



Climate and parent material controls on organic matter storage in surface soils: A three-pool, density-separation approach

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ABSTRACT

Physically- and biochemically-distinct fractions of soil organic matter (SOM) can be separated by density to yield: (i) low-density plant detritus fraction easily separable from soil minerals (f-LF), (ii) low-density materials strongly associated with minerals (m-LF), and (iii) high-density fraction (HF) rich in microbially-processed organic matter strongly associated with minerals. The factors controlling the pool size and chemistry in these fractions, especially those in m-LF, are unclear. We examined the influence of climate and parent material on SOM in these fractions using two sets of forest soils (0–10 cm mineral horizon) developed from contrasting parent materials (metasedimentary vs. ultrabasic igneous rock) along an altitudinal gradient in Mt. Kinabalu, Borneo. From 700 m to upper altitudes (1700, 2700 m), where mean annual temperature decreases from 24 to 12 °C with roughly constant rainfall, surface soil C stocks on both parent materials increased from 2.6–2.8 to 5.4–7.5 kg m⁻² with progressively greater proportions in m-LF and, to less extent, fresher plant detritus fractions. In HF, C and N concentrations increased with altitude though their stocks were roughly constant due to progressively lower bulk density. Labile C assessed by laboratory incubation of bulk soils correlated better with C concentrations in the two LF pools than in HF, especially at the lowest altitude where most SOM is strongly associated with minerals as HF. These results suggest that LF pools are more labile and more sensitive to altitude-induced, climate differences as compared to the HF pool that is protected by the mineral matrix. The C:N ratios of HF on metasedimentary rock increased with altitude (11 to 17) while those on ultrabasic rock remained essentially constant (14–15), implying interactive influence of altitude and parent material on stoichiometry. Unusually high C:N and alkyl-C in m-LF were found in mid-altitude soils, suggesting selective preservation of inherently recalcitrant materials in addition to, or instead of, mineral coating or aggregate occlusion of partially-degraded LF by microbial metabolites.

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1. Introduction

Soil organic matter (SOM) is a significant carbon reservoir on the earth's surface and plays a fundamental role in ecosystem functioning. Yet long-term responses of SOM to environmental gradients remain uncertain. This uncertainty is largely due to complex feedbacks among soil, biota, climate, and parent material at landscape scale and intimate interactions between diverse organic compounds and mineral phases at microscopic scale (e.g., Sollins et al., 1996; Davidson and Janssens, 2006).

Particle density allows separation of SOM pools of varying degrees of microbial alteration and mineral association (Greenland and Ford, 1964; Sollins et al., 1999). Low-density, fresh plant detritus is relatively

free of soil mineral particles (free light fraction, f-LF) and microbially-processed organic materials are more strongly bound to soil minerals (high-density fraction, HF) (Christensen, 1996). HF accounts for major fractions of SOM in most soils, but still contains a wide range of organic materials including the decaying plant detritus that is loosely associated with mineral particles and/or occluded within aggregates. Golchin et al. (1994) developed a technique to further separate low-density materials associated with soil mineral matrix (i.e. in conventional HF) by ultrasound dispersion in a liquid (1.60–1.65 g cm⁻³) which is slightly denser than mineral-free organic materials in soil. They termed the liberated low-density fraction as occluded LF. Instead, here we use the term mineral-associated LF (m-LF) to include the detritus not only occluded in aggregates but also associated with mineral particles in other ways (e.g., attachments and coating of mineral particles, e.g., Baisden et al., 2002). The mineral-associated LF is considered to form through the fragmentation and degradation of f-LF (especially preferential degradation of O-alkyl-C, i.e. carbohydrates), concurrent association of remaining LF with mineral particles

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and aggregates through microbial metabolites, and thus comprise an intermediate stage of decomposition between f-LF and HF in Golchin et al.'s (1997) often-cited conceptual model. Reported chemical characteristics of m-LF appear to have higher variability than those of f-LF and HF, possibly due to methodological variation and/or heterogeneity in the organic compounds isolated as m-LF (Wagai et al., in review-a). For instance, m-LF can have 20-unit higher or 6-unit lower C:N ratio compared to f-LF (Parker et al., 2002; Rasmussen et al., 2005) and has faster or slower turnover times compared to HF (Baisden et al., 2002; Swanston et al., 2005; Rasmussen et al., 2005). While m-LF can account for more than a quarter of total SOM standing stocks (some of above studies), little is known about the variation in the chemistry and constituents of this pool or the factors controlling its formation and fate.

Our study objectives were to (i) assess the controls of climate and parent material on SOM storage in the three density fractions and (ii) elucidate functional relationships among these fractions. We studied a series of undisturbed tropical forest soils developed on two contrasting parent materials, acidic metasedimentary vs. ultrabasic igneous rock, along a climate gradient on a mountain slope (altitude range of 700–2700 m with little change in annual rainfall) in Borneo. Given adequate moisture, the temperature sensitivity of microbial degradation should be the highest in f-LF among the density fractions (e.g., Trumbore et al., 1996) because of high substrate quality and accessibility (lack of mineral armoring). Thus, we hypothesized that, from a warm to cool climate gradient, soil C and N storage in HF would change little while that in both f-LF and m-LF would increase, because the formation of m-LF is likely regulated by the abundance of f-LF which is sensitive to climate. We focused on surface mineral soils (0–10 cm) where major pools of SOM are present and climate exerts stronger control than at depth.

2. Methods

2.1. Study area

We studied soils on the eastern and southern slopes of Mt. Kinabalu (4095 m, 6°05'N, 160°33'E) located in northeastern Borneo, under primary rain forests within Kinabalu Park. We selected six sites on the environmental matrix of two parent materials × three altitudes (at ca. 700, 1700, and 2700 m above sea level, Table 1), which is a subset of long-term ecological study sites described in Kitayama and Aiba (2002). The climate is humid tropical with weak influences of the Asiatic monsoon. Mean annual air temperature (MAT) decreases strongly with altitude (Table 1), with a mean lapse rate of 0.0055 °C m⁻¹, and intra-annual variations are <2 °C (Kitayama, 1992). Mean annual rainfall is relatively constant (2300–2400 mm yr⁻¹) with altitude, although inter-annual variation can be large (e.g., 1800–3300 mm yr⁻¹ during 1996–1999, Kitayama and Aiba, 2002). Air and soil moisture generally increase with altitude due to cooling and more frequent cloud cover at higher altitudes. Species-rich, broad-leaved tropical rain forests cover the mountain slope up to ca 3500 m altitude. With increasing altitude, tree height, forest biomass and productivity all decline, especially on the ultrabasic parent material presumably due to greater limitation in nutrients such as phosphorus (Kitayama and Aiba, 2002). Conifer trees intermix with broad-leaved trees with greater abundance with increasing altitude. The rainforest at the lowest altitude (700 m) is dominated by Dipterocarpaceae (mostly *Shorea* spp.), which is one of the most diversified dominant families of the region (Aiba and Kitayama, 1999). The sites at higher altitudes (1700–2700 m) are characterized as montane rain forests where Myrtaceae and Podocarpaceae (esp. genus, *Leptospermum*, *Syzygium*, *Tristaniopsis*, *Dacrycarpus*, and *Dacrydium*) become dominant (Aiba and Kitayama, 1999). Altitude showed stronger influence on forest composition than parent material did (Aiba and Kitayama, 1999).

