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Incubation of soil with agricultural lime and phosphorus enhances biological nitrogen fixation and yield of soybean (*Glycine max* (L.) Merrill) in an ultisol

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ABSTRACT

Low pH and phosphorus are among major soil chemical constraints to Biological Nitrogen Fixation (BNF) and soybean production in sub-Saharan Africa. A screenhouse pot experiment was conducted during the 2016/2017 season at Sokoine University of Agriculture- Morogoro Tanzania, to examine the ameliorating effect of liming to pH, phosphorus uptake, BNF and yield of soybean grown on an ultisol. The experiment was a 3^3 factorial-laid out as a split-plot with two replications. Source of nitrogen (inoculation, non-inoculation and applied inorganic nitrogen) was main plot factor, while the subplots received combinations of lime and phosphorus. The levels of lime used for the incubation experiment were 0, 5 and 10 tons/ha while those of P were 0, 50 and 100 kg/ha. A commercial inoculant-Legumefix, was used for inoculation. Results indicated that liming significantly (P<0.05) raised the soil pH and that the interaction of lime and phosphorus showed significant (P<0.05) effect on nodulation and N_2 fixation. Overall, application of 5 tons of lime/ha and 100 kg P/ha gave the best-case scenario in terms of pH amelioration, P availability and BNF enhancement by the soybean-Bradyrhizobium symbiosis.

Keywords: BNF, lime, pH, phosphorus, ultisol

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a leguminous vegetable plant of the Pea family, *Fabaceae* (Singh and Shivakumar, 2010), which grows under a wide range of environments notably from tropical and subtropical to temperate climates. It is a rich source of inexpensive proteins (Binang et al., 2013), dietary fiber, minerals and other bioactive compounds such as isoflavones (Macák and Candráková, 2013). Soybean has multiple economic uses as it can be sold as food grain and/or in processed forms like soy nuts, soy milk, soy pulp and oil (Akibode and Maredia, 2011). Its other product lines include edible oils, printing ink and biodiesel. As a result, soybean serves as a major foreign exchange earner for countries that produce it (Singh and Shivakumar, 2010; Aniekwe and Mbah, 2014).

Agronomically, soybean is a good rotation and/or inter-mixed crop especially with cereals, where in addition to reducing pathogen infestation rates, it improves overall soil fertility mainly through its capacity to symbiotically fix atmospheric nitrogen to plant available forms of nitrogen (Akibode and Maredia, 2011; Chianu et al., 2011).

Biological nitrogen fixation by a soybean-rhizobia association, can however, be affected by a myriad of factors including the soil reaction, soil N content, soil phosphorus, and the native rhizobial population in a particular soil (Giller and Wilson, 1991). For example, both saline-sodic, sodic (pH≥9) and very strongly acidic

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(pH≤5) soil conditions reduce the survival of rhizobia thus inhibit nodulation and biological N fixation (Mabrouk and Belhadj, 2012) mainly because rhizobia are neutrophils in nature preferring slightly acidic to slightly alkaline (pH 5.1-8.9) conditions for their optimum growth and activity (Slonczewski et al., 2009). On the other hand, soils rich in plant available nitrogen (≥30 mg N/kg in the 0-30 cm layer) have been found to subdue the biological nitrogen fixation process through retardation of root infection by rhizobia, nodule development and the extent of nodulation (Singh and Shivakumar, 2010; Chianu et al., 2011; Weisany et al., 2013). Furthermore, in very acidic pH conditions such as those found in ultisols, another important plant nutrient, phosphorus tends to be fixed to plant unavailable forms making supplemental P fertilization a necessity to enhance N fixation.

Since ultisols, which are characterized by low soil fertility attributed to low pH, are widespread in Africa (Giller and Wilson, 1991), such soils are, therefore, associated with pronounced low yields of important crops such as soybean (Chianu et al., 2011). Liming and phosphorus application have been shown to enhance BNF in ultisols (Chianu et al., 2011; Binang et al., 2013; Njeru et al., 2013). However, information on the optimum rates of liming materials and phosphorus for BNF and soybean production in Sub Saharan Africa (SSA) is scanty, hence need to generate such information is both urgent and crucial. In the current study, conducted during the year 2016/2017, optimal amounts of phosphorus and calcite that should be applied to an ultisol for enhanced BNF and soybean yield at the Magadu section of the Sokoine University of Agriculture (SUA) farm were determined.

MATERIALS AND METHODS

Description of study area, soil sampling and analysis

This study was designed and conducted as a glasshouse pot experiment during 2016/2017 growing season based on an extremely acidic (pH<4.5) ultisol from Magadu section of the Sokoine University of Agriculture (SUA) farm. The Magadu section of the SUA farm is located at the foot slopes of the Uluguru Mountain in Morogoro municipality, Morogoro region, Tanzania. The farm lies between latitude 06° 50' 24.7" S and longitude 37° 38' 59.8" E and at an elevation of 526 m above sea level (Msanya et al., 2003). The rainfall distribution is bimodal with the first rainy season running from March to May while the second rainy season runs from November to January. Twenty soil sample portions of 10 kg were collected randomly from 3 ha area at the rooting depth of 0-30 cm, thoroughly mixed, air dried, ground and sieved through a 6 mm sieve and mixed again to make one composite sample.

From the sieved composite sample, about one-kilogram soil was sieved again through a 2 mm sieve for laboratory analysis on chemical, physical and biological properties of the soil. Particle size distribution was determined by the hydrometer method after dispersion with 5% sodium hexametaphosphate (Gee and Bauder, 1986) and soil textural class was determined using the United State Department of Agriculture (USDA) soil textural class triangle (United State Department of Agriculture, 1975). Soil pH was determined electrometrically in 1:2.5 soil:water and soil:0.01 M $CaCl_2$ suspensions (Thomas, 1996). CEC and exchangeable bases (Ca^{2+} Mg^{2+}, K^+ and Na^+) were determined by the 1 M (pH 7) NH_4-acetate saturation method followed by displacing adsorbed NH^{4+} using 1 M KCl. Exchangeable Ca^{2+} and Mg^{2+} were quantified using an atomic absorption spectrophotometer (AAS) and exchangeable K^+ and Na^+ by the use of a flame photometer (Thomas, 1996). Organic carbon was determined by the Walkely and Black wet oxidation method (Nelson and Sommers, 1996). Total N was determined by the Kjeldahl digestion-distillation method as described by Bremner (1996). Available P was extracted by the Bray-1 method (Kuo, 1996) and quantified spectrophotometrically at 884 nm. Plant available micronutrients (Fe, Cu, Zn and Mn) were extracted by the DTPA reagent method (Lindsay and Norvell, 1978) while boron was determined by hot water extraction method (Bremner, 1996) and exchangeable acidity by the use of titration method (Thomas, 1996).

