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Original article

Long-term effects of leguminous cover crops on biochemical and biological properties in the organic and mineral layers of soils of a coconut plantation

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Abstract

The primary aim of the study was to determine the long-term (12 years) effects of leguminous cover crops like *Atylosia scarabaeoides, Centrosema pubescens, Calopogonium mucunoides* and *Pueraria phaseoloides* on important soil biochemical and biological properties and their interrelationships in the organic (fresh litter layer, F and fermented + humus layer, F + H) and mineral (0–10 and 10–20 cm) layers of soils of a 19-year-old coconut plantation.

The total biomass production (above-ground) for the 12-year period varied significantly between the cover crops and ranged from 34.86 (calopo) to 90.43 (pueraria) Mg ha^{-1}. Total N and C additions at the cover cropped (CC) site for the 12-year period were 0.97–3.07 Mg ha^{-1} and 16.90–43.34 Mg ha^{-1}, respectively. Irrespective of layers, the levels of organic C, total N, organic substrates viz., dissolved organic C and N, labile organic N, water soluble carbohydrates, and light fraction organic matter-C and were markedly higher in the CC site compared to the control. Consequently, the levels of microbial biomass-C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC}), net N mineralization rates, CO_2 evolution, metabolic quotient (qCO_2) and the activities of L-asparaginase, L-glutaminase and β-glucosaminidase were significantly higher in the CC site compared to the corresponding levels in the control site. Between layers, the levels of various chemical, biochemical and microbial parameters were consistently higher in the organic layers compared to the mineral layers at all the sites including control. Among the ratios of various microbial indices, the ratios of C_{MIC}: organic C and C_{MIC}: P_{MIC} did not differ significantly between the layers and sites. However, the ratio of C_{MIC}: N_{MIC} was relatively higher in the mineral layers and control site. The variation in individual soil properties between layers and sites reflected the concomitant changes occurring in soil organic matter content. Apparently, microbial activity was limited by the supply of biologically available substrates in the mineral layers and the control site. Contrarily, the more direct supply of nutrients from decomposing plant litter and the indirect supply of nutrients from the mineralization of organic matter led to significantly higher levels of microbial biomass in the organic layers.
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Keywords: Cover crops; Soil biological properties; Soil organic matter pools; CO_2 evolution; Net N mineralization rates; Wet humid tropics

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1. Introduction

In tropical Asia, cover crops are frequently planted in oil palm [13] and coconut plantations [2]. Cover crops reduce soil erosion and therefore soil nutrient and organic matter losses [37]. Besides, they fix atmospheric N, recycle nutrients and affect soil nutrient availability [38]. The total benefit of leguminous cover intercropped with oil palm was found to be 239 kg N ha^{-1} $year^{-1}$ [1]. Similarly, a mixture of cover crops (calopo, centrosema, pueraria, atylosia) grown in the interspaces of a 19-year-old coconut plantation was found to contribute 3.8–10.2 Mg ha^{-1} $year^{-1}$ of biomass. Annual incorporation of this biomass over a period of 10 years increased soil organic C by 1.9–4.6 g kg^{-1}, total N by 0.52–0.78 g kg^{-1}, Bray P by 1.3–2.3 mg 100 g^{-1} and extractable K by 3.2–4.2 mg 100 g^{-1} [19,21]. However, relatively limited information is available concerning biochemical and biological properties and their interrelationships in the organic and mineral layers of soils repeatedly incorporated with leguminous cover crops.

We, therefore, conducted a study with the primary aim of determining the long-term (12 years) effects of leguminous cover crops on various biological properties (microbial biomass C, N and P, net N mineralization rate, CO_2 evolution and qCO_2) and their inter-relationships in the organic and mineral layers of soils of a 19-year-old coconut plantation. The effects of these cover crops on a few important enzymes involved in C and N cycling (L-asparaginase, L-glutaminase and β-glucosaminidase) and various pools of soil organic matter (SOM) involved in nutrient supply like dissolved organic carbon (DOC), dissolved organic nitrogen (DON), labile organic nitrogen (LON) and light fraction organic matter (LFOM) were also studied. These SOM pools have different functional roles in soils, respond more rapidly to management than SOM content [8] and provide a measure of subtle or early effects of management on soil quality [7].

2. Materials and methods

2.1. Study site

The study site ($10^{\circ}30^{l}$–$13°42^{l}$ N latitude and $92^{\circ}14^{l}$–$94^{\circ}16^{l}$ E longitude) was located on a coconut farm near Port Blair (S. Andaman, India). The mean annual precipitation is 3100 mm spread over 7 months from May to November. The dry season lasts from January to April. The mean annual temperature is 30.1 °C and the region has a true maritime climate with least variation in maximum and minimum temperatures throughout the year. The soil of the study site is a fluventic sulfaquent made of alluvial and marine deposits. At 0–30 cm the soil is light brown, sandy clay loam with medium permeability, firm and sticky.

