






Preservation of organic matter in soils of a climo-biosequence in the Main Range of Peninsular Malaysia

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Abstract: Limited information is available about factors of soil organic carbon (SOC) preservation in soils along a climo-biosequence. The objective of this study was to evaluate the role of soil texture and mineralogy on preservation of SOC in the topsoil and subsoil along a climo-biosequence in the Main Range of Peninsular Malaysia. Soil samples from the A and B-horizons of four representative soil profiles were subjected to particle-size fractionation and mineralogical analyses including X-ray diffraction and selective dissolution. The proportion of SOC in the 250-2000 μm fraction (SOC associated with coarse sand) decreased while the proportion of SOC in the <53 μm fraction (SOC associated with clay and silt) increased with depth. This reflected the importance of the fine mineral fractions of the soil matrix for SOC storage in the subsoil. Close relationships between the content of SOC in the <53 μm fraction and the content of poorly crystalline Fe oxides [oxalate-extractable Fe (Fe_o) – pyrophosphate-extractable Fe (Fe_p)] and poorly crystalline inorganic forms of Al [oxalate-extractable Al (Al_o) – pyrophosphate-extractable Al (Al_p)] in the B-horizon indicated the importance of

poorly crystalline Fe oxides and poorly crystalline aluminosilicates for the preservation of SOC in the B-horizon. The increasing trend of Fe_o - Fe_p and Al_o - Al_p over elevation suggest that the importance of poorly crystalline Fe oxides and poorly crystalline aluminosilicates for the preservation of SOC in the B-horizon increased with increasing elevation. This study demonstrates that regardless of differences in climate and vegetation along the studied climo-biosequence, preservation of SOC in the subsoil depends on clay mineralogy.

Keywords: Particle-size fractionation; Poorly crystalline minerals; Soil organic matter; Soil texture

Introduction

Considerable amounts of soil organic carbon (SOC), though at lower concentrations compared to the organic layers and topsoil, can be stored in the subsoil, particularly B-horizons (Kaiser et al. 2002). Dick et al. (2005) reported between 54% and 66% of the SOC in soil profiles from different Brazilian Ferralsols were found below the A-horizon. In another study, Rumpel et al. (2002) found up to 75%

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of SOC in the mineral soils of two profiles in temperate regions were stored below the A-horizon. The importance of subsoil for carbon (C) storage has also been shown by C inventories (e.g. Batjes 1996; Jobbágy and Jackson 2000). Recent studies suggested that C dynamics in the subsoil and topsoil may be governed by different factors (environmental and/or pedological) (Salomé et al. 2010).

In natural ecosystems, dynamic equilibrium between C input and loss determines the amount of SOC, which is controlled by the soil forming factors (e.g. climate and vegetation) and soil properties (e.g. texture and mineralogy) (Tsui et al. 2013). The influence of the former is greater than the latter particularly across broad geographic regions (Zinn et al. 2007). Subsoil is less responsive to climatic influences (Dalmolin et al. 2006); thus, with increasing soil depth the role of soil mineral matrix for the storage of SOC becomes prominent (Kaiser et al. 2002). Studies have been recently done in Eastern Australia showed that the importance of parent material (including clay content and mineralogy) to SOC storage increased with increasing soil depth (Hobley et al. 2015; Gray et al. 2015). Larger proportion of SOC can be associated with fine mineral fractions in the subsoil than topsoil (Kaiser et al. 2002).

Various protective mechanisms of soil organic matter (SOM) against biological attack offered by the soil mineral matrix are dependent on the chemical and physical nature of soil minerals as well as the architecture of the soil matrix (Baldock and Skjemstad 2000). The interaction of SOM with clay minerals and pedogenic oxides has been recognized as one of the principal processes responsible for the preservation of SOM (Oades 1988; Sollins et al. 1996; Baldock and Skjemstad 2000; Lützwow et al. 2006). Several studies in the past investigated the importance of Fe and Al oxides, hydroxides, and oxyhydroxides (termed as Fe oxides and Al oxides hereafter) for the preservation of SOM (Kaiser et al. 2002; Eusterhues et al. 2005; Spielvogel et al. 2008; Pronk et al. 2011; Thaymuang et al. 2013). Due to their high reactivity and large specific surface area, oxides have the potential to stabilize large amounts of SOM (Schwertmann and Taylor 1989; Grand and Lavkulich 2011). Oxides are usually differentiated based on their crystallinity

(Schwertmann and Taylor 1989). A number of authors have emphasized the importance of poorly crystalline phases in protecting SOM in subsoil (e.g. Kleber et al. 2005; Mikutta et al. 2006; Spielvogel et al. 2008; Lorenz et al. 2009).

The Main Range of Peninsular Malaysia extends over the central part of the peninsula, following NW/SE orientation. Gradients of soil forming factors such as climate and vegetation in the Main Range, which is composed of fairly uniform parent material (Paramanathan 1977), form various soil types with different potential to preserve SOC. This group of soil forms a climo-biosequence. Climo-biosequences has been used to study the effects of climate and vegetation on soil properties if all state-factors except climate and vegetation are relatively constant (Jenny 1941). Although recent studies have addressed mineral-associated preservation of SOC along chronosequences (e.g. Chenu and Plante 2006; Cui et al. 2014; Wang et al. 2014) and elevation gradients (e.g. Wagai et al. 2008; Podwojewski et al. 2011), little information is available on controlling factors of SOC preservation in the topsoil and subsoil along a climosequence and/or climo-biosequence (e.g. Egli et al. 2007; Zeraatpishe and Khormali 2012). The research question is whether preservation of SOC in both the topsoil and subsoil along a climo-biosequence is the consequence of association with soil mineral matrix. Therefore, the role of soil texture and mineralogy for the preservation of SOC in the topsoil and subsoil along a climo-biosequence in the Main Range of Peninsular Malaysia was evaluated. It is hypothesized that soil texture and mineralogy affect preservation of SOC in the subsoil. To test this hypothesis, the clay+silt-associated SOC, representing preserved SOC, in both the topsoil and subsoil was estimated by particle-size fractionation. The relations between the content of SOC in this fraction and the content of clay, clay+silt and indicators of Fe oxides and allophane-type aluminosilicates were analyzed.

