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Growth and nodulation response of six indigenous trees and two shrubby legumes to phosphorus and nitrogen fertilizers in two soils of Ghana

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Abstract

Fast growth and high N_2-fixation make multipurpose trees attractive in agroforestry. We assessed the effects of N and P fertilizer application on nodulation and growth of *Millita thonningii* native to West Africa and compared its attributes with five known multipurpose leguminous trees *Acacia auriculiformis, A. mangium, Albizia lebbeck, Albizia zygia, Leucaena leucocephala,* and two shrubby legumes, *Cajanus cajan* and *Crotalaria ochroleuca* in Hatso and Toje soils. They all were nodulated without inoculation, with nodulation being higher in Toje soil. Up to 90 kg P ha $^{-1}$ enhanced nodulation and growth, but further increase to 120 kg P ha $^{-1}$ caused from 53% to 600% reduction in nodules on *M. thonningii* and *A. zygia*, respectively and significant reduction in tree growth. Nitrogen fertilization decreased nodulation in the tree legumes more in the two shrubs and also more when the N fertilizer was combined with 90 kg P ha $^{-1}$. *M. thonningii* had highest yield in both un-amended and amended soils, almost 3 and 7 times more than the lowest*, A. zygia,* in the un-amended Toje and Hatso soils, respectively. *M. thonningii* used less P for growth, on average 22 and 18 mg P g $^{-1}$ dry matter in the Toje and Hatso soils, compared to 50 and 39 mg P g $^{-1}$ for the six trees. Thus *M. thonningii* with its high yield, nodulation and P use efficiency has great potential in agroforestry.

Key words: Agroforestry, Nodulation, Tree legumes, *Millitia thonningii*

Introduction

Nitrogen fixing trees in particular, are essential for the establishment of sustainable agroforestry practices by resource-poor and/or environmentally conscious farmers (Awonaike et al., 1996). However, the growth of nitrogen - fixing trees is often limited by the available supply of phosphorus in soil, and any factor limiting growth may also limit rates of N_2 fixation (Binkley et al., 2013)

Legumes in general require larger amount of P than other plants and its effects are complex acting on nodulation, nitrogen fixation and plant growth (Pereira and Bliss, 1987). Several workers (Gate and Wilson, 1974; Jenkinsen et al., 1987; Olofintoye, 1986) have reported that a high phosphorus level is needed for maximum nodulation and nitrogen fixation in legumes, but the amounts required for optimum nodulation and N_2 fixation differ widely among genotypes (Pereira and Bliss, 1987). Danso et al. (1992) have also reported the effect of phosphorus on nodulation, root proliferation and its overall beneficial effect on legumes. Deficiencies in nutrients like P, essential for the growth of not only plant but also of bacteria can cause reduction in the numbers and size of nodules as well as the amount of N_2 fixed (Giller and Wilson, 1991). It has been suggested by Hayman (1986) that, as nodulated plants often have less well-developed root systems than unnodulated plants, the ability of nodulated plants to capture nutrients, particularly P is decreased and most legumes therefore depend heavily on mycorrhizae

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for efficient uptake of phosphorus.

Another soil nutrient whose low availability limits the growth of many plants in most tropical soils is N (Sanginga et al., 1986). Biological nitrogen fixation (BNF) in legumes has therefore been recommended as a promising alternative to the use of inorganic N fertilizers in the tropics where fertilizers are either not easily available, or are not affordable by many of the peasant farmers (Sanginga et al., 1989). Besides, in contrast to P, BNF is severely inhibited in N-rich soils or where inorganic N fertilizers have been added to soil (Gibson, 1971).The capacity for nitrogen fixation by a nodulated legume is influenced in at least two ways by mineral nitrogen. Firstly, although the process of nodulation may be promoted by relatively low level of available nitrate or ammonia, higher concentrations almost always depress nodulation (Dixon and Wheeler, 1986). Secondly, the rate of N_2 fixation by an active, growing and well nodulated legume is suppressed by high inorganic N (Davidson and Robson, 1986). It was suggested that nitrogen fertilizers added to soil may delay the symbiotic process through decreased multiplication of free-living rhizobia (Dart, 1974). Recent reports indicate that, N fertilizers inhibit at least three phases of legume nodulation; i.e., through reduction in root hair infection (Abdel-Wahab et al*.,* 1996), nodule initiation, growth and development (Atkins et al*.,* 1984; Imsande, 1986), decreased nitrogenase activity (Sanginga et al., 1989; Arreseigor, 1997) and by promoting premature nodule senescence (Gibson and Harper, 1985; Abaidoo et al*.,* 1990). Sanginga et al. (1989) reported that N fertilization at a rate of 40-80 kg N ha^{-1} reduced the nodule mass and N_2 fixation in *Leucaena leucocephala*. Peoples et al*.,* (1994) reported that the $^{15}N/^{14}N$ ratio in *L. leucocephala* leaves declined with plantation age and Dommergues (1995) inferred that this pattern indicated a decline in N_2 fixation as soil N supply increased.

In this study, six species of common indigenous leguminous trees and two shrubs were assessed for ways of enhancing their growth, nodulation and nitrogen fixing ability on two different soil types that occur in the coastal savannah zone of Ghana. Our main objective was to assess to what extent nodulation and growth of *Millitia thonningi* native to West Africa can be improved by phosphorus application and compared it with already known indigenous agroforestry tree legumes and shrubs in N-poor as well as N-rich soils.

Materials and Methods

Location of the study area

All the studies were carried out in small nursery bags at the screen house behind Ecological Laboratory, University of Ghana, Legon.

Soil and site characteristics

Two soil types used for the studies were taken from the Accra plains (05^{o} 39.627^{1} N, 0011.619$^{1'}$ W). The soils belong to the Toje and Hatso series (Local Names) (Brammer, 1967). Toje and Hatso series are classified as Rhodic lixisol and Haplic lixisol respectively according to FAO (2006). The two soils are widely cultivated by resource poor farmers in the area and occur on the same soil catena with Toje series being at the top and Hatso series being at the middle slope.

