Soil Organic Matter Dynamics in Particle Size Fractions as Revealed by the $^{13}C/^{12}C$ Isotopic Ratio in Tropical Soil

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ABSTRACT

This s In order to better understand the dynamics of soil organic matter (SOM) in tropical soils and the impact of converting native forest into plantation, we studied the dynamics of different physically separated SOM pools at different depths under natural conditions and 7 years of pasture (Pancium Maximum) cultivated via the replacement of the native C (C3-derived) and pasture C (C4-derived).Organic C stocks of the secondary forest, was (15±3 kg m-2)and pasture (19±3 kg m-2) were significantly different, which was attributed to the high biomass production of the tropical pasture and the protective effect of the high clay content (<138.15 g kg-2). We observed that 72.5-50% of the total organic C accumulated in the clay and silt fraction. The replacement of forest -derived C by pasture-derived C was in average 44%, 34%, and 33% for 0-30, 30-60, and 60-90 cm depth, respectively, suggesting a fast turnover rate of organic C regardless of the high clay content. The replacement decreased in the order: coarse, sand and increase silt, clay fraction. In conclusions, after 7 years of pasture, about 56% of the total organic C in the clay in the topsoil was still from C3, indicating that a significant part of this fraction was relatively recalcitrant

الملخص العربي

من أجل فهم ديناميكية المواد العضوية للتربة (S O M) بشكل افضل في التربة المدارية وتاثير تحويل الغابات الاصلية إلي مزارع المراعي (Pancium Maximum)) تم فصل عينات التربة التي تم تجميعها من أعماق مختلفة فيزيائيا و قياس نسبة الكربون العضوي في كل جزئية من جزيئات التربة المفصولة فيزيائيا في كلا من منطقة الغابات الاصلية (C_3) ومنطقة نباتات المراعي (C_4) التي تم استزارعها من سبع سنوات بدلا من اشجار الغابات. كان مخزون الكربون العضوي في التربة الغابات مختلف كثيرا (15± 3كجم $^-$ 2) وفي تربة المراعي (19± 3كجم $^-$ 2) ويعزي ذلك الي ارتفاع انتاج الكتلة الحيوية لنباتات المراعي المدارية والتاثير الوقائي لمحتوي الطين العالي. حيث انه لوحظ ان مابين 50 – 72% من مجموع الكربون العضوي تراكم في جزيئات الطين والطمي.وكان معدل استبدال الكربون المشتق من نباتات المراعي في المتوسط 44% – 34% و33% عند العمق 0– 30و 30–60 و 60– 90 سم علي التوالي. نستنتج أنه بعد 7 سنوات من استبدال أشجار الغابات بنباتات المراعي المدارية 56 % من اجمالي الكربون العضوي في جزيئات الطين والطمي عند العمق 0 –30 سم لا يزال من اشجار الغابات (C_3) مما يشير الي دور جزيئات الطين في حماية المادة العضوية من التحلل.

INTRODUCTION

Land-cover conversion is the second most important source of anthropogenic greenhouse gases (GHG) emissions, generating around 7–14% of the total carbon dioxide (CO_2) emissions around the world (Harris *et al.*, 2012). Deforestation in the tropics has been

estimated at ~1.1 million km2 during the period (Hansen *et al*., 2013) and conversion from forests to pasture has become the most common land use change globally (Change, 2006).

The loss of soil organic carbon by conversion of natural vegetation to cultivated use is well known. Various land uses result in very rabid declines in soil organic matter (Zhu *et al*., 2016). Much of this loss in soil organic carbon can be attributed to reduced input of organic matter, increased decomposability of crop residues, and tillage effects that decrease

the amount of physical protection to decomposition. Soil organic carbon includes plant, animal and microbial residues in all stages of decomposition. The turnover rate of the different soil organic carbon compounds varies due to the complex interactions between biological, chemical, and physical processes in soil. Many factors (climate, land use, soil moisture, soil texture, clay content, mineral, erosion, leaching, fire, etc.) play a role in regulating the accumulation and loss of SOC storage (Chen *et al*., 2009).