The metasedimentary rocks found in the study area (Trusmi Formation) have experienced low-grade metamorphism and inten-

Table 1
Climatic, ecosystem, and general soil characteristics of Mt. Kinabalu study sites

	Metasedimentary rock series			Ultrabasic rock series		
	700 m	1700 m	2700 m	700 m	1700 m	2700 m
Actual altitude of each site (m)	650	1560	2590	700	1860	2700
Mean annual air temperature (°C) ^a	24	19	13	23	17	12
Mean annual rainfall (mm) ^b	2392	2380	2256	2392	2380	2256
Rainforest type ^c	Hill dipterocarp	Lower montane	Upper montane	Hill dipterocarp	Lower montane	Upper montane
Aboveground biomass (kg m ⁻²) ^c	48.1	28.0	29.5	54.2	21.3	10.8
ANPP (g m ⁻² yr ⁻¹) ^c	1913	1222	780	1715	813	725
O-horizon thickness (cm)	2.0	6.8	3.4	2.8	3.5	4.0
Dithionite-extr Fe (mg FeOOH g ⁻¹) ^d	33	13	6.2	492	73	48
Clay content (%)	37.8	9.5	14.8	45.6	16.6	24.3
Mineralogy of clay (<2 μm) fraction ^e	Kao, Gib	Kao, Illt, Qrt	Kao, Illt	Hem, Gth	Chl, Tlc, Gth	Chl, Tlc, Illt, Gth
Soil type (suborder level) ^f	Humult	Tropalf (tropept/tropod)	Tropept	Perox	Tropalf	Tropept

Soil data are from 0–10 cm surface mineral horizon.

^a Based on the weather station at 2700 m and the lapse rate of 0.0055 °C per meter (Kitayama, 1992).

^b From a weather station at each elevation (Kitayama, 1992).

^c From Kitayama and Aiba (1998) at the same set of sites. ANPP refers to aboveground net primary production.

^d From Wagai and Mayer (2007).

^e Kao = kaolinite, Gib = gibbsite, Illt = illite, Qrt = quartz, Hem = hematite, Gth = goethite, Chl = chlorite, and Tlc = talc.

^f Tentative classification of soils at each site according to US Taxonomy (Soil Survey Staff, 1999).

sive tectonics, and consist of Eocene argillite, slate, phyllite, siltstone, and sandstones (Jacobson, 1970; Hutchison, 2005). The ultrabasic igneous rocks consist mostly of peridotite with varying serpentinization, characterized by high concentrations of MgO, FeO, and heavy metals (Jacobson, 1970). Parent rock above 3000 m is largely granitic massif. All six sites were located on 11–22° slopes facing south or east. The two sites at 2700 m were <1 km apart whereas the other sites are 7–17 km apart from one another (for map, see Aiba and Kitayama, 1999). Soil profiles showed no indication of colluviation. In addition, preliminary elemental analysis of surface soils from the metasedimentary sites showed little cobalt and nickel, indicative elements for ultrabasic material (data not shown).

The margin of former glaciers in the area is unclear. Areas around 2700 m were likely either glaciated or received peri-glacial deposits, whereas the 700 m altitude had no evidence of glacial influence (Jacobson, 1970). Soils at the 700-m sites are more weathered than the upper-altitude sites as indicated by heavy texture, deep solum, high pedogenic iron content, and advanced clay mineralogies (Table 1). The greater weathering of the 700-m soils may reflect longer weathering due to their older soil surface age (compared to the 1700 and 2700 m sites), or simply higher temperature (faster chemical weathering) over the same period of soil formation. Nevertheless, SOM storage in surface soils as well as forest composition have likely reached a quasi steady state at all of our sites after the last glaciation because SOM accretion significantly slows down after the initial several centuries of pedogenesis on non-volcanic parent materials (Birkeland, 1999).

2.2. Soil sampling and handling

After an initial reconnaissance, we collected five soil samples inside or adjacent to the permanent vegetation plot established previously (Aiba and Kitayama, 1999; Kitayama and Aiba, 2002) at

each site. The samples were taken at least 10 m apart. At the 1700-m metasedimentary site, two samples were taken at 2-m intervals due to intense change in soil color at this scale. At each sampling location, the O-horizon was sampled with knife and then 0–10 cm mineral-soil sample was taken by a core sampler (diameter of 3.7 cm) three times and mixed to make one composite sample. The five samples from each site were sieved (4 mm) to reduce disturbance effect on incubation experiment and visible roots were hand-picked for 30 min per sample. Coarser plant detritus (>4 mm detritus) and gravels were separately washed, dried at 75 °C, and weighed to correct for standing C stock (see below). After sieving, each of the five samples (bulk soil) was separately homogenized and subsets were (i) air-dried for density separation, (ii) kept moist in a refrigerator less than a month for soil pH and laboratory incubation experiments, and (iii) oven-dried at 105 °C or freeze-dried for other analyses including mineral surface area and organic coverage of surfaces (Wagai et al., in review-b). To measure bulk density for standing C stock calculation, another set of field soils were sampled at each site ($N=4-6$). O-horizon materials were sampled from a defined ground area (10×10 or 15×15 cm²) and the average depths of O-horizon were measured. After O-horizon removal, 0–10 cm mineral soils were sampled using the same core sampler. Samples were not sieved this time. O-horizon and mineral-soil samples were dried at 75 °C and 105 °C, respectively, for gravimetric water content and bulk density calculation.

2.3. Density separation

We separated surface mineral-soil samples into three density fractions based on Golchin et al. (1994) with slight modification. Ten g of sieved, air-dried samples were gently turned over 20 times by hand in 40 mL tubes filled with 30 mL of 1.60 g cm⁻³ sodium polytungstate solution followed by centrifugation (35,000 g) for 30 min. The free low-density fraction (f-LF) was floated and transferred into a Buchner funnel placed with a glass-fiber filter (Whatman GF/C) and rinsed with deionized water until the electrical conductivity of the rinse water reached <50 µS, and then oven-dried at 60 °C. The remaining soil slurry was treated with ultrasound (656 J mL⁻¹) using a Fisher-Artek-Dynatech Model 300 sonicator with a 19-mm diameter tip while keeping the 40-mL tube in ice water. The sonicator was calorimetrically calibrated (North, 1976). The material floating after sonication (mineral-associated low-density fraction, or m-LF) was recovered using the same procedure as the f-LF recovery. Finer detritus materials stuck in the filter were largely recovered by rinsing the filter with water. Sediments in the heavy liquid after the sonication (high-density fraction – HF) were rinsed several times (conductivity <50 µS), and freeze-dried. Water contents in the air-dried bulk soils were corrected when presenting the C and N concentrations in density fractions on bulk soil mass basis.

Morphological characteristics were observed by a dissecting microscope for all isolated density fractions after the drying. To assess the mineral associations of plant detritus, we also examined selected f-LF materials without drying. Using one field-moist bulk soil each from 1700-m metasedimentary and 1700-m ultrabasic sites, f-LF was isolated as described above. Moist subsamples of the isolated fraction were quickly mounted on glass slides and observed by light microscopy (model BX51, Olympus, Tokyo, Japan).

2.4. Solid-state ¹³C NMR spectroscopy

For solid-state CPMAS ¹³C NMR spectroscopy, equal aliquots from five replicates were combined for each density fraction at each site. For the metasedimentary soils, one O-horizon sample was prepared in the same way for each site. For the ultrabasic soils, HF fractions were not analyzed due to high iron concentrations. Solid-state ¹³C NMR spectra were obtained on a Bruker DSX 200 spectrometer at a ¹³C resonance frequency of 50.3 MHz using the cross-polarization magic

angle spinning technique (Wilson, 1987). During the contact time of 1 ms, a ramped 1H pulse that was shaped from 100% to 50% was used to circumvent Hartmann–Hahn mismatches (Peersen et al., 1993; Cook et al., 1996). Depending on the sensitivity of the samples, between 9700 and 373,000 scans were accumulated using a pulse delay of 100–400 ms. Line broadenings between 50 and 150 Hz were applied prior to Fourier transformation. The ¹³C chemical shift scale is referenced to tetramethylsilane (=0 ppm). The NMR spectra were divided into five major chemical shift regions: alkyl-C (–10 to 45 ppm), O/N-alkyl-C (45 to 110 ppm), aromatic C (110 to 160 ppm), carbonyl C (160 to 220 ppm) (Knicker and Luüdemann, 1995). The relative carbon distribution was determined by integration of the signal intensity in the different chemical shift regions using an adapted integration routine supplied with the instrument software.