Soil Analysis

Soil pH was determined electrometrically (Peech, 1965) in distilled water at a soil: solution ratio of 1:1 using Pracitronic M.V 88 pH electrometer. Organic carbon was determined using the wet combustion method of Walkley and Black (1934) whiles Bray and Kurtz (1945) method was used to measure available phosphorus. Total nitrogen was determined by the distillation and titration method of Bremner (1965). The CEC of the soil was determined by extraction of the exchangeable bases using neutral ammonium acetate (NH4OAc, pH 7.0) and the aliquot used to determined Ca, Mg, K and

Na. The soil texture was determined by the particle size analysis using the modified Bouyoucos hydrometer method as describe by Day (1965).

Phosphorus response studies

The response of six tree legumes namely, *Acacia auriculiformis, Acacia mangium, Albizia lebbeck, Albizia zygia, Leucaena leucocephala* and *Millitia thonningii,* to phosphorus fertilizer was evaluated in Toje and Hatso soil series.

The experiment was conducted in polyethene bags (30 cm high, 15 cm diameter at top and bottom). Each nursery bag was filled with 2 kg of dried and sieved soil (0.2 mm diameter). The treatments were:

i. No P fertilizer (control)
ii. P fertilizer at 30, 60, 90 and 120 kg P ha^{-1} in the form of Triple Super Phosphate (TSP)

The fertilizers were thoroughly mixed with each soil before filling into the polyethene bags.

Pre-germinated seeds were planted in each polyethene bag and later thinned to fifteen days after germination when the seedlings were in two leaf stage.

The experiment was arranged in a randomized complete block design with four replicates. The plants were watered daily until harvest. The plants were harvested 12 weeks after planting by cutting the stem at the soil level. The plant roots were carefully washed from the soil in excess water. Nodules were then separated from the roots and counted and the shoot dry weights determined after drying in an oven for 3 days at a temperature of 70^0C until constant weights were achieved. Total P in the shoot was determined and the physiological P use efficiency (PPUE) calculated using the modified formular of Elliot and White (1994)

$$PPUE = \frac{\text{Shoot P content (mg)}}{\text{Shoot dry weight (g)}}$$

Nitrogen response studies

The response of four trees and two shrub legumes to nitrogen application was evaluated in two different experiments. The first experiment involved using phosphorus as a basal treatment for all the pots @ 90 kg P ha^{-1}, whilst the second experiment did not include P treatment. In the first experiment, the four tree legumes *(Acacia auriculiformis, Acacia mangium Leucaena leucocephala,* and *Millitia thonningii*) and two shrub legumes (*Cajanus cajan and Crotalaria ochroleuca*) were used. Each nursery bag was filled with 2 kg of soil and mixed thoroughly with 90 kg P ha^{-1} as basal treatment.

The treatments were:

i. No P fertilizer and nitrogen fertilizer (control)
ii. 90 kg P ha^{-1} fertilizer and no nitrogen fertilizer
iii. 90 kg P ha^{-1} fertilizer and five different levels of nitrogen fertilizers at 40, 80, 120, 180 and 240 kg N ha^{-1}.

Pre-germinated seeds of each legume tree were planted in the nursery bags. Nitrogen fertilizer in the form of urea (21% N) was applied to the nursery bags at a depth of 1cm. The nitrogen fertilizer was applied one week after emergence of the tree legumes. The tree legumes were allowed to grow for 12 weeks and watered daily after which they were uprooted. The seedlings were chopped at the soil surface and the shoot dry weights determined after drying in an oven for 3 days at a temperature of 70^0C. The roots were carefully washed of sand and the nodule numbers determined after excising from the roots.

The second nitrogen response study was carried out just like the first one above but in this case, phosphorus was not applied as basal treatment.
The treatments were:

i. No N fertilizer (control)
ii. Five levels of N fertilizer at 40, 80,120,180 and 240 kg N ha^{-1}.

Statistical Analysis

The data collected from the different trials were subjected to general Analysis of Variance using GenSTAT (9th edition) software and the means obtained were compared by lsd at 5 % level of significance using Duncan Multiple Range Test. Microsoft Excel program was used to generate graphs for data presentation

Results and Discussion

Soil properties

The characteristics of the soils used in this study are presented in Table 1. Hatso series, a sandy soil was lower in total N, organic carbon and available P, whereas Toje series, a sandy clay loam was lower in pH and CEC values.

Table 1. Chemical properties of Toje and Hatso soils

Parameters	Soil Types	
	Toje	Hatso
pH	5.3	6.0
Total N (g kg $^{-1}$)	0.59	0.34
Organic carbon (g kg $^{-1}$)	6.5	3.7
CEC (cmol kg $^{-1}$)	5.84	7.40
Available P (mg kg $^{-1}$)	7.52	3.76
Texture	Sandy clay loam	Sandy

Influence of phosphorus and nitrogen fertilizer application on nodulation of six tree legumes in the Hatso and Toje soils.

