Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

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To cite this article: Rukia Bakari , Nancy Mungai , Moses Thuita & Cargele Masso (2020) Impact of soil acidity and liming on soybean (*Glycine max*) nodulation and nitrogen fixation in Kenyan soils, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 70:8, 667-678, DOI: 10.1080/09064710.2020.1833976

To link to this article: https://doi.org/10.1080/09064710.2020.1833976

Published online: 22 Oct 2020.

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE
2020, VOL. 70, NO. 8, 667–678
https://doi.org/10.1080/09064710.2020.1833976

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Impact of soil acidity and liming on soybean (*Glycine max*) nodulation and nitrogen fixation in Kenyan soils

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ABSTRACT

There is a wide application of rhizobia inoculants to legume crops in Africa, irrespective of the soil acidity, though the latter limits the effectiveness of inoculants. Two trials were conducted in a controlled environment to determine suitable soil pH and impact of liming on soybean nodulation and nitrogen fixation to inform proper application of the rhizobia-inoculant technology on acid soils. In the first trial; soil, variety and inoculation had significant influence ($p < 0.05$) on weighed nodule effectiveness (WNE) and N fixation. Strongly acidic soils recorded low WNE and N fixation. In the second trial, WNE and N fixation significantly increased with co-application of lime and inoculation ($p < 0.05$). The results showed that soybean inoculation is effective in increasing nodulation and N fixation in moderate acidic soils, contrarily to strongly acidic soils. Interestingly, co-application of lime and inoculation has potential of increasing nodulation and N fixation in strongly acidic soils. The WNE is recommended as a robust formula to report nodule effectiveness, compared to the current percentage method.

ARTICLE HISTORY
Received 23 July 2020
Accepted 5 October 2020

KEYWORDS
Acidic soils; nodule effectiveness; biological nitrogen fixation; inoculation; nutrients; soybean; soil liming

Introduction

Soybean (*Glycine max*) is a legume of tropical to subtropical origin and an important source of food and income (Maingi et al. 2006). Inoculation of soybean throughout the world is between $12–20 \times 10^8$ ha year^{-1}, which leads to the establishment of rhizobia population in the rhizosphere and thus an improvement in nodulation and biological nitrogen fixation (BNF) (Hassen et al. 2014).

Rhizobia are not significantly present in soils and those present are often not highly effective, thus it is necessary to inoculate legumes to assure effective nodulation. Rhizobia inoculants are widely applied in fields where rhizobia populations are low, especially if legumes have barely been grown in that field. The Rhizobia inoculants are one of the biofertilisers made from selected strains of beneficial soil microorganisms i.e. nitrogen-fixing bacteria that take part in BNF. Soil acidity is one of the limiting factors of nitrogen fixation by the legume-rhizobia symbiosis (Van Zwieten et al. 2015), though several development projects in Africa have been promoting legume inoculation whithout taking into consideration the limitations related to soil pH.

In Kenya, acidic soils occur in high rainfall areas, including highlands of Rift Valley, and it occurs in about 13% of Kenyan land area (Kisinyo et al. 2014). Acidic soils are deficient in phosphorus (P), magnesium (Mg), calcium (Ca), molybdenum (Mo), and potassium (K) with a high concentration of iron (Fe), aluminium (Al), hydrogen (H), copper (Cu) and manganese (Mn) ions (Keino et al. 2015). Soybean production in Kenya is low and this could be due to its sensitivity to low soil pH. Soil pH below 5.2 and above 6.5 does not favour soybean growth, hence poor yields are experienced under such conditions (Peters et al. 2004). High levels of aluminium and low levels of phosphorus in acidic soils affect the growth of symbiotic nitrogen-fixing bacteria. Soil pH < 5.0 limits soybean nodulation due to toxicity effects of Al and Fe ions causing poor nodules formation and functioning (Nisa et al. 2012). Acidic soils also face reduced organic matter breakdown, nutrient cycling by microorganisms, reduced uptake of nutrients by plant roots and inhibition of root growth (Fageria et al. 2013). Soybean requires high nutrients, with P and K being most crucial for optimal production (Sikka et al. 2012). Acidic soils have a high concentration of Al and Fe ions in solution and these

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cause P sorption making it unavailable for plant use (Keino et al. 2015). At low soil pH, soybean nodulation and BNF is limited and this has been attributed to the low P level at pH < 5.5 due to its sorption by Al and Fe (Kisinyo et al. 2014). Application of P, K, and inoculation of soybean with Legumefix in Western Kenya did not produce high yields probably due to soil acidity (Keino et al. 2015).

Soil liming increases the availability of basic cations; it reduces the concentration of toxic levels of Al and increases P availability, hence increasing soil pH (Kisinyo et al. 2014). Different lime products are available in the market and the most common being agricultural lime. It is effective in improving yield when used alone as well as in combination with fertilisers (Nekesa et al. 2011). Lime use by farmers in Kenya is limited by the cost, accessibility, costs of application (i.e. labour), and low demand by farmers due to lack of awareness about the benefits of liming. Liming of acidic soils in Kenyan Rift valley resulted in a reduction of exchangeable acidity, an increase in available P and increased yields (Kisinyo et al. 2014). In acid soils, liming generally improved crop production (Nuwamanya 1984) and increased microbial activity (Badole et al. 2015). However, little has been done to assess the optimal soil pH for effective nodulation and biological N fixation, and the potential of liming materials to enhance nodulation in low pH soils. There is an overdue need to provide local scientific-evidence to inform the various development projects promoting the use of rhizobia inoculants, irrespective of the soil acidity. Thus, this study investigated the optimal pH range for soybean inoculation and the impact of liming acidic soils on soybean nodulation and nitrogen fixation, and developed a powerful approach to determine the nodule effectiveness.

Materials and methods

Experimental soils, handling and analyses

Two trials were set up at the International Institute of Tropical Agriculture (IITA) Nairobi, Kenya. The first trial aimed at assessing optimal soil pH for effective nodulation of soybean under inoculation. The soils used in this study where from regions with high agricultural potential, low soil pH conditions and with no history of soybean growing. Ten soil samples collected from various locations in Kenya, specifically from Kuresoi (0.2993°S, 35.5302°E), Mauche (0.3316°S, 35.9449°E), Murang'a (Kangema) (0.7957°S, 37.1327°E), and Kitui (Kyangwithya East) (1.3751°S, 37.952°E) were used. Two samples were collected from each location, except for Mauche where four samples were taken i.e. two samples from each side of the road. Soils from these regions have a pH range of 4.3–6.3, with those from Kuresoi (Humic Andosol) and Murang'a (Humic Nitosols) being strongly acidic, while Mauche (Vitric Andosol) and Kitui soils (humic Cambisols) have moderate acidity (Table 1).

