Research Article Open Access

Amir Hossein Jafarzadeh-Haghighi, Jusop Shamshuddin*, Jol Hamdan, and Norhazlin Zainuddin

Structural composition of organic matter in particle-size fractions of soils along a climo-biosequence in the Main Range of Peninsular Malaysia

DOI 10.1515/geo-2016-0034
Received November 28, 2015; accepted March 21, 2016

Abstract: Information on structural composition of organic matter (OM) in particle-size fractions of soils along a climo-biosequence is sparse. The objective of this study was to examine structural composition and morphological characteristics of OM in particle-size fractions of soils along a climo-biosequence in order to better understand the factors and processes affecting structural composition of soil organic matter. To explore changes in structural composition of OM in soils with different pedogenesis, the A-horizon was considered for further analyses including particle-size fractionation, solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and scanning electron microscopy (SEM). Due to the increase in the thickness of organic layer with increasing elevation, the A-horizon was situated at greater depth in soils of higher elevation. The relationship between relative abundances of carbon (C) structures and particle-size fractions was examined using principal component analysis (PCA). It was found that alkyl C (20.1-73.4%) and O-alkyl C (16.8-67.7%) dominated particle-size fractions. The proportion of alkyl C increased with increasing elevation, while O-alkyl C showed an opposite trend. Results of PCA confirmed this finding and showed the relative enrichment of alkyl C in soils of higher elevation. Increase in the proportion of alkyl C in 250-2000 μm fraction is linked to selective preservation of aliphatic compounds derived from root litter. SEM results showed an increase in root contribution to the 250-2000 μm fraction with increasing elevation. For the <53 μm fraction, pedogenic process of podzolization is responsible for the relative enrichment of alkyl C. This study demonstrates that changes in structural composition of OM in particle-size fractions of soils along the studied climo-biosequence are attributed to site-specific differences in pedogenesis as a function of climate and vegetation.

Keywords: alkyl C, O-alkyl C, pedogenesis, scanning electron microscopy, solid-state ^{13}C CPMAS NMR spectroscopy

1 Introduction

Climatic factors and vegetation types are believed to control structural composition of soil organic matter (SOM) [1]. Climate and vegetation are considered as two independent variables in the state-factor model [2]. According to this model, climo-biosequences allow evaluating effects of climate and vegetation on soil development [3]. Climo-biosequences can also be used to examine changes in the structural composition of SOM as influenced by climate and vegetation. In addition, climate-gradient studies (i.e. climosequences) by substituting space for time offer further insight into the effects of climate on such structural alterations occurring over longer periods of time [4]. Studies on structural composition of SOM along the climatic and bioclimatic gradients have focused on bulk soil, e.g. [1, 5, 6] and often disregarded fractions of SOM.

Several chemical and physical fractionation schemes have been proposed to isolate SOM fractions with similar chemical/physical composition [7]. Physical fractionation of soil based on the size and/or density of particles leads to the separation of uncomplexed organic matter (OM) and organo-mineral complexes [8, 9]. Such fraction-

***Corresponding Author: Jusop Shamshuddin:** Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, E-mail: shamshud@upm.edu.my; phone number: +60389474865
Amir Hossein Jafarzadeh-Haghighi, Jol Hamdan: Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Norhazlin Zainuddin: Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ation procedure yields OM fractions that are more chemically homogenous compared to the bulk soil [10]. Particle-size fractionation aims to separate OM associated with primary particles (i.e. sand, silt, and clay) after complete disaggregation [11]. SOM pools obtained from particle-size fractionation are directly related to the structure and functions of SOM in situ [12]. For example, of the OM pools isolated via particle-size fractionation, the OM in the sand fraction known as particulate organic matter (POM) shows the most structural similarities to the original plant input [10]. This normally labile fraction provides a source of energy and nutrients for microorganisms [13]. Solid-state ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy has been widely used to examine the structural composition of OM in particle-size fractions, e.g. [14–19]. This method is non-destructive and doesn't require extraction of OM [20]. The ^{13}C CPMAS NMR spectra, which are run under similar experimental conditions, allow semi quantitative characterization of OM in particle-size fractions [15]. Generally, the proportion of alkyl C would increase and O-alkyl C decrease with decreasing particle-size [21].

Little is known about changes in structural composition of OM in particle-size-fractions of soils along a climo-biosequence. Examining soils within a mountain range is considered as a common form of climosequence and/or climo-biosequence [3]. The Main Range of Peninsular Malaysia, the backbone mountain range located in the center of the peninsula, extends northward into the Peninsular Thailand and southward to the Indonesian tin islands [22]. Gradients of soil forming factors such as climate and vegetation on fairly uniform parent material in the Main Range [23] enables us to investigate such alterations in structural composition of OM. The research question is whether changes in structural composition of OM in particle-size fractions of soils along a climo-biosequence can be ascribed to differences in climate and vegetation or pedogenesis as a function of the two variables. Our objective was to examine structural composition and morphological characteristics of OM in various particle-size fractions of soils along a climo-biosequence in order to better understand the factors and processes affecting structural composition of SOM. We hypothesized that pedogenesis as a function of climate and vegetation controls structural composition of OM in particle-size fractions of soils along a climo-biosequence. To test this hypothesis, samples from the A-horizon of soils were fractionated into three particle-size fractions (250-2000 µm, 53-250 µm, and <53 µm). The structural composition and morphological characteristics of OM in each particle-size fraction were analyzed by ^{13}C CPMAS NMR spectroscopy and scanning electron microscopy (SEM), respectively.

2 Materials and methods

2.1 Study area and soil sampling

The study area was located along an elevation gradient in the Main Range of Peninsular Malaysia (04°14'–04°31'N, 101°18'–101°23'E) (Figure 1). Variation in climate and vegetation was a major cause of differences in pedogenesis along the respective elevation gradient. After preliminary field studies, a group of four soil profiles (P1-P4), representative of major elevation zones, were examined (Table 1). According to the state-factor model [2], this group of soils formed a climo-biosequence in which two of the state factors (climate and vegetation) were allowed to vary and the other three were held constant as much as possible.