1 Materials and Methods

1.1 Study sites

After preliminary field studies, four representative soil profiles (P1-P4) in the major

elevation zones along an elevation gradient in the Main Range of Peninsular Malaysia were selected and studied (Figure 1). Variations in climate and vegetation are responsible for the differences in pedogenesis and soil properties along the respective elevation gradient. According to the state-factor model of Jenny (1941), attempts were made to hold other three state factors (i.e. parent material, relief, and time) constant. Therefore, the group of soils studied was called a climo-biosequence. P1 (4°14'N, 101°18'E) was a Vetic Cutanic Acrisol, located in the foothill zone under hill dipterocarp forest. P2 (4°24'N, 101°23'E) was a Vetic Acrisol, situated in the lower montane zone under upper dipterocarp forest. P3 (4°29'N, 101°23'E) was also located in the lower montane zone but under myrtaceous forest. P3 was classified as Albic Follic Podzol. P4 (4°31'N, 101°23'E) was located in the upper montane zone in the forest dominated by ericaceous genera. P4 was classified as Sapric Ombric Histosol.

Based on meteorological records over 10 years

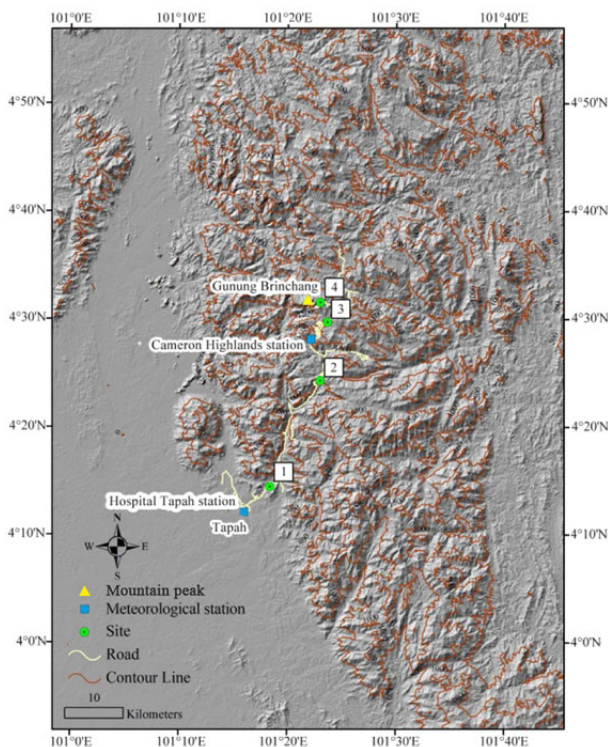


Figure 1 Location of the investigated sites (1-4) along an elevation gradient in the Main Range of Peninsular Malaysia from near Tapah to Gunung Brinchang, Cameron Highlands. The map was prepared using ArcGIS software [data source for contour lines and hillshade: SRTM DEM with 90 m resolution; road: topographic map (1:50000); points: GPS surveying].

(2003-2012), mean annual rainfall was 3282 mm at the Hospital Tapah station (4°12'N, 101°16'E; 35.1 m a.s.l.) and 2977 mm at the Cameron Highlands station (4°28'N, 101°22'E; 1545 m a.s.l.). The occurrences of fog and duration as an additional water input increased with increasing elevation (Kumaran 2008). The mean annual temperature decreased from 26.9°C (recorded at Hospital Tapah) to 18.0°C (recorded at Cameron Highlands). The slope inclinations varied between 20%-30% and all sites were located on similar geomorphic position. The geomorphic position of all sampling sites was mountain flank. All soils were derived from granitic saprolite (Paramananthan 1977). The age of the granitic bedrock in the Main Range of Peninsular Malaysia falls within the range 200-230 Ma (Triassic period) (Cobbing et al. 1992; Ghani 2009). Accordingly, it was assumed that all the sampling sites had similar geomorphic age. A brief characterization of the study sites is given in Table 1.

1.2 Soil sampling

Bulk soil samples were taken from all genetic horizons which were described according to the standard procedure (FAO 2006). Samples were air-dried, ground gently, and passed through 2 mm sieve prior to the physical, chemical, and mineralogical analyses. Subsamples were ground to <250 µm for the determination of total carbon (TC) by dry combustion method using a LECO TruMac elemental analyzer.

1.3 Soil chemical, physical, and mineralogical analyses

Soil pH was measured potentiometrically in a supernatant suspension of 1:2.5 soil/water ratio (van Reeuwijk 2002). Particle-size distribution was determined gravimetrically by the pipette method (Gee and Bauder 1986). Organically-bound iron (Fe_p) and aluminum (Al_p) were obtained from 16 hours shaking by 0.1 mol L⁻¹ sodium pyrophosphate solution at pH 10 (McKeague 1967). The organically complexed and poorly crystalline iron (Fe_o) and aluminum (Al_o) oxides as well as Si_o were extracted by acid ammonium oxalate (adjusted to pH 3) in darkness (McKeague and Day 1966). Free iron (Fe_a) oxides, which consist of

Table 1 Characterization of the study sites along the climo-biosequence in the Main Range of Peninsular Malaysia

Site/soil profile	Elevation (m a.s.l.)	Elevation zone	Soil moisture-temperature regime ^a	Vegetation ^b	Geological formation ^a	Soil classification (WRB) ^c
P1	155	Foothill (<750 m a.s.l.)	Udic-Isohyperthermic	Hill dipterocarp forest	Quartz monzonite (Adamellite)	Vetic Cutanic Acrisol (Alumic, Hyperdystric, Clayic, Chromic)
P2	1129	Lower montane (750-1200 m a.s.l.)	Perudic Isohyperthermic	Upper dipterocarp forest	Granite	Vetic Acrisol (Alumic, Humic, Hyperdystric, Clayic, Chromic)
P3	1567	Lower montane (1200-1800 m a.s.l.)	Perudic-Isothermic	Myrtaceous forest	Granite	Albic Folic Podzol
P4	1946	Upper montane (>1800 m a.s.l.)	Perudic-Isomesic	Ericaceous forest	Granite	Sapric Ombric Histosol (Dystric)

Notes: ^a From Paramanathan (1977); ^b From Burgess (1969); ^c IUSS Working Group WRB (2015)

crystalline and poorly crystalline oxides, were determined by dithionite-citrate extraction method (Holmgren 1967). The concentrations of Fe, Al, and Si were determined by ICP-OES (Perkin Elmer Optima 8300). The $Fe_d - Fe_o$, $Fe_o - Fe_p$, Fe_o/Fe_d ratio, $Al_o - Al_p$, and $(Al_o - Al_p)/Si_o$ represent crystalline Fe oxides, poorly crystalline Fe oxides, crystallinity index, poorly crystalline inorganic forms of Al, and poorly crystalline aluminosilicates, respectively.

