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Effect of tillage on biological nitrogen fixation and yield of soybean (*Glycine max* L. Merril) varieties

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Abstract

Soil properties, plant characteristics, agronomic practices and environmental factors often influence biological nitrogen fixation of legumes. Tillage method used in a specific agro-ecological zone is among these factors thus, the aim of this study was to determine the effect of tillage and no tillage on biological nitrogen fixation and grain yields of three soybean varieties. The study was conducted at four agro-ecological zones of Western Kenya where treatments were replicated thrice using randomized complete block design in split plot arrangement. No tillage and conventional tillage were the main plots whereas, soybean varieties: Nyala, SB19 and SB20 were the sub-plots. Amount of N fixed was determined using ^{15}N abundance method and quantity of nitrogen fixed under no tillage practice exceeded conventional tillage at all sites however, soybean varieties fixed same amount of N. Interaction effect of tillage method and variety on amount of N fixed was varying at every agro-ecological zone though, interaction between no tillage and Nyala fixed more nitrogen at LM 3 than at other agro-ecological zones. Soybean grain yield was similar between tillage methods and also among varieties in a combination of all sites. Practicing no tillage encourages biological nitrogen fixation and its longtime operation leads to yield increase as it improves most soil properties.

Keywords: Till; no till; biomass; N_2-fixation.
Abbreviations: Asl_Above the sea level, BNF_Biological nitrogen fixation, IITA_International Institute for Tropical Agriculture, LM 1_Lower midland sugarcane zone, LM 2_Marginal sugarcane zone, LM 3_Lower midland marginal cotton zone, LM 4_Lower midland cotton zone, TGx_Tropical Glycine Cross.

Introduction

Poor soil fertility has been acknowledged as a major hindrance to high crop yield (Hilhorst et al., 2000). Researchers have devised ways of alleviating this problem, some of which comprise application of organic and inorganic fertilizers. However, use of inorganic fertilizers by small holder farmers in sub-Saharan Africa is inadequate (Bationo et al., 2006) due to high costs, unavailability and sometimes lack of knowledge on usage. Materials for organic fertilizers are also difficult to acquire as farmers prefer supplying stovers to livestock rather than leaving them in the field to decay and consequently release nutrients (Baijukya, 2004). These challenges have led to exploitation of other economical ways of supplying nutrients to the crops and one of these ways is biological nitrogen fixation. Biological nitrogen fixation (BNF) in legumes has for a longtime been a component of many farming systems throughout the world. Soybean for example, a crop which is currently being promoted as an alternative source of proteins, cooking oil, income to farmers and for soil fertility improvement in Kenya (Misiko et al., 2008), has the capacity to obtain its full nitrogen requirements through symbiotic nitrogen fixation and contribute surplus N to the soil reserves for successive crops (Salvagiotti et al., 2008). Sanginga et al., (2003) reported that some soybean varieties biologically fix 44 to 103 kg N ha^{-1} annually. However, this biological nitrogen fixation (BNF) process is primarily controlled by four principal factors: effectiveness of rhizobia-host plant symbiosis, ability of the host plant to accumulate N, amount of available soil N and environmental constraints (Van Kessel and Hartley, 2000). Soil as a medium of plant growth has greater bearing on nitrogen fixation, plant growth and final yield hence, management of its environment is of great importance. Several factors such as: acidity, salinity, alkalinity, temperature, moisture, fertility and structure (Hungria and Vargas, 2000) influence soil environment. Tillage is among the major practices that influence physical, biological and chemical properties of the soil environment and subsequently affects nitrogen fixation (Kihara et al., 2012). Conventional tillage (tillage) has been practiced over a longtime around the globe (Fowler and Rockstrom, 2001) due to its numerous advantages which consist of loosening of the soil leading to increase in drainage, root development and acceleration of organic matter decomposition by soil micro-organisms and improvement of aeration (Moussa-Machraou et al., 2010). However, its sustainability has been questioned over time because of depreciation of natural resources and climate changes (Hobbs and Gupta, 2003). This has become the foundation as to why most researchers are now advocating for no tillage. Contrary to conventional tillage, no tillage minimizes soil and nutrient losses through leaching and erosion (Shipitalo et al., 2000; Schillinger, 2001), it increases soil water storage (Malhi et al., 2001) and has reduced production cost (Singh et al., 2008). The merits and demerits of both conventional tillage and no tillage all impinge on the amount of nitrogen fixed, growth and yield of a crop. It has also been reported that effect of tillage methods depend on crop species, climate, site and time of tillage (Martinez et al., 2008). Western Kenya has several agro-ecological zones which are characterized by unreliable rainfall distribution (Rockstrom et al., 2007) and farmers practice various methods of tillage. There is need for a tillage method that is site-specific to enhance nitrogen fixation, increase growth and improve grain yield of soybean. Therefore, the main objective of this study was to determine effect of tillage and no tillage, as they are the major tillage methods practiced in Western Kenya (Gicheru et al., 2004), on biological nitrogen fixation and yield of different soybean varieties at different agro-ecological zones of Western Kenya.