2.5. Incubation experiments

To quantify the amount of readily-degradable C (i.e. labile C), we conducted laboratory incubation of bulk soils at field capacity (Zak et al., 1990; Townsend et al., 1997). Using subsamples of sieved, refrigerated, field-moist soils, the equivalent of 10-g dry soils were transferred to funnels with 1-mm mesh lining, sprayed with water to bring to field capacity, and allowed to drain for 3 h. The soil-containing funnels were then placed in incubation jars (355 mL), sealed with pre-boiled, polystyrene stoppers, and kept dark at 22–25 °C for one month. The concentration of CO₂ in the headspace was measured every 4–8 days. The headspace gas was mixed 8 to 10 times with a 20-mL syringe, then sampled with a 0.5-mL syringe, and injected into a gas chromatograph with a thermal conductivity detector (Hewlett-Packard 5700A). After the CO₂ measurements, jars were opened and flushed with ambient air. Water loss during the gas exchange was accounted for by normalizing to the weight before opening. During the experiment, the headspace CO₂ concentration was kept <4% by volume. To calculate the total amount of C respired in 30 days, simple linear regression was applied to the cumulative CO₂ data (mg C g⁻¹) from each sample with time as explanatory variable. Natural log transformation of the CO₂ data improved the model fit in some of the lower-altitude soils, but when the difference in labile C estimates was minor (<5%) the transformation was not used.

2.6. Other soil analyses

Total C and N concentrations were analyzed with a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer, Norwalk, CT, USA) for each density fraction, bulk soil, >4-mm detritus, and O-horizon. A pilot test showed the absence of carbonate in the studied soils. Soil pH in deionized water and 0.01 N calcium chloride was measured using subsets of field-moist soils at a soil:solution ratio of 1:5 (g:mL) after 0.5–1.0 h equilibration (Carter, 1993). Soil texture was determined by standard sieving and sedimentation techniques (Gee and Bauder, 1986) for one bulk sample from each site. Sedimentation rate was calculated from Stoke's Law assuming particle density of 4.76 g cm⁻³ (mean of goethite and hematite particle density) for the 700-m ultrabasic sample and 2.60 g cm⁻³ for the other samples. Isolated clay fraction was used to determine mineralogy by X-ray diffraction (Rigaku Miniflex) with CuKα and variable slits using randomly-oriented samples.

2.7. Data analysis

For standing C stock estimate, total C concentrations for the 0–10 cm mineral soils were calculated by summing the C concentration of the bulk soil (<4-mm fraction) and that of >4-mm detritus after correcting for >4-mm gravels (that accounted for 0–20% of the dry mass of unsieved mineral soils). We included the coarser detritus that is not typically regarded as a part of SOM because its ecological importance (Fernandez et al., 1993) and dependence on climatic

Table 2
Chemical characteristics and soil water content of 0–10 cm mineral soils from each site

Parent material	Metasedimentary rock series						Ultrabasic rock series					
	700 m		1700 m		2700 m		700 m		1700 m		2700 m	
Total organic C (mg g ⁻¹)	29.8 ^c	(4.3)	49.1 ^{bc}	(7.0)	142 ^a	(18)	30.3 ^c	(2.8)	62.1 ^{bc}	(8.1)	83.0 ^b	(7.7)
Total N (mg g ⁻¹)	2.5 ^c	(0.3)	2.8 ^{bc}	(0.5)	7.0 ^a	(0.9)	2.1 ^c	(0.2)	3.1 ^{bc}	(0.4)	4.9 ^{ab}	(0.5)
C:N ratio	12 ^d	(0.4)	18 ^{ab}	(0.69)	20 ^a	(0.73)	15 ^c	(0.77)	20 ^a	(0.67)	17 ^{bc}	(0.2)
PH in deionized water	3.88 ^c	(0.10)	4.13 ^c	(0.11)	3.86 ^c	(0.13)	4.83 ^b	(0.11)	5.45 ^a	(0.10)	4.87 ^b	(0.15)
PH in CaCl ₂	3.48 ^{bc}	(0.03)	3.36 ^c	(0.06)	3.09 ^c	(0.05)	4.01 ^{ab}	(0.09)	4.59 ^a	(0.25)	4.19 ^a	(0.20)
Soil water content (%)	34.5 ^{cd}	(2.3)	53.7 ^{cd}	(5.0)	136 ^b	(11)	27.2 ^d	(2.2)	59.0 ^c	(2.0)	172 ^a	(12)
Bulk density (g cm ⁻³) [#]	0.89 ^{ab}	(0.16)	1.09 ^a	(0.12)	0.48 ^c	(0.15)	0.83 ^b	(0.02)	0.62 ^c	(0.06)	0.51 ^c	(0.06)

N=5 (1 SE).

For each row, mean values with different superscripts are significantly different ($\alpha=0.05$).

[#]For bulk density, a separate set of 0–10 cm soils were used (see text). N=4–6.

factors (Bird et al., 1996). The total C concentration was multiplied by the mean bulk density (Table 2) to estimate the standing C stock, which was then partitioned into the density fractions using the mass partitioning ratios among the four fractions (three density fractions and >4-mm detritus). We did not quantify the amounts of larger rocks as those were not common in the surface soils. It is, however, possible that we overestimated the SOM stock by not accounting for larger rocks. Because soil C and bulk density data came from different set of soils, we calculated error propagation from the relative standard deviation (RSD) for each term as follows:

$$\text{RSD}_{\text{total soil C stock}} = \text{square root of } \left[(\text{RSD}_{\text{OC concentration}})^2 + (\text{RSD}_{\text{bulk density}})^2 \right].$$

Using the total standing C stock in O-horizon plus 0–10 cm mineral horizon and annual C input to soil, mean residence time of C was estimated assuming steady state. Annual C input to the soil at each site was calculated as the sum of litterfall rate and the belowground C input (fine root turnover). The latter was assumed to be 20–60% of aboveground net primary productivity (ANPP) based on values from temperate forests (Waring et al., 1998; Norby et al., 2004). Thus the final mean residence time was shown as a range. We used the litterfall and ANPP values from Kitayama and Aiba (2002).

We treated altitude and parent material as observational treatments and the five samples per site as replicates of each pair of altitude and parent material. We performed two-way factorial ANOVA to test for the difference in C and N concentrations and C:N ratios in bulk soil and density fractions between altitudes, parent materials, and their interactions. Residuals were checked for normality and constant variance and, if necessary, natural log transformed. Tukey–Kramer HSD test was performed to compare means among the six sites as ad hoc test. For soil pH and soil water content, we simply compared means using the Tukey–Kramer test. All references to statistical significance were based on 5% probability level unless otherwise noted. Simple linear regression analysis was performed to assess the relationship between the C pools among the isolated density fractions (mg C g⁻¹ bulk soil) and labile C (mg CO₂-C g⁻¹ bulk soil), i.e. respired C during the 30-day incubation. All statistical analyses were conducted using JMP version 6.0.3 (SAS Institute Inc.).

3. Results

3.1. General characteristics of soil and density fractions

O-horizon thickness tended to increase with increasing altitude (Table 1) presumably reflecting slower decomposition under cooler and wetter conditions. No Oa horizon (H layer) was developed at 700-m sites. O-horizons at upper altitudes (1700–2700 m) consisted of 0–5 cm of Oi horizon and 2–7 cm of Oe+Oa horizon. Parts of the 1700-m metasedimentary site had a particularly thick Oe horizon. A-horizon thickness also tended to increase with altitude (field observation).

