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Study on measurement and statistical analysis of adherent soil chemical compositions of leguminous plants and their impact on nitrogen fixation

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Abstract

The most important step of this study is to conduct the careful assessment of the interaction of leguminous plants with soil chemical compositions. The amount of chemical components as well as total nitrogen in adhered soil of ten species of leguminous plants and two species of non-leguminous plants with normal soil (controls) were measured at a site of Jhenidah district in Bangladesh. On average, the adjacent soil of leguminous plants was slightly alkaline; amount of nitrogen was low; phosphorus, zink and organic matter were medium; sulpher and boron were remained optimum. Interestingly, potassium was extremely high (1.11±0.37 Me/100g). The amount of nitrogen was soared to 35.89% in the adherent soil of *Dalbergia sissooo* compared with control soil. In this way, total nitrogen was accrued in the soil by the all ten leguminous plants. According to the regression analysis, for 12 ppm increase of phosphorus, there was only 0.01% rise in amount of nitrogen on average that affect greatly to the total soil nitrogen. However, soil pH has a less influence on this issue. The higher amount of nitrogen in soil sample indicating that leguminous plants have significant effect on amount of nitrogen in soil. Thus cultivation of legume crops might reduce the utilization of nitrogen based fertilizers.

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Introduction

Nitrogen is generally considered as one of the major growth limiting factors for plants. For higher production, crops such as wheat, rice, and maize need 20 to 40 kg soil N ha^{-1} to satisfy the N requirements (Peoples *et al.*, 1992). To meet such high demand, farmers must either apply inorganic synthetic nitrogen or rely on biological nitrogen fixation (BNF) by legumes (Caroline *et al.*, 2011; Postage, 1982).

Although nitrogen is an essential nutrient for plant growth and development but it is unavailable in its most prevalent form as atmospheric nitrogen. Plants usually depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide ecological problems as well as affects the human health (Vitousek, 1997). Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants.

Biological nitrogen fixation (BNF) is the cheapest and environment friendly procedure in which nitrogen fixing micro-organisms, interacting with leguminous plants, fix aerobic nitrogen into soil (Franche *et al.*, 2009). Leguminous plants naturally increase nitrogen in field and reduce the amount of nitrogen fertilizer application for next cultivation of any plants (Herridge and Bergersen, 1988; Peoples and Herridge, 1990). Legumes are often considered to be the major nitrogen-fixing systems, as they may derive up to 90% of their nitrogen from N2. The rate of nitrogen fixation ranges from 200 to 300 kg N/ha/crop can be obtain for most species, but this largely depends on cultivars and culture conditions (Peoples *et al.*, 1995).

Various soil chemical compositions also influence the growth of leguminous plants and nitrogen fixation. Soil pH is a significant factor of them. It affects the soil's physical, chemical, and biological properties as well as plant growth. The nutrition, growth, and yields of most crops decrease where pH is low and increase as pH rises to an optimum level. At very acid or alkaline pH levels, organic matter mineralization is slowed down or stopped because of poor microbial activity linked to bacteria. Nitrification and nitrogen fixation are also inhibited by low pH (Smith *et al.*, 1996).Total carbon and nitrogen, available ammonium, and extractable phosphorus, as well as soil basal respiration, all significantly affects the biological nitrogen fixation process (Caroline *et al.*, 2011). Subsequently these influence the total nitrogen in soil.

Biological nitrogen fixation is usually carried out by specialized group of microbes. These microorganisms utilize the nitrogenase enzyme to catalyze the conversions of atmospheric nitrogen to ammonia. Plants can readily assimilate ammonia to produce nitrogenous biomolecules. The increase in nitrogen uptake by leguminous plants is likely to create more favorable environment for symbiotic nitrogen fixing bacteria and free living microbes. However, nitrogen fixation depends on the systems, plant cultivars, soil and many other parameters. The main objective of this work is to explore the effect of leguminous plants and their soil parameters (phosphorus and soil pH) on total nitrogen in soil. To investigate we measured the amount of chemical components as well as total nitrogen in adhered soil of ten species of leguminous plants and two species of non-leguminous plants with normal soil. The amount of nitrogen was 35.89% higher in the adherent soil of *Dalbergia sissooo* compared with control soil. Thus legume crops might reduce the application of nitrogen fertilizers for next crop cultivation. Further depth research encompassing the interactions among legumes, their soil parameters and microbial community must be made for potential improvement of our agriculture.

