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REGULAR ARTICLE

Studies on litter characterization using ^{13}C NMR and assessment of microbial activity in natural forest and plantation crops' (teak and rubber) soil ecosystems of Kerala, India

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Abstract The leaf litter is the major source of soil organic matter in natural and many plantation crop ecosystems. Quantity and quality of organic matter in a soil ecosystem is of utmost importance in regulating the soil health. Hence assessment of quality of organic matter input, viz., litter is important and is attempted in this study. The leaf litter of rubber (*Hevea brasiliensis*), pueraria (*Pueraria phaseoloides*), mucuna (*Mucuna bracteata*), teak (*Tectona grandis*) and forest (mixed species) were analyzed using solid state ^{13}C nuclear magnetic resonance (NMR) to study the relative abundance of different carbon compounds present. The spectra revealed that litter of all species studied contain relatively larger amounts of polysaccharides compared to other C containing compounds. Also it could be observed that the alkyl-C to O-alkyl-C ratio of rubber litter was much higher compared to that of others. Aromatics and carbonyl compounds were also present in all litter species. The resource quality based on alkyl-C to O-alkyl-C ratio of the litter samples studied can be arranged in the order pueraria > teak > mucuna > forest > rubber. The respiration rate, substrate induced respiration rate and biomass-C (*Cmic*) of the litter samples were estimated. It could be observed that litter associated microbial activity decreased as alkyl-C to O-alkyl-C ratio increased. Resource quality derived from the NMR spectra and the litter biological properties were complementary. Soil samples (0–15 cm) from the five soil ecosystems (rubber, pueraria, mucuna, teak and forest) were analyzed for respiration rate, substrate induced respiration rate, *Cmic*, total-C and total-N. The forest soil had higher respiration rate, total-C and total-N compared to cultivated soil systems. Pueraria, mucuna and teak soils were comparable for their biological properties while rubber soil recorded comparatively lower microbial activity.

Keywords Litter quality · ^{13}C NMR · Microbial activity · Soil organic matter · Rubber

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Introduction

Soil organic matter management in arable soils is important as it regulates the physical, chemical and biological properties of soil (Weil and Magdoff 2004) and is essential to sustain soil quality (Wander 2004).

Plant litter is the major source of soil organic matter in natural as well as many of the cultivated soil systems (Kogel-Knabner 2002). Litter quality influences the decomposition process of soil organic matter amongst other factors such as activity and variety of decomposer organisms present and the prevailing environmental conditions. However, as litter quality is the factor which is more amenable to management options (Giller and Cadisch 1997; Krull et al. 2003), its assessment in every soil ecosystem is of great importance in maintaining or restoring soil quality.

C-to-N ratio was considered as a general index of plant litter quality as early as 1920s (Waksman 1924; Waksman and Tenney 1928), however, in the last few decades, many papers have questioned its reliability to predict C and N mineralization in soil (Heal et al. 1997; Herman et al. 1977). The different carbon compounds present in litter are varying in nature and structure, and cannot be treated as a single entity—total-C, especially when their interaction with decomposer organisms widely differs. For example, decomposition rate is positively correlated with polysaccharides, hemicelluloses and pectin (Swift et al. 1979) while negatively correlated with lignins and tannins in litter (Dighton 1978; Rayner and Boddy 1988; Slapokas and Granhall 1991).

Plant litter is the primary source of food and energy for soil biota and its quality is of critical importance in regulating microbial biomass (Wardle and Lavelle 1997). Though many biological indicators of soil quality have been proposed (Elliot et al. 1996; Pankhurst et al. 1997), microbial biomass measurement through substrate induced respiration method is often preferred as it can be applied to a range of soils and due to easiness in its measurement (Webster et al. 2001).