Experimental soils were collected at a depth of 20 cm and 10 subsamples randomly collected per location using hoe for digging out soils, to mimic the surface layer generally representing the soybean rhizosphere. The soil samples from each site were homogenised and the composite samples obtained, put in 50 kg capacity sack and transferred to the screenhouse at IITA Nairobi. The samples were then air-dried for 48 h and sieved through a 2 mm sieve and a subsample (50 g) used in physical and chemical analyses of the soils.

Chemical analysis of the soil was determined following the procedure described in Okalebo et al. (2002) to assess nutrient composition including soil pH (soil pH-water using glass electrode pH meter), total N (Kjeldahl), C (Walkley-black). Available P, exchangeable bases (Ca, Mg, and K) and micronutrients (Fe, Cu, Zn) extracted using Mehlich 3 method. Available P was then determined using ammonium vanadate method and amount determined using a spectrophotometer, while the amounts of extracted exchangeable bases and micronutrients were determined using atomic absorption.

From the soil analyses, the study soils differed in fertility level with a wide range of coefficients of variation 13.69 to 269.22% (Table 1). Soils S1, S2, S3 and S4 with pH 4.3, 4.8, 4.6 and 4.7 respectively were considered as strongly acidic. Soils S5, S6, S7, and S8 were considered as moderately acidic (pH: 5.6–5.9) and soils S9 and S10 (pH: 6.2 and 6.3 respectively) were considered as slightly acidic (Table 1). Available P ranged from medium to high in the moderate and slightly acidic soils, while the strongly acidic had low level of available P based on the classification by Okalebo et al. (2002). Basic cations in the very strongly acidic soils were very low, implying also low cation exchange capacity (CEC).

Evaluation of optimal soil pH range for effective soybean nodulation and nitrogen fixation

Experimental setup

Two soybean varieties: Nyala and TGx1740-2F (SB19) were inoculated with Biofix and Legumefix. Soybean variety SB19 is promiscuous and medium maturing with high biomass yield, while Nyala is early maturing, non-promiscuous and does well as an intercrop

Table 1. Physical and chemical characteristics of soils collected from various regions in Kenya.

Soil pH (H_2O)	Total N	Organic C	Available P	K	Ca	Mg	Fe	Cu	Zn	Soil texture	Soil code
	%					$mgkg^{-1}$					
4.3 ± 0.5	0.07 ± 0.07	0.42 ± 0.14	6 ± 4.36	0.77 ± 0.65	1.09 ± 0.25	ND	134.4 ± 12.1	ND	ND	SL	S1
4.8 ± 0.5	0.27 ± 0.17	1.76 ± 0.67	16 ± 7.21	1.09 ± 0.47	1.17 ± 0.48	ND	228.7 ± 34.9	ND	20.6 ± 4.12	SL	S2
4.6 ± 1.2	0.23 ± 0.15	2.97 ± 1.51	6 ± 2.65	1.5 ± 0.62	2.41 ± 1.23	ND	371.3 ± 21.95	ND	11.6 ± 2.42	L	S3
4.7 ± 1.1	0.2 ± 0.06	2.3 ± 0.7	20 ± 11.36	1.5 ± 0.5	3.66 ± 2.22	ND	281.7 ± 17.16	ND	11.3 ± 0.46	L	S4
5.8 ± 0.9	0.2 ± 0.05	2.97 ± 0.1	36 ± 21.17	2 ± 1.32	11.46 ± 0.88	17.11 ± 7.22	357.6 ± 40.72	ND	40.2 ± 2.36	L	S5
5.6 ± 0.9	0.27 ± 1.13	3.21 ± 0.83	50 ± 26.46	1.5 ± 0.72	14.59 ± 6.30	1.83 ± 0.52	282.8 ± 2.57	ND	38.5 ± 6.61	L	S6
5.7 ± 0.7	0.24 ± 0.12	3.15 ± 0.44	26 ± 12.17	1.5 ± 0.95	13.37 ± 3.28	2.56 ± 0.53	302.5 ± 5.05	ND	44.4 ± 4.67	L	S7
5.9 ± 1.1	0.26 ± 0.15	3.27 ± 0.56	66 ± 14.93	1.51 ± 1.29	14.48 ± 3.89	2.47 ± 0.63	301.4 ± 2.93	ND	9.03 ± 1.42	L	S8
6.2 ± 1.4	0.09 ± 0.07	0.85 ± 0.44	40 ± 11.14	0	10.42 ± 2.54	1.83 ± 0.32	27.53 ± 4.24	1.79 ± 0.76	3.17 ± 0.3	SCL	S9
6.3 ± 1.3	0.13 ± 0.03	0.81 ± 0.45	30 ± 2.53	1.34 ± 0.29	7.79 ± 4.00	2.08 ± 0.2	39.41 ± 6.90	10.59 ± 2.94	5.11 ± 0.85	SCL	S10

Note: S1 and S2 are soils from Murang'a, S3 and S4 are soils from Kuresoi, S5, S6, S7 and S8 are from Mauche, S9 and S10 are soils from Kitui. Total N is total nitrogen, C, carbon; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; N, nitrogen, Fe, Iron; Cu, copper; Zn, zinc. SCL is sand clay loam, L-loam and SL is sandy loam. ND is Not Detected.

(ICRISAT 2013). The two varieties differ in their N fixation and pod formation (Thuita et al. 2012). The commercial inoculants differ in strain content; Legumefix manufactured from UK (Legume Technology LTD-UK) contains *Bradyrhizobium japonicum* strain 532C and Biofix manufactured by MEA LTD Kenya contains *Bradyrhizobium diazoefficiens* strain USDA110. Biofix is a widely used inoculant in Kenya due to its availability (local formulation). Recent studies in Kenya indicated the effectiveness of the Legumefix inoculant, which has been imported to Kenya (Thuita et al. 2018).

Soybean inoculation and planting

The soils were filled in 2 kg capacity containers designated as S1 to S10 and nutrient solution was applied 2 days before planting, except for control and reference crop pots. Ten millilitres of the standard nutrients solution containing (KH_2PO_4 (P),$CuSO_4.5H_2O$ (Cu), $ZnCl_2$ (Zn), $Na_2B_4O_7.10H_2O$ (B), $Na_2MoO_4.2H_2O$ (Mo)) at a concentration of 300 mg P, 0.06 mg Cu, 0.2 mg Zn, 0.04 mg B and 0.008 mg Mo L^{-1}were mixed with soil before packing in the pots and the soils maintained at 80% field capacity.

