one year of maize in rotation was present this was the only year in which it made sense to analyze the effect of rotation on maize. Otherwise, treatment and year effects would be confounded. Maize was grown in a rotation in 2017, and soybean in 2018, the effect of crop sequence and fertilization was analyzed. The model used for maize was: Grain yield 2017 ~ Maize Fertilization * Rotation * Location + (1|Replicates), for soybean: Grain yield 2018 ~ Soybean Fertilization * Rotation * Location + (1| Replicates). To assess the effect of fertilizer additions and crop sequence CropFert was used for soybean, specified for 2017 and 2018 for soybean (e.g. Grain yield ~ CropFert2017 * Location + (1|Replicates), and Grain yield ~ CropFert2018 * Location + (1|Replicates)). Additionally, the effect of fertilization in previous year(s) within the rotational treatments was tested, for maize 2017 and soybean 2018. This was done through creating a subset with only the rotational treatments. For maize 2017 in this subset soybean fertilization was tested and for soybean 2018 maize fertilization was tested.

To test the effect of inoculation on soybean grain yield a comparison between two treatment was made; CS+ and CS+ 1xInoc. The effect of this difference in inoculation was tested for the grain yield of soybean in general, for 2017 and 2018 (e.g. Grain yield ~ Inoculation * Location * Year + (1|Replicates)). CS+ 1xInoc was only used for this comparison.

For the soil nutrient content, the %Ndfa and amount of N, only data from 2018 is available. Tests were performed to analyse if fertilization and crop rotation overall made a difference (e.g. %Ndfa ~ Rotation * Fertilization * Location + (1|Replicates)). For the soil nutrient content a choice was made to only analyse CM+ and CM-, since these two treatments were expected to be most different in their influence on soil nutrient content (the model used was e.g. P ~ Treatment * Location + (1|Replicates)). In the N and P balance, (with and without crop residues, dataset in Appendix II) all treatments from Pawe are taken into account. From Bako biomass data was incomplete, thus calculations were not made for Bako. Statistical analyses of N and P balances and the costs, benefits, profits and BCR's was not performed. It was deemed wrong practice to perform statistical analyses on these calculations, with a strong theoretical basis.

3 Results

3.1 Grain yield

The effect of the two locations and the three trial years are assessed. The two crops are discussed separately, since the expected maize yield is always higher than soybean grain yield ($p < 0.001$). This is intrinsic to the crop characteristics. The locations are discussed separately, since interaction between location and rotation ($p = 0.04$) and location and fertilization ($p = 0.006$) was found.
To illustrate the differences the average values for grain yield (kg ha^{-1}) are given in Table 6a and 6b, for Pawe and Bako.

Table 6a Average grain yield (kg ha^{-1}) for maize and soybean in Pawe indicated per year and per crop and grouped per treatment: with the total indicating total grain yield per year per crop, SEM[4] in parentheses.

Grain Yield

	2016		**2017**		**2018**	
Treatment	**Maize**	**Soybean**	**Maize**	**Soybean**	**Maize**	**Soybean**
CM+	6472 (125)		4809 (297)		7628 (433)	
CM-	3856 (559)		4032 (312)		3277 (253)	
CS+		3299 (216)		2036 (50)		1901 (137)
CS+ 1xInoc		3264 (269)		2187 (71)		1865 (153)
CS-		2878 (110)		1776 (195)		1484 (206)
RS+M+		3086 (220)	5738 (175)			2266 (78)
RS+M-		2874 (136)	4952 (695)			2108 (118)
RS-M+		2773 (470)	5423 (506)			1961 (155)
RS-M-		3025 (282)	4331 (518)			1729 (403)
Crop average	**5164 (561)**	**3028 (96)**	**4881 (204)**	**2000 (65)**	**5453 (854)**	**1889 (82)**

Table 6b Average grain yield (kg ha^{-1}) in Bako indicated per year and per crop and grouped per treatment: with the total indicating total grain yield per year per crop, SEM in parentheses.

Grain Yield

	2016		**2017**		**2018**	
Treatments	**Maize**	**Soybean**	**Maize**	**Soybean**	**Maize**	**Soybean**
CM+	8965 (2047)		6602 (735)		5511 (680)	
CM-	7940 (2438)		6071 (1053)		3808 (96)	
CS+		2751 (663)		2384 (247)		1276 (176)
CS+ 1xInoc		2949 (404)		2510 (98)		1237 (82)
CS-		3172 (37)		2257 (354)		1043 (183)
RS+M+		2834 (237)	8141 (250)			1231 (97)
RS+M-		3202(140)	7396 (475)			1355 (323)
RS-M+		3124(134)	10888 (886)			335 (37)
RS-M-		3410 (100)	8286 (252)			1849 (818)
Total	**8452 (1442)**	**3063 (111)**	**7897 (442)**	**2384 (132)**	**4660 (489)**	**1182 (145)**

For maize the effects are discussed in 2017 only, since this was the only year with maize in a rotation. In both 2016 and 2018 soybean was grown in a rotational cropping sequence. Therefore statements for rotation, fertilization and re-inoculation for soybean can be made for the data in Table 6a & b. For

[4] Standard error of the mean

soybean; neither rotation (p = 0.7 Pawe; p = 0.07 Bako), nor fertilization (p = 0.1 Pawe; p = 0.06 Bako), nor re-inoculation (p = 0.7) was found to be significant, either for Pawe or for Bako. Each consecutive year the soybean yield decreases. In Pawe, 2016 is higher in yield than 2017 (p = 0.001) and 2018 (p < 0.001). In Bako the yield significantly decreases between 2016 and 2018 (p = 0.003).
In Table 6a and 6b it is can be seen that yield for maize and soybean is higher in Bako than in Pawe for 2016 and 2017, this is not the case for 2018. In 2018 both crops have a lower yield in Bako than in Pawe.

To further delve into the effects for maize seen from crop sequence and fertilization. A subset of the maize grain yield was made in 2017. Again, the locations will be discussed separately, since rotation and locations showed a significant interaction effect (p = 0.03).

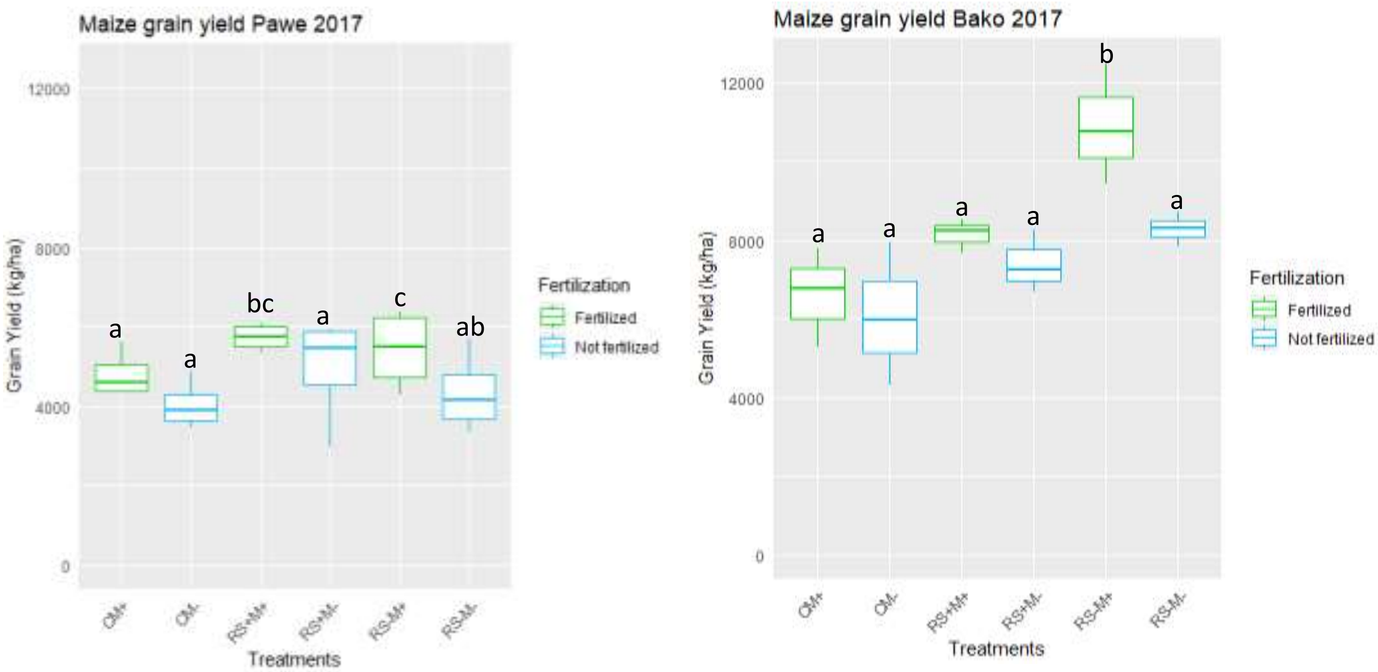


Figure 5a & b Maize grain yield in Pawe and Bako (respectively) for 2017, with indication for fertilization and significance between the treatments (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).

For Pawe, rotation does not show a significant effect (p = 0.06). Fertilization (p = 0.02) positively affect maize grain yield and fertilization within the rotation had a significantly positive effect (p = 0.009). In Bako rotation had a significantly positive effect (p = 0.007). Fertilization did not show a significant difference (p = 0.15). Fertilization within the rotation influenced maize yield positively (p = 0.009). Prior soybean fertilization had no significant effect at either location.
As Figure 5b indicates, in Bako the only significant difference is between RS-M+ and the other treatments. RS-M+ showed exceptionally high yields. The differences between the treatments in Pawe is smaller. Here, RS-M+ was significantly higher yielding than all other treatments except RS+M+. The hypothesis for maize that yield would be highest in a fully fertilized rotation (RS+M+) is not the case for Pawe or Bako.

Effects of location, rotation, fertilization and re-inoculation were tested on soybean grain yield in 2018. Pawe was generally higher in soybean yield than Bako ($p < 0.001$). Except that, there were no effects found either of rotation ($p = 0.2$), fertilization (0.1) or re-inoculation ($p = 0.1$), either of the current year, or of previous years. There were no significant differences between the treatments, as Figure 6 also shows.

The hypothesis, that continuous fertilization and inoculation would increase yield, was not met.

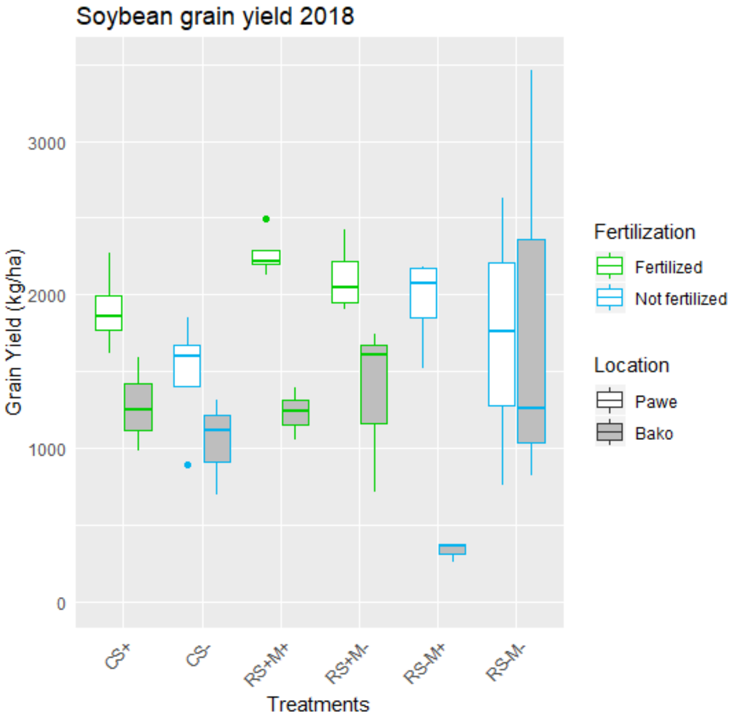


Figure 6 Soybean grain yield (kg ha-1) as found in 2018, fertilized or unfertilized for Pawe or Bako with treatments indicated (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).