The clay fraction (<2 μ m) was separated by sedimentation after destruction of SOM with hydrogen peroxide (H_2O_2)(30%) and chemical dispersion by sodium hexametaphosphate (NaHMP). X-ray diffraction (XRD) analyses were performed on glass slides, which contained oriented clays using X-ray diffractometer (Philips PW 3440/60 X'Pert PRO), equipped with Cu-K α tube operating at 40 kV and 30 mA. The diffractometer recorded clay minerals pattern from 3-50 $^{\circ}2\theta$ using the step size of 0.02 $^{\circ}2\theta$ and a count time of 1.25 s per step. PAN analytical X'Pert HighScore Plus 3.0 software was used to identify XRD reflections, their d-spacing, and relative intensity. The OriginPro 9.1 software (Originlab Corporation, Northampton, USA) was used to plot and stack the XRD patterns by Y offset. The patterns were interpreted according to Brown and Brindley (1980).

1.4 Particle-size fractionation

Particle-size fractionation is considered as a useful approach for isolation of SOC pools associated with particles of different size and

therefore of different mineralogy which are, in turn, different in composition and function (Christensen 1992). Particle-size fractions were obtained based on the modified method of Sanderman et al. (2011). As the fractionation procedure is laborious, eight soil horizons involving the A and B-horizons of all soil profiles were selected for particle-size fractionation. For those soil profiles with two subdivisions for B-horizon (P1 and P4), the upper subdivision was selected due to the higher concentration of SOC.

The samples were fractionated in triplicate to get enough materials and determine the repeatability of the method. Briefly, 10 g of samples <2 mm were dispersed with 40 mL NaHMP (5 g L⁻¹) in a 50 mL centrifuge tube. The samples were vortexed for 30 s in order to wet the soils completely. Based on preliminary tests, 10 glass beads (5 mm) were added for the complete dispersion of soils. The samples were then shaken overnight on a rotary shaker set to 60 rpm. The dispersed soil samples were passed through the stacked sieves of 250 and 53 μ m and rinsed with distilled water (DI) until the suspension passing the sieve became clear. The materials retained on the sieves were washed into pre-weighed 500 mL polypropylene beakers with the aid of a wash bottle.

The soil slurry containing fractions <53 μ m was collected in the tray and then transferred to 1 L cylinder. Afterwards, the slurry was flocculated using saturated aluminum sulfate, kept overnight, siphoned off the extra water, and centrifuged to obtain the fractions <53 μ m. The fractions <53 μ m were washed three times with DI to remove extra aluminum sulfate. Using this approach, three

particle-size fractions were obtained including: (1) 250-2000 μm (coarse sand); (2) 53-250 μm (fine sand); and (3) <53 μm (clay+silt). All fractions were oven-dried at 65°C, homogenized, and weighed to calculate the mass recovery. The target was mass recovery more than 98%. A subsample from each fraction was ground to pass 250- μm mesh size in order to analyze TC.

1.5 Organic carbon determination

The concentration of TC in the ground bulk soil samples and three particle-size fractions were determined by dry combustion using a LECO TruMac elemental analyzer. All samples were run in triplicate. Working standard (Bungor Series) was run at the beginning, at the end and after every 10 unknown samples for calibration. The accuracy of working standard was tested against certified standard (BBOT) by repeating analysis at four different masses according to procedure proposed by Sanderman et al. (2011) (data not shown). The mean relative standard deviation ($RSD = \text{standard deviation}/\text{mean} \times 100$) of the working standard for TC was 2.04%. The target recovery for TC was between 85% to 115%. All soil samples were free of carbonate as tested by 4 mol L⁻¹ hydrochloric acid (HCl); therefore, the concentration of TC was equal to the concentration of organic carbon.

The SOC enrichment factor ($E_{SOC} = \text{g C kg}^{-1} \text{ fraction} / \text{g C kg}^{-1} \text{ bulk soil}$) for each particle-size fraction was calculated to compare C distribution among the particle-size fractions separated from different soils along the climo-biosequence (Christensen 2001; Schöning 2005). The SOC size pools were calculated based on the concentration of SOC in each particle-size fraction and the content of the respective particle-size fraction in the soil.

1.6 Scanning electron microscopy

Fine fractions (<53 μm) were spread out on the scanning electron microscope (SEM) stub and fixed by an underlying thin layer of high conductivity paint (Acheson Colloids Company, Plymouth). The samples were gold-coated using sputter coater machine and imaged with SEM (LEO 1455 VPSEM). Imaging was performed at the tension voltage of 10 kV. The elemental composition of samples was estimated by energy

dispersive X-ray (EDX) detector (Oxford Instruments Analytical Ltd, UK) attached to the electron microscope.

1.7 Statistical analysis

Means, standard errors, and RSDs were calculated in Microsoft® Excel® 2010 (Microsoft, Redmond, USA). Multiple regression analyses were conducted to determine the relationship between the content of SOC in <53 μm fraction and the content of clay, clay+silt, Fe_o – Fe_p, and Al_o – Al_p in the A and B-horizons. The statistical significance of R-squared (R²) was tested by the F test. Regression analyses were carried out using R v3.3.3 software for windows (R core team 2017). In some cases, data were square-root transformed to reduce the skewness and produce more closely the normal distribution.