In order to check soil erosion and associated nutrient losses, a study involving leguminous crops grown as soil cover in the interspaces of a 19-year-old coconut plantation was initiated in 1991 at the Central Agricultural Research Institute, Port Blair. The study comprises treatments involving leguminous crops as natural soil cover in separate plots (1 0 × 10 m) demarcated by live bounds of lemon grass *(Cymbopogon flexuosus).* Four cover crops viz., *Atylosia scarabaeoides, Centrosema pubescens, Calopogonium mucunoides* and *Pueraria phaseoloides* were grown individually during the rainy season and incorporated into the soil towards the end of monsoon (in December every year). Incorporation was through zero-tillage, wherein the cover crops were cut 5 cm above the soil surface and the biomass was left as such in the respective plots for in situ decomposition. The study also consists of appropriate controls without any cover crop or live bound. The common weeds encountered in the study area were *Mikania cordata*, *Eupatorium odoratum* and *Euphorbia hirta*. The weeds in the control plots were also incorporated as described above towards the end of monsoon. The experiment had four replications laid out in a randomized block design. No chemical fertilizers or pesticides were applied to any of the plots.

2.2. Estimation of biomass and plant analysis

The biomass was harvested at maximum growth period (before incorporation of the cover crops) in the inner 2 m^2 area of each plot and yield estimated. The fresh weight of above- and below-ground biomass was determined and then oven dried (60 ± 5 °C) for dry weight determination and chemical analysis. Subsamples of each plant material were analyzed for carbohydrate, carbon, nitrogen, lignin and polyphenol content. Organic C concentration was determined by the Walkley–Black method [41], assuming that all plant C was oxidized during digestion. Carbohydrate concentration was determined by digesting 0.5 g of the plant sample with 10 ml of 2.5 M H_2SO_4 at 100 °C in a water bath followed by estimation of carbohydrate as described by Brink et al. [11]. Nitrogen concentration in the plant material was determined by digesting 0.4 g of the sample with 5 ml of concentrated H_2SO_4 and 7 ml of 30% H_2O_2 at 400 °C for 1 hour, followed by steam

distillation and titration [10]. Lignin was determined by the acid detergent fiber method [27] and polyphenol was determined using the Folin–Denis method wherein 70% methanol, 0.5% formic acid and 0.05% ascorbic acid are used as extractants and the Folin–Ciocalteu reagent and tannic acid are used as standards [5].

2.3. Soil sampling

The different organic layers could be distinguished morphologically into fresh litter layer (F) and fermented + humus layer (F + H). During sampling, 10 random cores (25 cm height, 7 cm diameter) were taken from each plot at the end of the 12th year (after the end of the monsoon). The cores were then divided into organic (F and F + H) and mineral (0–10 and 10–20 cm) layers. The samples were immediately transferred to the laboratory, where living plant material and coarse roots were removed, sieved (< 2 mm for mineral layer, 5 mm for organic layer) analyzed for their moisture content on the day of collection and stored at 4 °C. The samples were always drawn from the inner two-thirds of each plot and bulked to give one composite sample per layer per plot. For bulk chemical analyses, the samples were dried at 65 °C (organic soil) or 105 °C (mineral soil) and ground with a grinder. Other measurements were done using fresh material. All analyses were carried out within 1 month after sampling.

2.4. Soil chemical/biochemical/microbiological analyses

Organic C was determined by the Walkley–Black method [41] and total N using an automated N analyzer. The soil pH was measured in the suspension with 1:2.5 (mineral soil) and 1:10 (organic soil) of soil and distilled water (or 0.01 M $CaCl_2$), respectively. Soil mineral N was extracted using 2 M KCl and NH_4-N and NO_3-N were determined by the method described by Bremner and Mulvaney [10].

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined by incubating samples at constant temperature (20 °C) and moisture (55% of WHC) for 3 weeks. Water extractions were made initially and after the incubation. The soil samples were shaken in 1.5 l ultra-pure water for 2 h (200 rpm), and the suspensions were filtered through a series of filters comprising of Whatman GF/D and Scleicher and Schuell GF 52 glass filter and millipore filter (0.45 μm) using a vacuum system [53]. In the extract, the total N was determined by the alkaline persulfate oxidation [14] and organic C as mentioned earlier. Labile organic nitrogen (LON) was determined by subtracting initial mineral N content from mineral N accumulated following a 2-week incubation of 100 g portion of the sample at 20 °C in an aerobic incubation chamber [6]. Light fraction organic matter (LFOM) was separated using a 1.7 g cm^{-3} solution of NaI according to Strickland and Sollins [57] and its C and N content determined as mentioned earlier.