Indigenous rhizobial presence was checked by conducting the Most Probable Number (MPN)-plant infection technique (Giller and Wilson, 1991) to determine the potential of the soybean crop to form effective soybean-rhizobia symbiosis with indigenous populations or that inoculation would be required.

Screenhouse pot experiment and total N determination

The study was a factorial experiment laid down in a split plot design with two replications. The main plot factor was the source of nitrogen at three levels namely (i) inoculation with legumefix commercially available inoculant, (ii) non-inoculation where BNF if any would only have resulted from indigenous rhizobial species and (iii) inorganic N applied at a rate of 200 kg N/ha. The sub-plots factor involved the different combinations of lime and phosphorus also at three levels of 0, 5 and 10 tons/ha and 0, 50 and 100 kg/ha of lime and phosphorus, respectively. Pure calcite ($CaCO_3$) was used as the agricultural liming material and triple super phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) was used as the source of phosphorus and urea ($CO(NH_2)_2$ as source of N

Experimental growth medium was prepared by mixing four kg of composite soil which had previously passed through a 6 mm sieve with desired combinations of lime and P and placed into 5-liter capacity plastic pots. Several holes were made at the bottom of each pot and loosely plugged with cotton wool to facilitate drainage. The pots were maintained at field capacity moisture status and incubated for 40 days. The post-incubation pH of each pot was measured electrometrically in 1: 2.5 soil: water suspension as described by Thomas (1996). Two pre-germinated soybean seeds were then sown at a depth of about 15 mm in each pot. To appropriate pots, LegumeFix was used to inoculate the germinated seedlings by pipetting 1 ml of the inoculant to each seedling two days after emergence. With all the recommended agronomic practices observed, the plants were allowed to grow for 40 days post-planting.

Following the 40 days period of vegetative growth, a sharp knife was used to cut off each plant at the base just above the soil level. The above ground shoots of the sampled plants were cleaned of any debris, packed in paper envelopes and kept in ventilated oven at 60-70 °C to constant weight prior to dry matter determination using an electronic balance. The dried plant samples were milled into powder and wet digested by Kjeldahl method and the total N accumulation was determined by distillation-titration method as described by Peoples et al. (1989). N_2 fixed was estimated as total N balance as follows:

Total N balance = (final N in soil + N in plant material) – (initial N in soil)

To recover the below-ground biomass, the growth medium containing the roots and associated nodules was carefully placed in small sized sieve enough to trap any dry matter from being washed away. The roots were then washed thoroughly with water to remove soil particles and organic debris. From the clean root system, nodules were carefully plucked out and both their numbers and fresh volumes determined. Nodule numbers were determined by manual counting per plant while fresh nodule volumes were determined by the volume displacement technique as explained by Solomon et al. (2012). Briefly, all nodules from a single plant were cleaned, blotted dry and then placed in a 10 ml capacity plastic measuring cylinder half filled with water. The volume of water displaced by the nodules obtained from the sampled plants was recorded, and the average was considered as nodule volume per plant. Nodule dry weight was determined by measuring weight of the nodules using an electronic balance after drying the nodules at 70 °C to constant weight.

Quantification of soybean content of Ca, P and total dry matter yield

Following 40 days of vegetative growth, soybean plants were carefully harvested, processed and subjected to total plant analysis for determination of phosphorus and calcium contents. Briefly, samples were oven-dried, ground and sieved through a 0.5 mm sieve. The sample powder was further subject to dry combustion (ashing) method as described by Anderson and Ingram (1989). Phosphorus was determined by spectrophotometer and atomic adsorption spectrometer analysis was used for Ca determination.

For dry matter yield estimates, soybean plants were sampled at flowering stage of growth and the above ground shoot of the sampled plants was used to determine dry matter yields (DMY). Plant samples were cleaned of any debris and then packed in envelopes and dried in ventilation oven at 60-70 °C to constant weight. The dry matter yields were determined using an electron balance.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using GenStat 14th edition. Treatment means were separated using New Duncan's Multiple Range Test (NDMRT) at 0.05 probability level.

RESULTS AND DISCUSSION

Selected properties of the soil used in this study

Table 1 shows selected properties of the soil used in this study. Accordingly, the textural class of the soil from the study area was loam (United State Department of Agriculture, 1975), which would enhance the growth and production of soybean due to its good aeration, moisture and nutrient retention capabilities among others. However, the pH of this soil (pH 4.6), which corresponds to very strongly acidic soil reaction, is not suitable for growth and development of most annual crops including soybean. This is because low soil pH impairs plant development and affects availability of various plant nutrients such as phosphorus and exchangeable bases (Marschner, 1995). Various studies have reported more than 90% reduction in nodule numbers and up to 50% reduction in nodule weight due to low soil pH (Alva et al., 1990; Evans et al., 1990). Furthermore, the strongly acidic soil reaction has been shown, elsewhere, to adversely impair BNF as most N_2-fixing symbionts prefer slightly acidic (pH 5.1) to slightly alkaline (pH 8.9) soil conditions (Bhagwat and Apte, 1989; Graham et al., 1994).

The total N of the soil was very low (<0.1%), according to Landon (1991), indicative of requisite levels of soil N for the legumes to amply fix nitrogen. It has been reported elsewhere that while high concentrations of mineral N either as nitrate or ammonium or ammonium nitrate significantly suppress nodule numbers, nodule dry weight and total N_2 fixed per plant of nodulated soybeans (Gulden and Vessey, 1997; Gan et al., 2004), too low concentrations of nitrogen in growth media are equally detrimental to BNF especially at initial stages of growth before the plant can reliably fix its own nitrogen by symbiotic BNF.

The experimental soil had low available phosphorus (P) (<15 mg/kg soil) which necessitated application of P fertilizer to enhance soybean N_2 fixation. P is highly required in energy transfer processes involved in BNF (Rotaru and Sinclair, 2009a, b) and both abrupt removal of P supply and suboptimal presence of P in a growth medium have been shown to severely arrest nodule growth and substantially reduce nitrogenase activity leading to ultimate reduction in N_2 fixation (Hog-Jensen et al., 2002).

Exchangeable calcium (Ca) of the soil under study was low (<4 cmol/kg soil). However, in this study, the calcium problem was addressed by the type of phosphatic fertilizer used ($Ca(H_2PO_4)_2 \cdot H_2O$) as it would supply Ca in addition to phosphorus in the equation. Both exchangeable magnesium and exchangeable potassium readings were high (>0.5 cmol/kg soil and ≥0.4 cmol/kg soil, respectively) according to the categorization by Landon (1991) hence needed no corrective measures for the current study.

