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Dynamics of native and introduced rhizobia under different cropping sequences and soil fertility levels in a Siaya County, Kenya

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To address the problem of decreasing food production and livelihoods resulting from declining soil fertility in Kenya, the conservation and sustainable use of soil micro organisms is critical. To realize this purpose, effective and infective commercial rhizobial inoculants have been evaluated and identified. However, the absence or presence of infective indigenous rhizobia in soils requires to be demonstrated alongside effective commercial inoculants under different cropping sequences, seasons and inoculation regimes. We therefore laid out a completely randomized block design in four replicates in soils of low indigenous rhizobia with 7 cropping sequences and a selected strain of commercial *B. japonicum* (532c) for four seasons. The initial soil characterization revealed that the soils were low in native rhizobia (CFU g^{-1} soil), confirming the need for current interventions by this study. During the short rain (SR) 2014 season, data revealed significant increase in nodulation in the inoculated plots when compared to the uninoculated plots. There was a drastic seasonal increase in nodule weight under the uninoculated mono soybean, an indication of buildup of the native rhizobia with the presence of the host legume. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis distinguished 3 intergenic spacer groups (IGS) denoting low diversity of the native rhizobia able to nodulate soybeans under these conditions. The IGS I corresponded with that of the commercial inoculant applied while IGS II and III were dominant in the uninoculated treatment demonstrating the competitive competence of the indigenous rhizobia. The distribution of the different IGS groups within the experiment was more affected by season, cropping system and treatment (inoculated or uninoculated) rather than site. IGS group II and III had almost similar proportions of nodule occupancy in the first season under the uninoculated sequences. However, with season IGS II predominated indicating its possible adaptation to the local conditions and hence better competitive ability. We conclude that the use of promiscuous soybean varieties in soils with some background rhizobia will increase nodulation and rhizobia diversity as the host legume is included in the cropping sequence. There is need for extensive agro ecological zone evaluation since the native strains could be site specific. Characterization of total bacterial diversity and evenness in the study site is equally recommended

Keywords: IGS groups, seasons, infective, effective, Dynamics.

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INTRODUCTION

Nitrogen (N) is one of the most abundant elements on earth. However, it is one of the most limiting nutrient in growth and production of crops. Nitrogen can be utilized by crops when it is reduced to ammonia. It can be reduced by chemical fixation through industrial production and/or biological fixation involving microorganisms. Inoculating soybean with effective and efficient *Bradyrhizobium* spp may have a role in increasing biological nitrogen fixation and crop yields if properly exploited. Soils may vary considerably in the nature of their inherently established rhizobia populations. Fixation of N can only be achieved in the presence of efficient rhizobia strains, which can be native to the soil or introduced in form of inoculants. Inoculation of legumes with effective rhizobia can improve grain yields (Giller, 2001; Nkwiine and Rwakaikara-Silver, 2007; Thuita et al., 2012). Inoculation of legumes is necessary in absence of compatible rhizobia and when rhizobia populations are low or inefficient in fixing N (Fening and Danso, 2002; Abaidoo et al., 2007).

Studies on soybean nodulation with commercial inoculants have been shown to be inconsistent (Abaidoo et al. 2000; Pule-Meulenberg et al. 2011). These field results suggest that soybean production in Africa may still require inoculation with rhizobium bacteria, if N_2 fixation, plant growth and grain yields are to be optimized. This therefore facilitates the need to monitor the size and the dynamics of native and introduced rhizobia under different cropping sequences for effective agricultural management. Successful establishment of the need for inoculation under field conditions will result to improved nitrogen management which will in turn optimize economic returns to farmers and minimize environmental concerns associated with nitrogen use (Bundyand Andraski, 2005). This would further improve contributions of biological nitrogen fixation by soybean to sustainability of cropping sequences in Kenya. The objective of this study was to assess the dynamics of the native and introduced Bradyrhizobium japonicum strain under different cropping systems and soil fertility This will in turn result to delineation of better cropping sequences for improved crop yields.

MATERIALS AND METHODS

Siaya is one of the counties that comprise the former Nyanza Province. The County combines the former Siaya, Bondo, Rarieda and Ugunja districts. It borders Busia, Kakamega, Vihiga Kisumu, and Homabay counties. It also borders Lake Victoria, the second largest fresh water lake in the world. Figure 1 shows the study sites.

The study was carried out in Ugunja, Wagai and Ukwala sites in Siaya County and their brief description is given in Table 1.

Description of experimental layout and treatments

A randomized complete block design experiment was set up with all treatments replicated 4 times. It was set up in 3 different farms starting from the SR 2014 for four cropping seasons.

The different cropping sequences and inoculation regimes are presented in Table 2. The plot sizes were measuring 4.5M x 5M with a 1 m inter-plot spacing that was planted with sweet potatoes to act as a buffer between plots to reduce inter-plot contamination by the inoculants and mineral fertilizers.

Management of experimental trials

The experiment was on-farm and researcher-managed. However, most of the operations were carried out by local people, following farmers' practices. Initial land preparation was done using an animal-drawn plough followed by hand hoes for all subsequent seasons. In plots where the test crop was maize, hybrid variety 306 from Western Seed Company commonly known as IR (Imidazolinone-resistant) maize was used. The IR maize technology is based upon inherited resistance of maize to systemic herbicide (imazapyr) and combines low-dose imazapyr seed coating applied to maize seed (AATF, 2007). Two seeds of maize were planted per hill. Two weeks after planting, thinning was done to ensure one plant per hill (53 000 hills ha^{-1}). The inter and intra spacing for maize was 75cm x 25cm respectively.

For soybeans, the promiscuous variety TGx1740-2F (SB19), a medium-maturing variety (95–100 days) was used as the test crop. It was planted at 0.75m × 0.05m (266 000 seeds ha^{-1}). Rhizobia inoculant *Bradyrhizobium japonicum* strain 532c (Legumefix) was the commercial strain used in the different cropping sequence as shown in Table 2. It was applied at the rate of 10 g/kg of seed. Legumefix has a peat-based formulation with a minimum concentration of 1×10^9 CFU mL^{-1} of Bradyrhizobium. Inoculation was done at planting as a seed coating following the instructions given on products by the producing company. Inter and intra row spacing for soybeans was 50cm x 5 cm respectively.