The fumigation-extraction method [58] was used to determine soil microbial biomass- C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC}) using k_{EC} of 0.45 [31], k_{EN} of 0.54 [33] and k_{EP} of 0.40 [12], respectively. For measuring CO_2 evolution, the organic layers were fragmented in a blender for 3–5 s to a particle size of < 25 mm^2 and the soil from the mineral layers was sieved to < 2 mm. Then, 100 g (oven-dry basis) of moist sample was weighed into polythene jars and adjusted to 55% of its water-holding capacity. Each jar was covered with parafilm, pricked with holes to allow aeration and placed in an incubator at 20 °C and 70% relative humidity for a 7 day pre-incubation. After the pre-incubation, each jar, together with another small jar containing 20 ml 1 M NaOH was placed into a 2 l airtight glass jar and incubated at 20 °C for 7 days. The jars were flushed with air every 1–2 day to ensure aerobic condition. After the incubation, excess 1.5 M $BaCl_2$ was added to the NaOH solution to precipitate carbonate and the remaining NaOH was titrated with HCl using phenolphthalein as indicator. The metabolic quotient (qCO_2) was calculated as follows: (μg CO_2-C evolved in 7 days g^{-1} soil)/(μg biomass C g^{-1} soil)/ 7 days × 1000 = mg CO_2-C g^{-1} biomass C per day.

Net N mineralization rates of soils were determined through a 16 week incubation experiment. About 80 g of the sample and 80 g of silica-sand mixture were thoroughly mixed and transferred to PVC leaching tubes (40 cm height, 3.5 cm Ø). The sample-sand mixture was supported in the tube on a glass wool pad above a one-hole stopper fitted with a glass drainage tube. The mineral N initially present was leached from the system using 100 ml of 0.01 M $CaCl_2$ in small increments (10 ml at a time) followed by 25 ml of N-free nutrient solution prepared with KH_2PO_4, K_2SO_4, $MgSO_4$ and $CaSO_4$ containing 100, 24, 113, 0.5 and 4 mg l^{-1} of Ca, Mg, S, P and K, respectively; its pH was approximately 7. The tubes were stoppered, the moisture potential was brought to 0.01 MPa by overnight equilibration on a suction manifold apparatus, and the tubes were incubated at 35 ± 1 °C. Mineralized N (NH_4-N and NO_3-N) was determined following 1, 2, 4,

8, 10, 12, 14 and 16 weeks of incubation. After each extraction, the moisture potential was brought to 0.01 MPa and the tubes returned to the incubator. NH_4-N and NO_3-N in the leachate were determined as described by Keeney and Nelson [36].

L-asparaginase and L-glutaminase activities were determined by using L-asparagine and L-glutamine, respectively, as the substrate [24,25] and β-glucosaminidase activity was assayed by using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as the substrate [43].

2.5. Statistics

Analyses were performed on all soil samples and the values reported are means of four replications expressed on an oven-dry soil basis. The significance of treatment effects was determined by one-way analysis of variance. Where the F values were significant, post hoc comparisons were made using the least significant difference test at the 0.05 probability level. The net N mineralization rates were calculated using linear regression analysis. Differences in net N mineralization rates were studied using the *t*-test with the Bonferroni adjustment. Pearson correlations were performed with STATISTICA 5.1 [55].

3. Results and discussion

3.1. Soil chemical and biochemical characteristics

Soil pH, in general, did not differ significantly between layers and sites (Table 2) and varied in a narrow range of between 4.3–5.2 (H_2O) and 3.8–4.3 ($CaCl_2$). However, all the other variables varied significantly between layers and sites, though the precise relationships between layers and between sites varied among the different parameters. At the cover cropped (CC) site, organic C and total N levels were markedly higher in the organic layers (mean 41.4 and 1.86%, respectively) compared to the mineral layers (mean 3.4 and 0.30%, respectively; Table 2). Between sites, organic C and total N levels were markedly lower in both the organic (20.9% and 1.2%, respectively) and mineral layers (1.02% and 0.14%, respectively) of the control site compared to the corresponding levels in the CC site. Apparently, organic C levels increased twofold in the organic layers and threefold in the mineral layers of the CC site compared to control. Likewise, total N increased 1.6-fold in the organic layer and twofold in the mineral layers at the CC site. Also, the average levels of various microbial substrates viz., carbohydrates, DOC, DON, LON etc were markedly higher in the organic layers compared to the mineral layers at all sites. Between sites, the accumulation of these microbial substrates was at significantly greater levels in all layers of the CC site relative to control (Table 2).

3.2. Soil microbial biomass

At the CC site, C_{MIC}, N_{MIC} and P_{MIC} levels were significantly higher in the organic layers (mean 5.5 g/kg, 642 and 199 mg/kg, respectively) compared to their respective levels in the mineral layers (0.36 g/kg, 31 and 13 mg/kg, respectively; Table 3). Such variations could be ascribed to the variations in quantity and quality of organic substrates accumulated in various layers. For instance, marked decrease in the concentration of carbohydrates, DOC, DON, LON led to simultaneous reduction in the levels of microbial biomass in the mineral layers (Table 3). Substrates like organic C, carbohydrates and dissolved organic matter represent a very active soil organic component [9] and a readily available source of energy for soil microorganisms [17]. Between sites, the microbial biomass levels were significantly higher in all layers of the CC site. Compared to control, C_{MIC}, N_{MIC} and P_{MIC} were 2–3-fold higher in the organic and mineral layers of the CC site. This is due mainly to greater accumulation of substrates like organic C, carbohydrates, DOC, DON etc, which accumulate through leaching from fresh litter, plant re-

