Biogeochemistry of mineral–organic associations across a long-term mineralogical soil gradient (0.3–4100 kyr), Hawaiian Islands

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Abstract

Organic matter (OM) in mineral–organic associations (MOAs) represents a large fraction of carbon in terrestrial ecosystems which is considered stable against biodegradation. To assess the role of MOAs in carbon cycling, there is a need to better understand (i) the time-dependent biogeochemical evolution of MOAs in soil, (ii) the effect of the mineral composition on the physico-chemical properties of attached OM, and (iii) the resulting consequences for the stabilization of OM. We studied the development of MOAs across a mineralogical soil gradient (0.3–4100 kyr) at the Hawaiian Islands that derived from basaltic tephra under comparable climatic and hydrological regimes. Mineral–organic associations were characterized using biomarker analyses of OM with chemolytic methods (lignin phenols, non-cellulosic carbohydrates) and wet chemical extractions, surface area/porosity measurements (N2 at 77 K and CO2 at 273 K), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and differential scanning calorimetry (DSC). The results show that in the initial weathering stage (0.3 kyr), MOAs are mainly composed of primary, low-surface area minerals (olivine, pyroxene, feldspar) with small amounts of attached OM and lignin phenols but a large contribution of microbial-derived carbohydrates. As high-surface area, poorly crystalline (PC) minerals increase in abundance during the second weathering stage (20–400 kyr), the content of mineral-associated OM increased sharply, up to 290 mg C/g MOA, with lignin phenols being favored over carbohydrates in the association with minerals. In the third and final weathering stage (1400–4100 kyr), metastable PC phases transformed into well crystalline secondary Fe and Al (hydr)oxides and kaolin minerals that were associated with less OM overall, and depleted in both lignin and carbohydrate as a fraction of total OM. XPS, the N2 pore volume data and OM–mineral volumetric ratios suggest that, in contrast to the endmember sites where OM accumulated at the surfaces of larger mineral grains, topsoil MOAs of the 20–400-kyr sites are composed of a homogeneous admixture of small-sized PC minerals and OM, which originated from both adsorption and precipitation processes. The chemical composition of OM in surface-horizon MOAs, however, was largely controlled by the uniform source vegetation irrespective of the substrate age whereas in subsoil horizons, aromatic and carboxylic C correlated positively with oxalate-extractable Al and Si and CuCl2-extractable Al concentrations representing PC.
aluminosilicates and Al-organic complexes ($r^2 > 0.85$). Additionally, XPS depth profiles suggest a zonal structure of sorbed OM with aromatic carbons being enriched in the proximity of mineral surfaces and amide carbons (peptides/proteins) being located in outer regions of MOAs. Albeit the mineralogical and compositional changes of OM, the rigidity of mineral-associated OM as analyzed by DSC changed little over time. A significantly reduced side chain mobility of sorbed OM was, however, observed in subsoil MOAs, which likely arose from stronger mineral–organic bindings. In conclusion, our study shows that the properties of soil MOAs change substantially over time with different mineral assemblages favoring the association of different types of OM, which is further accentuated by a vertical gradient of OM composition on mineral surfaces. Factors supporting the stabilization of sorbed OM were (i) the surface area and reactivity of minerals (primary or secondary crystalline minerals versus PC secondary minerals), (ii) the association of OM with micropores of PC minerals (via ‘sterically’ enhanced adsorption), (iii) the effective embedding of OM in ‘well mixed’ arrays with PC minerals and monomeric/polymeric metal species, (iv) the inherent stability of acidic aromatic OM components, and (iv) an impaired segmental mobility of sorbed OM, which might increase its stability against desorption and microbial utilization.

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1. INTRODUCTION

Up to >90% of a soil’s carbon inventory resides in intimate association with minerals (Baisden et al., 2002; Basile-Doelsch et al., 2007; Crow et al., 2007). Organic C (OC) bound to minerals is considered relatively stable (Torn et al., 1997; Keil et al., 1998; Kalbitz et al., 2005; Mikutta et al., 2006) and controls the long-term sequestration of organic matter (OM) in terrestrial and marine ecosystems. The ability of ecosystems to sequester atmospheric carbon thus depends on the partitioning of soil and sediment carbon into more stable fractions (IPCC, 2001) such as mineral–organic associations (MOAs). Organic matter stabilization by minerals has been envisioned as a combined effect of the chemical properties of mineral sorbents (surface area and charge) and the structural properties of the OM involved, with aromatic structures being most resistant towards biodegradation (Kalbitz et al., 2005; Mikutta et al., 2007).

Most primary minerals in soils are in disequilibrium with extrinsic environmental factors like the temperature and hydrochemical regime. Thus, weathering triggers the formation of thermodynamically more stable mineral phases such as Fe and Al (hydr)oxides and aluminosilicate clay minerals. As a result, the inventory of clay and secondary metal (hydr)oxides in soils can increase substantially over time spans of thousands of years (e.g., Harden, 1987; Masiello et al., 2004). Such mineralogical shifts affect the amount of soil OC stored (Torn et al., 1997; Brenner et al., 2001; Basile-Doelsch et al., 2007), but little is known about compositional variations of mineral-associated OM (MOM) in different mineralogical settings. Batch experiments reveal that OM often sorbs to minerals selectively due to chemical and molecular size fractionation (Aufdenkampe et al., 2001; Chorover and Amistadi, 2001; Feng et al., 2005). In soils, Fe oxides exhibit a strong affinity for highly oxidized aromatic, lignin-derived OC (Kaiser et al., 1997; Chorover and Amistadi, 2001; Mikutta et al., 2007), whereas river sediments and kaolinite were reported to retain N-containing compounds selectively (Aufdenkampe et al., 2001). A mineralogical impact on OM composition has also been tentatively suggested by analysis of OM composition (lignin, cutin and suberin acids) of mineralogically diverse density fractions of an Andic Dystrudept A horizon (Sollins et al., 2006). Straightforward evaluation of a mineralogical control can, however, be compromised by the fact that MOM is subjected to in-situ microbial transformations, which may mask the mineral-induced chemical imprint (Keil et al., 1998). Most studies related to sorptive fractionation and differential stabilization of OM at mineral surfaces have been conducted in sediments (Keil et al., 1998; Arnarson and Keil, 2001; Dickens et al., 2006; Arnarson and Keil, 2007), thus limiting our understanding of long-term compositional changes of MOM in soil environments.