The continuous mono maize plot received P and N at 60 kg ha^{-1} at planting and top dressing respectively for

Figure 1: Map of the study sites

Table 1: Locational, climatic characteristics and cropping history of experimental sites Parameter

	Ugunja	**Wagai**	**Ukwala**
Agro climatic zone	LM1	LM1	LM1
Southing	00^{0} 14 $883^{'}$	00^{0} 04 $299^{'}$	00^{0} 04 $561^{'}$
Easting	034^{0} 22 $595^{'}$	034^{0} 27 $133^{'}$	034^{0} 32 $465^{'}$
Total annual rainfall (mm)	1600	1800	1400
Mean temperature (˚C)	22.3-21.4	22.3-21.4	22.7-220.
Soil type	Ferralsol	Ferralsol	Ferralsol
Crop grown in LR 2014	Maize	Maize	Maize
Crop grown in SR 2013	Maize + Beans	Fallow	Maize + Beans
Crop grown in LR 2013	Cassava	Maize +Beans	Maize

Agro-ecological zones (Jaetzold et al., 2006); LM: lower midland.
ǂ Inoculation was done only in the first season (SR 2014)

the four cropping seasons (FURP, 1994). The P and N source for maize was Sympal (NPK 0:23:15 + 10 CaO + 4S + 1 MgO) and Mavuno top (26% N, 10% CaO and 5% S) fertilizer respectively. Top dressing was done at 4 weeks after planting when the maize had attained the height of about 45 cm. Under the rotational sequence, maize was only fertilized with P at planting and the P source was Sympal. Under this sequence, nitrogen was

Table 2: Treatment structure for the different cropping sequence, inoculant and fertilizer application in the study site

S No.	Cropping sequence	Inoculation (Soybean)	Fertilizer application (Maize)
1	Continuous soybean	+	-
2	Continuous soybean	-	-
3	Continuous soybean ‡	-	-
4	Maize-Soybeans rotation	+	60 Kg P
5	Continuous Maize	-	60 kg P and 60 Kg N
6	Soybeans-Maize rotation	+	60 Kg P
7	Soybeans-Maize rotation	-	60 Kg P

Table 3: Initial soil physical and chemical properties of the study sites

	Division						
Soil Parameter	**Ugunja**	**Ukwala**	**Wagai**	**Mean**	**LSD$_{(0.05)}$**	**CV (%)**	**F Pr**
pH ($H_2$0)	4.1a	4.2a	4.3a	4.2	0.53	6.4	NS
Extractable P (ppm)	28.7a	23.7a	26.76a	26.3	10.1	19.2	NS
Exchangeable K (cmol$_c$kg^{-1})	0.12a	0.05a	0.21a	0.13	0.22	26.2	NS
Exchangeable Ca(cmol$_c$kg^{-1})	0.16a	0.91ab	1.12a	0.62	0.88	46.2	0.05
Exchangeable Mg (cmol$_c$kg^{-1})	1.1b	0.49c	1.95a	1.46	0.42	1.9	0.02
Mn (mgkg^{-1})	0.30b	0.59b	1.38a	0.7	0.83	58.9	0.03
Fe (mgkg^{-1})	24.a	42.0a	18.7b	29.4	25.1	42.6	0.08
Cu (mgkg^{-1})	1.9c	3.8b	7.3a	4.1	1.6	20.1	0.01
Zn (mgkg^{-1})	0.04b	0.13b	6.3a	2.2	1.2	24.5	0.01
N (%)	0.12a	0.08a	0.12a	0.1	0.2	24.2	NS
C (%)	1.3a	0.9a	1.3a	1.1	0.4	21	NS
Sand (%)	46	28	22				
Clay (%)	46	52	54				
Silt (%)	8	20	24				
Textural Class	Sandy clay soil	Clay soil	Clay loam				

NS means not significant

expected to be supplied to the maize crop from the preceding soybean crop. Standard agronomic practices such as weeding, pest and disease control were carried out uniformly in all plots. These practices always started from the uninoculated plots to avoid soil contamination with rhizobia.

Soil sampling and determination of the physico-chemical properties

Soil samples were taken up to a depth of 20cm in each replicate for characterization. The soils were submitted for analysis at MEA laboratories limited. The soils were submitted for analysis at MEA limited laboratories using methods described in Okalebo et al. (2002). The analyzed parameters are presented in Table 3.

Assessment of above ground biomass accumulation

All soybean plots were sampled to determine the nodulation and biomass production. The samples were collected at mid pod filling stage by destructive sampling from a 0.5 m row long section leaving at least 0.5 m from each end of any of the two net plot rows. The number of soybean plants within the section were counted and recorded. The plants were excavated and the entire roots mass including nodules were collected. Nodules were thoroughly cleaned and then their fresh weight was determined. The fresh weight of the above ground biomass was determined and later dried at 65˚c and the dry weight was recorded.

Nodule analysis

Before analysis each nodule was surface sterilized with 96% ethanol for 30 seconds and rinsed with sterile water, then surface sterilized with 3.5% $(CaClO)_2$ for 3 minutes and rinsed 3 times with sterile distilled water. Each nodule was crushed in 150 µl of sterile water and DNA was extracted. Total Genomic from 8 nodules per plot were extracted separately. DNA was extracted as described by Krasova-Wade et al. (2003) with modifications: precipitation was done with 100 µl of cold iso-propanol at -20°C overnight. Samples were then be centrifuged for 15 min at 4 °C and the pellets cleaned with 70% (v/v) ethanol before being air-dried and re suspended in 50 µl of sterile micropure water.