Results

Dry weight and percentage of active nodules

Figure 1 illustrates that dry nodule weight between tillage methods was different at LM 1 and LM 3 whereas, among varieties it was higher at LM 3. The figure further shows that at agro-ecological zones where dry nodule weight was different, no tillage was superior while among varieties SB20 was the best. Percent active nodules were similar between tillage methods and different among varieties. The difference in percent active nodules among varieties was at LM 1 and LM 3 where SB20 and Nyala had higher percentages respectively (Figure 2).

Nitrogen fixed

Nitrogen fixed biologically differed significantly between tillage methods at LM 1 and LM 3 but, was similar at LM 2 and LM 4. More nitrogen was fixed in No tillage than tillage at LM 1 and LM 3 (Table 1). There were significant differences in nitrogen fixed among the soybean varieties at each site (Table 1). The amount of nitrogen fixed by various varieties was site dependent as shown by SB20 and SB19 fixing more nitrogen at LM 1 and at LM 2 respectively whereas, Nyala fixed more nitrogen at LM 3 and LM 4 (Table 1). The results of significant interactive effect of tillage method and variety on amount of N fixed at all sites are presented in Table 2. At LM 1, Nyala fixed the lowest amount of N under tillage management, while SB19 fixed the lowest N under no tillage management. The values in both cases were only significantly inferior to those of SB20. At LM 2, the highest amount of N was fixed by SB19 under the two tillage conditions while SB20 performed minimally. At LM 3, there were no significant differences in the amount of N fixed by all varieties under tillage condition, though, under no tillage Nyala fixed significantly more N than the other two varieties. At LM 4, there were significant differences among the varieties in the amount of N fixed under tillage condition and Nyala was the top fixer. Nyala and SB19 fixed similar amounts of N with values significantly better than SB20 under no tillage condition.

Shoot biomass and grain yield

There were significant differences in the values of root biomass, shoot biomass and dry grain yield among the sites of evaluation (Table 4). Root biomass, shoot biomass and grain yield were not significantly different between the two tillage methods (Table 4). However, there were significant differences among varieties on root and shoot biomasses (Table 6) and SB20 expressed the highest root and shoot biomasses.

Results of tillage and variety interactive effects on yield parameters in Table 5 show that there were no significant differences in root biomass among the varieties under tillage condition, while SB20 produced significantly more root biomass than the other two varieties under no tillage condition. Interaction effects on shoot biomass revealed that both Nyala and SB19 produced similar shoot biomass values which were significantly inferior to that of SB20 under tillage condition, whereas under no tillage condition Nyala's shoot biomass was the lowest and SB20 the highest. SB19 produced the highest dry grain yield which was significantly better than the other varieties under tillage condition. However, there were no appreciable differences in dry grain yields among the three soybean varieties under no tillage condition.