Materials and methods

Sample Collection, Plants Selection and Experimental Site

Soil samples were collected from the root associated soil of plants. Ten leguminous plants and two non-leguminous plants species were selected for soil sample collection (Table 1). The experimental site was at Jhenidah district in Bangladesh that is located at 23⁰-15'-00''N to 23⁰-45'-00''N and 88⁰-45'-00''E to 89⁰-45'-00''E (LGED, 2012).

Measurement of Chemical components of the Root Associated Soil Samples

Chemical components of the root associated soil samples, such as; nitrogen, phosphorus, potassium, sulpher, zink, boron, acidity (pH) and organic matter were measured as previously described methods. The procedures for measurement of chemical components were maintained according to "Fertilizer Recommendation Guide -2005" (Muslem *et al.*, 2005).

A wet-oxidation method was used for determining nitrogen from soil sample and the values were represented as percentage (%) (Sikora and Stott, 1996).For highly calcareous soils (pH greater than 7.4), the modified Olsen sodium bicarbonate method was used for measurement of phosphorus (Allan and Killorn, 1996) and the results were reported as parts per million (ppm). Potassium was extracted from soil by ammonium acetate method and values were represented as Me/100g (Allan and Killorn, 1996). The organic carbon of the soil was determined volumetrically by wet oxidation method of Walkley and Black as described by Imamul and Alam (2005). P^H of soil sample was measured electrochemically using a glass electrode pH meter at a soil: water ratio of 1: 2.5 as described by Imamul and Alam (2005). The Sulfer, Zink, Boron content of the soil sample were subsequently determined by calcium bi phosphate, DTPA and calcium chloride extraction methods.

Statistical analysis

Analysis of Variance (ANOVA) was performed using SPSS version 20. The statistical significance was set at the P<0.05 confidence level. Test of significance of the means was by the Least Significance Difference (LSD). We calculated the exact urea fertilizer required for specific amount of nitrogen in a field, following the formula of "Fertilizer Recommendation Guide - 2005" (Muslem *et al.*, 2005).

$F_r = U_f - C_i / C_s \times (S_t - L_s)$.

Where,

F_r = Fertilizer nutrient required for given soil test value.

U_f= Upper limit of the recommended fertilizer nutrient for the respective soil test value interpretation (STVI) class.

C_i = Units of class intervals used for fertilizer nutrient recommendation.

C_s = Units of class intervals used for STVI class.

S_t = Soil test value.

L_s = Lower limit of the soil test value within STVI class.

Results and discussion

Chemical features of the adjoining soil of leguminous and non-leguminous plants

The results of chemical properties of soil, such as nitrogen, phosphorus, potassium, sulpher, zink, boron, acidity (pH) and organic matter were shown in Table 2. According to results the highest level of nitrogen was found in the root associated soil of Rosewood (0.13±0.01), while the lowest amount was available in the normal soil sample (control soil) with 0.08±0.00. The nitrogen fixation capacities of leguminous plants were much higher than the non-leguminous plants. The maximum amount of phosphorus (20.9±0.05) and potassium (1.11±0.37) were found in leguminous plant adjoining soils than the non-leguminous plant adjoining soil and normal soil. The higher amount of phosphorus helps nitrogen fixation also previously reported by reed *et al.* (2007). The soil content of sulpher, zink, boron, p^H and organic matter were nearly in the same range in all the samples.

Homogeneity test of control samples

The chemical properties of three controls (without root associate soil, root associate soil of Black Mustard and Asian palm) were tested by ANOVA (Single Factor) in the Table 5. Since the F-statistic value was smaller than F-critical value, the null hypothesis could be accepted at a 95% confidence level. Thus, the data was not significant (p=0.99) and there was no significant variation amongst the chemical compositions of control soil (having no plant interaction) and adjacent soils of non-leguminous plants. Therefore, it seemed that they could be good controls in our study. We used Analysis of variance (ANOVA) to test the homogeneity of controls, because this method is used to compare measurements to determine if the measurements are sampled from the same or different distributions (Gelman, 2005).

Table 1 . List of the leguminous plants and non-leguminous plants species.