Altitude had a significant effect on total C and N concentrations in the 0–10 cm surface mineral soils ($p<0.001$) whereas parent material did not. The interaction of altitude and parent material, however, showed moderate significance ($p=0.013$). With the increase in altitude, surface soil organic matter concentrations progressively increased on both metasedimentary and ultrabasic parent materials, and bulk density correspondingly decreased (Table 2). Bulk soil C:N ratio was significantly influenced by altitude, parent material, and their interactions ($p<0.005$). At 2700 m, metasedimentary soils had significantly higher C and N concentrations and C:N ratios than ultrabasic soils (Table 2). At 700 and 1700 m, on the other hand, total C and N were similar but C:N ratio was significantly lower in metasedimentary soils than their ultrabasic counterparts. Soil pH was significantly lower by roughly 1 unit in metasedimentary than ultrabasic soils at each altitude (Table 2).

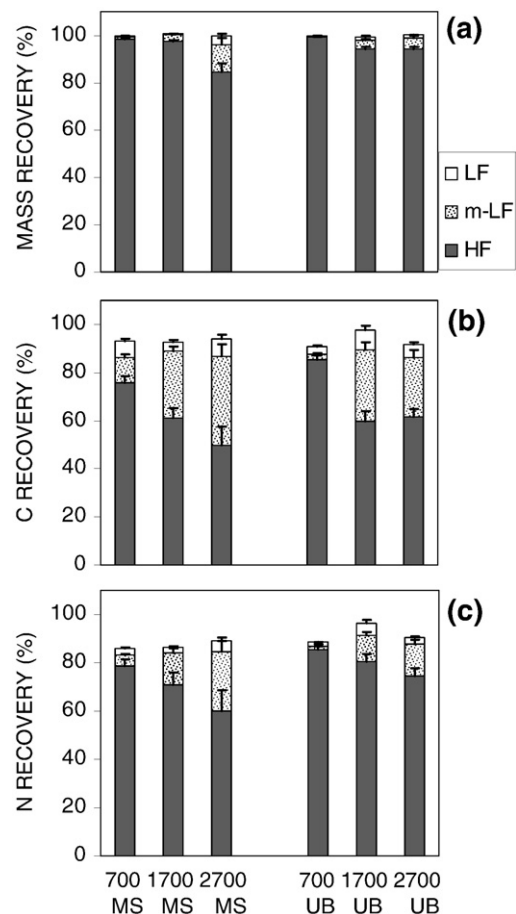


Fig. 1. Recovery and partitioning of mass (a), carbon (b), and nitrogen (c) to the three density fractions. Recovery was calculated relative to the values from bulk soil sample. Error bar=1 SE.

Table 3
Concentration of C and N in the isolated density fractions on fraction (upper) and bulk soil basis (lower)

Parent material	Metasedimentary rock series			Ultrabasic rock series		
	700 m	1700 m	2700 m	700 m	1700 m	2700 m
C in f-LF (mg g ⁻¹ fraction)	364 ^a (9)	354 ^a (9)	343 ^a (7)	357 ^a (20)	355 ^a (16)	325 ^a (13)
C in m-LF	480 ^{ab} (12)	507 ^a (13)	472 ^{ab} (7)	481 ^{ab} (20)	494 ^{ab} (12)	439 ^b (12)
C in HF	22 ^c (2)	31 ^c (5)	80 ^a (10)	26 ^c (2)	36 ^{bc} (3)	54 ^b (4)
N in f-LF (mg g ⁻¹ fraction)	13.9 ^a (1.2)	12.3 ^a (0.5)	10.1 ^a (1.0)	13.9 ^a (1.0)	10.8 ^a (0.7)	10.6 ^a (0.4)
N in m-LF	16.0 ^b (0.6)	12.8 ^{bc} (1.6)	14.8 ^b (0.7)	20.4 ^a (1.5)	8.9 ^c (0.5)	13.6 ^b (0.9)
N in HF	1.9 ^c (0.2)	2.0 ^c (0.4)	4.8 ^a (0.6)	1.8 ^c (0.2)	2.5 ^{bc} (0.2)	3.8 ^{ab} (0.3)
C in f-LF (mg g ⁻¹ bulk soil)	2.1 ^b (0.5)	1.8 ^b (0.3)	11.8 ^a (4.3)	1.0 ^b (0.1)	5.5 ^{ab} (2.1)	4.6 ^{ab} (0.8)
C in m-LF	3.2 ^b (0.8)	14.0 ^b (2.7)	54.0 ^a (11.1)	0.7 ^b (0.2)	17.9 ^b (3.2)	20.6 ^b (3.3)
C in HF	21.9 ^c (2.1)	29.7 ^c (4.5)	67.3 ^a (7.7)	25.7 ^c (2.0)	33.9 ^{bc} (2.2)	50.4 ^{ab} (3.6)
N in f-LF (mg g ⁻¹ bulk soil)	0.08 ^{ab} (0.02)	0.06 ^{ab} (0.01)	0.36 ^a (0.15)	0.04 ^b (0.01)	0.17 ^{ab} (0.07)	0.15 ^{ab} (0.02)
N in m-LF	0.11 ^{bc} (0.03)	0.39 ^{ab} (0.11)	1.77 ^a (0.44)	0.03 ^c (0.01)	0.33 ^b (0.06)	0.66 ^{ab} (0.13)
N in HF	1.92 ^c (0.15)	1.94 ^c (0.34)	4.07 ^a (0.53)	1.76 ^c (0.18)	2.34 ^{bc} (0.17)	3.61 ^{ab} (0.23)
C:N ratio in f-LF	27.3 ^c (2.8)	28.9 ^{abc} (1.2)	35.0 ^a (3.2)	25.8 ^{bc} (0.8)	33.3 ^{ab} (2.2)	30.6 ^{abc} (1.0)
C:N in m-LF	30.2 ^{bc} (1.2)	43.0 ^{ab} (6.4)	32.2 ^{bc} (1.9)	24.1 ^c (1.8)	56.5 ^a (4.4)	32.8 ^{bc} (2.0)
C:N in HF	11.4 ^c (0.2)	15.6 ^{ab} (0.7)	16.7 ^a (0.3)	14.8 ^{ab} (0.8)	14.6 ^b (0.4)	14.0 ^b (0.4)

N=5 (1 SE).

For each row, mean values with different superscripts are significantly different ($\alpha=0.05$).

Our density separation showed almost full recovery of soil mass (Fig. 1a). Both C and N were, however, slightly lost during the separation process (Fig. 1b,c), possibly as dissolved matter, fine colloids and/or fragmented detritus materials stuck on the glass-fiber filter during the m-LF rinse step. Greater loss of N might have resulted from the loss of low C:N organic material or inorganic N. HF accounted

for 50–85% of total soil C and 60–85% of N across the studied sites (Fig. 1b,c). Most of the remaining SOM was accounted for by m-LF (2–37% of C and 1–25% of N). Along the metasedimentary transect, the partitioning of SOM shifted from HF to m-LF with increasing altitude (Fig. 1b, c). Specifically, HF accounted for 76% ($\pm 3\%$), 61% ($\pm 4\%$), 50% ($\pm 8\%$) and m-LF accounted for 10% ($\pm 1\%$), 28% ($\pm 2\%$), 37%

