Litter from different sources influences the nutrient availability, biological and enzyme activity in the rubber growing soils in Kerala, India

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Abstract

Plants and microorganisms are key players within the soil ecosystem and are responsible for many important soil cycling processes, such as carbon mobilization and nitrogen mineralization. Since rubber (*Hevea brasiliensis*) plantations are deciduous in nature and most of the plantation floors have either a leguminous cover crop of *Mucuna bracteata* or *Pueraria phaseoloides* and they contribute a lot to the soil biomass. Soil cellulase, dehydrogenase and nitrogenase activity is an important aspect to estimate soil biological properties as it acts as a biological indicator towards soil fertility. The specific influence of litter addition on the dynamics of cellulase, dehydrogenase and nitrogenase enzymes in the plantation floor when monitored continuously for three years though a field experiment indicated that cellulase enzyme values ranged from 38.35 to 99.85 µg $g^{-1}$of glucose hydrolyzed g^{-1} of soil 24 h^{-1} and dehydrogenase enzyme level ranged from 61.70 to 293.37 µg of TPF hydrolyzed g^{-1} of soil 24 h^{-1} and the nitrogenase enzyme values ranged from 13.76 to 498.07 µ moles of ethylene produced g^{-1} of soil h^{-1}. Among the three litter sources, *Pueraria* significantly lowered the pH and was brought to the extremely acidic range and coinciding with that reduced availability of K, Ca and Mg was recorded with *Pueraria* treatment. Among the soil properties, effect of pH on enzyme activity was more pronounced and cellulase exhibited positive correlation and dehydrogenase and nitrogenase exhibited negative correlation with soil pH. *Mucuna* litter addition was capable of enhancing the bacteria, fungi and actinomycetes populations in soil compared to *Pueraria* and rubber litter.

Keywords: *Hevea brasiliensis*, Leguminous cover crops, Litter degradation, Microbial population, Mineralization, Soil enzymes.

Introduction

Soil enzymes play a substantial role in maintaining soil health and its environment and enhance the reaction rate at which plant residues decompose and release plant available nutrients. Soil enzymes regulate ecosystem functions and play a critical role in nutrient cycling (Makoi and Ndakidemi, 2010). Enzymes in the soil are mainly of microbial origin and main sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Microorganisms play key roles in nutrient cycling (Salazar et al., 2011) structure formation, organic matter decomposition, N fixation, and toxin removal (Gomez et al., 2003; Acosta-Martinez et al., 2010). Soil microorganisms are sensitive to environmental stress and are thus useful indicators of soil quality and soil health (Doran and Zeiss, 2000; Salazar et al., 2011). Usually, enzymes are specific to a substrate and have active sites that bind with the substrate to form a temporary complex from where they perform many biochemical processes that results in the attendant stabilization of soil structure, decomposition of organic wastes, organic matter formation, and mineralization of nutrient contained in the substrate

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and ultimately help in nutrient cycling. Rubber plantations are one of the ideally suited areas where many kinds of enzymatic reactions can be thought of in view of the deciduous nature of rubber trees and rich accumulation of biomass at the plantation floor. 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). But it is to be noted that not much detailed study had gone into comparing the influence of rubber litter and accumulated litter from common leguminous cover crops like *Mucuna* or *Pueraria* in modulating the microbial flora and more specifically the enzyme activities of cellulase, dehydrogenase and nitrogenase in the litter beds particularly in a traditional rubber tract with tropical humid climate and sandy clay loam texture. The study assumes importance when all the litters under study offer quite different leaf chemistry and degradation pattern.

Cellulase is a group of extra cellular enzymes which hydrolyze the insoluble cellulose polymers into soluble sugars and act as primary agents of organic matter degradation. Cellulase enzyme activities can be more influenced by type of organic matter than the quantity of organic matter (Pancholy and Rice, 1973). Kanzawa and Miyashita (1987) reported that the activity of cellulase decreased with increasing soil depth, even though the soils were rich in humus. Burns (1982) reported that the major portion of cellulase enzyme was bound to and protected by soil colloids.