Rubber, a tree native of the Amazon forests was introduced to India about a century ago. It is now very widely planted and occupies about 16% of the total cultivated area in the Kerala State of India. Most of the initial rubber plantations were on forest cleared areas. Rubber plantations are cycled by new trees in about 30 years and many of the plantations in Kerala have entered the third replanting cycle (Karthikakuttyamma et al. 2000). Like rubber, initial teak plantations were also raised in cleared forest areas and some fields are under continuous plantations for more than a century. In rubber plantations during the initial 7 to 8 years of cultivation the fields are usually planted with leguminous cover crops such as, mucuna or pueraria and the litter turn over is estimated to be 3–6 tonnes (Mathew et al. 1989; Krishnakumar and Potty 1992). Subsequently rubber leaf litter itself becomes the principal organic matter source in rubber plantations and its turn over is estimated to be about 6 tonnes/ha/year (Krishnakumar and Potty 1992). In case of teak plantations in Kerala the litter fall is reported to be in the range of 5.5 to 12.1 tonnes/ha/year (O'Connell and Sankaran 1997) and in case of forest in the region, the litter fall is reported to be in the range 12.18 to 14.43 tonnes/ha/year (Mohankumar and Deepu 1992). Reports are available on the nutrient turn over from leaf litter of rubber, teak and forest and are not detailed here (Krishnakumar et al. 1991; Punnoose et al. 1994; Maharudrappa et al. 2000; Amponsah and Meyer 2000). However, studies on litter quality and comparative assessment of soil biological properties of these ecosystems viz. rubber, teak and forest are lacking and this study focuses on these aspects. As organic matter input differs in quality and quantity in these systems, associated microbial activity can vary. To test this hypothesis an attempt was made to assess the quality of leaf litter of rubber, teak, mucuna, pueraria and forest using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and assessment of microbial activity using respiration measurements.

The most important advantage of solid state ^{13}C NMR spectroscopy for litter and soil analyses is its ability of in situ characterization of organic C components. Incomplete extraction and risk of modification in the chemical nature of the compounds during the process are the major disadvantages of the conventional analyses (Hopkins and Chudek 1997; Skjemstad et al. 1997; Hopkins et al. 2000).

Materials and methods

Samples of soil and litter were collected from the following sites. The rubber fields were located in the Travancore Rubber Estate, Erumely, in the state of Kerala, India. The teak plantation and forest were within a few kilometer radius of the rubber fields at the Kalaketty range in Kanamala forest division. All these sites are located between 76° 52′ and 77° E longitude and 9° 25′ to 9°30′ N latitude.

1. Rubber: The plants were 10 years of age and belonged to the third cycle of cultivation. The area was under monocrop cultivation of rubber for more than 75 years. In mature rubber plantations though sparsely grown weeds were noticed, litter turn over from these species was very low compared to that of rubber.
2. Rubber in association with the leguminous cover crop, pueraria: The rubber plants were 3 years old. Thick, luxuriant growth of legume existed in the field. This area was also under continuous cultivation of rubber for more than 70 years. The litter turn over from young rubber plants was negligibly low compared to that of legume, pueraria, in this field.
3. Rubber in association with the leguminous cover crop, mucuna. The rubber plants were 3 years old and thick, luxuriant growth of the legume existed and this area had also been under continuous rubber cultivation for more than 70 years. Here also the litter turn over from young rubber plants was negligibly low compared to that of legume, mucuna.
4. Teak: The area was under continuous teak cultivation for more than 60 years with natural growth of undercover vegetation. The major species existed was *Tectona grandis* and its litter contributes largely to the total litter turn over in this field. The existed under flora were shrub type and the major species were, *Diospyros malabarica*, *Sida acuta*, *Sida cordata*, *Heliateres isora* and *Melastoma malabathricum*. The field was located adjoining to the natural forest experimental field.
5. Virgin forest. The area was a reserve forest and no human activity was noted. The forest area where the soil samples were taken consists of thick flora of tree species as well as under cover shrubs. The major tree species were, *Ceiba pentandra*, *Dalbergia latifolia*, *Xylia xylocarpa*, *Terminalia paniculata*, *Lagerstroemia microcarpa* and the major shrub species were, *H. isora*, *Spermacoce latifolia*, *Eclipta prostrata*, *M. malabathricum*, *D. malabarica*, *S. acuta*, *S. cordata*, *Adhatoda vasica*, *Strobilanthus ciliatus* and *Hibiscus tiliaceus*.

Litter samples

Litter samples from rubber, pueraria, mucuna, teak and forest sites were collected. Fallen litter that showed no obvious symptoms of physical disintegration or decomposition were hand picked. Samples were collected from ten different points, each of 1 m^2, pooled, from an area of about 5 ha and a representative sample was selected for further analysis.