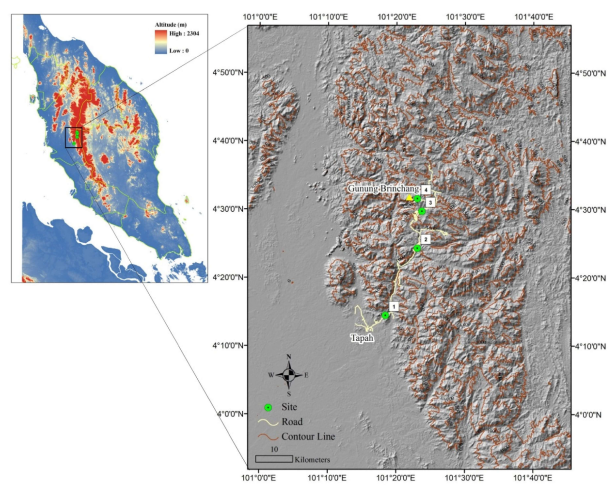

Figure 1: Map of the study area showing the location of the investigated sites (1-4) along an elevation gradient in the Main Range of Peninsular Malaysia (cited from Jafarzadeh-Haghighi et al. [38])

With increasing elevation, mean annual temperature decreased from 26.9 °C recorded at Hospital Tapah meteorological station (04°12'N, 101°16'E; 35.1 m asl) to 18 °C recorded at Cameron Highlands meteorological station (04°28'N, 101°22'E; 1545 m asl). The mean annual rainfall was 3282 mm at the Hospital Tapah station and 2977 mm at the Cameron Highlands station. The occurrence and duration of fog as an additional water input increased with increasing elevation [24]. As a function of climatic factors, four vegetation zones occur with increasing elevation, progressing from hill dipterocarp forest (<750 m asl)

through upper dipterocarp forest (750-1200 m asl), myrtaceous forest (1200-1800 m asl), and ericaceous forest (>1800 m asl) [25]. All sampling sites were situated on similar geomorphic position with slopes ranging from 20 to 30%. All soils were developed from granitoid parent material [23]. The granitoid bedrock in the Main Range of Peninsular Malaysia is of Triassic age (200-230 Ma) [22, 26]. This claim had been proven by U-Pb zircon emplacement which indicated the age of granite ranging from late Triassic (230 ± 9 Ma) to early Jurassic (207 ± 14 Ma) with a peak around 210 Ma [27]. Accordingly, the geomorphic age was assumed similar for all sampling sites.

Soil samples were collected by pedogenic horizons [28], air-dried and passed through a 2-mm sieve in order to remove coarse fragments. In the current study, to explore changes in structural composition of OM in soils with different pedogenesis, the A-horizon was considered for further analyses including particle-size fractionation, solid-state ^{13}C CPMAS NMR spectroscopy, and scanning electron microscopy (SEM). The thickness and situation of the A-horizon in the studied soil profiles are illustrated in Figure 2.

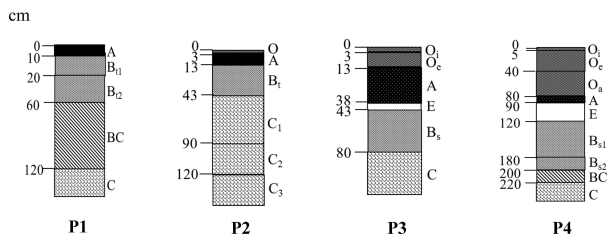

Figure 2: Schematic diagram of the studied soil profiles showing master horizons, their sequence and thickness

2.2 Soil analysis

Soil pH was measured in 1:2.5 soil to water ratio [29]. Total carbon (TC) was determined by dry combustion method using LECO TruMac elemental analyzer. The absence of inorganic carbon (C) was confirmed by 4 mol L^{-1} hydrochloric acid (HCl) [30]; therefore, the concentration of TC was equal to organic carbon (OC). Total nitrogen (N) was analyzed by Kjeldahl digestion [31].

2.3 Particle-size fractionation

The particle-size fractionation procedure described in Sanderman et al. [7] was modified to use in this study. Soil samples were subjected to chemical dispersion by adding 40 mL sodium hexametaphosphate (5 g L^{-1}) to 10 g of the soils (<2 mm) that were previously weighed in 50 mL centrifuge tubes. Soil samples and dispersant were mixed for 30 s using vortex mixer. Based on preliminary experiment, 10 5-mm glass beads were added to each centrifuge tube to aid in complete soil dispersion. Samples were then shaken for 16 hours on a rotary shaker set to 60 rpm. Wet sieving using stacks of 250 and 53 µm sieves separated 250-2000 µm (coarse sand) and 53-250 µm (fine sand) fractions. Thereafter, the remaining particles including silt and clay were flocculated using saturated aluminum sulfate, kept overnight, and centrifuged to obtain the <53 µm fractions. The <53 µm fractions were washed three times with distilled water to remove extra salt. All fractions were oven-dried at 65 °C, homogenized and weighed to calculate mass recovery. Particle-size fractionation was carried out in triplicate to get enough materials for further analyses. After fractionation, between 98.1 and 99.5% of initial masses were recovered. A finely ground (<250 µm) subsample from each fraction was analyzed for TC by LECO TruMac elemental analyzer. The TC recovery ranged from 92.4 to 109.6%.

2.4 Sample preparation for solid-state ^{13}C CPMAS NMR analysis

OC in the 250-2000 µm fraction had to be concentrated prior to solid-state ^{13}C CPMAS NMR analysis by reducing the amount of sand particles in this fraction. To this end, the 250-2000 µm fraction was subjected to sodium polytungstate (SPT) (Sometu, Germany) solution adjusted to a density of 1.85 g cm^{-3}. Density of 1.85 g cm^{-3} was chosen based on the study of Cambardella and Elliot [32]. Particles in sediment of density higher than 1.85 g cm^{-3} were mostly composed of sand grains while the OM in SPT solution was floated. The floated materials were separated with the aid of Büchner funnel fitted with a 0.7 µm glass fiber filter (Sartorius, Germany) and washed with 100 mL distilled water to remove the SPT. The OM on the filter was oven-dried at 65 °C for 24 hours. The organic materials were brushed off from the surface of the filter and kept in plastic vial for ^{13}C CPMAS NMR analysis. The 53-250 µm fractions were taken for ^{13}C CPMAS NMR analysis without any preparation.