Whereas the chemical composition of soil OM has been subject to numerous studies, little is known about the time-dependent alteration of the physical properties of mineral-attached OM in natural environments. Soil OM undergoes structural changes upon aging (Schaumann, 2006; Hurrass and Schaumann, 2007). Differential scanning calorimetry experiments indicate a decrease in segmental mobility of OM over time, likely as a result of water- or metal-mediated cross-linkings between OM chains (Schaumann, 2005). The flexibility of OM bound directly to mineral surfaces is expected to decrease as more ligands are involved in mineral attachment, and as such, flexibility should depend on the type of minerals present and on the amount and composition of OM accumulated. Steric rearrangements of OM at mineral surfaces leading to the neoformation of organic–organic (Collins et al., 1995) or organic–mineral bonds (Kaiser et al., 2007) are thus supposed to confer a greater stability to sorbed OM. In consequence, mineralogical changes over time affect mineral–OM interactions in at least three aspects: (i) by the development of reactive surfaces

Abbreviations used: MOA, mineral–organic association; A-MOA and B-MOA, mineral–organic associations from A and B horizons, respectively; LSAG, long substrate age gradient; MOC and MN, mineral-associated organic carbon and total nitrogen; MOM, mineral-associated organic matter; OC, organic carbon; PC, poorly crystalline; SSA, specific surface area; TEM–EDS, Transmission electron microscopy–energy dispersive spectrometry; XPS, X-ray photoelectron spectroscopy.
that will enable OM to interact with minerals, (ii) by creating conditions that allow for sorptive fractionation processes of OM, and (iii) by changing the physical properties of sorbed OM.

This study investigates the changing nature of mineral–organic associations during progressive basalt weathering (Pliocene to Holocene) in the Hawaiian archipelago. We complement previous research on mineral–OM relationships in soils of this weathering gradient known as ‘long substrate age gradient’ (LSAG) where the transformation of mineral matter over 0.3–4100 kyr causes a differential storage of OM (Torn et al., 1997, 2005; Chorover et al., 2004; Vitousek, 2004). The main objective of this study is to examine the time-dependent formation and characteristics of MOAs and the consequences for the chemical and physical properties of MOM, and thus to contribute to the understanding of OM stabilization in soils. We hypothesize that the time-dependent transformation of primary silicates dominating in juvenile basaltic lava into more mature secondary minerals is accompanied by distinct changes in the physico-chemical properties of MOM. The LSAG is most suitable to trace the impact of minerals because each site has a similar geological setting, climate, and plant species composition, but different soil mineral composition. The similar plant species assemblage at each site is critical to the study because it minimizes the influence of primary organic resources on the chemical variability of MOM. Mineral–organic associations were isolated densimetrically and examined along the LSAG according to three aspects: (i) changes in mineralogical composition, surface area and porosity were elucidated by selective extractions, X-ray photoelectron spectroscopy (XPS), gas adsorption ($N_2$ at 77 K and $CO_2$ at 273 K), and transmission electron microscopy coupled with energy dispersive spectroscopy (TEM–EDS); (ii) the chemical transformation of OM in MOAs was examined by XPS and molecular biomarker analyses (lignin phenols, non-cellulosic carbohydrates), and (iii) the alteration of the physical state of OM in MOAs over time was investigated by differential scanning calorimetry (DSC).

2. MATERIALS AND METHODS

2.1. Study site and sample collection

Detailed descriptions of the geological, climatic and morphological settings of the LSAG sites are given in Vitousek (2004) and Chorover et al. (2004). All soils were formed from basaltic materials composed primarily of tephra overlying lava or a lava-ash mixture. Parent material age, measured using $^{14}C$ and $K/Ar$ radiometric techniques, ranges from 0.3 to 4100 kyr (Fig. 1 and Table 1). The sites are located at 1130–1500 m elevation above sea level with a mean annual temperature of 289 K and a mean annual precipitation of 2500 mm. At each site the montane rainforest is dominated by *Metrosideros polymorpha* (>75%) while the subcanopies are more diverse across the sites, including different native tree ferns in the genus *Cibotium*. Soils of the six sites have been classified as (1) Thaptic Udivitrand (Thurston), (2–4) Aquic Hydrudand (Laupahoehoe, Kohala, Pololu), (5) Aquic Hapludand (Kolekole), and (6) Plinthic Kandiudox (Kokee) (Chorover et al., 2004). Source vegetation, organic layers, and mineral A and B horizons (0–20; 10–100 cm; Table 1) were sampled in June 2007 analogous to the sampling scheme described in Chorover et al. (2004). Samples were carefully mixed in the field, double packed in Ziploc bags, and stored in the laboratory coolers on blue ice. High priority-shipping to Germany was accomplished at a temperature <278 K within four days. In the laboratory, soil samples were immediately pushed through a 2-mm sieve, roots, visible

![Fig. 1. The Hawaiian archipelago and study sites (ESRI data base).](image-url)
Table 1
Site age, origin, sampling depth and pH of soils, and basic properties of isolated mineral–organic associations (MOAs) from A and B horizons of the long substrate age gradient.