Seeds were surface sterilised with sodium hypochlorite for 1 min and rinsed five times with sterilised distilled water. Planting of the negative control (non-inoculated) was first to avoid cross-contamination. Soybean seeds inoculation was at a rate of 1 g of inoculant $(100\ g)^{-1}$ of seeds following instruction on each pack of the inoculants. Three healthy seeds were planted pot^{-1}, and thinned to one plant pot^{-1} on the 10^{th} day after planting. Sorghum was used as a reference crop in the determination of BNF using the N-difference method (Viera-Vargas et al. 1995).

Data collection

On the 10^{th} week after planting, shoots from each pot were cut using a clean knife at 1 cm above the soil surface, and pots emptied into a 2 mm sieve washing away the soil and roots collected. Nodules were removed from the roots, counted, and weighed to determine their fresh weight, which was used in the determination of the weighed nodule effectiveness. From each soybean plant, 10 nodules were randomly selected, and then cut into two pieces and the colour observed and recorded for determination of nodule effectiveness. The weighed nodule effectiveness (WNE) was determined as shown in Equation (1). The approach outlines the importance of nodule fresh weight for the overall effectiveness per plant. It estimates the weighed nodule effectiveness (g $plant^{-1}$) taking into consideration the total weight of the nodules. Equation (1) is a significant innovation, as the traditional nodule effectiveness is misleading when the percentage of effective nodules out the sample of 10 nodules is high, while their fresh weight is low.

$$\text{WNE (g plant}^{-1}) = \frac{\text{ENOT} \times \text{TNFW}}{10} \quad (1)$$

where ENOT and TNFW stand for effective nodules out of 10 per plant, and total nodule fresh weight (g plant^{-1}), respectively.

The shoots were oven-dried at 60°C until constant weight (approximately 48 h), weighed to determine the shoot dry weight. Nitrogen fixation was determined using the N difference method (Unkovich et al. 2008). The dry shoot of soybean and sorghum shoots were ground and used to determine tissue N concentration using Kjeldahl method (Rutherford et al. 2008). The amount of shoot N and shoot dry weight were used in the determination of N_2 fixation (Viera-Vargas et al. 1995).

Evaluation of the effect of liming on soybean nodulation and nitrogen fixation

The second trial aimed at determining the effect of liming on soybean performance under inoculation. Two acidic soils: S2 (pH 4.8) and S4 (pH4.7) from the first trial, were incubated with agricultural lime for two weeks before planting of soybean. The lime rates for the two soils were determined in a laboratory incubation study. From the incubation study, agricultural lime was more effective in increasing soil pH than Minjingu phosphate rock (data not shown). The lime was added based on its effective calcium carbonate equivalence (ECCE) and adjusted based on moisture content following the Shoemaker, McLean and Pratt (SMP) lime requirement method (Thomas 1996). Agricultural lime used had an ECCE of 78.9% with >50% of its particles passing through a 0.25 mm sieve. Liming rate were 30 t ha^{-1} and 34 t ha^{-1} of lime to raise the pH to 6.0 for soil S2 and S4 respectively. All the other treatments and data collection were done the same way as in the first trial.

Data analysis

For the first trial, all data of the WNE, shoot dry weight, P and N uptake and nitrogen fixation were subjected to analysis of variance (ANOVA) at $p < 0.05$ level of significance using the mixed procedure of SAS system (SAS Institute Inc 2014). The second trial was considered as a three-way factorial (soybean varieties, lime, and inoculation) with ANOVA performed separately for each soil. The effects of the different treatments and their interactions were compared using the standard error of the difference (SED) of the mean. A *T*-test was done for soils S2 and S4 on all the measured parameters in the second trial.

Results

Weighed nodule effectiveness

In the first trial; soils, inoculation and soybean varieties interaction significantly influenced the WNE $p < 0.05$ (Table 2). Moderate acidic soils with soybean variety SB19 inoculated with Legumefix had highest WNE. Soybean inoculation with Biofix and Legumefix resulted in high WNE, while control plants had minimal to insignificant nodulation. Control plants (non-inoculated) in the slightly acidic soils had nodules; however, the WNE was low compared to that of inoculated plants (Table 2; Figure 1).

In the second trial, liming of strongly acidic soils improved nodulation. There was no significant difference between soil S2 and S4 for the WNE (Table 2). In both soils, lime, variety and inoculation interaction significantly increased the WNE (Table 2; $p < 0.05$). Soybean variety SB19 inoculated with Legumefix had the highest WNE in both soils (Figure 1(b,c)). Inoculation with Legumefix resulted in much higher WNE compared to Biofix in both limed and unlimed treatments. The non-inoculated control plants in both limed and unlimed treatments did not produce any nodules. On average, soybean variety SB19 had high WNE compared to Nyala variety.

Shoot biomass

In the first trial, soil and inoculation had a significant influence on shoot dry weight $p < 0.05$ (Table 2). Moderate and slightly acidic soils had high shoot dry weight compared to the strongly acidic soils (Table 2; Figure 2(a)). Biofix and Legumefix inoculants significantly increased shoot dry weight, while non-inoculated control plants had the least shoot dry weight (Table 2; Figure 2(b)).

A *t*-test indicated no significant difference on the shoot dry weight between soil S2 and S4 in the second trial (Table 2). Co-application of lime and inoculation had a significant influence in increasing shoot dry weight in soil S2. Inoculated soybean in limed soil produced high shoot dry weight compared to inoculated soybean in unlimed soil. Application of lime and Legumefix inoculant increased shoot dry weight by 7.17 g plant^{-1} more than in soybean inoculated with Biofix. The plants that did not receive inoculation for the limed and unlimed treatments had the lowest shoot dry weight compared to the inoculated plants (Figure 2(c)). In soil S4, soil liming increased shoot dry

Table 2. Summary of Analysis of variance (ANOVA) for soybean nodulation, shoot dry weight, nitrogen uptake and fixation and phosphorus uptake.