Hydrofluoric acid (HF) treatment was applied to the <53 µm fractions before ^{13}C CPMAS NMR analysis in order to enhance the signal to noise ratio of the NMR spectra through concentrating OC and reducing the interference from paramagnetic compounds [33]. HF treatment procedure was adapted from Sanderman et al. [7]. Briefly,

Table 1: Characterization of the study sites and soil profiles

Site/soil profile	Elevation (m asl)	Elevation zone	Soil moisture-temperature regime[a]	Vegetation species[b]	Soil classification (Soil Taxonomy[c] and WRB[d])
1/P1	155	Foothill (<750 m asl)	Udic-Isohyperthermic	*Shorea leprosula*	Typic Paleudult; Vetic Cutanic Acrisols
2/P2	1129	Lower montane (750–1200 m asl)	Perudic-Isohyperthermic	*Shorea platyclados*	Typic Haplohumult; Vetic Acrisols
3/P3	1567	Lower montane (1200–1800 m asl)	Perudic-Isothermic	genus of *Syzygium*	Typic Haplorthod; Albic Folic Podzols
4/P4	1946	Upper montane (>1800 m asl)	Perudic-Isomesic	Ericaceous genera (e.g. *Rhododendron*)	Terric Haplosaprist; Sapric Ombric Histosols

[a] From Paramananthan [23] [b] B. Perumal, personal communication [c] Keys to Soil Taxonomy [45] [d] IUSS Working Group WRB [46]

37.5 mL of 2% HF was added to the 2.5 g (<250 µm) <53 µm fractions (soil/solution ratio of 1/15), thoroughly mixed using vortex mixer and shaken in the sequence of 5 times for 1 hour, 3 times for 16 hours, and 1 time for 48 hours. At the end of each shaking stage, samples were centrifuged at 2000 rpm for 15 min. The supernatant of the first three treatments was filtered using 0.7 µm glass fiber filter to collect the light fractions floated in HF, which were recombined with final samples at the end of the treatment. Subsequently, the samples were washed three times with distilled water to eliminate extra HF, transferred to preweighed 500 mL polypropylene beaker, and oven dried at 65 °C. Those samples that dispersed during the washing stage were flocculated with saturated aluminum sulfate.

2.5 Solid-state ^{13}C CPMAS NMR analysis

The structural composition of OM in particle-size fractions were examined by solid-state ^{13}C CPMAS NMR spectroscopy. The spectra were acquired on a JEOL ECA 400 spectrometer (JEOL NMR, JEOL RESONANCE, Inc., Japan) equipped with 9.4 T (400 MHz) magnetic field operating at a ^{13}C resonance frequency of 100.53 MHz. Finely ground samples were loaded into a 4 mm-diameter silicon nitride rotor with Vespel® cap and bottom, packed down firmly, and spun at 6 kHz. RAMP-CP was employed to compensate the cross polarization (CP) inefficiencies at high fields and high spinning rates. The acquisition parameters for CP analysis were set as follows: a 5 s relaxation delay, 2.75 µs proton 90° pulse length, 1.5 ms contact time, and 50.9 ms acquisition time. The number of scans for all samples was 15000. The chemical shift was referenced to high frequency adamantine peak (38.55 ppm) spectrum [34] which was measured independently with identical spectrometer setting. The measurement data (free induction decay) was processed with Delta v5.0.3 software (JEOL RESONANCE, Inc.). One-dimensional (1D) NMR data processing included DC balance, zero filling to 8 k, and single exponential line broadening of 50 Hz, fourier transform, phase and baseline correction. The spectra were plotted between -100 and 300 ppm and stacked by Y offset using the OriginPro 9.1 software (Originlab Corporation, Northampton, USA). Four chemical shift regions were apparent on the spectra as follows: 0 to 45 ppm (alkyl C), 45 to 110 ppm (oxygenated alkyl C, abbreviated as O-alkyl C in further text), 110 to 165 ppm (aromatic and phenolic C), and 165 to 215 ppm (carbonyl C including carboxylic, ester, amide and ketone C). The relative abundances of C structures were determined by integration of the spectra using the chemical shift limits and expressed as a percentage of total signals. To evaluate the extent of decomposition of OM in particle-size fractions, alkyl C/O-alkyl C ratio was calculated [35]. Variation in relative intensities due to 1D NMR data processing was less than 6%.

2.6 Scanning electron microscopy

Unground samples of 250-2000 and 53-250 µm fractions and ground samples of <53 µm fractions were placed on SEM stub that was covered with a thin layer of high conductivity paint (Acheson Colloids Company, Plymouth) to fix the samples. The samples were gold-coated prior

to SEM analysis. Gold-coated samples were imaged with SEM (LEO 1455 VPSEM) with both quadrant back scattering detector (QBSD) and secondary electron detector (SE). The electron beam acceleration voltage of 10 kV was used to avoid charging of the particles. Spectrograms were recorded using energy dispersive X-ray (EDX) detector (Oxford Instruments Analytical Ltd, UK) attached to the electron microscope to investigate the elemental composition of the samples. The atomic O/C ratios of POM in 250–2000 µm fractions were calculated as the criteria reflecting the oxidation state of organic particles.