3.2 Soil nutrient content

The effect of the three management aspects on soil nutrient content is answered in two parts: the first part consists of a soil analysis of two treatments, and the second part consists of nutrient balances. The soil analysis focusses on the effect of maize fertilization and location. The nutrient balances for N (kg ha^{-1}) and P (kg ha^{-1}) take all treatments into account and focus on all three management aspects (rotation, fertilization and re-inoculation). Nutrient balances are either with or without crop residues and will be analyzed separately.

3.2.1 Soil analysis

As shown in Table 7 and 8, the two treatments selected, CM+ and CM-, were often not significantly different from each other. Although, CM+ receives 88 kg N ha^{-1} yr^{-1} there is no difference found between the CM+ and CM-, neither for Pawe ($p = 0.2$) nor for Bako ($p = 0.6$). Actually, total N values are very uniform between fertilized and unfertilized maize and between the two locations. In Bako the only significant difference between CM+ and CM- was found in mg P kg^{-1}. Here CM- is significantly higher than CM+ ($p = 0.03$), despite not receiving any fertilizer for the last three years (38 kg ha^{-1} yr^{-1}). Differences due to fertilization are not present. Although, these differences, and other values in Table 7 and 8 do indicate heterogeneity of the field. For example, the differences in Sand Clay and Silt percentages in Pawe for CM- and CM.

The locations showed differences more often than the two treatments did, with especially significant differences between Pawe and Bako for Ca (mmol kg^{-1}) Mg (mmol mg^{-1}) , Na (mg kg^{-1}), organic matter (OM in %), CEC (mmol kg $^{-1}$) and Sand (%). P (mg kg^{-1}) and Silt (%) also differed between the two locations, with Bako being lower in average values for all of these, expect Na (mg kg^{-1}) (Table 7 and 8).

Table 7 Average values per treatment (CM+ or CM-, continuous maize with or without fertilization) are indicated (n = 3), including averages per location (n = 6). Significance between treatments and locations is shown when present.

Location	*Treat*	*Reps*	*Total N g kg^{-1}*	*P mg kg^{-1}*	*Ca mmol kg^{-1}*	*Mg mmol kg^{-1}*	*K mmol kg^{-1}*	*Na mg kg^{-1}*
Pawe	*CM+*	3	1.6	3.3	48.4	28.8	3.1	23
	CM-	3	1.5	2	54.0	32.7	3.2	22.3
p-value			NS	NS	NS	NS	NS	NS
Average Pawe		6	1.6	2.7	51.2	30.8	3.2	22.7
Bako	*CM+*	3	1.6	3	26.4	10.4	3.4	27.3
	CM-	3	1.6	4.7	28.0	10.8	3.8	24.3
p-value			NS	0.03	NS	NS	NS	NS
Average Bako		6	1.6	3.8	27.2	10.6	3.6	25.8
Significant differences between locations			NS	0.01	<0.001	<0.001	NS	0.008

Table 8 Average values per treatment (CM+ or CM-, continuous maize with or without fertilization) are indicated (n = 3), including averages per location (n = 6). Significance between treatments and locations is shown when present.

Location	*Treat*	***Reps***	**pH**	**OM (%)**	**CEC (cmol (+) kg^{-1})**	**Sand (%)**	**Clay (%)**	**Silt (%)**
Pawe	*CM+*	3	5.2	4.5	24.3	33.9	28.4	37.7
	CM-	3	5.3	4.5	26.2	14.9	41.2	43.9
p-value			NS	NS	NS	NS	NS	NS
Average Pawe		6	6	4.5	25.5	24.5	34.8	40.7
Bako	*CM+*	3	5.0	3.5	14.2	29.9	21.9	48.2
	CM-	3	5.1	3.5	14.6	24.1	19.5	56.4
p-value			NS	NS	NS	NS	NS	NS
Average Bako		6	6	3.5	14.4	27.0	20.7	52.3
Significant differences between locations			NS	0.01	<0.001	NS	0.03	NS

3.2.2 Soil nutrient balance (for N and P).

Another way the soil nutrients were analyzed, was through testing the effects of rotation and fertilization on soil balances. Two balances were made for N and P each, with and without crop residues (Figure 7a and 7b for N, and 8a and 8b for P). The N balance without crop residues (Figure 7a), indicated that most datapoints in this balance were negative. When looking at Figure 7a a minimal difference between the rotational treatments and CS+ CS- and CM- is to be seen. CM+ was relatively highest in all three years. Maize grain is lower in N content than soybean (1.5% N in maize grain and

6.1% N content in soybean). So per kg ha^{-1} of maize less nitrogen is taken off, which made the balance less negative respectively. It is visible from Figure 7a that the (partially) unfertilized treatments, CM-, CS-, RS+M-, RS-M+, RS-M-, have a downward trend throughout the three years.
The N balance including crop resiudes (Figure 7b) overall, was less negative than the N balance without crop residues (Figure 7a). CM+ showed the most positive trend and indicated the highest value for N in 2018 (155.3 kg ha^{-1}). RS+M+ and RS-M+ also indicated a clear positive trend with values in 2018 being above 0. Overall, this indicated that maize fertilization has the most positive influence on the N balance with crop residues incorporated. This was to be expected since maize receives more N fertilizer; 88 kg ha^{-1} for maize and 9.5 kg ha^{-1} for soybean. And maize was higher in biomass yield, adding to the increase. Although, soybean fixation of N is incorporated in the balances, this did not seem to alleviate the difference of influence of the two crops. On average 87.6 kg N ha^{-1} was fixed in Pawe in 2018. This bridges the difference in amount of fertilizer added.

Both P balances, with and without crop residues (Figure 8a and 8b), are a more spread around 0 than the N balances with and without crop residues, respectively. This is due to the lower P content in maize and soybean grain. Additionally, more P (kg ha-1) was added through fertilization, than N (kg ha-1), especially for soybean (9.5 kg ha-1 N and 19.5 kg ha-1 P).
First, the P balance without crop residues (Figure 8a) is discussed. Fertilization either for or for soybean was higher in P values than without fertilization. When looking at Figure 8a CM+, CS+ and RS+M all show a positive trend with average values in 2018 above 0. The rotational treatments did not seem to perform better or wors than the continuous treatments, the differences found were more due to fertilization.
Figure 8b had most positive datapoints out of all four of the nutrient balances. Each cropping system with fertilization, either for maize or for soybean exceeded 0. Clearly, fertilization either for maize or for soybean had a positive influence. When looking at Figure 8a, there was no difference between the rotational and continuous treatments. Again this was more due to fertilization. CM+, CS+, RS+M+, RS+M-, RS+M-, RS-M+ all showed a positive trend with average values in 2018 above 0. The highest average values were from CM+ (68.3 kg P ha-1) and RS+M+ (69.4 kg P ha-1). RS+M- (27.6 kg P ha-1) and RS-M+ (31.7 kg P ha-1) were fairly equal in 2018, although different crops receive fertilizer. Over all three years the two treatments received almost equal amounts of P fertilizer; RS+M- receives 2x 19.5 kg ha-1 P, RS-M+ recieves 1x 38 kg ha-1. All average values for the treatments are given in Appendix II Table 1a & b.

The main differences in the P balance were due to fertilization. This seemed the trend for all four nutrient balances. Generally the hypothesis that RS+M+ would be highest can be put to rest. Soybean had a more negative effect on the N and P balances than maize, with two years of soybean and one year of maize grown in a rotation. This made the rotational treatments more negative than the continuous maize treatments.

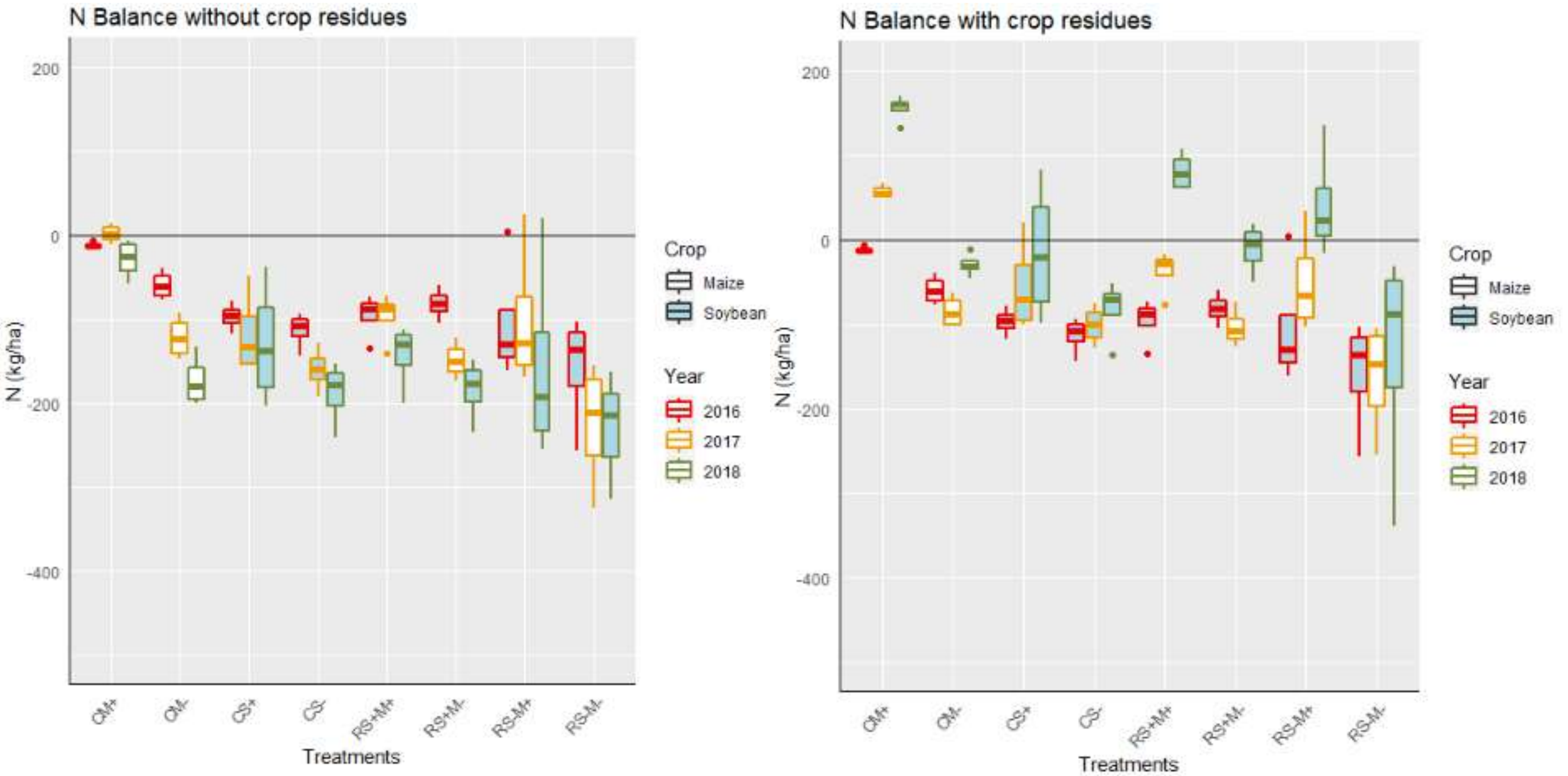
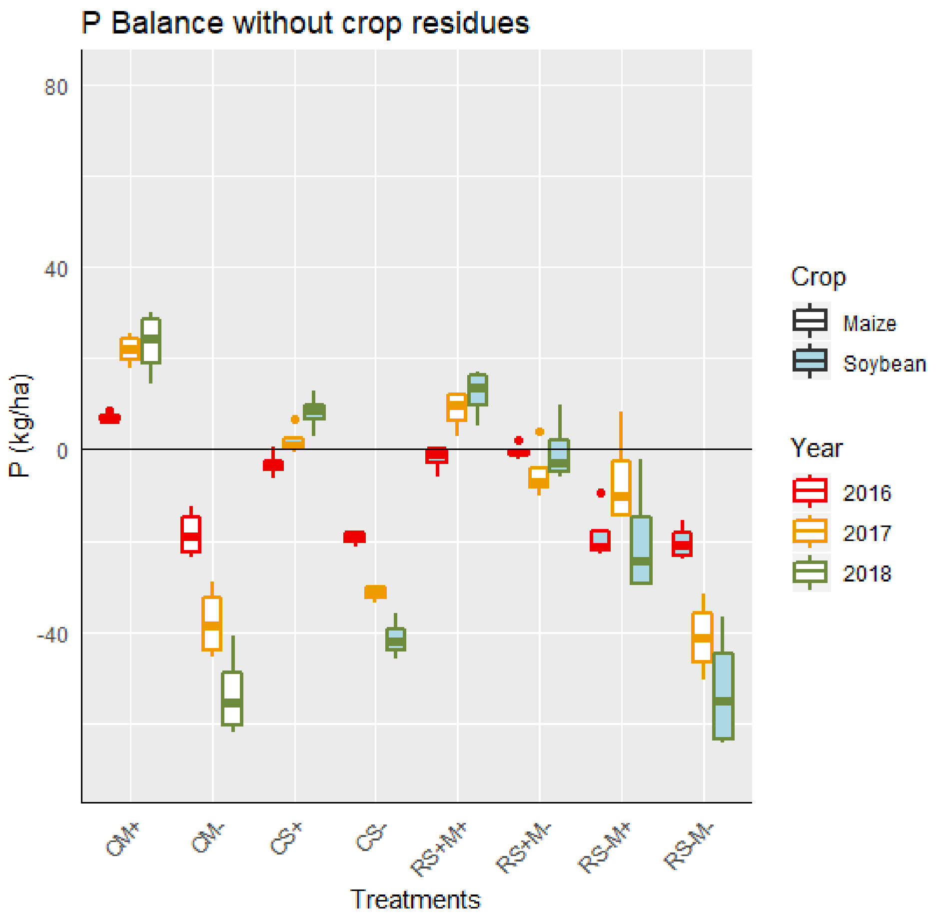


