Rubber Science, **30**(1): 56-65, 2017 RESEARCH ARTICLE

UREASE AND ACID PHOSPHATASE ENZYME MODULATION IN RUBBER PLANTATIONS UNDER THE INFLUENCE OF COVER CROPS AND RUBBER LITTER

Geetha Jose and Mercykutty Joseph

Rubber Research Institute of India, Rubber Board, Kottayam-686 009, Kerala, India

Received: 15 November 2016 Accepted: 17 January 2017

Jose, G. and Joseph, M. (2017). Urease and acid phosphatase enzyme modulation in rubber plantations under the influence of cover crops and rubber litter. *Rubber Science*, **30**(1): 66-75.

Soil urease and phosphatase enzymes play a major role in the mineralization process of organic substrates and their activities were influenced by numerous factors of which soil properties play a key role. The influence of litter from cover crops (*Pueraria phaseoloides* and *Mucuna bracteata*) and rubber (*Hevea brasiliensis*) on the dynamics of urease and acid phosphatase enzymes in the plantation floor of rubber in the traditional region of cultivation was monitored continuously for three years through a field experiment. Pre-calculated quantities of different litter were subjected to natural decomposition in soil and the soil samples were retrieved and activities of urease and phosphatase were monitored twice in a year. The results indicated that both enzyme activities were higher in plots with *Pueraria* than *Mucuna* or rubber. The urease enzyme activity ranged from 77.4 to 179.5 ppm of urea hydrolyzed g^{-1} of soil hr^{-1} and the phosphatase enzyme activity ranged from 230.5 to 482.0 µg of p-nitrophenol released g^{-1} of soil hr^{-1}. Urease maintained significant negative correlations with pH in all the treatments. Positive significant correlations were recorded for urease and acid phosphatase activity with total N in the case of leguminous cover crops.

Key words: Cover crops, Litter degradation, Mineralization, Soil enzymes

INTRODUCTION

Enzymes in a soil system determine to a large extent the biochemical activities and soil quality. Enzymes which originate within the cells of soil organisms or plant roots (endo-cellular) or which accumulate outside them (extra-cellular) regulate the mineralization process (Speir and Ross, 1978). Since these components respond very sensitively to environmental stresses, natural and anthropogenic disturbances *etc.* their levels in soils fluctuate with the soil system. Any healthy soil could necessarily support high content of organic biomass, biological and enzyme activities which support various nutrient cycles to operate (Sajjad *et al.*, 2002). Leguminous cover crops are widely accepted for their contribution to soil quality through biomass addition and enrichment of soil C and N (Fageria *et al.*, 2005). As phosphorus is often the most limited nutrient in tropical soils, inclusion of leguminous cover crop on tropical soils may seem counter-intuitive. Leguminous cover crops influence the soil P forms through the addition of soil organic

Correspondence: Geetha Jose (Email: gjosein@yahoo.co.in)

matter, deep soil mining, and microbial priming (Brady and Weil 1999; Dinesh *et al.*, 2004).

Urease and phosphatase are two major enzymes that are identified in any productive soils which regulate the supply of two major nutrients namely N and P to crops. Urease enzyme in soil originates mainly from plants (Polacco, 1977) and microorganisms and found as both intra and extra-cellular enzymes (Mobley and Hausinger, 1989).

Urease enzyme is basically involved in the hydrolysis of added urea sources to the soil which finally get converted to NH_3 and CO_2 with the concomitant rise in soil pH (Byrnes and Amberger, 1989). It has been seen that urease enzyme extracted from plants or microorganisms rapidly got degraded in soil by proteolytic enzymes. Significant fraction of ureolytic activity in the soil is carried out by extracellular urease, which is protected and stabilized in soil through immobilization on organic and mineral colloids in soil (Pettit *et al.*, 1976; Zantua and Bremner, 1977). Mohammadi (2011) reported that extracellular urease enzyme remained protected in soil through adsorption on clay particles or through encapsulation with humic complexes. Park and Seaton (1996) reported that the activity of urease in soil was very much related to the availability of organic sources in that soil.