2.7 Statistical analysis

Calculations of mean and standard deviation were performed using Microsoft® Excel® 2010 (Microsoft, Redmond, USA). The relationship between relative abundances of C structures (alkyl C, O-alkyl C, aromatic C, and carbonyl C) as variables, indicating changes in structural composition of OM, and a total of 12 particle-size fractions separated from the A-horizon of four soil profiles was examined using principal component analysis (PCA). Two principal components were extracted. These components represented 90.9% of variance in structural composition of OM in particle-size fractions. PCA was performed using OriginPro 9.1 software (Originlab Corporation, Northampton, USA).

Table 2: Selected chemical properties for the A-horizon of the studied soil profiles

Soil profile	pH in H_2O	OC	N
		g kg^{-1}	
P1	4.54	22.0	2.5
P2	4.56	33.3	2.9
P3	4.00	13.3	1.0
P4	4.13	43.2	1.6

3 Results and discussion

3.1 Soil characterization

The soils under study were classified as Typic Paleudults (P1), Typic Haplohumults (P2), Typic Haplorthods (P3) and Terric Haplosaprists (P4) (Table 1). All soils were acidic with pH values varying from 4.00 to 4.56 (Table 2). The soils exhibited high OC concentration ranging from 13.3 to 43.2 g kg^{-1} (Table 2). Table 3 shows the distribution of particle-size fractions, OC size pools and their distribution in the bulk soil samples from the A-horizon of the studied soils. The <53 µm fraction was the dominant fraction in P1 and P2, followed by 250-2000 and 53-250 µm fractions. However, in P3 and P4, the dominant fraction was 250-2000 µm which was followed by <53 and 53-250 µm fractions. The OC content in the <53 µm fraction was larger than the other two fractions in all soils. This fraction accounted for 61.5-92.3% of the total OC.

3.2 Structural composition of organic matter in particle-size fractions

Figure 3 shows the solid-state ^{13}C CPMAS NMR spectra acquired for particle-size fractions from the A-horizon of the studied soils. Resonances around 30, 72, 105, and 173 ppm were observed in all ^{13}C CPMAS NMR spectra. Other common resonances in the spectra collected from studied soils are around 56, 65, 88, 130, and 150 ppm. The resonance near 30 ppm was typical for methylene C in long chain aliphatic compounds (i.e. fatty acids and waxes). The peak near 56 ppm corresponded to methoxyl carbon [36]. The resonance around 72 ppm was assigned to C2, C3, and C5 and that near 105 ppm to anomeric C1 in cellulose and hemicellulose, which were accompanied by the shoulders around 65 and 88 ppm corresponding to C6 and C4 carbons, respectively [37]. The signals near 130 and 150 ppm were attributed to C- or H-substituted and O-substituted (e.g. phenolic C) aromatic carbon, respectively, in lignin. Carboxylic, amide, and ester carbon occurred near 173 ppm [14].

Table 4 shows the distribution of C structures in the particle-size fractions separated from the A-horizon of the studied soils. Alkyl C (20.1-73.4%) and O-alkyl C (16.8-67.7%) dominated the particle-size fractions (more than 80% of the total signals), which agrees with the findings of Schöning and Kögel-Knabner [18] who reported the dominance of alkyl C and O/N-alkyl C in particle-size fractions throughout the Cambisol and Luvisol profiles under forests in France and Germany. Compared to alkyl C and O-alkyl C, low proportion of aromatic C and carbonyl C were observed in most samples. Figure 3 and Table 4 clearly demonstrate that structural composition of SOM was strongly influenced by particle size. The proportion of alkyl C increased, while the proportion of O-alkyl C decreased from 250–2000 to <53 µm fractions. A decrease in the O-alkyl C with decreasing particle-size indicated the preferential decomposition of carbohydrates in plant

Table 3: Distribution of particle-size fractions, organic carbon (OC) size pools and their distribution in the bulk soil samples from the A-horizon of the studied soils [values are mean with standard deviation in parentheses (n=3)]

Soil profile	Particle-size fraction µm	Particle-size distribution % of total dry weight	OC content g kg_{soil}^{-1}	OC distribution % of total OC
P1	250–2000	37.6 (0.8)	3.1	14.0
	53–250	15.2 (0.6)	2.0	9.1
	<53	45.3 (1.4)	15.6	70.7
P2	250-2000	28.5 (1.1)	4.2	12.7
	53–250	6.9 (0.1)	2.6	7.7
	<53	63.1 (1.1)	24.0	72.0
P3	250–2000	59.1 (1.4)	3.4	25.7
	53–250	16.2 (0.7)	1.8	13.8
	<53	24.2 (0.7)	8.2	61.5
P4	250–2000	56.5 (3.8)	5.6	13.0
	53–250	12.2 (2.0)	1.8	4.3
	<53	30.7 (1.9)	39.9	92.3