	DF	Weighed nodule effectiveness (g plant^{-1})	Shoot dry weight (g plant^{-1})	N uptake (g plant^{-1})	Ndfa %	P uptake (mg plant^{-1})
Greenhouse experiment 1						
Soil (S)	9	***	***	***	***	***
Varieties (V)	1	*	ns	ns	ns	ns
Inoculation (I)	2	***	***	***	***	***
S×V	9	ns	ns	ns	ns	ns
S×I	18	***	ns	***	***	*
V×I	2	ns	ns	ns	ns	ns
S×V×I	18	***	ns	*	**	ns
Greenhouse experiment 2 (Soil S2)						
Lime (L)	1	*	*	*	*	*
Varieties (V)	1	ns	ns	ns	ns	ns
Inoculation (I)	2	***	***	***	***	***
L×V	1	ns	ns	ns	ns	ns
L×I	2	***	*	ns	*	ns
V×I	2	ns	ns	*	ns	ns
L×V×I	2	*	ns	ns	ns	ns
Greenhouse experiment 2 (Soil S4)						
Lime (L)	1	*	*	***	*	*
Varieties (V)	1	**	ns	ns	**	ns
Inoculation (I)	2	***	***	***	***	**
L×V	1	*	ns	ns	*	ns
L×I	2	*	ns	*	*	ns
V×I	2	ns	ns	ns	*	ns
L×V×I	2	*	ns	ns	*	ns
***T*-test (S2–S4)**						
(p Value)	70	ns	ns	ns	ns	ns

Note: ANOVA is Analysis of variance; ns: not significant at $p < 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

weight by 28.27% than in unlimed soil (Table 2; Figure 2(d)), while inoculation with Legumefix increased shoot dry weight by 81.09% and inoculation with Biofix increased shoot dry weight by 17.08% (Figure 2(e)).

Phosphorus uptake

In the first trial, soil and inoculation interaction had significant influence on P uptake $p < 0.05$ (Table 2). Highest amount of P uptake was in the moderate acidic soil with soybean inoculated with Legumefix (Figure 3(a)).

There was no significant difference in P uptake between soils S2 and S4 at $p < 0.05$ in the second trial (Table 2). Lime and inoculation significantly influenced P uptake ($p < 0.05$). On average, soil liming increased P uptake by 8.06 and 6.51 mg P kg^{-1} in soils S2 and S4 respectively (Table 2; Figure 3(b,d)). Soybean Inoculation with Legumefix increased P uptake by 15.74 and 9.33 mg P kg^{-1}, while inoculation with Biofix increased P uptake by 10.24 and 9.01 mg P kg^{-1} above control in soils S2 and S4 respectively (Figure 3(c,e)).

Nitrogen uptake

Nitrogen uptake was significantly influenced by the interaction of soil, variety and inoculation in the first trial (Table 2; $p < 0.05$). Highest levels of N uptake were in the moderate and slightly acidic soils, while the strongly acidic soils had low levels of N uptake. Inoculated plants had high level of N uptake compared to the non-inoculated control plants (Figure 4(a)).

A *t*-test between S2 and S4 indicated no significant difference in N uptake (Table 2). The interaction of inoculation and soybean variety was significant for N uptake in soil S2 (Table 2; $p < 0.05$). Inoculation of Nyala and SB19 with Legumefix resulted in high N uptake compared to inoculation with Biofix (Figure 4(b)). Co-application of inoculation and lime significantly increased N uptake in soil S4 (Figure 4(c)). Legumefix + lime resulted in high N uptake compared to Biofix + lime. Control plants in both lime and without-lime treatments had the least N uptake levels.

Biological nitrogen fixation

Nitrogen fixation was significantly influenced by the interaction of soil, variety and inoculation $p < 0.05$ (Table 2). Moderate and slightly acidic soils had highest level of N fixation compared to the strongly acidic soils. Inoculation also improved nitrogen fixation; soybean inoculated with Legumefix had a high level of nitrogen fixation compared to inoculation with Biofix. The strongly acidic soils had the low level of N fixation for both varieties under inoculation, while the moderate and slightly acidic soils had the highest levels of N fixation for both Nyala and SB19 under inoculation (Figure 5(a)). Nitrogen fixation in non-inoculated control plants was low for both Nyala and SB19 varieties.