Results of the study indicated that the two *Acacia* species, *A. auriculiformis* and *A. mangium* were the poorest nodulators, followed by *L. leucocephala* and *A. zygia* and best nodulators being *M. thonningii* and *A. lebbeck* (Fig.1 & Fig. 2) in both soils. Nodulation of all six tree species increased with phosphorus application to the Hatso soil, with the highest nodulation in each tree occurring with 90 kg P ha^{-1} application, and being in all cases significantly higher than the zero P controls. For the 60 kg P ha^{-1} level, the increases in nodulation relative to the zero P controls were significant only for the two *Albizia* species, *A. lebbeck* and *A. zygia*. However, increasing phosphorus application from 90 kg P ha^{-1} to 120 kg P ha $^{-1}$ resulted in significant decrease in the number of nodules formed in all the tree legumes (Fig. 1) except *A. auriculiformis.* In the Toje soil, *A. mangium* and *M. thonningii* nodulated better than they did in Hatso soil, while the reverse was the case for the remaining four species. Similarly, in the Hatso soil, nodule formation was enhanced by P fertilization, but to a lesser degree than in the Toje soil. Although highest nodulation in both soils occurred at the 90 kg P ha^{-1} level, in the Toje soil, these increases when compared with their respective controls reached significant level ($p = 0.05$) only in the *A. zygia* and *M. thonningii* species, compared to five of the six tree species in the Hatso soil. Increasing P application from 90 to 120 kg P ha^{-1} resulted in decreases in nodulation of all six tree species grown in the Toje soil, to the extent that nodulation was completely eliminated in *A. mangium* at the 120 kg P ha^{-1} level.

The tree legumes varied in their nodulation responses to phosphorus. For instance, while the application of 90 kg P ha^{-1} to *L. leucocephala* resulted in over 850% and 113% increases in nodules formed in Hatso and Toje soils respectively, *M. thonningii* at that same rate experienced only 48% and 29% increase in the Hatso and Toje soils respectively.

In contrast to P, increasing N fertilizer rates resulted in lower nodulation on the four trees (*L. leucocephala, A. auriculiformis, A. mangium* and *M. thonningii*) and two shrubby (*C. ochroleuca* and *C. cajan*) legumes examined in both Hatso (Fig. 3) and Toje (Fig. 4) soils. Nodulation of the tree species was more sensitive to increasing levels of soil N than the shrubby legumes in both Hatso and Toje soils. Thus while the two shrubs formed nodules up to the highest level of 240 kg N ha^{-1} rate in the Hatso soil, for the tree legumes, except for *M. thonningii* which was able to form some nodules at the 40 kg N ha $^{-1}$ rate and not beyond, the three

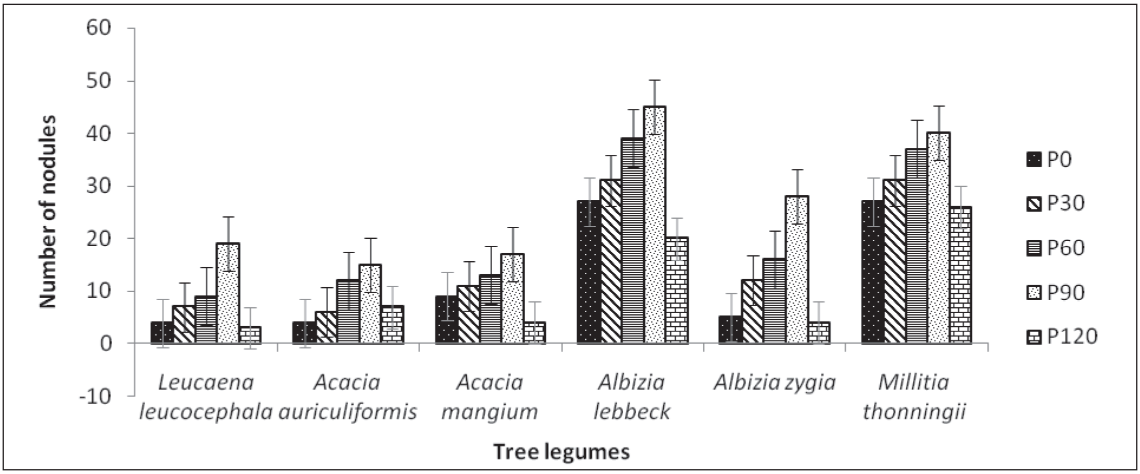

Figure 1. Effect of five rates of phosphorus fertilizer on the nodulation of six tree legumes in Hatso soil.

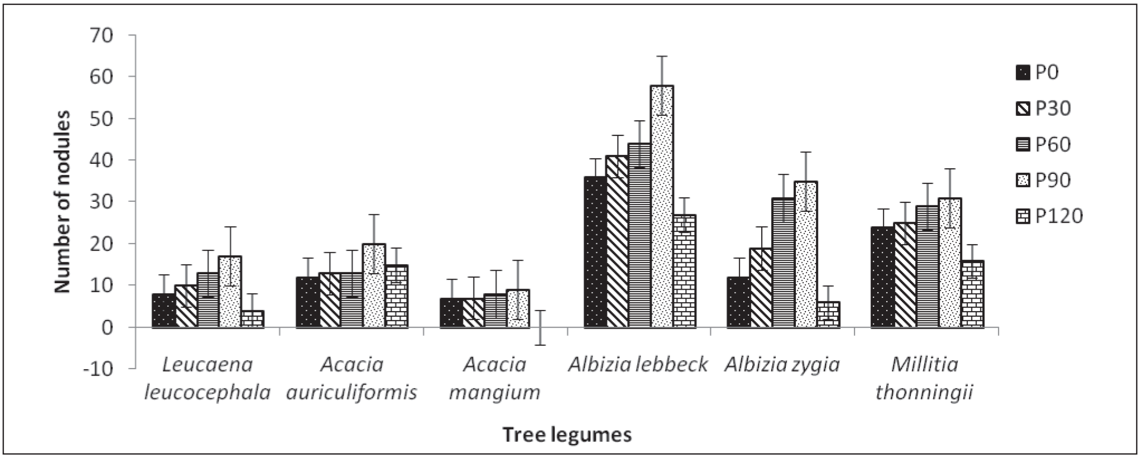

Figure 2. Effect of five rates of phosphorus fertilizer on nodulation of six tree legumes in Toje soil.