Table 1
Biomass production, relevant organic and inorganic characteristics of the cover crops and nutrient additions for the 12-year period

	Biomass [a] ($Mg\ ha^{-1}$)	Composition ($g\ kg^{-1}$)					C/N	Addition ($Mg\ ha^{-1}$)				
		Total C	Total N	Total carbohydrate	Lignin	Polyphenol		Total C	Total N	Total carbohydrate	Lignin	Polyphenol
Calopo	34.86b	484.8	27.8b	114.3b	75.2a	34.6a	17.4	16.90d	0.97b	3.99c	2.62c	1.21b
Pueraria	90.43a	479.3	33.9a	128.4a	62.3b	22.8c	14.1	43.34a	3.07a	11.61a	5.63a	2.06a
Centrosema	49.18b	488.4	27.4b	132.6a	73.5a	31.4a	17.8	24.02c	1.35b	6.52b	3.61b	1.54b
Atylosia	77.67a	462.8	35.2a	131.4a	66.4b	27.2b	13.1	35.95b	2.73a	10.20a	5.16a	2.11a

Within each column, means followed by the same letter are not statistically significant at $P < 0.05$.
[a] Sum of 12 years; includes only above-ground biomass.

Table 2
Chemical and biochemical characteristics of the organic and mineral layers of soils amended with leguminous cover crops

Location	Layer	pH ($CaCl_2$)	pH (H_2O)	Organic C (%)	Total N (%)	C/N	DOC	DON	LON	LFOM-C	LFOM-N	Water soluble carbo-hydrate
							(mg kg^{-1})					
Control	F	4.2	4.3	21.6c	1.27c	17.0	421.6c	43.9c	8.67c	581c	15.6c	16.3b
	F + H	3.8	4.4	20.2c	1.03c	19.6	586.9c	48.6c	7.48c	553c	16.3c	11.2c
	0–10	4.1	4.3	1.18d	0.18c	6.5	206.4c	21.2c	3.62c	202c	6.8c	3.4c
	10–20	4.1	4.6	0.86c	0.11c	7.8	183.6c	21.6c	2.87c	193c	6.9b	3.0c
Calopo	F	3.9	5.0	36.4b	1.56b	23.3	983.4b	81.2a	10.84b	1154a	48.4a	27.3a
	F + H	3.9	4.9	35.1b	1.62b	21.7	962.4b	84.8b	11.63b	1049a	43.2a	16.4b
	0–10	3.8	4.8	2.9c	0.23b	12.6	452.7a	44.9b	5.96b	798a	30.6a	4.9b
	10–20	4.2	4.9	2.1b	0.23b	9.1	402.8b	31.8b	6.04b	604a	32.4a	5.3b
Pueraria	F	3.8	4.9	51.9a	2.27a	22.9	1106.8a	83.6a	13.64a	1214b	54.6a	27.8a
	F + H	3.8	4.8	43.2a	2.20a	19.6	1212.9a	104.8a	14.12a	1008b	51.4a	24.2a
	0–10	4.0	4.3	5.3a	0.49a	10.8	512.8a	48.3ab	6.83a	746b	25.6b	8.3a
	10–20	4.3	4.9	4.2a	0.28ab	15.0	483.2a	40.4a	7.12a	635b	31.4a	7.9a
Centrosema	F	4.2	5.2	38.6b	1.64b	23.5	963.4b	74.6b	11.63b	1164a	51.4a	26.2a
	F + H	3.9	4.8	34.3b	1.42b	24.0	994.6b	86.9b	12.24b	1036ab	51.6a	15.6b
	0–10	3.8	4.9	2.7c	0.29b	9.3	432.7b	42.4b	5.65b	796a	40.8a	5.6b
	10–20	4.1	5.0	2.7b	0.23b	11.7	420.1b	38.6a	5.21b	612a	31.3a	5.9b
Atylosia	F	4.3	4.9	46.7a	2.32a	20.0	1208.6a	84.7a	14.23a	1212b	59.3a	28.4a
	F + H	3.8	4.3	44.8a	1.87ab	24.0	1125.3ab	111.9a	13.21a	1019b	46.2b	21.3a
	0–10	4.0	4.6	4.1b	0.32b	12.8	486.2ab	49.3a	7.14a	783b	38.4a	7.2a
	10–20	4.1	4.9	3.1ab	0.29a	10.7	412.3b	38.4a	6.13b	602b	31.3a	6.5b

Within each horizon, column means followed by the same letter are not statistically significant at $P < 0.05$.