Overall, based on the soil analytical data presented in Table 1, the Ultisol of the study area is rated as of low fertility status for soybean production. The soil fertility limitations being very low soil pH, low total N and low available P among others. Enhanced and sustainable soybean production at the study site (Magadu section of SUA farm) can be attained if the following are taken on board, namely liming the soil, application of phosphorus fertilizer and inoculation of soybean seeds before planting. Liming the soil to increase the soil pH to at least 5.5 can enhance cation exchange capacity of the soil and increase mineralization of soil N through decomposition

Table 1. Chemical, physical and biological properties of the soil (Ultisol) from the study area

Soil parameter	Value
Clay (%)	25
Silt (%)	30
Sand (%)	45
Soil textural class	Loam
pH in water (H_2O)	4.6
pH in $CaCl_2$ (0.01 M $CaCl_2$)	4.2
Organic carbon (%)	0.64
Organic matter (%)	1.1
Total N (%)	0.08
Available P Bray 1 (mg/kg)	7.42
Cation exchange capacity ($cmol_c$/kg)	13.51
Ca^{2+}	1.6
Mg^{2+}	0.81
K^+	0.4
Na^+	0.07
Base saturation (%)	21.32
Indigenous rhizobia count	(2 to 79 cells/g soil) = low

of organic matter as the microbial activities increase. Application of phosphorus fertilizer can also enhance and sustain soybean production through BNF (Mahamood, 2008). Phosphorus acts as an energy source for N2 fixation because it is required in biosynthesis of adenosine triphosphate (ATP). Up to 16 molecules of ATP are converted to adenosine diphosphate (ADP) as each molecule of N_2 is reduced to NH_3.

The observed low numbers of indigenous rhizobia capable of nodulating soybean could be attributed to the fact that the Magadu section of the SUA farm has not been under soybean or other related legumes for a long time. (Morel et al., 2012). This called for inoculation of soybean seeds as soybean will likely respond to inoculation if the number of *Bradyrhizobia* in the soil is greater than 50 cells/g soil (Mishra et al., 2013).

Raising pH of the ultisol through liming led to enhanced calcium and phosphorus content in soybean plant shoots

Results on pH level, calcium and phosphorus content of the soil following incubation of the acidic soil with a mixture of agricultural lime (calcite) and phosphorus for forty days maintained at field capacity are presented in Tables 2 and 3. Soil pH increased significantly ($P<0.05$) as the rate of liming increased from 0 to 5 to 10 tons/ha regardless of the level of phosphorus applied. pH increased from 4.6 to 6.8 when lime applied was increased from 0 to 10 tons/ha. Similar results on pH increase due to lime application have been reported elsewhere (Bekere, 2012; Ayodele and Shittu, 2014). Furthermore, there was a general tendency for the pH to decrease, but non-significantly, with increasing amounts of P applied at a constant liming rate (Table 2).

Results show that calcium content of soybean shoots, was significantly ($P<0.05$) different among the main plot treatments (Table 3). Further analysis of the interaction between lime and phosphorus application revealed that at any level of P applied, the calcium content of soybean shoots increased with increasing liming rate from 0 to 5 to 10 t/ha in inoculated, non-inoculated and N applied plots. The inorganic N applied plants had higher Ca content followed by inoculated plants while the non-inoculated plants had the least shoot Ca content at nearly all levels of combinations of lime and P applied (Figure 1).

Following concurrent application of lime and phosphorus and subsequent growing of soybean plants, no meaningful trend on the levels of shoot P content was observed among the three main plot treatments of inoculated, non-inoculated and inorganic N applied (Table 3), regardless of the level of lime and/or phosphorus applied. This observation was also supported by the results of the analysis of the interaction effects of lime and phosphorus application on P content of soybean shoots (Figure 2).

This could be attributed to the fact that P was also used for energy storage and transfer from the leaves to the roots for the symbionts in the inoculated plot (Weisany et al., 2013). However, this observation is not

Table 2. Effect of lime and phosphorus on pH and Ca content soybean shoots

Water parameters and bulk density					
Lime (tons)	Phosphorus (kg)	pH	% Ca in the main plots		
			Inoculated	Inorganic N	Non-inoculated
0	0	4.6[a]	0.212[a]	0.326[abcde]	0.25[abcd]
	50	4.6[a]	0.236[ab]	0.33[abcdef]	0.354[abcdefg]
	100	4.5[a]	0.297[abcde]	0.312[abcde]	0.245[abc]
5	0	5.9[b]	0.515[cdefghij]	0.605[ghijk]	0.373[abcdefgh]
	50	6[b]	0.638[hijk]	0.652[ijk]	0.397[abcdefghi]
	100	5.7[b]	0.491[bcdefghij]	0.695[jkl]	0.402[abcdefghi]
10	0	6.8[c]	0.558[efghijk]	0.913[l]	0.425[abcdefghij]
	50	6.8[c]	0.52[defghijk]	0.913[l]	0.468[abcdefghij]
	100	6.7[c]	0.6[fghijk]	0.785[kl]	0.686[jkl]
CV (%) = 50.5					

[a, b, c, d, e, f, g, h, i, j, k, l] Values in the same column followed by different letters are significantly different according to Duncan's New Multiple Range Test at P=0.05.

Table 3. Effect of lime and phosphorus on P content in soybean shoots

Water parameters and bulk density					
Lime (tons)	Phosphorus (kg)	pH	Shoot P (%) in the main plots		
			Inoculated	Inorganic N	Non-inoculated
0	0	4.6	0.0778[ab]	0.2991[fg]	0.1257[abcd]
	50	4.6	0.113[abcd]	0.464[h]	0.1779[abcdef]
	100	4.5	0.2272[cdefg]	0.4696[h]	0.1708[abcdef]
5	0	5.9	0.0553[a]	0.2723[efg]	0.1116[abcd]
	50	6	0.1018[abc]	0.3033[fg]	0.1511[abcde]
	100	5.7	0.1342[abcde]	0.2794[efg]	0.0919[abc]
10	0	6.8	0.1807[abcdef]	0.2554[defg]	0.1765[abcdef]
	50	6.8	0.1891[abcdef]	0.2526[defg]	0.2216[bcdef]
	100	6.7	0.3681[gh]	0.1891[abcdef]	0.2737[efg]
CV (%) = 50.5					

[a, b, c, d, e, f, g, h] Values in the same column followed by different letters are significantly different according to Duncan's New Multiple Range Test at P=0.05.

