Investigating the nutrient status of “non-responsive” soils across Rwanda, using a nutrient omission trial with soybean (*Glycine max)*

Report about an internship in the N2Africa project on a research station of the Rwanda Agriculture Board (RAB) in Rubona

by

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1 Summary

A nutrient omission trial according to “A double pot technique for rapid soil testing” (JANSSEN, 1974) with soybean (*Glycine max*) as test plant has been conducted on a Rwandan research station to investigate the nutrient status of four Rwandan soils. These soils were identified as low responding to P-fertilisation and inoculation, when growing soybean and climbing bean (*Phaseolus vulgaris*), respectively. The soil samples were taken across Rwanda from three different provinces (1x Northern province, 1x Eastern province, 2x Southern province), representing three different Agro-Ecological-Zones (AEZ). Every macronutrient apart from Ca (N, P, K, Mg, S) was tested separately; micronutrients were tested combined in one treatment. Lime treatments were integrated as a third experimental factor for two acid soils (pH < 5). Following indicators were used to evaluate the performance and growth of soybean: Visual observations, aboveground biomass, stem height, Sufficiency Quotient (derived from Relative-Growth-Rate), nodulation, activity of nodules and plant tissue analysis. Every measurement was taken at three growth stages: 14 days after emergence (DAE), 26 DAE and 34 DAE.

Plants grown in every soil showed lean performance and minor growth when K was omitted. Clear potassium deficiency symptoms were detected on every -K treatment. Aboveground biomass, stem height and nodulation were significantly reduced already in early growth stages. On average the final biomass of -K treatments was reduced by 71.4 *%* compared to control treatments with full nutrient supply (nut no N) and by 91.9 *%* compared to N-fertilised treatments. A laboratory analysis classified all soils to have a “low” potassium content. These results demand an increased attention to potassium fertilisation and prevention of K losses.

In general N-fertilised treatments had an unexpected high biomass production. Their growth exceeded the growth of all other treatments for many times. Even treatments with complete nutrient supply (but no N)and seed inoculation, creating favourable conditions for biological nitrogen fixation (BNF), could not compete with fertiliser-N-supplied treatments. A coherent explanation for this unexpected difference in biomass production is still missing.

2 Introduction

The project called “Putting nitrogen fixation to work for smallholder farmers in Africa” (N2Africa) aims to improve the production of legumes and thus biological nitrogen fixation (BNF) in different countries of sub-Saharan Africa. This improvement is driven by different activities, such as integration of new crop varieties, development and production of qualitative inoculant, training of farmers, etc.. A main constraint to biological nitrogen fixation are so called non-responsive soils which occur on several sites of the project. On these soils, yield does not respond to recommended management, being mineral fertilisation with a P-based fertiliser and inoculation in soybean. Soils can be non-responsive because “deficiencies in [...] nutrients essential for the growth of bacteria or plants can cause reductions in the number and size of nodules formed and in the amount of N2 fixed” (GILLER, 2001). In Rwanda, non-responsive soils have been identified through different experiments, conducted in 2010.

Having an average of 411.4 citizens living per square kilometre, Rwanda is the most densely populated country on the African continent (United Nations – Population Division, 2013). This number illustrates the extraordinary demographic pressure, which is imposed upon the soils of Rwanda to ensure food security. Keeping in mind this responsibility beard by Rwandan soils, the issue of non-responsiveness reveals to be more than an unfavourable classification but rather a dramatic liability for the farmer. Therefore it is imperative to investigate and fix the causes of non-responsiveness.

The central question of this research is: Which nutrients are insufficiently supplied and are thus a limiting factor for biological nitrogen fixation and plant growth? Further on, other than nutritional soil properties will be taken into consideration. Finally conclusions can be drawn about which factors inhibit optimum crop production.

3 Material and methods

3.1 The double pot technique

The double pot technique has been developed by JANSSEN (1974) and represents a method for easy and "rapid identification of the nutrients which are in short supply in soils." The principle of this technique is to provide two different sources for nutrient-uptake, which the plant can access simultaneously. The first source is the test-soil itself; the second one is a defined nutrient solution. By omitting one selected nutrient in the solution, the plant is forced to draw this nutrient from the soil. If the soil does not supply this omitted nutrient the plant will suffer from deficiency symptoms, such as limited growth and leaf-chlorosis. These symptoms will be visible already in early growth stages, so that conclusions about further development and yield can be inferred already after a few weeks.

An upper pot (pot 1, figure 1) contains the test-soil. It is located upon the pot of the nutrient solution (pot 2). Pot 1 has holes in its bottom to let the roots penetrate into the nutrient solution; whereas pot 2 has a lid to support the weight of pot 1. Between the surface of the nutrient solution and the bottom of pot 1 there is an air space of approx. 1cm to supply oxygen for the roots.

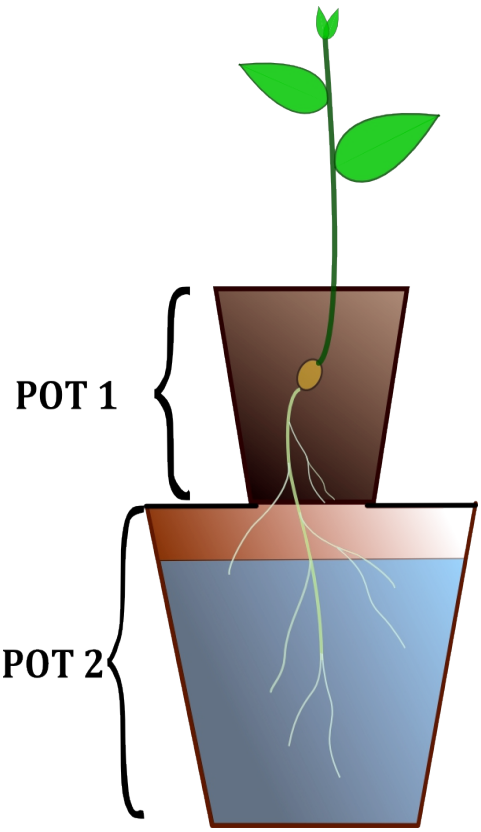

Figure 1: Illustration of the double pot design

3.2 Experimental design

The trial was set up in a greenhouse of the Rwanda Agriculture Board (RAB) of the southern province in Rubona (Huye). It had a completely randomised block design with four replicates and altogether it contained 432 treatments. Parts of a common sewage pipe with 9 cm diameter served as upper pots. Small pieces of mosquito-net were taped to their bottom, to prevent the soil from falling into the solution, but providing passage for the roots. As bottom pots small plastic pots of 2 l volume were used. These pots were available in four different colours and each colour represented one replication. On February 9th three seeds of soybean were sown in each pot and

five DAE they were reduced to a single plant per pot. During this time the pots were covered with a sheet of white paper to reduce evaporation. Seeds were inoculated with *Legumefix* inoculant by *Legume Technology Ltd.*. After thinning I added 40 g (80 g for Kawangire) of gravel, which had been obtained by sieving the soils. The variety of soybean I used is called TGX 1740-2F(SB19). A germination test revealed a germination rate of 94.6 %. The nutrient solutions have been renewed according to the harvests on February 27th and on March 11th. The pH of the solutions was adjusted by using a 10 % HCl for acidic solutions (-K treatments) and a 0.1 molar NaOH for alkaline solutions (complete+N, complete, complete+lime, -P, -P+lime, -S and -MICRO treatments); the exact amounts and pH measurements are given in Appendix III. To provide equal conditions for all treatments it was necessary to keep the soils constantly at field capacity. To do so, the initial plan was to weigh five pots of each soil from every block every second day and thus to measure the evaporation and the missing amount of water. But the conditions in the greenhouse did not allow to wait two days between the watering and forced us to weigh them every day. The plants grew very fast and soon they started to influence the measurements. Thus they were not weighed anymore, but the average amount of water of the last measurements was simply added every day. The soil of Cyabingo (chapter 2..3.1) was somehow very problematic regarding the procedure of watering. It became too fast too dry and was simply too firm to soak again. So some holes were bored in the soil to increase its surface and thus allow more water to enter the compact aggregate. In early March we observed small, pale scars along leaf veins which we identified after some time as damages of thrips. To prevent further damage and influence on the experiment, we applied *LAMBDA CYHALOTHRIN 100 EC* all over the greenhouse.