Table 3
Biological properties of the organic and mineral layers of soils amended with leguminous cover crops

Location	Layer	C_{MIC} (g kg^{-1})	N_{MIC} (mg kg^{-1})	P_{MIC} (mg kg^{-1})	CO_2 evolution (mg CO_2-C kg^{-1} d^{-1})	L-Asparaginase (mg NH_4-N kg^{-1} h^{-1})	L-Glutaminase (mg NH_4-N kg^{-1} h^{-1})	β-Glucosaminidase (mg *p*-nitrophenol kg^{-1} h^{-1})
Control	F	2.32c	242c	86.4d	41.3d	26.4c	518.2c	436.4c
	F + H	2.06c	213d	64.6c	35.6c	23.3d	361.7c	438.3c
	0–10	0.18c	15d	6.9c	2.4c	3.6d	121.3d	40.6c
	10–20	0.17c	14c	6.2c	2.1c	2.7c	124.2c	36.4c
Calopo	F	6.02b	811b	224.2bc	162.5c	34.2b	763.4b	637.4b
	F + H	3.57b	356c	121.6b	83.8b	27.8c	621.8b	512.4b
	0–10	0.34bc	28c	11.9b	5.1b	6.4c	183.6bc	57.3b
	10–20	0.30b	25b	10.2b	4.9b	7.1b	142.1b	59.8b
Pueraria	F	8.09a	1024a	284.2a	215.6a	46.4a	1123.6a	802.6a
	F + H	4.69a	421ab	184.3a	115.6a	42.1a	832.7a	681.4a
	0–10	0.49a	35a	14.7a	7.6a	8.1b	217.4ab	81.2a
	10–20	0.39a	32a	16.3a	6.1a	11.2a	186.6a	62.3ab
Centrosema	F	6.21b	802b	220.8c	156.5c	36.4b	745.6b	632.4b
	F + H	3.56b	364bc	124.8b	89.5b	32.3b	624.7b	502.6b
	0–10	0.32c	31bc	10.8b	6.2b	8.2b	172.8c	56.2b
	10–20	0.31b	31a	11.9b	5.1b	7.4b	155.3b	57.8b
Atylosia	F	7.64a	936a	273.8ab	186.3b	47.8a	1024.8a	812.7a
	F + H	4.38a	422a	158.6a	114.6a	40.2a	836.9a	672.4a
	0–10	0.41ab	36a	16.2a	7.5a	11.7a	219.4a	83.2a
	10–20	0.32b	30a	15.3a	4.6b	7.2b	136.2b	63.2a

Within each horizon, column means followed by the same letter are not statistically significant at $P < 0.05$.

Table 4
Ratios of various microbial indices in the organic and mineral layers of soils amended with leguminous cover crops

Location	Layer	C_{MIC}: Org. C	C_{MIC}: N_{MIC}	C_{MIC}: P_{MIC}	qCO_2 (mg CO_2-C g^{-1} biomass C per day)
Control	F	1.07a	9.6a	26.8a	17.8b
	F + H	1.02a	9.7a	31.9a	17.6c
	0–10	1.52a	12.0b	26.1b	13.0c
	10–20	2.0a	12.1a	27.4b	12.2b
Calopo	F	1.65a	7.4b	26.8a	27.0a
	F + H	1.02a	10.0a	29.4a	23.5b
	0–10	1.17b	12.1b	28.6b	14.9b
	10–20	1.43b	12.0a	29.4a	16.2a
Pueraria	F	1.56a	7.9b	28.5a	26.6a
	F + H	1.08a	11.1a	25.4b	24.6a
	0–10	0.92b	14.0a	33.3a	15.5b
	10–20	0.93b	12.2a	23.9c	15.4a
Centrosema	F	1.61a	7.7b	28.1a	25.2a
	F + H	1.04a	9.8a	28.5a	25.1a
	0–10	1.18b	10.3b	29.6b	19.1a
	10–20	1.15b	10.0a	29.0a	16.3a
Atylosia	F	1.64a	8.2b	27.9a	24.4a
	F + H	0.98a	10.4a	27.6a	26.2a
	0–10	1.00b	11.4b	25.3c	18.3a
	10–20	1.03b	10.7a	20.9c	14.2a

Within each horizon, column means followed by the same letter are not statistically significant at $P < 0.05$.

sidue decomposition [46,48] as well as humified organic matter and the plant rhizosphere [35]. Regular incorporation of cover crops, therefore, provided a steady supply of substrates to support the microbial community thus confirming the observation that when different treatments from the same site or soils under similar conditions are compared, the microbial biomass might be related to the steady-state substrate availability [51]. This is well reflected by the existence of a strong correlation (Table 6) between microbial biomass and related parameters like organic C, carbohydrates, DOC, DON, LON etc. This is expected since microbial biomass is more quickly influenced by organic inputs [48] than by changes in soil organic matter [42].