Discussion

High amount of nitrogen fixed under no tillage condition at LM 1 and LM 3 could have been due to minimal disturbance of soil leading to increase in rhizobial activity. In addition, Zhang et al., (2012) stated that rhizobial population was high under no tillage condition. Ferreira et al., (2000) further affirmed that rhizobia isolates from no tillage condition plots fixed more atmospheric nitrogen and Van Kessel and Hartley, (2000) in their review also asserted that no tillage leads to stimulation of nitrogen fixation. These arguments, supported by the high dry nodule weight under no tillage condition justifies the high amount of nitrogen fixed at LM 1 and LM 3. Low amount of nitrogen that was similar between tillage methods at LM 2 and LM 4 could be attributed to low pH and high sodium content (Table 3). Furthermore, low initial soil nitrogen at LM 4 could have accentuated minimal N fixation. These factors possibly affected rhizobia activities at LM 2 and LM 4. The effect of these factors on N fixation conforms to Belnap and Lange, (2001) review on the same. The difference in nitrogen fixed among soybean varieties could have been due to differences in soil moisture within their plots which probably enhanced activity of rhizobia at different sites. In Van Kessel and Hartley, (2000) review they reported that increased soil moisture increases the potential of biological nitrogen fixation. Besides soil moisture, the genetic ability of different varieties to fix nitrogen could have also caused the differences. High amount of nitrogen fixed at LM 1 by SB20 planted under no tillage was perhaps due to high percentage of active nodules on the roots compared to the other varieties. At LM 2 and LM 4, SB19 and Nyala under tillage and no tillage fixed high amount of nitrogen possibly due to their adaptability to those agro-ecological zones. Nyala fixed outstanding amount of nitrogen under no tillage at LM 3 probably due to high soil moisture content as argued by Malhi et al., (2001) and the fact that no tillage usually have rhizobia isolates which fix more atmospheric nitrogen than tillage according to Ferreira et al., (2000). Root biomass that was high at LM 3 could have been due to sufficient soil nutrients such as total nitrogen and phosphorus (Table 3) that are known to boost root growth. This vast root biomass was perhaps the reason for massive shoot biomass leading to increased photosynthesis and translocation of assimilates

Table 1. Effects of soybean variety and tillage method on the amount of nitrogen fixed (kg ha^{-1}) at different sites.

Source	Nitrogen fixed (Kg ha^{-1})			
	LM 1	LM 2	LM 3	LM 4
Soybean variety				
Nyala	6.5^{b}	13.5^{ab}	19.3^{a}	16.6^{a}
SB19	7.9^{b}	16.9^{a}	6.0^{b}	13.7^{ab}
SB20	14.0^{a}	6.4^{b}	7.0^{b}	5.4^{b}
Tillage method				
Tillage	6.5^{b}	5.7^{a}	8.1^{b}	5.8^{a}
No tillage	12.4a	6.8a	13.8a	6.4a

Means with different letters are significantly different at p<0.05 within a column of a source.

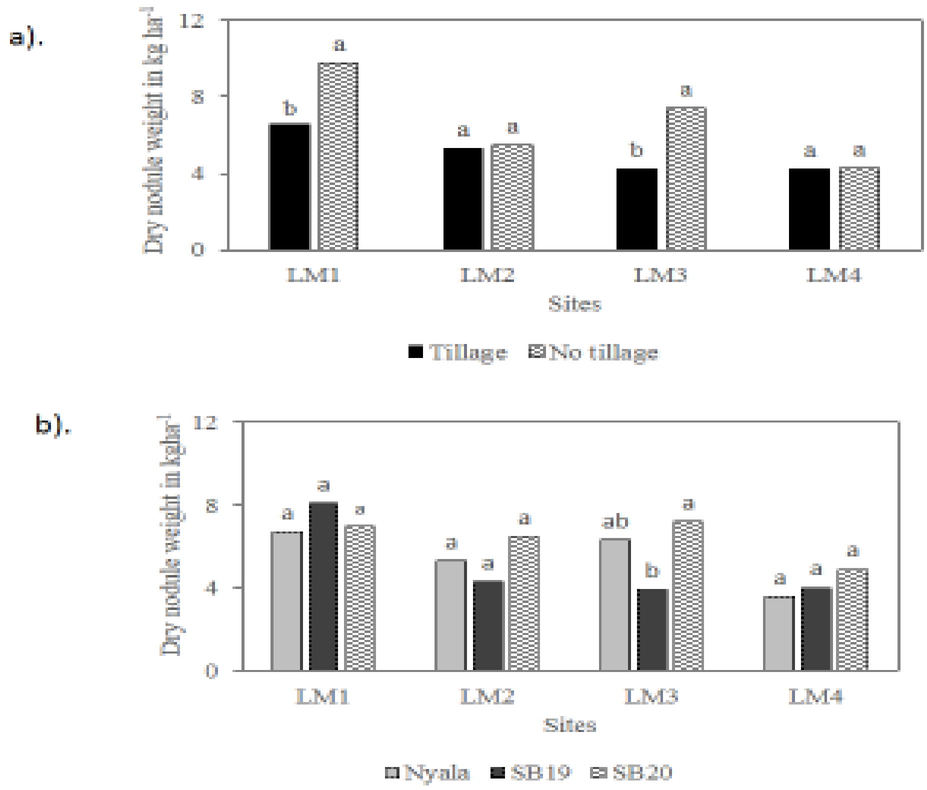

Fig 1. Effect of: a). tillage method; b). variety on soybean dry nodule weight (kg ha^{-1}) at different sites. Means with different letters are significantly different at p<0.05 within a site.