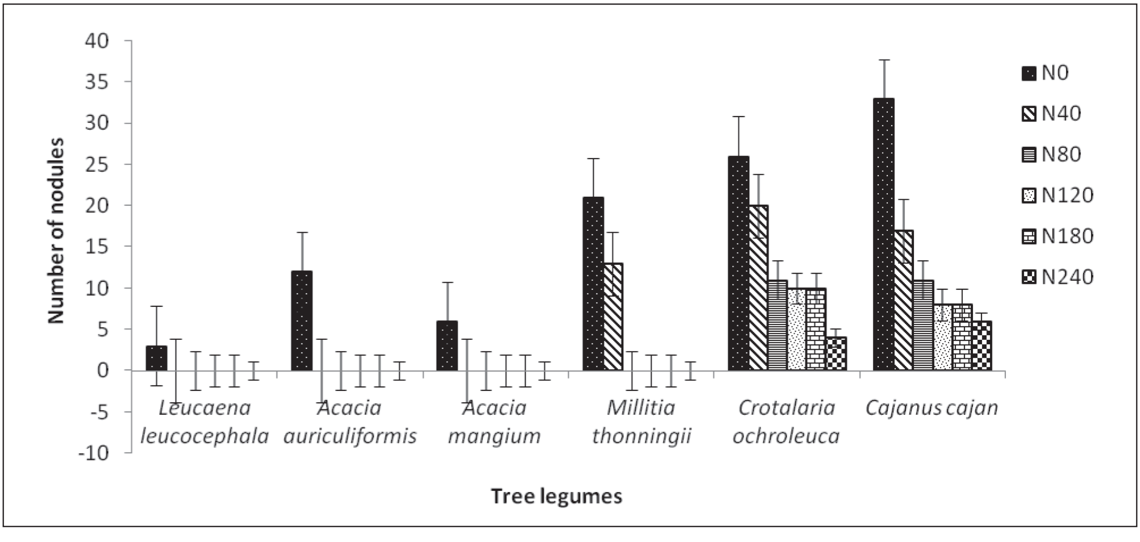

Figure 3. Effect of six rates of nitrogen fertilizer on nodulation of four trees and shrubby legumes in Hatso soil

other legumes (*L. leucocephala, A. auriculiformis,* and *A. mangium*) did not form any nodule once fertilizer N was applied at the rate of 40 kg N ha^{-1}. The contrasting inhibitory effect of inorganic N on nodulation between the tree and shrubby legumes was similarly evident in the Toje soil (Fig. 4). In contrast, although nodulation in the two shrubby species *C. ochroleuca* and *C. cajan* was also sensitive to increasing N levels in the Toje soil, they formed some nodules even at the highest N level, 240 kg N ha^{-1}. In the Toje soil, two of the trees*, A. auriculiformis* and *M. thonningii* did not form any nodules once fertilizer N was applied at the 40 kg N ha^{-1} rate, while the other two *L. leucocephala* and *A. mangium* formed a few nodules at the 40 kg N ha^{-1} rate but not beyond (Fig. 4). The two shrubs, *C. ochroleuca* and *C. cajan* showed far greater tolerance to N in terms of its effect on nodulation.

The combined effect of the individual contrasting effects of N and P on nodulation reported above for the four tree and two shrub legumes are presented in Fig. 5 and Fig. 6 for the Hatso and Toje soils, respectively. With the P levels for nodulation fixed at 90 kg P ha^{-1}, the optimum reported in Fig. 1 and 2, increasing the rates of only N application resulted in decreases in numbers of nodules formed on all the tree and shrub legumes with each succeeding increase in N level in the two soils. However, compared to the soil N application, supplemental P significantly mitigated the severe depressive effect of N on nodulation, in particular by increasing the range of soil N levels within which the tree legumes in particular could form nodules. Similar reductions in the suppressive effect of N on nodulation were obtained for the tree and shrubby species grown in the Toje soil once 90 kg P ha^{-1} was applied to the soil and is best illustrated by the results obtained for the two *Acacia spp* and *L. leucocephala* (Fig. 4 compared to Fig. 6)

Shoot dry matter yield (g) response of tree legumes to phosphorus and nitrogen fertilizers in two soil types

Except for *A. zygia* in the Toje soil, the application of phosphorus fertilizer at all rates resulted in significant increases (p=0.05) in shoot dry matter yield of the tree legumes (Table 2 & Table3). Increasing phosphorus application rates resulted increase in shoot dry matter yield till 90 kg P ha^{-1} but beyond which shoot dry matter yield decreased significantly (p=0.05). Thus, for all trees, the highest growth response to P in both soils occurred at the 90 kg N ha^{-1} level and was close to or more than three times better than the control for *A. auriculiformis*, *A. zygia, L. leucocephala* in Hatso soil (Table 2) and about two times for *A. lebbeck*,

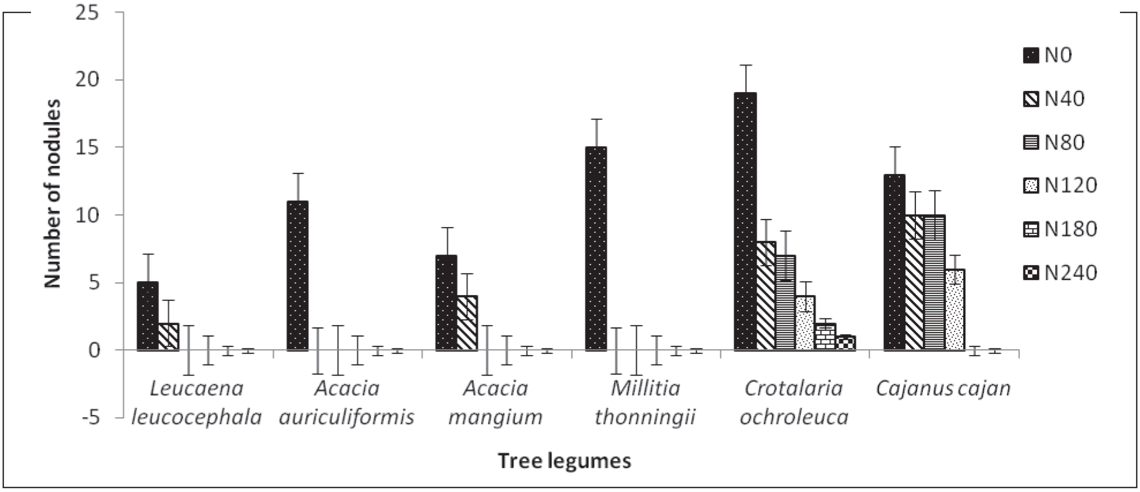

Figure 4. Effect of six rates of nitrogen fertilizer on nodulation of four tree and two shrubby legumes in Toje soil.