The ratios of various microbial variables viz., C_{MIC}: organic C, C_{MIC}: N_{MIC} and C_{MIC}: P_{MIC} (Table 4) were calculated from the data in Table 3. The ratio of C_{MIC}: organic C reflects the availability of substrates to soil microflora [4]. At the CC site, the mean C_{MIC}: organic C ratio did not differ significantly between layers and sites and varied in a narrow range of between 1.10–1.32%. Similarly, the mean C_{MIC}: P_{MIC} ratio also exhibited little variation between layers and sites (range 27.8–29.4). However, the C_{MIC}: N_{MIC} ratio exhibited variations between layers and sites. At the CC site, C_{MIC}: N_{MIC} ratio was on an average 9.0 in the organic layer, while in the mineral layer the ratio increased to an average value of 12.0. Between sites, the mean C_{MIC} : N_{MIC} ratio was 9.0 and 11.6, respectively, in the organic and mineral layers of the CC site, while the corresponding levels in the control site increased slightly to 9.6 and 12.1, respectively. Higher C_{MIC}: N_{MIC} ratio in the control site is presumably due to high organic matter availability coupled with relatively low N availability as observed by Salamanca et al. [49] in some secondary tropical forest sites of Philippines. Increased MBC:MBN ratios in incubation experiments with complex soil microbial populations [15] have been attributed to low N availability in combination with high C availability.

3.3. Soil enzyme activities

We also determined the activities of a few important enzymes (L-asparaginase, L-glutaminase and β-glucosaminidase) involved in C and N cycles in soils (Table 3). L-asparaginase and L-glutaminase are two of the most important enzymes involved in hydrolysis of native and added organic N to soils, which specifically act on C–N bonds in linear amides other than peptides [24,25]. Similarly, β-glucosaminidase is considered to be important in C and N cycling because it participates in the processes whereby chitin is converted to aminosugars, which is one of the major sources of mineralizable N in soils [22]. However, studies on activities of these enzymes in soils are very few. In the present study, the enzyme activities were markedly higher in the organic layers of all sites (Table 3). Mean activities of L-aspar-

Table 5
Net N mineralization rates, ammonification and nitrification in various layers and chemical composition of the organic layers of soils amended with leguminous cover crops

Location	Layer	Net N mineralization (mg ($NH_4 + NO_3$)-N kg^{-1} d^{-1})			Ammonification[b]	Nitrification[b]	Lignin (%)	Hemicellulose (%)	Cellulose (%)	Polyphenol (%)
		Rate	SE[a]	Goodness of fit (R^2)						
Control	F	11.6c	2.1	0.96	53	47	36.3	14.3	12.8	18.4
	F + H	9.3d	2.3	0.93	72	28	27.4	13.1	12.2	18.2
	0–10	1.2d	1.3	0.53	76	NS				
	10–20	1.8c	1.0	0.54	75	25				
Calopo	F	15.4b	1.2	0.51	94	6	27.2	10.4	15.4	12.2
	F + H	14.9bc	1.7	0.56	76	NS	24.1	9.7	15.3	12.8
	0–10	2.2c	0.9	0.54	74	36				
	10–20	4.0b	0.9	0.61	70	30				
Pueraria	F	19.8a	3.1	0.92	58	42	31.4	10.4	16.7	14.6
	F + H	14.3c	2.0	0.56	83	17	26.6	8.1	15.1	13.8
	0–10	3.5b	1.4	0.68	91	NS				
	10–20	5.6a	1.8	0.42	95	5				
Centrosema	F	15.1b	2.7	0.78	90	10	27.8	10.5	15.1	12.6
	F + H	14.0c	4.2	0.45	74	26	23.2	8.2	15.7	12.9
	0–10	3.8b	0.9	0.65	96	NS				
	10–20	5.3a	1.0	0.68	94	NS				
Atylosia	F	18.1a	3.2	0.54	64	36	31.2	10.4	16.3	15.8
	F + H	15.9ab	3.3	0.56	77	NS	24.3	7.3	14.7	13.0
	0–10	4.6a	1.5	0.46	72	28				
	10–20	4.5b	1.9	0.49	88	12				

[a] Standard error of linear regression through the NH_4-N and NO_3-N content.
[b] Ammonification and nitrification rates were calculated as the regression through the NH_4-N and NO_3-N content, respectively, and represented as % of net N mineralization rate.

Table 6
Pearson's correlation coefficients ($P < 0.05$) between various soil properties ($N = 80$)