Figure 7a & b N balance (kg ha^{-1}) either with or without crop residues, per treatment indicated per year and per crop (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).



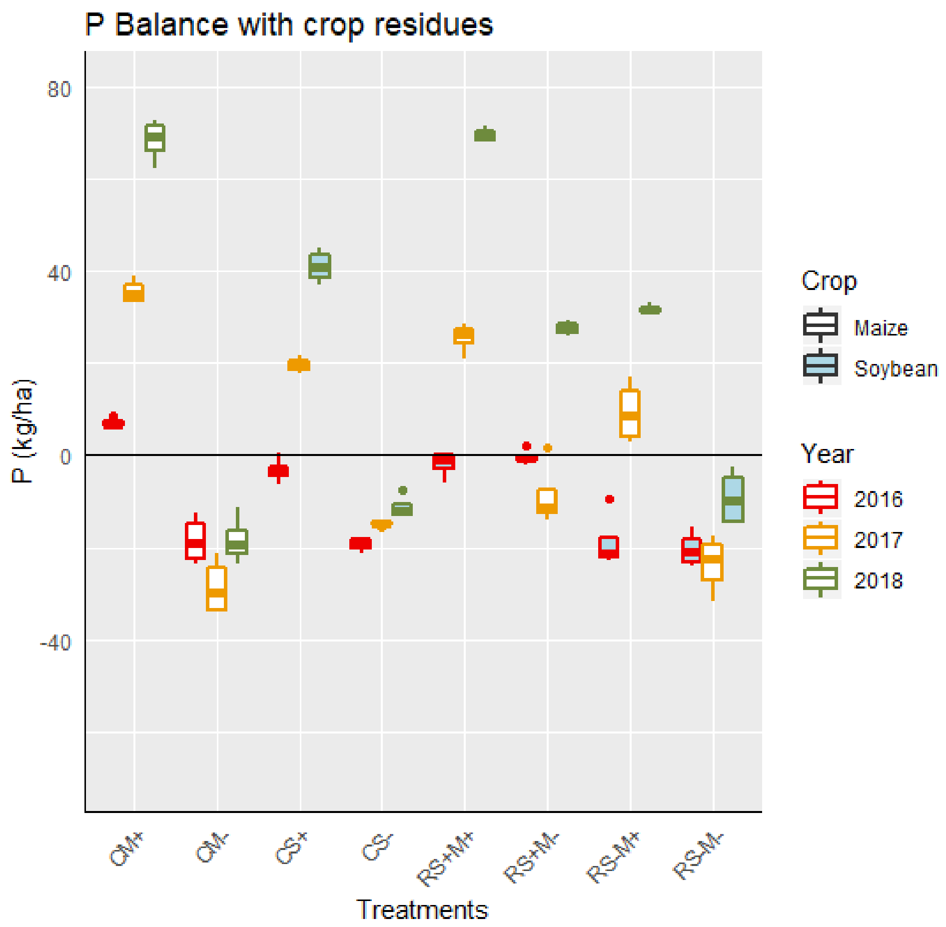


Figure 8a & b P balance (kg ha-1) either with or without crop residues, per treatment indicated per year and per crop (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).

3.3 Ndfa and amount N_2 fixed

Table 9a and b give the proportion of total N and δ ^{15}N of the soybean and the reference weeds per treatment. With these values the Ndfa (%) was calculated and the amount of N fixed (kg ha^{-1}).

Table 9a & b Biomass (kg ha $^{-1}$), N (%), total N (kg ha^{-1}), δ^{15}N for soybean and the reference weeds (‰), Ndfa (%) and the amount of N fixed (kg ha^{-1}) for Pawe and Bako, respectively. SEM is indicated in parentheses, (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).

Pawe

Treatments	Biomass (kg ha^{-1})	N (%)	Total N (kg ha^{-1})	δ^{15} N soybean (‰)	δ^{15} N reference weeds[5] (‰)	Ndfa (%)	N fixed (kg ha^{-1})
CS+	5069.4 (281.3)	2.9 (0.1)	146.0 (9.1)	1.0 (0.5)	4.3 (0.5)	64.8 (10.7)	94.6 (17.9)
CS+ 1xInoc	5138.9 (279.1)	2.8 (0.1)	141.9 (7.3)	1.1 (0.7)	5.2 (0.4)	68.0 (11.7)	98.1 (19.8)
CS-	4166.7 (491.4)	2.7 (0.2)	113.5 (18.6)	1.6 (0.4)	4.8 (0.4)	59.9 (4.5)	66.8 (9.7)
RS+M+	6365.7 (389.9)	2.6 (0.1)	167.0 (12.3)	1.5 (0.2)	3.9 (0.4)	49.9 (3.8)	82.5 (5.3)
RS+M-	6088.0 (326.0)	3.0 (0.2)	181.0 (17.1)	1.7 (0.3)	4.1 (0.2)	47.7 (4.6)	84.1 (3.3)
RS-M+	5324.1 (493.6)	2.9 (0.1)	155.3 (15.5)	1.7 (0.5)	3.8 (0.4)	33.9 (4.6)	51.9 (6.7)
RS-M-	4537.0 (922.1)	2.5 (0.2)	118.3 (32.6)	1.4 (0.3)	3.7 (0.8)	54.9 (7.7)	58.5 (10.9)
Average	5241.4 (217.4)	2.8 (0.1)	146.1 (7.4)	1.4 (0.2)	4.3 (0.2)	54.1 (3.2)	76.6 (5.1)

Bako

Treatments	Biomass (kg ha^{-1})	N (%)	Total N (kg ha^{-1})	δ^{15} N soybean (‰)	δ^{15} N reference weeds[4] (‰)	Ndfa (%)	N fixed (kgha^{-1})
CS+	4096.4 (522.1)	2.0 (0.2)	78.9 (5.6)	-0.9 (0.4)	5.3 (0.3)	80.7 (5.8)	64.4 (9.4)
CS+ 1xInoc	4044.7 (308.7)	1.9 (0.1)	78.6 (5.3)	-1.4 (0.3)	5.9 (0.3)	87.9 (3.6)	69.0 (4.8)
CS-	4070.6 (33.7)	2.2 (0.1)	89.3 (5.1)	-1.3 (0.4)	5.8 (0.6)	86.4 (4.1)	76.9 (3.0)
RS+M+	4123.4 (263.2)	2.2 (0.2)	90.8 (13.4)	0.2 (1.5)	4.9 (0.3)	85.3 (3.9)	66.3 (4.9)
RS+M-	3016.6 (907.9)	2.0 (0.1)	43.2 (0.4)	-2.0 (0.4)	5.6 (0.1)	94.7 (5.0)	40.9 (1.8)
RS-M+	960.2 (55.6)	2.2 (0.1)	21.3 (1.9)	-2.0 (0.2)	4.6 (0.9)	93.1 (3.6)	19.9 (2.4)
RS-M-	4051.2 (71.2)	2.5 (0.2)	100.6 (5.3)	-1.4 (0.0)	6.4 (0.1)	88.3 (0.0)	88.9 (4.7)
Average	3483.9 (281.0)	2.1 (0.1)	71.8 (2.1)	-1.1 (0.3)	5.5 (0.2)	87.7 (1.7)	60.5 (5.3)

[5] A mixed and bulked sample of different reference weeds were used. In the Appendix I a list of reference weed species used, is given.

The $\delta^{15}N$ of soybean in Pawe was higher for all treatments than the $\delta^{15}N$ of Bako. The average in Pawe was 1.4 ‰, while that of Bako was -1.1 ‰ for soybean. The negative $\delta^{15}N$ indicate that the main source of N was derived from mineral soil N. Whereas a $\delta^{15}N$ more around 0 would indicate that atmospheric N pools were more used than soil N pools. The percentage Ndfa was higher in Bako (87.7) than in Pawe (54.1), although these differences are not seen in the amount of N fixed. Percentage Ndfa (Figure 9) and amount of N fixed (Figure 10a & 10b) were further analysed.

Inoculation was not of influence on either location ($p = 0.2$) , either for the full dataset, or between CS+ and CS+ 1xInoc ($p = 0.5$). Rotation had a significant interaction with location ($p = 0.048$), thus tests were split for the both locations.
In Pawe, no differences were found for rotation ($p = 0.1$) or fertilization ($p = 0.2$). Although, there are differences shown in Figure 8 these are not significant[6].
In Bako the rotational cropping sequences were significantly higher in %Ndfa than the continuous treatments ($p = 0.03$). No significant effect of fertilization was found ($p = 0.3$), although when looking at Figure 10, it seems like unfertilized treatments are higher in %Ndfa. The hypothesis that %Ndfa would be highest under full fertilization and inoculation is not supported. The %Ndfa responded very differently at both locations. Probably due to different soil values, P was significantly higher in Bako, while the amount of total N was similar for both locations.