C isotope analysis is a powerful tool for the study of C turnover in the complex soil decomposer system. Important considerations in such studies are the effect of local environmental conditions on the δ13C value of carbon input to the soil from live vegetation, as well as the effects of soil physical parameters on the evolution of the $\delta^{13}C$ value of SOC with time (Balesdent *et al*., 1996). Delta ^{13}C ($\delta^{13}C$) values have been utilized to document vegetation change and to quantify soil organic matter turnover, the majority of the tropical forests and wood plantation are C3 with $\delta^{13}C$ values in the range of −22% and −35%. Meanwhile, grass is C4-plants with $\delta^{13}C$ values in the range of −8% to −18%. Delta ^{13}C values are not altered significantly during de- composition and soil organic matter formation, essentially into the fine-silt-sized fraction (Balesdent *et al*., 1998). Consequently, $\delta^{13}C$ values of SOC reflect the relative contribution of plant species to net primary production. Although there may be a continuum of soil organic carbon compounds in terms of

their decomposability and turnover time. Physical fraction techniques are often used to define and delineate various relatively discrete soil organic carbon pools. Physical defined fractions, while containing diverse array of organic compounds, integrate structural and functional properties of soil organic carbon (Post& Kwon, 2000), emphasizes the role of soil minerals and soil structure in SOC turnover, and relates more directly to SOC dynamics in situ than classical wet chemical SOC fraction, and increasing attention has focused upon the responses of C and N pools in different soil particle–size fractions to climate Change (Nian-Peng *et al*., 2014). As in terrestrial ecosystems the amount of carbon in soil is usually greater than the amount in living vegetation. It is therefore important to understand the dynamics of soil carbon as well as its role in terrestrial ecosystem carbon balance and the global carbon cycle by using the advanced methods.

MATERIALS AND METHODS

The study was conducted in the pasture (*Panicum Maximum*) site at University Putra Malaysia (UPM), Serdang, Selangor, and the secondary forest one hundred years old at Puchong, Selangor. Figure 1. The UPM plantation lies between (Latitude: 101° 43 32.14" E, Longitude: 002° 58 57.65" N). The secondary forest lies between (latitude: 101° 38 44.12"E, longitude: 003° 00 42.21"N). The soil classified as Serdang at UPM and Puchong. The soil texture at Serdang series is sandy clay. An experimental plot of 100×100 m with three replicates was designed for the field experiment, and the period of study was in March 2015.

Soil sampling and analysis:
Soils were sampled using steel rings soil sampler. The depth intervals sampled were 0–30, 30-60, and 60-90cm. Samples were collected from 3 replicate for each sites, homogenized and dried prior to analysis. For each depth interval, physical fraction (Sistla,

2016) , organic matter (OM) by loss on ignition method (Heiri *et al*., 2001), total carbon and nitrogen and $\delta^{13}C$ stable isotopes were determined according to modified methods by (Zulkifli *et al*., 2014)
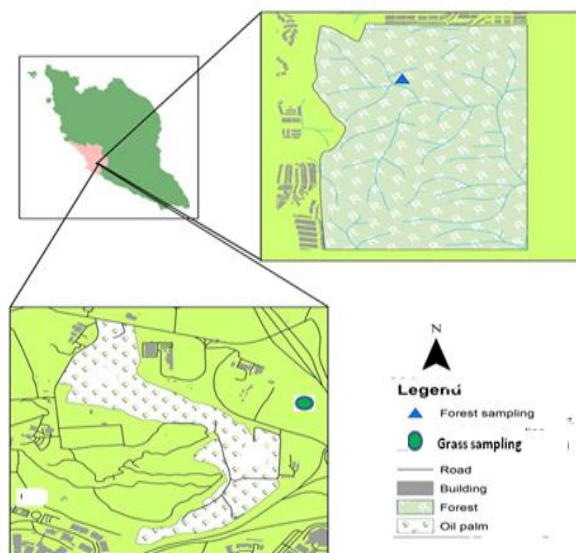

Figure 1 Map of the study area: showing secondary forest and pasture area in the UPM

Identification of $\delta^{13}C$ stable isotopes was done using an elemental analyzer connected on-line to Continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the Stable Isotope Laboratory (Nuclear Malaysia). Isotopic ratios are expressed in conventional delta (δ) notation in parts per thousand:

$$\delta\,X = (R\ sample/\ R\ standard^{-1}) \times 1000,$$

Where X = r ^{13}C and R= $^{13}C/^{12}C$.
The international standard is V-PDB for ^{13}C.
The precision of triplicate measurements was, 0.2% for $\delta15N$, and, 0.1% for $\delta^{13}C$.