2 Results and Discussion

2.1 Soil characterization

Table 2 shows selected physical and chemical characteristics of the soils studied. The soils were acidic throughout all the profiles with pH values varying from 3.95 to 5.99. At all sites, organic horizons contained the largest concentration of SOC. The thickness of the organic layer increased with increasing elevation. The concentration of SOC in the mineral horizons ranged from 1.7 to 63.9 g kg⁻¹. At the P1 and P2 sites, the concentration of SOC in the mineral horizons decreased with soil depth. By contrast, at the P3 and P4 sites, the SOC accumulated in the B_s horizons due to downward movement of SOC during the process of podzolization.

2.2 Distribution of particle-size fractions in the bulk soil

Table 3 shows the distribution of particle-size fractions in the bulk samples from selected horizons of the soil profiles and the RSD for each particle-size fraction in each soil horizon. There was close correspondence between size fractions obtained by particle-size fractionation procedure (Table 3) and the texture using the pipette method (Table 2). The <53 μm fraction increased with

depth, whereas the 250-2000 µm fraction decreased with depth in all the soil profiles. Also, the proportion of <53 µm fraction in the B-horizon increased with increasing elevation along the climo-biosequence. The mean mass recovery for each horizon ranged from 98% to 99.5%. The RSD values for each particle-size fraction in each horizon ranged from 1.6 to 17.4%. The mean RSD of the 250-2000, 53-250 and <53 µm fraction (n = 8) were 5.4%, 5.9%, and 3.2%, respectively. Low values of RSD reflected the repeatability of the fractionation procedure for the given soils.

2.3 Organic carbon in particle-size fractions: the effect of soil texture on preservation of organic carbon

Table 4 shows the E_{SOC} for each particle-size fraction from selected horizons of the soils studied. All of the <53 µm fractions were enriched, whereas the majority of 250-2000 and 53-250 µm fractions were depleted in SOC. The highest concentrations of SOC were found in the <53 µm fractions. The <53 µm fraction in the A-horizon of P3 and P4 with enrichment factors of 2.54 and 3.01, respectively, showed the highest values within all soils. Results indicated that those <53 µm fractions with higher SOC enrichment factor had lower content of clay+silt particles in the bulk soil and vice versa. This was probably attributed to the dilution effect (Amelung et al. 1998; Zinn et al. 2007). In other words, higher content of clay+silt dilutes the SOC concentration and decreases the enrichment factor. The enrichment of SOC in the <53 µm fraction reflected the strong organo-mineral interaction in the fine fractions. The enrichment of SOC in the fine fractions (particularly clay) was reported in the previous studies (e.g. Christensen 1992; Bayer et al. 2006).

The SOC size pools and their distribution for each soil horizon are shown in Table 5. The <53 µm fraction with more than 60% of the total SOC was the main SOC pool in all soil horizons. This result is in accordance with the study of Zinn et al. (2007) who found clay fraction as the main SOC pool in three soils from the Brazilian Cerrado. In all soils, A-horizons contained the largest proportion of SOC in the 250-2000 µm fraction due to their close proximity to the organic horizons and soil surface where high amounts of C input were derived from

plant residues (leaf and/or root litter). The portion of SOC in the 250-2000 µm fraction decreased from 12.7%-25.7% in the A-horizon to 2.1%-7.4% in the B-horizon.

Table 2 Physical and chemical characteristics of the soils studied

Profile	H _z	Depth (cm)	pH	SOC (g kg ⁻¹)	Clay (%)	Silt (%)	Sand (%)
P1	A	0-10	4.54	22.0	28.4	17.9	53.5
	B _{t1}	10-30	4.85	8.5	41.1	15.6	43.2
	B _{t2}	30-60	4.87	6.8	43.3	20.1	36.4
	BC	60-120	5.10	2.6	33.1	23.8	42.8
	C	>120	5.19	1.8	23.8	25.6	50.4
P2	O	0-3	4.84	427.9	n.d ^a	n.d	n.d
	A	3-13	4.56	33.3	36.2	26.1	37.6
	B _t	13-43	4.45	13.2	43.5	22.8	33.6
	C ₁	43-90	4.62	7.3	17.9	37.7	44.3
	C ₂	90-120	4.79	1.7	17.7	37.7	44.4
P3	C ₃	>120	4.77	2.0	13.7	39.5	46.7
	O _i	0-3	4.41	432.0	n.d	n.d	n.d
	O _e	3-13	4.21	195.8	n.d	n.d	n.d
	A	13-38	4.00	13.3	5.1	16.1	78.6
	E	38-43	4.09	13.1	24.9	21.2	53.7
P4	B _s	43-80	4.22	29.2	20.1	35.0	44.7
	C	>80	4.67	2.5	11.8	36.6	51.4
	O _i	0-5	4.16	495.0	n.d	n.d	n.d
	O _e	5-40	3.95	505.3	n.d	n.d	n.d
	O _a	40-80	4.24	266.0	n.d	n.d	n.d
P4	A	80-90	4.13	43.2	10.9	11.5	77.3
	E	90-120	4.59	8.8	15.3	17.9	66.7
	B _{s1}	120-180	5.99	63.9	12.2	54.2	32.7
	B _{s2}	180-200	5.47	26.5	20.5	36.7	42.4
	BC	200-220	4.97	3.9	18.2	31.4	50.3
P4	C	>220	5.02	1.8	4.9	25.2	69.6

Notes: H_z: Horizon; ^a Not determined.

Decrease in the proportion of SOC associated with the coarse sand and increase in the proportion of SOC associated with the clay and silt in the subsoil showed the importance of soil matrix for the storage of SOC and the intimate association of SOC with clay-sized mineral particles in the subsoil. Kaiser et al. (2002) reported an increase in the portion of SOC in the clay fraction with depth in Podzols and Cambisols from northern Bavaria, Germany. The SEM image of the <53 µm fractions combined with EDX spectra supported the above assumption and confirmed the association of SOC with mineral particles (Figure 2). By observing the particles <2 µm at high magnification, Chenu and Plante (2006) found that most of the mineral particles were in fact organo-clay complexes.