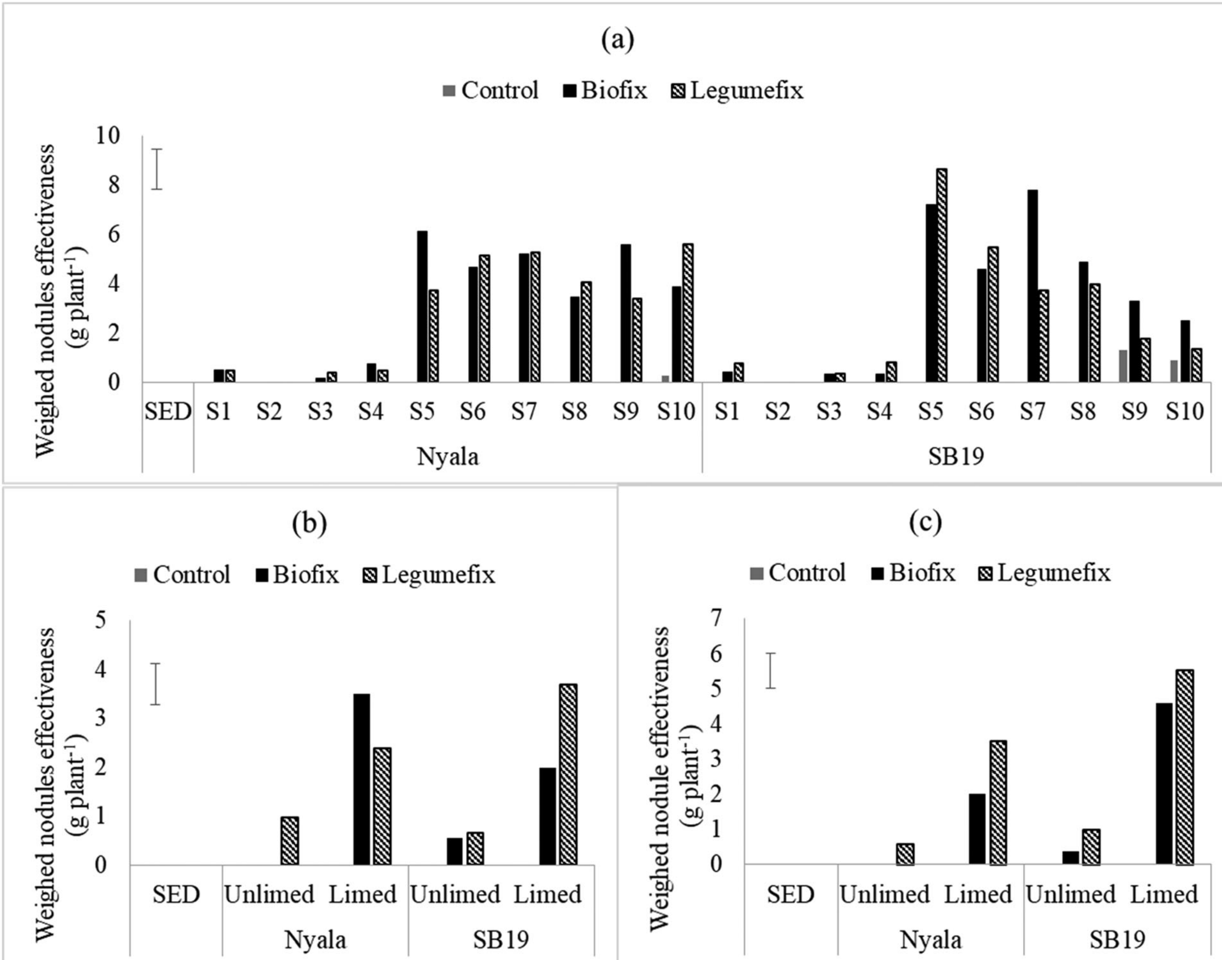

Figure 1. Weighed nodule effectiveness in soybean as influenced by (a) soil, variety and inoculation interaction, in the first trial; and (b and c) the interaction of lime, inoculation and variety in the second trial, for soil S2 and S4 respectively (as indicated in Table 2). The error bars represent standard error of the difference of the means (SED).

Co-application of lime and inoculants had a significant influence on Ndfa (Table 2; $p < 0.05$). Soybean inoculation in limed soil had high levels of N fixation compared to without-lime (Figure 5(b)). Soybean inoculated with Legumefix in limed soil resulted in high N fixation levels compared to inoculation with Biofix. Lime, variety and inoculation, as well as their interaction significantly influenced Ndfa in soil S4 ($p < 0.05$). Co-application of Legumefix and lime for SB19 variety increased N fixation compared to inoculation with Biofix in limed soils (Figure 5(c)). There was no significant difference in N fixation between the two soils (i.e. S2 and S4) at $p < 0.05$.

Discussion

This study has developed a robust formula (i.e. WNE) that takes into consideration the fresh weight of all the nodules per plant (Equation (1)) to estimate nodule effectiveness. Originally, nodule effectiveness was based on the percentage of pink nodules out of 10 randomly sampled nodules (FAO 1993). The approach represents a bias when variable number of nodules are found per plant (i.e. plants with several nodules versus plants with a few nodules). The method underestimates the nodule effectiveness when there are a high number of nodules per plant, when very few out of the sampled nodules show the pink colour. Conversely, it overestimates the nodule effectiveness when there are a low number of nodules per plant, while most of the sampled nodules show the pick colour. The WNE improves the estimation of the nodule effectiveness, building on the FAO's (1993) method, but taking into consideration the total nodule fresh weight per plant, and indirectly the total nodule number per plant.

The soils, inoculation, soybean varieties, as well as their interactions, significantly increased the WNE, N uptake, and nitrogen fixation, as shown in the ANOVA summary in Table 2. Moderate and slightly acidic soils had high rates of the measured parameters compared

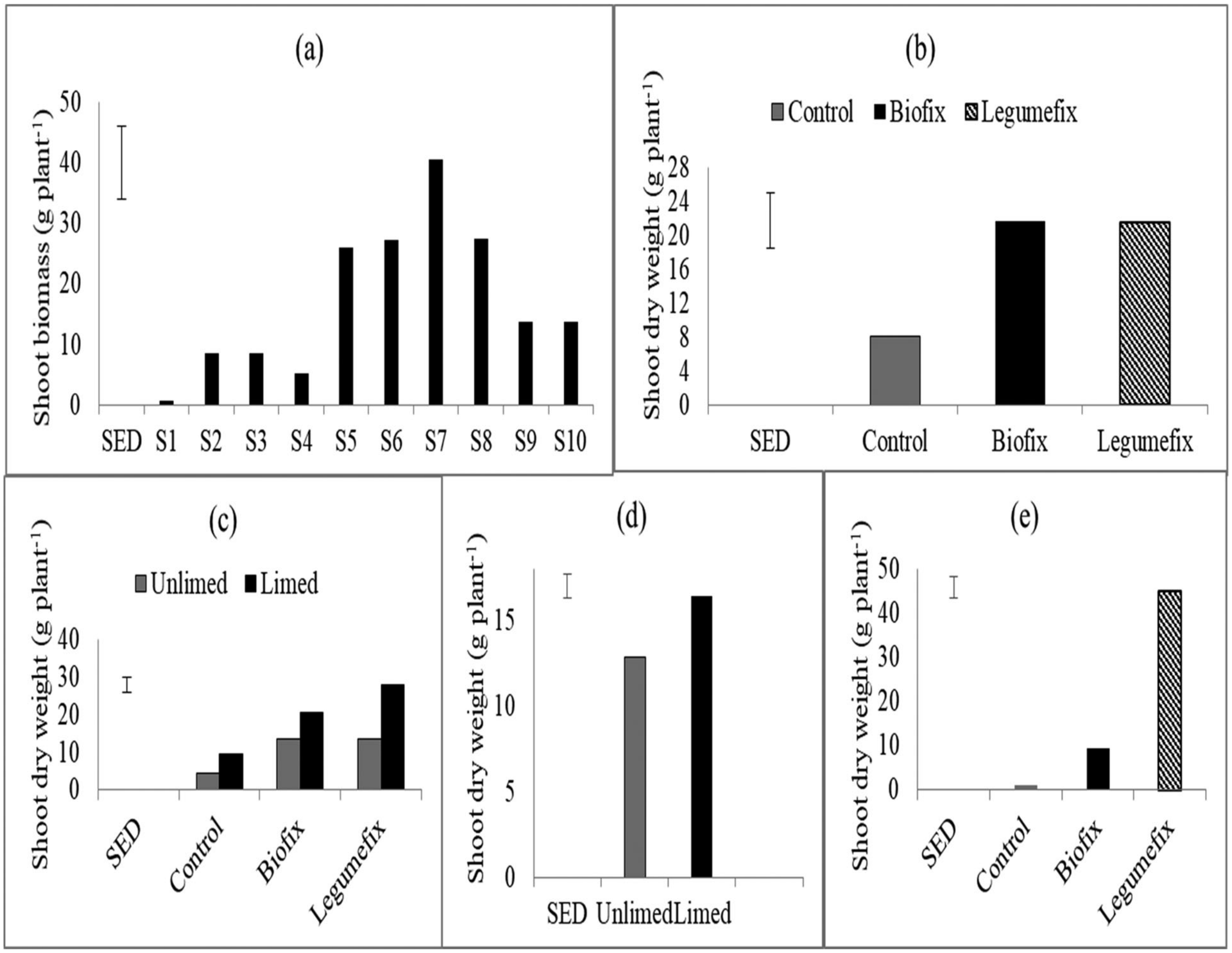

Figure 2. Shoot dry weight as influenced by (a) soils and (b) inoculation effects in the first trial; (c) interaction of lime and inoculation in soil S2 and (d and e) lime and inoculation effects in soil S4 in the second trial (as indicated in Table 2). The error bars represent standard error of the difference of the means (SED).