to the sinks (grains) and consequently high dry grain yield. Similarity in root biomass, shoot biomass and grain yield between tillage methods in the two seasons could be attributed to the argument that growth and yield differences in no tillage and tillage always become evident after several years of cropping (Malhi et al., 2006). SB20 developed massive roots and shoots under the two tillage conditions while Nyala's were small under no tillage possibly due to genetic traits of the varieties (Tefera, 2011). Grain yield of SB19 was excellent under tillage probably due to its trait of medium maturity. This trait could have enabled it utilize available soil moisture and attain maximum growth before the onset of minimum or lack of rainfall.

Materials and methods

Experimental sites

The experiment was conducted for two seasons: March to August, 2011 and September to December, 2011 in four sites representing four agro-ecological zones of Western Kenya. The sites were: Kanduyi in Bungoma county (0˚ 35´ N, 34˚ 35´ E) which lies on agro-ecological LM 1 – Lower midland sugarcane zone. The area receives annual rainfall of 1600 – 1800 mm, average temperature of 21 – 22 ˚C and the soils are well drained, deep to very deep, red to dark brown and friable sandy clay (classified as Ferralo – orthic acrisols). The second site was Ugunja in Siaya county (0˚ 09´ N, 34˚ 18´ E) which receives annual rainfall of 1450 – 1600 mm, average temperature of 21 – 22 ˚C and lies on agro-ecological zone LM 2 – marginal sugarcane zone. The soils at LM 2 are well drained, deep, dark red (classified as Orthic – rhodic ferralisols). The third site was Kari in Alupe, Busia County (0˚ 28´ N, 34˚ 07´ E). It receives 1100 – 1450 mm, rainfall annually, average temperature of 22 – 23 ˚C and the soils are well drained, deep and of low fertility (classified as Ferralo – orthic acrisols). It lies on agro-ecological zone LM 3 - Lower midland marginal cotton zone. The last site was Asembo in Rarieda, Siaya County (0˚ 08´ N, 34˚ 23´ E). This lies on agro-ecological zone LM 4 – Lower midland cotton zone,

Table 2. Interactive effects of tillage and soybean variety on the amount of nitrogen fixed (kg ha^{-1}) at LM 1, LM 2, LM 3 and LM 4.

Tillage	Variety	LM 1	LM 2	LM 3	LM 4
Tillage	Nyala	3.8^{c}	6.3^{b}	10.0^{b}	7.9^{a}
	SB19	7.0^{bc}	7.7^{a}	6.2^{b}	6.5^{b}
	SB20	10.7^{ab}	3.2^{c}	5.7^{b}	2.1^{c}
No tillage	Nyala	11.3^{ab}	7.4^{ab}	28.7^{a}	8.8^{a}
	SB19	8.8^{b}	8.9^{a}	5.8^{b}	7.3^{ab}
	SB20	17.2^{a}	3.5^{c}	7.0^{b}	3.4^{c}

Means with different letters are significantly different at p<0.05 within a column.

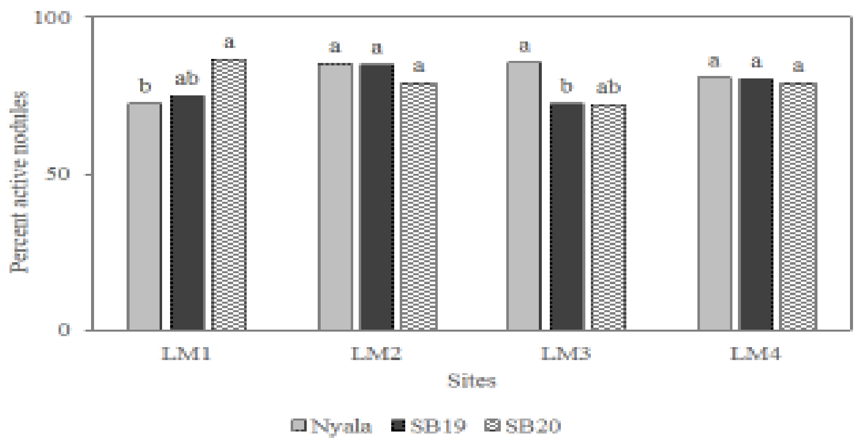

Fig 2. Effect of variety on percent active nodules of soybean at different sites. Means with different letters are significantly different at p<0.05 within a site.

receives annual rainfall amount of 900 – 1100 mm, average temperature of 22 – 23 ˚C and the soils are well drained, very deep and dark red (classified as Orthic feralisols) (Jaetzold et al., 2005).