Phosphatase is a broad group of enzymes that hydrolyzes esters and anhydrides of phosphoric acid. Soil phosphatase enzymes play critical and major roles in the mineralization process of organic P sources (Speir and Ross, 1978; Tabatabai, 1994). According to Tarafdar and Marschner (1994), phosphatase enzyme may originate from the plant roots, associated mycorrhiza or other fungi or from bacteria. Tarafdar and Junk (1988) observed that acid and alkaline phosphatase activities were higher in the rhizosphere and particularly near the root surface. Rubber growing soils are acidic and the organic P status of the rubber growing soils ranges from 64 to 86 per cent of the total P (Prasannakumari *et al.*, 2008; Joseph, 2016). Harrison (1983) observed a positive relationship between phosphatase and organic matter content since the enzyme was mostly seen associated with humic-protein complex. Baligar *et al.* (1998) observed a decline in phosphatase activity with increasing sampling depth which they attributed to low organic matter content at lower depth. Activities of these enzymes in rubber plantation, with the protection of its plantation floor with leguminous cover crops or natural litter fall monitored for understanding the possible enzyme dynamics and its relationship with N and P availability.

MATERIALS AND METHODS

A field experiment was conducted in Travancore Rubber Estate, Erumely in Pathanamthitta district which represents a typical and traditional rubber growing tract in Central Kerala. The experimental area (latitude 9° 27′ N and longitude 76° 52′ E) had a mean annual rainfall of 338 cm with tropical humid climate, sandy clay texture with the soil Ustic Haplohumults. Three-year-old rubber plantations were selected which had exclusive establishment of leguminous cover crops *Mucuna bracteata* (T_1-field with *Mucuna* litter) and *Pueraria phaseoloides* (T_2-field with *Pueraria* litter). Adjacent to these plots, another plantation with 12-year-old rubber trees with natural cover was also selected to assess the impact of rubber leaf litter (T_3-field with rubber litter). The experiment was conducted for

three years in the same location. The average quantity of leaf litter from 10 different locations in each plot was computed for calculating the litter inputs every year particularly during February when maximum leaf fall occurs. During February, the pre-calculated quantities of dry leaf litter were placed in the designated area of one square meter plots in the field on a net laid on the ground after clearing and levelling the specified area to permit exclusive and natural degradation of added litter in soil. Further, litter fall from any other source to these specific treatment areas was prevented totally by spreading another bigger net over these sites at a height of one foot above.

Soil samples (0-15 cm depth) were collected from each treatment twice a year (in February and October) continuously for a period of three years. Soil samples were dried in shade, sieved through two mm sieve and analysed for different soil parameters. The pH of the soil samples was measured in a suspension of soil in water (1:2.5 ratio) with a pH meter (Model- ELICO- LI-612). Organic carbon content of the soil samples was determined by the wet digestion techniques of Walkley and Black (1934), as described by Jackson (1973). Total N in soil was determined by modified micro Kjeldahl method (Jackson, 1973). Available P in soil was extracted using Bray No. II reagent (Bray and Kurtz, 1945) and was determined colorimetrically by the chlorostannous reduced molybdophosphoric blue colour method in hydrochloric acid medium.

For enzyme assay, soil samples were collected during pre-monsoon (February) and post-monsoon (October) and the samples were maintained at soil moisture levels under 50 per cent field capacity. The urease activity in soil was determined by the standard procedures as outlined by Broadbent *et al.* (1964). The acid phosphatase activity in soil was determined by following the procedure described by Eivazi and Tabatabai (1977).