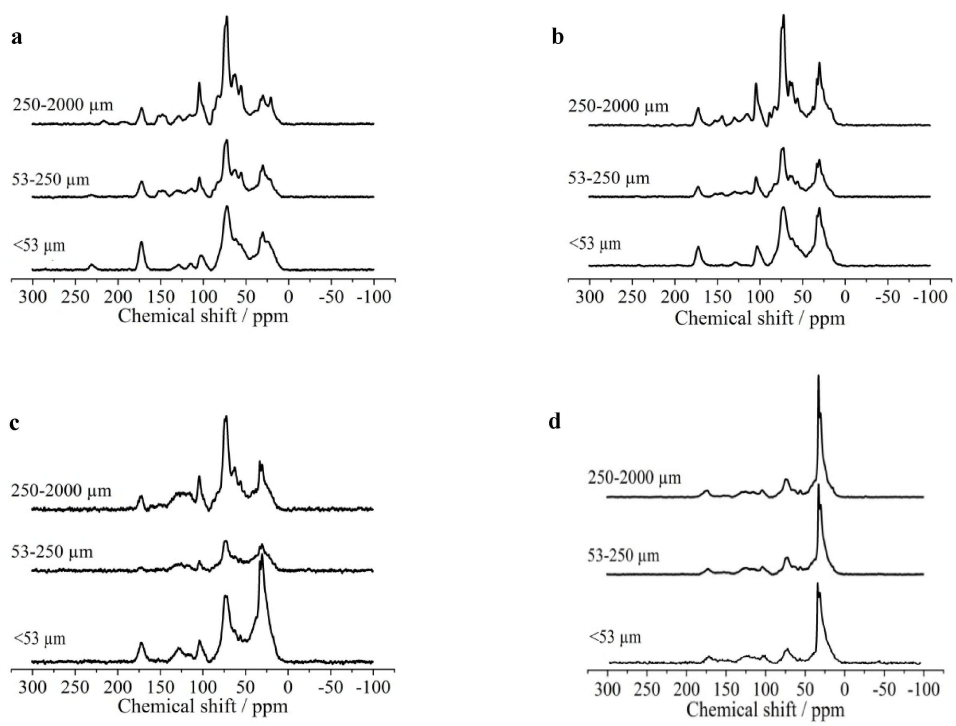

Figure 3: Solid-state ^{13}C CPMAS NMR spectra of particle-size fractions from the A-horizon of the studied soils: (a) P1; (b) P2; (c) P3; and (d) P4

Table 4: Distribution of carbon (C) structures in particle-size fractions separated from the A-horizon of the studied soils [values are mean with standard deviation of data processing replicates (n=3)].

Soil Profile	Fraction µm	Alkyl C (0–45 ppm) %	O-alkyl C (45–110 ppm)	Aromatic C (110–165 ppm)	Carbonyl C (165–215 ppm)	Alkyl C/O-alkyl C
P1	250–2000	20.1 (0.5)	67.7 (0.4)	8.5 (0.1)	3.8 (0.1)	0.30
	53–250	25.5 (0.4)	57.1 (0.3)	11.2 (0.3)	6.3 (0.3)	0.45
	<53	30.5 (0.0)	57.3 (0.2)	3.7 (0.1)	8.4 (0.1)	0.53
P2	250–2000	27.9 (0.1)	60.0 (0.1)	8.3 (0.0)	3.7 (0.0)	0.46
	53–250	30.7 (0.3)	57.0 (0.9)	8.4 (0.5)	3.8 (0.2)	0.54
	<53	38.3 (0.0)	54.2 (0.2)	1.5 (0.0)	6.0 (0.2)	0.71
P3	250–2000	23.2 (0.3)	58.2 (0.2)	16.2 (0.3)	2.4 (0.1)	0.40
	53–250	38.7 (0.4)	50.2 (0.2)	11.1 (0.2)	tr[a]	0.77
	<53	47.0 (0.7)	41.3 (0.5)	6.9 (0.1)	4.1 (0.2)	1.14
P4	250–2000	63.8 (0.3)	23.4 (0.1)	8.5 (0.3)	4.2 (0.2)	2.72
	53–250	59.0 (1.3)	25.9 (0.6)	11.5 (0.6)	3.7 (0.2)	2.28
	<53	73.4 (0.5)	16.8 (0.2)	9.8 (0.3)	tr	4.37

[a] trace

residues [37]. The accumulation of alkyl C in the fine fractions of the studied soils could be due to both the selective preservation of aliphatic compounds in organic residues and in situ synthesis of alkyl-type products by microorganisms as stated by Baldock et al. [14]. Aromatic C was mainly concentrated in the 53–250 µm fractions likely due to the usage of easily decomposable carbohydrates by microorganisms leading to selective preservation of more recalcitrant compounds. Baldock et al. [14] reported a similar dominance of aromatic C in the fine sand and silt fractions of soil samples from Mollisols and Oxisols. The contribution of aromatic C to the fine fractions was rather small. This occurs if the soil has not been subjected to regular burning [18]. Differences in the proportion of carbonyl C were variable and did not follow the consistent trend in all soils.

The results of ^{13}C CPMAS NMR in this study are in general agreement with the findings of previous studies, which used ^{13}C CPMAS NMR analysis to characterize the structural composition of OM in particle-size fractions, e.g. [14, 18, 19, 37]. In each soil and from the same parent vegetation, differences in structural composition of OM between fractions of physical fractionation were possibly ascribed to a different degree of decomposition as stated by Golchin et al. [9]. The extent of decomposition in the respective particle-size fractions followed the model formulated by Baldock et al. [14]. According to this model, decomposition of plant residues followed a continuum from not degraded plant residues in the larger fraction (250-2000 µm) to partially decomposed plant residues in the intermediate fraction (53-250 µm) to degraded residues in the finest fraction (<53 µm). In this regard, the alkyl C/O-alkyl C ratio as an index of the extent of decomposition [35] increased from 250–2000 to <53 µm fraction in all studied soils (Table 4). Consequently, OM associated with coarse sand consisted of labile plant residues, while stable carbon tended to be associated with clay+silt particles.

In comparing soils along the climo-biosequence, the proportion of alkyl C increased, whereas the proportion of O-alkyl C decreased with increasing elevation. The proportion of aromatic C and carbonyl C did not show any trend along the climo-biosequence. Results of PCA verified the above-mentioned finding (Figure 4). The biplot showed the effectiveness of the first component, mainly composed of O-alkyl C and alkyl C, in separating particle-size fractions of soils at low and high elevations. In this biplot, particle-size fractions of P4 were concentrated near the vector of alkyl C showing relative enrichment of alkyl C in soils of higher elevation. These changes are explained by differences in pedogenesis as a function of climate and vegetation. A decrease in temperature with rising elevation slows down the decomposition of SOM [3], providing conditions for the accumulation of OM on the soil surface. In addition, poor degradability of litters from plants with high waxes such as genera of ericaceous and mosses (abundant in the forest above 1800 m asl) could retard the process of decomposition in areas of high elevation. In another study, we found organic horizons of soil under eri-

