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N:P Stoichiometry of Soybean (*Glycine max L.*) as influenced by nitrogen and phosphorus fertilizers on Lithosols

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ABSTRACT
Field experiments were conducted in 2010 and 2011 rainy seasons at the teaching and research farm of the Department of Crop Science, Adamawa state University, Mubi to assess the effect of nitrogen and phosphorus fertilizers on the N:P stoichiometry of soybean (*Glycine max* L.). Four rates of N (0, 25, 50, 75 kg N/ha) and 4 rates of P (0, 30, 60, 90 kg P/ha) were factors combined, replicated three times (n = 96) and laid out in a randomized complete block design. To characterize N:P stoichiometry, actual ratios and slopes of functions relating nitrogen uptake (N_u) and phosphorus uptake (P_u) were used. Variability in N:P ratios and N:P stoichiometry of soybean as influenced by N and P fertilizers were assessed. Results showed that N:P ratios varied between 24 the maximum and 1.9 the minimum. The relationship between N:P ratios and N uptake was significant ($P<0.05$) with R^2 = 0.19 while that of N:P ratio and P uptake was 0.20 indicating that P uptake is the major source of variability in the N:P stoichiometry. Maximum nitrogen uptake was 66 kg N/ha with phosphorus uptake at half maximum nitrogen uptake of 4.1 kg P/ha. Frequency distribution shows that over 25% of soybean attaining maximum yield had N:P ratios between 4 and 6. Grain protein concentration was also a major source of variation in N:P ratios.
Keywords: Stoichiometry, Nitrogen, Phosphorus, Fertilizers, Yield, Soybean

INTRODUCTION

An important component of environmental control is the nitrogen (N) and phosphorus (P) ratio of the soil solution (Gusewell, 2004). The extent to which the plant N:P ratio reflects that of the soil solution is a function of the homeostatic regulation exerted by the plant over its N:P stoichiometry (Garrish *et al*. 2010). Approaches for the diagnosis and management of crop nutrition often targets individual nutrients. However, there is an increasing interest in integrated nutrient management.

Analysis of N:P ratios in plant biomass offers a simple and cost effective tool for accessing constraints on terrestrial plant productivity associated with N and P availability (Koerselman and Meuleman, 1996; Tessier and Raynal, 2003; Gusewell, 2004). Recent theoretical and experimental studies to understand and manage crop responses to multiple nutrients were conducted by Prasad *et al*. (2002) and Kho (2000). In continuation of these attempts, focus on N:P stoichiometry and co-limitation which are two important ecological concepts that can contribute to understand N-P relationships in crops were made the topic for research in fertilizer management and modelling. Furthermore, N:P ratios have been proposed as a diagnostic tool for nutrient limitation in natural vegetation (Koerselman and Meuleman, 1996; Gusewell *et al*., 2003). It was suggested that N:P ratios <14 indicate limitation by N and those >16 indicate limitation by P (Koerselman and Meuleman, 1996). Ratios outside these ranges do not always respond to fertilization with the nutrient indicated to be in limiting supply (Gusewell, 2003; Craine *et al*., 2008). In addition, significant variation has been observed in N:P ratios among species growing at a common site presumably with access to similar N and P supplies in the soil environment (Agren, 2008; Cernusak *et al*., 2010). In agreement with these theories, Sterner and Elser, (2002) gave a definition to the optimum N:P for a specie as the N:P ratio where its growth is equally limited by N and P.

Studies of crop N:P stoichiometry and co-limitation are scarce. It is in line with this that this study was carried out to investigate the N:P stoichiometry of soybean with specific reference to variability in N:P ratios in response to N and P fertilizer additions.