Polymerase chain reaction (PCR) amplification of bacteria

Primers FGPS1490-72 (5'-TGCGGCTGGATCCCCTCCTT-3') (Normand et al., 1996) and FGPL132-38 (5'-CCGGGTTTCCCCATTCGG-3') (Ponsonnet and Nesme, 1994) were used for PCR amplification of the 16S–23S rDNA spacer region. PCR reactions was carried out in a 25 µl volume containing 12.5 µl of Faststart PCR Master Mix (Roche), 8.5 µl of sterile micropure water, 10 pmol of each primer and 2 µl of template DNA. PCR cycling conditions was as follow: initial denaturation for 5 min at 94°C, 35 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 58°C) and extension (30s at 72°C) and a final extension for 7 min at 72°C. Size and quality of amplification products was confirmed by horizontal electrophoresis on a 2% (w/v) agarose gel in TBE buffer (1.1% (w/v) Tris–HCl; 0.1% (w/v) Na_2EDTA, $2H_2O$; 0.55% (w/v) boric acid). Gel was pre-stained with 0.12µ g/ml of Ethidium Bromide and visualized under UV illumination (Biorad Gel Doc system). 10 µ l of PCR products was digested for 2 h at 37°C with 5 µl of the restriction endonucleases Hae III (Roche). Restriction fragments were run on a 3% (w/v) agarose gel as described earlier. Profiles were determined depending on the number of bands and the size of each band after digestion. Samples presenting the same profile were classified into the same IGS profile group.

Estimation of Bradyrhizobium populations in the study soils

A most probable umber (MPN) experiment in sterile sand using dilutions from soil in the 3 study sites was carried out according to Brockwell (1963) to estimate the indigenous rhizobia populations in the soil. Serial dilutions were done to obtain a fourfold serial dilution of 1:50, 1:250, 1: 1250, and 1:6250 with four replicates per dilution for the 3 sites. Sand was used as a growth medium and was washed thoroughly, dried and autoclaved at 121^0c for 1 hour and placed in sterile PVC planting pots.

Statistical analysis

Experimental data was subjected to analysis of variance (ANOVA) to generate the means and least significant differences at 5% significance level using GENSTAT 14th edition. Least significant difference (LSD) values were used to compare means where significant differences between the means were detected. Maize and soybean yield data is reported in a different publication (Mutegi et al., in press).

RESULTS

Rainfall characteristics of the study sites

The daily and cumulative rainfall characteristics for Ugunja, Ukwala and Wagai sites over the cropping seasons are presented in the Appendix. The cumulative rainfall for Ugunja was 355.9, 475.2, 491.6 and 331.4mm for SR 14, LR 15, SR 15 and LR 16 respectively and 82.2 % of the rains falling before 50% podding in LR 2016. Ukwala site received the lowest cumulative amount of rainfall but it was properly distributed within the planting season except in LR 2016 where 83.9% was received before 50% podding. In Wagai, the highest cumulative rainfall amount was recorded in SR 15 (424.6 mm) while the lowest was recorded in LR 16 (322.1 mm) in which 85.2% was received before 50% podding stage.

Initial soil characterization at the study sites

Table 3 shows initial soil chemical and physical characteristics of the study sites.

The soils of the study sites were acidic with the pH averaging 4.2. The sites differed significantly ($P<0.05$) in exchangeable Ca, Mg, Mn, Fe, Cu and Zinc (Table 3). The exchangeable cations were in the ranges of 0.01-0.28, 0.49-1.97 and 0.07-1.65 $cmol_c kg^{-1}$ for K, Mg and Ca respectively. The pH, extractable P, exchangeable K and total N and C were not significantly different ($P<0.05$) within the sites. Wagai site however, had higher amount of exchangeable cations in comparison to other sites (though not significantly different $P<0.05$). Wagai site also had the highest pH and the lowest Fe levels. Ugunja site was classified as a clay soil.

The population estimates of indigenous rhizobia in soil was 0.364×10^3,0.436×10^3 and 0.328×10^3per gram of soil in Ugunja, Wagai and Ukwala respectively (Table 1).

Nodulation

During the SR 2014 season, site, treatment and site × treatment interaction had significant ($P<0.05$) effects on nodule weight. Statistical analysis showed significant effect ($p<0.05$) of inoculation on nodulation. The highest nodule weight was in Wagai (1.10 g plant^{-1}), followed by Ugunja (0.761 g plant^{-1}) while the lowest was in Ukwala (0.602 g plant^{-1}) (Figure 2). Under the uninoculated control, nodule weight decreased in the order of Wagai>Ugunja>Ukwala (0.385, 0.12 and 0.092 g plant^{-1} respectively). This was an equivalence of 64.1%, 71.6% and 51% increase in nodule weight relative to the uninoculated control for Ugunja, Wagai and Ukwala respectively (Figure 2).

During the LR 2015 season, maize-inoculated soybean rotational sequence had the highest nodule weight (0.82, 0.68 and 0.58 g plant^{-1}) for Wagai, Ugunja and Ukwala respectively. The differences in nodule weight between the inoculated and the uninoculated treatments were not significantly different during the LR 2015 season.

Nodule weight ranged from 0.677 g plant^{-1}in maize-inoculated soybean rotation and 1.086 grams plant^{-1} under continuous uninoculated sequence during the SR 2015 season. Significant differences in nodule weight were observed with 23.4% and 27.8% increases under the uninoculated relative to the inoculated treatments for Ugunja and Ukwala respectively.

During the LR 2016 season, site and cropping systems × site were significant ($p < 0.05$) in nodule weight with Wagai, Ugunja and Ukwala recording 0.476, 0.403 and 0.128.9 g plant^{-1} respectively (Figure 2). There was a 7.3% and 0.83% increase in nodule weight in the uninoculated treatments than in comparison to the uninoculated treatments. However, this season recorded the lowest nodule weight and biomass yields in comparison to the other seasons. On average, Wagai site recorded significantly higher nodule weights across all seasons (Figure 3). There was a drastic seasonal increase in nodule weight under the uninoculated mono soybean except for LR 2016 (Figure 3).The short rain seasons also yielded higher nodule weight than the LR seasons with the highest weights being recorded in SR 2015 season.