RESULTS AND DISCUSSION

The analytical results of soil samples collected from the experimental field with respect to pH is presented in Table 1. A close scrutiny of the data on soil pH indicated that different treatments induced variations in pH values. Though pH values were significantly influenced by treatments during the first observation, the same effect was not seen during the second and third year of the study. The mean values ranged from 4.2 to 5.4 indicating an acidic soil reaction. In general, it was seen that *Pueraria* had significantly lowered the pH of soil in all the seasons when compared to other treatment counterparts. The observed variations in pH may be attributed to the difference in the relative efficacy of the added organic matter in controlling the

Table 1. **Effect of litter sources on soil pH**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	5.1	5.3	4.7	4.9	4.8	4.7
Pueraria	4.8	5.1	4.7	4.7	4.7	4.2
Hevea	5.1	5.4	4.8	4.8	4.8	4.7
CD (P=0.05)	0.1	0.1	NS	0.1	NS	0.2

Table 2. **Effect of litter sources on organic carbon content (%)**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	1.6	2.1	2.2	1.8	1.8	1.7
Pueraria	2.5	2.5	2.6	2.1	2.5	2.1
Hevea	2.6	2.6	2.6	2.4	2.4	2.3
CD (P=0.05)	0.2	0.4	0.2	0.3	0.3	0.2

protonation-deprotonation mechanisms leading to the varied activity of hydrogen ions in the soil. Further, the efficacy of organic envelop derived from different cover crops might have favoured as a decisive factor in chelating active iron and aluminium ions in soil which ultimately determined the hydrogen ion activity (Silva *et al.*, 2000). Nature of the cover crop, quantity of organic matter, its rate of degradation and climatic parameters which persisted during the experimental period ultimately might have determined the soil reaction. The present observation is in conformity with the observations reported by Martinez and Tabatabai (2000). Pooled analysis of the data on pH (Table 5) also indicated significant effect of treatments.

Organic carbon values recorded from different treatments remained significant on account of the effect of various treatment applications (Table 2). Highest mean value of 2.6 per cent was registered from rubber litter. Pooled analysis also showed similar trend with the highest value of 2.5 per cent from rubber (Table 5). The higher organic carbon content noted in rubber fields may be attributed to the slow decomposition rates of rubber leaves. Thus, the quantity of organically active phase derived from rubber leaf litter is higher than the same obtained from cover crops. Another possible reason that could be attributed to the existence of variation in organic carbon content in soil could be the variations in rhizosphere activity, variations in the root exudations and associated involvement of different microbial population in the degradation process (Vitousek *et al.*, 2002). Specific substrate preference has been reported for many microorganisms involved in the degradation process (Girisha *et al.*, 2003) also might have resulted in variations in organic carbon content noted in plots receiving organic inputs. Among the cover crops, *Pueraria* was found to contribute more

Table 3. **Effect of litter sources on total nitrogen content (%)**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	0.24	0.18	0.25	0.24	0.24	0.26
Pueraria	0.31	0.23	0.30	0.25	0.25	0.31
Hevea	0.36	0.23	0.28	0.27	0.32	0.30
CD (P=0.05)	0.03	0.04	0.03	0.02	0.03	0.03

Table 4. **Effect of litter sources on available phosphorous content (mg kg^{-1})**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	24.2	30.1	36.7	22.5	19.3	30.4
Pueraria	30.8	59.0	27.7	26.6	41.3	30.1
Hevea	32.9	30.9	35.5	14.2	33.3	21.6
CD (P=0.05)	NS	NS	NS	NS	10.3	NS

organic carbon than *Mucuna*. In the pooled analysis (Table 5) also same trend was observed. The possible reason for this observation might be the relative ease with which *Pueraria* undergoes decomposition in the field compared to *Mucuna* as reported by Philip and Abraham (2009).