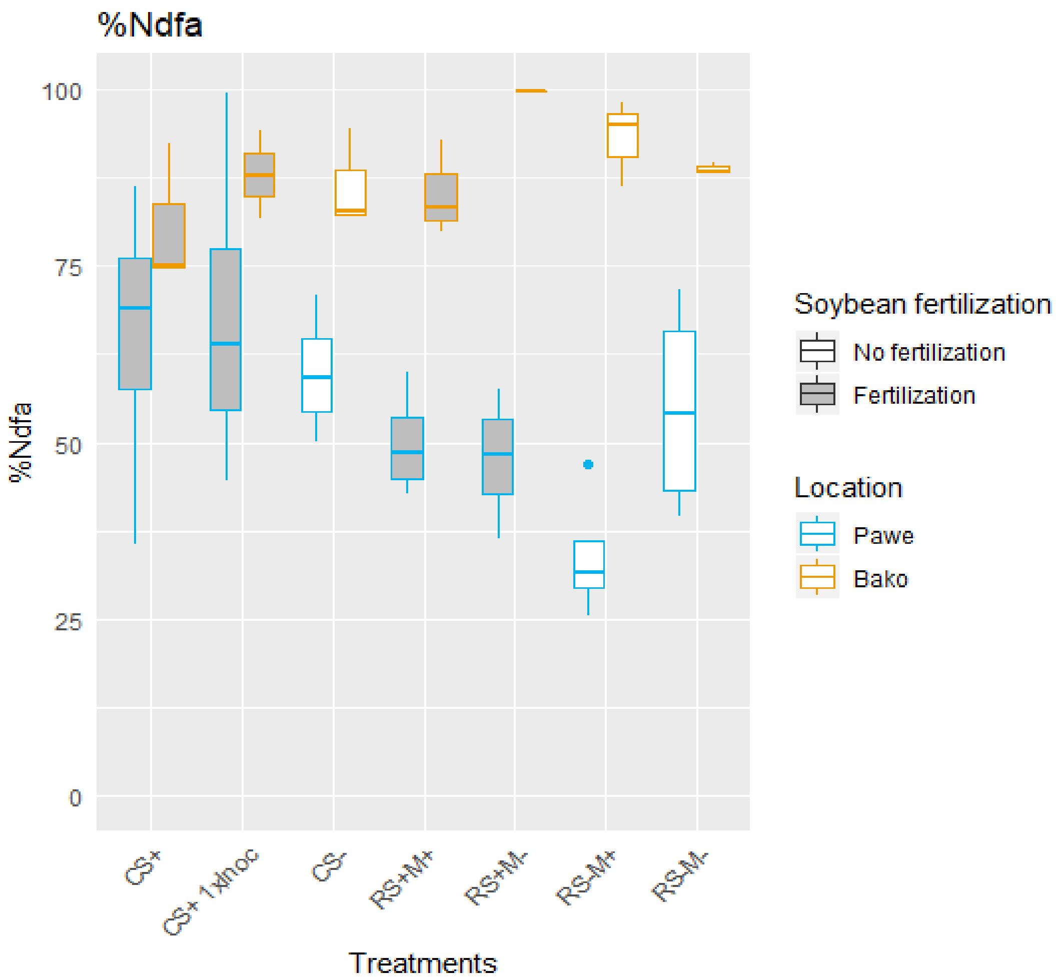
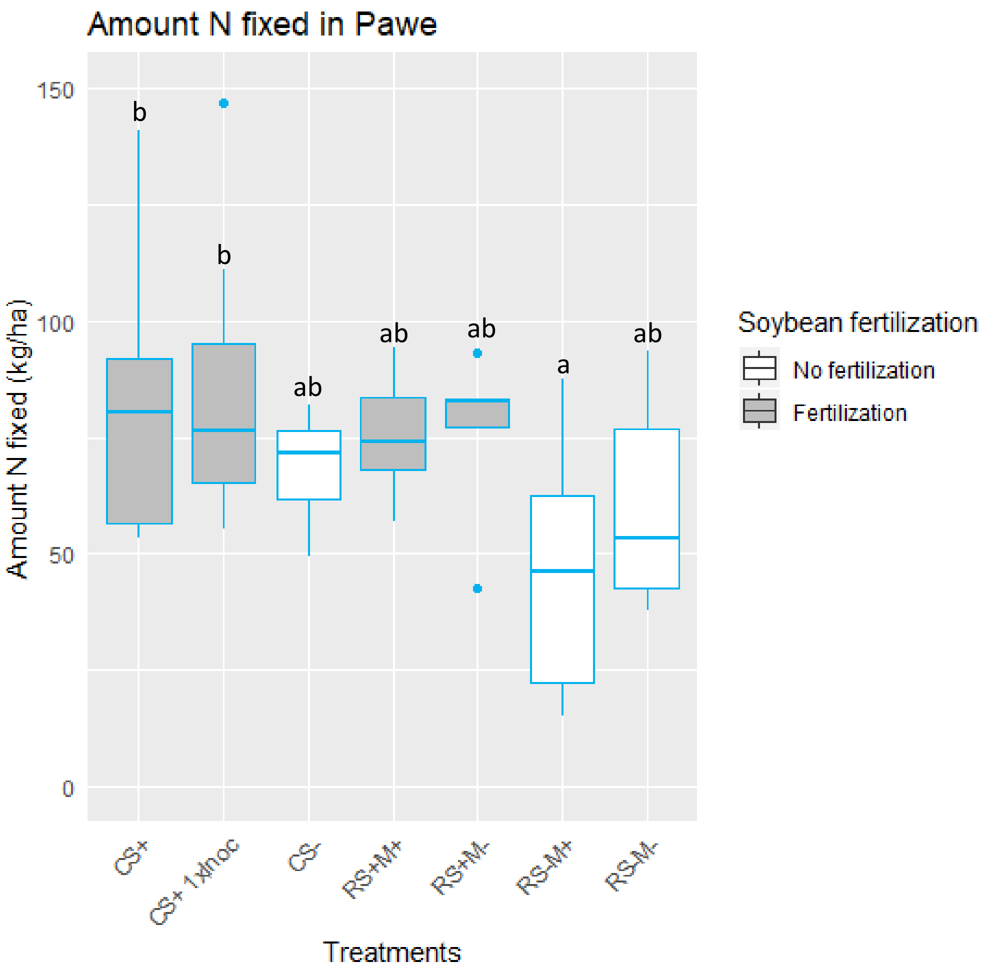


Figure 9 %Ndfa per treatment in Pawe (n=4) and Bako (n=3), C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized, all fertilization inputs include yearly inoculation for soybean, except for the treatment indicating 1xInoc which only received inoculation in 2016.

[6] There is a significant difference between CS+ 1xInoc and RS-M+ however this is not valuable difference, since CS+ 1xInoc was only used to test the re-inoculation, and no re-inoculation effect was found.



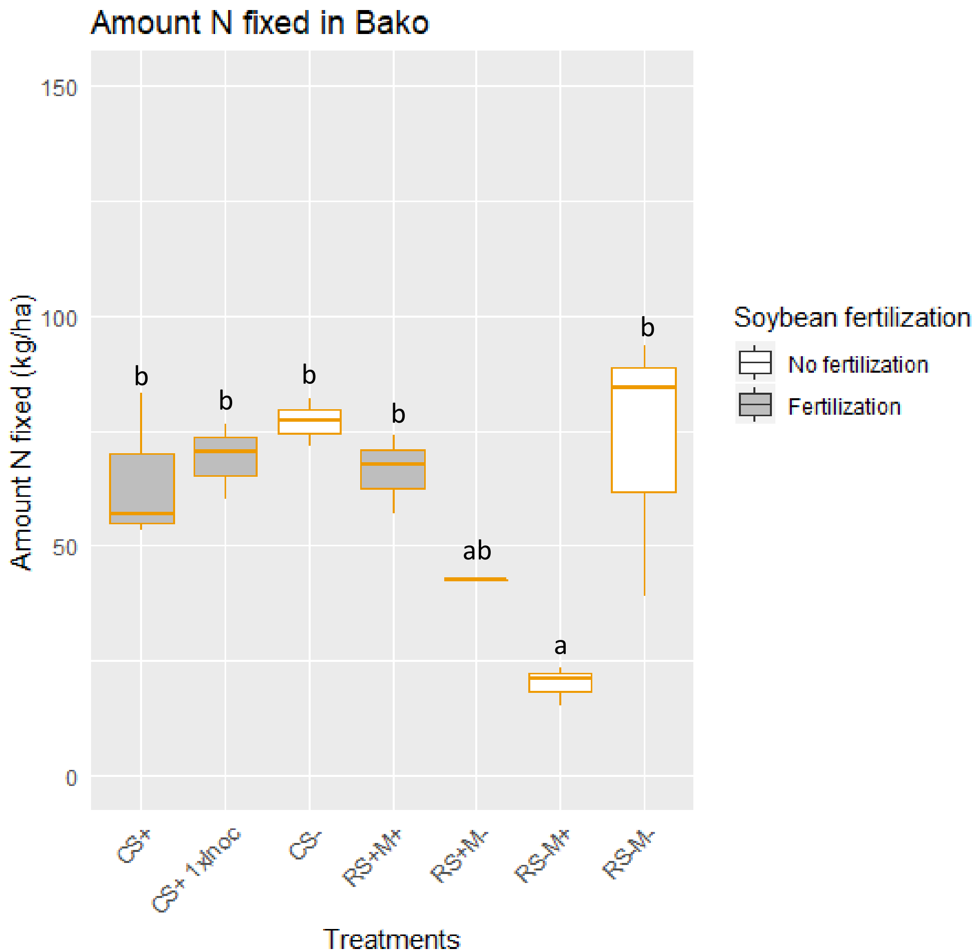


Figure 10a & b Amount N (kg ha-1) fixed per treatment in Pawe (n=4), and in Bako (n = 3), with indications for significant differences between the treatments. C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized, all fertilization inputs include yearly *inoculation for soybean, except for the treatment indicating 1xInoc which only received inoculation in 2016.*

The amounts of N fixed in Pawe and Bako was more equal than %Ndfa (for Pawe and Bako). Although %Ndfa in Bako was high, the biomass yield was lower in Bako than in Pawe (Table 10a & b). Re-inoculation did not have an effect on the amount of N fixed at either location. Interaction between fertilization and location (p = 0.005) dictated that further analysis is done per location. In Pawe fertilization (p = 0.1) and crop sequence (p = 0.6) are not of influence on the amount of N fixed (kg ha^{-1}). Between specific treatments differences were found; RS-M- is significantly lower than CS+ or CS+ 1xInoc (p = 0.01, p = 0.006), as indicated in Figure 10a.
In Bako fertilization significantly increased the amount N (kg ha^{-1}) fixed (p = 0.005).This is mainly caused by the low biomass yield of RS-M+ (Table 9b). RS-M+ was significantly lower than all continuous soybean (CS+ p = 0.004, CS+ 1xInoc $p < 0.001$, CS- 1xInoc $p < 0.001$). RS-M+ was also significantly lower than RS+M+ (p = 0.002) and than RS-M- ($p < 0.001$). The significant differences between the treatments are indicated in Figure 10b. Rotation was not of significant influence (p = 0.2).
Here the hypothesis, that CS+ would be highest, was shown. For both locations amount N (kg ha^{-1}) was highest in CS+ among other treatments.

3.4 Cost-benefit analysis

Table 10a and b give an indication of the possible costs, benefits, profits and the BCR for the treatments discussed in this study. Although maize fertilization was higher in cost than that of soybean, it also had higher yields. The cost of planting soybean (without fertilization) was higher than maize (without fertilization) due to the higher seeding rate of soybean. Sale of the crops did not differ a lot in price; maize was sold for 6 Birr kg^{-1} and soybean for 7.5 Birr kg^{-1}. Because of this, benefits from maize in USD[7] ha^{-1} were generally higher than for soybean. All in all the cost, benefits and profits from maize with fertilization (M+) were highest. Therefore, at both locations, both for cumulative benefits as for cumulative profits CM+ was highest. When looking at the cumulative benefits, for Pawe, RS+M+ and RS-M+ were second and third highest, respectively. RS+M+ was also second most profitable cumulatively in Pawe. However, since costs for CM- were lower than for RS-M+, CM- becomes third most profitable cumulatively. In Bako the cumulative benefits were second highest for CM-, and third highest for RS-M+. RS-M+ had very high yields in 2017. In this year the benefits were also highest for RS-M+. When looking at the cumulative profits in Bako, CM- and RS-M- were second and third, respectively. Maize yield overall was high in Bako, even without fertilizer inputs.

If, the cost of maize fertilization cannot be afforded, the rotations, especially RS-M+ or RS+M- form a good alternative in Pawe.