MATERIALS AND METHODS

Study area

This research was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Adamawa State University, Mubi, during the 2010 and 2011 cropping seasons. Mubi is located 10^{o} 11'N and longitude 13^{o}19'E at an altitude of 696 m above sea level. Rain fall is the critical climatic factor in this area and the pattern is unimodal. Staple crops grown are maize, sorghum, rice, groundnut and cowpea (Shehu *et al.*, 2007)

Experimental design

A 4 x 4 factorial experiment consisting of four (4) rates of Nitrogen (N) fertilizer (0, 25, 50, and 75 kg N/ha) and four rates of phosphorus (P) fertilizer (0, 30, 60 and 90 kg P/ha) was adopted and applied as urea and single supper phosphate (SSP), respectively. The treatment combinations was replicated three times and laid out in a randomized complete block design (RCBD). The particle size analysis of the soil of the experiment area is sandy-loam comprising of 53.8% sand, 32.5% silt and 13.7% clay (Table 1).

Grain yield per unit nutrient uptake

Grain yield (Y) per unit nutrient uptake (N_u, P_u) is the nutrient utilization efficiency (Janssen, 1998). It was assessed with both slopes of regressions and ratios between the variables. In reporting uptake in grain (N_g) and non-grain (N_{ng}) components, grain yield per unit nitrogen uptake was divided into two components, Y/N_g, which is the inverse of grain nitrogen concentration and nitrogen harvest index (NHI).

Where $NHI = \frac{N_g}{N_g + N_{ng}}$

Thus

$$\frac{Y}{N_u} = \frac{Y}{N_g}\frac{N_g}{N_g + N_{ng}} \quad (1)$$

N:P stoichiometry

Relationship between N uptake (N_u) and P uptake (P_u) were characterised using actual N:P ratios and parameters of fitted models as both approaches are commonly used (Duivenbooden *et al.*, 1996; Sterner and Elser, 2002). Actual N:P ratios were analysed using SAS, (2010) and scatter plots of N_u and P_u to highlight the maximum and minimum ratios.

Relationship between N_u and P_u for achieving maximum yield was investigated with linear models and non- linear functions. Log transformation was also used to test relationships.

$$N_u = N_o + a\left(1 - e^{-bP_u}\right) \quad (2)$$

Where N_o, a and b are parameters which was estimated with sigma plots. Equation 2 was used to test the zero intercept hypothesis ($N_0 = 0$). Equations 1 and 2 were adopted as described by Sadras (2006).

RESULTS AND DISCUSSION

The data set in Tables 2 and 3 is presented to provide an agronomic background for N:P stoichiometry. Year differences did not significantly (P=0.05) affect seed yield, shoot contents and uptake of N and P. However, concentration ranges of both N and P employed in this experiment induced pronounced variations in seed yield (Table 2). Seed yield, uptake and shoot contents of N and P were significantly (P<0.005) influenced by P fertilizers. This agrees with the findings of Soliman and Farah (1985) who reported that several investigators reported marked yield increase by nitrogen application and positive correlation existed between phosphorus application rates and yield. Their interaction were also significant except for shoot P. Table 3 shows that both year and fertilizers did not significantly (P=0.05) influence grain N and P, protein contents and harvest index of N and P. Interaction of the fertilizers had similar response.

Grain yield and nutrient uptake

The major source of variation in yield and nutrient uptake in soybean is nutrient availability which is affected by variation in soil and fertilizers applied (Shehu *et al*., 2007). Log-transformation improved R^2 for grain yield as a function of N_u from 0.05 to 0.07 at non- zero intercept while that of grain yield and P_u was from 0.02 to 0.04.

Grain yield was more closely related to uptake of N than uptake of P (Fig. 1a and b). Grain yield per unit nutrient uptake as quantified by both slopes of regressions and ratios was much less in soybean than the one reported by Sadras (2006) for cereals but similar to the one in oil seed crops. Similar response was reported by Penning de Vries *et al*. (1974), Whitfield et al, (1989), Hall *et al*. (1990) and Del Pozo *et al*. (2000) and attributed it to higher specific rates of respiration and high production value of seeds.

Equation (1) and Figs. 2a and b were used to analyse N uptake in grain, non-grain components and yield per unit N uptake. Yield per unit grain N was related to nitrogen harvest index (P>0.0007) and weak for phosphorus harvest index (Fig. 3a and b). Nitrogen uptake was the main source of variation in yield per unit N uptake while grain N accounted for 35% of the variation in nitrogen harvest index. Grain P is the major source of variation in yield per unit P uptake compared to N uptake (Fig. 4a and b). Variation in grain N concentration was 20 folds the variation in nitrogen harvest index. Maximum nitrogen uptake was 66 kg N/ha with phosphorus uptake at half maximum nitrogen uptake of 4.1 kg P/ha.