The total N content varied significantly between treatments during each sampling period (Table 3). Pooled analysis of the data on total N (Table 5) also indicated highly significant effect of treatments. The mean values ranged from 0.18 to 0.36 per cent. The highest value (0.36%) was recorded for rubber during February in the first year. Highly significant effect of treatments observed with respect to total N could be attributed to the variations in the mineralization of organic N from organic sources particularly from cover crops and rubber leaf litter. The observed variation could be justified by the reports of Orimoloye *et al.* (2010) wherein they indicated that in rubber plantations, N could easily be lost through several processes in the soil leading to spatial variations in N levels. Fatondji *et al.* (2009) reported that when organic amendments were applied to the field, prior to the rainy season, nutrient release rate strongly exceeded plant nutrient uptake, which finally led to leaching losses especially for N and to a lesser extent for K. This also directly justifies the reason for the observed higher total N content in rubber soils where slow decomposition and gradual build up of soil N might have occurred compared to a situation where faster decomposition and substantial loss of N was likely from easily decomposable *Mucuna* and *Pueraria* added plots. On exclusive consideration of cover crops for addition of N in soils, the highest contribution of soil total N was observed in the case of *Pueraria*.

Table 4 provides data on available P which ranged from 14.2 to 59.0 mg kg^{-1}. Not much of difference could be seen in the available P status between treatments for both individual and pooled analysis (Table 5). The highest value of 59.0 mg kg^{-1} was noted in *Pueraria* during the post-monsoon of the first year and the lowest value of 14.2 mg kg^{-1} was noted from rubber during the fourth observation period. Phosphatase activity observed was significant both in individual and pooled analysis and was found to be

Table 5. **Pooled analysis of the effect of litter sources on pH, OC, total nitrogen and available phosphorous**

Litter sources	pH	organic carbon (%)	Total N (%)	Available P (mg kg^{-1})
Mucuna	4.91	1.88	0.24	27.1
Pueraria	4.69	2.38	0.28	35.9
Hevea	4.92	2.47	0.29	28.0
CD (P=0.05)	0.07	0.19	0.02	NS

the highest for *Pueraria* during the post-monsoon sampling in the first year. No consistent results could be obtained in general with respect to P. Perhaps, this could be attributed to the faster decomposition and subsequent utilization of the released P by plants or through losses which might have occurred through fixation process (Fatondji *et al.*, 2009). The release of P is mainly dependent on the rate of decomposition and easiness with which the added litter disappears from soil. In the case of rubber, though litter addition was substantial, the slow rate of decomposition and occurrence of P as a structural constituent of the tissues might have resulted in a slow release of P to the soil system leading to low available P. This is indirectly evident from the highly significant phosphatase activity observed in all the plots.

Urease activity noted from the experimental site at periodic interval over a period of three years is presented in Table 6. The highest mean value of 179.5 ppm of urea hydrolyzed g^{-1} of soil hr^{-1} was noted for *Pueraria* in the last phase of observation in the third year while its corresponding pre-treatment values in February in the first year was 77.4 ppm of urea hydrolyzed g^{-1} of soil hr^{-1}. The seasonal effect was not pronounced in the last observations invariably in plots receiving cover crops and these plots registered higher values than rubber. This can be attributed to the relatively narrow C:N ratio and a higher percentage of easily degradable fractions present in cover crop litter (Jose, 2014). Palm and Sanchez (1991) made exactly similar observations while comparing the decomposition pattern of grasses and leguminous cover crops.

Ureolytic microorganisms and urease activity were influenced mostly by higher organic matter, moisture regime, and total N content and dissolved ionic concentration of soil (Shan *et al.*, 2008). The highest activities have been noted in summer compared to winter in the prescuit study. Excessive moisture or low temperature might have been the cause for the noted reduction of the urease activity in the post-monsoon season. Fenn *et al.* (1992) reported enhanced enzyme activity in surface soil with an increase in organic matter. Further, the observations made by Cepeda *et al.* (2007) reaffirms that the enzyme activity always followed an increase with enhancement in organic matter content, but the activity gets altered with the type of land use and vegetative cover and thus substantiating the enrichment of urease activity in agricultural and forest soil. Thus, the higher activity of urease noted in the case of plots receiving litter from cover crops, *Mucuna* and *Pueraria* is well justified. Root exudates of the cover crop coupled with the microclimate available in the soil