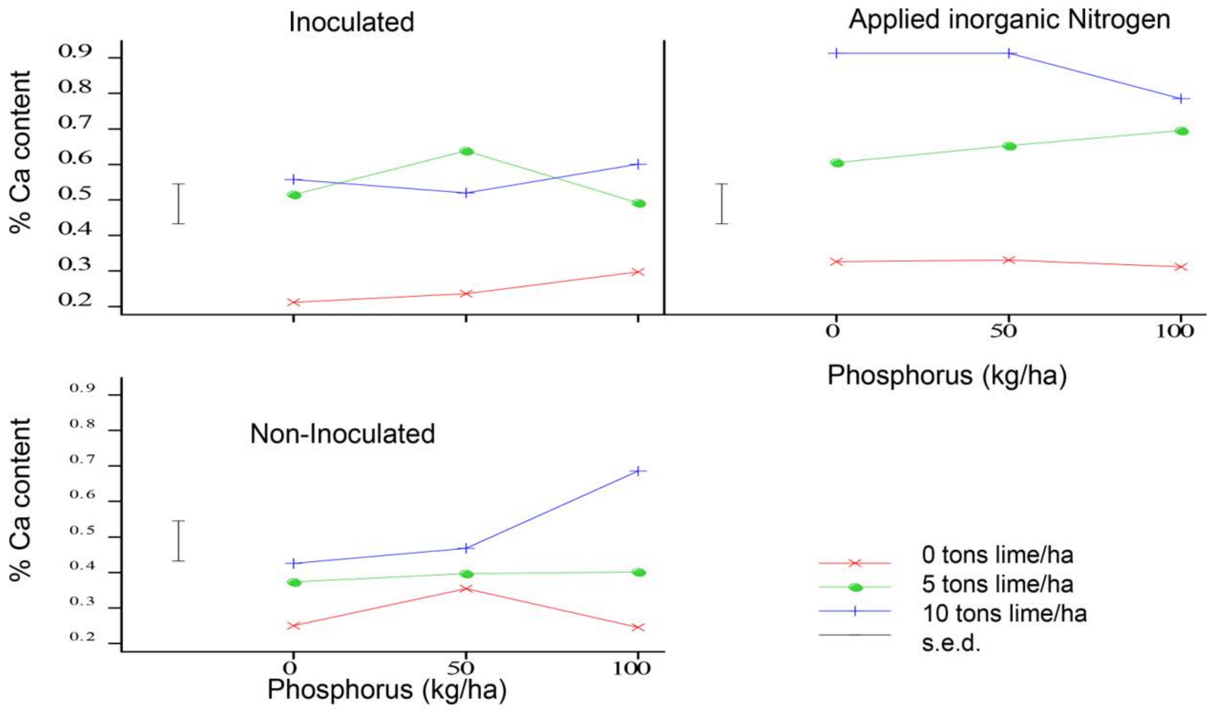

Figure 1. Profile plots showing interaction effect of liming and phosphorus application on Ca content of soybean shoots (For the determination of Ca content of the soybean plants, samples were oven-dried, ground and sieved through a 0.5 mm sieve. The sample powder was further subjected to dry combustion (ashing). Ca content of the dry ash sample was determined using an atomic adsorption spectrometer (Varian).)

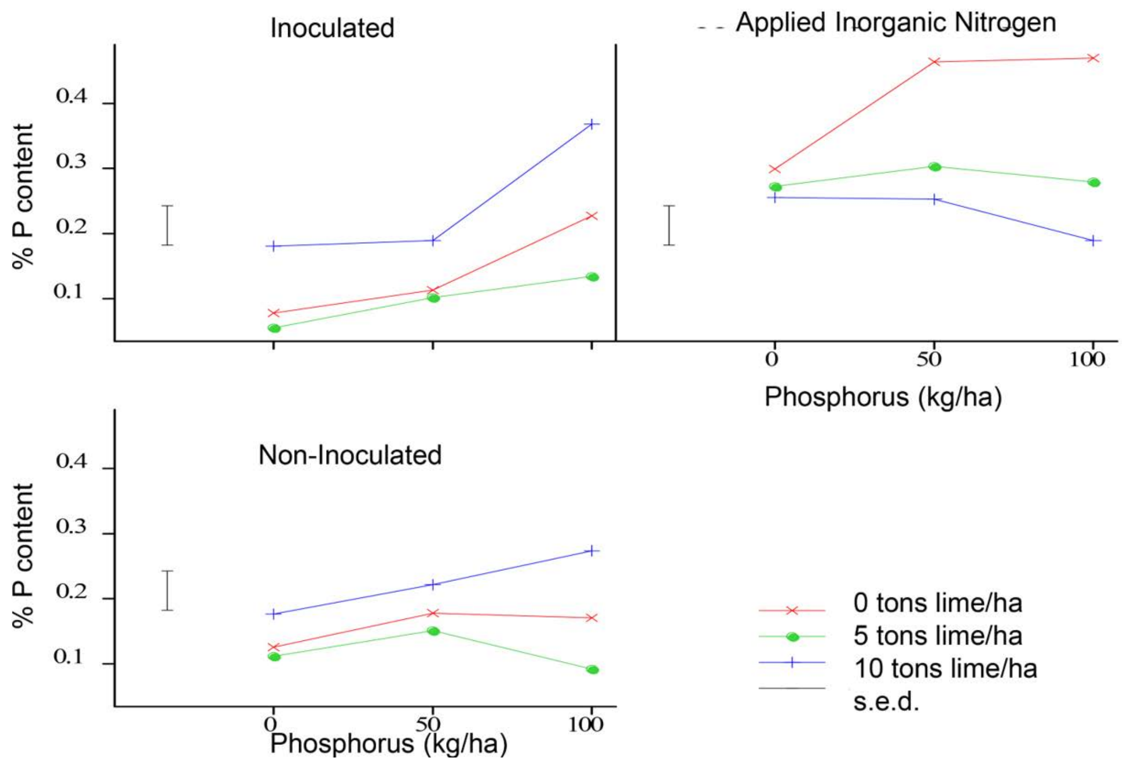

Figure 2. Profile plots showing interaction effects of lime and P application on P content of soybean plant shoots (Plant samples were oven-dried, ground and sieved through a 0.5 mm sieve. The sample powder was further subjected to dry combustion (ashing), allowed to cool, and dissolved in dilute HCl prior to analysis. For Phosphorus quantification, a colour development method using the ammonium molybdate/ vanadate mixed reagents was followed as described by Okalebo et al. (2002). The colour intensity of the deep blue complex was determined using a UV- visible spectrophotometer (Biomate6, Thermoscientific).)

consistent with the findings by Basu et al. (2008) who reported that non-inoculated control plants accumulated more P than the inoculated treatments.