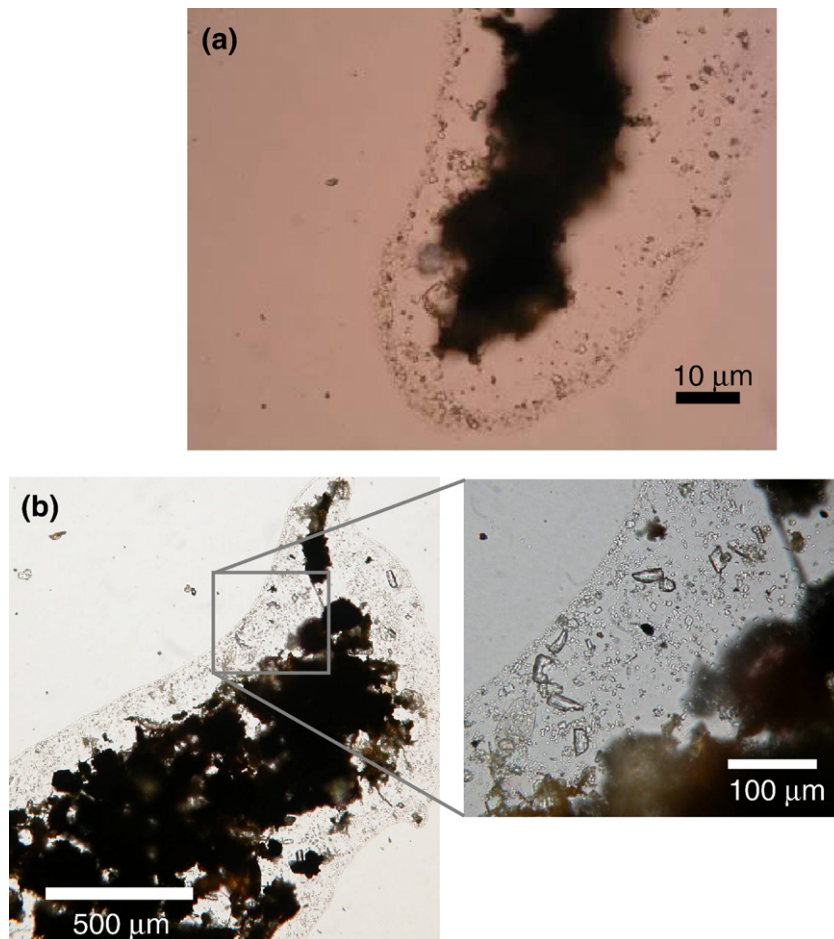


Fig. 2. Examples of mineral coatings on f-LF materials from 1700-m metasedimentary (a) and 1700-ultrabasic soil (b). In the photograph (b), dark matter is a clump of organic detritus fragments of 1–2 mm size. While the mineral coat appears $>100\ \mu\text{m}$ in thickness (left image), the magnified image showed that it was not packed with mineral particles but rather dispersed (right image) presumably because coated particles washed off during its separation and placement onto glass slide. The actual mineral coating must thus be much thinner, perhaps several μm thickness, and consisting of fine particles (clay and fine silt) with occasional coarse silt and sand grains.

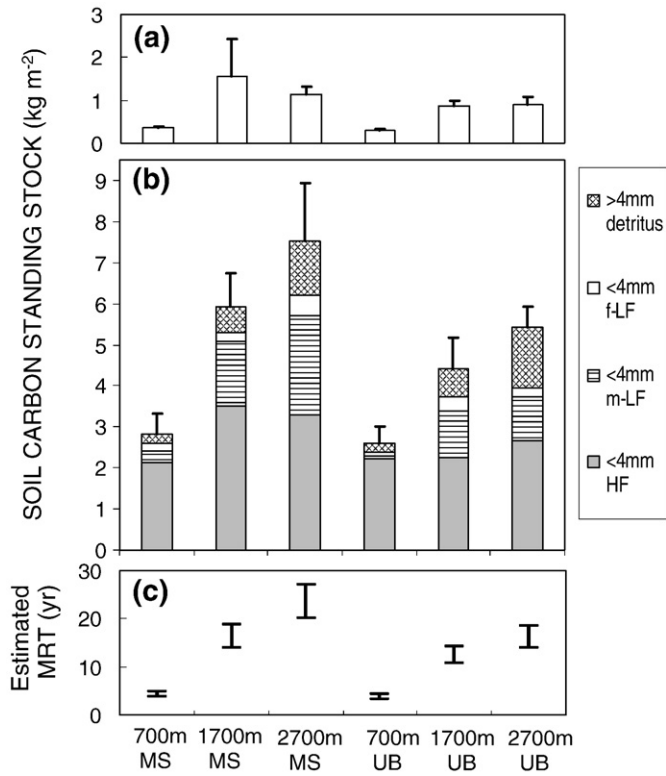


Fig. 3. Soil carbon standing stocks in O-horizon (a) and 0–10 cm mineral horizon (b). Error bar = 1 SE. (c) Mean residence time of the C in the surface soil (O-horizon + 0–10 cm mineral horizon) estimated from litterfall and ANPP data with steady-state assumption (see text).

(±5%) of total soil C at 700, 1700, and 2700 m, respectively (Fig. 1b). A similar trend was found along the ultrabasic transect except for lack of difference between the upper two sites. Nitrogen showed similar partitioning patterns to C (Fig. 1c).

As expected, C and N were much more concentrated in f-LF and m-LF than in HF at all sites (Table 3). While C and N concentrations in the two LF fractions showed no altitudinal trend, those in HF progressively increased with increasing altitude by 2.1–3.6 fold on both parent materials (Table 3). When normalized on a bulk soil basis, altitude significantly influenced the C and N concentrations in all three density fractions whereas parent material and the interaction of altitude and parent material were significant only for m-LF. With the increase in altitude from 700 m to 2700 m, the C and N concentrations in m-LF (on bulk soil basis) increased by 17–29 fold whereas those in HF increased by 2–3 fold (Table 3).

3.2. Microscopic observations

Materials recovered as f-LF were largely coarse plant detritus (generally >100 μm) with relatively intact morphology. Much of the plant detritus in f-LF appeared to be covered with fine mineral particles. In contrast, m-LF was dominated by more fragmented plant detritus (rarely larger than 100 μm) that had little recognizable morphology, except for some pollen- or spore-like materials that were no more than a quarter of the total volume recovered as m-LF (visual observation). Pollen and spores were also observed in this fraction in other studies (Golchin et al., 1994; Baisden et al., 2002). High-density fractions contained no recognizable plant detritus and were instead dominated by mineral particles. These morphological features were consistent across all soils from the six sites, and are in accord with previous studies (Golchin et al., 1994; Sohi et al., 2001; Baisden et al., 2002).

A few fragments of charred material (1–4 mm) were found only in 700-m metasedimentary soils and removed prior to the density separation. We thus tested for black carbon (C left after 375 °C muffling for 24 h, Nguyen et al., 2004) for the 700-m metasedimentary and ultrabasic sites where fire was more likely among the studied sites although we found no trace of fire at any of our sites. The black C was <2.5% of total soil C of both soils. The charred material found at the 700-m metasedimentary soil was likely eolian deposits from small fires associated with local agricultural activity near the park.

Detailed examination of f-LF materials (at moist condition) from the 1700-m metasedimentary and ultrabasic soils showed great heterogeneity in detritus–mineral associations. Much of the detritus, including clumps, appeared to be coated with mineral particles and other translucent materials (Fig. 2). Typical mineral-coat thicknesses were several to tens of μm. On the other hand, the recovered m-LF looked free of mineral particles, presumably due to the detachment of mineral grains from plant detritus by the ultrasound treatment when isolating the m-LF. Assuming that organic matter concentration equals two times the organic C concentration, the f-LF fractions were about two thirds organic matter by weight, with the remainder being presumably mineral matter, while the m-LF fractions were essentially pure organic matter (Table 3). Higher C contents in m-LF than f-LF have been found in many other forest and agricultural soils (Golchin et al., 1994; Parker et al., 2002; Kölbl and Kögel-Knabner, 2004; Swanston et al., 2005; Rasmussen et al., 2005). Note that the original m-LF materials (in field condition), however, must have been associated with mineral particles and aggregates prior to sonication to the same (or greater) extent as f-LF, to have had net density >1.6 g cm⁻³ prior to the initial density-separation step (see Discussion 4.4).