Nodule occupancy

All the nodules (from inoculated and uninoculated plots) gave a PCR product with the expected size (930–1,050 bp) and were therefore considered for restriction. Three main RFLP profiles were obtained after digestion with HAE III restriction enzyme. Strains were classified in 3 different IGS groups (IGS 1, IGS II and IGS III) based on their respective RFLP profiles. Table 4 shows summary of nodule occupancy that was obtained after analysis of the nodules. The profile of the used commercial inoculant had previously been identified (Thuita et al., 2012) and corresponds to what was identified as profile 1 in the current study. IGS group II and III were obtained from nodules that corresponded with plots that were not inoculated. Nodule occupancy for IGS group II and III was consistently recorded in the uninoculated plots with SR 2014 having the lowest occupancy (Table 4).

Soybean inoculated with legumefix strain showed a dominance of the strain during the entire experimental period irrespective of the cropping sequence. IGS II and IGS III occurred in almost similar proportions during the first season. However, the trend changed with IGS II dominating with seasons in comparison to IGS III. Under inoculated soybean-maize rotation, IGS I had higher nodule occupancy relative to IGS II and IGS III. Notably also, under uninoculated sequence, IGS II had a very high proportion of occupancy relative to IGS III as from the second season (Table 4).

DISCUSSIONS

Rainfall

The rainfall amounts and distribution varied largely between season with SR 15 receiving the highest and LR 16 receiving the lowest (491.6 mm and 331.4 mm respectively). During LR 16 season, 85% of the rainfall was received before flowering and it could therefore have interfered with the fertilization and root development during flowering stage of the crops and consequently reducing yield. Our results agree with Balarios and Edmaedes (1993) who reported that drought stress occurs with different intensity and at any plant development stage from germination to physiological maturity, and flowering is the most critical stage in maize.

Report by Staton M (2012) indicate that soybean yield losses will be the greatest when moisture stress occurs between the middle of the R4 growth stage and the middle of the R5 growth stage .Stress at this time reduces the number of pods per plant as the plants are no longer able to produce new blossoms and pods. This is the major source of the lost yield. However, the number of seeds per pod and the size of the seed can also be reduced at this time. Leaf loss will continue in severely stressed plants.

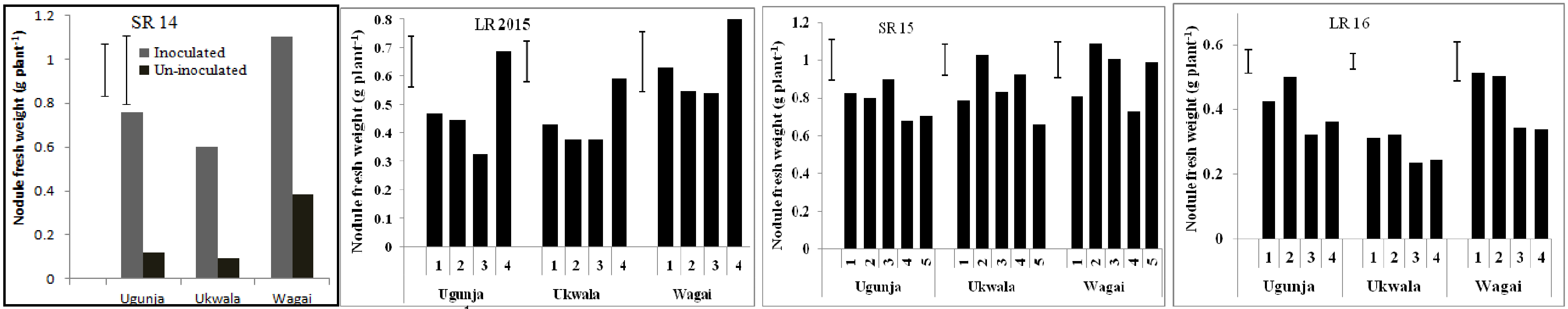
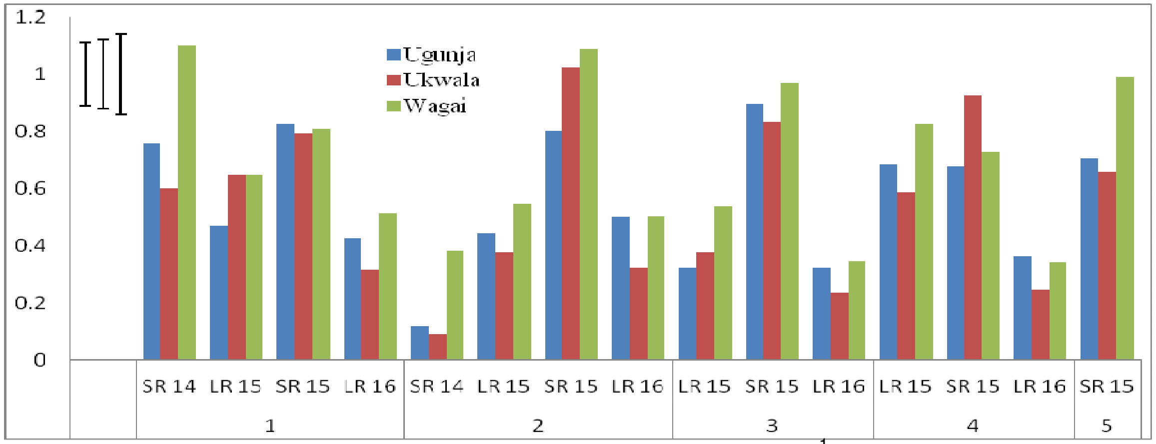


Figure 2: Soybean nodule fresh weight (g plant^{-1}) at 50% podding as infuenced by different cropping sequences in the study sites for the four seasons



1=Continuous soybean (Inoculated)
2=Continuous soybean (Uninoculated)
3=Continuous soybean*
4=Maize-soybean (Inoculated)
5=Maize-soybean (Uninoculated)
*Inoculated only in SR 2014 season

Figure 3: Seasonal changes in soybean nodule fresh weight (g plant^{-1}) with cropping sequences at 50% podding

The low rainfall is likely to explain the poor nodulation. Moisture stress has a strong effect on nodulation (Zahran, 1999) and in studies conducted in Southern Africa, nodulation of promiscuous soybean has been shown to be correlated with the amount of rainfall, being higher with high rainfall, or at least when moisture stress is not affecting crop development (Mpepereki et al., 2000; Musiyiwa et al.,2005a,b).