Table 6. **Effect of litter sources on urease activity (ppm of urea g^{-1} soil hr^{-1})**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	89.1	63.9	81.4	64.6	117.6	110.4
Pueraria	77.4	51.6	55.9	70.9	115.7	179.5
Hevea	20.4	33.2	48.6	73.7	118.1	38.6
CD (P=0.05)	15.6	16.1	10.7	NS	NS	26.2

Table 7. **Effect of litter sources on acid phosphatase activity (µg of p-nitrophenol g^{-1} soil hr^{-1})**

Litter sources	Period of observation					
	First year		Second year		Third year	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Mucuna	367.5	268.5	387.0	374.5	300.0	306.0
Pueraria	482.0	230.5	361.0	447.0	344.0	370.0
Hevea	456.0	383.5	305.5	368.0	365.0	404.0
CD (P=0.05)	39.5	84.6	41.3	40.1	48.62	40.0

surface might have promoted a higher ureolytic microbial population and activity (Palm and Sanchez, 1991).

Data on phosphatase activity observed from different treatments in the experiment is provided in Table 7. From the data it is clear that, there were fluctuations in the enzyme levels during the experimental period. Compared to pre-treatment levels, the enzyme levels were lower in the second phase of sampling. Subsequently in the next two sampling periods, the enzyme levels generally increased in all the treatments except in rubber. Mean values ranged from 230.5 to 482.0 µg of p-nitrophenol released g^{-1} of soil hr^{-1} and the highest enzyme activity was recorded by *Pueraria* during February in the first year. The significant and higher levels of phosphatase enzyme activity noted in all treatments might be due to the variations in the chemical composition of the accumulated litter and associated build up of microbial population coupled with the possible protection of the enzyme from getting denatured or destroyed through adsorption and stabilization mechanisms brought about by fairly higher levels of organic colloids (Nannipieri *et al.*, 1990) at the sampling sites. In this context, the observations of Girisha *et al.* (2003) highlighting the need and importance of substrate specificity in promoting enzyme activity is relevant. Thus it is more or less evident that the source of organic input is irrelevant and insignificant in ushering the phosphatase activity.

Correlation analysis between enzyme activities and soil parameters (Table 8) indicated positive significant correlations between both urease and acid phosphatase activity with total N in the case of leguminous litter addition. Urease enzyme maintained significant negative correlations with pH in all the treatments. Phosphatase

Table 8. **Correlation between enzyme activities and soil parameters**

Litter sources	Enzyme	Soil chemical parameters			
		pH	Organic carbon	Total N	Available P
Mucuna	Urease	-0.35 *	-0.13	0.30 *	-0.05
	Phosphatase	-0.20	0.17	0.47 *	-0.17
Pueraria	Urease	-0.72 *	-0.21	0.29 *	-0.14
	Phosphatase	-0.29 *	-0.05	0.41 *	-0.36 *
Hevea	Urease	-0.35 *	-0.14	0.01	-0.10
	Phosphatase	0.14	0.15	0.23	0.09

registered negative correlations with pH and available P in the leguminous litter addition but was significant only for *Pueraria*.

CONCLUSION

The study concluded that the leguminous cover crop litter underwent faster decomposition than rubber litter and *Pueraria* decomposed faster than *Mucuna*. Among the cover crops, *Pueraria* contributed more organic carbon than *Mucuna*. On exclusive consideration of cover crops for addition of N in soils, the highest contribution of soil total N was observed in the case of *Pueraria*. Available P content was also found to be higher in *Pueraria* litter. It was observed that soil pH was lower in *Pueraria* compared to *Mucuna* and rubber litter addition. Urease enzyme activity was found to be higher in *Pueraria* treated plots. Urease enzyme had significant positive correlation with total N in the case of two leguminous cover crops and not with rubber litter. Urease enzyme maintained significant negative correlations with pH in all the treatments.