Dehydrogenase is considered as one of the best indicators of microbial activity since it occurs only within living cells, unlike other enzymes which are mostly extra cellular (Burns, 1978). This enzyme exists as an integral part of intact cells and is considered to be an essential component of the enzyme systems of all microorganisms. Being a respiratory chain enzyme, they play a major role in the energy production of microorganisms. Dehydrogenase plays an essential role in the initial stages of the oxidation of soil organic matter (Ross, 1971) by transferring hydrogen and electrons from substrates to acceptors. These processes, being a part of respiration pathways of soil microorganisms, are also closely related to the availability of soil air and moisture and hence seasonal influences are reflected in this enzyme activity (Glinski and Stepniewski, 1985). Hence, dehydrogenase activity can be relied as an indicator of redox systems operating in soil. Doran (1990) reported that various tillage practices affected the microbial biomass and resulted in attendant decrease in dehydrogenase activity. Baligar et al. (1997) reported that the major soil factors that regulate the activity of the enzyme were, content of moisture, quantum of OC, total N, soil texture, forms of P and S, CEC besides existing ratio of Mg to Ca in the soil. According to Cooper and Warman (1997) the application of well rotten compost was found to enhance the dehydrogenase activity in a silty clay soil when compared with the independent application of either other manures or fertilizers. Fraser et al. (1998) observed that in a sandy loam soil, increasing plant cover which indirectly provided greater amount of litter for incorporation into the soil supported higher dehydrogenase activity and they indicated that it was more linked with the levels of organic carbon in soils.

In any N fixation mechanism, the reduction of molecular N to NH_3 is catalyzed by the nitrogenase enzyme. Nitrogenase is extremely sensitive to the presence of oxygen. Leghaemoglobin binds to oxygen and facilitate oxygen free areas within the roots of plants where the bacterial nitrogenase can become active. Roper et al. (1994) reported that the application of nitrogenous fertilizer depressed nitrogenase activity and that cultivation encouraged its activity compared to the zero tillage conditions. Seneviratne and Jayasinghearachchi (2005) identified that the presence of low sulphate ions concentration in the soil favored better nitrogenase activity. This paper provides an insight into the dedicated role of different naturally added litter sources in the rubber growing soils of Kerala and associated nutrient bioavailability and three major

enzyme dynamics.

Materials and Methods

A field experiment 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 90 27' N and longitude 760 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). 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 (OC) content of the soil samples was determined by the wet digestion techniques of Walkley and Black (1934), as described by Jackson (1973). Total nitrogen (N) in soil was determined by modified micro Kjeldahl method (Jackson, 1973). Available phosphorus (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. Available potassium (K) in soil was extracted with neutral normal ammonium acetate (1:5 ratio) and the content in the leachate was estimated using a flame photometer (Model-Systronics) (Jackson, 1973). Available calcium (Ca) and available magnesium (Mg) in soil was extracted using ammonium acetate as extractant and is determined using Atomic Absorption Spectrophotometer (Piper, 1970). The clay content was determined by the hydrometer method as suggested by Gupta and Dakshinamoorthy (1980). The cation exchange capacity (CEC) was estimated using neutral normal ammonium acetate (Black, 1965).

Soil samples were collected during pre-monsoon (February) and post-monsoon (October) and maintained at soil moisture levels under 50 per cent field capacity and used for the soil enzyme assay and microbiological analysis. The cellulase activity was estimated according to the method described by Pancholy and Rice (1973). The dehydrogenase activity was assayed as per the method outlined by Casida et al. (1964). The nitrogenase activity was estimated according to the method described by Turner and Gibson (1980). Serial dilution and plate count techniques were employed to enumerate micro flora of soil (Timonin, 1940). Correlations between enzyme and soil properties were calculated as per Panse and Sukhatme (1967).