Soil physico-chemical properties

The Organic Carbon (C %) and total nitrogen (N %) in the study soils ranged from 0.79-1.68% and 0.08-0.17% respectively. Tekalign (1991) rated

Table 4: Summary of IGS groups from nodule occupancy under different cropping sequences and inoculation

			Nodule occupancy in the IGS groups (%)															
Cropping system	**Inoculation**	**Site**	**SR 2014**				**LR 2015**				**SR 2015**				**LR 2016**			
		Profile	**I**	**II**	**III**	**N^{a}**	**I**	**II**	**III**	**N^{a}**	**I**	**II**	**III**	**N^{a}**	**I**	**II**	**III**	**N***
Continuous soybean	+	Ugunja	89	10	1	22	90	10	0	30	83	17	0	23	82	18	0	18
		Wagai	75	15	10	21	100	0	0	27	100	0	0	27	86	14	0	22
		Ukwala	86	5	9	22	95	5	0	20	92	8	0	13	85	15	0	24
Continuous soybean	-	Ugunja	0	55	45	22	0	74	26	22	0	86	14	21	0	77	23	13
		Wagai	0	55	45	28	0	62	38	30	0	81	19	26	0	78	22	32
		Ukwala	0	61	39	21	0	63	37	21	0	60	40	25	0	91	9	23
Continuous soybean**	-	Ugunja	91	9	0	22	94	6	0	17	16	67	17	16	0	95	5	18
		Wagai	71	19	10	21	86	14	0	21	22	7	71	14	0	82	18	17
		Ukwala	82	2	16	22	74	0	26	19	0	76	24	12	0	95	5	19
Soybean-Maize rotation	-	Ugunja	0	83	17	12	N/A	N/A	N/A	N/A	9	91	0	11	N/A	N/A	N/A	N/A
		Wagai	0	76	24	25	N/A	N/A	N/A	N/A	6	86	8	13	N/A	N/A	N/A	N/A
		Ukwala	0	78	22	16	N/A	N/A	N/A	N/A	0	78	22	22	N/A	N/A	N/A	N/A
Maize-Soybean rotation	+	Ugunja	N/A	N/A	N/A	N/A	82	18	0	17	N/A	N/A	N/A	N/A	84	5	11	19
		Wagai	N/A	N/A	N/A	N/A	88	12	0	12	N/A	N/A	N/A	N/A	61	39	0	14
		Ukwala	N/A	N/A	N/A	N/A	73	27	0	15	N/A	N/A	N/A	N/A	79	21	0	20
Soybean-Maize rotation	+	Ugunja	91	9	0	22	N/A	N/A	N/A	N/A	80	15	5	20	N/A	N/A	N/A	N/A
		Wagai	71	21	8	21	N/A	N/A	N/A	N/A	45	33	22	19	N/A	N/A	N/A	N/A
		Ukwala	86	0	14	22	N/A	N/A	N/A	N/A	76	20	4	23	N/A	N/A	N/A	N/A

N*- the total number of nodules analysed

soils with a measured value of C at 0.5-1.5% and N at 0.05-0.12% as low. The low inherent total nitrogen could have resulted to enhanced nodulation and biomass yield in the SR 2014 season in comparison to the control in the 3 study sites. These findings are in consistence with Hungira et al. (2003) who noted that low level of N in the soil can enhance nodule formation and increase grain yields. When the nitrogen levels in the soil are high, nodule formation and biomass formation is negatively affected. If soybean has a source of nitrogen readily available, there is no incentive to signal rhizobia to form nodules and thus the rhizobia do not create the nod factor. Once the carry over nitrogen is used up, the plant then signal to the rhizobia, but the whole nodulation process then becomes delayed or the signaling window can be missed, resulting to little or no nodulation by the soybean plants. Inhibitory effects of added nitrogen fertilizer to nodulation and nitrogen fixation have been reported by other investigators (Floor, 1985; Chemining'wa et al., 2004; Taylor et al., 2005).

The mean soil pH values of the study sites was extremely acidic ($pH<4.5$) according to grading levels for soil acidity described by Kanyanjua et al. (2002). This impedes the rhizobia from creating the nod factor and form nodules. Low soil pH is generally accepted as an indicator of conditions under which some other soil properties may limit crop growth rather than as a primary cause of poor growth.Under these conditions, important micro nutrients including molybdenum that are cofactors for nitrogen fixation may become unavailable. Strong soil acidity is associated with Aluminium (Al),Hydrogen (H), Iron (Fe) and Manganese (Mn) toxicities to plant roots in the soil solution and corresponding deficiencies of the available P, Molybdenum (Mo), Ca, Mg and K (Giller and Wilson, 1991; Jorge and Arrunda, 1997). These acidic conditions could have resulted in low nodulation of the native soil rhizobia during the SR 2014 seasons.

In acid soils with a $pH<5.0$, Al minerals hydrolyze to form octahedron hexahydrate (Al^{3+}) and mononuclear hydroxides ($Al(OH)^{2+}$ and $Al(OH)_2^{+}$ which are responsible for P sorption (Kinraide, 1991; Kochian, 1995).

The value for the exchangeable cations were not significantly different between sites expect in Wagai where the Mg^{2+} levels were significantly higher than Ugunja and Ukwala. The average exchangeable K^{+}, Ca^{2+}, and Mg^{2+} were 0.13, 0.62 and 1.46 $cmol_c kg^{-1}$ respectively. The ranges for exchangeable cations in the three study sites could be rated as very low to low according to Okalebo et al. (2002). Calcium deficiency affects attachment of rhizobia to root hairs, nodulation and nodule development (Alva et al., 1990). Calcium-spiking phenomenon is initiated in root-hair cells of legumes by nodulation factors and rhizobia, suggesting that Ca^{2+} plays a pivotal role in symbiotic interactions at the molecular level. Poor nodulation of soybeans in acid soil has been attributed to an Al induced Ca deficiency.