<table>
<thead>
<tr>
<th>Site age (kyr)</th>
<th>Island</th>
<th>Depth (cm)</th>
<th>Soil pH (H₂O)</th>
<th>Absolute density (MOA) (g/cm³)</th>
<th>MOCᵃ</th>
<th>MNᵇ</th>
<th>C/N</th>
<th>MOC₉OHᵇ</th>
<th>XAD-8ᶜ</th>
<th>Fe₉₀ᵈ</th>
<th>Fe₉₀ᵈ</th>
<th>Fe₉₀/Fe₉₀ᵈ</th>
<th>Al₉₀ᵈ</th>
<th>Al₉₀ᵇ</th>
<th>Si₉₀ᵈ</th>
<th>Clay mineral compositionᶠ</th>
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</thead>
<tbody>
<tr>
<td><strong>A horizons</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Thurston 0.3</td>
<td>Hawaii</td>
<td>2–9</td>
<td>5.5</td>
<td>2.9</td>
<td>18.7 (0.8)</td>
<td>2.3 (0.3)</td>
<td>8</td>
<td>70</td>
<td>56</td>
<td>5.0</td>
<td>4.3</td>
<td>0.9</td>
<td>2.6</td>
<td>1.5</td>
<td>0.8</td>
<td>P, F, a</td>
</tr>
<tr>
<td>Laupahoehoe 20</td>
<td>Hawaii</td>
<td>0–10</td>
<td>4.1</td>
<td>2.4</td>
<td>177.5 (0.2)</td>
<td>9.9 (0.1)</td>
<td>18</td>
<td>47</td>
<td>66</td>
<td>153.6</td>
<td>84.2</td>
<td>0.5</td>
<td>3.3</td>
<td>0.9</td>
<td>0.3</td>
<td>F, A, m, q</td>
</tr>
<tr>
<td>Kohala 150</td>
<td>Hawaii</td>
<td>0–7</td>
<td>3.9</td>
<td>2.1</td>
<td>187.6 (0.1)</td>
<td>13.2 (0.1)</td>
<td>14</td>
<td>63</td>
<td>68</td>
<td>46.9</td>
<td>37.4</td>
<td>0.8</td>
<td>18.5</td>
<td>11.1</td>
<td>0.8</td>
<td>A, F, v, hiv, q</td>
</tr>
<tr>
<td>Pololu 400</td>
<td>Hawaii</td>
<td>0–10</td>
<td>3.9</td>
<td>1.8</td>
<td>290.4 (0.0)</td>
<td>19.4 (0.1)</td>
<td>15</td>
<td>66</td>
<td>81</td>
<td>43.7</td>
<td>40.0</td>
<td>0.9</td>
<td>7.6</td>
<td>6.1</td>
<td>0.1</td>
<td>A, F, v, hiv, k, q</td>
</tr>
<tr>
<td>Kolekole 1400</td>
<td>Molokai</td>
<td>0–8</td>
<td>4.2</td>
<td>2.3</td>
<td>131.1 (0.1)</td>
<td>9.7 (0.1)</td>
<td>13</td>
<td>60</td>
<td>65</td>
<td>40.1</td>
<td>15.4</td>
<td>0.4</td>
<td>4.2</td>
<td>1.9</td>
<td>0.1</td>
<td>K, Gi, F, he, q</td>
</tr>
<tr>
<td>Kokee 4100</td>
<td>Kauai</td>
<td>0–16</td>
<td>4.8</td>
<td>3.2</td>
<td>40.0 (0.1)</td>
<td>2.8 (0.2)</td>
<td>14</td>
<td>74</td>
<td>49</td>
<td>241.3</td>
<td>44.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.2</td>
<td>0.0</td>
<td>K, Gi, Go, he</td>
</tr>
<tr>
<td><strong>B horizons</strong></td>
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</tr>
<tr>
<td>Thurston 0.3</td>
<td></td>
<td>15–23</td>
<td>6.1</td>
<td>2.8</td>
<td>23.2 (0.1)</td>
<td>2.5 (0.1)</td>
<td>9</td>
<td>46</td>
<td>51</td>
<td>11.5</td>
<td>10.7</td>
<td>0.9</td>
<td>8.1</td>
<td>1.5</td>
<td>4.1</td>
<td>P, F, a</td>
</tr>
<tr>
<td>Laupahoehoe 20</td>
<td></td>
<td>45–67</td>
<td>5.3</td>
<td>2.3</td>
<td>113.6 (0.1)</td>
<td>5.6 (0.2)</td>
<td>20</td>
<td>86</td>
<td>46</td>
<td>100.8</td>
<td>77.9</td>
<td>0.8</td>
<td>113.5</td>
<td>7.6</td>
<td>29.5</td>
<td>F, A, m, q</td>
</tr>
<tr>
<td>Kohala 150</td>
<td></td>
<td>41–60</td>
<td>4.0</td>
<td>2.1</td>
<td>120.6 (0.1)</td>
<td>4.4 (0.1)</td>
<td>27</td>
<td>92</td>
<td>37</td>
<td>15.4</td>
<td>16.9</td>
<td>1.1</td>
<td>137.8</td>
<td>15.9</td>
<td>44.7</td>
<td>A, F, hiv, q</td>
</tr>
<tr>
<td>Pololu 400</td>
<td></td>
<td>44–64</td>
<td>4.9</td>
<td>2.5</td>
<td>81.3 (0.0)</td>
<td>3.6 (0.2)</td>
<td>23</td>
<td>88</td>
<td>46</td>
<td>124.1</td>
<td>56.6</td>
<td>0.5</td>
<td>50.5</td>
<td>9.2</td>
<td>4.9</td>
<td>A, F, v, hiv, k, q</td>
</tr>
<tr>
<td>Kolekole 1400</td>
<td></td>
<td>30–49</td>
<td>4.9</td>
<td>2.6</td>
<td>19.9 (0.1)</td>
<td>2.0 (0.1)</td>
<td>10</td>
<td>100</td>
<td>29</td>
<td>53.3</td>
<td>14.4</td>
<td>0.3</td>
<td>14.0</td>
<td>2.4</td>
<td>2.0</td>
<td>K, Gi, f, he, q, m</td>
</tr>
<tr>
<td>Kokee 4100</td>
<td></td>
<td>50–100</td>
<td>5.2</td>
<td>3.1</td>
<td>12.1 (0.1)</td>
<td>1.6 (0.2)</td>
<td>7</td>
<td>75</td>
<td>21</td>
<td>143.9</td>
<td>2.3</td>
<td>0.0</td>
<td>2.1</td>
<td>0.4</td>
<td>0.1</td>
<td>K, Gi, Go, he, m</td>
</tr>
</tbody>
</table>

ᵃ MOC and MN = mineral-associated organic C and N.
ᵇ Fraction of the mineral-associated OC extractable in 0.1 M NaOH.
ᶜ Fraction of the NaOH-extractable OC that adsorbs to XAD-8 resin after acidification to pH 2.
ᵈ Fe₉₀ = dithionite-extractable Fe; Fe₉₀, Al₉₀, Si₉₀ = oxalate-extractable Fe, Al, Si.
ᵉ Al₉₀ = CuCl₂-extractable Al.
ᶠ Data compiled from Chorover et al. (2004): upper case letters indicate major constituents, lower case letters indicate minor constituents; P, plagioclase; A, short-range-ordered Al gels or aluminosilicates (e.g., allophane); F, ferrihydrite; Q, quartz; V, vermiculite; HIV, hydroxyl-interlayered vermiculite; K, kaolin minerals (kaolinite and/or halloysite); Gi, gibbsite; He, hematite; Go, goethite; M, magnetite.
plant and animal remains were removed, and the material was stored field-moist in the dark at 277 K until use.