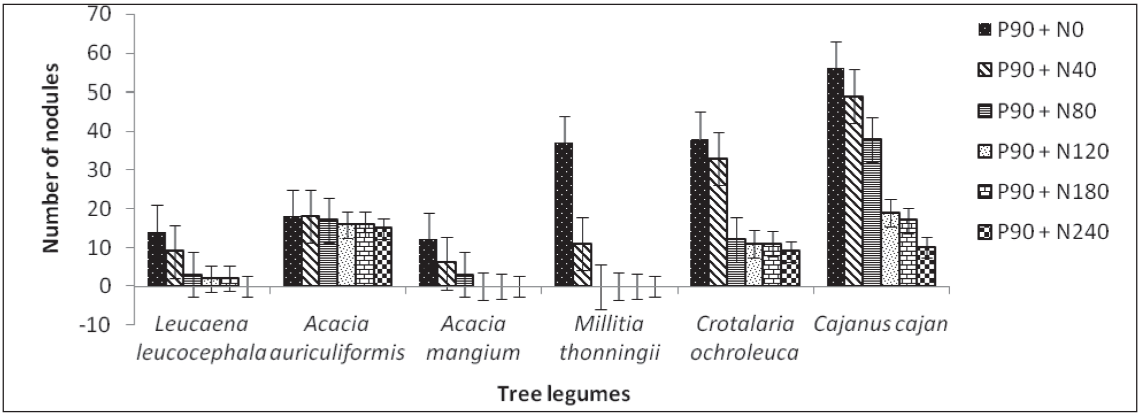

Figure 5. Effect of six rates of nitrogen fertilizer on the nodulation of four trees and two shrubby legumes grown in Hatso soil amended with 90 kg P ha^{-1} phosphorus fertilizer.

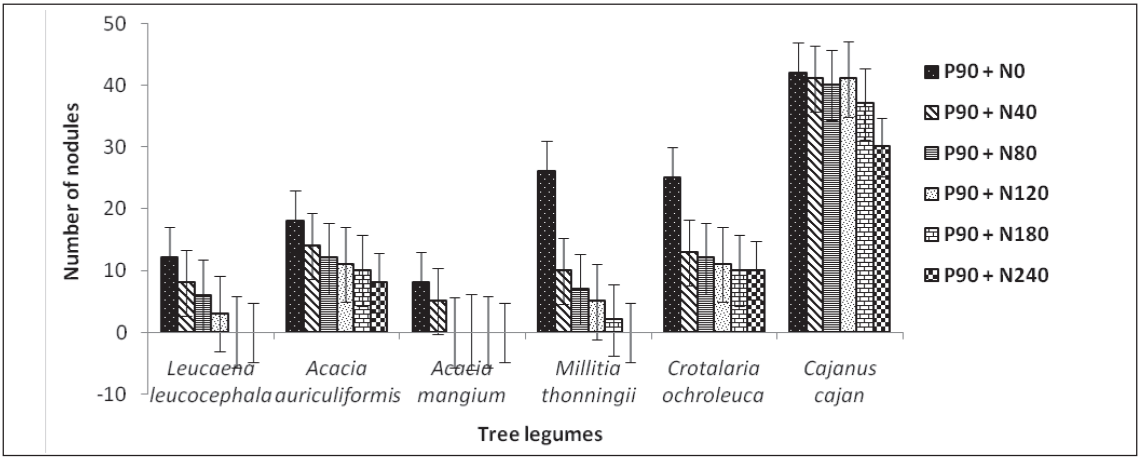

Figure 6. Effect of six rates of nitrogen fertilizer on the nodulation of four trees and two shrubby legumes grown in Toje soils ammended with 90 kg P ha^{-1} phosphorus fertilizer.

with the lowest response. *M. thonningii* was on average 78% higher at 90 kg N ha^{-1} than the control. Generally, application of 30 kg P ha^{-1} in all the soils resulted in the highest percent shoot dry matter yield increase compared to subsequent application rates that ranged from 6% in the case of *A. zygia* in Toje

Table 2. Effect of phosphorus application on shoot dry matter yield (g) of six tree legumes in Hatso soil

P-rates Kg ha^{-1}	*Acacia auriculiformis*	*Acacia mangium*	*Albizia lebbeck*	*Albizia* zygia	*Leucaena leucocephala*	*Millitia thonningii*
	Yield (g plant^{-1})					
P_0	0.24c	0.12b	0.57c	0.11b	0.34d	0.78c
P_{30}	0.50b	0.24a	1.02b	0.17b	0.72c	0.89bc
P_{60}	0.54ab	0.27a	1.11ab	0.26ab	0.89b	0.94ab
P_{90}	0.62a	0.32a	1.21a	0.31a	1.08a	1.05a
P_{120}	0.25c	0.08b	0.46c	0.13b	0.41d	1.05a
Plt average	0.43	0.21	0.87	0.20	0.69	0.94

Lsd (5%) : Phosphorus=0.1 Tree=0.06 Soil=0.06 Pho*Tree=0.12
Means followed by the same letter under the same column are not significantly different.