Physical fraction:
Three replicate treatments were carried out on the three equivalent shares of each soil. Mineral particle-size distribution was obtained after H_2O_2 oxidation of SOM, using conventional sieving and sedimentation techniques.

Particle–size fractionation was completed by disrupting soil aggregates of bulk soil samples using ultrasonic energy and separating the particle–size fractions by a combination of wet sieving and continuous flow centrifugation (Sista, 2016). Briefly, 40 g of sieved soil (< 2 mm) was suspended in 250 ml of deionized water (the floating visible debris was removed) with 5mm diameter glass beads and the aggregates ruptured by mechanical shaking overnight (Balesdent *et al*., 1991).
The fractions >50μm were recovered by wet sieving. As the mechanical shaking did not allow the complete breakdown of aggregates, the >50 μm fractions were sonified by using a probe-type ultrasonic cell disrupter system (scientz-IID) operating for 15 min in the continuous mode at 361 W. We used a sieve to separate sand (particle size, 53–2000 μm) by manual wet sieving method with deionized water. Particles which consisted of silt (2– 53 μm) and clay (< 2 μm) passing through the sieve during the wet sieving process were collected. In order to separate slit from clay, the mixture of particles and water was poured into a 500 ml centrifuge bottles and centrifuged at 682 rpm for 5 min. During this procedure, only the silt fraction sinks to the bottom, while the clay fraction remains suspended. The silt fraction was then re-suspended in 200 ml deionized water and re-centrifuged at 476 rpm; this procedure was repeated 5 times to optimize separation. The clay fraction was obtained by transferring the suspensions into new centrifuge bottles and centrifuging them at 4000 rpm for 30 min. All the fractions were dried at 50 ∘C and then ground for further chemical analysis.

Organic C and $\delta^{13}C$:
Organic C was analysed for all samples and fractions, using an Elemental Analyzer EA1180. 13C abundance was determined after the conversion of total C to CO_2, Results were expressed as $\delta^{13}C$ (‰).
For the calculations, we used a mass balance Eq. (1), according to (Balesdent *et al*., 1996).

$$C_T\delta13_T= Cc_3\delta13c3 + Cc_4\delta13c4 \quad (1)$$

Where CT is the total soil organic carbon, Cc3 is the soil organic carbon derived from C_3 vegetation, Cc4 is the soil organic carbon derived from C_4 vegetation, δ13T is the δ13C of total soil organic carbon, δ13c3 is the δ13C of soil organic carbon derived from C_3 vegetation, and δ13c4 is the δ13C of soil organic carbon derived from C_4 vegetation.

Note: δ13litter is the δ13C of the SF litter were (-30.64) and (-13) for pasture.

RESULTS AND DISCUSSION

Results:
Physiochemical properties:

The results of the effect of soil depths on the bulk density, soil texture, water content, OC of the SF and pasture respectively are summarized in Table 1. As seen in Table 1, the deepest part of the soil i.e. the subsoil 60-90 cm depth and the (30-60 cm depth) had significantly greater bulk density (1.62 g/cm3, 0.95g/m3) respectively than 0-30 cm depth (0.88 g/cm3). Similarly, the pasture site represented the same trend.

In the same manner, water content and clay percentage were significantly increased gradually by increasing the soil depth. The relative distribution of organic C among the fractions was similar for all depth Table 2 and Fig. 2. Organic C concentrated in the clay fraction for all depth of the profiles. The fraction of organic C present in the clay fraction in relation to the total organic C increased with depth from about 30% to 80%, mostly as a consequence of a decrease in coarse fractions Fig.2.