The BCR was highest with high outputs and low inputs. Therefore unfertilized maize was generally seen to have the highest value. Over the years both soybean and the rotational cropping sequences (generally) became more of interest as well. From the rotational cropping systems, cumulatively in Pawe and Bako RS-M- was highest, RS+M- was second.

[7] Conversion of 1 Birr = 0.00393 USD was used (Exchange Rates, 2018).

Table 10a & b The costs (USD ha^{-1}) , benefits (USD ha^{-1}), profits (USD ha^{-1}) and a benefit cost ratio (BCR) calculated for 2016, 2017 and 2018) for Pawe and Bako with the sum over the three years per treatment indicated (C = continuous, R = rotation, M = maize, S = soybean, + = fertilized, - = not fertilized).

Pawe

Treatments	Cost '16	Cost '17	Cost '18	Cumulative costs	Benefit '16	Benefit '17	Benefit '18	Cumulative benefits
CM+	122	122	122	366	1318	979	1553	3849
CM-	16	16	16	48	785	821	667	2273
CS+	67	67	67	202	840	518	484	1842
CS-	43	43	43	130	732	452	378	1562
RS+M+	67	122	67	256	785	1168	577	2530
RS+M-	67	16	67	150	731	1008	537	2276
RS-M+	43	122	43	209	706	1104	499	2309
RS-M-	43	16	43	103	770	882	440	2091

Treatments	Profit '16	Profit '17	Profit '18	Cumulative profit	BCR'16	BCR'17	BCR'18	Average BCR
CM+	1196	857	1431	3484	11	8.0	12.7	10.5
CM-	769	805	651	2225	49	51.5	41.8	47.5
CS+	772	451	417	1640	12	7.7	7.2	9.1
CS-	689	409	334	1432	17	10.4	8.7	12.0
RS+M+	718	1046	509	2274	12	9.6	8.6	9.9
RS+M-	664	992	469	2126	11	63.2	8.0	15.1
RS-M+	662	982	456	2100	16	9.1	11.5	11.1
RS-M-	726	866	397	1989	18	55.3	10.1	20.3

Bako

Treatments	Cost '16	Cost '17	Cost '18	Cumulative costs	Benefit '16	Benefit '17	Benefit '18	Cumulative benefits
CM+	122	122	122	366	1825	1344	1122	4291
CM-	16	16	16	48	1616	1236	775	3628
CS+	67	67	67	202	700	607	325	1631
CS-	43	43	43	130	807	574	265	1647
RS+M+	67	122	67	256	721	1657	313	2692
RS+M-	67	16	67	150	815	1506	345	2665
RS-M+	43	122	43	209	795	2217	85	3097
RS-M-	43	16	43	103	868	1687	470	3025

Treatments	Profit '16	Profit '17	Profit '18	Cumulative profit	BCR'16	BCR'17	BCR'18	Average BCR
CM+	1703	1222	1000	3925	15.0	11.0	9.2	11.7
CM-	1600	1220	759	3580	101.4	77.5	48.6	75.8
CS+	633	540	257	1430	10.4	9.0	4.8	8.1
CS-	764	531	222	1517	18.6	13.2	6.1	12.6
RS+M+	654	1535	246	2435	10.7	13.6	4.7	10.5
RS+M-	748	1490	278	2515	12.1	94.4	5.1	17.7
RS-M+	752	2095	42	2888	18.3	18.2	2.0	14.8
RS-M-	824	1671	427	2922	20.0	105.8	10.8	29.4

4 Discussion

This study explored the possibility of the use of three management aspects, rotation, fertilization and inoculation, to sustainably intensify maize and soybean cultivation in Ethiopia. This discussion first focuses on how the trial set-up may have influenced the results. Second, findings of the four research questions are discussed in relation to relevant literature. Finally, some recommendations for future research are given.

4.1 Set up field trial

All yield data was converted into hectares from test plots with a size of 4.5m by 6m (in Pawe), or 5.1m by 4.5m (in Bako). Measuring inaccuracies, field fluctuations (in the soil and climate) and other factors, like pests and diseases, may only indicate small influences in crop measurements per plot, but can carry through more significantly differences once converted to kg ha^{-1}. This possibly results in yield variability between the treatments that is not due to the management aspects.

A key difference between the two research stations (Pawe and Bako) is that the field experiment in Bako does not have a RCBD set-up. This field experiment has three equal management practices replicated side by side (Figure 3, paragraph 2.3). Therefore, field variability and treatments are confounded. The choice for a non-RCBD set up was made in order to keep contamination between the plots at a minimum. Especially in the rain-season it can be difficult to keep the soil from the different plots separated, additionally ridging between the plots is used. Contamination is expected to be low in Bako, also because the distance between the plots within the rows is larger than in Pawe. Although, especially the yield of RS-M+ is exceptionally high for all three replicates. This cannot be due to contamination since this plot is at the end of the field beside CS-. However this could be caused by field heterogeneity.
The field experiment in Pawe does have a RCBD layout, with four replicates. Here contamination is minimized through ridging between the plots, and between the plots and the path: The path only runs between the rows of plots and is slightly lower than the actual plots. Clay content in the soil in Pawe is relatively high, and clearly clumped together when wet. The soil easily stuck to shoes and clothing. This increases contamination risk by workers crossing from plot to plot. The field workers are involved in a multitude of experiments, and are not specifically aware of the individual fertilization or inoculation applications performed previously. Contamination is expected to be likely between the plots in Pawe. However, there are no indications for contamination in the data.

With nine treatments in the field trial (Table 1) it was difficult to test the effect of repeated inoculation. CS+ and CS+ 1xInoc were the only two treatments that could be compared to test this. CS- did also receive inoculation in the first year, however there was no unfertilized treatment without inoculation to compare this with. Except those three treatments the yearly inoculation was always paired with fertilizer additions.

Currently, only one year of maize in a rotational cropping is present. This makes it unclear to which degree the effects are due to seasonal, locational or possibly random effects. Especially when maize yield, for specific treatments (RS-M+ in Bako) was as high as it is. It is also difficult to analyze the possible effect of soil nutrient values and N_2 fixation on maize, since soil samples, and the N_2 fixation samples were conducted in 2018, while maize was cultivated in rotation in 2017.

4.2 Yield differences

The first research question is: how do crop sequence, fertilizer and inoculation inputs affect the grain yield of maize and soybean. In answering this question the main focus is on yield response in maize, because maize was more responsive to the above-mentioned inputs than soybean yield was. Maize grain yield was significantly positively influenced by prior soybean cropping at both locations. At Pawe a significant yield increase was shown through added fertilization either in continuous or rotational cropping. The maize yield varied between the two locations with Bako having a significantly higher yield than Pawe. Soybean was unresponsive to either cropping sequence or fertilization and yields steadily decreased throughout the years for all treatments. In the following two sub-paragraphs the observed differences will be further discussed per crop.

4.2.2 Maize grain yield

As said, in this study, maize yield responded positively to the rotational crop sequence at both locations. This is in line with previous research, which indicates yield improvements for a soybean-maize rotation (Lupwayi et al., 2011; Franke et al., 2018), although the effect of prior soybean cropping on maize yield is situation specific (Abera et al., 2015). Therefore, it would be interesting to validate the yield increase found in this study, with multiple years of rotational cropping.
In Pawe fertilization additionally improved yield. Since fertilization overall improved the yield, it also improved yields within the rotation, which made RS+M+ and RS-M+ the two highest yielding treatments. As seen in Figure 4 (paragraph 3.1) RS+M+ was higher in yield than RS-M+. However, this was a marginal difference between the two treatments. In Bako RS-M+ was very high yielding relative to all other treatments. Which would be in line with a common farmer practice of planting maize on more fertile fields and more often receives fertilizer inputs, than legumes do. It is situation dependent how maize grain yield responds to this farmer practice, with soil type and nutrient content very much playing a role (Zingore et al., 2008).

Maize yield in Bako could be increased due to the significantly higher level of P in Bako (3.8 mg kg^{-1}) than in Pawe (2.7 mg kg^{-1}). Although Bako is found to be significantly lower in other soil parameters than Pawe (e.g. Ca, Mg, CEC and OM). All in all, it is difficult to explain the yield difference on the basis of the soil values found in this study. The yield difference between the two locations, is more probably because two hybrid maize varieties were used; BH-540 in Pawe, and BH-660 in Bako. These varieties were grown at the two locations for all three years included in this study. BH-540 has a moderate adaptation for Pawe (and Bako), while BH-660 is shown to be best adapted for the Bako area (Kelemu & Mamo, 2002). This probably explains the higher yield in Bako than in Pawe for all treatments.
For Pawe, maize yield fluctuated between the years, 2016 had on average 5.2 t ha^{-1}, 2017 4.9 t ha^{-1}, and 2018 5.5 t ha^{-1}. The yield from all years is low for an on station trial. Earlier research for on station trials in Bako found values between 8.0 – 9.0 t ha^{-1} for BH-540 (Legesse et al., 2011) and an average of 7.3 t ha^{-1} for five EIAR locations for BH-540 (Twumasi-Afriyie et al., 2011). The yield data of this study is far below these on-station trials, and more in the range, or even below the range, of yield from farmer's fields: 5.0 – 6.5 t ha^{-1} (Legesse et al., 2011). At least in 2016 (8.5 t ha^{-1}) and 2017 (7.9 t ha^{-1}) BH-660 yielded well in Bako, in this study. Although yields are lower than earlier on-station trials (9.0 – 12.0 in Bako (Legesse et al., 2011), and 9.8 on average for five EIAR locations (Twumasi-Afriyie et al., 2011)), they are above or in the upper range of yield from farmer's fields (6.0 – 8.0 t ha^{-1}, Legesse et al., 2011). However, yield in Bako in this study was very low for 2018 (4.7 t ha^{-1}) relative to 2016 and 2017, and in comparison to the literature (Legesse et al., 2011; Twumasi-Afriyie et al., 2011). Differences compared with the references could also be due to the different years analysed, Legesse et al. (2011) is based on a National Maize Research Project from 1988–2010. And Twumasi-Afriyie et al. (2011) is based on data from five EIAR research stations from 2001 - 2011. While data from this study is from 2016 – 2018.

The sudden decrease in yield in Bako from 2017 to 2018 could be based on seasonal changes. When looking at climate data for temperature and rainfall for 2016, 2017 and 2018 in Bako, the temperature seems to be higher in 2018 than in earlier years, mainly the minimum temperature shows a difference (Appendix IV Figure 1). Rainfall for these three years do not give a clear reason for the lower yield in 2018 (Appendix IV Figure 2).

4.2.2 Soybean yield

The answer for soybean is clearly, that neither rotational cropping, nor fertilization, nor re-inoculation were of influence on grain yield at either location. The lack of effects on soybean-yield under fertilization and re-inoculation was not expected, previous research indicates a positive yield response on mineral fertilizers and/or from manure (Zingore et al., 2008; Rurangwa et al., 2018). Earlier farm trials from N2Africa similarly indicate that additions of P and/or inoculants (as used in this field trial) increase yields between 8 – 70% (Abebe et al., 2019).