Table 3 Distribution of the particle-size fractions in the bulk samples from selected horizons of the soil profiles [values are mean with standard error in parentheses ($n = 3$)] and the relative standard deviation (RSD) for each particle-size fraction in each soil horizon

Profile	Horizon	Distribution of the particle-size fractions (% of total dry weight)			Recovery (%)	RSD for the particle-size fractions (%)		
		250-2000 (μm)	53-250 (μm)	<53 (μm)		250-2000 (μm)	53-250 (μm)	<53 (μm)
P1	A	37.6 (0.5)	15.2 (0.3)	45.3 (0.8)	98.1	2.2	3.6	3.0
	B _{tl}	28.4 (0.9)	10.1 (0.2)	59.5 (0.8)	98.0	5.5	3.7	2.2
P2	A	28.5 (0.6)	6.9 (0.1)	63.1 (0.6)	98.5	3.8	1.7	1.7
	B _t	26.4 (0.3)	7.7 (0.2)	65.3 (0.6)	99.4	1.9	3.4	1.6
P3	A	59.1 (0.8)	16.2 (0.4)	24.2 (0.4)	99.5	2.4	4.5	3.0
	B _s	25.6 (2.5)	6.4 (0.2)	66.0 (2.3)	98.0	17.4	6.5	5.8
P4	A	56.5 (2.2)	12.2 (1.2)	30.7 (1.1)	99.4	6.7	16.0	6.0
	B _{st}	5.2 (0.1)	22.3 (1.0)	70.6 (1.0)	98.1	2.9	8.1	2.5

Table 4 Concentration of soil organic carbon (SOC) [values are mean with standard error in parentheses ($n = 3$)] and SOC enrichment factor (E_{soc}) for each particle-size fraction from selected horizons of the studied soils

Profile	Horizon	SOC concentration for the particle-size fractions ($\text{g kg}^{-1}\text{fraction}$)			E_{soc} for the particle-size fractions		
		250-2000 (μm)	53-250 (μm)	<53 (μm)	250-2000 (μm)	53-250 (μm)	<53 (μm)
P1	A	8.2 (0.02)	13.1 (0.91)	34.3 (0.09)	0.37	0.60	1.56
	B _{tl}	1.7 (0.16)	2.1 (0.28)	13.7 (0.24)	0.20	0.25	1.61
P2	A	14.8 (0.76)	37.1 (0.87)	38.0 (0.28)	0.44	1.11	1.14
	B _t	2.7 (0.08)	9.0 (0.27)	16.5 (0.09)	0.20	0.68	1.25
P3	A	5.8 (0.14)	11.3 (0.23)	33.8 (0.14)	0.44	0.85	2.54
	B _s	2.3 (0.14)	6.8 (0.12)	37.8 (0.12)	0.08	0.23	1.30
P4	A	9.9 (0.26)	15.1 (0.45)	129.8 (1.15)	0.23	0.35	3.01
	B _{st}	91.1 (1.77)	64.1 (1.01)	72.7 (0.08)	1.43	1.00	1.14

Table 5 Soil organic carbon (SOC) size pools and their distribution for selected horizons of the soils studied

Profile	Horizon	SOC concentration for the particle-size fractions ($\text{g kg}^{-1}\text{soil}$)			SOC distribution for the particle-size fractions (% of total SOC)			Recovery (%)
		250-2000 (μm)	53-250 (μm)	<53 (μm)	250-2000 (μm)	53-250 (μm)	<53 (μm)	
P1	A	3.1	2.0	15.6	14.0	9.1	70.7	93.8
	B _{tl}	0.5	0.2	8.2	5.7	2.5	96.0	104.2
P2	A	4.2	2.6	24.0	12.7	7.7	72.0	92.4
	B _t	0.7	0.7	10.8	5.4	5.2	81.8	92.4
P3	A	3.4	1.8	8.2	25.7	13.8	61.5	101.0
	B _s	0.6	0.4	25.0	2.1	1.5	85.5	89.1
P4	A	5.6	1.8	39.9	13.0	4.3	92.3	109.6
	B _{st}	4.7	14.3	51.3	7.4	22.4	80.3	110.1

The protective role of clay+silt particles on SOC has been established in previous studies (Feller and Beare 1997; Six et al. 2002; Jolivet et al. 2003). Six et al. (2002) and Jolivet et al. (2003) suggested that the proportion of clay+silt fraction defines the maximum capacity of the soil to preserve SOM. Although both the content of SOC in the <53 μm fraction and soil content of clay+silt in the B-horizon increased with increasing elevation (Figure 3c), weak correlation between the content of SOC in the <53 μm fraction and the content of clay+silt particles in the B-horizon was

observed (Figure 3a). This shows that changes in the content of SOC in the <53 μm fraction may not be associated with changes in the clay+silt content. This result is in contrast to the findings of Hassink (1997) who found close relationship between the amounts of SOC associated with clay+silt (<20 μm) and the percentage of soil particles in this size fraction.

On the other hand, negative relationship between the content of SOC in the <53 μm fraction and the content of clay particles in the B-horizon was observed (Figure 3b). According to the Figure 3b