A higher level of acid phosphatase enzyme activity was observed in *Pueraria* litter addition. Phosphatase registered negative correlations with pH and available P in the leguminous litter addition but was significant only for *Pueraria*. However, it was noted that this enzyme had significant positive correlation with total N in the case of leguminous litter addition.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R. Kothandaraman, Deputy Director (Rtd.), Plant Pathology Division, Dr. C. Kuruvilla Jacob, Director of Training (Rtd.), Dr. Jacob Mathew, Joint Director (Rtd.), Sri. Sabu P. Idicula, Joint Director (Crop Protection) Plant Pathology Division, Dr. Joshua Abraham, Scientist C, Agronomy/Soils for extending help and support during this investigation. Thanks are due to Sri. Remesh B. Nair, Joint Director (S&P) for the valuable advice and help in statistical analysis of the data.

REFERENCES

Baligar, V.C., Wright, R.J. and Smedley, M.D. (1998). Acid phosphatase activity in soils of the Appalachian region. *Soil Science Society of America Journal*, **52**: 1612-1616.

Brady, N.C. and Weil, R.R. (1999). *The Nature and Properties of Soils*. Prentice-Hall, Inc. 881 p.

Bray R.H. and Kurtz, L.T. (1945). Determination of total organic and available forms of phosphorous in soils. *Soil Science*, **59**: 39-45.

Broadbent, F.E., Hill, G.N. and Tyler, K.B. (1964). Transformation and movement of urea in soils. *Soil Science Society of America Proceedings*, **22**: 303-307.

Byrnes, B.H. and Amberger, A. (1989). Fate of broadcast urea in a flooded soil when treated with N-(n-butyl) thiophosphoric triamide, a urease inhibitor. *Fertilizer Research*, **18**: 221-231.

Cepeda, C.T., Sotres, F.G. and Leiros, M.C. (2007). Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biology and Biochemistry*, **39**(1): 311-319.

Dinesh, R., Suryanarayana, M. A., Chaudhuri, S. G. and Sheeja, T. E. (2004). Long-term influence of leguminous cover crops on the biochemical properties of sandy clay loam Fluventic Sulfaquent in a humid tropical region of India. *Soil and Tillage Research*, **77**: 69-77.

Eivazi, P. and Tabatabai, M.A. (1977). Phosphatase in soil. *Soil Biology and Biochemistry*, **9**: 167-172.

Fageria, N.K., Baligar, V.C. and Bailey, B.A. (2005). Role of cover crops in improving soil and row

crop productivity. *Communications in Soil Science and Plant Analysis*, **36**(19&20): 2733-2757.

Fatondji, D., Martius, C., Zougmore, R., Vlek, L.G., Bielders, C.L. and Koala, S. (2009). Decomposition of organic amendment and nutrient release under the zai technique in the Sahel. *Nutrient Cycling in Agro ecosystems*, **85**: 225-239.

Fenn, L. B., Tipton, J. L. and Tatum, G. (1992). Urease activity in two cultivated and non-cultivated acid soils. *Biology and Fertility of Soils*, **13**(3): 152-154.

Girisha, G.K., Condron, L.M., Clinton, P.W. and Davis, M.R. (2003). Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. *Forest Ecology and Management*, **179**(1&3): 169-181.

Harrison, A. F. (1983). Relationship between intensity of phosphatase activity and physico-chemical properties in woodland soils. *Soil Biology and Biochemistry*, **15**: 93-99.

Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice Hall of India Private Ltd., New Delhi. 498 p.

Jose, G. (2014). *Studies on soil enzymes and available nutrients in relation to litter decomposition in rubber plantations*. Ph.D. Thesis, Mahatma Gandhi University, Kottayam, Kerala, India, 116 p.