Table 1: Soil properties for different depth under secondary forest (SF) and pasture (P).

Site	Depth (cm)	Bulk density (g cm⁻³)	Soil Texture				Water content (%)	OC (g kg⁻¹)	ON (g kg⁻¹)
			coarse (g kg⁻¹)	Sand (g kg⁻¹)	Silt (g kg⁻¹)	Clay (g kg⁻¹)			
SF	0-30	0.88	29.52	32.30	15.46	22.72	20.14	**90.135**	2.97
	30-60	0.95	25.88	29.39	14.7	30	21.96	51	1.16
	60-90	1.26	20.76	28.0	16.878	34.24	23.46	49.5	1.001
P	0-30	0.9	26.32	25.99	22.35	25.24	20.46	**138.15**	3.16
	30-60	1.10	18.75	15.41	29.23	36.58	22.4	63	3.2
	60-90	1.2	14.5	16.02	32.4	37.05	22.96	40.5	1.01

Table 2: Organic C contents in relation to total soil (g kg-1 of soil) for different depth under secondary forest (SF) and pasture (P), and δ13C secondary forest (SF) and pasture (P) at (0-30 cm)

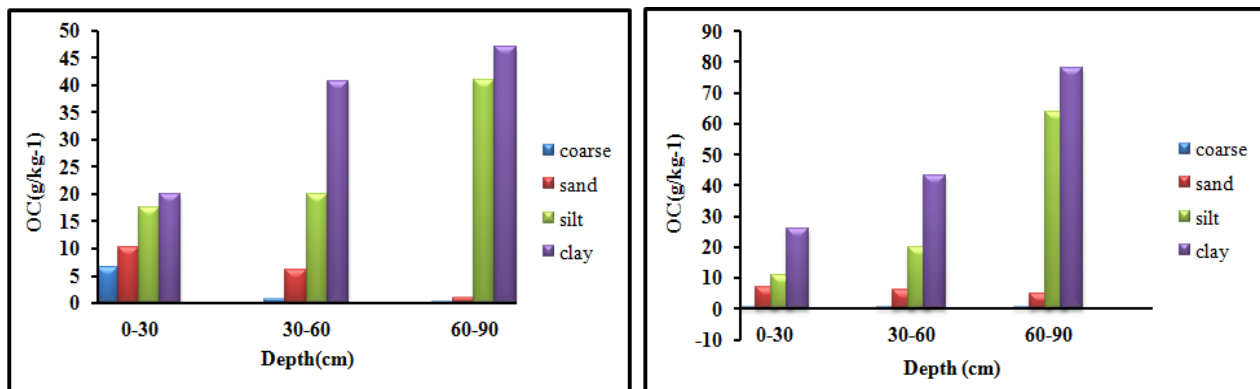| Site	Depth (cm)		Fractions				δ¹³C
			Coarse (250-2000μm)	Sand (50-250 μm)	Silt (2-50 μm)	Clay <2 μm)	
SF		0-30	0.6	6.2	10.9	22.0	-30.1
		30-60	0.4	2.4	4.8	19.6	-29.5
		60-90	0.1	0.9	3.7	17.6	-29.4
	δ¹³C		-	-30.6	-30	-29.5	
P		0-30	0.6	4.5	16.9	45	-13.4
		30-60	0.3	1.7	10.8	47	-20.6
		60-90	0.1	1.0	6.4	50	-29.6
	δ¹³C		-	-25	-15.4	-15	

Fig. 2. Organic C distribution (g/kg) of the total organic C among the particle size fractions in different depth. "a'' Secondary forest (SF),'b'' pasture (P)

δ^{13}C of soil and fractions:

In the SF profiles, δ13C increased from 0-30 cm to 30-60 cm and remained stable towards the bottom 60cm, the increases was of 1.9‰, The average stable values was -30.1,-29.5 and -29.4 ‰ for 0-30, 30- 60 and 60-90cm respectively. In pasture, the values of δ^{13}C decreased continuously with depth by 3.37%. A larger difference in δ^{13}C values was observed between pasture profiles than between SF profiles. Related to fractions, sand tended to be depleted in ^{13}C compared to the other fractions and bulk soil. Those fractions showed δ13C values closed to the litter of SF (-30.6). The silt fraction was in general slightly depleted and the clay fraction slightly enriched in ^{13}C with respect to the bulk soil. For all the fractions changes with depth were similar to those in the bulk soil. In pasture profiles sand was remarkably depleted in ^{13}C compared to the other fractions, and clay was enriched with ^{1}3C.