The low levels of exchangeable cations reported in the study sites imply that the soils have very low percent base saturation and high exchangeable acidity on the cation exchange sites. This scenario could however be explained by the fact that ferralsols are highly weathered and the dominant clay type is kaolinite which has very little capacity to hold the exchange cations. These findings are in agreement with Howieson and Ballard (2004) who noted that low rhizobia efficacy could be attributed to soil acidity ($pH < 5$) and low cation exchange capacity (< 10 cmol kg^{-1}).

As reported in this study, ferralsols are one of the major soil groups in Siaya County in Western Kenya (Sombroek et al., 1982) with low available P (Njui and Musandu, 1999; Wasonga et al., 2008). Tidsale et al. (1990) associated the low levels of P to high levels of P sorption through their reaction with phosphate ions to form insoluble compounds. The infection of a leguminous root by rhizobium/bradyrhizobium and N_2 fixation has high energy requirements. The nitrogenase activity is dependent on ATP for the reduction of atmospheric N_2 to ammonia; approximately 21 mol of ATP are converted to ADP per mol N_2 reduced (Shanmugan et al., 1978). This high energy requirement commonly stored by plants in high-energy P bonds, explains why the scarcity of soluble P in soil is a critical limiting factor for legumes because it not only affects plant growth but also nodulation and N_2 fixation (Hallworth, 1958);Gates and Wilson, 1974).

The soils could be described as having a fine texture (more than 40% clay) as described by the USDA soil textural groups reported in Okalebo at al.(2002). Despite the high clay content in the soils, the exchange cations are low and this is contrary to the expectation.

The soils of the study site had clay > sand >silt respectively. It has been reported that soils with high clay loam contents are suitable for maize/ legumes production because of their moderate capacities to retain plant nutrients and soil water (De Datta, 1981). The high clay loam contents in these soils would further allow the moderate percolation of water through the soils, hence encouraging roots respiration and other biological processes in the soils including symbiotic BNF process.

The study sites were not previously cultivated with soybeans for the last 3 seasons. Such soils will seldom contain sufficient population of native Bradyrhizobium japonicum to ensure satisfactory nodulation (Mohammadi et al., 2012). Other studies conducted under greenhouse conditions with promiscuous soybean variety obtained indigenous rhizobial populations of less than 10^3 rhizobia g^{-1} of soil (Maingi et al., 2006; Atieno et al., 2012; Thuita et al., 2012).

Most probable numbers and nodulation

The present study revealed the bradyrhizobial counts in the study soils was not high for effective nodulation during the SR 2014 season. This is supported by the suggestion by Danso (1992) that population range of 10^3 to 10^4 rhizobia per gram of soil should by most standard be adequate for high nodulation. The higher nodule weight in the first season in the inoculated treatment confirmed presence of low in effective native rhizobia in the study soils which could not compete with the commercial inoculant. The low nodule weight in the uninoculated plots could also be ascribed to the cropping history of the study sites (Table 2). The fields had not been cropped with soybeans for the past 2 years. This in turn implies that the naturalized rhizobia had become less in effective and/or infective. The importance of cropping history on soil rhizobial populations has also been demonstrated elsewhere (Karanja et al., 1995; Mendes and Bottomley, 1998). Venkateswarlu et al. (1997) reported that cropping history had a more critical influence on the abundance of native rhizobial populations than soils or climatic factors. The low nodule weight in the uninoculated control during the SR 2014 season could further be attributed to the low indigenous rhizobia populations recorded in the study soils, thus showing the competitive advantage of the inoculated rhizobium over the indigenous rhizobia population. Other authors have reported similar trends of nodulation and biomass yield with different legume pulses (Cheminingwa and Vessey. 2006; Leidi et al., 2000). In soils with low rhizobial population, rhizobium inoculation alone produced significantly higher nodule weights than the uninoculated treatments in the first season. The synergetic effects of low total N in the soils together with inoculation resulted in increased nodule weight. Similar finding was confirmed by Hungria et al. (2003) and Vargas et al. (2003) through rhizobia inoculation and improved nodule occupation. Egamberdiyeva et al. (2004b) reported that the yield of soybean varieties was 48% higher for inoculated than for the uninoculated plants, while Okereke et al. 2004) reported increase in seed yields after bradyrhizobia inoculation ranged between 1200 and 2180 kg ha^{-1} against the uninoculated plants which had seed yields of 1050 kg ha^{-1}

The high nodule weight in the maize- inoculated soybean rotation system during the LR 2015 could be attributed to the residual effects of P and other micro nutrients that were applied as Sympal to maize during the previous season. Besides provision of P and K to maize, Sympal is formulated with additional elements (CaO, S and MgO). The CaO in Sympal could have amended the low pH in the study soil through liming and consequently improving nodulation and biomass yields. Lime, when applied in soils reacts with water leading to the production of OH^- and Ca^{2+} ions which displaces H^+ and Al^{3+} ions from soil adsorption sites resulting in an increase in soil pH (Kisinyo et al., 2015). As calcium concentrations in the treatments were greater, it seems likely that the growth and survival of native rhizobia were interrupted by the interactions of soil factors. Increased concentration of calcium can significantly improve the survival of S. Meliloti strain under acidic conditions (Dilworth et al., 1999). Nazih et al. (1993) found that liming of acidic soils increased survival of native or inoculated R. leguminosarum bv. Trifolii. The CaO could also have resulted to decreased exchangeable Al with a significant increase in exchangeable Ca and Mg concentrations.

The better performance in terms of nodule weight in the rotational sequences than the mono cropped sequences during the LR 2015 could be could be attributed to other benefits accrued from the rotational sequences. These non-N benefits effects, according to some authors include enhancement of soil microbial activity, improved soil physical and chemical properties, elimination of phytotoxic substances, addition of growth promoting substances and reduced disease incidence (Peoples and Crasswell 1992; Jackson et al. 1993; Reeves and Wood 1994; Giller 2001)

After the SR 2014 season, the uninoculated control had higher nodule and biomass weight than under the inoculated cropping. This could be attributed to build up of resident rhizobia due to continuous planting of the host legume (soybeans) and thereby increasing their competitive competence.