N:P stoichiometry

Nitrogen and phosphorus ratio can be defined as a direct function of N uptake and an inverse function of P uptake (Sadras, 2006). Scatter plots of N uptake as a function of P uptake (Fig. 5a) indicated a variance between 24 the maximum and 5 the minimum. The relationship between N_u and P_u in soybean agrees with the finding of Rashid and Salim (1991) who reported that phosphatic fertilizers increased the efficiency of nitrogen fertilizers and also increase the total uptake of N and P. Nitrogen and phosphorus ratios of soybean were lower and less variable than ratios reported by Elser *et al*. (2000) for foliage of terrestrial autotrophs. Relationship between N:P and N uptake (Fig. 5b) and the inverse relationship between N:P and P uptake (Fig. 6a) were evident in soybean. Relationship between actual N:P ratio and uptake of N and P in soybean shows that P uptake contributes more than N uptake to the variation in N:P ratio. Log-transformations improved R^2 at $P<0.05$. Twenty five percent of soybean attaining maximum yield had N:P ratio between 4 and 6 (Fig. 6b). This agrees with the findings of Guswell and Koerselman (2002) who reported that research with plants strongly supports the idea that P uptake contributes more than N uptake to the variation in N:P ratios.

Factors contributing to the greater role of P as a source of variation in the N:P ratios include relatively less plant N concentration and more variable resorption of P than N from senescing leaves (Aerts and Chapin, 2000; Koerselman, 2002). The value of P as a source of variation in plant N:P ratio may also have a molecular component, as the P content of major biomolecules is more variable than their N content (Sterner and Elser, 2002).

The impact of residuals of the linear regression of N_u and P_u on grain protein concentration showed a strong relationship (Fig. 7). Nutrient management strategies that improve the uptake of nitrogen and phosphorus will increase grain protein concentration of soybean. Thus, the relevance of determining the influence of grain protein on N:P ratio is based on the assumption presented by Agren (2004) that proteins account for most of the organisms nitrogen pool whereas ribosomes accounted for much of the phosphorus pool. In addition, decoupling of growth and nutrient content and distortion of stoichiometry related to excess nutrients in plants have been considered with emphasis on cell level compartmentalisation (Leigh and Wyn Jones, 1984; Sterner and Elser, 2002).

CONCLUSION

The concentration ranges of both nitrogen and phosphorus used in this study induced variation in N:P ratios. Weakness of N was more pronounced as P uptake contributes more to the variation in N:P ratio than N uptake. Relationship between nitrogen uptake and Phosphorus uptake can be assessed by non-linear functions and improved by log-transformation thereby revealing the impact of N:P stoichiometry on soybean yield. Therefore, N:P soichiometry need to be considered in planning soybean production nutrient management practices.

Table 1: Some physical and chemical properties of soil of the experimental site

Parameter	Value
Physical	
Sand (%)	53.8
Silt (%)	32.5
Clay (%)	13.7
Chemical	
Organic carbon g kg^{-1}	3.70
Total N (g kg^{-1})	0.17
Available P (mg kg^{-1})	6.68
Potassium [Cmol (+)kg^{-1}]	0.45
Calcium [Cmol (+)kg^{-1}]	1.90
Magnesium [Cmol (+)kg^{-1}]	0.41
Sodium [Cmol (+)kg^{-1}]	0.35
CEC [Cmol (+)kg^{-1}]	3.32

Table 2. Effect of N and P fertilizers on yield, N and P uptake and shoot contents of soybean