Results and Discussion

The effect of litter sources on pH and availability of nutrients during the third year both at the pre-monsoon and post-monsoon sampling is presented in Table 1. The pH values ranged from 4.19 to 4.83 indicating an acidic soil reaction. It is seen that *Pueraria* had significantly lowered the pH of soil

during the post-monsoon sampling 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 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 favored as a decisive factor in chelating active Fe and Al ions in soil which ultimately determined the hydrogen ion activity. 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). The available P was not influenced by treatments (Table 1). This could be attributed to the faster decomposition and subsequent utilization of the released P by plants or through fixation process (Fatondji et al., 2009). Significant difference due to treatment effect was recorded on the availability of K. Higher available K was recorded in the pre-monsoon sampling compared to the post-monsoon sampling. Among the litter sources, significantly high K availability was recorded by rubber litter during the pre-monsoon sampling. However, during the second sampling the values were on par between rubber litter and *Mucuna* litter and was significantly lower with *Pueraria* litter. High K availability recorded in the pre-monsoon sampling in rubber litter and *Mucuna* litter may be due to the slow decomposition rate of these two compared to the *Pueraria* litter. However, in the second sampling, the K availability was on par between rubber and *Mucuna* litter and was significantly lower with *Pueraria* litter addition. Among the three litter sources, *Pueraria* litter might have decomposed more quickly and the cation content of the leaves was reported to be low compared to *Mucuna* litter (Philip et al., 2005; Philip and Abraham, 2009) which might be the reason for low K availability in *Pueraria* treatment. The K ions liberated though the decomposition of litter might have leached and removed from the surface soil during the heavy rainfall resulted in low K availability during the post monsoon sampling.

The available Ca content indicated that there was significant difference between *Pueraria* and others in the first season sampling (Table 1). In the second season, *Mucuna* registered high value compared to rubber and *Pueraria* litter. Though there is significant difference in the case of available Ca content in soil due to application of cover crops, no specific trend could be discerned. This might be due to mobilization and re-absorption of Ca by roots. Greggio et al. (2008) endorsed this view and stated that factors like heat, moisture and action of decomposing agents favored the release of chemical elements contained in the litter mass which could get subsequently mobilized and re-absorbed by roots. The Ca content was observed to be significantly high in rubber and *Mucuna* in the first sampling which might mainly be attributed to the higher Ca content of the litter derived from *Mucuna* and rubber, respectively. In the post-monsoon sampling, significantly higher available Ca was recorded by *Mucuna* compared to the two other sources which were on par with each other. The innate higher content of Ca in *Mucuna* and its faster

Table 1. Effect of litter sources on soil pH and available nutrients

Litter sources	pH		Available nutrients (mg kg^{-1})							
			P		K		Ca		Mg	
	I	II	I	II	I	II	I	II	I	II
Mucuna	4.75	4.70	19.3	30.4	129.5	123.0	163.9	164.7	33.7	35.1
Pueraria	4.68	4.19	41.3	30.1	151.0	107.5	88.9	101.4	19.4	23.3
Rubber	4.83	4.70	33.3	21.6	194.5	122.5	193.7	117.1	54.3	26.9
CD (P=0.05)	NS	0.17	10.3	NS	27.8	12.8	56.9	28.4	8.5	4.7

I. Pre-monsoon season II. Post-monsoon season

decomposition in soil perhaps might have supported enhanced levels of Ca status in *Mucuna* treated plots. Regina and Tarazona (2001) reported that greater source of residues and chemical elements in soils come from the organic matter decomposition of leaf litter that gets accumulated on the soil surface.

The Mg content in plots differed significantly (Table 1) due to treatment effects. In the first sampling, difference with the litter source was very wide with very high value recorded by rubber litter. In the second season, significantly high value was recorded by *Mucuna* litter and between *Pueraria* and rubber litter the values were on par. In the case of cover crop treated plots, as time advanced, the Mg status increased. The higher values of Mg observed in the case of litter derived from *Mucuna* could be attributed to the comparatively high content of Mg in *Mucuna* litter.

Organic carbon values (Table 2) remained highly significant on account of the effect of various treatment applications. Highest mean value of 2.63 per cent was registered from rubber and was on par with *Pueraria* litter (2.48 per cent). The existence of variation in OC 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 and Turner, 1994). 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 OC compared to *Mucuna*.

The total N content varied significantly between treatments during each sampling period (Table 2). 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 lead 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 litter treatment where slow decomposition and gradual buildup of soil N might have occurred compared to a situation. On exclusive consideration of cover crops for addition of N in soils, highest contribution of soil total N was observed in the case of *Pueraria*.