3.3 Experimental Factors

3.3.1 Description of the sites

The experiment was designed as a two-factorial experiment. The first factor to be tested was the soil, which had four different treatments. Four different soils, from three different AEZ of Rwanda were collected and tested. One soil from the northern province around Rwaza (A), another one from the eastern province around Kayonza (D) and two soils from the southern province around Kamonyi (B, C). The samples were taken from the top soil (0–30 cm) in a W-pattern to ensure a representative sample of the tested field.

- A: Cyabingo – Rwaza (northern province): S1° 34' 2.352" E29° 40' 44.22"
- B: Musambira – Kamonyi (souther province): S1° 59' 31.308" E29° 51' 43.956"

- C: Nyarubaka – Kamonyi (southern province): S2° 6' 25.992" E29° 48' 52.74"
- D: Kawangire – Kayonza (eastern province): S1° 48' 29.196" E30° 27' 0.972"

Chemical and physical analysis of each soil were done by *Crop Nutrition Laboratory Services Ltd* at Nairobi in April 2013. Phosphorous content was assessed using the *Olsen*-method, nitrogen through *Kjeldahl*-digestion and other nutrients by *Mehlich* three stock solution. On every site, apart from Cyabingo, the trials to identify non-responsiveness of soil were conducted with soybean. Since the most important legume in the northern region is climbing bean, this was the test crop for the experiment in Cyabingo.

Cropping histories on the sites after the trials were as shown below:

Cyabingo: maize → peas → fodder crops

Musambira: pineapple

Nyarubaka: maize intercropped with bush bean; cassava

Kawangire: maize intercropped with beans → Irish potatoes → sorghum → soybean; cassava

3.3.2 Nutrient treatments

The second factor were different nutrient solutions, used to reveal nutrient deficiencies in the soil. Eight different nutrient treatments were integrated, namely:

1. Control (only distilled water)
2. Complete + nitrogen
3. Complete
4. -P
5. -K
6. -Mg
7. -S
8. -Micronutrients

Only the second treatment (complete + N) provided an additional nitrogen source, all other treatments had a nitrogen free solution. This was due to indications of nitrogen deficiencies in preliminary double-pot experiments (van der Starre, 2012; Foli, 2012). A Ca omitting treatment has not been integrated, since plant roots do not grow into Ca deficient solutions (JANSSEN, 1970). Besides, most Rwandan soils were described as sufficiently supplied with Ca (VANDER ZAAG et. al, 1983). The concentration of nutrients is derived from a standard *Hoagland solution* in a half

dilution and modified for the specific use with soybeans (PARADISO et al. 2012); the ion concentration in the complete+N treatment was (in mM): N 7.5, P 0.5, K 3.0, Ca 2.5, Mg 1.0, S 1.0; (in µM): Fe 60.0, Mn 7.4, Zn 0.96, Cu 1.04, B 7.13, Mo 0.01. All other treatments have the same composition, apart from their specific omitted nutrient; their exact concentrations and amounts per pot are shown in appendix. II.

In addition to the given factors, separate lime treatments were integrated for soils with a pH below 5 (Cyabingo and Musambira). The lime was only applied in the complete treatment and the -P treatment, since P was expected to be a major restricting factor and it may be a reason for non-responsiveness. Lime treatments were marked with a star:

3* Complete + lime

4* -P + lime

To determine the exact pH of the soils I have conducted own measurements with a 0.01molar solution of $CaCl_2$. On the basis of the measured values (Appendix III), lime was added to the soils of Cyabingo and Musambira. To calculate the necessary amount of lime a buffer curve as described by JOHNSTON and ASKIN (2005) was created (Appendix III). A second pH measurement at the end of the trial revealed an increase of the pH to 5.4 for the soil of Cyabingo and to 5.3 for Musambira, respectively.

3.4 Measurements

To determine relative growth rate, destructive biomass measurements were done at three growth stages. The first harvest date was at 14 DAE, the second at 26 DAE and the final harvest at 34 DAE. For each harvest date the following measurements were taken: stem height, aboveground biomass, root biomass, number of nodules and the number of active nodules out of four randomly chosen. Stem height has been measured from the base of the first root to the growing tip. The plants were oven dried at 70 °C for 48 h and afterwards their roots and shoots were weighed separately. The plain number of nodules was counted, regardless of their position and size. Then four nodules were randomly chosen and visually checked, if they were active or not. Nodule activity was assessed by slicing the nodules and noting their colour; active nodules have a deep red or pink colour, whereas inactive nodules are greyish (FAO, 1984). From 10 DAE on, we made visual observations of deficiency symptoms and recorded them. To analyse the nutrient content of the plants, four replicates of each treatment were collected, grind to a homogeneous sample and

analysed by *Crop Nutrition Laboratory Services Ltd* at Nairobi for all nutrients using *ICP* analysis. The nutrient contents in the plants allow to compare the availability of single nutrients.

3.5 Mathematics and statistical analysis

According to JANSSEN (1974) the single nutrient treatments should be compared to the complete treatment, which has theoretically the highest biomass accumulation, due to optimal conditions for growth. The parameter to express the relationship between nutrient treatments and complete treatment is called "sufficiency quotient" (SQ). This quotient is derived from the "relative growth rate" (RGR), which reflects the relative net growth during a certain period of time and is calculated by using equation (1) (RGR = R_S, S = aboveground biomass; t = time). Equation (2) is the integration of equation (1) and shows the mean value of the RGR within the period of two dates. The ratio of the RGR of a nutrient treatment ($(R_S)_{-K}$; in this case -K treatment) and the RGR of the complete solution ($(R_S)_C$) is called the sufficiency quotient (SQ_p) and shown in equation (3). If the sufficiency quotient is multiplied by 100, it reflects the percentage growth of a treatment compared to the complete treatment.

(1) $$R_S = \frac{1}{S}\frac{dS}{dt}$$

(2) $$R_S = \frac{(lnS_2 - lnS_1)}{(t_2 - t_1)}$$

(3) $$SQ_p = \frac{(R_S)_{-K}}{(R_S)_C}$$

The experiment was randomised using random numbers of OpenOffice.org Calc (version 3.4.1). The statistical analysis and the graphics were made with the programming language R (version 2.15.1). The packages for graphical illustration were "Hmisc" (version 3.10-1.1) and "gplots" (version 2.11.0). Every tested factor was analysed separately for each soil with a univariate ANOVA with a significance level of $\alpha = 0.05$. The normality and the homogeneity of variance of the residuals have been tested with the Shapiro-Willk-Test and the Levene-Test, respectively. In case of violating the homogeneity of variance a *log(y)*- or *sqrt(y)*-transformation was made, to adjust the data. If there was only a violation of normality, data were not transformed, since the experiment was completely randomised and hence normality generally assumed (STEVENS, 1999). Outliers were not considered in the ANOVA, but they may be the reason for extraordinary width of error bars. Outliers were defined as shown in equation (4): x = value of data, Q_1 = lower quartile Q_3 = upper quartile, IQR = interquartile range.