Compared to literature soybean yield in 2016 (3028 kg ha^{-1} in Pawe and 3063 kg ha^{-1} in Bako) was above the range found for expected possible yield of farmer's fields: 1700 – 2900 kg ha^{-1} (Soil Health Consortium, 2014), but not in a high yield range for demonstration trials (3300 – 4000 kg ha^{-1}, Abebe et al., 2019). Independently from the management treatments soybean yields decreased significantly throughout the years. Yield in 2017 was 2000 kg ha^{-1} in Pawe and 2384 kg ha^{-1} in Bako, and in 2018 this was 1889 kg ha^{-1} in Pawe and 1182 kg ha^{-1} in Bako. In 2018 TGx-13-3-2644 was cultivated in Pawe, while in Bako Belessa-95 continued to be grown. For both varieties yields were lower than the expected yields of 2000-2500 kg ha^{-1}, and 1700-2900 kg ha^{-1}, for TGx-13-3-2644 and Belessa-95 respectively (Africa Soil Health Consortium, 2014). The yield of 2018 in Bako was below the target yield of 1274 kg ha^{-1} (Bajukya et al., 2013). The high yield of 2016, possibly, was too high to sustain. However the continuous drop in 2017 – 2018 with yields bellow the expected possible, or target yields (Baijukya et al., 2013; Africa Soil Health Consortium, 2014), would indicate that there is a limiting factor unrelated to the management treatments. The limiting factors will be discussed in size of probability (high – low). The first possibility is, that although fertilizer is added the amount of N, P and S is still too low for a stable soybean grain yield (kg ha^{-1}). Additionally, other (micro)nutrient could deficient. Generally in Ethiopia soil fertility is poor, with earlier studies finding deficiencies of N, P, K, Zn and Cu for example (Woldeab & Mamo, 1991; Haileslassie et al., 2005; Merkeb et al., 2016; Abdulkadir et al., 2017; Jembere et al., 2017; Abebe et al., 2019). Soil data from this study also support this as a possibility for the decreasing soybean yields. The soil nutrients are further discussed under 4.3 Soil properties. Secondly, it is possible that there are increasing seasonal elements limiting soybean growth (increasing dry spells, or temperature e.g.). Especially, yield in Bako is lower in 2018 than in previous years, for soybean and for maize. As stated under 4.2.1, climate data gave no clear indication for this.

For 2016 and 2017 Belessa-95 was grown at both locations, while in 2018 Belessa-95 was grown at Bako, but TGx-13-3-2644 was grown at Pawe. 2018 was the first year in which Pawe (1889 kg ha^{-1}) had higher yields than Bako (1182 kg ha^{-1}). Literature also indicates that TGx-13-3-2644 is higher yielding than Belessa-95 (Africa Soil Health Consortium, 2014).

If there are external limiting factors for the yield such as low (micro)nutrient levels or an unfavorable climate, this could be the cause of the lack of re-inoculation response seen in soybean yield. However, when looking at the high to normal range of %Ndfa, this seems less the case. Possibly there was still rhizobia present from previous trials, or contamination between the plots, made the differences in re-inoculation less clear.

4.3 Soil properties

It is very difficult to draw a clear conclusion on the second research question: what is the effect of crop sequence and fertilizer inputs on the soil nutrient content. For both the data from the soil analysis as the N and P nutrient balances have their downsides. However when combining the trends and differences found and taking into account their possible faults it can be said that fertilization and the incorporation of crop residues increases the soil nutrient values. For further conclusions on the performance of the management aspects it is probably more reliable to look at the other variables (grain yield, N2 fixation or an economic indication).

4.3.1 Soil analysis

The soil values, at least between the two treatments CM+ and CM-, did not indicate significant differences for most of the soil parameters (only for P a significant difference was found). In general, it is uncertain to what degree these soil values would represent managements techniques over a three year period. In order to actually see differences in soil values longer trials are necessary. Literature on soil studies found, varied between 5 and 60 years, with a common duration of the trial being 20 years (Kraemer & Hermann, 1979; Mercik & Nemeth, 1985; Visser & Parkinson, 1992; Hatfield & Cambardella, 2001; Warman, 2005, Merkeb et al., 2016). Indicating that probably the three years included in this study is not enough to be able to see (significant) differences.

Values found in this research are very much in line with earlier findings, that Ethiopian soils are poor in soil fertility. Especially N and P being limiting for crop production (Woldeab & Mamo, 1991; Haileslassie et al., 2005; Abebe et al., 2019). A surprising find in wheat research over the last 20-30 years was crop responsivity to K. Ethiopian soils were thought to be rich in potassium (K), but the crop response indicates that potassium could be limiting as well. Micronutrients, like Zn and Cu, were found to be limiting as well. With Fe and Mn less often being limiting for crop growth (Abdulkadir et al., 2017). A study conducted in Pawe using six soil types found that all soil types were consistently deficient in nitrogen, phosphorus, sulfur and boron (Jembere et al., 2017). Merkeb et al. (2016) also indicated that (extractable) P was below critical values, while here the values for OM (4.2%) were in a medium range and total N (0.15%) was high (according to the range classifications of Tekalign (1991)). The situation in Bako was found to be similar, with study sites showing poor nutrient fertility especially for N and P (Chimdessa, 2016). In this study, like in Merkeb et al. (2016), total N (0.16%) was found to be high (0.12 – 0.25%, Tekalign (1991) and P was low (2.7 mg kg^{-1} for Pawe and 3.8 mg kg^{-1} for Bako).Values for K were very low (3.2 mmol kg^{-1} Pawe, 3.6 mmol kg^{-1} Bako).

The differences found between the data of this study and earlier research can be due to local heterogeneity, seasonal fluctuations, measurement inaccuracies, or due to the somewhat empirical nature of soil analyses especially for micronutrients (Fairhurst, 2012). Values are very dependent on the extraction methods deployed, which is already seen between Belete et al. (2019) and Merkeb et al. (2016), using the Mehlic and the Olsen extraction method, respectively. Additionally, large ranges of soil values are also found within studies (Chimdessa, 2016; Merkeb et al., 2016). For micronutrients it is difficult to get hard values, because of interdependency of soil factors and micronutrient availability. Such as, multiple soil factors impacting the micronutrients, or one parameter affecting the availability of multiple micronutrients, e.g. pH influence on Mo and Mn (Sillanpää, 1982).

4.3.2 N and P balance

The N and P balances indicate that including crop residues improves the cropping system, in the sense that the management trials tested become less negative in soil nutrients. Maize overall decreases N values less than soybean, with maize fertilization relatively improving the N-balance. Maize grain (1.5 %) is lower in N content than soybean (6.1 % N). So, although maize yields are higher than soybean-yields, the offtake in N is lower for maize. The average offtake for CM+ and CM- over three years is 77.7 kg N $ha^{-1} yr^{-1}$, while for soybean the average, for CS+ and CS-, is 140.3 kg N $ha^{-1} yr^{-1}$. When looking at P both maize and soybean fertilization improve the P-balance, and actually increase the amount of P. Crop-choice between soybean or maize is less of influence on the P-balance, because maize and soybean grain are more equal in P content; 0.48% P for maize, 0.68% for soybean. Making the average offtake 24.1 kg P ha^{-1} yr^{-1} for maize, and 15.5 kg P ha^{-1} yr^{-1} for soybean. The annual fertilizer rates for maize and soybean, respectively are; 19 kg N ha^{-1} & 38 kg P ha^{-1}, 8 kg N ha^{-1} & 19 kg P ha^{-1}. Especially for N the difference between the offtake and fertilizer input was large. The incorporation of crop residues is a good practice, but does not make the N balance predominantly positive. Manure additions could improve this nutrient balance, with literature finding improvements for N and P (Bedada, 2015; Abdulkadir et al., 2017; Belata et al., 2019). For P fertilization was a sufficient compensation for the removed grain.

The amount fixed by soybean is included as an N input. It is difficult to assess how much of the amount N fixed will be available in the soil. However, it is necessary to add the amount of N fixed in the balance, otherwise the amount of N taken off (in the calculation) is larger than the actual amount of N taken off from the soil. Because the grain yield (in amount N), including the amount of N fixed (and assimilated in grain), is used as the offtake. The incorporation of this improves the N balance, on average (both locations combined) for around 68 kg ha^{-1}.

Haileslassie et al. (2005) uses two approaches (Universal Soil Loss Equations (USLE) and Landscape Process Modelling at Multidimensions and Scales (LAPSUS)) to calculate the soil nutrient balances, with both approaches the nutrient balances show a yearly decrease for both N and P; -123 kg N ha^{-1} yr^{-1} (USLE), -49 kg N ha^{-1} yr^{-1} (LAPSUS), -20 kg P ha^{-1} yr^{-1} (USLE), -5 kg P ha^{-1} yr^{-1}, with data from Benishangul (the region in which Pawe lies). Haileslassie et al. (2005) does not include crop residues, but does include N fixed by legumes. Values are a national average for all cultivated crops. Yearly averages from this study were calculated, combining values for maize and soybean. First a yearly average was calculated, then the values for all three years were averaged; -218 kg N ha^{-1} yr^{-1} (without crop residues), -10 kg N ha^{-1} yr^{-1} (with crop residues), -147 kg P ha^{-1} yr^{-1} (without crop resiudes) and 7 kg P ha^{-1} yr^{-1} (with crop residues). The averages from this study are more negative without crop residues and more positive with crop residues than values from Haileslassie et al. (2005). Values from this study are difficult to compare to Haileslassie et al. (2005). Hailselassie et al. (2005) includes five indicators for in- and output, including leaching, denitrification and erosion. None of these aspects are included in the balances above. The inclusion of more variables into the balances made in this study, may have made the effect of crop residues less clear. The addition of more variables into the balance, would make it more accurate. Haileslassie et al. (2005) was made based on CSA[8] data 1999/2000, while this study has data from 2016 – 2018.

Hailselassie et al. (2005) find negative balances for both N and P, so although the size of the negative values is still debatable, the overall negative values of the N and P balances presented in this study can be taken seriously. And, for this study, indicate that although fertilizer was added, soil mining was still present under the average of the treatments. Which is further supported by the FAO (2000) seeing soil degradation in Ethiopia generally as being very high, as seen in Figure 11, which compares the SSA countries on the level of soil degradation. Additionally, plenty of research indicates that N and P are limiting nutrients in general for Ethiopia (Haileslassie et al., 2005; Kraaijenvanger & Veldkamp, 2012; Abdulkadir et al., 2017, Abebe et al., 2019; Belete et al., 2019)

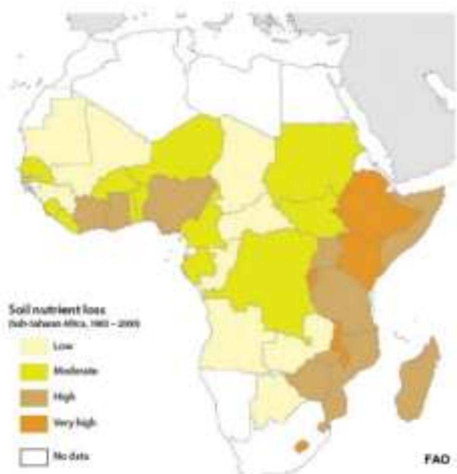


Figure 11 Soil nutrient mining in SSA 1983 – 2000 (FAO, 2000).

4.3.3 Legume choice

Soybean could be an inefficient choice of legume when N return to the soil is one's goal. Compared to common bean, cowpea and

[8] Central Statistical Agency

groundnut, soybean had the highest percentage N concentration in grain from %Ndfa, and second lowest in stover (groundnut was lower) (Franke et al., 2013). Indicating low returns from %Ndfa to the soil. Soybean has been bread to transfer most N to grain, so even if crop residue is left maybe not that much N is returned (Buresh et al., 1997). On the other hand, this does mean that dietary benefits from soybean are higher than of other legumes. The choice of legume in this retrospect very much depends on the goal at hand.

4.4 Ndfa and amount N

The third research question is: what is the effect of the crop sequence, rhizobia and fertilizer input on %Ndfa and amount N fixed by soybean. With no clear effect of cropping sequence, fertilizer and inoculation additions on %Ndfa or amount of N found for Pawe in 2018. Although from Figure 10 and 11 (paragraph 3.3) it seems that continuous soybean growth is higher in %Ndfa and amount of N, especially when fertilized. This is not the case for Bako, where rotational soybean cropping increased %Ndfa in 2018. And from Figure 10 it seems that this is stimulated in unfertilized treatments. While fertilizer increases the amount of N fixed and when looking at Figure 11 the rotation seems to decrease the amount of N fixed. It is unexpected that fertilizer and rhizobia inputs do not influence %Ndfa or amount of N since earlier research found influences from these two management aspects (Abdulkadir et al., 2017), though variance was seen from AEZs. With especially the addition of manure and P fertilizer being advise for smallholder farmers (Rurangwa et al., 2018).