Table 3. Effect of phosphorus application on shoot dry matter yield (g) of six tree legumes in Toje soil.

P-rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Albizia lebbeck*	*Albizia zygia*	*Leucaena leucocephala*	*Millitia thonningii*
	Yield (g plant^{-1})					
P_0	0.37c	0.21c	0.66c	0.30b	0.52d	0.81c
P_{30}	0.55ab	0.37b	1.21b	0.32b	0.95b	0.98bc
P_{60}	0.59ab	0.43ab	1.34a	0.33ab	0.99b	1.05b
P_{90}	0.64a	0.51a	1.44a	0.44a	1.41a	1.23a
P_{120}	0.48b	0.19c	0.41d	0.24b	0.76c	1.05b
Plt average	0.53	0.34	1.01	0.33	0.93	1.02

*Lsd (5%) : Phosphorus=0.1 Tree=0.06 Soil=0.06 Pho*Tree=0.12*
Means followed by the same letter under the same column are not significantly different.

soil to 112% for *L. leucocephala* in Hatso soils. Growth response to P application was influenced by soil type with the growth increases being generally higher in Toje soil than in the Hatso soil.

Data presented in Tables 4 and 5 show that, the efficiencies with which the various tree species used their absorbed P for dry matter production varied and was influenced by the species and the available amount of P in the soil. Without exception, the amount of P used to produce a gram of shoot dry matter increased as the P fertilizer rate increased. In addition, the plant genotypes varied considerably in their P use efficiencies, with *M. thonningii* being the most outstanding in its ability to convert P to shoot dry matter.

The effect of nitrogen fertilizer on dry matter yield of four tree and two shrub legumes is presented in Tables 6 and 7. Nitrogen fertilizer application

Table 4. Influence of rates of P applied on the physiological P used efficiency(PPUE) (mg P g $^{-1}$) of six tree legumes in Hatso soil.

P-rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Albizia lebbeck*	*Albizia* zygia	*Leucaena leucocephala*	*Millitia thonningii*	Average
	Yield (mg P g $^{-1}$)						
P_0	18.79	35.08	15.35	38.91	14.85	11.79	22.46
P_{30}	19.46	42.13	15.60	57.47	24.17	18.00	29.47
P_{60}	24.33	52.75	23.64	57.85	27.19	23.10	34.81
P_{90}	26.15	56.65	22.94	61.64	28.83	26.88	37.68
P_{120}	79.92	288.63	73.85	186.63	83.49	28.91	123.57
Plt average	33.73	95.05	30.28	80.50	35.71	21.74	

Table 5 Influence of rates of P applied on the physiological P used used efficiency (PPUE) (mg P g $^{-1}$) of six tree legumes in Toje soil.

P-rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Albizia lebbeck*	*Albizia zygia*	*Leucaena leucocephala*	*Millita thonningii*	Treatment Average
	Yield (mg P g^{-1})						
P_0	18.95	23.86	15.48	20.37	16.05	13.99	18.11
P_{30}	22.25	35.97	16.29	27.91	36.19	18.48	23.68
P_{60}	25.90	38.23	20.98	52.76	29.43	22.26	31.59
P_{90}	31.86	41.22	25.85	52.23	28.98	27.73	34.65
P_{120}	51.96	149.84	100.93	115.86	63.70	36.42	86.45
Plt average	30.18	57.82	35.91	53.83	32.87	23.78	

generally resulted in significant (p=0.05) increases in shoot dry matter yield of all the tree legumes at all treatment levels except *C. ochroleuca* and *C. cajan* which recorded a significant (p=0.05) decrease in dry matter beyond an application of 80 kg N ha^{-1} (Table 6). The effect of the nitrogen fertilizer on dry matter though significant at all treatment levels, decreased with increasing N application in all the soils. Nitrogen application at all treatment levels for the legume species gave significant higher dry matter yields in Toje soil than Hatso soil.

Tables 8 and 9 provide information on the influence of six levels of nitrogen fertilizer on the shoot dry matter yield of four tree and two shrubby legumes in two soil types amended with 90 kg P ha^{-1}. Nitrogen application at all treatment levels except *C. ochroleuca* and *C. cajan* for which a further nitrogen application beyond 120 kg N ha^{-1} resulted in significant (p=0.05) decrease in shoot dry matter in Hatso soil (Table 8) but in Toje soil the decrease occurred beyond 120 kg N ha^{-1} in the case of *C. ochroleuca* and beyond 180 kg N ha^{-1} in the case of *C. cajan* (Table 9). The tree legumes responded variably to nitrogen application in the two soils. For instance, an application of 40 kg N ha^{-1} to *C. cajan* resulted in more than 125% and 142% increases in dry matter in Hatso and Toje soils, respectively and also less than 2% and more than 72% in the case of *M. thonningii* in Hatso and Toje soils, respectively. Comparing the results of the two studies on dry matter yield response of the tree legumes to nitrogen fertilizer on the two soils with or without phosphorus, nitrogen effect on dry matter yield was more pronounced when P was added to the soils than not.

Compared to when only N fertilizer was applied to the soil, the supplementary addition of 90 kg P ha^{-1} produced a synergistic effect on the shoot dry matter yield of all the six plant species examined, in the Hatso (Table 8) as well as the Toje soil (Table 9) and at each of the six N rates. Differences in the stimulation of growth through P supplementation were however more pronounced among the tree species than between the two soils. The data also show that the stimulatory effect of P was at each of the N levels was far more pronounced for the two shrubs, *C. cajan* and *C. ochroleuca* than for the four tree species. Among the tree species themselves, genetic differences were high, with the growth of *M. thonningi* benefitting the least from the extra P addition, especially at rates higher than 40 kg N ha^{-1}.