Year	Yield (kg/ha)	N uptake (kg/ha)	N shoot content (%)	P uptake (kg/ha)	P shoot content (%)
2010	613.42	31.50	2.67	4.08	0.33
2011	620.10	33.85	2.70	3.98	0.37
LSD	42.66	5.16	0.22	0.74	0.03
P	NS	NS	NS	NS	NS
N (kg/ha)					
0	558.79	31.04	2.59	4.01	0.32
25	644.56	30.08	2.65	3.90	0.33
50	669.61	35.42	2.80	4.08	0.31
75	594.09	34.17	2.69	4.12	0.31
LSD	60.33	7.30	0.32	2.70	0.05
P	0.002	NS	NS	NS	NS
P (kg/ha)					
0	506.68	26.79	2.45	3.47	0.28
30	632.87	29.38	2.56	3.36	0.35
60	615.87	34.46	2.86	4.39	0.33
90	711.64	40.08	2.85	4.90	0.36
LSD	60.33	7.30	0.32	1.05	0.05
P	0.0001	0.002	0.02	0.01	0.04
Interaction					
N x P	**	**	*	*	NS

Means in the same column of a treatment is separated by $LSD_{0.05}$

NS= not significant

*= Significant at 5% level of probability

**= Significant at 1% level of probability

Table 3: Effect of N and P fertilizers on grain N and P, harvest index and protein contents of soybean

Year	Grain N (kg/kg)	Grain P (kg/kg)	Protein content (%)	NHI	PHI
2010	4.93	0.42	30.78	0.60	0.56
2011	3.94	0.43	24.61	0.59	0.56
LSD	2.18	0.06	13.65	0.02	0.01
P	NS	NS	NS	NS	NS
N (kg/ha)					
0	3.79	0.43	23.67	0.59	0.55
25	5.92	0.43	36.97	0.61	0.56
50	4.11	0.41	25.69	0.60	0.57
75	3.91	0.45	24.45	0.59	0.56
LSD	3.09	0.09	19.31	0.02	0.02
P	NS	NS	NS	NS	NS
P (kg/ha)					
0	3.56	0.41	22.27	0.59	0.58
30	3.81	0.46	23.80	0.60	0.57
60	6.19	0.40	38.65	0.60	0.55
90	4.17	0.44	26.05	0.59	0.56
LSD	3.09	0.09	19.31	0.02	0.02
P	NS	NS	NS	NS	0.01
Interaction					
N x P	NS	NS	NS	NS	NS