During the SR 2015 season, Ukwala site outperformed the other sites in terms of biomass yield. This could be attributed to the higher clay contents than the other sites. High clay content aids in retention of cation and therefore increased nutrients availability. The non significant differences in nodule weights reported in the SR 2015 season further affirms that the native rhizobia was equivalently effective and infective in the third season after continuous soybean cropping. The better growth of soybean could be a mechanism that that enhance larger populations of rhizobia resulting from senescing nodules, or stronger stimulatory rhizosphere effect.

During the LR 2016 season, the adverse effects of water stress on the survival of rhizobia could have reduced the nodule numbers and biomass yields recorded. Sporadic and erratic rainfall was recorded during that growing season (Appendix). This adverse effect has previously been reported in other studies (Mnasri et al. 2007, Marino et al. (2007) showed drought to induce a decline in nodule water potential that result in a cell redox imbalance in legume plants. The low rainfall during the LR season could have caused water stress on the plants giving very few nodules. The better performance of the un inoculated plots could be an indication of buildup of competitive resident rhizobia as a result of continuous planting of the host plant. Other studies have reported that continuous cultivation of the host plant could increase the native rhizobia population (Vlassak et al., 1996).

The low population levels of soybean indigenous rhizobia in the study sites could be attributed to the limited integration of soybean into cropping systems within the study area. This is evidenced by the cropping history given in table 1. The low populations could also be as a result of the low pH and P deficiencies that have been shown to adversely affect both survival of rhizobia and nodulation process in legumes (Graham 1992). Nodules are reported to be strong sinks for P than the roots, shoots and even mature leaves (Graham 1992).

The continuous cropping of soybean allowed the indigenous strains to increase and stabilize in the soil.

On average, Wagai site had higher nodule weight in all the seasons (Figure 3). This could be ascribed to relatively higher CFU of the native rhizobia g^{-1} of soil, higher pH value and higher levels of exchangeable cations relative to other sites (Table 2).

Nodule occupancy

The two IGS groups (II and III) of the native strains were identified in the study soils. This denotes low diversity of the native rhizobia able to nodulate soybean. Low diversity of the native strains could be explained by the low carbon contents in the study soils (Table 3). Soil organic carbon has been shown to be one of the primary drivers of overall microbial community size, diversity and structure (Sul et al., 2013), as well as influences on the soil rhizobia population. Soil OM is important for the saprophytic survival of rhizobia when they are not in the presence of a host legume (Rinaudi et al., 2006). It is also associated with improved water holding capacity and aggregation, both of which are associated with supporting microbial activity (Lagomarsino et al., 2012; Sul et al., 2013).

During the SR 2014 season, the IGS group III was shown to have the similar intrinsic nodulating competitiveness with IGS group II but, over time, this was to some extent offset probably by its poor colonisation of the root surface, where it could have been outgrown by IGS group II.

The observed differences in the behaviour of IGS II and IGS III could be ascribed to the lower competitive ability of IGS III strain compared with that of IGS II, to the reduced resistance of IGS III to poor soil conditions of the study sites and to the strong plant choice of the more suitable strains. There may also be mechanisms where the host plant has a system by which it can accept infection by more compatible rhizobia, rejecting (sanction mechanisms) those that are less compatible ones (Simms et al. 2006; Marco et al. 2009) in certain environmental conditions (Kanu and Dakora 2012; There could be a possibility that there was a high degree of intra strain competition between IGS II and IGS III probably due to their environmental adaptations.

The promiscuous soybean variety used was TGx1740-2F (SB19), a medium-maturing variety (95–100 days) that has been bred for nodulation with indigenous Bradyrhizobium spp. at IITA-Ibadan, Nigeria (Abaidoo et al., 2007) thus eliminating the need for inoculation. This variety showed better nodulation with a range of native rhizobia than other local varieties in different parts of Kenya (Wasike et al., 2009). It's important therefore to include the variety into the different cropping sequences if we have to realize its importance.

CONCLUSIONS AND RECOMMENDATIONS

The symbiotic performance of promiscuous soybeans depends upon the population size, effectiveness and survival of indigenous or introduced rhizobia in the field. The presence of the host legume and the history of the land use impact the size of the indigenous rhizobia population as well as its diversity. Cropping sequences that include the host legumes will therefore enhance the populations of the indigenous rhizobia with the well adapted strains being dominant. An understanding of rhizobia interactions in the rhizosphere is required in order to optimize plant–rhizobial interactions and plant growth promotion. Inclusion of host legumes in a cropping sequence represent alternative management practices to mineral fertilizers, and may have a role to play in the restoration and the maintenance of soil fertility in highly N depleted soils in Africa.

From our discussions, it is evident that the promiscuous-nodulating soybean variety used in the study area exhibited a marked response to inoculation and the background soil rhizobia in increasing nodulation shoot biomass and yields of both maize and soybean under different cropping sequences. We confirmed presence of two strains of the study soils with one strain outperforming the other. Further, the native inoculants performed better or similarly with the commercial inoculants and therefore have a great potential of being further developed to provide cheap and efficient inoculum to small holder farmers in the study areas and beyond. Inclusion of soybean into different cropping sequences can therefore stimulate the populations of native rhizobia and agronomic productivity. They may represent a promising way of minimizing the utilization of mineral N fertilizers. These studies, moreso, form an important step towards the development of affordable and efficient rhizobial inoculants which are well adapted to the local conditions. Further studies on rhizobia diversity and richness are imperative for us to make conclusive decisions.