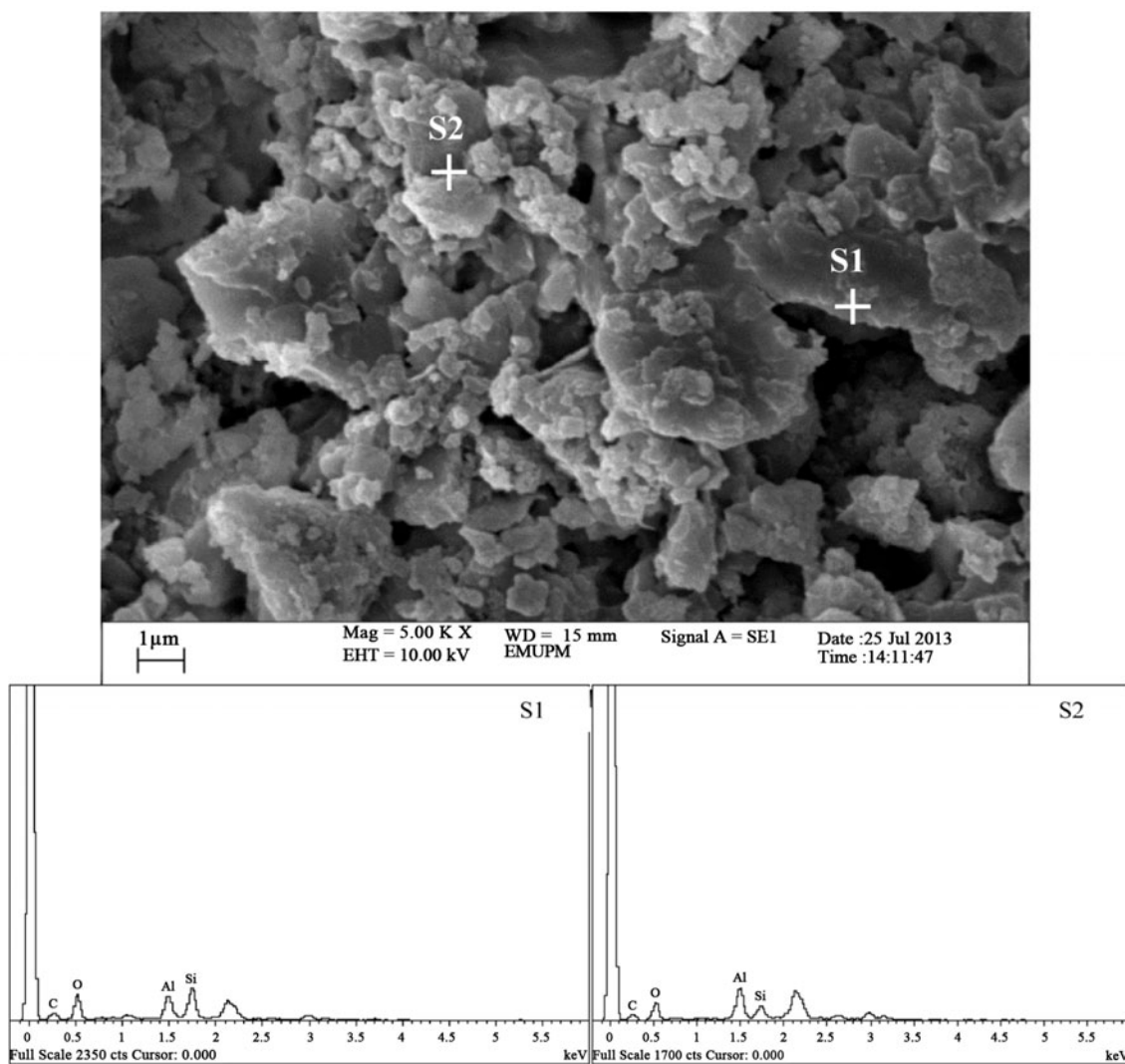


Figure 2 Scanning electron micrograph of the <53 μm fraction from the B_{s1} horizon of P4 combined with energy dispersive X-ray (EDX) spectra indicates the association of SOC with mineral particles. EDX spectra are displayed at the two areas by S1 and S2. The large peak at the beginning of the spectra, which was not pointed out on the figure, is assigned to the gold element from coating material.

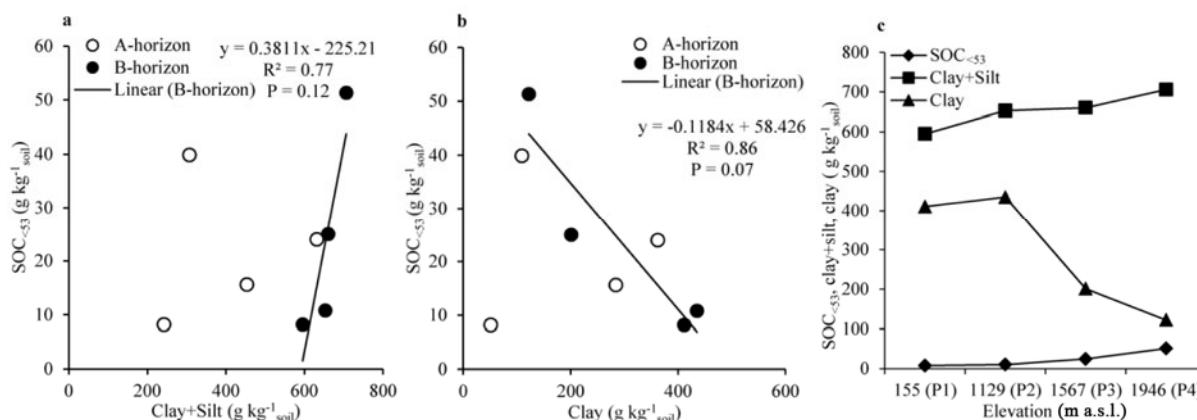


Figure 3 Relationship between (a) the content of SOC in the <53 μm fraction and soil content of clay+silt, (b) the content of SOC in the <53 μm fraction and soil clay content from the A and B-horizon of the soils studied, and (c) the change of SOC_{<53}, soil content of clay+silt, and soil clay content in the B-horizon over elevation.