to the strongly acidic soils (Figure 1). The strongly acidic soils had low levels of P, Mg, Ca, and K (Table 1); contributing to low WNE, shoot dry weight, P and N uptake, and nitrogen fixation. Inoculation of soybean in the moderate and slightly acidic soils also increased WNE, shoot dry weight, P and N uptake, and nitrogen fixation (Figures 1–5). It was concluded that inoculation ineffectiveness was mainly due to rhizobia sensitivity to low soil pH, which is generally associated with Al and Fe toxicity as previously reported by Fageria et al. (2013) and low nutrients availability.

There was also a significant difference among the two inoculants used (Table 2). Legumefix was more effective in increasing the measured parameters like shoot dry weight (Figure 2(b,e)) compared to Biofix and non-inoculated plants. This could be attributed to the strain in the Legumefix inoculant being more tolerant to acidic soils compared to the strain in the Biofix inoculant. Inoculation of soybean variety SB19 with Legumefix in the moderately and slightly acidic soils resulted in the high levels of WNE, N uptake, and nitrogen fixation (Figures 1(a), 4 (a) and 5(a)). This was related to the fact the promiscuous soybean variety SB19 has a high N fixing ability compared to Nyala, as previously reported by Thuita et al. (2012). High level of N uptake and fixation in the moderate and slightly acidic soils can be attributed to nutrients availability like cations (Table 1), which are crucial in N fixation. Nitrogenase activity increases with increasing K in the soil (Keino et al. 2015). Calcium plays a major role in the rhizobia-legume symbioses, it is used for adhesion by rhizobia; hence, its deficiency in acidic soils affects rhizobia attachment, and infection thread formation, thus nitrogen fixation is negatively affected (Meng-Han et al. 2012).

In this study, moderate and slightly acidic soils had high shoot dry weight compared to the strongly acidic soils (Figure 2(a)). This is attributed to low levels of essential nutrients at pH < 5.5 resulting in stunted growth. Low levels of Mg in the strongly acidic soils compared to the moderate and slightly

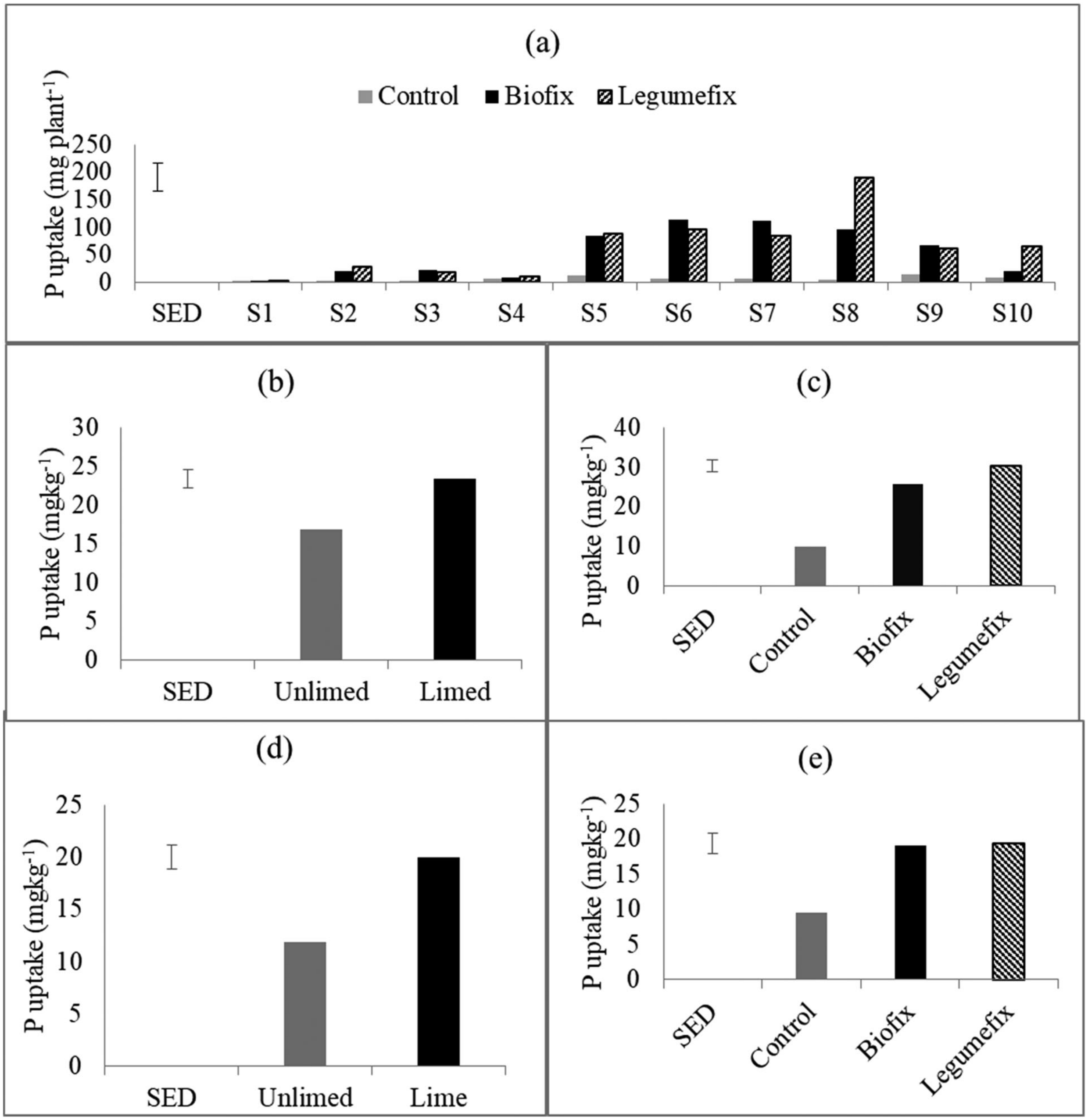

Figure 3. Phosphorus uptake as influenced by (a) soil and inoculation interaction in the first trial; and (b and c) lime and inoculation single effects respectively, in soil S2 and (d and e) lime and inoculation effects in soil S4 respectively for the second trial (as indicated in Table 2). The error bars represent standard error of the differences of the means (SED).