caceous forest (P4) were rich in aliphatic structures [38]. Therefore, thickness of the organic layer increased with increasing elevation. Accordingly, the A-horizon was located at greater depth with increasing elevation (Figure 2). For example, the A-horizon of P4 was overlain by 80 cm organic horizons with sequence of O_i-O_e-O_a. The aboveground organic input had little influence on the OM composition in soils of higher elevation where the A-horizon was situated at greater depth. At greater depth OM was almost derived from root litter. The aliphatic nature of POM in 250–2000 and subsequently 53–250 μm fractions from the A-horizon of P4 might originate from root-derived suberin. Rumpel et al. [39] and Nierop [40] found an important contribution of root-derived suberin to SOM composition at greater depth in which POM was mainly derived from root litter. The presence of root fragments in the 250–2000 μm fraction from the A-horizon of P4 was confirmed by electron microscopy (Figure 5d). In the <53 μm fraction of the A-horizon from the respective soil, in addition to in situ synthesis of aliphatic structures by microorganisms, podzolization was responsible for the relative enrichment of alkyl C by transporting hydrophilic O-alkyl and carboxyl C to the subsoil. Podzolization was the active pedogenic process in soils of higher elevation (P3 and P4) in the Main Range of Peninsular Malaysia (A.H. Jafarzadeh-Haghighi, unpublished data). The OM in the <53 μm fraction are often in association with clay and silt particles. The clay+silt particles provide remarkable degree of biological protection [41, 42].

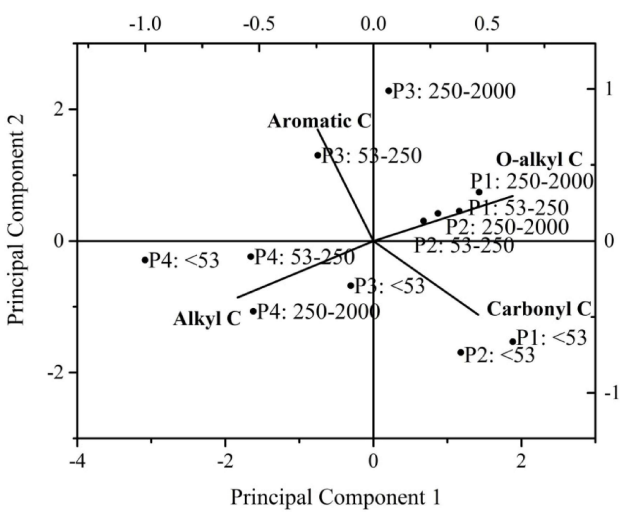

Figure 4: Principal component analysis for carbon (C) structures derived from NMR spectra of particle-size fractions separated from the A-horizon of the studied soils. Black circles represent the particle-size fractions and vectors indicate the C structures

3.3 Morphological characteristics of organic matter in particle-size fractions

The shape of organic particles and their surface characteristics may depend on the original plant species and the type of fragment (e.g. leaves and roots) as well as the degree of decomposition that the organic particle has undergone [43]. SEM micrograph of the 250–2000 μm fractions indicated similarities between POM in this fraction and the original plant fragments. For example, cell alignment (Figure 5b, 5c, and 5d) was easily recognizable in this fraction. This is similar to the findings of Kaiser et al. [43] for different coarse organic particles ranging from 315 to 2000 μm and Golchin et al. [9] for the free light fractions (<1.6 g cm^{-3}). Inspection of this fraction under higher magnification clearly showed the presence of fine mineral particles with an angular shape on the surface of organic particles (Figure 5b). This phenomenon indicated possible interactions of soil mineral particles with products of the microbial metabolism as stated by Kaiser et al. [43]; however, some were free of mineral components. In terms of visual appearance, the POMs in 250–2000 μm fraction become more rounded and blackish with increasing elevation. For example, several discrete spheres were observed in 250–2000 μm fraction from the A-horizon of P4. These particles were amorphous under SEM. By applying EDX to the randomly selected POMs in 250–2000 μm fraction from the A-horizon of all studied soils, POM of P4 showed lower atomic O/C ratio compared to its counterpart in other soils revealing higher degree of decomposition (Figure 5). SEM and EDX results supported the findings of ^{13}C CPMAS NMR and helped to interpret the higher alkyl C/O-alkyl C ratio for 250–2000 μm fraction of P4 compared to the other soils. SEM and EDX findings revealed that in addition to selective preservation of alkyl C derived from root litter, the contribution of alkyl C to 250-2000 μm fraction of P4 increased due to increase in decomposition of plant residues at greater depth which, in turn, resulted in high alkyl C/O-alkyl C ratio for the respective fraction.

The smaller POM particles appear to be more decomposed than the larger particles [32]. The 53–250 μm fractions contained smaller POM compared to that of the 250-2000 μm fraction. The POM in the 53–250 μm fractions bore little resemblance to the parent vegetation fragment, and seemingly more decomposed than POM in 250–2000 fraction (Figure 6a and 6b). However, undecomposed plant debris and fine roots can also be observed in some samples. Similar results were reported for fine sand fraction (20-250 μm) by Amelung et al. [44]. It is believed that OM in <53 μm fraction consisted of microbiologically altered plant residues and microbial residues. In this fraction,

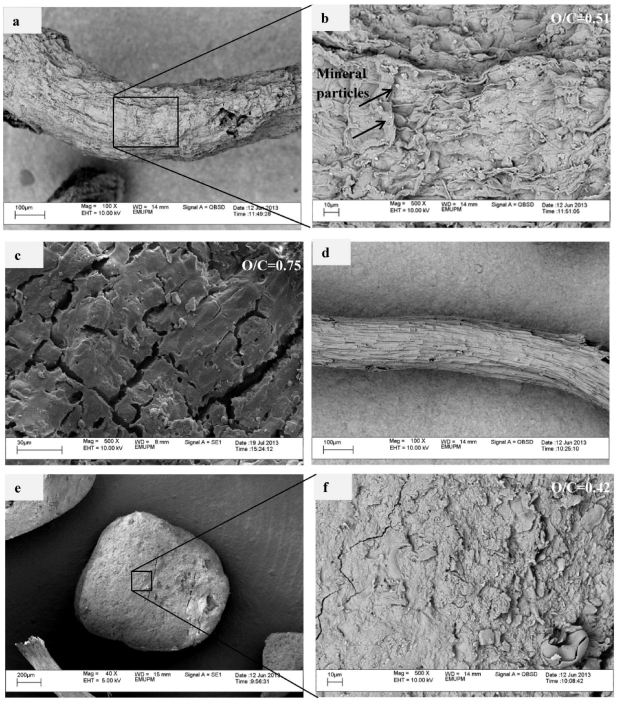

Figure 5: Scanning electron micrograph of POM in 250–2000 µm fractions from the A-horizon of P1 (a and b), P3 (c) and P4 (d, e, and f). The atomic O/C ratios of POM in 250-2000 µm fractions of the respective soils are shown at the top right-hand corner of the micrographs b, c, and f