Discussion:

Organic C contents in soil and fractions:

Table (1) shows that, there was significant change in organic C stock occurred after pasture cultivation. Nevertheless, a change of organic C distribution with depth was observed, which is probably due to a higher contribution of below roots than above ground litter in pasture. The high contents of clay in the studied soil Table (1) may be responsible for the maintenance of the organic C stocks after conversion to pasture, due to the protective effect of clay (Yonekura *et al*., 2013). At the same time, the high biomass production (especially roots) of the tropical C grass *panicum maxima* (Roscoe *et al*., 2001) was probably responsible for maintaining an organic C supply comparable to that in pasture site. The relationship between organic C in the clay faction and the total organic C in the soil (Fig. 2) suggested that the ratio of clay organic matter increased with depth was in agreement with (Roscoe *et al*., 2001) who showed that the physical capacity of soil to preserve SOM (defined as the maximum amount of C that can be associated with clay and silt particles) against decomposition. Clay and silt fractions in soil usually have higher C and N concentrations and stocks than that of sand fraction, and thus soils with higher clay and silt contents generally have higher soil organic C (SOC) and N stocks (Follett *et al*., 2012).

δ^{13}C of soil and fractions:

The SF profile was typical for soils under C3 vegetation, where δ13C$_3$ values of total soil usually increase with depth (Balesdent *et al*., 1996). This increase has been attributed to (i) isotopic discrimination during decomposition, (ii) differences in decomposition rates of organic compounds with different δ13 C

signature, and (iii) decrease in $^{13}C/^{12}C$ isotopic ratio of atmospheric CO_2 over the last 150 years, as a result of fossil fuel burning and additional biosphere mineralization (Balesdent *et al*., 1996) However, the enrichment in 13C with depth was observed only from 0-30 to 30-60. In SF the lower amount of organic C in the Coarse than in the silt and clay fractions (Table 1) indicated that most of the carbon was present as organ mineral complexes. This suggested a high degree of humification already in this horizon. Since the studied soil is very homogeneous to 1-m depth (Table 1). The same behavior was reported for similar soils under origin plants (Roscoe *et al*., 2001). The difference between the $\delta^{13}C$ values of the SF profiles was in average 1.9%. This difference may be attributed to the variability of the organic material added. Since the $\delta^{13}C$ tends to increase with decomposition. The variability could be the result of different distribution of C with different degrees of humification.

In pasture, the values of $\delta^{13}C$ were significantly higher than in SF throughout the profile and for all fractions table 2. A decreasing trend in these values with depth was also observed. These results suggested that substantial addition of pasture organic C (richer in ^{13}C) occurred in all fractions and was highest in the first depth. The difference between profiles was higher than for SF, which could be attributed to an additional source of variation.

Replacement of organic C:

In general, a high replacement of organic C from SF by pasture material was observed in the soil (Table 2), suggesting that organic C dynamic is relatively fast, notwithstanding the high clay content (Table 1). These findings were in accordance with previous (Roscoe *et al*., 2001) studies, suggesting a fast turnover rate of SOM in tropical oxisols. The low replacement of C in sand fraction may attribute to present the chemically recalcitrant, or its location in the soil matrix preserved SOM against decomposition

CONCLUSION

After 7 years of pasture, the levels of organic carbon in the first 30 cm of the studied soil change significantly, partly due to the high biomass production of the tropical grasses, and especially to the protective effect of the high clay content. Nevertheless, a substantial replacement of the original organic C was observed, especially in the topsoil, which suggests a high turnover rate of C in this system.

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