Soil samples

Four surface soil samples (0–15 cm) were collected randomly from each site. The samples were air dried and sieved (2 mm) and stored in plastic containers for subsequent analysis. The soils of the sampled area, developed under tropical humid climate (average annual rainfall is about 4,150 mm and temperature is about 27°C) were deep, gravelly sandy clay with strong acid reaction (pH 4.5 to 5.5), low cation exchange capacity and low base saturation. These low activity clay soils belong at subgroup level of soil taxonomy to Ustic Haplohumults (NBSS & LUP 1999). No clear 'O' horizons were observed at any location, all samples were taken from mineral horizon after removal of fresh or partly decomposed leaves.

^{13}C NMR analysis

The collected litter samples were dried at 50°C for about 8 h in an oven and powdered using a ball mill before analysis by solid-state ^{13}C NMR using a Chemagnetics CMX LITE 300 MHz spectrometer at 4 kHz magic angle spinning (MAS), 1 ms contact time and 1 s relaxation delay with tetramethyl silane as the external reference. From the shift ranges assigned to alkyl-C (0–45 ppm) and O-alkyl-C (45–90 ppm), their ratio was calculated for all the samples analysed (Wilson 1987). An image analyzer was used to process the NMR spectra to calculate the peak area and ratio.

CO_2 measurements

Respiration rates of all the soil and litter samples were determined by measuring the evolved CO_2 from a wet sample (50% water holding capacity) incubated in a plastic container for 120 h. The measurements were made using a gas chromatograph (Varian 90) fitted with a porapak Q column and a thermal conductivity detector. The substrate induced respiration rate measurements were carried out 6 h after glucose addition. Glucose was added at the rate of 2 μg/g of sample and mixed well. The samples were kept at room temperature (15–17°C). All the estimations were done in triplicate. Microbial biomass-C was calculated for all the samples by the method of Anderson and Domsch (1978) with minor modifications described by Hopkins and Ferguson (1994).

Total C and total N determinations

Carbon and nitrogen of all the soil and litter samples were determined by dry combustion method using a CHN analyzer (Carlo Erba).

Statistical analysis

The parameters, respiration rate, SIR, biomass-C (*Cmic*), total C and total N of the litter and soil samples from the five different sites were subjected to ANOVA by general linear model in SPSS (version 11) to see for significant differences among the litter/soil samples of different systems using LSD at 5%. Standard error of the mean (SEM) and critical difference (CD) were worked out for each parameter.

Results

^{13}C CPMAS NMR spectra of the litter samples viz. rubber, pueraria, mucuna, teak and forest are shown in Fig. 1. Strong resonances were observed between 60–90 ppm and 90–110 ppm in the spectra of all litter samples indicating the presence of O-alkyl-C and di-O-alkyl-C groups respectively (Fig. 1). Intensity of signals in the 10–45 ppm region widely varied among the litter samples. These are attributable to the aliphatic hydrocarbons such as lipids and waxes (Hopkins and Chudek 1997). Signal intensities in these region were weak compared to the corresponding O-alkyl signals in the spectra of the pueraria and teak samples. The rubber litter had the strongest aliphatic-C signal while the forest and mucuna litters had also shown its moderate presence. The areas under signals of alkyl-C and O-alkyl C were measured and rubber litter had distinctly higher value for alkyl-C to O-alkyl-C ratio and it followed among the litter samples studied in the order rubber > mucuna > teak > pueraria (Table 1). Relative signal intensities of