2.2. Density fractionation and chemical analyses

Density fractionation: Mineral–organic associations with \( \rho > 1.6 \, \text{g/cm}^3 \) were obtained by suspending the field-moist (20–74 wt.% \( \% \text{H}_2\text{O} \)) low-aggregated samples in sodium polytungstate (SPT) solution. Contaminant OC and N concentrations in the original SPT salt were at the detection limit (\( \approx 0.001\% \text{ OC and } \approx 0.001\% \text{ N} \)). Briefly, between 31 and 98 g field-moist soil (25 g dry weight after freeze-drying at 0.3 mbar) were mixed with 125 mL SPT giving a final density of \( 1.6 \pm 0.01 \, \text{g/cm}^3 \). Following vigorous agitation on a horizontally shaker (300 rpm) for 30 min, the suspensions were left to stand for 30 min and then centrifuged at 4500 g for 15 min. If dispersion was incomplete, the shaking period was extended. A 1.6-g/cm\(^3\) density cutoff was chosen because MOAs, e.g., those derived from coprecipitation of OM with Al, can exhibit very low absolute densities (1.7 g/cm\(^3\)) (Kaiser and Guggenberger, 2007a). Ultrasonic dispersion was avoided because of the risk of transfer MOAs containing poorly crystalline (PC) minerals into the density fraction \(<1.6 \, \text{g/cm}^3\) (Kaiser and Guggenberger, 2007a). The floating particulate OM was transferred on 0.7-μm glass microfibre filter (GF/F Whatman) and vacuum filtered. The procedure was repeated until no floating particulate OM was observed. The particulate OM and MOA fractions were washed with distilled water to an electric conductivity of <50 dS/m. In some cases, colloids started to settle. The particulate OM and MOA fractions were washed with distilled water to an electric conductivity of <50 dS/m. In some cases, colloids started to settle. This procedure was primarily important for the youngest sample (Thurston), which still contains larger primary mineral particles. Then samples were excited with non-monochromated Al K\( \alpha \) radiation (\( E_{\text{exc}} = 1486.6 \, \text{eV} \)) at an incident angle of 45° and an electron beam spot size of 800 μm (~500 μm\(^2\)). Surface composition analyses of MOAs up to a depth of ~3–5 nm included a survey element scan and a high-resolution scan at the C\( _{1s} \) edge. The bulk element composition of MOAs was revealed by scanning from 0 to 1100 eV using a pass energy of 93.90 eV, a channel width of 0.4 eV/step, and a measure time of 20 ms/step (exposition time = 10 min); in the high-resolution mode, MOAs were scanned at the C\( _{1s} \), edge using a pass energy of 11.75 eV, a channel width of 0.1 eV/step and measure time of 100 ms/step (exposition time = 30 min). Significant X-ray-induced alteration of MOM that could cause false structural C assignments is expected only for exposure times >30 min (Dengis et al., 1995; Zubavichus et al., 2004). Vacuum during measurements was \( \approx 3 \times 10^{-9} \, \text{mbar} \). Contamination rate by adventitious C was estimated to be 0.1 atom%/h. Sample charging during analysis lead to peak shifts of \( \approx 3 \, \text{eV} \), which was corrected based on the maximum principal C\( _{1s} \) sub-peak centered at 285 eV. Data analysis was accomplished with the instrument software package (Multipak V6.1A; Physical Electronics) using manufacturer-based sensitivity factors whereas graphics were generated using Unifit for Windows, Version 2008 (Hesse et al., 2003). Identification of binding energies was done according to Moulder et al. (1992). The chemical composition of MOM was revealed by deconvoluting the C\( _{1s} \) peak into sub-peaks by fitting Gaussian–Lorentzian functions (85%/15%) that are assigned to different C environments (NIST XPS database, Monteil-Rivera et al., 2000). The full-width-at-half-maximum (FWHM) of fitted sub-peaks was allowed to vary between 0 and 1.5. Additionally, we analyzed the chemical composition of MOAs from A horizons along depth profiles in one representative sample area (~500 μm\(^2\)) immedi-
ate after sputtering with Ar+ ions for 15 min ($t_{15}$) and 30 min ($t_{30}$), respectively [ion fluence = $1.6 \times 10^{14}/(\text{cm}^2 \cdot \text{s})$]. Thirty minutes were set as time limit for Ar+ gassing to avoid the reduction of C1s signals below that acceptable for peak processing. Etching velocity was $\sim 1.4 \text{ nm/min}$ for pure SiO2. While the sputter efficiency for OM and minerals differs and hence the actual sputter depth remains unknown, this procedure is appropriate to reveal shifts in the vertical composition of OM at particle surfaces (Amelung et al., 2002).