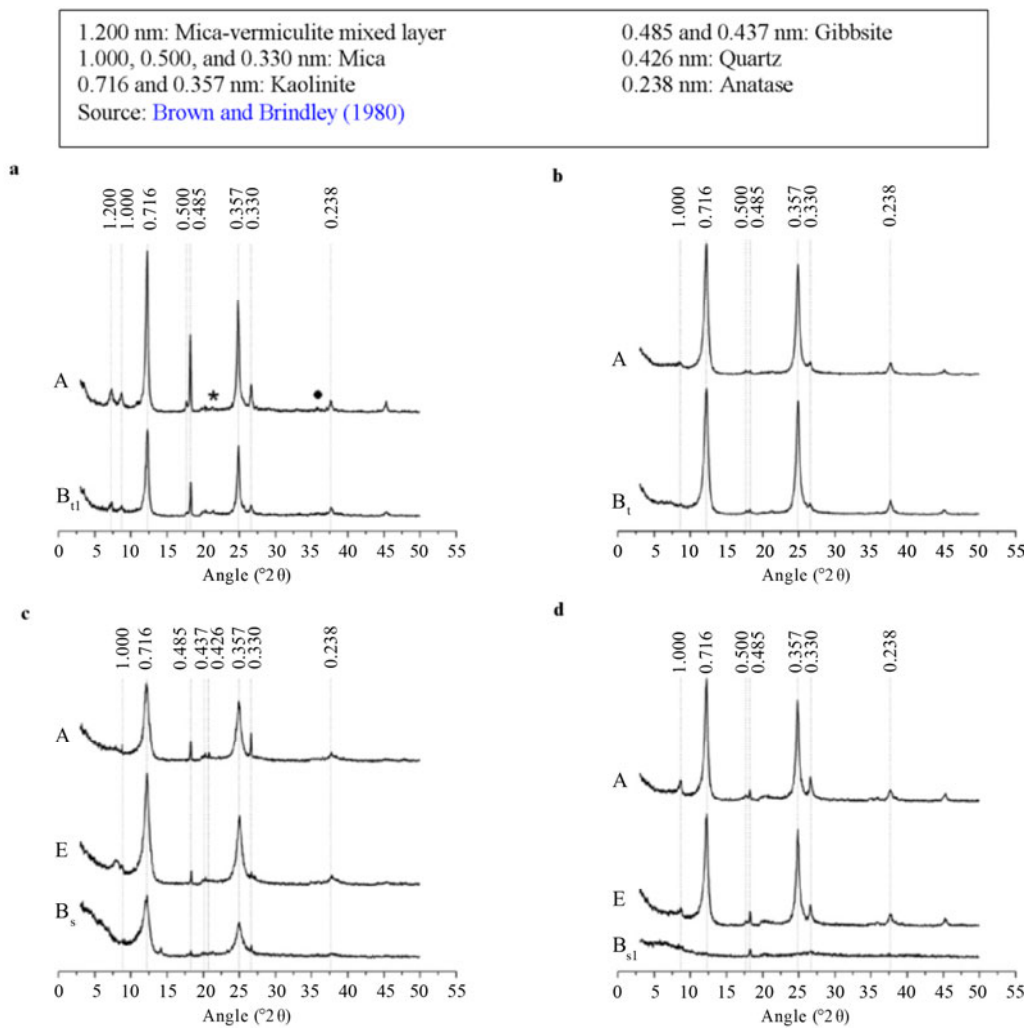


Figure 4 X-ray diffraction patterns of the oriented clays from selected horizons of the soils studied: (a) P1; (b) P2; (c) P3; and (d) P4. Dotted vertical lines show d-spacing in nm. Star and circle indicate reflections at 0.416 and 0.251 nm, which are assigned to goethite and hematite, respectively.

and [Figure 3c](#), soils with smaller content of clay particles contained higher amounts of SOC associated with clay+silt. This can be explained by the higher contribution of silt-sized particles ([Table 2](#)) and, in turn, silt-associated SOM in the B_s horizon of P3 and P4 and/or the presence of clay-sized minerals with high potential to preserve SOC (e.g. poorly crystalline Fe oxides and poorly crystalline aluminosilicates) in the B-horizon of the respective soils. Therefore, not only clay content but also clay type can influence the preservation of SOC in the B-horizon. There was no relationship between the content of SOC in the <53 μm fraction and both the content of clay+silt particles and the content of clay particles in the A-horizon ([Figure 3](#)). Results suggest that texture is not the main controlling factor of SOC preservation in the soils

studied.

2.4 The effect of soil mineralogy on preservation of organic carbon

X-ray diffraction patterns of the separated clay fractions from selected horizons of the four soils showed the mineralogical composition ([Figure 4](#)). Kaolinite was the most widespread silicate clay mineral in the study area that was identified in the clay fraction of all soil horizons by the sharp reflections at 0.716 and 0.357 nm ([Brown and Brindley 1980](#)). Various amounts of other minerals including gibbsite (reflection at 0.485 nm), mica (reflections at 1.000, 0.500, and 0.330 nm), mica-vermiculite mixed layers (reflection at 1.200 nm), anatase (reflection at 0.238 nm), and Fe oxides

Table 6 Fe, Al, and Si in various extracts as well as the crystalline Fe oxides, poorly crystalline Fe oxides, poorly crystalline inorganic forms of Al, and crystallinity index for selected horizons of the soils studied

Profile	Horizon	Fe _d ^a	Fe _o ^b	Fe _p ^c	Al _o ^d	Al _p ^e	Si _o ^f	Fe _d -Fe _o	Fe _o - Fe _p	Al _o - Al _p	Fe _o /Fe _d
		(g kg ⁻¹)									
P1	A	18.3	2.2	3.3	1.5	1.6	0.1	16.1	-1.1	-0.1	0.12
	B _{ti}	29.0	1.9	1.8	1.6	1.2	0.1	27.1	0.1	0.4	0.07
P2	A	18.1	3.3	3.5	2.2	1.8	0.1	14.8	-0.2	0.4	0.18
	B _t	21.1	3.4	2.8	2.3	1.4	0.1	17.7	0.6	0.9	0.16
P3	A	0.2	0.1	0.1	0.7	0.7	tr ^g	0.1	0.0	0.0	0.50
	E	1.9	0.8	0.5	2.9	2.4	0.1	1.1	2.3	0.5	0.42
	B _s	16.2	9.9	7.2	6.9	5.3	0.1	6.3	2.7	1.6	0.61
P4	A	0.1	0.1	0.1	3.4	3.3	1.0	tr	0.0	0.1	1.00
	E	0.4	0.4	0.4	3.0	2.3	1.0	tr	0.0	0.7	1.00
	B _{si}	9.6	8.8	1.5	86.3	8.3	35.6	0.8	7.3	78.0	0.92

^a Dithionite extractable Fe; ^b Oxalate extractable Fe; ^c Pyrophosphate extractable Fe; ^d Oxalate extractable Al; ^e Pyrophosphate extractable Al; ^f Oxalate extractable Si; ^g Trace

(weak peaks at 0.416 and 0.251 nm reflecting the presence of goethite and hematite, respectively) (Brown and Brindley 1980) were present in the clay fraction. The XRD results revealed that expandable clay minerals (e.g. smectite) were absent in soils of the study area. Therefore, intercalation of SOM by the internal surfaces of phyllosilicates does not contribute to the preservation of SOM in the soils studied.