(4) $(Q_3 + 1.5 * IQR) < x < (Q_1 - 1.5 * IQR)$

Successively a multiple paired comparison of the means was performed with the Tukey-HSD-Test and illustrated with letters using the package “multcompview” (version 0.1-5). A non-linear regression showed the relation of aboveground biomass and stem height. No ANOVA was performed for the data of activity of nodules, since they were not assessed as metric values. Lime treatments were only compared to the corresponding treatment without lime and not to other nutrient treatments, since liming is another experimental factor. Nevertheless, lime treatments are shown in every figure and table to display their performance.

4 Results

4.1 Soil analysis

Table 1 reflects the soil characteristics assessed by the analysis of *Crop Nutrition Laboratory Services Ltd* at Nairobi. The analysis of the laboratory was conducted in de-ionised water only and reveals a pH below 5 only for Musambira. In contrast, the analysis at RAB Rubona conducted with a pH-meter in a 0.01 mol/l solution of KCl showed a pH below 5 for Musambira and Cyabingo. According to the FAO (2006) the soil textures are classified as sandy clay loam (SCL) for Cyabingo and Nyarubaka, sandy clay (SC) for Musambira and clay (C) for Kawangire. However, during the experiment I had the impression, that Cyabingo was the soil hardest to handle and seemed to have the highest clay content. The soil analysis report included an interpretation of the given data, which classified all soils to have a "low" potassium content. Besides, the soils of Cyabingo and Musambira were classified to be insufficiently supplied with phosphorous, calcium, magnesium and nitrogen; additionally Musambira had a "low" organic carbon content. The Ca:Mg ratio has been evaluated as "low" for every soil.

Table 1: Nutrient contents and soil properties

Sites	pH (H_2O)	P	K	Ca	Mg	Na	EC(S)	C	N	CLAY	SAND	SILT
		ppm					uS/cm			%		
Cyabingo	5.01	8.4	119	1300	216	26.5	93	2.33	0.12	32.9	45.1	22.0
Musambira	4.61	3.2	93.7	78.4	22.5	19	56	1.42	0.09	40.9	55.1	4.0
Nyarubaka	6.14	61.0	61.3	513	144	27.8	65	2.27	0.20	20.9	67.2	12.0
Kawangire	5.99	82.6	116	2620	509	32.2	95	3.48	0.21	48.9	37.1	14.0

4.2 Visual observations

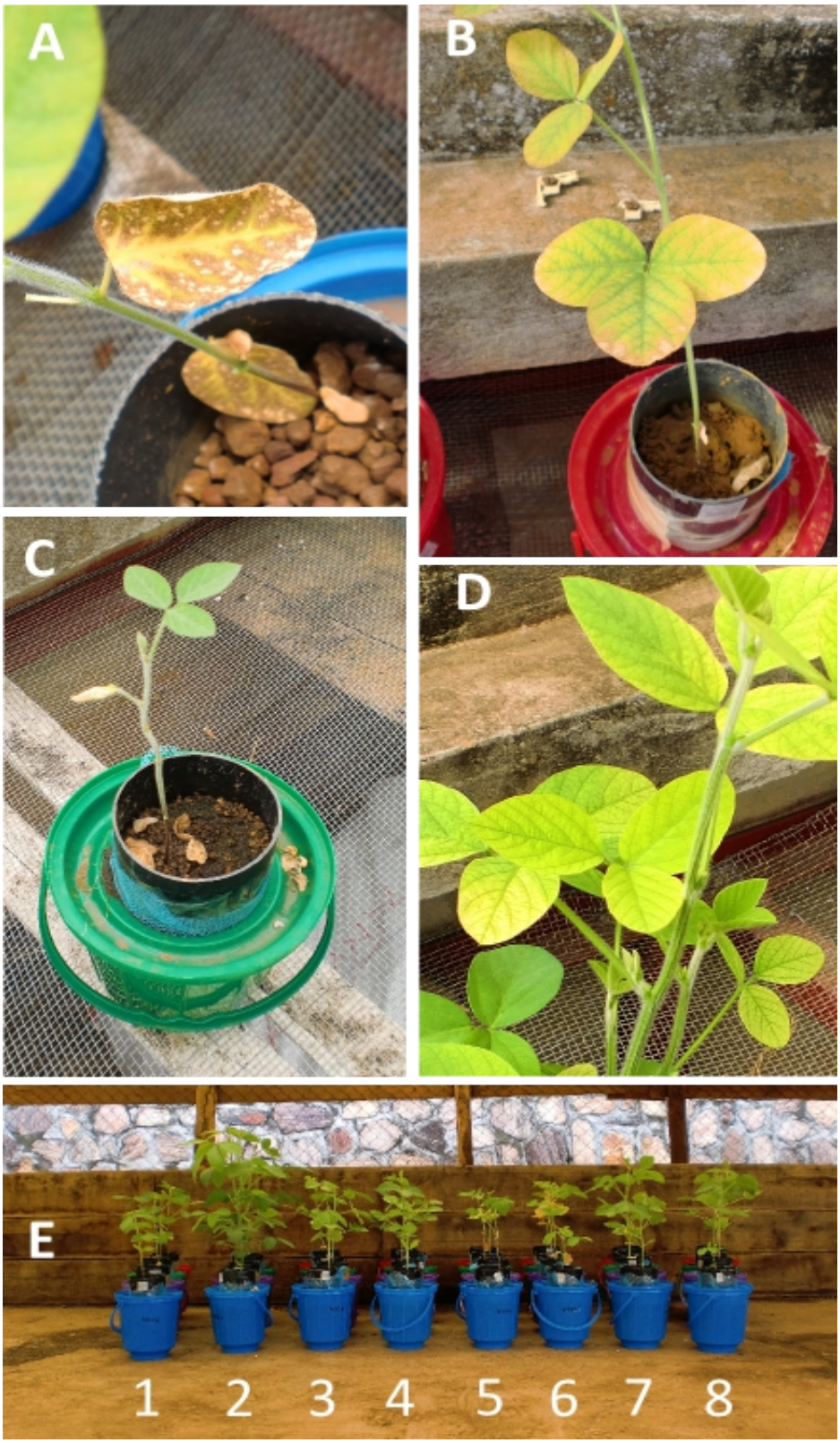

Figure 2: Deficiency symptoms; *A (-K, Kawangire), C (-K, Nyarubaka): Potassiom deficiencies, B (-Mg, Cyabingo): Magnesium deficiency, D (complete+N, Nyarubaka): Nitrogen defeciency, E (Kawangire): Comparison of treatments*

First visual symptoms occurred 12 DAE in -K treatments and were constant during the whole experimental period. Those were visible as pale and yellow chlorosis on the whole leaf and strong necrosis on the leaf edges (figure 2); finally old leafs were shed. These symptoms occurred across all -K treatments of every soil and even scattered across other treatments; especially -Mg treatments. Generally K deficiency symptoms were also visible in -Mg treatments and the other way around. Mg deficiency symptoms occurred in almost every -Mg treatment and were strongest in treatments of Cyabingo and Musambira. Different symptoms, which were identified as Nitrogen and Magnesium deficiency symptoms occurred scattered among different treatments, but strongest in complete+N treatments, due to its excessive growth.

4.3 Plant tissue analysis

The analysis of nutrient contents in plant tissue allows to compare the nutrient availability of the different treatments. To compare the availability of one particular nutrient, it is important to consider the complete treatment, which has the highest availability for every nutrient, the control, which reflects the nutrient availability of the testsoil and the specific treatment, which omits this nutrient (table 2). Regarding the first, the nitrogen concentrations shows that -K treatments performed extremely badly in acid soils. Their nitrogen concentration was even lower than the control treatment and the complete treatment was 143 % and 81 % higher for Cyabingo and Musambira, respectively. The lowest nitrogen content from Nyarubaka had the control treatment and from Kawangire the -Micro treatment. Comparing the nitrogen contents of the complete+N and the complete treatments displays, that the nitrogen fertilized treatment has a higher nitrogen concentration only in one case (Musambira), in another (Kawangire) it is even lower. The analysis of phosphorous content revealed extremely low values in -P treatments of every soil. The complete treatment of Musambira has a 24.57 times higher concentration than the -P treatment. Liming had no observable effect on the availability of phosphorous. The full results of ICP analysis, including micronutrients are shown in appendix V.