Plant materials

Three soybean varieties that were planted are: Nyala, SB19 and SB20. Nyala matures within a period of 83 – 93 days, grows in an altitude of 1200 – 2400 m asl and its average grain yield is 0.7 – 2.5 t ha^{-1}. It can also grow in areas with minimum rainfall of 300 mm annually. It is a local variety which is susceptible to rust disease and nodulates with specific rhizobia strains (Myaka et al., 2005). This variety was chosen because it is dominant among local farmers (Myaka et al., 2005). The other two varieties were new introductions from IITA. One of them is TGx 1740-2F which is also known as SB19. It matures within a period of 92 – 96 days, has more pods per plant up to the top of the plant, performs well under poor and erratic rainfall, and has better lodging resistance. Its grain yield is between 1761 – 2232 kg ha^{-1} (Tefera, 2011). The other soybean variety is TGx 1448-2E which is better known as SB20. It matures within a period of 115 – 117 days and has grain yield ranging between 2403 – 2458 kg ha^{-1} (Tefera, 2011).

Experimental procedures

The experiment was replicated thrice in randomized complete block design under split-plot arrangement. Nyala, SB19 and SB20 were the soybean varieties tested on no tillage and conventional tillage (tillage). No tillage and conventional tillage were the main plots while soybean varieties were the sub plots. Main experimental plots measured 13 m by 11 m while sub-plots measured 4m by 3m. Conventional tillage was conducted using hoes of 20 cm length and 15 cm width while no tillage was done using glyphosate at 1.5 litres in 100 litres of water per hectare two weeks before planting. A basal rate of fertilizer was applied to all treatments in form of Triple Superphosphate (TSP) at a rate of 30 kg P ha^{-1} and potassium in form of Muriate of Potash (MOP) at a rate of 30 kg K ha^{-1} in furrows of 5 cm depth and 3 cm away from the planting lines and covered with soil. All soybean seeds were inoculated using biofix inoculants strain USDA 110 from Mea Limited – Kenya at 10g kg^{-1} of seeds and planted at a spacing of 50 by 5 cm. Maize stovers which had 60% moisture content were chopped at 10 – 15 cm length and applied at a rate of 4000 kg ha^{-1} between the rows after emergence in both tillage and no tillage plots. Rust control was done using armistar Xtra from Syngenta at a rate of 1l ha^{-1} three times after flowering (this is the stage when the plants are highly susceptible) at an interval of two weeks. Weeding in no tillage was done by hand pulling depending on appearance of weeds whereas in conventional tillage it was done using hoes after every two to three weeks.

Soil characterization and data collection

Soil samples were taken for analysis of organic carbon content, total nitrogen, available phosphorus and potassium, pH, particle size according to standard procedures outlined by Okalebo et al., (2002). Plants for biomass and N accumulation and assessment were randomly sampled in an area of 0.1m^{2} within the net plot at 50% flowering stage (at

Table 3. Top soil (0-20 cm) chemical and physical characteristics of the experimental sites.

Site	pH	Olsen P	C.E.C	K	Ca	Mg	Na	clay	sand	Silt	Soil texture	Total N	Total C
		ppm	Meq/100g	Meq /100g	Meq /100g	Meq/10 0g	Meq /100g	%	%	%		%	%
LM 1	5.3	12	8.39	0.27	3.66	0.94	0.05	24.8	69.6	5.6	sandy clay loam	0.08	1.04
LM 2	4.8	13	6.90	0.29	1.60	0.87	0.14	28.9	55.5	15.6	Sandy clay loam	0.11	1.29
LM 3	5.7	20	4.70	0.16	2.11	0.92	0.05	36.8	57.6	5.6	Sandy clay	0.12	1.12
LM 4	6.0	20	3.31	0.31	1.74	0.47	0.06	10.9	85.5	3.6	Loamy sand	0.04	0.40

Table 4. Effect of experimental site, tillage methods and variety on root biomass (kg ha^{-1}), shoot biomass (kg ha^{-1}) and dry grain yield (kg ha^{-1}) of soybean.