2.5. Surface area and porosity analyses

Gas adsorption and desorption was measured with a Nova 4200 analyzer (Quantachrome Corp., Boynton Beach, USA) using N2 and CO2 as adsorbates. For N2 adsorption, about 100–1000 mg of solid was weighed into the sample cell to ensure sufficient surface area for analysis. Adsorbed water was removed by outgassing the sample at 313 K and 10–3 mbar for 48 h. After re-weighing, N2 adsorption–desorption isotherms were recorded at 77 K in the partial pressure region $2.5 \times 10^{-3}–0.99 \ P_0$ using 40 adsorption and 40 desorption points. $P_0$ was remeasured after every 20th adsorption/desorption point to account for short-term alterations of atmospheric pressure. Thermal equilibration was 90 s, equilibration time was set minimal 240 s and maximal 400 s, and pressure tolerance was 0.1 mm Hg. The specific surface area ($SSA$) was determined from the relative pressure range of 0.05–0.3 by the BET equation (Brunauer et al., 1938). In the BET model, the $C$ constant expresses the energy of adsorption to a surface and relates to the net enthalpy of N2 adsorption ($\Delta H_{\text{ads}}$) as follows (Rouquerol et al., 1998; Mayer, 1999)

$$C_{\text{BET}} \approx \exp[(\Delta H_{\text{ads}} - \Delta H_{\text{cond}})/RT] \approx \exp(\Delta H_{\text{ads}}/RT)$$

(1)

where $\Delta H_{\text{ads}}$ is the adsorption enthalpy of the gas directly on the surface, $\Delta H_{\text{cond}}$ is the enthalpy of gas condensation, $R$ is the universal gas constant, and $T$ is the temperature of gas adsorption. The mineral $SSA$ of MOAs still accessible to N2 ($SSA_{\text{corr}}$) was estimated by correction of gas adsorption data for OM contents according to:

$$SSA_{\text{corr}} = SSA/(1 - \text{MOM}/1000)$$

(2)

where $SSA$ denotes measured specific surface area values (m2/g), and MOM refers to the organic matter content (2 x MOC; mg/g). The volume of mesopores was determined by the Barrett–Joyner–Halenda model (Barrett et al., 1951) using the adsorption leg of the isotherm; the total pore volume was estimated at $P/P_0 = 0.99$.

Carbon dioxide adsorption was performed at 273 K in an ice bath from 2 x $10^{-6}$ to 0.03 $P/P_0$ using 38 adsorption points. Outgassing and measuring conditions were equal to the N2 adsorption experiment. Before determination of the weight of the water-free sample and to avoid contamination with CO2, the sample was backfilled with N2. For both adsorbates, micropore volumes were determined by the Dubinin–Radushkevich equation (Gregg and Sing, 1982).

The absolute density of MOAs (Table 1), as required by the instrument software, was measured in triplicate with a He pycnometer (Micromeritics ACCUPyc 1330, Norcross, USA). The experimental error was $<1\%$. All surface area and porosity analyses were carried out at least in duplicate.

2.6. Transmission electron microscopy (TEM)

Before TEM analysis, MOA samples from A horizons were suspended in methanol and sonicated for 5 min. One drop of the suspensions was placed onto a porous carbon film (Agar Scientific Ltd., Stansted, UK) on a Cu TEM grid and left to dry at room temperature for 5 min. Particle aggregates covering holes in the support film were imaged on a FEI CM200 instrument (FEI Company, Eindhoven, The Netherlands) equipped with a field-emission gun operated at an acceleration voltage of 200 kV. Energy dispersive X-ray spectroscopy (EDS) analyses were performed on MOA particles for areas of various size (20–200 nm) using a UTW ISIS EDX analyzer with an ultrathin window allowing detection of light elements (Oxford Instruments, United Kingdom).

2.7. Differential scanning calorimetry (DSC)

To characterize the degree of rigidity of MOM we used DSC and determined the glass transition-like step transition temperature ($T^*$) of OM as a measure for the matrix rigidity (Hurrell and Schaumann, 2005; Schaumann and LeBoeuf, 2005). Three to six replicates of each sample (2–8 mg MOA) that were equilibrated for at least two weeks at 293 K and 31% humidity were weighed into standard Al pans. The pans were sealed hermetically prior to the DSC experiment. Differential scanning calorimetry experiments were performed using a DSC Q1000 calorimeter (TA Instruments, Alzenau, Germany) with TZero technology® and a refrigerated cooling system, and N2 as a purge gas. The samples were abruptly cooled in the DSC instrument to 223 K and then heated at a rate of 10 K/min from 223 K to 383 K and 403 K for samples from A and B horizons, respectively. This first heating cycle was followed by a second abrupt cooling and a subsequent heating cycle. The DSC data were analyzed using Universal Analysis software Version 4.1 (TA Instruments). Baseline correction was performed by subtracting the thermogram of the second heating cycle from that of the first one to separate the non-reversing from the reversing effects as discussed by Hurrell and Schaumann (2005). The glass transition-like step transition is indicated by an inflection point in the thermogram (Hurrell and Schaumann, 2005; Schaumann and LeBoeuf, 2005). Operationally, three tangent lines were applied and $T^*$ was defined as the temperature at the curve inflection. In order to verify that transitions are solely due to OM, we also analyzed the thermal behavior of a suite of pure minerals at 31% humidity including 2-line ferrhydrite, allopahne, goethite, PC Al(OH)$_3$, gibbsite, vermiculite, and kaolinite (clay minerals <2 μm, Na+-saturated). We found no significant transition in the temperature range examined. Only ferrhydrite revealed a small transition at 363–373 K but neither the transition temperature nor the tran-
sition intensity in the MOA samples correlated with the ferricyanide content (~oxalate-extractable Fe), suggesting that minerals do not significantly contribute to the observed step transitions.

2.8. Chemical analysis of mineral-associated organic matter

Lignin-derived phenols: Lignin-derived phenols were quantified in duplicate after CuO oxidation. Briefly, 25–500 mg of organic layer and MOA samples were mixed with 15 mL 2 M NaOH, 50–100 mg Fe(NH4)2(SO4)2 6H2O, 250–500 mg CuO and 50 mg glucose and oxidized for 2 h at 443 K (Amelung et al., 1999). The lignin-derived phenols were extracted from the acidified solution (pH 1.8–2.2) using C18 solid-phase columns (Mallinckrodt Baker Corp., Phillipsburg, NJ), eluted with ethyl acetate and derivatized with a 1:1 mixture of pyridine and N,O-bis(trimethylsilyl)trifluoroacetamide. The derivatives were separated and quantified using a gas chromatograph mass spectrometer (GCMS-QP 2010, Shimadzu Corp., Tokyo, Japan) and a SPB-5 fused silica column (30 m, 0.25 i.d., 0.25-µm film; Supelco, Bellefonte, PA). Injector temperature was 523 K and the temperature programme included 373 K for 1 min, 15 K/min to 523 K, 523 K for 10 min, 30 K/min to 573 K, 573 K for 7 min. Helium was used as carrier gas with a constant pressure of 1 bar. As internal recovery standards, ethylvanillin was added prior to CuO oxidation and phenylacetic acid prior to derivatization. The recovery of ethylvanillin from MOA samples averaged 115 ± 2%, and those of litter samples averaged 100 ± 2%, and those of O samples averaged 92 ± 2%, and phenylacetic acid prior to derivatization. The recovery was set to 523 K and the FID to 573 K. The temperature program comprised the following steps: heating to 433 K for 4 min, heating to 458 K (rate: 8 K/min), 1.5 min at 458 K, heating to 523 K (rate: 4 K/min), 0.5 min at 458 K, heating to 573 K (rate: 50 K/min) and 5 min at 573 K. The recovery of myo-inositol from MOA samples averaged 102 ± 15%, those of litter samples averaged 115 ± 8%, and those of O samples averaged 116 ± 4%.