Cation exchange capacity values (Table 2) indicated that all treatments had significantly influenced the buildup of CEC in the pre-monsoon sampling with rubber litter registering the highest values. During the post-monsoon sampling *Pueraria* and rubber litter additions registered on par values. The highest mean value of 11.82 cmol (p+) kg^{-1} was recorded by rubber. The first sampling was significantly superior in rubber litter treatment compared to *Pueraria*. However, in the second sampling, significantly high values were recorded by *Pueraria* and rubber litter treatment and were on par. The variations in the nature and content of organic

Table 2. Effect of litter sources on soil properties

Litter sources	OC (%)		Total N (%)		CEC (cmol(p+) kg^{-1})		Clay content (%)	
	I	II	I	II	I	II	I	II
Mucuna	1.78	1.74	0.24	0.26	7.67	8.33	33.05	32.38
Pueraria	2.48	2.12	0.25	0.30	10.69	10.91	32.95	34.40
Rubber	2.63	2.25	0.32	0.31	11.66	11.82	35.22	35.50
CD (P=0.05)	0.27	0.24	0.03	0.03	0.58	1.20	2.24	1.89

I. Pre-monsoon season II. Post-monsoon season

colloids derived from the litter sources and their interaction with inorganic soil components forming similar exchange complex with varying ion exchange properties might be the reasons for the observed variations in CEC values. Similar effects have been reported by Suvannang et al. (2010) in a study on the biomass production and decomposition pattern of two cover crop residues in a rubber tree plantation in Thailand.

The clay content (Table 2) during the first season was on par between *Mucuna* and rubber litter additions and was on par between *Pueraria* and *Mucuna* litter during the second sampling season indicative of the influence of litter addition on retaining the inorganic colloidal fraction irrespective of the litter sources and preventing them from leaching to lower layers. The strong and stable clay-humus complexes so formed might have effectively retained the finer inorganic fractions in the upper layer from leaching justifying the observations. Bandick and Dick (1999) corroborated such a contention and further indicated that these complexes, promoted the presence of microbial communities in their vicinity leading to the possibility of generation of microbial gum in stabilizing the complexes.

The absolute values for bacterial count (Table 3) was $42x10^5$ cfu g^{-1} during pre-monsoon season and $46x10^5$ cfu g^{-1} in soil during the post-monsoon season for *Mucuna,* while the corresponding values for *Pueraria* was $39x10^5$ cfu g^{-1} for pre-monsoon season and $43x10^5$ cfu g^{-1} in soil and for post-monsoon sampling and for rubber litter incorporation, the values were $35x10^5$ cfu g^{-1} in soil during pre-monsoon sampling and $39x10^5$ cfu g^{-1} in soil during the post-monsoon sampling. Fungal population also indicated more or less same trend as in the case of bacteria with higher values getting in the second observation. The population values were $28x10^4$ cfu g^{-1} for *Mucuna* during the pre-monsoon sampling and $32x10^4$ cfu g^{-1}during the post- monsoon sampling. For *Pueraria* it was $25x10^4$ cfu g^{-1} during the pre-monsoon sampling and $28.4x10^4$ cfu g^{-1} during the post-monsoon sampling. For rubber litter the values were $22x10^4$ cfu g^{-1} in soil during the pre-monsoon sampling and to $24.1x10^4$ cfu g^{-1} in soil during the post-monsoon sampling. The population of actinomycetes observed from different treatments showed wide variation and a different trend compared to bacteria and fungus. The population was $16.5x10^4$ cfu g^{-1} and $14.5x10^4$ cfu g^{-1}, respectively during pre-and post-monsoon sampling for *Mucuna* and it was $11x10^4$ cfu g^{-1} for pre-monsoon samples and $10.2x10^4$ cfu g^{-1} for post- monsoon samples for *Pueraria*. However, the population was $14.2x10^4$ cfu g^{-1} for the pre-monsoon sampling and $9.8x10^4$ cfug^{-1} for post-monsoon sampling for rubber litter

Table 3. Effect of litter sources on the microbial population

Litter sources	Bacteria (10^5) cfu g^{-1}		Fungi (10^4) cfu g^{-1}		Actinomycetes (10^4) cfu g^{-1}	
	I	II	I	II	I	II
Mucuna	42	46	28	32	16.5	14.5
Pueraria	39	43	25	28.4	11	10.2
Rubber	35	39	22	24.1	14.2	9.8