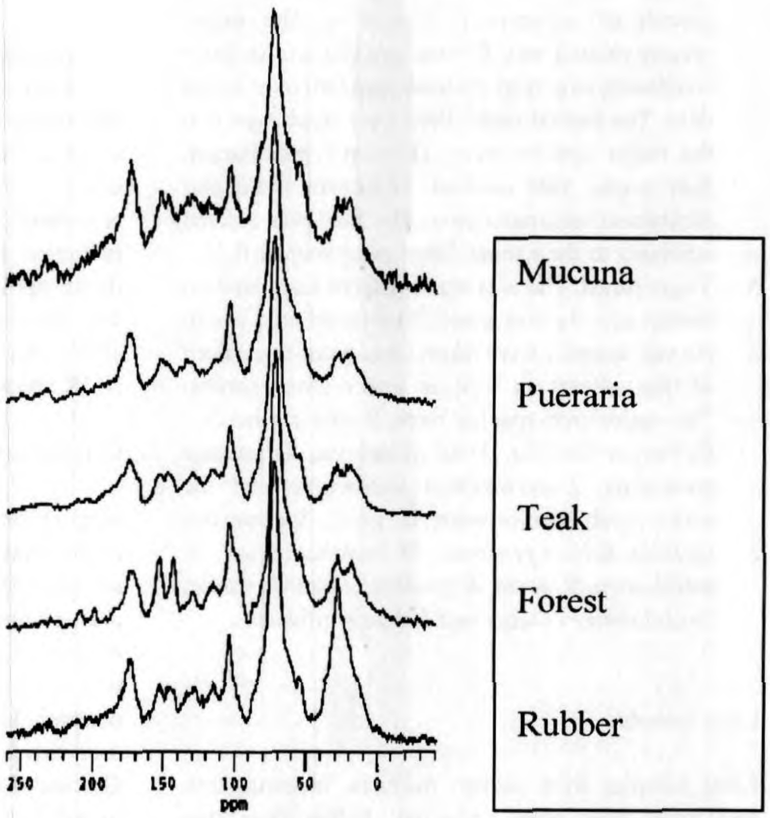

Fig. 1 ^{13}C NMR spectra of litter samples

Table 1 Respiration rate, substrate induced respiration rate (SIR), microbial biomass (*Cmic*), total N, total C, C-to-N ratio and alkyl-C to O-alkyl-C ratio of litter samples

Litter samples	Respiration rate (μmol CO_2/g/h)	SIR (μmol CO_2/ g/h)	*Cmic* (mg C/g litter)	Total C (%)	Total N (%)	C-to-N ratio	Alkyl C to O-alkyl C ratio
Mucuna	1.863	33.980	37.667	43.600	2.98	14.693	0.35
Pueraria	5.356	45.001	49.884	44.467	2.45	18.20	0.26
Teak	3.741	36.317	39.45	49.207	0.86	57.42	0.31
Forest	0.493	6.211	6.885	48.680	0.96	50.95	0.36
Rubber	0.373	2.046	2.754	54.770	1.45	38.08	0.64
SEM	0.2781	3.2128	0.7889	0.0931	2.3091	3.0832	–
CD	0.8762	10.1228	2.4857	0.2933	7.2755	9.7145	–

SEM Standard error of the mean, *CD* critical difference

the resonances observed in the 110–160 ppm region, representative of aromatic-C (Hopkins and Chudek 1997), also varied among the species studied. These signal intensities were weak compared to other C group signals in pueraria and teak spectra while strong in rubber, mucuna and forest samples.

The respiration rate, SIR, *Cmic*, total C, total N and C-to-N ratio of the litter samples studied are given in Table 1. Each sample was triplicated and the mean values are shown in the table. The respiration rate, SIR and *Cmic* followed the decreasing order—pueraria > teak > mucuna > forest > rubber (Table 1) while the alkyl-C to O-alkyl-C ratio followed the reverse order. The respiration rate of rubber litter was significantly lower to other litters except to that of forest litter while the pueraria litter had a significantly higher respiration rate than that of all other litter species studied. Except to that of rubber, forest litter had a significantly lower rate of respiration. SIR also followed a similar trend among the different litters as in the case of respiration rate. The rubber litter had a distinctly lower SIR compared to other litter samples while pueraria had a significantly higher rate than the other litter samples. Though a higher value for forest litter is noticed compared to rubber it is not significant. Between teak and mucuna litters though SIR value was numerically higher for teak it was not significant. *Cmic* followed exactly the same trend as that of SIR among the different litter samples.

Total C content of rubber litter was significantly higher to all other litter species while pueraria and mucuna had comparable values and were significantly lower than that of other litter samples. Teak and forest samples were also having similar values. Total N content was significantly higher for mucuna litter. Next to mucuna, pueraia and then rubber had higher N content while forest and teak litters were having lower values and were similar. The C-to-N ratio was significantly higher for teak litter compared to all other litter samples except to that of forest litter. Though not significant, teak litter had a higher value than forest litter. Mucuna and pueraria litters were having significantly lower values compared to other litter samples.