2.9. Terminology and statistics

In the following, mineral–organic associations from A horizons are termed ‘A-MOAs’, those from B horizons ‘B-MOAs’, respectively. The Pearson correlation coefficient was calculated based on z-transformed variables using Statistica 5.1 (StatSoft Inc., Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1. Time-dependent transformation of mineral–organic associations

3.1.1. Mineralogical composition at different weathering stages

The mineral composition of LSAG soils varies substantially over 4100 kyr of basalt weathering causing significant transformation of mineral matter (Torn et al., 1997; Chorover et al., 2004; Ziegler et al., 2005). Despite rock weathering being a continuous process, the most significant transformations of minerals in MOAs with time occur within three operationally defined weathering stages (I–III). Stage I corresponds to the 0.3-kyr site (Thurston) and is characterized by rapid weathering of basaltic tephra, thus causing a substantial loss of non-hydrolyzing cations (K+, Na+, Ca2+, Mg2+) and the creation of Al and H+ acidity (Chorover et al., 2004). Primary minerals like olivine, pyroxene, plagioclase (andesine and anorthite) present at the 0.3-kyr site (Table 1) are weathered almost completely before 20 kyr giving rise to metastable minerals that dominate during stage II (20–400 kyr). Ferricyanide/nanocrystalline goethite, Al gels, and allophane constitute the dominant mineral phases during phase II of the <2-µm fraction (Chorover et al., 1999) whereas quartz and Ti-bearing minerals (anatase, ilmenite) comprise the larger grain sizes (not shown). A significant portion of Al dissolved from minerals during weathering stage II is held in complexes with OM as shown by the large CuCl2-extractable Al concentrations (Table 1). Moreover, at the youngest and intermediate-aged sites (0.3–400 kyr; stage I and II), a predominant portion of the secondary Fe exists in the form...
of PC Fe (hydr)oxides as indicated by high Fe reactivity ratios (Fe$_{ox}$/Fe$_{hydr}$ ~ 0.8). In stage III (1400 and 4100 kyr), the metastable mineral phases are progressively converted into more crystalline Fe oxides in case of ferrihydrite (Ko-kee: Fe$_{ox}$/Fe$_{hydr}$ = 0), and into gibbsite, kaolinite and halloysite in case of Al gels and allophane (Chorover et al., 2004; Vitousek, 2004; Ziegler et al., 2005). Fig. 2 displays three representative XP scans of mineral–organic associations, showing that across all weathering stages the suite of detected elements at particle surfaces was comparable. Aluminum, Si, Fe, and Ti were the major inorganic elements, whereas basic cations (Ca, Mg) were only detected at the 0.3-kyr site (Table EA1). More Fe was present at the particle surfaces of the 20-kyr and 4100-kyr MOAs and a weak but significant correlation between the atom percentages of Fe and Ti ($r^2 = 0.36; p < 0.05; n = 12$) suggests a partial coexistence of Fe and Ti minerals. Across the LSAG, Al$_{ox}$ concentrations did not covary with Fe$_{ox}$ but covaried strongly with Si$_{ox}$ concentrations ($r^2 = 0.94; p < 0.001; n = 12$), reflecting the contribution of allophane during stage II. The abundance of PC minerals (allophane, (hydr)oxides) as early products of basalt weathering is, however, not restricted to wet tropical conditions but has also been observed for a dry soil chronosequence at the Canary Islands (Lanzarote) covering substrate ages of <40 kyr and a mean annual precipitation of only ~140 mm (Jahn et al., 1992).

3.1.2. Yields and extractability of mineral-associated organic matter

Shifts in mineral composition were closely related to the concentration of MOC and mineral-associated N (MN). MOAs contained between 18 and 290 mg MOC/g corresponding to an average of 79 ± 4% of total OC, and 2–19 mg MN/g, averaging 88 ± 4% of total N (Table 1). This means that the main proportion of OM in LSAG soils is associated with minerals; a consequence of the acidic pH (Table 1) that favors sorptive interactions between OM and variable charge minerals. The MOC versus MN correlation coefficient was close to unity ($r^2 = 0.92; p < 0.001; n = 12$), suggesting a predominant organic origin of N. Both MOC and MN concentrations increased during weathering stage I and II concurrent with the formation of PC mineral phases (Torn et al., 1997). Assuming that at least in A horizons, the MOC contents reflect differences in the capacity of minerals and monomeric/polymeric metals to bind OM, the sorption capacity increased from the youngest site towards a maximum at 400 kyr by a factor of 16 and then subsequently declined again by 86% towards the oldest site. Hence, the MOC accumulation proceeds faster than its decline into later weathering stages (1400–4100 kyr), which is linked to the rapid formation of metastable minerals and metal–organic complexes during weathering stage II and the subsequent slow transformation of PC phases into more stable minerals.

On average 63 ± 9% OC was extracted by 0.1 M NaOH from A-MOAs whereas more OC was liberated from B-MOAs (82 ± 19%; Table 1). The larger MOC extractability in subsoil MOAs indicates a larger extent of direct mineral–organic bindings compared to the A-MOAs. For A-MOAs, the majority of the extracted MOC was adsorbed to XAD-8 resin (Table 1), suggesting a large contribution of aromatic substances. In contrast, the released OC from B-MOAs was slightly less hydrophobic (Table 1). Notably, the fraction of XAD-8 adsorbable MOC was smallest in weathering stage III (1400 and 4100 kyr), suggesting that the crystalline minerals present at the oldest sites are associated with more hydrophilic compounds than the minerals at the other sites (Table 1).