The %Ndfa found for Bako (on average 87.7%) was significantly higher than for Pawe (on average 54.1%), and also relatively high when compared to reference literature. Between 5 – 74% was found by Ronner & Franke (2012) in literature between 2000 – 2012. However, Ronner & Franke (2012) also indicate values between 65 – 89% for soybean, found by Giller et al. (1997). The reason that the values found for %Ndfa in Bako were relatively high, could be because only leaves were sampled (from soybean and reference weeds). While in Pawe the full plants were sampled, for both locations sampling was done at mid-podding (a growth stage of soybean). Partitioning of N in plants is dependent on the plant tissue, leaves partition relatively higher amounts of N than stems and pods combined (Bender et al., 2015). Possibly explaining why Bako is high in the range of values for %N. Sampling is most recommended at stages when legumes are at their peak biomass. This generally is from mid-flowering until pod setting (Unkovich et al., 1994), mid-podding is within this range.
The high %Ndfa values found are somewhat contradicting with the negative values indicated for $\delta^{15}N$ from soybean in Bako (-1.1 ‰). Although, this too could be because leaves were samples instead of the full plant. Leaf $\delta^{15}N$ was found to be negative in Kahmen et al. (2008). Generally, negative $\delta^{15}N$ indicate that the main source of N was derived from mineral soil N. Whereas a $\delta^{15}N$ more around 0 would indicate that atmospheric N pools were more used than soil N pools (Kahmen et al., 2008).

Not only the plant tissue and timing of the sampling is of importance. The calculation of %Ndfa is also very much dependent on a proper reference plant. It is preferred that the reference plants have the same growth period as the legume in question. The magnitude of this effect is dependent on uniformity of the soil profile (and thus connected to rooting depth) and on the uniformity of the $\delta^{15}N$ values of the reference plant over time. If both are reasonably stable the effect of the growing period of the reference plant is likely to be small (Giller, 2001). Reference weeds sampled at Pawe were likely younger than weeds sampled at Bako. Pawe encountered a lot more weeds in general, and the weeding management was not adequately stopped in order to be able to find reference weeds of the same growth period of soybean. All weeds were shorter than the soybean plants (at mid-podding). At Bako only very few weeds grew in the plots, most often all of these were sampled (around 5 plants per plot) although these were around the same height as the soybean plants (at mid-podding), it is uncertain what their growth period was. This makes the quality of the reference weeds and of the %Ndfa calculation more uncertain.

Additionally, in 2018 two different soybean varieties were grown TGx for Pawe and Belessa-95 for Bako. Not only influencing the %Ndfa (Hardarson et al., 1984), but also the amount of biomass (Baijukya et al., 2013; Afrika Soil Health Consortium, 2014; Ghiday, 2017), and thus the amount of N fixed. So, although confounded with location, Belessa-95 may have been more efficient in fixing N, although lower in biomass yield. The amounts of N fixed in Pawe and Bako were more equal, mainly because although the %Ndfa in Bako was higher on average than in Pawe, soybean yield was lower.

Data was only collected for 2018, therefore it is difficult to specify if difference were caused by heterogeneity in the test fields. Especially, for Bako, without the RCBD set-up of the field-trial, this could be the case. The data collected from the soil analysis does seem to point to field heterogeneity (especially in Pawe). With differences between CM+ and CM- fluctuating between soil parameters. In combination with the %Ndfa results it was surprising that N (mg kg^{-1}) and P (mg kg^{-1}) content did not differ significantly for the two locations. With N content being equal for both locations (N is 1.6 g kg^{-1}) and P even being higher for Bako than for Pawe (on average 2.7 mg kg^{-1} for Pawe, and 3.8 mg kg^{-1} for Bako). Other key elements for N_2 fixation, such as selenium, iron, molybdenum and copper, (Giller, 2001) did show convergence to the expectation that soil nutrient values would be lower in Bako than in Pawe, stimulating %Ndfa. All in all, though, it does not seem very probable that these soil values give an accurate indication for the differences in %Ndfa. Because of which it would be informative to collect data on %Ndfa and amount of N for following years.

4.5 Profitability and BCR

Smallholder farmers may not only be interested in an increase in yield, but also in the profitability of the management technique, or the financial inputs required. The fourth research question therefore went into the profitability of the different treatments, as well as the investment costs required. Cumulatively over the three years the rotational cropping systems perform relatively well. Although CM+ is the highest treatment in profitability (at both locations), in Pawe RS-M+ comes second. And RS+M+ is the third most profitable treatment for Pawe. In Bako this was different, CM+ was highest and CM- yields. Here the RS-M- treatment is third most profitable over the three years, with RS-M+ close behind. RS-M+, the common farmer practice, overall, performs well yield and profit-wise. Generally, treatments highest in yield were also highest in profit. Fertilized maize performed best for both. When compared to literature, Zingore et al. (2008) had equal findings. With especially maize fertilization being profitable, though Zingore et al. (2008) stated that “potential exists to increase income by targeting manure to soybean on the more fertile soils”. Fertilizer additions to soybean was probably not found as profitable in this study because of low soil fertility. Findings are very much dependent on the specific situation, with Zingore et al. (2008) also finding that economic advantages of the crops and fertilizer additions are soil type specific (sandy vs. granite soil), and dependent on the soil fertility. This could explain differences found between the two locations of this study.

A study into the profitability of soybean in Pawe indicated that main costs were weeding and labour. Due to quick sale after harvest, profits were lower, with a total average profit of 6461.8 ETB ha^{-1} (219.0 USD ha^{-1}) (Ayalew et al., 2018). Which is low compared to the calculations made in this thesis. The average profits of soybean in 2018 were 410 USD ha^{-1}. Probably because labour costs were not included in the analysis. Average labour cost for maize per treatment would be between 55 – 62 USD ha^{-1}, while for soybean it is estimated[9] to be between 39 - 40 USD ha^{-1}. Based on data obtained from Bako (Table 1, Appendix V).

Seeing the negative nutrient balances it is advised to incorporate fertilization in the cropping management. Although, the BCR indicated that the treatments without fertilizers are highest, this is

[9] Estimated from labour for seeding of common bean, soybean data was not present.

not advised, also because profit decreases through the years when looking at CM- (highest in BCR). In the cumulative total over the three years certain rotational treatments also become high in BCR; RS-M+ for Pawe, and RS-M- for Bako. If soybean yields could be improved, or at least, not decrease throughout the years, the rotational cropping sequences would be more beneficial. At the moment fertilizer always adds costs, thus lowering the BCR. However for farmers with a little more financial capacity it would be worthwhile to fertilize either soybean or maize in a rotational cropping sequence (RS+M- and RS-M+), when looking at the profits.

This data is created on the basis of the seed prices, seed rates and fertilizer prices, together with the grain yield data. Farmer profit's are very much dependent on the fluctuation of prices, with large regional and seasonal difference. Storage of produce (if not detrimental to the grain), and selling further after the general harvest period, could potentially increase farmer's profit (WFP Ethiopia, 2018). The price of inoculation for soybean has not been taken into account, if it had been soybean would be higher in costs and lower in profits. Since no effect of re-inoculation was seen.

4.7 Recommendations for future research

The recommendation is to keep soybean in the rotation, while working on improving soybean yield. Since positive effects on maize were already seen in one year of the rotational crop sequence. And previous N2Africa trials also showed a positive effect of soybean. Increasing soil coverage and organic matter content (Abebe et al., 2019), showing that the rotational cropping sequence with soybean is beneficial on the whole. Nonetheless management adaptations are needed to stabilize or improve soybean yield. Other than experimenting with different fertilization rates, possibilities to improve soybean yield could also be through the expansion of the crop rotation, for example with finger millet (Abebe et al., 2019). For maize it has been found that split-fertilizer input (of DAP or urea) increases yield and NUE (Tadesse et al., 2013; Abdulkadir et al., 2017). This could also have potential for smallholder farmers since no extra fertilizer inputs are needed.
All in all it is advised to analyze soil data from the two locations throughout the three years to obtain a more accurate view on the possible effects of the trial. The limited effect seen from inoculation may be due to low levels of soil (micro)nutrients. To elevate problems from low levels of (micro)nutrients manure additions could be beneficial. Positive results have been seen from manure additions, since this stimulate the soil structure and in some cases adds micronutrients as well (Bedada, 2015; Kraaijenvanger & Veldkamp, 2015; Abdulkadir et al., 2017; Belata et al., 2019).

5 Conclusion

Overall, two aspects are found to have a positive influence on grain yield, soil fertility and profit: cultivating maize with fertilization. This can either be in a continuous cropping system (CM+) or in a rotation (either RS-M+ or RS+M+). For maize in 2017 the rotations are higher yielding than continuous maize. RS+M+ is slightly higher for Pawe, than RS-M+. In Bako it is clear that RS-M+, the common farmer practice, is higher yielding than all other management aspects. This being said, at both locations CM+ overall has the highest yield and highest in cumulative profitability, and it also relatively performed best on the soil nutrient balances.

When looking at soil fertility it is difficult to give hard values. Therefore the trends indicated in the N and P balance are used for this conclusion. When crop residues are not incorporated, CM+ is the only treatment that indicates a positive trend for N, over the three years used in this study. When incorporating crop residues the rotational treatments, RS+M+ and RS-M+, are relatively positive as well. For P, CM+ is highest with or without crop residues. Although, in both cases RS+M+ has a positive influence as well, and is (almost) as positive as CM+.

For the amount of N fixed in 2018, the combined conclusion from both locations is that the continuous fertilized soybean treatment (CS+) is highest. When looking at %Ndfa there is a difference between the locations; in Pawe CS+ is highest, while in Bako this is RS+M+, with RS-M+ as a very close second.

Profit wise, again, the three treatments CM+, RS+M+ and RS-M+, are high when combining results from both locations.

Therefore, the overall conclusion is CM+ is best when all four variables are taken into account. However, in the long term it could well be that this continuous cropping becomes less favourable. RS-M+, the common farmer practice, and RS+M+, a fully fertilized rotation overall also perform well. These three treatments can be advised for farmers. RS-M+ is lowest in costs, subsequently RS+M+ is second lowest in cost. CM+ is highest in overall cost, also when compared to all other treatments.

This concludes, that the current common farmer practice performs well. Especially, when aiming for a (relatively) low cost management treatment, with high profits. For both locations more specific advice depends on which variable has priority.

6 Appendix

Appendix I – Reference weeds

The species names for the reference weeds were sometimes difficult to derive. Together with the research stations and the (possible) local names, the species names were derived: Stellaria media L.; Bidens frodonsa L.; Convolvulus arvensis L.; Bidens pilosa L.; Amaranthus spinosus L.; Celosia trigyna L.; Mentha pulegium L.; Eleusine indica L. Nicandra physalodes L.

Appendix II - N and P balance

The average of the N and P balances (kg ha^{-1}) both with and without crop residues, with a total per year, are given in Table 1a & b. Both balances are for Pawe (no balances were made for Bako, see 2.6.1).

Table 1a Average N balances (kg ha^{-1}) without crop residues and with crop residues per treatment, split per crop, with an average per year and SEM in parentheses.