Table 2: Nutrient concentration of plant analysis; *Compl= Complete; Contr= Control; Treat= Treatment; Values in the column "Treat" are the concentrations of those treatments, in which the particular nutrient was omitted.*

Nutrients	**Cyabingo**			**Musambira**			**Nyarubaka**			**Kawangire**		
	Compl	Contr	Treat	Compl	Contr	Treat	Compl	Contr	Treat	Compl	Contr	Treat
N [%]	2.41	1.36		2.46	1.62		2.67	1.62		3.47	2.36	
P [%]	1.21	0.09	0.18	1.72	0.12	0.07	1.14	0.30	0.16	1.12	0.32	0.23
K [%]	3.02	0.74	1.28	3.25	0.98	1.64	2.79	1.13	1.87	2.79	1.03	0.99
Mg [%]	0.39	0.51	0.23	0.42	0.15	0.09	0.39	0.48	0.24	0.44	0.79	0.39
S [ppm]	0.36	0.18	0.17	0.46	0.12	0.23	0.32	0.09	0.08	0.34	0.19	0.17

4.4 Stem height

The stem height should be an equivalent to the descriptions of JANSSEN (1974), who used the leaf length of maize to determine the sufficiency quotient. However, preliminary experiments have shown that the stem height is not an appropriate indicator for RGR (van der Starre, 2012). Nevertheless there are stable allometric dependencies between stem height and stem biomass of soybean (REDDY et al., 1998), which show its suitability as growth indicator. The data from this experiment and the relationship between stem height and aboveground biomass are well described by a logarithmic function (figure 3). A non-linear regression using the model formula $f(x) = a * ln(x) + b$ estimated the parameters a= 98.313 (P < 0.001) and b=308.369 (P < 0.001). However, there is no scientific principle for using this model, it has simply been chosen by visual estimation.

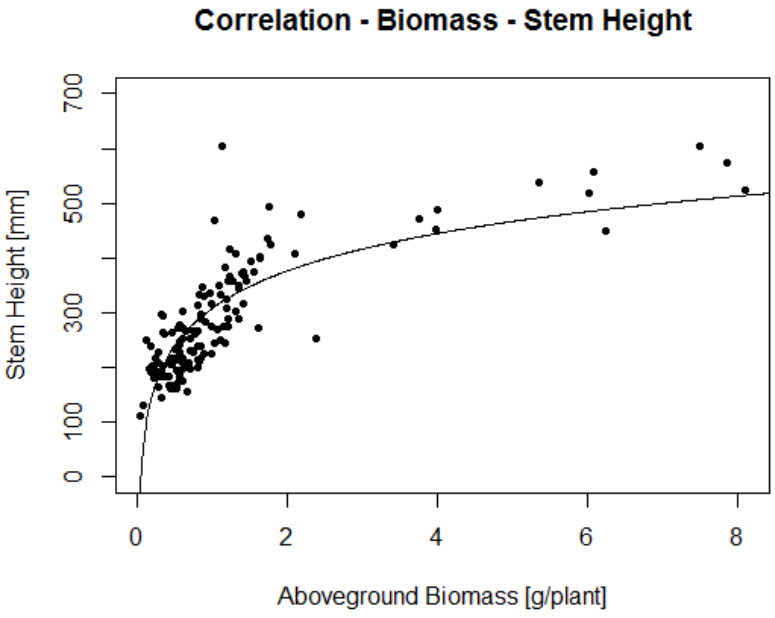

Figure 3: Non-Linear Regression of Biomass and Stem Height

Regarding the average stem height of all soils, complete+N treatments had a significantly increased height compared to all other treatments (table 3). The only soil which shows no significant differences between the treatments is the soil of Nyarubaka. Whereas the soil of Musambira does not show significant differences for complete, -P, -S and -Micro compared to complete+N. In the whole experiment, the -Micro from Cyabingo is the only treatment without Nitrogen, which is significantly higher than the control. All the treatments of the soil from Kawangire but -S are different from complete+N, which has the tallest average height of all treatments of the whole experiment (566 mm). The lowest value has -K from Musambira (148 mm). Liming did not have a significant effect but treatments of complete + lime had a taller stem than complete without lime in both soils.

Table 3: Stem Height; *Treatments which are marked with the same letter within a column are not significantly distinct from each other at α=0.05. Lime treatments (3*, 4*) were analysed separately and are marked with Greek letters.*

Treatment	Cyabingo	Musambira	Nyarubaka	Kawangire	Average
			(mm)		
control	193 c	225 ab	280 a	294 b	248 b
complete +N	463 a	375 a	404 a	566 a	452 a
complete	270 bc α	231 ab α	309 a	327 b	284 b
-P	216 bc α	206 ab α	299 a	304 b	299 b
-K	247 bc	149 b	227 a	247 b	213 b
-Mg	187 bc	188 b	270 a	307 b	237 b
-S	293 bc	216 ab	276 a	381 ab	271 b
-Micro	318 ab	235 ab	258 a	341 b	270 b
compl + lime	278 α	316 α			
-P + lime	179 α	194 α			

4.5 Nodulation

Since nodules are the motor for biological nitrogen fixation, they are mandatory to supply the plant sufficiently with nitrogen and to prevent growth depressions. According to HARDARSON et al. (1989) not only nodules at the crown root zone but also nodules at lateral roots and at deeper roots are responsible for the amount of N_2 fixed. Consequently the total number of nodules was counted to illustrate the nodulation exactly. On average only control, complete+N and -K treatments had significantly less nodules than complete treatments, whereas -Micro treatments - which had the highest value of 40.7 nodules - are significantly higher than control, complete+N, -K and -Mg treatments (figure 4). Liming had no effect on nodulation. Besides the number of nodules it is very important to consider their activity. By checking four nodules of each pot for their colour, we made a classification of five classes: 0%, 25%, 50%, 75% and 100% active. From these classes we derived the average activity of nodules. It is obvious that the complete + N treatments had the lowest average activity rate of all treatments (20.0%), whereas complete treatments had the highest average activity with 96.9% active nodules. But the average activities of control + lime, -P, -S and -Micro treatments were hardly lower than the one of the complete treatment. Besides the complete+N treatments, treatments of -P + lime, -K and -Mg showed an average activity below 75%.

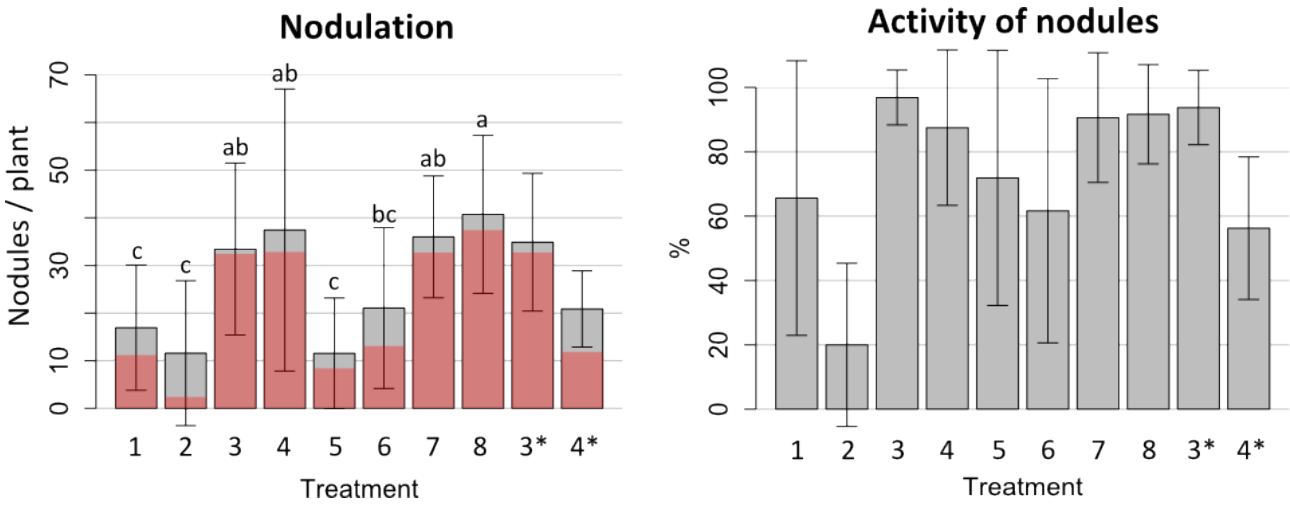

Figure 4: Average nodulation and estimated nodule activity of treatments; *Red shades illustrate the number of nodules multiplied by their estimated activity. Lime treatments (3*, 4*) were not considered in the anova.*