3.1.3. Specific surface area and mesoporosity

The specific surface area (SSA) and porosity of MOAs are displayed in Table 2. The A-MOA at the youngest site (0.3 kyr; weathering stage I) exhibits the lowest SSA and porosity due to the prevalence of primary silicates, consistent with SSA values reported for primary minerals such as olivine, anorthite, or diopside that not exceed ~2 m$^2$/g.

![Fig. 2. Representative X-ray photoelectron spectra of mineral–organic associations from A horizons of the long substrate age gradient. Spectra are stacked by a constant value.](image-url)
Table 2

Specific surface area (SSA), C\textsubscript{BET} constant, net enthalpy of N\textsubscript{2} adsorption (\Delta H\textsubscript{N2}), pore volume data, surface enrichment of C and N, and the volumetric ratio of OM and minerals in mineral–organic associations from A and B horizons of the long substrate age gradient.

<table>
<thead>
<tr>
<th>Site age</th>
<th>SSA (m\textsuperscript{2}/g)</th>
<th>C\textsubscript{BET}</th>
<th>\Delta H\textsubscript{N2} (KJ/mol)</th>
<th>SSA\textsubscript{corr, a} (m\textsuperscript{2}/g)</th>
<th>MIV\textsuperscript{b}</th>
<th>MEV\textsuperscript{c}</th>
<th>TPV\textsuperscript{d} (mm\textsuperscript{3}/g)</th>
<th>Surface enrichment\textsuperscript{e}</th>
<th>Volumetric ratio ( V_{OM}/V_{mineral} )</th>
<th>( \rho_{OM} = 1.4 ) g/cm\textsuperscript{3}</th>
<th>( \rho_{OM} = 1.7 ) g/cm\textsuperscript{3}</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>151</td>
<td>3.3</td>
<td>2.4</td>
<td>1.0</td>
<td>2.5</td>
<td>1.0</td>
<td>4.9</td>
<td>14.8</td>
<td>16.8</td>
</tr>
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<td>141</td>
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<td>84.4</td>
<td>23.5</td>
<td>25.3</td>
<td>32.5</td>
<td>44.7</td>
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<td>123</td>
<td>3.1</td>
<td>46.0</td>
<td>13.0</td>
<td>22.0</td>
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<td>2.7</td>
<td>45.3</td>
<td>7.5</td>
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<td>20.4</td>
<td>63.7</td>
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<td>27.4</td>
<td>8.5</td>
<td>11.0</td>
<td>10.3</td>
<td>52.8</td>
<td>115.6</td>
<td>1.7</td>
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<td>4.0</td>
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<td>8.8</td>
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<td>9.0</td>
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<td>80.0</td>
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<tr>
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<td>3.3</td>
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<td>33.5</td>
<td>84.4</td>
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<tr>
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<td>3.2</td>
<td>72.7</td>
<td>3.5</td>
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<td>39.4</td>
<td>141.7</td>
<td>265.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

\( a \) SSA corrected for OM content (see Section 2.5).

\( b \) Micropore volume (<2 nm).

\( c \) Mesopore volume.

\( d \) Total pore volume.

\( e \) Surface enrichment = C or N (XPS)/C or N (elemental analysis).

\( f \) Assumptions: \( m_{OM} = 2 \times m_{OC}, \rho_{OM} = 1.4 \) or \( 1.7 \) g/cm\textsuperscript{3}, \( V_{OM} = m_{OM}/\rho_{OM}, V_{mineral} = V_{MOA} - V_{OM}, \) where \( V_{MOA} = 1000 g/\rho_{MOA}, \rho_{MOA} \) data are listed in Table 1.
over a broad range of grain sizes (Brantley and Mellott, 2000). During 20 kyr of basalt weathering, SSAs increased 23-fold as a result of the neosynthesis of secondary mineral phases. Despite of its large OM content, significant mineral surface area (SSA\textsuperscript{coret} = \~84 m\textsuperscript{2}/g) and micropore volume (24 mm\textsuperscript{3}/g) of the 20-kyr A-MOA is still accessible to \textsuperscript{12}N\textsubscript{2}, confirming that, in the freeze-dried state, OM incompletely coats the mineral surfaces (Arnarson and Keil, 2001). After this initial SSA\textsuperscript{coret} increase, the SSA\textsuperscript{coret} of A-MOAs dropped during stage II (20–400 kyr) due to the sorption of OM, which is reflected by declining \textit{C}_{BET} constants (Table 2). In fact, the MOC concentrations correlated negatively with the \textit{C}_{BET} constants ($r^2 = 0.58; p < 0.01; n = 12$), confirming that natural OM when sorbed to mineral surfaces lowers the enthalpy of \textsuperscript{12}N\textsubscript{2} adsorption (Kaiser and Guggenberger, 2003; Mödl et al., 2007; Wagai et al., 2009). The larger \textit{C}_{BET} constants and SSA\textsuperscript{coret} values of B-MOAs (Table 2) reflect their smaller OM input and hence smaller OM contents in comparison to the A-MOAs. Finally in weathering stage III (1400–4100 kyr), the SSA\textsuperscript{coret} and the \textit{C}_{BET} constants of A-MOAs increased again because of the prevalence of minerals with smaller amounts of bound OM. Freeze-dried A-MOAs from stage II sites contained significantly more volume in <200-nm pores than MOAs from endmember soils, indicating a particularly small pore geometry of associations between OM and PC mineral phases. In contrast to A-MOAs, in B horizons the SSA\textsuperscript{coret}, \textit{C}_{BET} constants, and pore volume of MOAs show less variation (Table 2), but the overall pattern still reflects the cumulative effect of mineral alteration and OM sorption.