3.3. Organic matter standing stocks

Standing C stock in the O-horizon was lower at 700 m than upper altitudes on both parent materials (Fig. 3a). Standing total C stocks in the 0–10 cm mineral soil (including >4 mm detritus fraction) increased from the 700 m to upper-altitude sites (Fig. 3b). The mean C stock (±standard error) was 2.8 (±0.5), 5.9 (±0.8), and 7.5 (±1.4) kg C m⁻² for 700, 1700, and 2700 m, respectively, along the metasedimentary transect, and 2.6 (±0.4), 4.4 (±0.7), and 5.4 (±0.5) kg C m⁻²

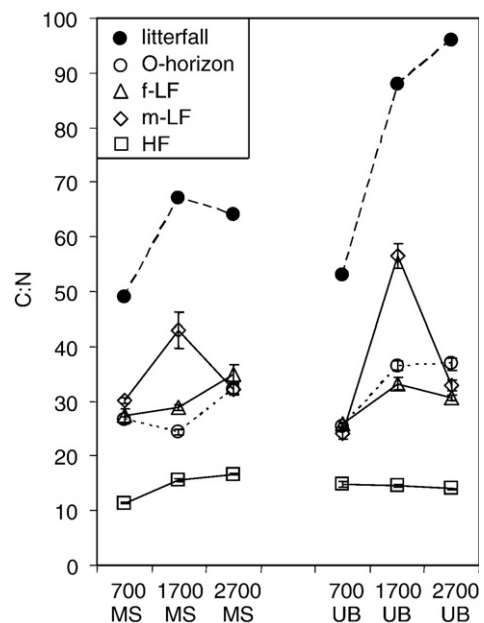


Fig. 4. Mean C:N ratios of the three density fractions at each site. Error bar = 1 SE. Litterfall data from the same sites (Kitayama and Aiba, 2002) were shown for comparison.

Table 4
Proportions of ^{13}C NMR chemical shift regions from isolated fractions

Site ID	Fraction	Percent of total signal intensity (%)						
		Carbonyl	Aromatic	O-alkyl	Alkyl	Alkyl O-alkyl	Aromatic O-alkyl	Aromatic alkyl
		160–220 ppm	110–160 ppm	45–110 ppm	–10–45 ppm			
700 m MS	O-horizon	10.7	19.9	49.1	20.2	0.4	0.4	1.0
	f-LF	11.9	23.9	45.5	18.7	0.4	0.5	1.3
	m-LF	10.1	17.4	33.4	39.1	1.2	0.5	0.4
	HF	11.6	9.0	42.0	37.3	0.9	0.2	0.2
1700 m MS	O-horizon	14.1	18.1	43.9	23.9	0.5	0.4	0.8
	f-LF	12.2	22.7	40.7	24.4	0.6	0.6	0.9
	m-LF	11.2	14.7	22.3	51.8	2.3	0.7	0.3
	HF	13.1	13.1	35.0	38.8	1.1	0.4	0.3
2700 m MS	O-horizon	9.3	23.8	47.2	19.7	0.4	0.5	1.2
	f-LF	10.4	26.2	42.4	21.0	0.5	0.6	1.2
	m-LF	9.3	19.8	40.4	30.6	0.8	0.5	0.6
	HF	9.5	9.9	55.0	25.5	0.5	0.2	0.4
700 m UB	f-LF	14.6	28.9	38.2	18.3	0.5	0.8	1.6
	m-LF	13.4	24.2	32.4	29.9	0.9	0.7	0.8
1700 m UB	f-LF	14.7	22.3	34.5	28.4	0.8	0.6	0.8
	m-LF	11.5	10.1	19.9	58.5	2.9	0.5	0.2
2700 m UB	f-LF	11.3	21.6	43.5	23.7	0.5	0.5	0.9
	m-LF	9.6	13.9	33.2	43.3	1.3	0.4	0.3

High-density fractions from the ultrabasic sites were not analyzed due to high iron contents.
O-horizons from the metasedimentary sites were analyzed for comparison with low-density fractions.

along the ultrabasic transect. Total N stocks in the mineral soil followed a similar pattern (data not shown). The increase in the total SOM stocks with increasing altitude, despite progressive decline in litterfall and aboveground net primary production along the same gradient (Kitayama and Aiba, 2002, Table 1), resulted in longer mean residence times of the surface soil C at higher altitudes (Fig. 3c). Standing C stock in the >4 mm detritus fraction increased with altitude on both parent materials (Fig. 3b). Despite the progressive increase in the C and N concentrations in the density fractions with altitude (Table 3), an altitudinal pattern in their standing stock was less clear due to the decrease in bulk density with altitude (Table 2). Nevertheless, C stocks in f-LF and m-LF appeared to increase from 700 m to the upper two altitudes on both parent materials, whereas the C stock in HF changed little (Fig. 3b). Thus, C stored as decaying plant detritus (>4 mm detritus, f-LF, m-LF) appeared to have higher response to the altitudinal gradient than C stored in HF.

3.4. C:N ratios and NMR results

The HF fraction consistently showed the lowest C:N ratios of 11.3–16.5, while the other fractions (litterfall in particular) were significantly higher across all sites (Fig. 4 and Appendix A). The C:N ratio ranged 24.3–35.6 in O-horizon, 26.9–35.8 in >4-mm detritus, and 25.8–35.0 in f-LF fraction. For these fractions, 2700-m sites showed higher C:N ratios than 700-m sites on both parent materials. The m-LF fraction had C:N ratios similar to other detritus fractions except for anomalously high values at both of the 1700-m sites (Fig. 4). The C:N ratios of HF significantly increased along the metasedimentary transect only (Table 3, Fig. 4). Between the two parent materials at each altitude pair, C:N ratio was significantly different only for the HF at 700 m and 2700 m (Table 3). The HF showed the lowest within-site

variability, while the m-LF fraction showed the greatest variability (Appendix A). Overall, altitude significantly affected the C:N ratio of all density fractions whereas parent material and the interaction of the two state factors showed significant influence only for HF.

^{13}C NMR spectra showed that the abundance of O-alkyl-C (carbohydrate groups) decreased in the following order: O-horizon > f-LF > m-LF in metasedimentary sites and f-LF > m-LF in ultrabasic sites (Table 4). Aromatic-C decreased from f-LF > m-LF > HF at all metasedimentary sites and from f-LF > m-LF in the ultrabasic sites (Table 4). Perhaps the most striking compositional trend from the ^{13}C NMR analyses was the elevated alkyl-C values found in the m-LF fraction (Table 4). The ratio of alkyl-C to O-alkyl-C was higher at the two 1700-m sites (2.3–2.9) than other four sites (0.8–1.3), consistent with the high C:N ratios in these samples (Fig. 4).

3.5. Microbial respiration (labile C) in relation to density fractions

Labile C, defined here as the amount of C respired during a 30-day laboratory incubation, significantly increased with altitude by 3–4 fold (Table 5), similar to the changes in total soil C (Table 2). The labile C normalized to total C was 15–22 mg C g C⁻¹ with no significant differences among sites (Table 5). Neither parent material nor the interaction of altitude and parent material had significant influence on labile C. To elucidate the relationship between the C pools in density fractions and microbially-available C, we correlated the labile C with the C present in each density fraction. We pooled the two sites at each altitude because C partitioning to the density fractions as well as the concentration and chemistry of organic matter in each fraction were similar between the two parent materials. The two LF pools, separately or in combination, had stronger positive correlation with the labile C than the HF pool at all altitudes (Table 6). The results

Table 5
Respired C after 30-day aerobic incubation of bulk soil samples

Parent material	Metasedimentary rock series			Ultrabasic rock series		
	700 m	1700 m	2700 m	700 m	1700 m	2700 m
Respired C after 30 days (mg C g ⁻¹)	0.62 ^c (0.07)	0.73 ^c (0.10)	2.67 ^a (0.43)	0.55 ^c (0.07)	1.18b ^c (0.27)	1.84 ^{ab} (0.20)
Normalized respired C (mg C g C ⁻¹)	21.5 ^a (2.7)	15.0 ^a (0.9)	18.8 ^a (1.3)	18.3 ^a (2.3)	18.3 ^a (1.9)	22.1 ^a (1.1)

N=5 (1 SE).

For each row, mean values with different superscripts are significantly different ($\alpha=0.05$).