4.6 Aboveground Biomass

The aboveground biomass reflects the vegetative growth and is the most important indicator for growth conditions and nutrient supply for the double-pot technique. During the experiment it was obvious that complete+N treatments of every soil had an extraordinary growth, which exceeded the growth of all other treatments for many times. The advantage of nitrogen fertilised treatments could already be observed at the first harvest date; their biomass production was significantly higher compared to most other treatments for the soils of Nyarubaka and Kawangire (figure 5). From the second harvest every complete+N treatment was significantly higher than any other treatment for every soil. Finally the third harvest displayed the clear dominance of every complete+N treatment. Another significant observation made was the bad performance and the minor growth of -K treatments, which could be verified as well. Every complete+N, complete, -P and -S treatment at 34 DAE shows significant differences compared to the -K treatment, for Cyabingo and Musambira also the -Micro treatments. One can easily observe, that complete+N has always the highest and -K the lowest value; for each soil both are significantly distinct from the complete treatment at 34 DAE. The highest value has complete+N treatment from Kawangire (6.32 g), where the lowest has -K from Musambira (0.12 g). Liming had no visible effect on the soil of Cyabingo, but for the soil of Musambira complete + lime was clearly higher than complete without lime; there was no significant effect on -P treatment. None of the soils showed a significant difference between control and complete treatment.

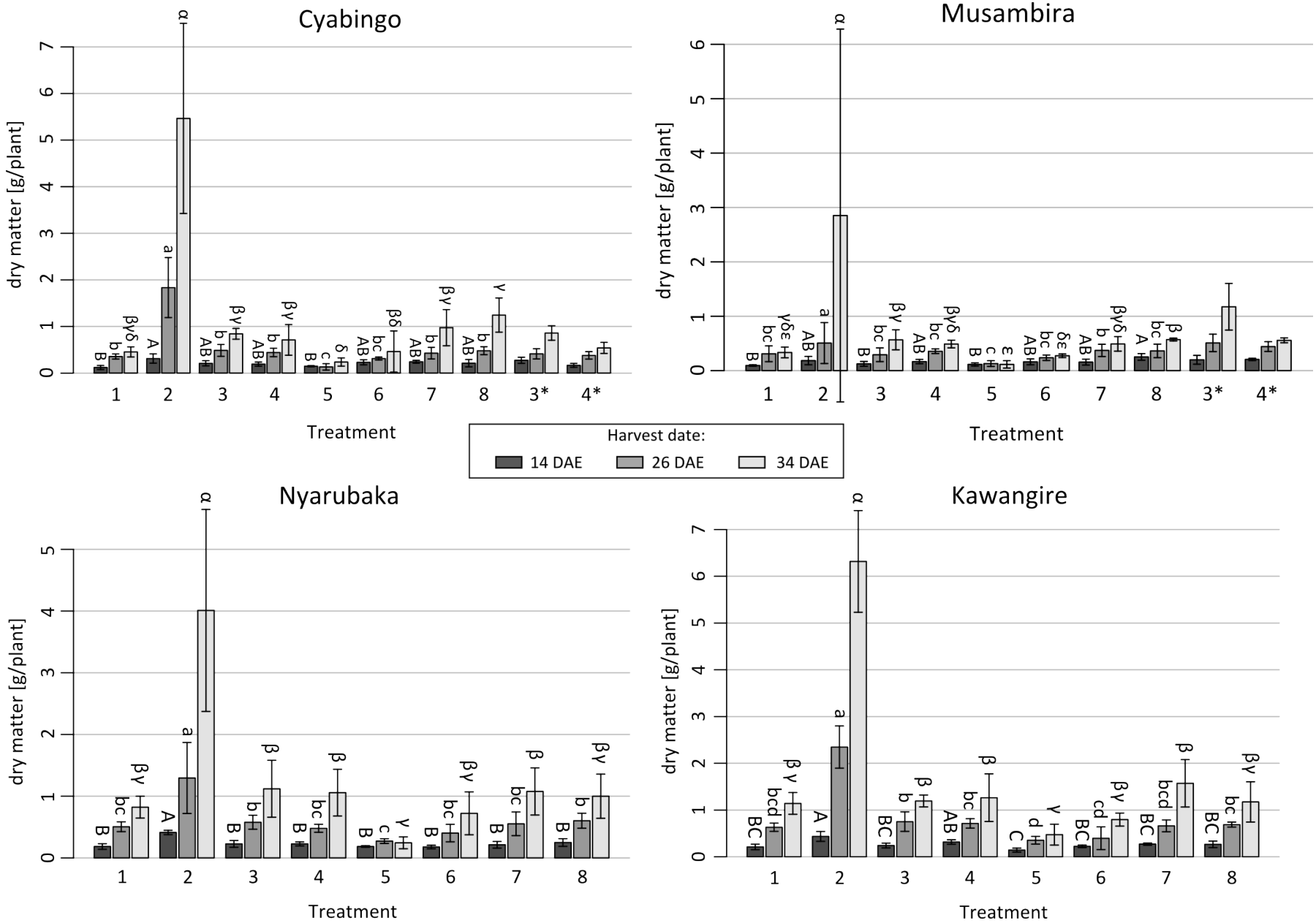

Figure 5: Aboveground Biomass of all treatments at each harvest date; *Treatments which are marked with the same sign within one plot and harvest date are not significantly different from each other at α=0.05 (capital letters: 14 DAE, lowercase letters: 26 DAE, Greek letters: 34 DAE). Lime treatments (3*, 4*) were not considered in the anova.*

4.7 Sufficiency Quotient

JANSSEN (1974) assumes that the relative growth rate, which reflects the relative growth of an individual plant between two points in time, serves as an indicator for an insufficient nutrient supply. Consequently a plant, which is sufficiently supplied with all nutrients, should have a maximum RGR. Following this principle, every treatment apart from complete+N and complete, should have a sufficiency quotient below 1. However this assumption has not always matched the results of this study (table 4). Several treatments show an average SQ above 1: -Micro from Cyabingo, -S from Nyarubaka and Kawangire and the control from Kawangire. Also remarkable is that the control never significantly distincts from the complete treatment. For every soil, apart from Cyabingo, -K treatments have the lowest values; the one from Musambira is even negative (-0.130), which means that the biomass even decreased during the growing period. This can be deduced from shedding of the leaves of several treatments. The -K treatments of Musambira and Nyarubaka are even significantly lower than the control. -Mg treatments are generally low as well; in the case of Cyabingo it has the lowest SQ and in all other soils it is the second lowest. Lime treatments did not have a significant effect on the sufficiency quotient, compared to their corresponding treatment without lime.