	Organic C	Total N	DOC	DON	LON	CO_2 evolution	LFOM-C	LFOM-N	Net N min.	L-Asp.	L-Glut.	β-Gluco.	C_{MIC}	N_{MIC}
Organic C														
Total N	0.81													
DOC	0.84	0.78												
DON	0.80	0.81	0.80											
LON	0.78	0.82	0.79	0.81										
CO_2 evolution	0.83	0.79	0.85	0.79	0.78									
LFOM-C	0.73	0.68	0.78	0.78	0.79	0.78								
LFOM-N	0.71	0.71	0.73	0.79	0.79	0.74	0.81							
Net N min.	0.78	0.79	0.83	0.81	0.84	0.81	0.77	0.74						
L-Asp.	0.87	0.81	0.84	0.83	0.81	0.83	0.73	0.76	0.81					
L-Glut.	0.81	0.80	0.84	0.83	0.80	0.83	0.76	0.74	0.79	0.80				
β-Gluco.	0.80	0.84	0.81	0.85	0.84	0.80	0.76	0.71	0.82	0.81	0.81			
C_{MIC}	0.85	0.81	0.86	0.88	0.81	0.88	0.78	0.76	0.84	0.81	0.84	0.80		
N_{MIC}	0.83	0.81	0.81	0.81	0.84	0.84	0.71	0.77	0.85	0.79	0.82	0.81	0.83	
P_{MIC}	0.80	0.83	0.84	0.84	0.80	0.86	0.78	0.76	0.81	0.78	0.86	0.80	0.82	0.80

DOC: dissolved organic C; DON: dissolved organic N; LON: labile organic N; LFOM-C and -N: light fraction organic matter-C and -N; Net N min.: net N mineralized; L-Asp: L-asparaginase; L-Glut: L-glutaminase; β-Gluco: β-glucosaminidase; C_{MIC}: microbial biomass C; N_{MIC}: microbial biomass N; P_{MIC}: microbial biomass P.

aginase, and L-glutaminase and β-glucosaminidase in the organic layers of the CC site were 38 mg NH_4-N kg^{-1} h^{-1}, 822 mg NH_4-N kg^{-1} h^{-1} and 657 mg *p*-nitrophenol kg^{-1} h^{-1}, respectively. Corresponding levels in the mineral layer were 8 mg NH_4-N kg^{-1} h^{-1}, 177 mg NH_4-N kg^{-1} h^{-1} and 65 mg *p*-nitrophenol kg^{-1} h^{-1}. Therefore the organic layers of the CC site exhibited a fourfold increase in L-asparaginase, and L-glutaminase activities and a 10-fold increase in β-glucosaminidase activity compared to the mineral layer.

Between sites, the enzymes were significantly activated to varying degrees in all the layers of the CC site compared to the control. Mean activities of L-asparaginase, L-glutaminase and β-glucosaminidase indicated a 1.5–2.0-fold increase in various layers of the CC site relative to control (Table 3). This is apparently a consequence of enhanced substrate and microbial biomass levels as reflected by the significant and positive correlation between the enzyme activities and parameters such as total N, organic C, DOC, DON, LON, C_{MIC}, N_{MIC}, P_{MIC}, net N mineralized etc (Table 6). This indicated that greater levels of labile substrates led to greater microbial proliferation and subsequently to greater enzyme synthesis and accumulation in the organic and mineral layers of the CC site.

3.4. CO_2 evolution and metabolic quotient (qCO_2)

Similar to microbial biomass levels, the CO_2 evolution rates were consistently higher in the organic layers of all sites. At the CC site, mean CO_2 evolution rate in the organic layers was 140.0 mg CO_2-C kg^{-1} d^{-1}, which decreased drastically to 6.0 mg CO_2-C kg^{-1} d^{-1} in the mineral layers (Table 3). Cover cropping significantly enhanced CO_2 evolution rates in the organic and mineral layers of soils. In general, mean CO_2 evolution rate at the CC site was 3–4-fold greater in the organic and mineral layers compared to control. Enhanced CO_2 production in the CC site, especially the organic layers can be attributed to greater levels of total organic carbon, which has been found to account for 75% and 81% of the variations in CO_2 production in the non-preincubated and pre-incubated soils, respectively [60].

The metabolic quotient (qCO_2) also exhibited a similar trend in various layers and sites. Mean qCO_2 levels in the organic layers of the CC site were consistently higher (25.3 mg CO_2-C g^{-1} biomass C per day) compared to the mineral layers (16.2 mg CO_2-C g^{-1} biomass C per day; Table 4). Likewise, the metabolic quotient in both the organic (range 23.5–27.0 mg CO_2-C g^{-1} biomass C per day) and mineral (range 14.2–19.1 mg CO_2-C g^{-1} biomass C per day) layers of the CC site was consistently greater than the corresponding levels in the control site (17.6–17.8 and 12.2–13.0 mg CO_2-C g^{-1} biomass C per day, respectively). The metabolic quotient indicates the maturity of a soil system [3] and higher levels in the CC site is more likely due to greater availability of soil organic matter [32] and organic matter additions [39,42]. Enhanced qCO_2 levels in the CC site would also be due to a changed microbial community structure [26,30]. However, this needs further investigation.