I. Pre-monsoon season II. Post- monsoon season

Table 4. Effect of litter sources on cellulase activity (µg $g^{-1}$of glucose hydrolyzed g^{-1} of soil 24 h^{-1})

Litter sources	Period of observation					
	First year		Second year		Third year	
	I	II	I	II	I	II
Mucuna	53.01	73.49	68.22	52.26	50.39	55.25
Pueraria	62.71	99.85	62.43	47.13	61.17	63.46
Rubber	38.35	67.66	63.93	50.30	52.91	55.62
CD (P=0.05)	7.72	19.46	NS	19.80	6.21	5.19

I. Pre-monsoon season II. Post-monsoon season

incorporation. The microbial activities were reduced during the post monsoon sampling period since these periods coincided with the summer months reiterating the fact that microbial activity associated with litter decomposition is limited by litter moisture. Again, this change in population also could be explained by the difference in the relative chemical composition of the litter added. The number of species involved in the decomposition of leguminous material is presumed to be higher compared to rubber litter. These observations are in conformity with the results reported by Becker et al. (1994).

The enzyme activity between treatments remained significantly different in the initial two observation periods (Table 4). During the third observation period there was no significant difference between treatments. The mean values ranged from 38.35 to 99.85 µg g^{-1} of glucose hydrolyzed g^{-1} of soil 24 h^{-1}. The highest value for enzyme activity was registered by *Pueraria* during the second phase sampling in the first year. However, during the remaining period of study the enzyme activity remained significantly higher in *Pueraria* when compared to the other two sources. *Pueraria* contains more of easily degradable substances with a narrow C:N ratio and relatively lower amounts of cellulose, lignin and polyphenols favors enhanced microbial activity (Philip and Abraham, 2009) thus a higher microbial load supported by the cover crops in the surface soil contributes to a high concentration of both ecto and endo enzymes. The variations in the leaf chemistry and ensuing differences in the organic sources originating from them might have differentiated the enzyme activity between treatments (Deng and Tabatabai, 1994). Thus, it is evident that the rate of degradation, the products formed and the interaction of the microbial enzymes with the degradation products of *Pueraria* have modified the reaction complex in favor of higher cellulase activity.

Dehydrogenase activity from the experimental site over a period of three years (Table 5) shows there was significant difference in the enzyme activity between treatments in almost all the six phases of study. The highest mean value of 293.37 µg of TPF hydrolyzed g^{-1} of soil 24 h^{-1} was observed in treatment *Mucuna* during the second phase of the third year while its corresponding pretreatment values at the start of the experiment was 115.59 µg of TPF hydrolyzed g^{-1} of soil 24 h^{-1}.

The enzyme activities noted towards the final observation were much higher when compared to its pretreatment levels. The observed disparity in activities in soils over the sampling periods is a clear reflection of the total range of oxidative activities taking place in the soil with the help of micro flora. The enhancement in activity might further be due to the significant role of the organisms play in the biological oxidation of organic sources through transfer of hydrogen ions and electrons to acceptors. Since, dehydrogenase activity occurs only within the living cell; any enhanced activity will only reflect the most active phase of micro flora. In this context, the significant difference in enzyme activity observed between treatments at different points of time may be justified with fluctuations in the microbial flora which depend mostly on the climate, composition and the degradability of the added

Table 5. Effect of litter sources on dehydrogenase activity (µg of TPF hydrolyzed g^{-1} of soil 24 h^{-1})

Litter sources	Period of observation					
	First year		Second year		Third year	
	I	II	II	I	II	II
Mucuna	115.59	154.13	148.35	112.30	144.04	293.37
Pueraria	125.76	134.23	61.70	88.79	119.71	144.68
Rubber	86.69	78.37	75.76	66.99	136.54	133.35
CD (P=0.05)	13.11	26.93	20.96	16.71	12.96	70.13

I. Pre-monsoon season II. Post-monsoon season

organic sources (Dick, 1994). In the present study, the added sources also remain quite different in its content and composition to induce variation in enzyme activity. Similar concern has been raised by Tian et al. (1992).