acidic soils contributed to the low shoot weight. Magnesium plays a vital role in plant growth; when it is deficient like in acidic soils, it interferes with photosynthesis and P reactions (Keino et al. 2015). Inoculated plants also had high shoot dry weight compared to non-inoculated plants (Figure 2(b,e)). Low shoot biomass in non-inoculated control plants had been reported in other studies, while the inoculated plants response to the inoculants can be attributed to low soil pH conditions, as previously reported by Goncalves et al. (2000). Nutrient deficiencies in the strongly acidic soils (Table 1) could explain the low nutrients uptake and consequently the stunt growth. This was confirmed with the low N and P uptake in the strongly acidic soils compared to the moderately and slightly acidic soils (Figures 3(a) and 4(a)), as previous reported by Fageria et al. (2013). Inoculation improved both N and P uptake in the moderately and slightly acidic soils (Figures 3(a) and 4(a)), which could be related to N fixation for N uptake, and the ability of *Rhizobium* to solubilise the precipitated P and make it available for uptake, as reported by Fatima et al. (2006).

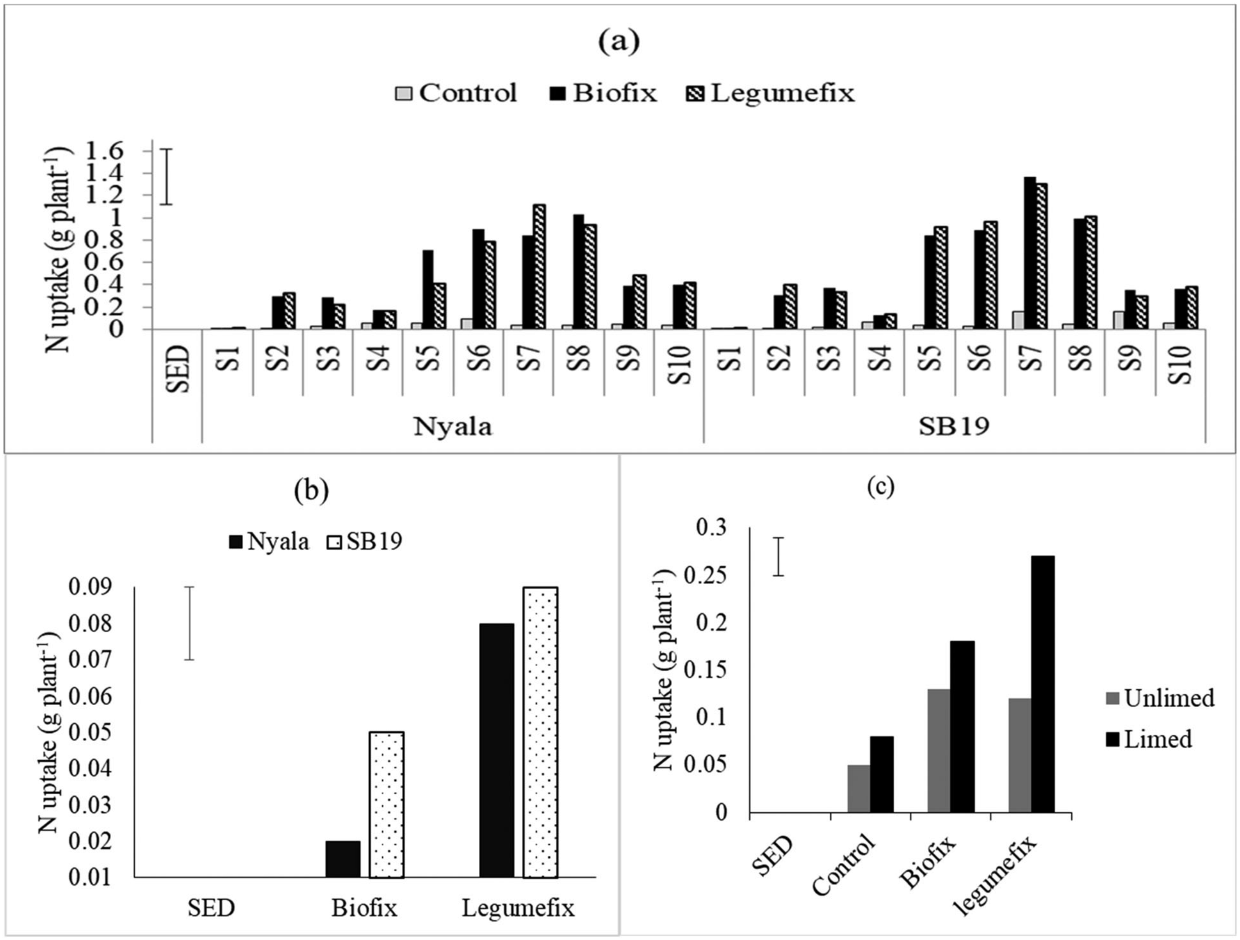

Figure 4. Nitrogen uptake as influenced by (a) soil, variety and inoculation interaction in the first trial, (b) variety and inoculation interaction in soil S2 in the second trial and (c) lime and inoculation interaction in soil S4 (as indicated in Table 2) . The error bars represent standard error of the differences of the means (SED).

Liming of the strongly acidic soils significantly increased the WNE, shoot dry weight, P and N uptake and N fixation (Table 2; Figures 1(b,c), 2(c,d), 3(b,d), 4(c) and 5(b,c)). In addition, there was no significant difference between the two-limed soils in increasing the measured parameters (Table 2). This indicates that strongly acidic soils require liming to improve the potential of biological nitrogen fixation. Most development initiatives in sub-Saharan Africa commonly promote rhizobia inoculants for legume crops like soybean, without provisions for correcting soil acidity, regardless of the pH levels. These findings would be useful for revisiting the recommendation for application of rhizobia inoculants in the region, particularly for strongly acidic soils. The improvement of nodule effectiveness, soybean growth, nutrients uptake, and biological nitrogen fixation, could be related to the improvement of nutrients' availability following application of lime, as previously reported by Meng-Han et al. (2012), Mullen et al. (2006), and Keino et al. (2015) and reduction of Al and Fe toxicity, as reported by Kisinyo et al. (2014).