Source	Root biomass (kg ha^{-1})	Shoot biomass (kg ha^{-1})	Dry grain yield (kg ha^{-1})
Site			
LM 1	60.0^{b}	306.6^{a}	921.8^{b}
LM 2	67.8^{ab}	248.7^{b}	661.5^{c}
LM 3	81.0^{a}	342.5^{a}	1543.0^{a}
LM 4	36.2^{c}	148.7^{c}	$703.7b^{c}$
Tillage method			
Tillage	63.1a	265.6a	1063.3a
No tillage	62.6a	273.2a	935.0a
Soybean variety			
Nyala	48.5^{b}	174.9^{c}	923.4^{a}
SB19	58.6^{b}	249.9^{b}	1115.0^{a}
SB20	78.9^{a}	359.5^{a}	943.7^{a}

Means with different letters are significantly different at $p<0.05$ within a column of a source.

Table 5. Interactive effects of tillage and variety on root biomass, shoot biomass and dry grain yield of soybean.

Tillage method	Variety	Root biomass (kg ha^{-1})	Shoot biomass (kg ha^{-1})	Dry grain yield (kg ha^{-1})
Tillage	Nyala	54.4bc	195.1bc	883.8b
	SB19	57.4bc	231.7b	1222.2a
	SB20	72.6ab	339.4a	925.5b
No Tillage	Nyala	42.1c	151.8c	753.1b
	SB19	55.1bc	246.3b	919.3b
	SB20	79.9a	350.6a	943.1b

Means with different letters are significantly different at p<0.05 within a column.

50% flowering it is assumed that N accumulation is maximum) (Unkovich et al., 2008). These plants were cut at the first node from the ground using a kitchen knife, packed in a well labeled polythene bag (17cm by 29cm by 30 microns) of known weight and their fresh weight determined using an electronic balance. At this stage, weeds from weedy fallow strips were also sampled in triplicate and their fresh weight determined. The plant and weed samples were oven dried at 65 ºC to a constant weight between 24 to 48 hours in a soil science laboratory and their dry weights determined. The dry plant and weed samples were ground separately in an electric grinder (model – Retsch SM 100 comfort), passed through 1 mm sieve, packed and labeled prior to N-fixation determination. Roots were excavated using a sharp spade and soil around them was carefully removed to recover nodules. Roots with nodules were packed in a polythene bag (17cm by 29 cm by 30 microns), kept in a cooler box then transported to the laboratory. The roots were detached from nodules, thereafter, nodules were counted and their colours assessed as either good (>75 % nodules per root system; pink in color), moderate (25% – 75% nodules per root system; pink in color) or poor (<25% nodules per root system; pink or white in color or >25% nodules but white in color) (Alemayehu, 2009). Finally both the roots and nodules were oven dried to determine their dry weights. Grain yield data was collected at physiological maturity within the net plot. Plants within the net plot were counted, uprooted and the roots cut away from the whole plant using a kitchen knife. Pods were separated from the haulms and their fresh weights taken. A subsample was picked from the pods and its fresh weight determined. The subsample was threshed and fresh weight of grains was taken. The grains were dried at 65 ºC in an oven (model – Memmert UNB 500) for a period of 24 – 48 hours and their dry weights measured. This was used to calculate grain yield kg ha^{-1}.

Determination of N_2-fixation

The ground plant samples were used to determine amount of nitrogen fixed using ^{15}N natural abundance method (Unkovich et al., 2008). Non N_2 fixing reference plants were three weed plants sampled from fallow strips. The weeds were: *Brassica napus*, *Sorghum sudanense* and *Oxalis corniculata*. Determination of N fixed using ^{15}N abundance was conducted at Wageningen University – Netherlands.

Statistical analysis

Data collected was subjected to analysis of variance (ANOVA) at 5% level of significance and the means were separated using Duncan's Multiple Range Test (DMRT) on SAS software version 9.00 (SAS, 2002).

Conclusion

No tillage should be encouraged to enhance amount of N fixed as exemplified at LM 1 and LM 3. This will further contribute to increase in soil fertility as it is also known that no tillage improves soil organic carbon among other soil benefits. Grain yield between tillage methods and among varieties was not significantly different in all the sites. This is expected because research has shown that different characteristics of tillage methods are visible over a long period of cropping, therefore, if this experiment could have gone beyond two seasons it could have vividly discerned the best tillage method for each site and the best adapted soybean variety for high nitrogen fixation and yield.

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