Treatment		*N without crop residues*			*N with crop residues*		
	Crop	2016	2017	2018	2016	2017	2018
CM+	Maize	-12.3 (1.9)	1.2 (5.6)	-29.1 (11.7)	-12.3 (1.9)	56.7 (4.0)	155.3 (7.9)
CM-	Maize	-59.8 (8.7)	-122.3 (12.8)	-173.1 (15.4)	-59.8 (8.7)	-84.7 (9.2)	-29.7 (7.0)
CS+	Soybean	-94.3 (6.3)	-117.3 (17.5)	-126.4 (25.0)	-94.3 (6.3)	-55.4 (19.4)	-8.9 (27.3)
CS-	Soybean	-113.5 (11.0)	-159.7 (13.3)	-188.2 (19.2)	-113.5 (11.0)	-101.7 (11.9)	-82.7 (18.5)
RS+M+	Maize		-97.2 (15.0)			-38.3 (13.3)	
	Soybean	-96.3 (13.2)		-143.5 (19.8)	-96.3 (13.2)		80.2 (11.2)
RS+M-	Maize		-149.0 (11.0)			-104.5 (11.5)	
	Soybean	-81.7 (9.3)		-184.1 (18.6)	-81.7 (9.3)		-10.5 (15.3)
RS-M+	Maize		-100.5 (43.8)			-50.1 (31.0)	
	Soybean	-104.4 (37.0)		-155.4 (61.6)	-104.4 (37.0)		-41.0 (33.2)
RS-M-	Maize		-225.4 (28.1)			-163.8 (34.2)	
	Soybean	-158.3 (34.2)		-304.6 (80.1)	-158.3 (34.2)		-136.7 (70.3)
	Yearly average	-90.6 (8.4)	-120.8 (12.0)	-158.4 (16.6)	-90.6 (8.4)	-66.34 (11.6)	-0.1 (16.9)

Table 1b Average P balances (kg ha^{-1}) without crop residues and with crop residues per treatment, split per crop, with an average per year and SEM in parentheses.*12*

Treatment		*P without crop residues*			*P with crop residues*		
	Crop	2016	2017	2018	2016	2017	2018
CM+	Maize	6.9 (0.6)	21.9 (1.7)	23.2 (3.6)	6.9 (0.6)	35.4 (1.3)	68.3 (2.3)
CM-	Maize	-18.5 (2.7)	-37.9 (4.0)	-53.6 (4.8)	-18.5 (2.7)	-28.7 (3.1)	-18.5 (2.6)
CS+	Soybean	-3.2 (1.1)	1.6 (1.1)	7.9 (1.6)	-3.2 (1.1)	19.3 (0.5)	41.4 (1.1)
CS-	Soybean	-19.4 (0.7)	-31.4 (0.9)	-41.4 (2.1)	-19.4 (0.7)	-14.8 (0.3)	-11.3 (1.3)
RS+M+	Maize		8.6 (2.2)			25.5 (1.7)	
	Soybean	-1.8		12.3	-1.8		69.4

		(1.5)		(2.7)	(1.5)		(0.7)
RS+M-	Maize		-5.2			-8.8	
			(3.2)			(3.5)	
	Soybean	-0.4		-0.4	-0.4		27.6
		(0.9)		(3.6)	(0.9)		(0.8)
RS-M+	Maize		-6.8			9.3	
			(5.3)			(3.4)	
	Soybean	-18.7		-20.0	-18.7		31.7
		(3.2)		(6.4)	(3.2)		(0.5)
RS-M-	Maize		-6.8			-23.6	
			(4.2)			(3.2)	
	Soybean	-18.7		-20.0	-20.4		-9.3
		(1.9)		(6.7)	(1.9)		(3.1)
	Yearly average	-8.7	-9.9	-13.0	-8.7	3.7	26.8
		(1.7)	(3.6)	(4.9)	1.7)	(3.7)	(5.3)

Appendix III – Grain yield

Table 1 Average grain yield (kg ha^{-1}) maize and soybean per treatment and specified per year, per crop and per location.

	Treatments										
		CM+	**CM-**	**CS+**	**CS+ 1xInoc**	**CS- (1xInoc)**	**RS+M+**	**RS+M-**	**RS-M-**	**RS-M+**	**Crop average**
2016											
	Maize	**7540.38**	**5606.02**								**6573.20**
	Pawe	6471.71	3855.88								5163.80
	Bako	8965.27	7939.54								8452.40
	Soybean			**3064.00**	**3129.28**	**3004.05**	**2977.81**	**3014.50**	**3190.25**	**2923.51**	**3043.34**
	Pawe			3299.09	3264.37	2877.86	3086.03	2873.69	3025.07	2773.14	3028.46
	Bako			2750.54	2949.16	3172.29	2833.51	3202.25	3410.49	3124.00	3063.18
2017											
	Maize	**5577.27**	**4905.99**				**6767.54**	**5999.21**	**6026.10**	**7765.18**	**6173.55**
	Pawe	4808.80	4032.00				5737.72	4951.97	4331.05	5423.21	4880.79
	Bako	6601.90	6071.32				8140.62	7395.52	8286.16	10887.81	7897.22
	Soybean			**2185.56**	**2325.50**	**1982.49**					**2164.52**
	Pawe			2036.49	2187.44	1776.36					2000.10
	Bako			2384.31	2509.57	2257.34					2383.74
2018											
	Maize	**6720.96**	**3504.67**								**5112.82**
	Pawe	7628.30	3277.17								5452.73
	Bako	5511.18	3808.02								4659.60
	Soybean			**1633.09**	**1573.57**	**1294.89**	**1822.07**	**1785.37**	**1780.28**	**1264.18**	**1593.35**
	Pawe			1901.25	1865.02	1483.83	2265.61	2108.39	1728.83	1961.33	1902.04
	Bako			1275.55	1184.96	1042.97	1230.69	1354.67	1848.87	334.64	1181.77

Appendix IV – Climate data Bako

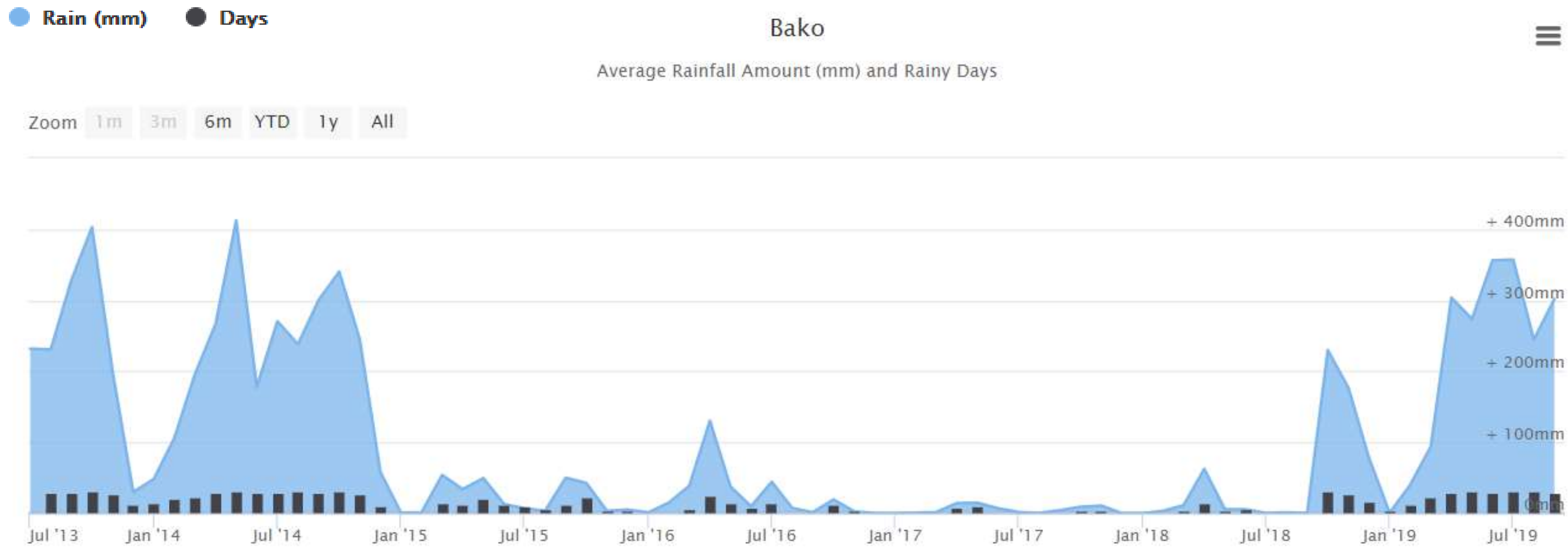


Figure 1 Rainfall data for Bako from July 2013 - July 2019 (World Weather Online, 2019).

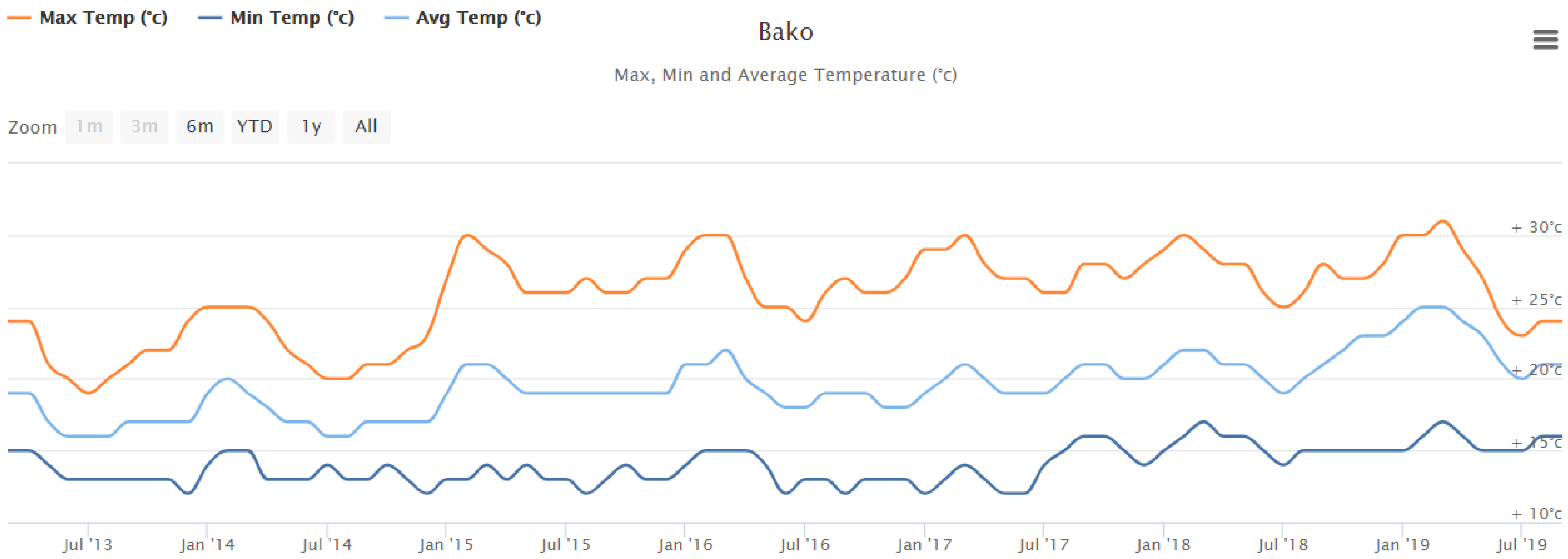


Figure 2 Maximum, minimum and average temperature for Bako from July 2013 - July 2019 (World Weather Online, 2019).

Appendix V – Seed price, sale price and labour cost

All data was received from N2Africa from Bako in 2017. Data for common bean is used as an estimate for soybean.

Table 1 Labour costs for maize and common bean received from N2Africa.

	Labour (days ha^{-1})	***Necessary labour***
for maize		
labour for land preparation	12	x
labour for sowing	4	x
labour for fertilizer application	6	x
labour for herbicide application	1	
labour for weeding	12	x
labour for harvesting	20	x
Total maize	52	
for common bean		
labour for land preparation	3	x
labour for sowing	4	x
labour for fertilizer application	1	x
labour for weeding	10	x
labour for harvesting	12	x
labour for treshing	4	X
Total common bean	34	

Since not all treatments receive fertilization, the labour days needed for maize were between 46 – 52 days ha^{-1}. For soybean this was 33 – 34 days ha^{-1}. The labour price per day was indicated as 35 Birr da^{-1}. Making the labour cost for maize 1610 – 1820 Birr ha^{-1} or 54.6 – 61.7 USD ha^{-1}, and for soybean 1155 – 1190 Birr ha^{-1} or 39.2 – 40.3 USD ha^{-1}. (Conversion of 1 Birr = 0.00393 USD was used (Exchange Rates, 2018).)

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