3.5. Net N mineralization rates

Net N mineralization rates of soils were determined from the slope of the linear regression fitted through the evolution of mineral N from various layers and sites over 16 weeks (Table 5). The R^2 values indicated that net N mineralization followed zero order kinetics and not first order kinetics. Probably, the mineralizable N pool was not sufficiently depleted to influence mineralization as observed by Vervaet et al. [59] in deciduous and coniferous forest soils. Besides, in soils where the substrate is continuously replaced by aboveground inputs, zero order kinetics was found to adequately describe the course of N mineralization [29]. At all the sites, net N mineralization rates were markedly higher in the organic layers and least in the mineral layers. Federer [23] and Vervaet et al. [59] also observed higher mineralization rates in the organic layer and the uppermost layers. These layers were found to account for 78% of the net N mineralization rates [44]. In our study, the organic layers accounted for 80% of the net N mineralization rates indicating that the age and resistance to decomposition of soil organic matter generally increases with depth in the soil profile. This coupled with a decrease in microbial activity reduced N mineralization rates [47].

Cover cropping enhanced N mineralization rates by 33–41% in the organic layers and by 45–67% in the mineral layers (Table 5). This can be attributed to higher organic matter levels in the former because N mineralization is a microbial process that is influenced both by the quantity and quality of soil organic matter [50]. Apparently the CC site accumulated greater levels of dissolved organic matter especially the labile fraction, which directly regulates N mineralization and nitrification rates and is the initial substrate for these N cycling pathways [34].

Though ammonification and nitrification did not vary consistently between layers and sites, ammonifica-

tion was the most important N mineralization process ranging from 53–96% (Table 5). Nitrification occurred mostly in the organic layers and sometimes in the mineral layers. Rapid NO_3-N immobilization leading to under estimation of net nitrification process [54] or low pH (4.3–5.0 (H_2O) and 3.8–4.3 ($CaCl_2$)) could be the possible reasons for low nitrification rates in our soils. Ste-Marie and Pare [56] observed no nitrification below a pH of 4.5 and Persson and Wirén [44] observed that no nitrification occurred below a pH of 3.95. While the contribution of ammonification decreased with increase in net N mineralization, at least in the organic layers, the contribution of nitrification process decreased with decreasing net N mineralization rates. The results, therefore, indicated the possibility of a positive correlation between nitrification and net N mineralization in accordance with the observations of Goncalves and Carlyle [29] who observed a linear increase in nitrification with mineralization. Similarly, Persson et al. [45] found a positive relationship between net N mineralization and net nitrification in mineral soils. Contrarily, lack of correlation between these two parameters has also been observed [40], possibly because net N mineralization and nitrification were influenced by different rates of immobilization of NH_4 and NO_3 [16]. We also observed a positive correlation between net N mineralization and CO_2 mineralized in our study (Table 6). This is common, as most heterotrophic C mineralizing microorganisms participate in the first stage of N mineralization (ammonification) and the heterotrophic microorganisms contribute more to net NH_4 accumulation than to NH_4 immobilization [40].

The N mineralization rates also varied considerably among the CC sites (Table 5). Pueraria and atylosia sites consistently registered greater rates compared to calopo and centrosema sites. Greater accumulation of dissolved organic matter especially the labile fraction [35] coupled with greater microbial activity [50] led to relatively higher net N mineralization rates in the organic and mineral layers of these sites compared to the corresponding layers in the calopo and centrosema sites. N mineralization rates have also been related to the quantity and quality of crop residue incorporated into the soil [18,20]. The lignin and polyphenol content of pueraria (62.3 and 22.8 g kg^{-1}, respectively) and atylosia (66.4 and 27.2 g kg^{-1}, respectively) were relatively lower than centrosema (73.5 and 31.4 g kg^{-1}, respectively) and calopo (73.5 and 31.4 and 75.2 and 34.6 g kg^{-1}, respectively; Table 1). However, the lignin and polyphenol levels in the organic layers of pueraria (26.6–31.4 and 13.8–14.6%, respectively) and atylosia (24.3–31.2 and 13.0–15.8%, respectively) sites were higher than the corresponding levels in the centrosema (23.2–27.8 and 12.6–12.9%, respectively) and calopo (24.1–27.2 and 12.20–12.8%, respectively) sites (Table 5). This is apparently due to greater levels of biomass incorporated over the years in the former and therefore larger additions of lignin and polyphenol to the soil (Table 1). The higher lignin and cellulose content in the organic layers of pueraria and atylosia sites would also explain the relatively higher levels of LFOM-C and -N at these sites (Table 2). LFOM primarily represents partially degraded plant litter inputs together with microbial and faunal debris/tissue [28] and is considered a moderately labile organic matter pool with amounts determining patterns of N mineralization [52].

Overall, incorporation of leguminous cover crops had a significant and variable influence on almost all the soil properties studied. The properties reflecting biological activity viz., C_{MIC}, N_{MIC}, P_{MIC}, CO_2 evolution, N mineralization, enzyme activities etc, in general, decreased in the mineral layers and were higher in the CC site compared to control. The variation in individual soil properties between layers and sites closely reflected the concomitant changes occurring in soil organic matter content. Apparently, microbial activity was limited by the supply of biologically available substrates in the control site. Within the CC site, variations in biological properties and their relationships were due mainly to variations in the quantity and quality of the present and past substrate, total dissolved organic C and N and labile soil organic matter.

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