Liming and phosphorus application enhanced nodulation of soybean

Effects of incubating the acidic ultisol with agricultural lime (calcite) and phosphorus on nodulation was examined in terms of nodule numbers, nodule fresh volume and nodule dry weight in a potted soil experiment for plants grown on inoculated, non-inoculated and inorganic N applied main plot treatments.

Results on nodule numbers are presented in Figure 3 (A). At all treatment combinations, there were significantly ($P<0.05$) higher nodule numbers in inoculated legumes than either inorganic N applied or non-inoculated legume pots. Results showed further that there was a clear interaction effect of the applied phosphorus and lime on nodule numbers. Accordingly, the best-case scenario was observed when the soil was limed at 5 tons/ha and P applied to 100 kg/ha under both inoculated and non-inoculated plots (Figure 3, B).

The interaction in inorganic N applied plot on nodule numbers was inconsistently small, probably due to high plant available N content in the soil solution which could have retarded root infection by the bradyrhizobia and subsequent nodule development. In a previous study Gan et al. (2004) reported that high concentrations of mineral N (≥10 mM), either as nitrate or ammonium or ammonium nitrate could significantly suppress nodule numbers, nodule dry weight and total N_2 fixed per plant of nodulated soybeans.

A similar trend was also observed with nodule fresh volume per plant. There were significantly higher fresh nodule volume values in inoculated legumes than either non-inoculated or inorganic N applied plants (Figure 4, A). Profile plot analysis indicated that in both inoculated and non-inoculated pots, the highest fresh nodule volume values were recorded when the soil was limed at 5 tones/ha and P applied at 100 kg/ha while values of nodule fresh volume per plant from the inorganic N applied plots were negligible and inconsistent (Figure 4, B).

Furthermore, results on effects of phosphorus and lime application on nodule dry weights are presented in Figure 5. Consistently higher dry nodule weights were recorded in inoculated plants compared to non-inoculated plants (Figure 5, A). Like with both nodule numbers and nodule fresh volume per plant, profile plot analysis indicated that highest nodule dry weights per plant were obtained when the soil was limed at 5 tones/ha and P applied at 100 kg/ha in the inoculated plants followed by the non-inoculated plots. Plants grown in pots receiving inorganic N gave negligible and inconsistent nodule dry weight values (Figure 5, B). The observed negative influence of inorganic N to nodulation and N2 fixation by soybeans is not surprising as numerous studies have shown that except for starter N applied at sowing, N fertilization of N_2-fixing legumes such as soybeans does depress BNF (Gan et al., 1997; Gan et al., 2004; Salvagiotti et al., 2009).

Liming and phosphorus application increased the amount of biologically fixed N and dry matter yield of soybean on an ultisol

Figure 6 shows data for effects of lime and phosphorus on the amounts of N_2 fixed by the soybean-rhizobia symbiosis tested under inoculated and non-inoculated conditions. Total N measurements were done as a proxy to the amount of biologically fixed N per plant. Results indicated that highest amounts of N_2 fixed were obtained when the soil was limed at 5 tons/ha and P applied at 100 kg/ha. Overall, inoculated plots had higher amounts of fixed N_2 compared to non-inoculated plot (Figure 6). This observation is also consistent with other studies such as that reported by Mahamood (2008) who observed an increase in N_2 fixed in inoculated plants with application of lime, P and the presence of adequate numbers of *Bradyrhizobium japonicum*. However, Giller (2001) reported non-significant results and concluded that the ability to form nodules is not always enough to obtain an effective nitrogen fixation symbiosis.

Dry matter content of plants was taken as a measure of yield as influenced by improvements in pH and subsequently P availability and N fixation. Results on