Means in the same column of a treatment is separated by $LSD_{0.05}$

NS= not significant

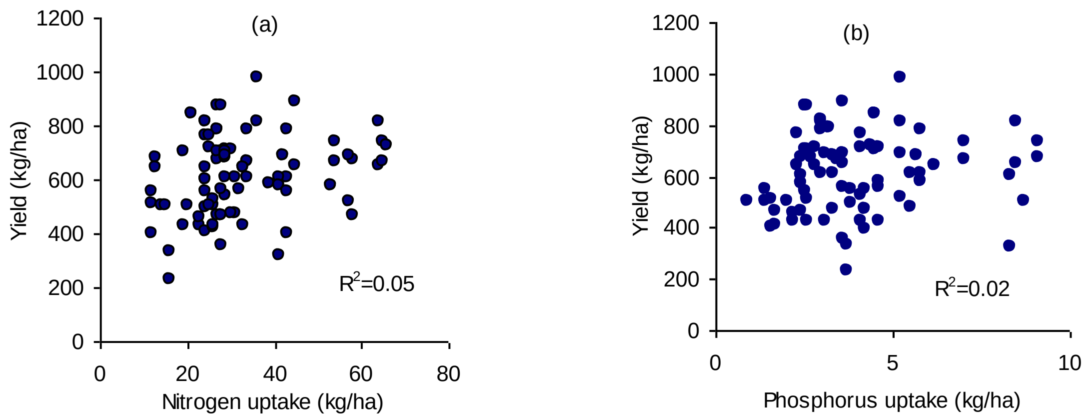

Fig. 1: Relationship between yield and (a) nitrogen uptake (b) phosphorus uptake

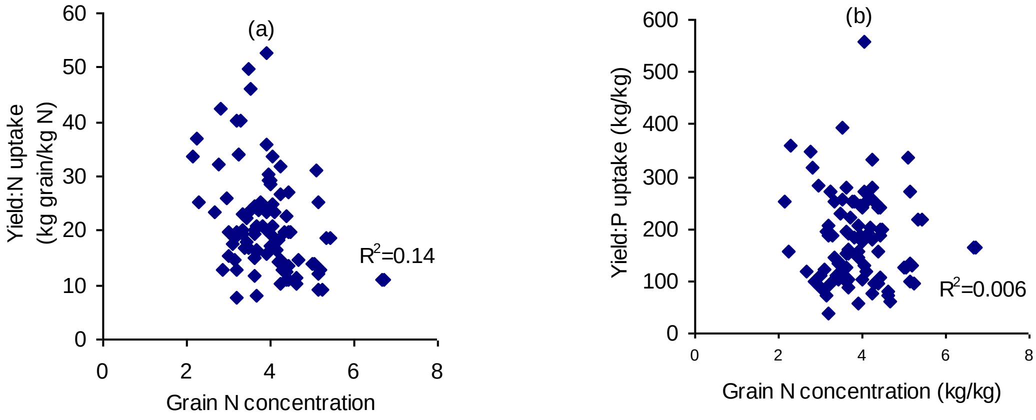

Fig. 2: Relationship between grain N concentration and (a) yield per unit nitrogen uptake (b) phosphorus uptake

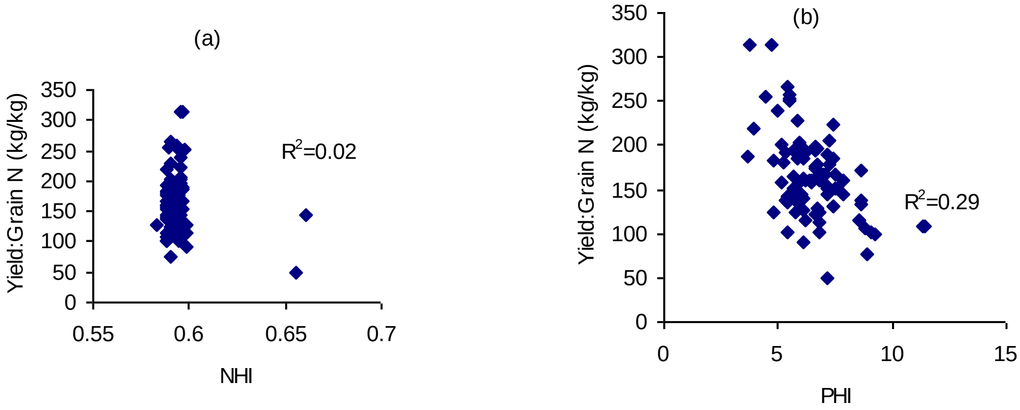

Fig. 3: Relationship between yield per unit grain N concentration and (a) nitrogen harvest index (b) phosphorus harvest index

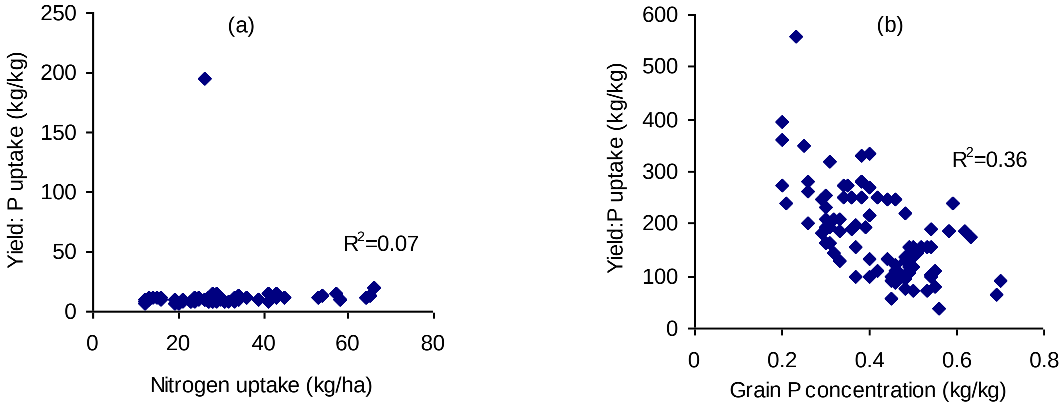

Fig. 4: Relationship between yield per unit P uptake and (a) nitrogen uptake (b) grain P concentration