Co-application of lime and inoculation improved the levels of the WNE, shoot dry weight, N uptake and nitrogen fixation (Table 2; Figures 1(b,c), 2(c), 4(c) and 5(b,c)). This shows that liming acidic soils improves the nodule effectiveness, and as results nitrogen fixation, soybean growth and nutrient uptake are enhanced, as previously reported by Appunu et al. (2014) and Bekere (2013). In addition to improved nutrient availability, this significant interaction between liming and inoculation could be related to creating a suitable environment for increased microbial activities in the rhizosphere, as reported by Nduwumuremyi (2013).

This study was conducted in controlled environment and showed promising results; therefore, there is a need to conduct similar studies in field conditions to cross-validate the findings. In the meantime, there is an overdue need to consider lime application to strongly acidic soils before application of rhizobia inoculants to legume crops like soybean to improve plant growth, and consequently yields when no other limiting factors are expected. Currently, there is a strong promotion of

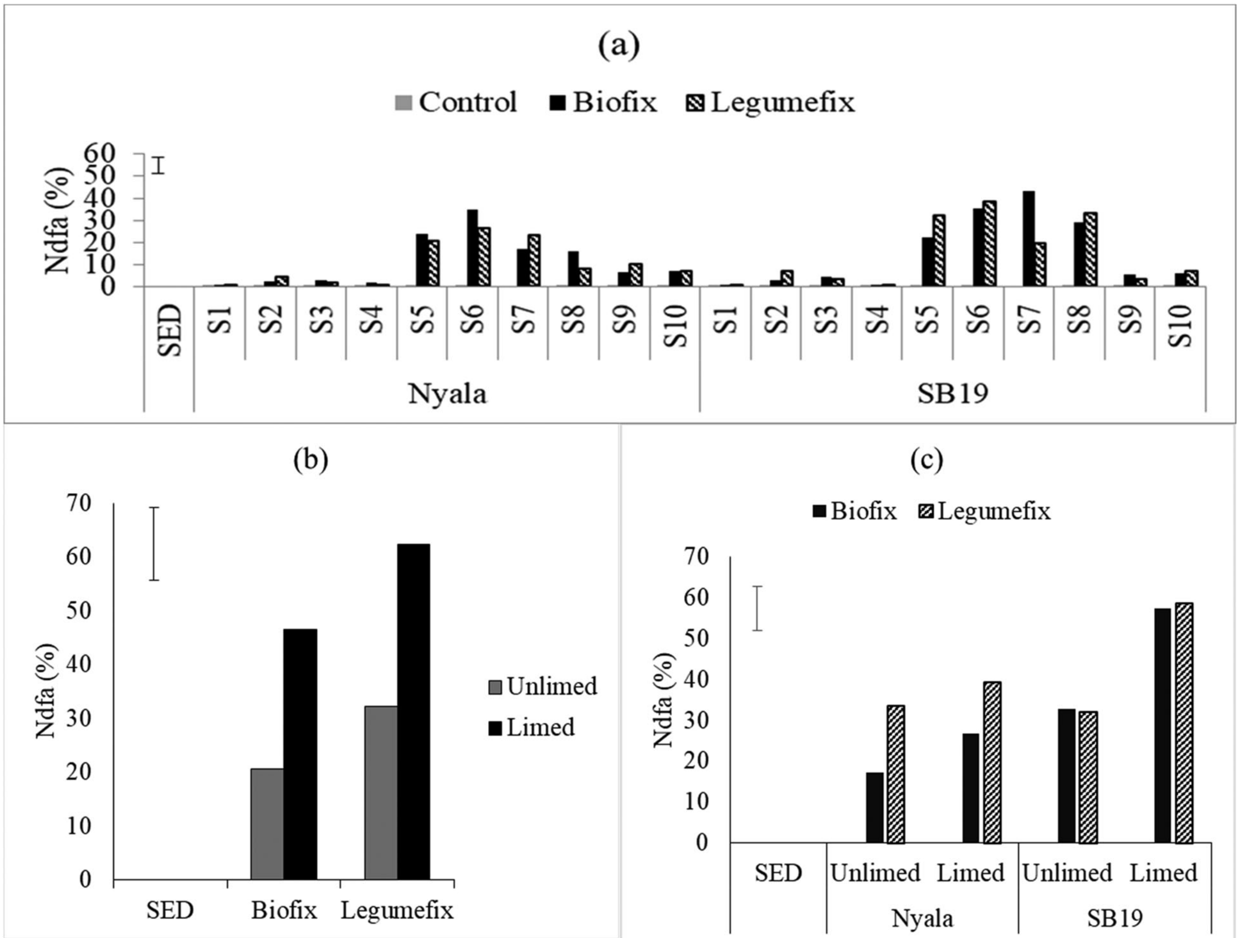

Figure 5. Nitrogen fixation as influenced by (a) soil, inoculation, and soybean varieties interaction in the first trial, (b) lime and inoculation in soil S2 in the second trial and (c) lime, variety and inoculation interaction in soil S4 (as indicated in Table 2). The error bars represent the standard error of differences of the means (SED).

rhizobia inoculants as in integral part of integrated soil fertility management in legume systems in sub-Saharan Africa, irrespective of the soil acidity; a practice that requires adjustment based on the findings of this study. When assessing the response of legume crops to rhizobia inoculation, it is recommended to use the weighed nodule effectiveness, as it corrects not only for the total number of nodules per par plant, but also the weight; compared to the current nodule effectiveness that uses the percentage of pink nodules out of a sample of 10 nodules per plant.

Acknowledgment

We are grateful to the International Institute of Tropical Agriculture (IITA) under the Commercial Products project (COMPRO-II) funded by the Bill and Melinda Gates Foundation (OPPGD1398) for financial support. We also acknowledge the contributions of Harrison Mburu, Elias Mwangi, Martin Kimanthi, and the laboratory staff of the International Centre for Tropical Agriculture (CIAT) for their technical assistance during laboratory and greenhouse work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

We are grateful to the International Institute of Tropical Agriculture (IITA) under the Commercial Products project (COMPRO-II) funded by the Bill and Melinda Gates Foundation [grant number OPPGD1398] for financial support.

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