Plant type	Local Name	English Name	Scientific Name
Leguminous	Sim	Field Beans	*Lablab purpureus* (L.) Sweet
	Masoor	Lentil	*Lens culinaris* Medic
	Cholai	Bengal Gram	*Cicer arietinum*
	Lojjaboti	Sensitive Plant	*Mimosa pudica* Linn.
	Shishu	Sisso/Indian Rosewood	*Dalbergia sissooo* Roxb.
	Babla	Indian Gum-Arabic Tree	*Acacia Nilotica* (L.)
	Sojne	Drumstick Tree	*Moringa olefeira*
	Tetul	Tamarind	*Tamarindus indica* Linn.
	Radhachura	Copper Pod Tree	*Peltophorum pterocarpum*
	Krisnachura	Peacock Flower	*Caesalpinia pulcherrima*
Non leguminous	Shorisha	Black Mustard	*Brassica Nigra*
	Tal	Asian palm	*Borassus flabellifer*

Table 2. Chemical properties of the adjoining soil samples of leguminous and non-leguminous plants.

Sample soils	Chemical components							
	N_2 (%)	P (ppm)	K (Me/100g)	Sulpher (ppm)	Zink (ppm)	Boron (ppm)	Acidity (p^H)	Organic Mater (%)
Normal soil	0.08±0.00	14.6±0.05	0.57±0.44	29.2±0.95	0.73±0.04	0.53±0.05	7.56±0.32	1.70±0.14
Mustard	0.08±0.00	14.5±0.20	0.37±0.23	23.3±0.76	0.77±0.12	0.49±0.08	7.7±0.10	1.79±0.05
Palm	0.08 ±0.05	16.8±0.34	0.64±0.31	32.3±0.87	0.82±0.08	0.60±0.07	7.45±0.25	2.05±0.11
Sensitive plant	0.11±0.01	17.5±0.46	0.69±0.22	28.3±0.15	0.82±0.24	0.57±0.04	7.63±0.05	2.29±0.38
Field Beans	0.12±0.01	17.6±0.05	1.05±0.13	35.3±0.41	1.00±0.19	0.63±0.18	7.46±0.25	2.39±0.24
Lentil	0.09±0.00	15.4±0.37	0.21±0.01	24.4±0.41	0.84±0.13	0.49±0.07	7.5±0.10	1.67±0.03
Bengal gram	0.09±0.00	16.5±0.16	0.22±0.05	20.0±0.58	0.70±0.02	0.43±0.06	7.73±0.05	1.70±0.02
Rosewood	0.13±0.01	15.2±0.89	1.11±0.37	25.6±0.55	0.89±0.21	0.57±0.07	7.63±0.32	2.62±0.28
Gum Arabic tree	0.10±0.01	15.1±0.87	0.47±0.36	23.3±0.11	0.99±0.12	0.59±0.18	7.10±0.36	1.95±0.24
Tamarind	0.11±0.01	20.9±0.05	0.80±0.17	35.9±0.31	1.01±0.16	0.69±0.10	7.13±0.05	2.28±0.29
Copper Pod tree	0.10±0.00	20.7±0.32	1.05±0.11	35.5±0.51	0.92±0.04	0.52±0.08	7.48±0.11	2.11±0.12
Peacock Flower	0.10±0.01	17.2±0.17	0.84±0.12	29.5±0.57	1.01±0.19	0.60±0.11	7.43±0.20	2.12±0.29
Drumstick tree	0.10±0.00	15.0±0.00	1.06±0.39	31.50±34	0.91±0.06	0.51±0.05	7.10±0.15	2.12±0.25

Value of three samples was calculated as mean ±SD.

Comparisons of chemical properties between adjoining soils of leguminous and non-leguminous plants

Comparisons of chemical properties between adjoining soils of leguminous and non- leguminous were showed in Table 3 with standard value. Interpretation of soil test values was performed based on critical limits for loamy and clayey soils of wetland crops. On average, the adherent soil of leguminous was slightly alkaline, amount of nitrogen was low, phosphorus zink and organic matter were medium, sulpher and boron were remained optimum. Interestingly, potassium was extremely high which was 0.70±.38 Me/100g. On the other hand, percentage of nitrogen and zink of sticky soil of non-leguminous plant was averagely very low in quantity. These results revealed that the root associated soil of leguminous plants have ability to fix nitrogen by interacting with nitrogen fixing bacteria (Kiers *et al.*, 2003).

Effect of nitrogen fixation of plants Determination of nitrogen fixation capacity of leguminous plants

To know whether any variation exists between the amount of nitrogen in the controls and ten leguminous plants, we performed t-test. Since the t-statistic value was greater than t-critical value, the null hypothesis could be rejected at a 95% confidence level (since alpha was set at 0.05). Hence, these data were significant ($p \leq 0.0004$) and there were differences in amount of nitrogen between three control soils (without root associate soil, root associate soil of Black Mustard and Asian palm) and adjoining soils of leguminous plants. A bit better result was shown by leguminous plants on average (Table 6). This means that nitrogen fixation was occurred by leguminous plants that affects the next cultivation largely.