Table 6

Correlation coefficient (r) and p -value from linear regression of labile C (mg C g^{-1}) from 30-day laboratory incubation against the amounts of C in each density fraction and bulk soil (mg C g^{-1} bulk) at each altitude ($N=10$)

Explanatory variable	700-m sites		1700-m sites		2700-m sites	
	R	p -value	R	p -value	R	p -value
f-LF	0.62*	0.054	0.92	<0.001	0.82	0.004
m-LF	0.51	0.130	0.89	0.001	0.79	0.007
f-LF+m-LF	0.59*	0.074	0.98	<0.001	0.81	0.005
HF	0.47	0.166	0.68	0.031	0.65	0.042
Bulk	0.56	0.090	0.91	<0.001	0.91	<0.001

* Natural log transformation improved the correlation coefficient to 0.75 ($p=0.013$) for “f-LF” and to 0.65 ($p=0.044$) for “f-LF+m-LF” at the 700-m sites.

support previous inferences that LF pools are more labile than HF (Golchin et al., 1997; Swanston et al., 2005; Rasmussen et al., 2005).

4. Discussion

4.1. Altitude (climate) influence

Higher concentrations and standing stocks of bulk SOM at higher altitudes (Table 1, Fig. 3b), despite progressive decline in productivity and C inputs to soil (Kitayama and Aiba, 2002, Table 1), are mainly attributable to the higher temperature sensitivity of decomposition rate than production rate by photosynthesis (e.g., Kirschbaum, 2000; Raich et al., 2006). The higher sensitivity of microbial decomposition may also explain non-linear response of SOM standing stock; significant increase from 700 m to 1700 m yet no clear difference between the upper two altitudes (Fig. 3a, b). Despite similar rainfall along the gradient, the soil moisture content was higher by 4–6 fold at 2700 m sites than at lower altitudes (Table 2) due to lower evapotranspiration and more frequent cloud formation. The higher soil water content might have further retarded decomposition in addition to the lower temperature, especially in the metasedimentary soils where water drainage was likely poorer due to higher bulk density (see Section 4.2).

Standing stocks of organic matter in the two LF pools (and >4-mm detritus) increased from 700 m to the upper altitudes (Fig. 3b). On the other hand, the C stock in HF remained relatively constant (Fig. 3b) despite the significant increase in its C and N concentrations with altitude (Table 3). This result stemmed from (i) the decrease in bulk density (Table 2) and (ii) lesser increases of C in HF relative to m-LF with altitude (Table 3). The smaller increase in HF with increasing altitude implies limited C flow from LF to HF due to reduced microbial activity under cooler climate and, possibly, due to lower abundance of reactive soil mineral components that can stabilize organic matter in HF. Minerals that enhance SOM stabilization, such as clay and iron (hydr)oxides, (e.g., Baldock and Skjemstad, 2000) were much less abundant at upper altitudes due to slower weathering (Table 1). Specific surface area of these soils decreased and was increasingly covered with organic matter towards the 2700-m sites (Wagai et al., in review-b), consistent with saturation of mineral protective capacity at upper altitudes. The proportion as well as the amount of C stored in m-LF was clearly smaller at lower altitudes (Table 3, Fig. 3), despite higher clay and iron contents compared to higher altitudes (Table 1). Thus, organic matter storage in m-LF appeared to be controlled by the availability of low-density detritus rather than mineral characteristics. The significant influence of clay content on m-LF pool size (Kölbl and Kögel-Knabner, 2004) may be masked at our sites by the over-riding climate control.

At the 1700 and 2700-m sites, where bulk soil C as well as the proportions of bulk C in the LF pools were much higher than 700-m soils (Fig. 1b, Tables 2 and 3), labile C assessed by laboratory incubation significantly correlated with the C concentration in all the fractions and bulk soil (Table 6). In contrast, at 700 m where bulk C was only

30 mg g^{-1} and 75–85% of it was strongly associated with mineral matrix as HF (Table 2, Fig. 1b), the variation in labile C was poorly explained by the C concentrations in the bulk or density fractions except for f-LF pool which showed a marginal significance (Table 6). These altitudinal changes in the relationship between labile C and the C pools in the density fractions suggest a shift in biological role of f-LF. LF materials became increasingly important limiting substrate for heterotrophs at lower altitudes where faster turnover rate (Fig. 3-c) kept the SOM level low and thus mineral protection of the remaining organic material was relatively more effective. Low C loading on mineral surfaces ($<1 \text{ mg C m}^{-2}$) found only at the 700-m sites (Wagai et al., in review-b) corroborates this idea. The correlation patterns (Table 6) and the partitioning of SOM into the density fractions (Fig. 1, 3) suggest that the two LF pools might have higher sensitivity to the altitude-induced, climate difference than the HF pool which is more protected by mineral matrix against microbial enzymatic attack.

Our inference agrees with studies that directly assessed C turnover using ^{14}C tracer along other climate gradients. Trumbore et al. (1996) showed that the mean residence time in LF (comparable to our f-LF) correlated negatively with MAT (ranging 4–25 °C) along altitude and latitudinal gradients, whereas that in HF (our “m-LF plus HF”) showed no significant correlation. Across non-water-stressed forests around the world, Bird et al. (1996) also showed significant covariation of plant detritus turnover in surface soil with temperature along a latitudinal gradient. It is therefore clear that the turnover of plant detritus having little association with minerals is highly sensitive to climate. Our results further suggest that the m-LF pool is also climate sensitive although the chemistry (and presumably factors constraining the decomposition rate) of m-LF varied drastically along Kinabalu climate gradient (see Section 4.4).

4.2. Parent material influence

The quantity and quality of SOM stored can vary according to the type of parent material; for example, rock chemistry can influence plant community and thus litter inputs to soil, and weathering products (i.e. soil minerals) vary in reactivity with organic matter. In the Sierra Nevada mountains, forest soils developed on basic parent materials accumulated more SOM than those on acid parent materials, presumably due to the difference in weathering rate, acidity, aggregate stability, and/or the amounts of clay-size minerals and poorly-crystalline phases of aluminum (Harradine and Jenny, 1958; Rasmussen et al., 2005). In contrast, for the surface mineral soils in Mt. Kinabalu, ultrabasic soils had similar or lower concentrations and standing stocks of organic matter than metasedimentary soils (Table 2 and Fig. 3b) despite much higher iron oxide content of the former (Table 1). The amounts of organic matter recovered as m-LF, which presumably includes aggregate-protected C, were also similar between the two parent materials (Table 3), although iron-rich soils can promote aggregation (Krishnamurti and Huang, 1987). Even in HF, where organic matter is most strongly bound to minerals, C and N contents were similar between the two parent materials (Table 3). Mineralogy nevertheless had some direct influence on SOM storage in HF. At the lowest altitude, where soil mineralogy was most different, (Table 1), sorptive association with iron oxide accounted for 47% and 14% of the C in HF in the ultrabasic and metasedimentary soils, respectively (Wagai and Mayer, 2007). Kaolinite dominates the mineral surface area of 700-m metasedimentary soils, but has at least an order of magnitude lower sorptive capacity than iron oxides (Tipping, 1981; Benke et al., 1999; Jardine et al., 1999; Nanbu and Yonebayashi, 2000; Kaiser and Guggenberger, 2007). Thus, sorption is likely a major mechanism of SOM storage in the 700-m ultrabasic soil. Other mechanisms are necessary to account for the storage in HF of the 700-m metasedimentary soil, such as hindering accessibility via aggregate formation, (Adu and Oades, 1978; Mayer, 1994) (see Section 4.3). While we focused on surface soil, the influence of parent material and

soil mineralogy on SOM storage is likely stronger at subsurface horizons.