Although several environmental factors affect nodulation, the absence of nodules on many N_2-fixing legumes has often been attributed to lack of compatible indigenous rhizobia in soil (Danso and

Table 6 Response of shoot dry matter yield of four trees and two shrubby legumes to six rates of nitrogen fertilizer application in Hatso soil.

N-rates Kg ha^{-1}	*Acacia auriculiformis*	*Acacia mangium*	*Cajanus cajan*	*Crotalaria ochroleuca*	*Leucaena leucocephala*	*Mellitia thonningii*
			Yield (g $plant^{-1}$)			
N_0	0.09c	0.11b	0.21bc	0.13bc	0.19b	0.38d
N_{40}	0.10c	0.14ab	0.25b	0.17b	0.25ab	0.44d
N_{80}	0.11c	0.14ab	0.35a	0.26a	0.26a	0.51c
N_{120}	0.15bc	0.16ab	0.34a	0.24a	0.27a	0.64b
N_{180}	0.18a	0.17ab	0.23b	0.14bc	0.27a	0.69b
N_{240}	0.23a	0.20a	0.16c	0.10c	0.30a	0.78a
Plt. average	0.14	0.17	0.26	0.15	0.26	0.57

Lsd (5%) : Treatment = 0.02 Tree = 0.02 Soil = 0.01 Soil*treatment = 0.04 Soil*tree = 0.04 Treatment*tree = 0.06 Soil*treatment*soil = 0.1

Means followed by the same letter under the same column are not significantly different.

Table 7 Response of shoot dry matter yield of four trees and two shrubby legumes to six rates of nitrogen fertilizer application in Toje soil

N-rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Cajanus cajan*	*Crotalaria ochroleuca*	*Leucaena leucocephala*	*Millitia thonningii*
			Yield (g plant $^{-1}$)			
N_0	0.32c	0.23f	0.26b	0.20a	0.38b	0.50d
N_{40}	0.35cd	0.31e	0.29b	0.22a	0.40b	0.62c
N_{80}	0.37bc	0.48d	0.30b	0.23a	0.42b	0.70b
N_{120}	0.42ab	0.55c	0.39a	0.24a	0.43b	1.72ab
N_{180}	0.43ab	0.62b	0.29b	0.19ab	0.52a	1.75ab
N_{240}	0.45a	0.69a	0.16c	0.13b	0.81a	1.78a
Plt. average	0.39	0.48	0.28	0.20	0.43	1.18

Lsd (5%) : Treatment = 0.02 Tree = 0.02 Soil = 0.01 Soil*treatment = 0.04 Soil*tree = 0.04 Treatment*tree = 0.06Soil*treatment*soil = 0.1

Means followed by the same letter under the same column are not significantly different.

Table 8. Effect of six rates of nitrogen fertilizer on shoot dry matter yield (g) of four trees and two shrubby legumes grown in the Hatso soil amended with 90 kg P ha $^{-1}$ phosphorus fertilizer.

P and N rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Cajanus cajan*	*Crotalaria ochroleuca*	*Leucaena leucocephala*	*Mellitia thonningii*
			Yield (g plant^{-1})			
P_{90}+N_0	0.23c	0.25e	1.30e	0.68d	0.48c	0.57d
P_{90}+N_{40}	0.31bc	0.30e	2.93a	1.30b	0.63b	0.58d
P_{90}+N_{80}	0.37b	0.49d	2.94a	1.49a	0.75a	0.61cd
P_{90}+N_{120}	0.43ab	0.93c	2.37b	1.57a	0.79a	0.69bc
P_{90}+N_{180}	0.46ab	1.08b	1.97c	0.86c	0.80a	0.76ab
P_{90}+N_{240}	0.48a	1.19a	1.48d	0.48e	0.84a	0.81a
Plt. average	0.38	0.71	2.17	1.06	0.72	0.67

Lsd (5%) : Treatment = 0.04 Tree = 0.04 Soil = 0.03 Soil*treatment = 0.06 Soil*tree = 0.06 Treatment*tree = 0.1Soil*treatment*soil = 0.14

Means followed by the same letter under the same column are not significantly different.

Table 9 Effect of six rates of nitrogen fertilizer on shoot dry matter yield (g) of four trees and two shrubby legumes grown in the Toje soil amended with 90 kg P ha $^{-1}$ phosphorus fertilizer.

P and N rates Kg ha $^{-1}$	*Acacia auriculiformis*	*Acacia mangium*	*Cajanus cajan*	*Crotalaria ochroleuca*	*Leucaena leucocephala*	*Mellitia thonningii*
			Yield (g plant $^{-1}$)			
P_{90}+N	0.43e	0.46d	0.64e	0.67f	0.55e	0.57d
P_{90}+N_{40}	0.53e	0.54cd	1.55d	1.50e	0.79d	0.98c
P_{90}+N_{80}	0.71d	0.57c	1.67c	1.68d	0.88d	1.46b
P_{90}+N_{120}	0.82c	1.01b	3.02b	2.75a	1.19c	2.00a
P_{90}+N_{180}	0.95b	1.46a	3.28a	2.54b	1.32b	2.03a
P_{90}+N_{240}	1.25a	1.48a	3.20a	2.17c	1.76a	2.05a
Plt. .average	0.78	0.92	2.23	1.89	1.08	1.52

Lsd (5%): Treatment = 0.04 Tree = 0.04 Soil = 0.03 Soil*treatment = 0.06 Soil*tree = 0.06 Treatment*tree = 0.1Soil*treatment*tree= 0.14

Means followed by the same letter under the same column are not significantly different.