Joseph, M. (2016). Rubber growing soils of India: An overview. *Rubber Science*, **29**(2): 119-139.

Martinez, V. and Tabatabai, M.A. (2000). Enzyme activities in limed agricultural soil. *Biology and Fertility of Soils*, **31**: 85-91.

Mobley, H.L. and Hausinger, R.P. (1989). Microbial ureases: significance, regulation and molecular characterization. *Microbiological Reviews*, **53** (1): 85-108.

Mohammadi, K. (2011). Effect of different fertilization methods on soil biological indexes. *Engineering and Technology*, **78**: 407-410.

Nannipieri, P., Grego, S. and Ceccanli, B. (1990). Ecological significance of biological activity in soil. In: *Soil Biochemistry*. Vol. 6. (Eds. Bollag J.M. and Stotzky G.). Marcel Dekker, New York, pp. 293-355.

Orimoloye, J.R., Ugwa, I.K. and Idoko, S.O. (2010). Soil management strategies for rubber cultivation in an undulating topography of Northern Cross River State. *Journal of Soil Science and Environmental Management*, **1**(2): 34-39.

Palm, C.A. and Sanchez, P.A. (1991). Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biology and Biochemistry*, **23**: 83-88.

Park, J. and Seaton, R.A.F. (1996). Integrative research and sustainable agriculture. *Agriculture Systems*, **50**: 81-100.

Pettit, N.M., Smith, A.R.J., Freedman, R.B. and Burns, R.G. (1976). Soil urease: Activity, stability and kinetic properties. *Soil Biology and Biochemistry*, **8**: 479-484.

Philip, A. and Abraham, J. (2009). Litter chemistry and decomposition in rubber plantations. *Natural Rubber Research*, **22**(1&2): 10-16.

Polacco, J.C. (1977). Nitrogen metabolism in soybean tissue culture. II. Urea utilization and urease synthesis require Ni^{2+}. *Plant Physiology*, 59: 827-830.

Prasannakumari, P., Joseph, M. and Nair, N.U. (2008). Organic phosphorous status of the major soil series under rubber cultivation in South India. *Natural Rubber Research*, **21**(1&2): 98-103.

Sajjad, M.H., Lodhi, A. and Azam, F. (2002). Changes in enzyme activity during the decomposition of plant residues in soil. *Pakistan Journal of Biological Sciences*, **5**(9): 952-955.

Shan, Q., Yu, Y., Yu, J. and Zhang, J. (2008). Soil enzyme activities and their indication for fertility of urban forest soil. *Frontiers in Environmental Science and Engineering*, **2**(2): 218-223.

Silva, I.R., Smyth, T.J., Moxley, D.F., Carter, T.E., Allen, N.S. and Rufty, T.W. (2000). Aluminum accumulation at nuclei of cells in the root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy. *Plant Physiology*, **123**: 543-552.

Speir, T.W. and Ross, D.J. (1978). Soil phosphatase and sulphatase. In: *Soil Enzymes*. Burns R.G. (Ed). Academic Press, London, UK, pp. 197-250.

Tabatabai, M.A. (1994). Soil enzymes. In: *Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties*. (Eds. Weaver *et al.*,). Soil Science Society of America Book Series No. 5, Madison, pp. 775-833.

Tarafdar, J.C. and Junk, A. (1988). Influence of humic substances on growth and physiological processes. *Biology and Fertility of Soils*, **3**: 199-204.

Tarafdar, J.C., and Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry*, **26**: 387-395.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B. and Sprent, J.I. (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry*, **57/58**: 1-45.

Walkley, A. and Black, I.A. (1934). An examination of Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, **63**: 251-263.

Zantua, M.I. and Bremner, J.M. (1977). Stability of urease in soils. *Soil Biology and Biochemistry*, **9**: 135-140.