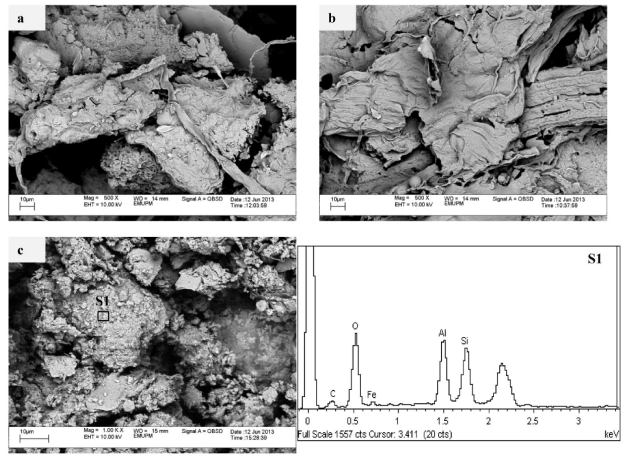

Figure 6: Scanning electron micrograph of POM in 53–250 µm fractions from the A-horizon of P1 (a) and P4 (b) and microaggregates in <53 µm fractions from the A-horizon of P1 (c). Energy dispersive X-ray (EDX) spectrum shows the presence of C in <53 µm fraction. EDX spectrum is displayed at one area by S1

OM was intimately associated with mineral particles. Due to reaggregation during the process of oven drying, this fraction showed several microaggregates having organo-mineral character (Figure 6c). Thus, compared to the other two fractions, OM particles in this fraction were not visible under electron microscope; however, the presence of C was identifiable by EDX spectrograms.

4 Conclusions

The following conclusions were obtained from this study:

1. Changes in structural composition of OM in particle-size fractions from the A-horizon of soils along the studied climo-biosequence are attributed to differences in pedogenesis as a function of climate and vegetation. Depth of the A-horizon, root litter input, and pedogenic processes have crucial impacts on structural composition of SOM in particle-size fractions;
2. An increase in the proportion of alkyl C in the coarse fractions (250–2000 and 53–250 µm) of the studied soils with increasing elevation is ascribed to selective preservation of aliphatic compounds derived from root litter. For the fine fraction (<53 µm), pedogenic process of podzolization is responsible for the relative enrichment of alkyl C; and
3. This study confirms that results of SEM/EDX could be a good complement for ^{13}C CPMAS NMR spectroscopy for studying the degree of decomposition of POM in 250-2000 and 53-250 µm fractions.

Acknowledgement: Thanks are given to Universiti Putra Malaysia (UPM) for providing technical and financial support for this study. This work was funded by the Research University Grant Scheme (RUGS) under Grant No. 01-02-12-1700RU (9327000) provided by UPM. We would like to express our gratitude to Dr Jeffrey Baldock at CSIRO Land and Water, Australia for providing helpful information on the method of particle-size fractionation, Siti Busyra Abu Hassan and Francis Voon Wai Thoo for their role in NMR analysis.

References

[1] Faz Cano A., Mermut A.R., Ortiz R., Benke M.B., Chatson B., ^{13}C CP/MAS-NMR spectra of organic matter as influenced by vegetation, climate, and soil characteristics in soils from Murcia, Spain. *Can. J. Soil Sci.*, 2002, 82, 403–411

[2] Jenny H., Factors of Soil Formation: A System of Quantitative Pedology. McGraw-Hill Book Company, Inc, New York, 1941

[3] Schaetzl R., Anderson S., Soils: Genesis and Geomorphology. Cambridge University Press, New York, 2005

[4] Djukic I., Zehetner F., Tatzber M., Gerzabek M.H., Soil organic-matter stocks and characteristics along an Alpine elevation gra-

dient. *J. Plant Nutr. Soil Sci.*, 2010, 173, 30–38

[5] Amelung W., Flach K.W., Zech W., Climatic effects on soil organic matter composition in the Great Plains. *Soil Sci. Soc. Am. J.*, 1997, 61, 115–123

[6] Dalmolin R.S.D., Gonçalves C.N., Dick D.P., Knicker H., Klamt E., Kögel-Knabner I., Organic matter characteristics and distribution in Ferralsol profiles of a climosequence in southern Brazil. *Eur. J. Soil Sci.*, 2006, 57, 644–654

[7] Sanderman J., Baldock J., Hawke B., Macdonald L., Massispuccini A., Szarvas S., National Soil Carbon Research Programme: Field and Laboratory Methodologies. CSIRO Land and Water, Urrbrae, 2011

[8] Christensen B.T., Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur. J. Soil Sci.*, 2001, 52, 345–353

[9] Golchin A., Oades J.M., Skjemstad J.O., Clarke P., Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. *Aust. J. Soil Res.*, 1994, 32, 285-309

[10] Norris C.E., Quideau S.A., Bhatti J.S., Wasylishen R.E., Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study. *Glob. Chang. Biol.*, 2011, 17, 480–494

[11] von Lützow M., Kögel-Knabner I., Ekschmitt K., Flessa H., Guggenberger G., Matzner E., et al. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.*, 2007, 39, 2183–2207

[12] Christensen B.T., Physical fractionation of soil and organic matter in primary particle size and density separates. *Adv. Soil Sci.*, 1992, 20, 1–90