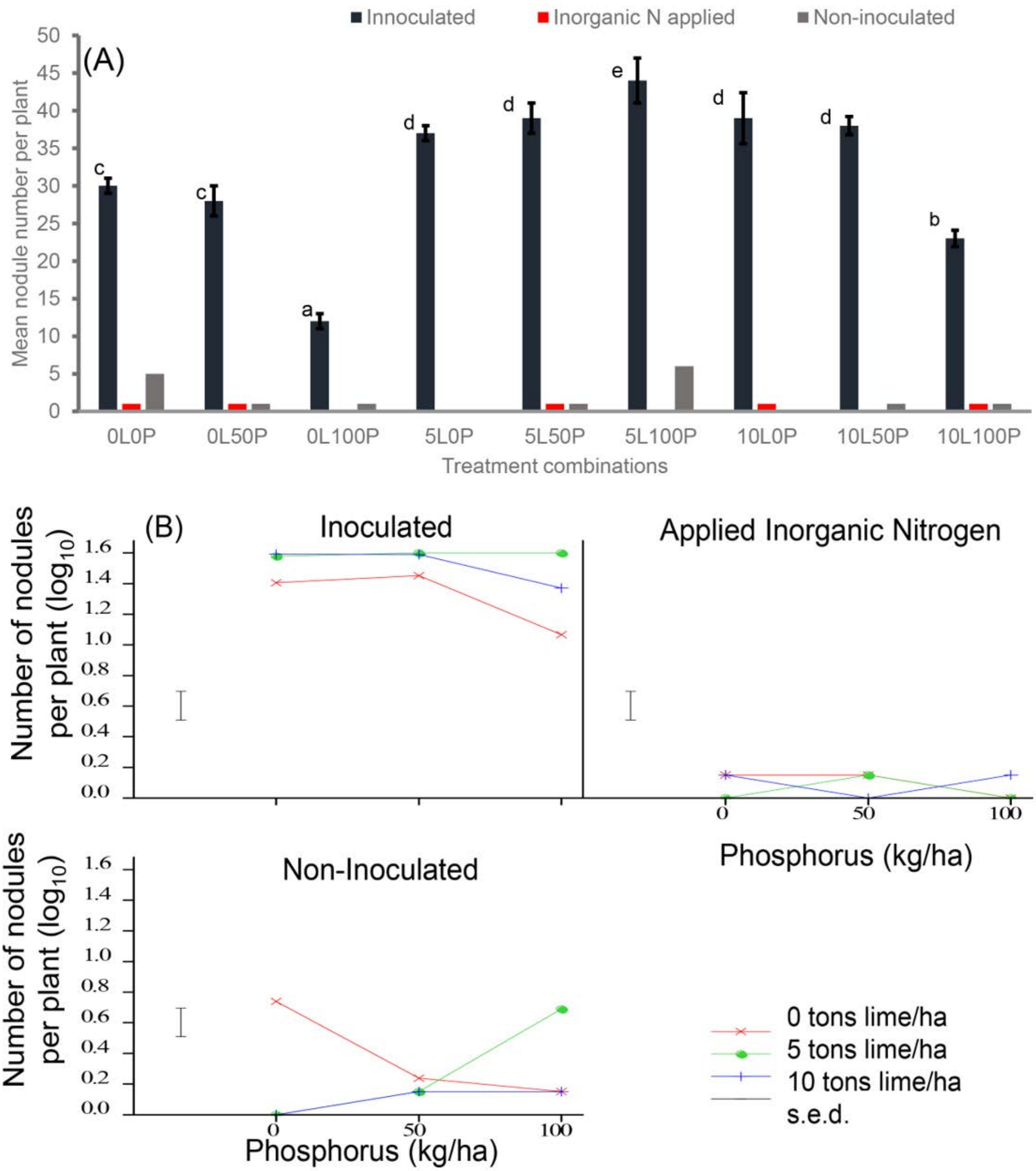

Figure 3. Main plot effects (A) and profile plots showing interaction effects (B) of the application of lime and phosphorus on nodule numbers of soybean plants (Following five weeks of growth, plants were carefully uprooted, roots gently washed to detach the soil from the root system and nodules counted manually. Nodule numbers were estimated per plant. No significant differences in nodule numbers were observed in inorganic N applied and non-inoculated pots.). Error bars indicate mean ± standard deviation. Bars of the same colour marked by same letters are not significantly (P≤0.05) different according to New Duncan's multiple range test.

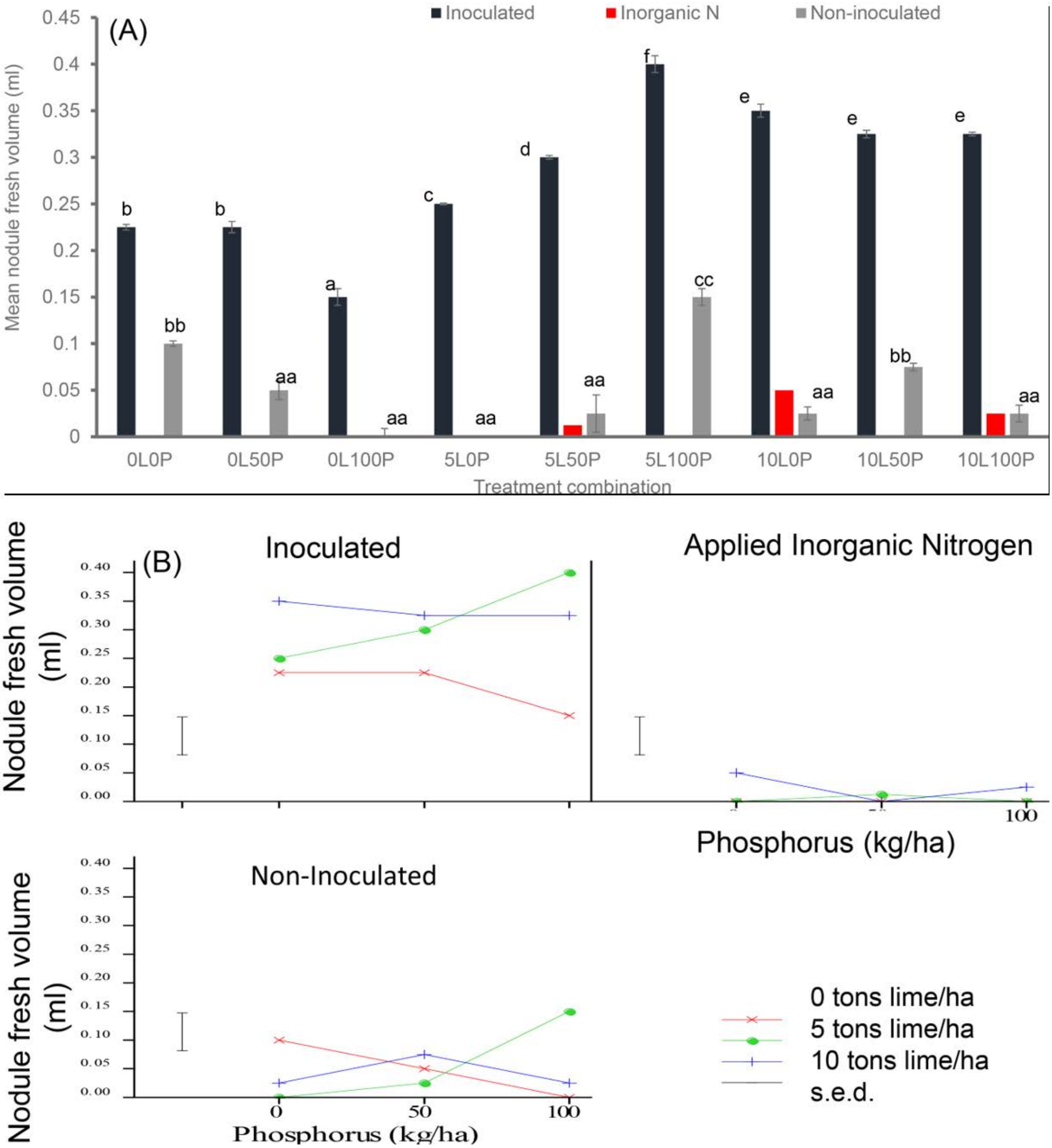

Figure 4. Main plot effects (A) and profile plots showing the interaction effects (B) of lime and phosphorus application on nodule fresh volume of soybean plants grown on a potted soil. Error bars indicate mean ± standard deviation. Bars of the same colour marked by same letters are not significantly (P≤0.05) different according to New Duncan's multiple range test.