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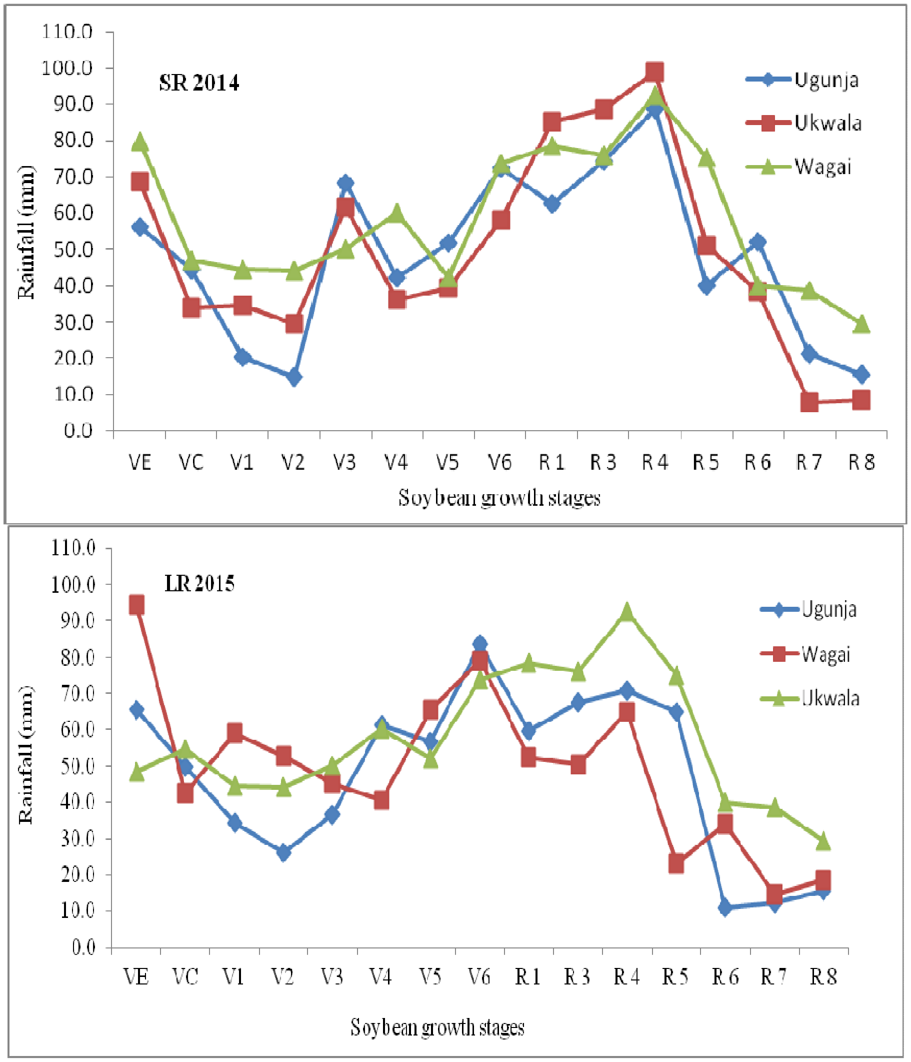
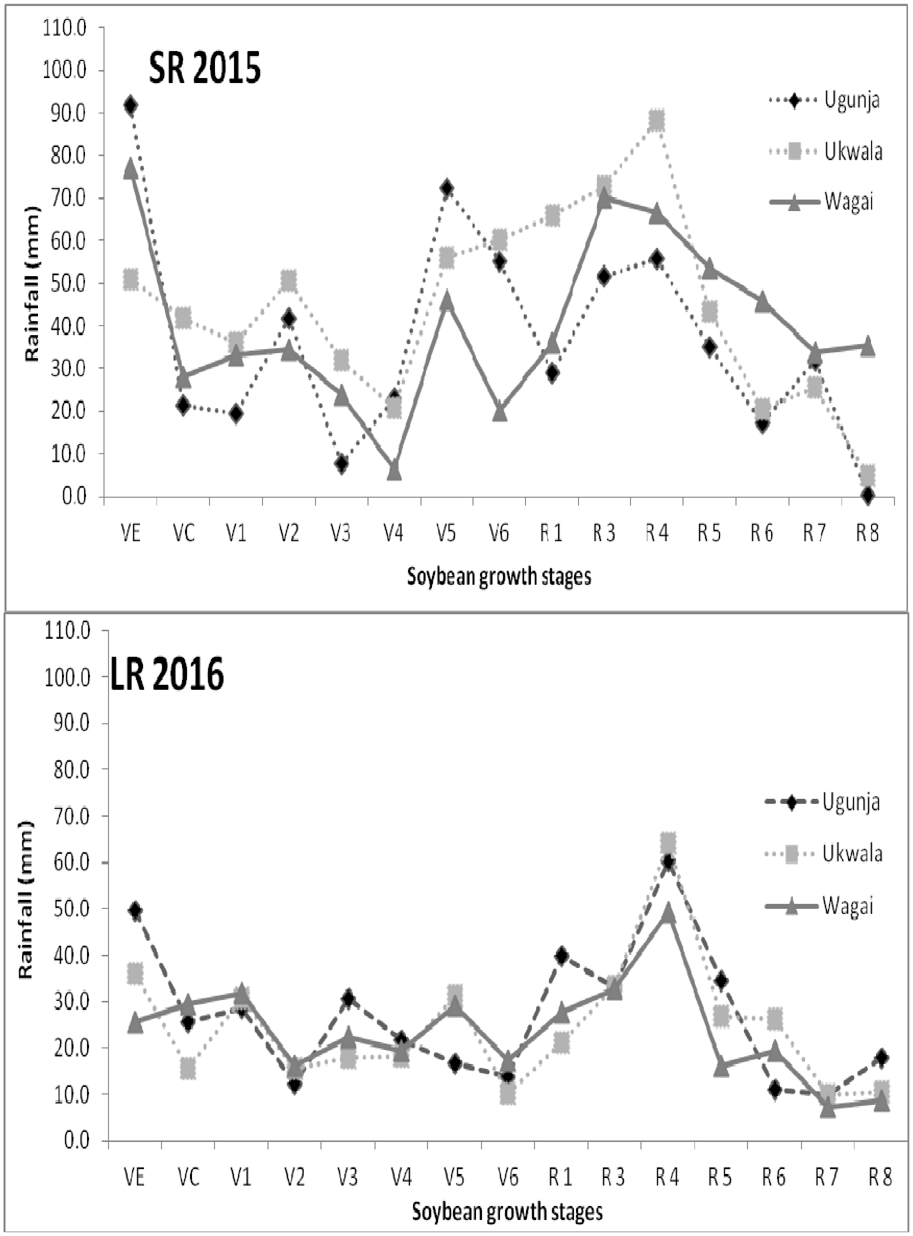
APPENDIX



Rainfall distribution of the study sites



Rainfall distribution of the study sites