[13] Baldock J.A., Skjemstad J.O., Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Org. Geochem.*, 2000, 31, 697–710

[14] Baldock J.A., Oades J.M., Waters A.G., Peng X., Vassallo A.M., Wilson M.A., Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. *Biogeochemistry.*, 1992, 16, 1–42

[15] Quideau S.A., Chadwick O.A., Benesi A., Graham R.C., Anderson M.A., A direct link between forest vegetation type and soil organic matter composition. *Geoderma.*, 2001, 104, 41–60

[16] Chen J.S., Chiu C.Y., Characterization of soil organic matter in different particle-size fractions in humid subalpine soils by CP/MAS ^{13}C NMR. *Geoderma.*, 2003, 117, 129–141

[17] Rumpel C., Eusterhues K., Kögel-Knabner I., Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. *Soil Biol. Biochem.*, 2004, 36, 177–190

[18] Schöning I., Kögel-Knabner I., Chemical composition of young and old carbon pools throughout Cambisol and Luvisol profiles under forests. *Soil Biol. Biochem.*, 2006, 38, 2411–2424

[19] Chen J.S., Chung T.L., Tian G., Chiu C.Y., Characterization of soil organic matter in perhumid natural cypress forest: comparison of humification in different particle-size fractions. *Bot. Stud.*, 2013, 54, 56

[20] Mathers N.J., Mao X.A., Xu Z.H., Saffigna P.G., Berners-Price S.J., Perera M.C.S., Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies. *Aust. J. Soil Res.*, 2000, 38, 769–787

[21] Preston C., Applications of NMR to soil organic matter analysis: history and prospects. *Soil Sci.*, 1996, 161, 144–166

[22] Ghani A.A., Plutonism. In: Hutchison C.S., Tan D.N.K. (Eds.), Geology of Peninsular Malaysia. University of Malaya and Geological Society of Malaysia, Kuala Lumpur, 2009, 211–232

[23] Paramananthan S., Soil genesis on igneous and metamorphic rocks in Malaysia. DSc Dissertation, State University of Ghent, Belgium, 1977

[24] Kumaran S., Hydrometeorology of tropical montane rainforest of Gunung Brinchang, Pahang Darul Makmur, Malaysia. PhD Thesis, Universiti Putra Malaysia, Malaysia, 2008

[25] Burgess P.F., Ecological factors in hill and mountain forests of the States of Malaya. *Malay. Nat. J.*, 1969, 22, 119–128

[26] Cobbing E.J., Pitfield P.E.J., Darbyshire D.P.F., Mallick D.I.J., The granites of the south-east asian tin belt. British Geological Survey, London, UK, 1992

[27] Metcalfe I., The Bentong-Raub suture zone. *J. Asian. Earth Sci.*, 2000, 18, 691-712

[28] Soil Survey Division Staff., Soil Survey Manual. Soil Conservation Service. USDA handbook 18, Washington, DC, 1993

[29] van Reeuwijk L.P., Procedures for Soil Analysis. International Soil Reference and Information, Wageningen, 2002

[30] Nelson D.W., Sommers L.E., Total carbon, organic carbon, and organic matter. In: Page A.L (Ed.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Inc and Soil Science Society of America, Inc, Madison, 1982, 539–577

[31] Bremner J.M., Mulvaney C.S., Nitrogen-total. In: Page A.L (Ed.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Inc and Soil Science Society of America, Inc, Madison, 1982, 595–624.

[32] Cambardella C.A., Elliot E.T., Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.*, 1992, 56, 777–783

[33] Simpson M.J., Preston C., Soil organic matter analysis by solid-state ^{13}C nuclear magnetic resonance spectroscopy. In: Carter M.R., Gregorich E.G. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, 2008, 681–692

[34] Earl W.L., Vanderhart D.L., Measurement of ^{13}C chemical shifts in solids. *J. Magn. Reson.*, 1982, 48, 35–54

[35] Baldock J.A., Oades J.M., Nelson P.N., Skene T.M., Golchin A., Clarke P., Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. *Aust. J. Soil Res.*, 1997, 35, 1061–1083

[36] Kögel-Knabner I., ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. *Geoderma.*, 1997, 80, 243–270

[37] Quideau S.A., Anderson M.A., Graham R.C., Chadwick O.A., Trumbore S.E., Soil organic matter processes: characterization by ^{13}C NMR and ^{14}C measurements. *For. Ecol. Manage.*, 2000, 138, 19–27

[38] Jafarzadeh-Haghighi A.H., Shamshuddin J., Hamdan J., Zainuddin N., Roslan I., Carbon contents and structural characteristics of organic matter in soils of a climo-biosequence in the Main Range of Peninsular Malaysia. *Asian J. Agric. Food Sci.*, 2015, 3, 529-541

[39] Rumpel C., Kögel-Knabner I., Bruhn F., Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. *Org. Geochem.*, 2002, 33, 1131–1142

[40] Nierop K.G.J., Origin of aliphatic compounds in a forest soil. *Org. Geochem.*, 1998, 29, 1009-1016

[41] Hassink J., The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant Soil.*, 1997,

191, 77–87
[42] Six J., Conant R.T., Paul E.A., Paustian K., Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil.*, 2002, 241, 155–176
[43] Kaiser M., Ellerbrock R.H., Sommer M., Separation of coarse organic particles from bulk surface soil samples by electrostatic attraction. *Soil Sci. Soc. Am. J.*, 2009, 73, 2118–2130
[44] Amelung W., Zech W., Zhang X., Follerr R.F., Tiessen H., Knox E., et al. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. *Soil Sci. Soc. Am. J.*, 1998, 62, 172–181
[45] Soil Survey Staff., Keys to Soil Taxonomy, 11th ed. USDA and Natural Resources Conservation Service, Washington, DC, 2010
[46] IUSS Working Group WRB., World Reference Base for Soil Resources 2006 World Soil Resources Reports No. 103, FAO, Rome, 2006.