Table 4: Sufficiency Quotients; *Treatments which are marked with the same letter within a column are not significantly distinct from each other at α=0.05. Lime treatments (3*, 4*) were analysed separately and are marked with Greek letters.*

Treatment	Cyabingo	Musambira	Nyarubaka	Kawangire	Average
control	0.954 bc	0.848 ab	0.962 b	1.064 ab	0.957 abc
complete+N	2.090 a	1.393 a	1.443 a	1.546 ab	1.623 a
complete	1.000 bc α	1.000 ab αβ	1.000 b	1.000 ab	1.000 ab
-P	0.880 bc α	0.714 ab α	0.955 b	0.837 ab	0.846 bc
-K	0.336 b	-0.130 c	0.149 c	0.698 b	0.263 d
-Mg	0.241 b	0.388 bc	0.860 b	0.785 ab	0.568 cd
-S	0.920 bc	0.757 ab	1.025 ab	1.163 ab	0.956 abc
-Micro	1.262 ac	0.577 abc	0.883 b	0.931 ab	0.913 bc
compl + lime	0.858 α	1.170 β			
-P + lime	0.821 α	0.666 α			

5 Discussion

5.1 Methodology

In order to get reasonable and representative results it is very important to follow the descriptions of JANSSEN (1974) accurately. However, not every detail of his descriptions has been considered as essential, when planning the experiment. We disregarded his method of watering the pots with plastic tubes filled with quartz sand, but simply assumed that it is sufficient to water them manually every second day. Though I realised very fast that it is necessary to water every day, our method did not seem to work properly for every soil (view chapter 3.1). JOHNSTON and ASKIN (2005) emphasized the importance of an accurate watering-system and several studies show the negative effects of an insufficient water supply: SPRENT (1971) reports of a significant reduction of root nodule activity, TANGUILIG et al. (1987) show a significant decline in root and shoot biomass production and nutrient uptake due to water stress; NITAMI et al. (2013) demonstrate the negative effect of water stress on yield components. With respect to these studies, it should be paid attention to the possibility that soil compactness (especially Cyabingo) and indistinct deficiency symptoms (limited growth) may have resulted from temporary drought effects.

Another experimental procedure, which should be reconsidered, is the sieving of the soils, with a 2 mm sieve. The intention to do so was to create equal conditions for every treatment. The soil of Kawangire, however, had a rock fragment content of 58 % and thus is classified as “class A (Abundant)” (FAO, 2006). It is questionable if the soil in the experiment still reflects the “original” soil and its properties, when 58% of its original weight was removed. This fact is important, since there might be other factors, not considered in the experiment, which could contribute to the non-responsiveness of soils. According to the *FAO Field Guide* (2006) the rootability of a soil is classified as “Poor”, when it shows a “high or very high content of coarse fragments (class A and D)”.

One further factor, which has not been integrated in the experiment, is the inoculation of seeds. In this experiment, simply every seed was inoculated with a commercial inoculant. As emphasized by DATE (2000), inoculation may have a strong impact on yield and growth components, depending on specific site properties. We tried to analyse the most important property, which is the occurrence of native rhizobium strains in the soils, by using the MPN method according to SOMASEGARAN (1994). Unfortunately the method failed two times and we have not gained any outcome.

5.2 Sufficiency Quotient

It is remarkable that the outcomes of the sufficiency assessment did not reflect the clear observations, made during the experimental period and verified in the biomass analysis. Most confusing are the facts, that the SQ associated with the control with distilled water hardly differs from the treatment with complete solution, and that some SQs exceed the value of 1. Recalculations of the solutions confirmed their correct composition and invalidated ideas of toxic effects, due to excessive concentrations. I still have not come to a reasonable explanation for these results. One reason for these indistinct results may be a bias in the method of calculating the RGR. HOFFMANN and POORTER (2002) propose to calculate the RGR from the mean natural logarithm-transformed plant weights (eqn. 5). In contrast to the RGR as described by JANSSEN (1974), the single values of one treatment are *ln*-transformed before averaging. Therefore the Sufficiency Quotient seems to be an inappropriate indicator to explain the results of this experiment.

$$(5) \quad R_S = \frac{\overline{\ln(S_2)} - \overline{\ln(S_1)}}{(t_2 - t_1)}$$

5.3 Deficiency Symptoms and biomass

The most striking result of this experiment is the limited growth and development in potassium omitted treatments. Every plant, which grew without K supply, showed lean performance and extremely inhibited growth. There are hardly any reports in literature of such severe potassium deficiency symptoms in soil-grown soybeans. However, there are several observations of a reduced vegetative growth, due to moderate potassium deficiencies (ITOH et al., 1997; PREMARATNE and OERTLI, 1994; SALE and CAMPBELL, 1987). Further on PREMARATNE and OERTLI (1994) report of a significant reduction of nodulation and similar visual symptoms as described in this study. This observation makes an ample application of K fertiliser and a sustainable conservation of the increased K level mandatory. Therefore the most important cropping factor, which needs to be taken into account, apart from K fertilisation, is the effective recycling of crop residues, so that fertilized potassium will return to the soil. Organic residues of several Rwandan crops contain considerable amounts of potassium, which can easily be accessed by the subsequent crop (LUPWAYI et al. 2005, MUBARAK et al. 2007, LINQUIST et al. 2007).

Though, it seems confusing that the control is never significantly distinct from the complete treatment. This observation shows that not only nutrient supply determines plant growth, but also

an appropriate composition of nutrients is mandatory. However, there was a clear demonstration of the growth potential by complete+N treatments, which had the same nutrient composition as complete treatments apart from nitrogen. The data suggest that even optimum nodulation is too inefficient to compete with nitrogen fertilizer. A coherent explanation for this unexpected difference in biomass production is still missing. In contrast to the given results and the wide difference between N-fertilized treatments and N-free treatments SALVAGIOTTI et al. (2008) conclude, that "BNF can provide the majority of the required N supply for soybean unless there are soil restrictions for normal nodule activity". These restrictions (e.g. insufficient water supply) may be part of an explanation. Another explanation, is the possibility that the formation of effective nodules is an "expensive deal" for the plant. The review of KASCHUK et al. (2009) demonstrates the costs for plants to gain nitrogen through rhizobia compared to costs of nitrate-reduction. In the case of soybean the nitrogen acquisition through nitrate-reduction was clearly more effective than through rhizobia.

5.4 Plant analysis

To interpret the outcome of the plant analysis correctly, it is necessary not only to consider the nutrient concentration in the plant tissue, but also to pay attention to the biomass production of each treatment. It seems surprising, that only in one case (Musambira) the complete+N treatment has a higher nitrogen concentration than the complete treatment, but given the extraordinary growth of plants in the complete+N treatment, the total amount of nitrogen taken up by the plant was several times higher in this treatment. The extremely low phosphorous concentrations were unexpected, considering the good performance of -P treatments. This observation makes clear that phosphorous is not optimally supplied by the soils, but still not a limiting factor for growth. However, the analysis of potassium concentrations confirmed the visual observations and provided further evidence that potassium is the major limiting factor for plant growth.

6 Conclusions

- K is a major limiting factor for growth in every soil assessed. It is imperative to improve the K supply at every site, in order to overcome non-responsiveness. Measures to improve K supply by the soil, e.g. by applying potassium fertiliser and improving K recycling through crop residues, are likely to be necessary.
- Plants grown in acid soils did not profit from lime application. The results suggest that, liming at planting cannot be considered as an appropriate measure, to counter non-responsiveness.
- Nitrogen fertilized treatments exceeded the growth of any other treatment several-fold. This indicates that other factors inhibited optimal nitrogen fixation in treatments having received no N fertiliser. Further investigations on physical soil properties may be needed to identify limitations to nitrogen fixation.
- The texture and structure of some soils seem to have had negative influences on plant growth. Particularly the high clay content in the soil of Cyabingo led to an extremely compact soil, which might have inhibited root development. The use of organic inputs can help to reduce bulk density of compact soils (BRONICK and LAL, 2005).
- The implementation of the double pot experiment should follow the recommendations of JANSSEN (1974) as closely as possible. Especially water management in a greenhouse under tropic conditions has to be done with great accuracy. Manual watering of pots with a pipette led to heterogeneously moistened soils and undesirable runoff.