Estimation of utilization of urea fertilizer on the basis of nitrogen fixation

The percentage of increased N_2 by leguminous plants was compared with normal soil where increase of N_2 is 0% (0.08-0.08). The highest amount of nitrogen was found in soil of Rosewood and this was 0.13%, whereas only 0.08% nitrogen was present in normal soil. Therefore, nitrogen fixation was increased 35.89% (0.13-0.08=0.05 unit; 0.05×100÷0.13=35.89%) in the soil of Rosewood compared with control soil (without root associate soil). In contrasting, only 3.84% nitrogen was increased in the adjacent soil of Black Mustard (non-leguminous plant used as control). For increased nitrogen (by leguminous plants),

application of urea in next cultivation was reduced in the fields of leguminous plants as compared to the fields of normal soils. The amount of urea application in next cultivation is usually predicted by Soil Research and Development Institute of Bangladesh. This forecasting has been employed in our research to show the benefits of nitrogen fixation of leguminous plants (Table 4). We calculated the exact urea fertilizer required for specific amount of nitrogen in a field, following the formula of "Fertilizer Recommendation Guide-2005" (Muslem *et al.*, 2005).

Table 3. Comparison of chemical properties between adjoining soils of leguminous and non- leguminous plants.

Chemical components	Leguminous plants soil	Non Leguminous Plants soil	Standard range*	Comments	
				LG	NL
Nitrogen (%)	0.09±0.00-0.13±0.01	0.08±0.00-0.08±0.05	< 0.09	High	Low
Phosphorus (ppm)	15.4±0.37-17.6±0.05	14.5±0.20-16.8±0.34	12.1-18.0	Medium	Medium
Potassium (Me/100g)	0.22±0.05-1.11±0.37	0.37±0.23-0.64±0.31	>0.375	Extreme	Extreme
Sulpher (ppm)	20.0±0.58-35.9±0.31	23.3±0.76-32.3±0.87	27.1-36.0	Optimum	Optimum
Zink (ppm)	0.70±0.02-1.01±0.19	0.77±0.12-0.82±0.08	0.91-1.35	Medium	Low
Boron (ppm)	0.43±0.06-0.69±0.10	0.49±0.08-0.60±0.07	0.451-0.6	Optimum	Optimum
Acidity (p^H)	7.10±0.15-7.73±0.05	7.45±0.25-7.7±0.10	7.4-8.4	Optimum	Optimum
Organic Mater (%)	1.67±0.03-2.62±0.28	1.79±0.05-2.05±0.11	1.8-3.4	Medium	Medium

*Source of standard range: Fertilizer recommendation guide, 2005.LG: Legume, NL: Non Legume. Values were represented as mean± SD of triplicate sample.

Impact of phosphorus and acidity on total nitrogen and soil chemical compositions Impact of phosphorus on total Nitrogen

Reed *et al.*(2007) found that the addition of phosphorus to soil more than doubles nitrogen fixation, due to the energy requirements of nitrogen fixation. For our study we carried out regression analysis where phosphorus in samples was used as independent parameter and nitrogen was used as dependent parameter. R Square value was only 0.07 ($p=1.48E-08$, $n=39$) which indicates that the correlation between these two parameters was very week and not suitable for strong prediction (Figure 1). The main cause for this, nitrogen fixation may not be strongly dependable on amount of phosphorus but for the nature of phosphorus (KH_2PO_4, Zn_3 (PO_4), Ca_3 $(PO_4)_2$ etc). However, with the lower R Square value, averagely, 22 ppm phosphorus was available for 0.10% nitrogen, whereas, 10 ppm phosphorus was available for 0.09% nitrogen. Hence, for 12 ppm increase of phosphorus, there was only 0.01% rise in nitrogen.

Table 4. Increased of nitrogen fixation by leguminous plants reduce urea application (in the field of paddy plant-Amon).