Respiration rate, SIR, *Cmic*, total N, total C and C-to-N ratio of surface soil samples (0–15 cm) of different systems are shown in Table 2. The values given in Table 2 for each system are the mean of four samples. Respiration rate of forest soil was much higher compared to all the other soils and rubber soil was having a significantly lower value except to that of mucuna soil. However, the trend was different for SIR values. Teak soil had significantly higher SIR compared to other soils except in the case of pueraria soil. Pueraria soil, though had a numerically higher value for SIR it was not significantly different to that of forest litter. Rubber soil was having a significantly lower SIR value compared to all the other soils. *Cmic* followed a similar trend as in the case of SIR.

Total C and total N were significantly higher in the case of forest soil compared to all the other soils. Mucuna soil had a slightly lower value for both total C and total N while other soils were having similar values. pH was highest for forest soil and lowest for rubber soil.

Discussion

The influence of litter quality on decomposition has been a subject of study and a quantum of literature

Table 2 Respiration rate, substrate induced respiration rate, microbial biomass, total N, total C and C-to-N ratio of soil samples of different eco systems

System	Respiration rate (μmol CO_2/g/h)	SIR (μmol CO_2/g/h)	*Cmic* (mg C/g soil)	Total C (%)	Total N (%)	pH	C-to-N ratio
Mucuna	0.025	0.237	0.263	3.543	0.223	4.95	15.9
Pueraria	0.036	0.317	0.352	4.448	0.250	4.99	17.8
Teak	0.040	0.358	0.397	4.303	0.295	5.19	14.6
Forest	0.112	0.276	0.306	6.360	0.378	5.51	16.9
Rubber	0.018	0.167	0.185	4.423	0.245	4.70	18.1
SEM	4.071×10^{-3}	0.0158	0.0158	0.2806	0.0224	0.1140	–
CD	0.0126	0.0476	0.0476	0.8456	0.0675	0.0343	–

SEM Standard error of the mean, *CD* critical difference

has come out in the last two decades. A comprehensive review on the subject is available by Heal et al. (1997). In terms of agronomical aspects litter quality refers to the relative easiness of decomposition by decomposing organisms and associated mineralization of nutrients (Paustian et al. 1997). On the other hand retardation in the rate of decomposition favours more resident period for the input C and this environmental aspect of litter quality is gaining importance in the soil C sequestration efforts to minimize the CO_2 build up in atmosphere (Janzen 2006). Ascertaining the litter quality or knowing the structural C species present in litter thus becomes very important in the management of soil C and its decomposition. ^{13}C NMR spectra of the five litter species studied reveals the relative abundance of different C species present in them. The polysaccharides are good substrates for microbes hence degrade rapidly in soil. The presence of species such as lignin/tannin tend to slow down the decomposition process of litter considerably and are reported to be the most recalcitrant materials among the naturally occurring organic compounds (Hammel 1997). Webster et al. (2001) and Hopkins et al. (2000) reported that as ratio of alkyl-C to O-alkyl-C increases the resource quality as a substrate for heterotrophic microorganisms declines.

The C species comprising polysaccharide C or carbohydrate C was invariably the largest group present in all the five litter species studied represented by the high peaks in the shift region 45–90 ppm (Fig. 1). However, the relative abundance of other C species such as alkyl C, aromatic C or carbonyl C to the respective O-alkyl C in each of the litter species varied widely which is clearly evident from the NMR spectra (Fig. 1). As alkyl C signal was very prominent in rubber litter and correspondingly the alkyl C to O-alkyl C ratio was also the highest among the different litter species studied. Natural rubber is a hydrocarbon (*cis* polyisoprene) with methyl groups and its presence in rubber litter might have contributed along with other alkyl C species present in the litter in giving an intense peak in 0–45 ppm region in the NMR spectra. Though not to the level at which alkyl C species were present, aromatic C species were also observed in rubber litter. These may result in increasing the recalcitrant nature of rubber litter. However, aromatic C signal intensities were more prominent for forest and mucuna litter, which might be due to the lignin sub units, may reflect on their decomposition nature. Compared to other litter species (excluding rubber), mucuna litter had intense alkyl C and aromatic C to the respective O-alkyl C signals. Though mucuna is a legume cover crop, based on biochemical nature of C species present, may not favour as a very good substrate for microorganisms. However, the other cover crop studied, pueraria, could be a very good substrate for decomposer species as the polysaccharide C signal was very intense while the other signals (including that of alky C and aromatic C) were very weak. Teak litter also had a similar nature as in the case of pueraria, however, aromatic C signal in comparison to the polysaccharide signal was reflected more in teak litter. Based on the NMR spectra information on the different C species present, especially in terms of alkyl C to O-alkyl C ratio, the litter quality in terms of decomposition may follow the order pueraria > teak> mucuna > forest > rubber on qualitative terms.