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Appendix I – Experimental plan

Replication / Block:

1 (purple pots)
2 (red pots)
3 (green pots)
4 (blue pots)

Soils:

A: Rwaza
B: Musambira
C: Nyarubaka
D: Kayonza

Nutrient treatment:

1: Control
2: Complete + N
3: Complete
4: -P
5: -K
6: -Mg
7: -S
8: -Micronutrients

Harvest date:

c: 14 DAE
b: 26 DAE
a: 34 DAE

Lime treatments are marked with a "*".

Labelling of treatments, e.g.: 3C4b -> Third replication, soil from Nyarubaka, -P treatment, Harvest date 26 DAE.

Location: Greenhouse of 'Soybean Program' at Rubona (Huye), Rwanda

Crop type: Soybean (*Glycine max* L., cv. TGx 1740-2F)

Sowing date: February 9th, 2013

Harvest of last pot: April 4th, 2013

Sowing density: Three seeds per pot (thinned to single plants after emergence)

Fertilization: According to nutrient treatments

1C5a	1A5b	1D3c	PATH	2C2a	2A4b*	2D6c
1C4a	1D5b	1D2c		2D6a	2A8b	2B7c
1A3a	1D7b	1B1c		2C4a	2D1b	2C1c
1D6a	1D4b	1A8c		2D5a	2B4b*	2C5c
1B1a	1B3b	1C4c		2A4a	2B3b	2B3c
1A4a	1B1b	1A7c		2D3a	2A1b	2A1c
1A7a	1D1b	1D4c		2A3a*	2C4b	2A2c
1D2a	1A7b	1C7c		2B3a*	2A7b	2D4c
1A3a*	1B4b*	1C1c		2B2a	2B5b	2B5c
1B8a	1A4b	1D8c		2B5a	2A3b	2C8c
1C6a	1A8b	1A3c*		2B4a	2D7b	2D7c
1A5a	1C3b	1B4c		2B8a	2C1b	2A7c
1B3a*	1A6b	1A3c		2A1a	2B3b*	2A3c
1D8a	1C2b	1B3c*		2C6a	2C3b	2A4c*
1D5a	1D8b	1A6c		2B3a	2B8b	2A5c
1D4a	1C1b	1C8c		2D2a	2B2b	2D5c
1D7a	1C5b	1A4c		2B7a	2D2b	2D2c
1C2a	1B6b	1C6c		2B6a	2A5b	2D1c
1B4a	1C7b	1D7c		2C1a	2C7b	2A6c
1C1a	1B5b	1D6c		2D7a	2A3b*	2C3c
1D3a	1B8b	1B4c*		2A7a	2A4b	2A3c*
1B7a	1B4b	1C3c		2A8a	2B1b	2B1c
1B6a	1A3b	1B8c		2A5a	2C8b	2C6c
1C7a	1A1b	1B5c		2B1a	2C5b	2B2c
1A4a*	1A4b*	1B2c		2C3a	2A6b	2B8c
1A6a	1D6b	1B7c		2A6a	2B6b	2B3c*
1D1a	1C4b	1D5c		2D1a	2C6b	2D3c
1A1a	1D2b	1A1c		2C5a	2B7b	2C7c
1A8a	1D3b	1B6c		2D8a	2A2b	2C2c
1B5a	1B3b*	1A4c*		2C7a	2D3b	2B4c*
1A2a	1C8b	1C2c		2A2a	2D6b	2D8c
1C3a	1B2b	1B3c		2B4a*	2B4b	2A4c
1B4a*	1A2b	1C5c		2D4a	2C2b	2A8c
1B2a	1A3b*	1A5c		2A4a*	2D5b	2B6c
1B3a	1B7b	1A2c		2A3a	2D8b	2B4c
1C8a	1C6b	1D1c		2C8a	2D4b	2C4c

3A8a	3B3b	3B5c	PATH	4C7a	4A3b	4D6c
3C2a	3C2b	3C1c		4B3a*	4B3b*	4C5c
3D8a	3D8b	3B6c		4B3a	4C6b	4D5c
3B5a	3C6b	3D1c		4B4a*	4C2b	4A7c
3C8a	3D4b	3C4c		4D6a	4A6b	4A5c
3C4a	3B6b	3C8c		4D8a	4D1b	4C6c
3C5a	3A2b	3B1c		4D1a	4D7b	4A8c
3A4a	3A4b*	3D4c		4C3a	4B3b	4C8c
3A7a	3A5b	3B2c		4B5a	4A1b	4B3c
3B7a	3C3b	3C3c		4B6a	4A4b*	4B4c*
3A2a	3C8b	3B4c		4A1a	4A8b	4A4c
3B8a	3A7b	3A6c		4A5a	4A7b	4C3c
3B2a	3B1b	3D5c		4A3a	4C5b	4B7c
3D2a	3B7b	3D7c		4A7a	4B5b	4B2c
3B6a	3D7b	3A3c		4C8a	4D4b	4B1c
3A1a	3C5b	3B3c*		4C6a	4D5b	4C2c
3C3a	3D3b	3B4c*		4A2a	4C1b	4A2c
3C6a	3A3b	3A5c		4D2a	4C7b	4B3c*
3B4a*	3B8b	3B7c		4B2a	4C8b	4D1c
3D5a	3D6b	3D8c		4D5a	4B1b	4C1c
3D6a	3C1b	3A2c		4C2a	4D3b	4A3c*
3A4a*	3B5b	3A3c*		4A4a*	4B6b	4B6c
3D1a	3B4b*	3B3c		4A6a	4B8b	4D2c
3B3a	3A6b	3C2c		4D3a	4A5b	4C7c
3A5a	3B2b	3D3c		4C4a	4C4b	4A4c*
3D3a	3D2b	3B8c		4C5a	4D2b	4B4c
3A6a	3C4b	3C7c		4A4a	4B4b*	4D8c
3B3a*	3A1b	3C6c		4D7a	4B2b	4D3c
3D7a	3A3b*	3A4c*		4D4a	4A3b*	4C4c
3A3a	3A8b	3A4c		4B1a	4C3b	4D7c
3B1a	3B4b	3A7c		4A8a	4B7b	4A6c
3D4a	3A4b	3C5c		4B8a	4D8b	4B8c
3C1a	3D5b	3D6c		4B7a	4A4b	4D4c
3B4a	3D1b	3D2c		4B4a	4A2b	4A1c
3C7a	3B3b*	3A8c		4A3a*	4B4b	4A3c
3A3a*	3C7b	3A1c		4C1a	4D6b	4B5c

Appendix II - Composition of nutrient treatments

	Molar weight (g/mol)	Desired concentration (mmol/l; µmol/l)	Amount per liter (mg)	Amount per pot (mg)
N		**7.5**		
NH_4NO_3	80	1.25	100.0	150.0
$Ca(NO_3)_2 \cdot (H2O)_4$	236,2	2.5	590.5	885.75
P		**0.5**		
H_3PO_4	98	0.5	49.0	73.5
K		**3.0**		
K_2CO_3	138.2	1.5	207.3	310.95
K_2SO_4 (only in -Mg)	s.b.			
Mg		**1.0**		
$MgSO_4 \cdot (H_2O)_7$	246.4	1.0	246.4	369.9
$MgCl_2 \cdot (H_2O)_6$ (only in -S)	203.3	1.0	203.3	304.95
S		**1.0**		
$MgSO_4 \cdot (H_2O)_7$	s.a.			
K_2SO_4 (only in -Mg)	174.3	1.0	174.3	261,45
Ca		**2.5**		
$CaCl_2 \cdot (H2O)_2$	147	2.5	368.0	552.0
Mn		**7.4**		
$Mn(II)Cl_2 \cdot (H2O)_2$	197.9	7.4	1.465	2.198
B		**7.13**		
H_3BO_3	61.8	7.13	0.441	0.662
Cu		**1.04**		
$Cu(II)SO_4 \cdot (H2O)_5$	249.7	1.04	0.260	0.390
Zn		**0.96**		
$Zn(II)SO_4 \cdot (H2O)_7$	287.6	0.96	0.276	0,414
Mo		**0.01**		
$Na_2MoO_4 \cdot (H2O)_2$	241.9	0.01	0.002	0,003