Further, de-polymerization of organic sources catalyzed by soil enzymes result in the production of low molecular weight and dissolved organic compounds which provide immediate energy, carbon and other nutrient substrates for microbial catabolic pathways, directly promoting microbial activity in soil. This version of thought had been amply substantiated by Tian et al. (2010). Thus, the addition of organic sources might have provided respectable amounts of carbon for energy source and sufficient mineral nutrients for the rapid proliferation of the micro flora which obviously brought in significant variations between treatments coupled with higher dehydrogenase activity. To be more specific, the litter derived from *Mucuna* and *Pueraria* contained easily degradable components with relatively higher nitrogen content, the enzyme values associated with these two treatments also remained higher and significant in most of the observations. However, the activity from *Mucuna* appeared to be significantly superior to *Pueraria* and this observation has been justified by Kothandaraman et al. (1989).

Nitrogenase enzyme activity from *Pueraria* and rubber remained statistically on par with each other in the third, fourth and fifth observations though there had been marginal variations in activity (Table 6). Invariably in all other phases, the enzyme levels remained significantly different from one another. The highest enzyme activity of 498.07 µ moles of ethylene produced g^{-1} of soil h^{-1} was noted in the second phase of the third year from *Mucuna*.

Compared to pretreatment levels there had been fluctuations in trends during the sampling periods and particularly towards the final phase of sampling with higher levels of activity. The catalytic action of the enzyme nitrogenase, results in the biological N fixation (Vitousek et al., 2002). Nitrogenase consists of two distinct proteins which contain molybdenum, iron, and sulphur. Because the nitrogenase proteins are denatured by exposure to oxygen (O_2), they can only operate successfully in an anaerobic environment. The observed activities in each phase, particularly after the first phase was not steady probably for want of a suitable environment for the enzyme to act. In this context, the observations made by García-Ruiz et al. (2008) emphasizing the need for suitable environment for the effective functioning of the nitrogenase enzyme in soils become relevant. However, the higher activity noted in the first two treatments compared to third might be due to the inherent ability of *Mucuna* and *Pueraria* crops in producing root nodules where the plant supplies carbon source and protects the oxygen sensitive nitrogenase enzyme. Stougaard (2000) substantiated similar contention for effective nitrogenase activity. The observed differences in activity among the nitrogen fixing crops particularly between *Mucuna* and *Pueraria* might possibly be due to the adaptations of organisms in establishing host specificity. The highest activity noted in the last phase of sampling in *Mucuna* might be due to a similar reason. The observed variations in chemical composition of

Table 6. Effect of litter sources on nitrogenase activity (µ moles of ethylene produced g^{-1} of soil h^{-1})

Litter sources	Period of observation					
	First year		Second year		Third year	
	I	II	I	II	I	II
Mucuna	62.90	66.05	43.00	109.05	134.55	498.07
Pueraria	228.17	71.89	31.57	53.18	109.21	286.68
Rubber	13.76	45.92	27.24	28.24	103.66	180.15
CD (P=0.05)	56.23	15.71	12.07	34.09	15.59	148.14

I. Pre-monsoon season II. Post-monsoon season

different plant species might have affected the microbial communities and biomass leading to changes in enzyme activity (Mille-Lindblom et al., 2006).

Correlation between enzyme activities and relevant soil parameters are presented in Table 7. Cellulase exhibited positive correlation with pH for the three litter sources and was significant for *Mucuna* and *Pueraria* litter addition. Dehydrogenase enzyme and pH were negatively correlated and the relation was significant for *Mucuna* and rubber. Similarly, nitrogenase enzyme was negatively correlated with pH and was significant for all the three litter sources. Cellulase recorded positive correlation with OC for *Mucuna* and *Pueraria* and the correlation was significant only for *Mucuna*. Nitrogenase enzyme was negatively correlated with OC for the three litter sources and was significant for *Mucuna* and rubber litter. No significant correlation was recorded between dehydrogenase activity and OC content. Cellulase enzyme exhibited negative correlation with total N and was significant for *Mucuna* and rubber litter. Dehydrogenase recorded positive correlation with total N for all the three litter sources and was significant for the treatment with rubber litter. Similarly, nitrogenase also registered positive relation with total N and was significant for *Pueraria.*