C-to-N ratio of litter of legume species, pueraria and mucuna were low as expected, less than 20. The lower values in these cases are more because of higher N content present in these litters.

Microbial activity associated with litter could be better linked to litter quality picture drawn out of NMR spectra. C-to-N ratio was not reflected on litter microbial activity (Table 1). The results supports the reports of Herman et al. (1977) and Berg (1986) that the influence of N on rate of decomposition of various C species in litter such as carbohydrates and lignin were different and C-to-N ratio alone could not help in predicting the decomposition rates. The distinctly higher value of alkyl-C to O-alkyl-C ratio in rubber litter and its lowest value for microbial activity (Table 1) support similar observations by Hopkins et al. (2000) in other species. Pueraria litter had comparatively lower alkyl-C to O-alkyl-C ratio and had significantly higher microbial activity. The results on microbial activity of the litter samples (Table 1) and the NMR interpretation on litter quality are complementary.

Significantly higher respiration rate of forest soil (Table 2) might be basically due to two reasons. The variety of species and huge quantity of litter available in forest compared to the cultivated systems. The quantity influence is not addressed in this study. Though the resource quality of forest litter was lower, the soil microbial activity was higher when compared to the other cultivated systems studied. In this case quantity of litter might have influenced more on microbial activity. Also other factors such as favorable soil conditions and microclimate might have influenced the soil microbial activity in forest ecosystem. The higher values for microbial activity and total C in forest soil might be due to the influence of enormous quantity of variety of litter and the resource quality respectively (Fig. 1). The total C content of rubber and teak soils were comparable. Both rubber and teak are plantation crops and soils are under 'no till' practice. However, the resource quality and microbial activity are distinctly different in these systems. Hence the mechanism of C storage or resident period can be different in these systems. In rubber soil, the resource quality and the monocrop cultivation could be major factors influencing the decomposition while in teak soil, under flora consisting many shrub species, higher duration of a plantation cycle (usually more than 100 years) and close proximity of forest ecosystem might be the factors. *Cmic* and SIR were highest in teak soils while that of rubber soils were lowest compared to other soils investigated (Table 2). All cultivated systems studied had significantly lower total-C compared to the natural system. Also forest soil had higher microbial activity. This might be due to the management practices adopted in the different cultivated systems such as monocrop cultivation, resource quality and quantity, application of chemicals etc. Among the cultivated systems the microbial activity differs significantly. Litter quality being different and the major organic matter source in these systems had influenced the microbial activity, which in turn can affect the decomposition process and soil properties.

Conclusions

Through ^{13}C CPMAS NMR studies, relative abundance of different C compounds present in litter samples of rubber, pueraria, mucuna, teak and forest could be obtained. Litter associated microbial activity was higher in legume and teak litters in terms of respiration rate. Forest and rubber litters were found to have lower litter associated microbial activity. An increase in microbial respiration rate was noted as the alkyl-C to O-alkyl-C ratio in litter decreased. If rubber is cover cropped with leguminous species during the initial years, organic matter input is mainly from cover crops and its quality especially that of pueraria may favour faster decomposition resulting in an increased active organic-C pool and microbial activity in soil. Generally this sort of situation is agronomically favoured in the active growing phase of a major crop (like rubber) as more nutrient release can occur. In subsequent years of rubber cultivation the organic matter input is through rubber leaf litter and its biochemical quality may favour a slow decomposition, thereby conservation of more C in the soil. Though soils of rubber plantations, as mono crop and pueraria cover cropped situations have comparable total-C and total-N contents to that of teak plantations with natural under growth, further in depth studies are required on different organic matter pools and assessment of stock organic carbon of these systems, which can give better in sight on decomposition process that can lead to refinement of policies to maintain soil health and quality in rubber plantation system.

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