Sample soil	Nitrogen (%)	Average N_2 increase (%)	Urea for Amon (Kg/ha)	Average urea decrease (kg/ha)
Normal soil	0.08±0.00	Lowest N2 fixation	202.5±0.00	Highest consumption
Mustard	0.08±0.00	3.84	200±4.33	2.5
Palm	0.08 ±0.05	4.56	197.25±6.23	3.2
Sensitive plant	0.11±0.01	26.47	180.83±10.92	21.66
Field Beans	0.12±0.01	30.55	176.25±7.50	105
Lentil	0.09±0.00	13.7	195.5±6.33	9.8
Bengal gram	0.09±0.00	13.79	192.5±4.33	10
Rosewood	0.13±0.01	35.89	168.75±12.99	33.75
Gum Arabic tree	0.10±0.01	27.60	179.50±8.25	23.3
Tamarind	0.11±0.01	28	178.33±7.93	24.167
Copper Pod tree	0.10±0.00	26.47	180.42±10.77	22.083
Peacock Flower	0.10±0.01	19.35	187.92±3.60	14.583
Drumstick tree	0.10±0.00	21.87	185.42±7.93	17.083

Values were given as mean± SD of triplicate sample.

Table 5. ANOVA (Single Factor analysis) for chemical substances of controls.

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	84.92628	8	10.61578	0.10868	0.998785	2.089185
Within Groups	6153.78	63	97.67905			
Total	6238.706	71				

Impact of acidity on soil chemical compositions

Consideration of soil acidity or alkalinity is important, because these conditions exert strong influences on root development as well as activity of soil bacteria and fungi, symbiotic nitrogen fixation by legumes and availability of a wide range of nutrients including phosphorous, iron, zinc, boron, manganese and copper (Muslem *et al.*, 2005).. The soil of the experimental area in our study was slightly alkaline. For fluctuation in soil pH range, the changes in relevant chemical compositions were tested in ANOVA (Single Factor). Since the F-statistic value was lower than F-critical value, the null hypothesis could be accepted at a 95% confidence level (since alpha was set at 0.05). Thus, it was not significant ($p=1$) and the variation was not existed among the chemical compositions of soil for the changes of pH values in this area. There were two possible rationales for this situation- firstly, the pH range was narrow and within the slightly alkaline conditions of soil, and thus it couldn't affect the soil chemical compositions noticeably. Secondly, not only the pH itself but also the sources of H^+ ions in soil solution are also the important factors to harness the chemical components of soil (Smith *et al.*, 1996)

Table 6. Variances analysis for nitrogen amount between the controls and ten leguminous plants (t-test: Two-Sample Assuming Unequal Variances).

Statistics	0.09(%)	0.1(%)
Mean	0.08875	0.105517241
Variance	6.96429E-05	0.000332759
Observations	8	29
Hypothesized Mean Difference	0	
df	26	
t Stat	3.732529682	
P(T<=t) one-tail	0.000467719	
t Critical one-tail	1.705617901	
P(T<=t) two-tail	0.000935438	
t Critical two-tail	2.055529418	

Impact of Acidity on Total Nitrogen

According to regression analysis (R^2 =8.80E-06, P=0.15, n=39), for soil pH 7.8 (the maximum pH in this area), the nitrogen was 0.10% on average. On the other hand, for soil pH 6.4 (the minimum pH in this area), the nitrogen was also 0.10% on average. This indicates that, for changes of pH in small range (6.4-7.8), in this area, there was no significant effect on amount of nitrogen. In our proposition, the change in small range of pH (6.4-7.8) had no effect on microbial community of nitrogen fixer, leguminous plants and their interactions in large amount, because the range was narrow and within the slightly alkaline conditions of soil. Thereby, there was no effect on total nitrogen. Smith *et al*. (1996) proposed the similar contention.

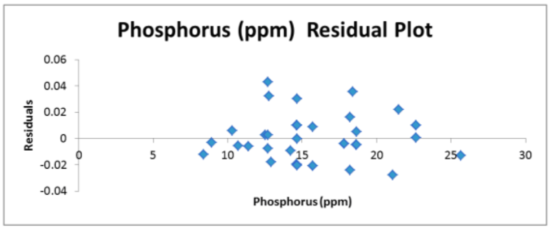

Fig. 1. Phosphorus (ppm) residual plot of regression analysis for phosphorus dependent nitrogen fixation.

Conclusion

In the present study we measured the chemical properties of adjoining soil samples of leguminous plants as well as non-leguminous plants. We found higher amount of nitrogen (35.89%) in adjoining soil sample of leguminous plant in contrast to non-leguminous plants. Moreover, legume plants contained high phosphorus content that helps to more biological nitrogen fixation. Thus cultivation of legume crops might reduce the utilization of N fertilizers in farm lands.

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