Owiredu, 1988). This was not the case in our study; all six tree legume species and the two multipurpose shrubs formed nodules without inoculation in both the Hatso and Toje soils, indicating that rhizobia capable of infecting all eight legume species resided and could possibly be endemic in the two soils. This observation is common for many tropical legumes grown in tropical soils (Dudeja and Khurana, 1989). In all cases however, it is obvious that nodulation in the natural soils was sub-optimal, with significant increases in nodulation resulting from the application of phosphorus to the tree legumes. Many reports have similarly indicated that high phosphorus levels are required for maximum nodulation and nitrogen fixation (Gate and Wilson, 1974; Olofintoye, 1986; Pereira and Bliss, 1987).

However, further increase in P application to 120 kg P ha^{-1} resulted in significant decreases in numbers of nodules formed on all trees, indicating that excessive P was detrimental to nodulation and consequently also to N_2 fixation. This observation is supported by the results of Tsvetkova et al. (2003) who reported decrease in nodule numbers of soybean by up to 35% of control with excess of P supply and a decrease in both nodule fresh weight and dry weight by almost 50% with over-supply of P. Similar observation was also reported by Uddin et al. (2007) for *A. lebbeck* in response to application of P-fertilizer.

Our data also showed that phosphorus fertilization in addition to promoting higher nodulation stimulated higher shoot dry matter production, which again is not surprising given the low-P soils we used. Maximum yields were in almost all cases achieved at 90 kg P ha^{-1}, the optimum for nodulation as well. Danso et al. (1992) also reported the positive effect of phosphorus on nodulation, root proliferation and the overall growth of legumes. However, similar to the detrimental effect of the 120 kg P ha^{-1} application on nodulation, the application of 120 kg P ha^{-1} resulted in decreased plant yield. Our results and those of others like Fletcher and Kurtz (1964) and Kadeba (1978) emphasize that excessive P application to legumes should be avoided, especially as it becomes economically and environmentally counter-productive. Our results for the P un-amended Hatso soil reveal a large genetic heterogeneity for yield, with a tree legume like *M. thonningii* giving about 7 and 3 times higher yield than for *A. mangium* and *A. auriculiformi*, respectively; for the averaged yields for the different P rates, the *M. thonningii* yield was more than 4 and 2 times against the same *A. mangium* and *A. auriculiformis*, respectively. This trend was repeated in the Toje soil too. Besides, *M. thonningii* was far less affected by the super optimal application of P than others like *A. lebbeck*, in both soils.

What is also obvious from this study is, what we would like to term "wastage with abundance of P". Without exception, as the rate of P applied increased, plants used relatively more of the P absorbed at the successive rate to produce an equivalent unit of shoot dry matter. This means the physiological phosphorus use efficiency (PPUE) deteriorated as more and more P fertilizer was supplied, to the point where averaged for all species at the 120 kg P ha^{-1} rate in Hatso soil, the plants required almost 124 mg P or more than 5 times the mean amount of P (about 22 mg P) used to produce a gramme of shoot dry matter at the zero-P rate. For the Toje soil, the equivalent ratio was close to below 5:1. *M. thonningii* once more was far more efficient in its usage of phosphorus in both soils.

The soil factor also played a role in P response, with the generally lower phosphorus response by the tree legumes to nodulation in Toje than the Hatso soil being possibly attributed to the lower pH and available P levels in the Hatso soil (Table 1).

Most reports agree that the effects of N fertilizer application on nodulation and plant growth go in opposite directions (Dixon and Wheeler, 1986; Davidson and Robson, 1986). Our observation that inorganic N fertilizer application resulted in decreased nodulation but increased yield in the tree and non-tree legumes examined is thus in line with this general observation (MacDicken, 1994). The inhibitory effect of nitrogen fertilizer was so strong that even at lowest rate (40 kg N ha^{-1}) significantly inhibited nodulation of the tree legumes. Thus although the inhibitory effect of inorganic N on nodulation and N_2 fixation is supported by the results of Sanginga et al. (1986), Arreseigor et al. (1997) and Solaiman, (1999) who also found the suppression of nodulation in agroforestry crops due to nitrogen fertilizer, it is inconsistent with findings by Afza et al. (1987) and Zahran, (1999) who reported that small doses/ levels of nitrogen fertilizer rather promoted nodulation of some crops.

Nitrogen application to the two soils resulted in significant increases in shoot dry matter yield of the four tree legumes up to the 240 kg N ha^{-1} rate, while for the two shrubs, *C. ochroleuca* and *C. cajan* significant decreases in dry matter occurred beyond 120 kg N ha^{-1}. Andrew (1977) attributed such genetic differences in amounts of N needed for maximum yield to differences in the critical concentrations of N for each genotype. Relative to the control, application of the lowest amount of N (40 kg N ha^{-1}) resulted in the highest increase in dry matter yield, as reported by Hardarson et al. (1984b). The increase in shoot dry matter yield with applied fertilizer nitrogen indicates that none of the tree legumes was able to fix enough N to satisfy its requirement during the period of growth. The superiority of *M. thonningii* was again shown in accumulating the highest amount of N at all N rates and in both soils

Application of basal phosphorus (90 kg P ha^{-1}) in the nitrogen response studies, showed a clear effect of P on the inhibition of nodulation by high levels of soil N. Phosphorus application resulted in mitigation of the negative effect of high N on nodulation of all the tree legumes in both soils. More studies, such as on i) whether this greater tolerance of nodulation to high levels of N is due to enhanced plant growth and consequent higher need for N by the bigger plants resulting from the application of P, or ii) is due to more root development and consequent increase in infection sites as reported by Danso et al., (1992).

With the demonstrated superiority of *M. thonningii* in nodulation, growth and P use efficiency relative to five widely used agroforestry trees and the presence of homologous rhizobia in the two soils studied and greater tolerance of nodulation to inorganic N in soil, we recommend a greater interest and wider examination of the possible unexploited potential of this tree in agroforestry. However, with the limited number of studies performed on this tree species, it is yet premature to conclude that it will similarly nodulate in many other tropical soils until further studies warrant such a generalisation.

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