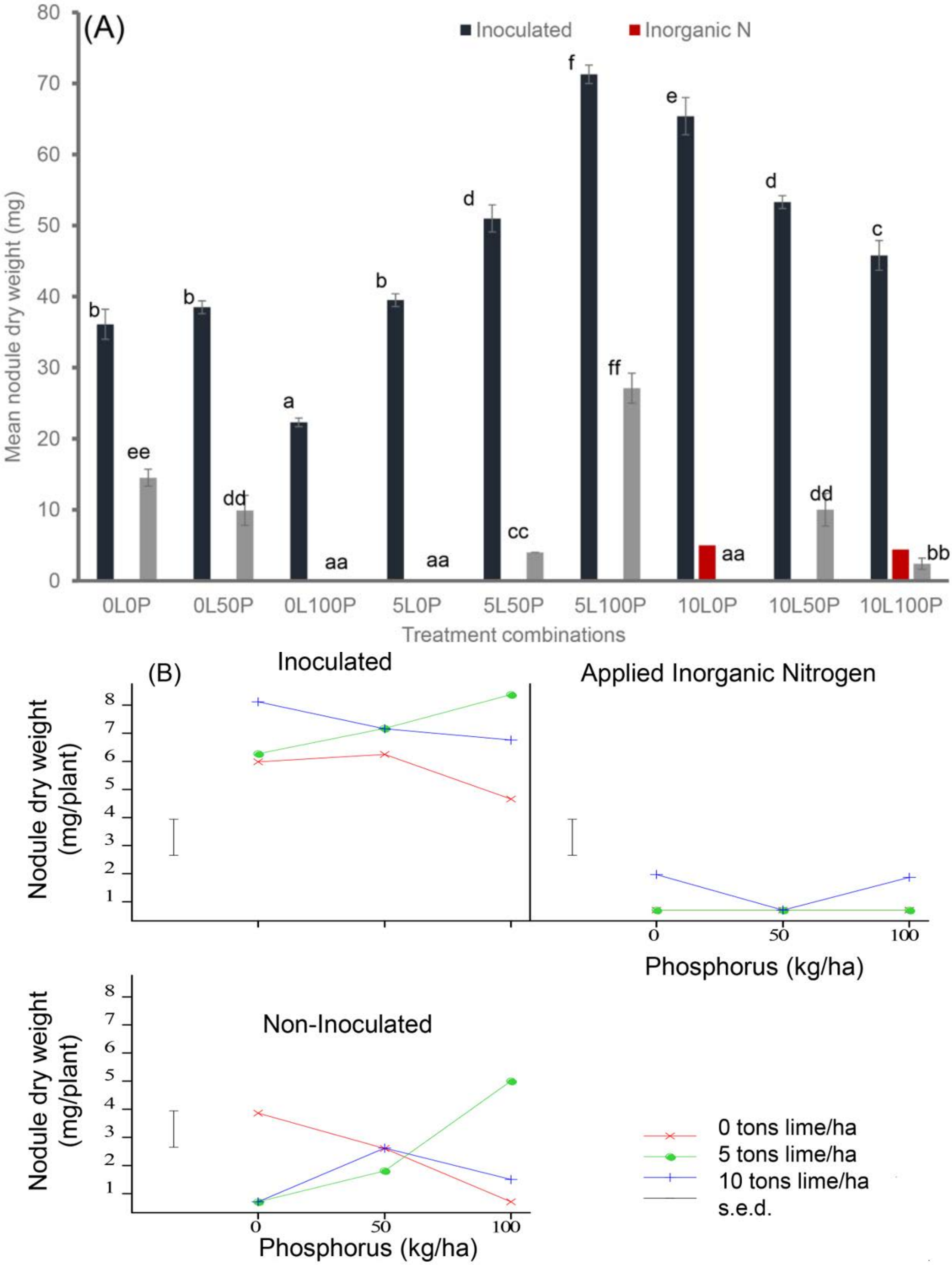

Figure 5. Main plot effects (A) and profile plots showing the interaction effects (B) of lime and phosphorus application on nodule dry weight of soybean plants grown on potted soil. Error bars indicate mean ± standard deviation. Bars of the same colour marked by same letters are not significantly (P≤0.05) different according to New Duncan's multiple range test.

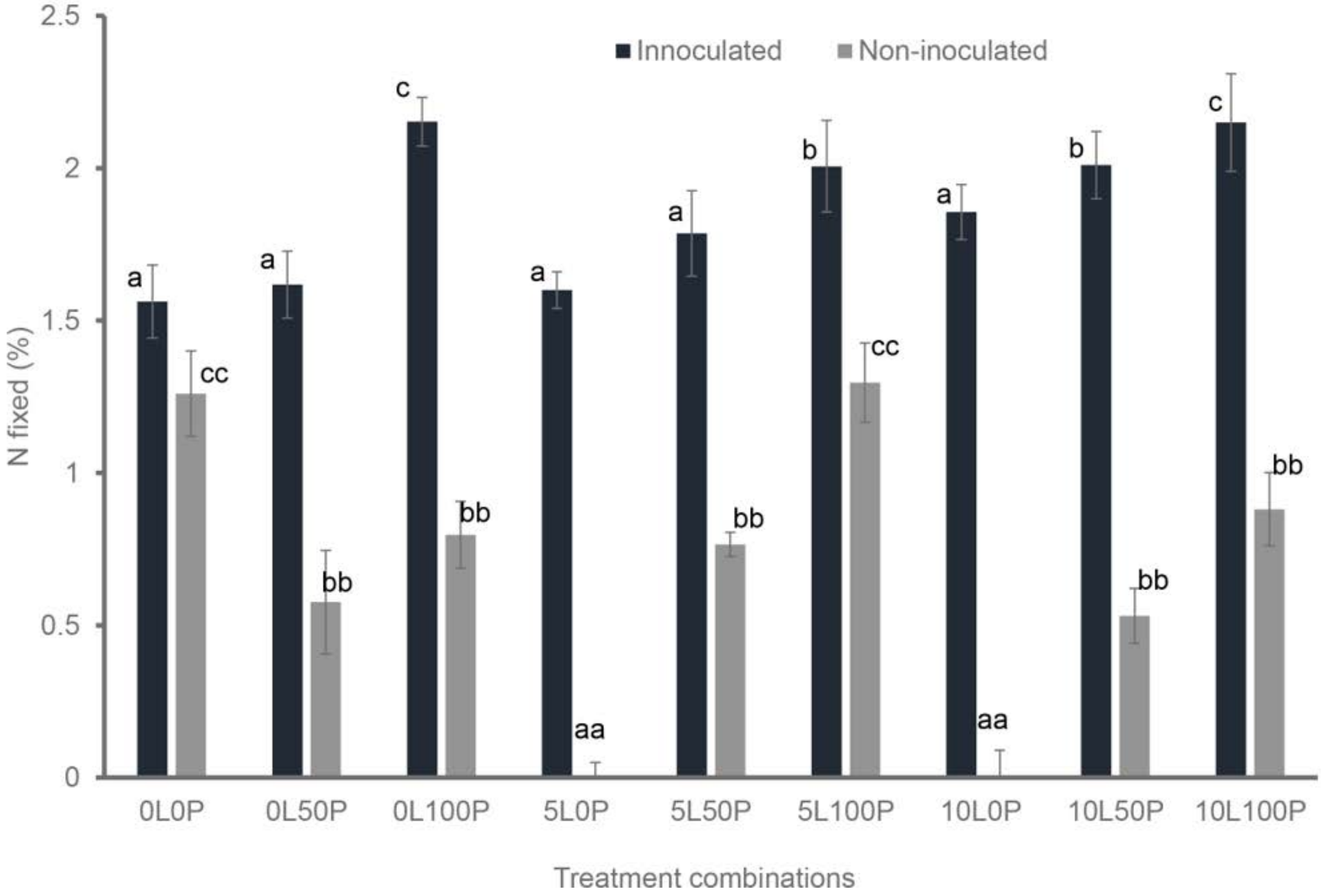

Figure 6. Effect of lime and phosphorus on the amount of N_2 fixed (For quantification of N, above ground biomass samples of soybean plants were dried, milled into powder, sieved to pass through a 0.5 mm sieve and wet digested by Kjeldahl digestion procedure. Total N accumulation was determined by distillation-titration method as described by Peoples *et al.* (1989). N_2 fixed was estimated as total N balance using the formula: Total N balance = (final N in soil + N in plant material) – (initial N in soil): Error bars indicate mean ± standard deviation. Bars of the same colour marked by same letters are not significantly (P≤0.05) different according to New Duncan's multiple range test.