At higher-altitude sites, differences in soil drainage, pH, and C input rate between the two parent materials may have had greater influence on SOM dynamics than soil mineralogy. The metasedimentary soils at 1700 and 2700 m had significantly lower redox potential and higher soil water content than the ultrabasic soils at two different sampling times (Kitayama et al., 1998; Hall et al., 2004) although our current samples didn't show such difference (Table 2). The iron-rich ultrabasic soils have more pedogenic iron oxides (Wagai and Mayer, 2007), which can structure soils to increase porosity and water infiltration rate (Uehara and Gillman, 1981). In addition, lower pH in the metasedimentary soils might have reduced decomposition (e.g., Motavalli et al., 1995). Lower forest productivity (Kitayama and Aiba, 2002, see Table 1) and thus lower C input to soil in the ultrabasic sites at higher altitudes might have also contributed to the lower SOM level. Thus parent material may have influenced SOM storage directly through mineralogical differences at the low altitude vs. alteration of soil pH, water content, and/or primary productivity at the higher altitudes.

4.3. Interactive influence of altitude and parent material

Stoichiometry of organic matter was affected by altitude, parent material, and their interactions. The increases in C:N and C:P ratios of litterfall material with increasing altitude is attributed to higher nutrient resorption efficiency of the trees at leaf senescence under more nutrient-limited soils, resulting in N- and P-depleted litter (Kitayama and Aiba, 2002), and likely contributed to the higher C:N of organic detritus pools fractions at higher altitudes (Fig. 4). The C:N in HF on metasedimentary series also showed significant increase in C:N ratio with altitude, whereas that on ultrabasic series were essentially constant despite increasing C concentration in HF with altitude on both parent materials (Table 3, Fig. 4). The difference in C:N trends may be related to the higher abundances of phyllosilicate clay minerals in the metasedimentary sites and iron oxides in the ultrabasic sites (Table 1). Due to the dominance of inert siloxane surfaces, phyllosilicate clays may protect organic matter from decay in a more physical fashion than more chemically reactive iron oxides (Gu et al., 1994; Kaiser and Guggenberger, 2007; Wagai and Mayer, 2007). Microaggregates (<2 μm) consisting of plant cell wall residues encrusted by fine clay particles (Fig. 6a in Chenu and Plante, 2006) may be an example. Clay-protected organic matter might therefore retain more of the character of its detrital precursor material, while the composition of organic matter associated with iron oxides is more constrained by the chemical nature of the sorptive or complexation interaction.

A major stoichiometric relationship among the organic matter pools across the six sites was the widening gap in C:N ratios between initial (litter) and end (HF) materials with increasing altitude (Fig. 4). This diverging trend suggests that similar amounts of N may be required to accumulate one unit mass of C into the HF at all altitudes, while the initial material (substrate) is increasingly N-depleted at higher altitudes. If true, then the microbial processes associated with organic matter transfer from LF pools to HF may be N-limited. Soil net mineralization and nitrification rates decreased almost to zero with increasing altitude, especially on the ultrabasic rock series (Kitayama et al., 1998; Hall et al., 2004). In an alpine meadow system, Neff et al. (2002) showed that fertilization with urea-N increased soil C storage in the HF, implying that N availability limited the C transfer from LF to HF. Parker et al. (2002), in contrast, found no significant increase in the C storage in HF after eight years of $(\text{NH}_4)_2\text{SO}_4$ application in cool temperate forest soils. N controls on SOM dynamics are complex (e.g., Swanston et al., 2004), but interactive effects of climate and parent material on the stoichiometry of HF, which represents the bulk of stable SOM, merits further investigation as it has significant implication for long-term ecosystem dynamics (e.g., Hobbie et al., 2002).

4.4. Nature of m-LF

Compared to f-LF and HF, the formation and ecological significance of m-LF is less clear as pointed out by Baisden et al. (2002) and Wagai et al. (in review-a). This fraction is commonly interpreted as an intermediate stage in the microbial processing between f-LF and HF and considered to form via preferential O-alkyl-C degradation of plant detritus by microbes and their synthesis of alkyl-C during which mineral particles and aggregates occlude the detritus (Golchin et al., 1997). In addition to mineral control of m-LF pool size (Kölbl and Kögel-Knabner, 2004), we suggest that selective preservation of recalcitrant biomolecules may be as important in m-LF formation in some soils based on three lines of observations. First, much higher C:N (Fig. 4) and aliphaticity (ratio of alkyl-C to O-alkyl-C) of m-LF compared to f-LF materials found at the two 1700-m sites (Table 4) is not necessarily consistent with the current model. Across all sites, the higher aliphaticity at the 1700-m sites was most clear in the m-LF but also apparent in f-LF (and O-horizon and HF for the 1700-m metasedimentary sites) (Table 4). These compositional trends suggest selective preservation of highly aliphatic source material in the m-LF. Dominant canopy trees in the 1700-m montane forest sites include the coniferous Podocarpaceae family and *Tristaniopsis* species that have tough and thick leaves (Aiba and Kitayama, 1999). Kitayama et al. (2004) showed that the concentration of ethanol-benzene extractable resin, a highly aliphatic fraction, in fresh litterfall materials was highest (18–24% by wt) at the 1700-m sites. Aliphatic biomolecules are highly recalcitrant and tend to accumulate in soil (Lorenz et al., 2007). Golchin et al. (1994) also found high aliphaticity of <1.6 g cm^{-3} occluded LF (equivalent to our m-LF) in soil under an acacia forest, whose litter is high in aliphatic-C, whereas the same fraction in soil under wheat-fallow rotation showed low aliphaticity. Higher C:N ratios of m-LF compared to that of f-LF have been reported in other forest, grassland, and agricultural soils (Parker et al., 2002; John et al., 2005; Rasmussen et al., 2005; Swanston et al., 2005). Second, low abundance of soil macrofauna at the mid-altitude sites in Mt. Kinabalu (Ito et al., 2002) might enhance accumulation of aliphatic plant residues. For instance, earthworms preferentially degraded aliphatic plant detritus in deciduous forest soils (Crow et al., in review). Third, the two 1700-m soils contained more endo-mycorrhizal spores ($4.5\text{--}11.0 \times 10^6$ spores m^{-2}) than the other four sites (T. Satomura, unpublished data). Fungal spores, like pollen, are coated with aliphatic compounds (Beilby, 1980).

Thus, plant detritus and/or heterotrophic soil organisms unique to the 1700-m sites might have contributed to the preservation of high C:N and aliphatic-rich compounds in m-LF. Variability in the C:N ratio, C structure, and/or mean residence time of m-LF appears greater compared to f-LF among the soils studied here and elsewhere (Golchin et al., 1994; Baisden et al., 2002; Parker et al., 2002; Rasmussen et al., 2005; Swanston et al., 2005), suggesting that m-LF may not be a homogeneous pool which forms solely via aggregate occlusion during the decomposition of f-LF materials (Wagai et al., in review-a). Further investigation on the formation, fate, and biodegradability of m-LF would help to improve current models of SOM dynamics.

5. Conclusion

Our study showed a strong altitudinal control on the concentration, standing stock and partitioning of SOM into the three density fractions in 0–10 cm mineral horizons. The two LF pools likely had higher apparent sensitivity to the altitude-induced, climate difference than the HF pool which is presumably more protected by mineral matrix against microbial enzymatic attack. Parent material control was more complex. Greater SOM storage in metasedimentary soils at the 1700 and 2700 m altitudes likely related to higher litter input and/or reduced decomposition under wetter or more acidic conditions. The C:N ratio of HF increased with altitude only along with the

metasedimentary transect, which likely reflected mineralogical differences between the two parent materials. The HF isolated here was, nevertheless, significantly different from the two LF pools with respect to morphology and chemistry. If we had not separated the m-LF from HF by the sonication step, then the standing stock of conventional HF (i.e. m-LF plus HF) would have shown a significant response to the altitudinal gradient and we wouldn't have found the consistently low C:N of, and the significant parent material influence on, the HF. The three-pool density-separation method (Golchin et al., 1994) used here was, therefore, effective in isolating ecologically-meaningful SOM pools.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.geoderma.2008.07.010.

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