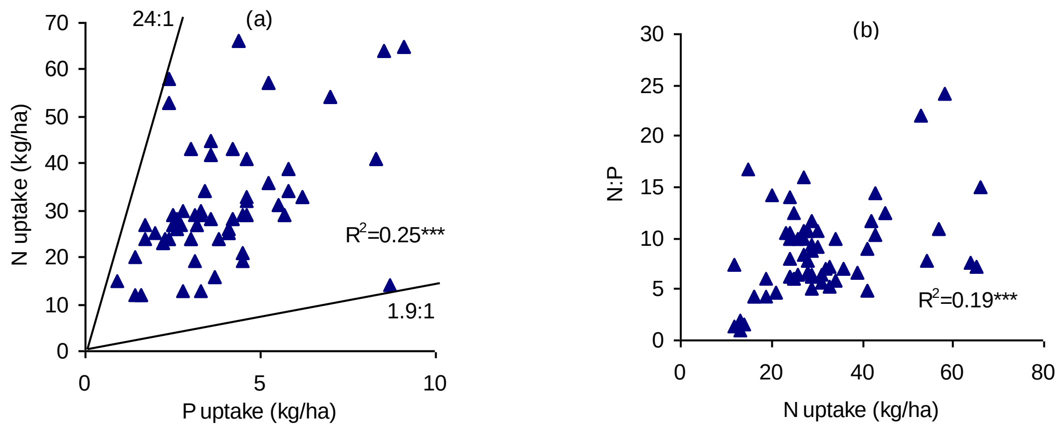

Fig. 5: Relationship between (a) P uptake and N uptake (b) N uptake and N:P ratios

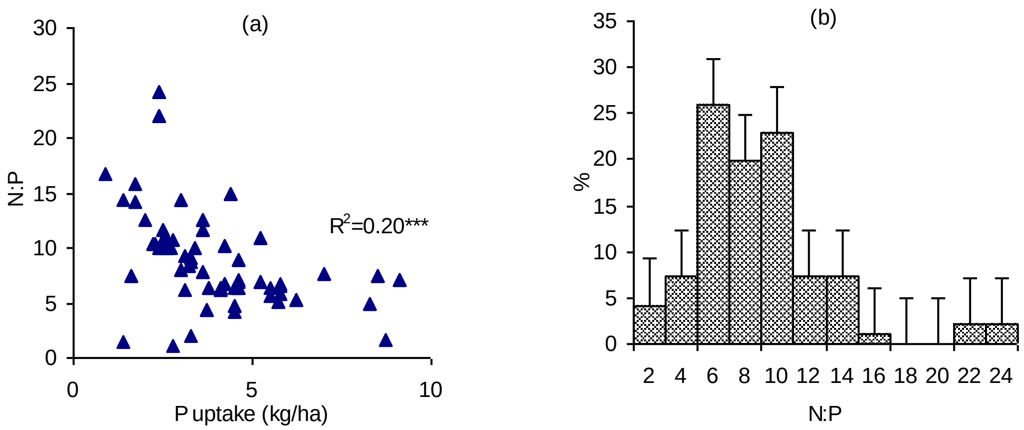

Fig. 6: Relationship between (a) P uptake and N:P ratio (b) Frequency distribution of N:P ratios

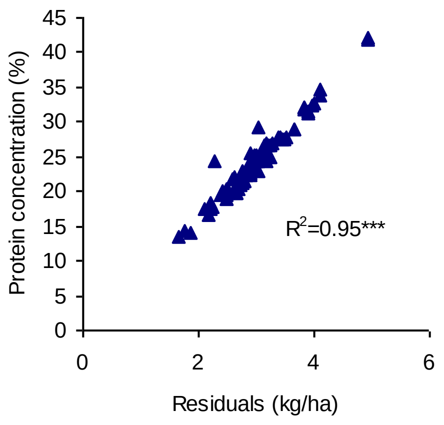

Fig.7: Relationship between protein concentration and residuals

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