shoot dry matter yield are presented in Figure 7. While no significant (P≤0.05) differences were observed among the inoculated, non-inoculated and inorganic N applied plants, profile analysis showed existence of interaction between lime and P application

Accordingly, liming the soil at 5 tons/ha coupled with P application at 100 kg/ha gave the best advantage in terms of total dry matter yield in all the main plot treatments of inoculated, non-inoculated and inorganic N applied (Figure 4 and Figure 7). Overall, under these conditions, inoculated conditions gave better dry matter yields than both the non- inoculated and inorganic N applied plots.

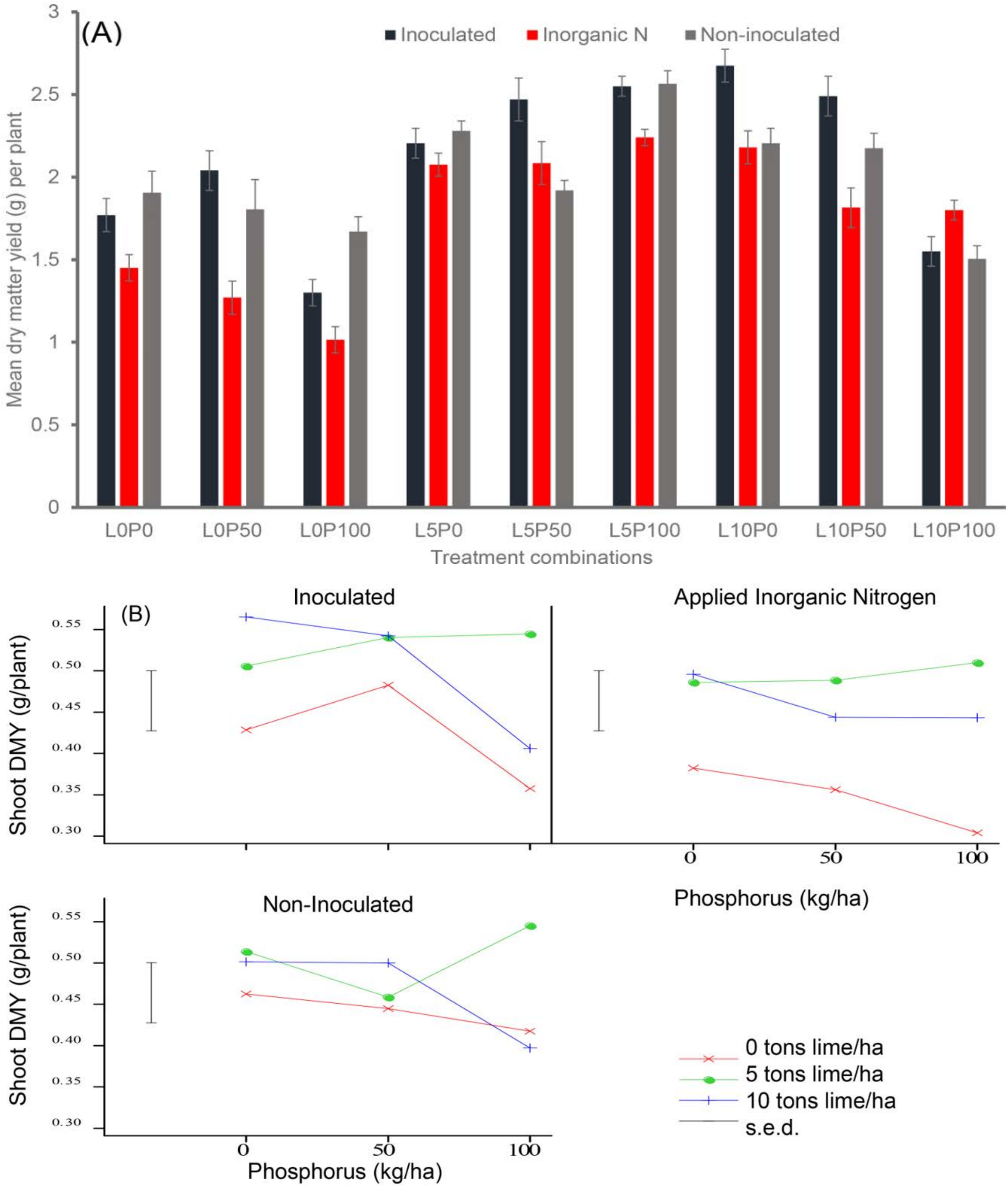

Figure 7. Main plot treatment effects (A) and profile plots showing the interaction effects (B) of liming and phosphorus application on shoot dry weights (Dry matter content of plants was taken as a measure of yield as influenced by improvements in pH. The dry matter yields were determined using an electron balance by measuring the weight in (g) of oven-dry plant shoot samples at representative samples for each treatment combination. Error bars indicate mean ± standard deviation. Bars of the same colour marked by same letters are not significantly (P≤0.05) different according to New Duncan's multiple range test.

CONCLUSIONS

The soil used in this study had a low population of the native *Bradyrhizobium*, therefore, sustainable soybean production at the Magadu section of SUA farm requires inoculation of the seeds with *Bradyrhizobium jopanicum* if one needs to capitalize on BNF. The soil pH of the experimental soil (Ultisol) increased with increase in the rate of liming. Liming at 10 tons per hectare increased the pH of the soil from 4.6 to about 6.8, in which microbial activity for nodulation and N fixation were enhanced.

No advantage is obtained on BNF by applying P on this soil in the absence of liming. Increasing liming rate to 5 tons/ha and phosphorus to the rate of 100 kg/ha improved the nodulation parameters namely nodule numbers, nodule fresh volume and nodule dry weight as well as N_2 fixed by soybean plants.

Therefore, it is recommended that lime and phosphorus application at the rate of 5 tons/ha and 100 kg/ha respectively, combined with inoculation should be employed for optimum BNF on the study soil at Magadu section of SUA farm that would ultimately increase soybean production. Field experimentation would still be required to augment observations made in the current study.

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