Appendix III - pH measurements and adjustment of nutrient solutions

Treatment	Average pH	Amount of HCl added (ml)	Amount of NaOH added (ml)
2	7.6	0.18	-
3 / 3*	7.7	0.18	-
4 / 4*	7.8	0.18	-
5	5.6	-	2.0
6	6.4	-	-
7	7.8	0.18	-
8	7.8	0.18	-

Buffer curve and lime calculations

pH-buffer curves with x ml of a saturated $Ca(OH)_2$-solution

Cyabingo		**Musambira**	
x	**pH**	**x**	**pH**
0.0	4.5	0.0	3.7
2.5	4.7	2.5	3.9
5.0	5.0	5.0	4.0
10.0	5.4	10.0	4.3
20.0	5.8	20.0	4.8
40.0	6.4	40.0	5.9
Volume which leads to pH 5.6:	15.0 ml		30.0 ml
Amount of lime per pot:	0.238 g		0.594 g

Formula to calculate the amount of lime per pot: $L = V * c/0.5 * M * S/20$

L: Amount of lime per pot (g)
V: Volume of $Ca(OH)_2$ needed to attain the desired pH (ml)
c: Concentration of $Ca(OH)_2$ in a saturated solution (= 0.038 mol/l)
M: Molar weight of $CaCO_3$ (= 100 g/mol)
S: Amount of soil per pot (= 250 g)

Appendix IV – Measurements of soil properties and watering management

	Cyabingo	Musambira	Nyarubaka	Kawangire
Stone content (%)	6	10	21	58
Water content at field capacity (%)	38	28	18	32
Amount of water given daily (ml)				
Block				
1	39	36	23	31
2	41	38	25	34
3	34	28	18	23
4	38	35	20	31

Each soil of each block was watered separately

Appendix V – ICP Analysis of plant tissue

		N	P	K	Ca	Mg	Mn	B	Zn	Fe	Cu	Na	S
Site	**Soil treatment**	**%**	**%**	**%**	**%**	**%**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**
Cyabingo	Control (distilled water)	1.36	0.09	0.74	1.10	0.51	439.00	40.70	41.00	136.00	4.19	27.60	0.18
	Complete + Nitrogen	2.57	0.63	2.64	1.79	0.42	190.00	32.70	17.80	139.00	4.79	28.80	0.28
	Complete	2.41	1.21	3.02	1.58	0.39	302.00	42.60	35.20	167.00	6.00	19.60	0.36
	complete + lime	2.62	1.11	2.93	1.62	0.37	111.00	40.50	27.00	158.00	6.99	24.80	0.37
	Phosphorus	2.31	0.18	2.16	1.37	0.38	264.00	43.70	41.10	144.00	6.50	33.30	0.24
	Phosphorus + Lime	2.62	0.07	1.69	1.41	0.39	106.00	45.60	43.60	172.00	9.07	31.80	0.20
	Potassium	0.99	0.89	1.28	1.80	0.63	467.00	51.70	38.20	484.00	7.37	101.00	0.33
	Magnesium	2.04	1.26	3.16	1.61	0.23	316.00	44.00	36.20	276.00	9.35	68.90	0.37
	Sulfur	2.31	0.69	2.33	1.83	0.38	270.00	45.70	33.70	171.00	6.16	42.20	0.17
	Micronutrients	3.04	1.24	3.06	1.73	0.38	252.00	27.30	19.10	249.00	5.04	35.10	0.35
Musambira	Control (distilled water)	1.62	0.12	0.98	0.43	0.15	1460.00	33.30	51.50	227.00	5.99	42.10	0.12
	Complete + Nitrogen	3.88	0.78	2.92	1.62	0.43	293.00	34.80	19.60	151.00	5.82	48.80	0.35
	Complete	2.46	1.72	3.25	1.88	0.42	722.00	52.00	44.20	185.00	10.60	56.10	0.46
	complete + lime	3.41	1.20	2.85	1.82	0.32	344.00	43.60	30.60	198.00	6.00	38.30	0.36
	Phosphorus	1.52	0.07	1.63	1.27	0.38	700.00	41.00	58.20	166.00	9.41	47.50	0.16
	Phosphorus + Lime	1.73	0.06	1.70	1.41	0.34	363.00	46.70	39.20	178.00	5.87	34.60	0.16
	Potassium	1.36	1.54	1.64	1.94	0.64	1170.00	56.80	71.40	1030.00	16.80	233.00	0.34
	Magnesium	2.46	1.61	3.72	1.63	0.09	955.00	69.70	42.60	254.00	13.30	71.50	0.32
	Sulfur	2.14	1.11	2.81	2.14	0.33	858.00	54.20	54.70	192.00	10.50	61.50	0.23
	Micronutrients	2.37	1.39	2.96	1.90	0.38	804.00	40.00	25.10	181.00	5.04	38.00	0.41
Nyarubaka	Control (distilled water)	1.62	0.30	1.13	1.19	0.48	82.70	29.30	29.60	108.00	3.25	27.50	0.09
	Complete + Nitrogen	2.67	0.72	2.75	1.71	0.43	56.60	36.70	18.80	119.00	5.37	31.80	0.23
	Complete	2.67	1.14	2.79	1.71	0.39	95.50	42.20	40.70	152.00	6.01	50.00	0.32
	Phosphorus	2.46	0.16	2.39	1.56	0.41	77.20	47.00	46.50	125.00	6.95	29.30	0.26
	Potassium	3.20	0.97	1.87	1.98	0.57	76.50	39.20	40.20	161.00	5.73	108.00	0.31
	Magnesium	1.94	1.42	3.04	1.87	0.24	109.00	53.10	31.30	210.00	6.30	36.50	0.32
	Sulfur	3.51	0.49	1.74	1.57	0.33	69.50	38.80	34.50	125.00	3.70	30.30	0.08
	Micronutrients	3.25	0.85	2.14	1.57	0.38	50.20	28.10	15.50	135.00	3.04	42.70	0.25
Kayonza	Control (distilled water)	2.36	0.32	1.03	1.44	0.79	121.00	63.60	36.60	147.00	4.67	27.60	0.19
	Complete + Nitrogen	2.10	0.55	2.60	1.70	0.44	65.40	38.30	15.50	113.00	6.55	25.60	0.22
	Complete	3.47	1.12	2.79	1.69	0.44	102.00	46.50	34.70	205.00	5.86	34.80	0.34
	Phosphorus	2.71	0.23	2.37	1.34	0.39	107.00	43.80	35.30	219.00	7.20	29.30	0.25
	Potassium	2.46	0.76	0.99	1.71	0.81	101.00	64.50	34.60	289.00	5.41	39.00	0.27
	Magnesium	2.94	1.41	2.75	1.83	0.39	130.00	52.00	38.70	241.00	8.32	39.10	0.37
	Sulfur	2.41	0.66	2.27	1.80	0.40	113.00	45.70	36.00	168.00	5.46	26.00	0.17
	Micronutrients	0.89	0.50	1.84	1.01	0.28	24.30	25.10	10.00	69.70	2.12	28.10	0.24