The amounts of the various forms of selective extractable Fe and Al are shown in Table 6. Along the climo-biosequence, Fe_d ranged from 0.1 to 29.0 g kg⁻¹, Fe_o from 0.1 to 9.9 g kg⁻¹, and Fe_p from 0.1 to 7.2 g kg⁻¹. Al_o varied from 0.7 to 86.3 g kg⁻¹ and Al_p from 0.7 to 8.3 g kg⁻¹. In the B-horizon, the concentration of Fe_d and the content of Fe_d - Fe_o decreased with increasing elevation along the climo-biosequence. On the other hand, the concentration of Fe_o and Al_o as well as the content of Fe_o - Fe_p and Al_o - Al_p in the B-horizon increased with increasing elevation (Table 6). In addition, Fe_o/Fe_d ratio as an active Fe fraction increased with increasing elevation, indicating a decreasing trend in the degree of crystallinity towards higher elevations (Table 6). This is in line with the findings of Schawe et al. (2007) who reported high Fe_o/Fe_d values for high-elevation soils of Bolivia. High Fe_o/Fe_d ratio in soils at high elevation implies the importance of poorly crystalline Fe oxides (i.e. ferrihydrite) for the preservation of SOC. According to the mineral assemblage for the soils studied, SOM can be mainly protected from decomposition through interaction with pedogenic oxides of Fe and Al and

kaolinite.

High concentration of Al_o in the B-horizon of P4 was probably due to the presence of poorly crystalline aluminosilicates. Subtracting Al_p from Al_o divided by Si_o can be used as a criterion for identification of allophane-imogolite complexes (Farmer et al. 1983; Parfitt and Childs 1988). This ratio for the B_{si} horizon in P4 was equal to 2.2, which lies in the range of 1.5-2.6 proposed by Child et al. (1983) for recognizing these complexes in New Zealand Spodosols. In addition, the presence of poorly crystalline aluminosilicates in the B_{si} horizon of P4 was revealed by the Al_p/Al_o ratio below 0.5, which is indicative of the presence of allophanic mineralogy as stated by Mizota and van Reeuwijk (1989). This suggests that poorly crystalline aluminosilicates might play an important role in preservation of SOC in the B-horizon of the P4 under ericaceous forest.

The relations between the content of SOC in the <53 μm fraction and the content of Fe_o - Fe_p and Al_o - Al_p in the B-horizon are shown in Figure 5. The proportion of SOC in the <53 μm fraction of the A-horizon was not correlated with indicators of Fe oxides and allophane-type aluminosilicates (data not shown due to the presence of negative values), while there were linear relationships between the content of SOC in the <53 μm fraction and the content of Fe_o - Fe_p (Figure 5a) and Al_o - Al_p (Figure 5b) in the B-horizon. These results demonstrated the importance of poorly crystalline Fe oxides and allophane-type aluminosilicates for the preservation of SOC in the B-horizon of soils along the studied climo-biosequence. The

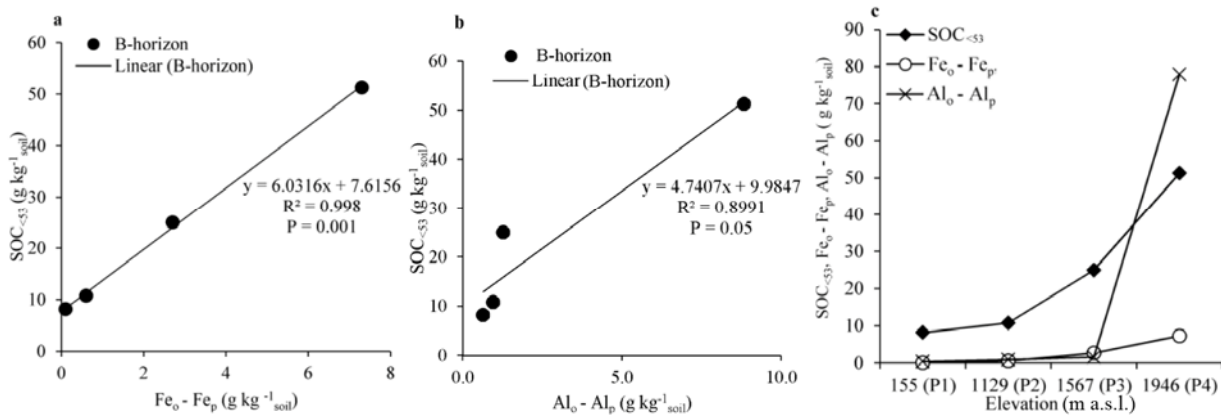


Figure 5 Relationship between the content of SOC in the <53 μm fraction and the content of (a) Fe_o - Fe_p, (b) Al_o - Al_p (data were square-root transformed) from the B-horizon of the soils studied, and (c) the change of SOC_{<53>}, Fe_o - Fe_p, and Al_o - Al_p in the B-horizon over elevation.

increasing trend of Fe_o - Fe_p and Al_o - Al_p over elevation suggest that the importance of poorly crystalline Fe oxides and allophane-type aluminosilicates for the preservation of SOC in the B-horizon increased with increasing elevation (Figure 5c).

Based on the results of the regression analysis, it is reasonable to infer that the preservation of SOC in the B-horizon of the soils studied is mainly controlled by clay mineralogy while in the A-horizon by other factors than soil texture and mineralogy. In another study, we found that the impacts of the soil forming factors of climate and vegetation on the C content of soils along the climo-biosequence was more pronounced in the surface horizons (Jafarzadeh-Haghighi et al. 2015). Zeraatphishe and Khormali (2012) reported the strong relationship between the SOC content and climatic factors in the topsoil (0-20 cm) along a climatic gradient.

3 Conclusions

The following conclusions were obtained from this study:

- 1) The amounts of preserved SOC in the subsoil increased with elevation;

- 2) Regardless of the differences in climate and vegetation along the studied climo-biosequence, preservation of SOC in the subsoil depends on clay mineralogy more specifically on amorphous Fe and Al phases; and

- 3) Based on the mineral assemblage of the soils studied, the amount of Fe oxides and their crystallinity as well as the amounts of poorly crystalline aluminosilicates are the important determinants of preservation of SOC in the B-horizon. The proportion of poorly crystalline form of minerals increased with elevation. Preserved SOC in soils at high elevation is mainly associated with poorly crystalline minerals.

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