Correlation analysis of enzyme activity with nutrient availability recorded negative and significant correlation between cellulase activity and available K for *Mucuna* and rubber litter and with Mg availability for *Pueraria* and rubber litter. Correlation between available P and cellulase activity was positive and significant for *Pueraria* litter. In general, though not significant, Mg availability and enzyme activity were negatively correlated for all the three enzymes for all the three litter sources except dehydrogenase activity in the treatment with rubber litter. Dehydrogenase registered negative correlations with available Ca in all treatments. Correlation between nitrogenase with available Ca was found to be negative for all the litter sources and significant for *Pueraria* litter. Cellulase enzyme recorded significant negative correlation with clay content in the case of *Mucuna* litter. Dehydrogensae and nitrogenase enzyme recorded positive correlation with clay content for all the litter sources and was significant for rubber litter. Positive significant correlations were found between both dehydrogenase and nitrogenase with CEC in the case of rubber litter addition.

Litter derived from the *Mucuna* and *Pueraria* contained relatively easily degradable components than rubber litter, and *Pueraria* decomposed faster than *Mucuna* and yielded relatively higher OC, total N and available P content. Among the cover crops the available Ca and Mg content in soil was found to be higher in *Mucuna* treated plots. Higher microbial activity was found during the wet season than the dry season. The absolute values for bacterial count remained the highest in the case of *Mucuna* followed by *Pueraria* and the least for rubber.

Table 7. Correlation between enzymes and soil parameters

Litter sources	Enzyme	pH	OC	Total N	Available nutrients				Clay content	CEC
					P	K	Ca	Mg		
Mucuna	Cellulase	0.29*	0.34*	-0.31*	0.07	-0.29*	0.12	-0.05	-0.30*	0.04
	Dehydrogenase	-0.27*	-0.05	0.19	0.11	0.12	-0.03	-0.18	0.15	0.22
	Nitrogenase	-0.37*	-0.25*	0.15	0.06	0.16	-0.13	-0.17	0.20	0.26*
Pueraria	Cellulase	0.44*	0.18	-0.12	0.40*	0.01	-0.20	-0.33*	-0.02	-0.08
	Dehydrogenase	-0.03	-0.05	0.02	0.17	0.06	-0.24	-0.27*	0.11	0.08
	Nitrogenase	-0.50*	-0.05	0.50*	-0.06	0.18	-0.38*	-0.31*	0.13	0.09
Rubber	Cellulase	0.10	-0.02	-0.60*	-0.01	-0.27*	0.08	-0.29*	0.09	0.05
	Dehydrogenase	-0.25*	-0.07	0.26*	0.02	0.26*	-0.03	0.07	0.40*	0.29*
	Nitrogenase	-0.31*	-0.33*	0.01	-0.09	-0.16	-0.21	-0.32*	0.45*	0.44*

*Significant at 5 per cent level

Fungal and actinomycetes population followed a similar pattern. The population of bacteria, fungi and actinomycetes in the rubber ecosystem is mainly controlled by the quantity and the quality of litter added and the relative ease with which they degraded.

Cellulase activity was found to be higher in cover crop *Pueraria* treated plots. The dehydrogenase activity was found to be significantly higher in *Mucuna* treated plots followed by *Pueraria* and rubber litter. A higher level of nitrogenase enzyme activity was observed in *Mucuna* litter addition. Enzyme activity was related with soil properties. Cellulase exhibited positive significant correlation with pH in the case of *Mucuna* and *Pueraria* litter addition. Dehydrogenase enzyme recorded negative correlation with pH and was significant for *Mucuna* and rubber. Nitrogenase enzyme had significant negative correlation with pH for all the three litter sources. Similarly, nitrogenase recorded negative relation with OC. While relating enzyme activity with the availability of nutrients, significant negative relation was recorded with available Mg. Enzyme activity was related with clay content and CEC. Positive significant correlations were found between both dehydrogenase and nitrogenase activity with clay content and CEC in the case of rubber litter addition.

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