The large \textit{C}_{BET} constants of all MOAs (Table 2) provide evidence that significant mineral surface area is bare of OM (Wagai et al., 2009). More extensive OM coatings/surroundings as indicated by smaller \textit{C}_{BET} values (72, 86) existed in A-MOAs of the 400-kyr and 1400-kyr site. Compared to other soils (Wagai et al., 2009), these \textit{C}_{BET} constants were still high owing to the presence of uncovered, microporous minerals. In accordance with the large \textit{C}_{BET} constants, also the atom% contribution of C to the bulk XP spectra of MOAs (hydrogen ignored) was <50% (14–49%; not shown) and also revealed that less C is associated with particle surfaces in B-MOAs compared to A-MOAs (Table EA1). Additionally, we adopted the empirical approach of Mayer (1999, 2005) which relates the difference in the net \textsuperscript{12}N\textsubscript{2} adsorption enthalpy between bare and organically coated mineral surfaces [$\Delta (\Delta H_{\text{ads}})$] to the OM fractional coverage according to $\Delta (\Delta H_{\text{ads}}) = \Delta H_{\text{bare}} - \Delta H_{\text{coated}} = 1.08$ (fractional coverage)$^{0.535}$, where $\Delta H_{\text{bare}}$ denotes the \textsuperscript{12}N\textsubscript{2} adsorption enthalpy for the bare mineral surfaces and $\Delta H_{\text{coated}}$ for the organically coated ones. Due to the dominating oxide-type mineral composition, 2-line ferrihydrite served as energetic reference for a blank mineral surface ($\textit{C}_{BET} = 239$ and $\Delta H_{\text{ads}} =$ $RT \ln \textit{C}_{BET} =$ $3.5$ KJ/mol). The assumption inherent to this approach is that all mineral surfaces are energetically homogeneous, which strictly is not valid for natural microporous samples. However, when using this approach, one would end up with no more than 50% of the \textsuperscript{12}N\textsubscript{2}-accessible mineral surface area covered by OM – in line with the high \textit{C}_{BET} constants and XPS data. The presence of significant inorganic surfaces, despite of the large OM contents, also agrees well with the high points of zero proton charge particular of stage-II soils (~6 in 1 mM LiCl electrolyte) (Chorover et al., 2004). In principle, mineral surfaces might remain bare of OM either by being located in microsites inaccessible to OM, e.g., in small aggregates, by electrostatic shielding by pre-existing sorbed OM, or by being chemically unreactive towards OM.

### 3.1.4. Importance of mineral microporosity

In soils, mineral micropores with diameters <2 nm can arise from crystal defects, the stacking edges of expandable 2:1 clay minerals, dead-ends of pores at domain boundaries of Fe (hydr)oxides or from interparticle spaces of PC minerals (Cabrera et al., 1981; Cornell and Schwertmann, 1996; Fischer et al., 1996; Aringhieri, 2004). The close proximity of pore walls render micropores favorable sorption sites for OM because of the high density of hydroxyl groups and the ability of OM to sorb to mineral surfaces through multiple bonds (Kaiser and Guggenberger, 2003). The \textsuperscript{12}N\textsubscript{2}- and CO\textsubscript{2}-detectable micropore volumes of MOAs are displayed in Table 2. The CO\textsubscript{2}-accessible micropore volume exceeded the \textsuperscript{12}N\textsubscript{2}-derived micropore volume in all MOAs by a factor of 1.1–2.9 (Table 2). This can be explained by the molecular sieving of \textsuperscript{12}N\textsubscript{2} at 77 K, whereas CO\textsubscript{2} at 273 K is capable of diffusing through the flexible organic network into microminerals (Kwon and Pignatello, 2005; Mikutta and Mikutta, 2006; Kaiser et al., 2007; Mikutta et al., 2008). The CO\textsubscript{2}-micropore volume of MOAs correlated positively with oxalate-extractable $\sum$(Fe, Al, Si) concentrations ($r^2 = 0.96; p < 0.001; n = 12$) whereas MOC failed to predict the CO\textsubscript{2}-micropore volume ($r^2 = 0.14$). Consequently, CO\textsubscript{2} particularly probed micropores of PC mineral phases such as Fe and Al (hydr)oxides and allophane rather than those of OM. Similarly, Kaiser et al. (2007) showed that the CO\textsubscript{2} sorption at 273 K to goethite and ferrihydrite is largely independent from the amount of sorbed OC even at extremely high OC concentrations comparable to the maximal OC concentrations in the MOAs studied (~300 mg OC/g). Also for synthetic coprecipitates, the reduction of the mineral micropore volume was much less intense for CO\textsubscript{2} than for N\textsubscript{2} if the OM content was increased (Mikutta et al., 2008). Therefore, the difference between the N\textsubscript{2}- and CO\textsubscript{2}-micropore volumes in the MOAs, particularly during weathering stage II, suggests that OM bound to PC minerals partly clogs the micropore entrances (Mikutta and Mikutta, 2006). Hence, at the vicinity of micropores, OM is ‘sterically’ stabilized by multiple bonds to the mineral surface (Kaiser and Guggenberger, 2007b), which would reduce OM desorption and thus biodegradation (Kaiser and Guggenberger, 2003, 2007b; Mikutta et al., 2007). Consequently, the association of OM with microporous mineral phases particularly in stage II is an additional factor that favors the accumulation and stabilization of OM in the LSAG soils.

### 3.1.5. Enrichment of organic matter at mineral surfaces and volumetric ratios

Table EA1 compiles the XPS-based element composition of MOA surfaces. Despite the low to moderate OM coverages, the surfaces of MOAs appear enriched with
OM as evidenced by the large OC concentrations (88–372 mg OC/g MOA) and MN concentrations (2–23 mg MN/g MOA; Table EA1). The XPS-based surface MOC concentrations are comparable with those observed for goethite reacted with dissolved OM in the sorption plateau region (276 mg OC/g; Kaiser and Guggenberger, 2007b) and with the maximum concentrations observed in different soils including Podzols, Andisols, Cambisols, and a Luvisol (Yuan et al., 1998; Gerin et al., 2003). The surface C and N enrichment was calculated as the ratio between XPS-derived MOC and MN concentrations and those derived from elemental analysis. Values >1 correspond to an enrichment, values <1 to a depletion of MOC and MN at particle surfaces relative to the bulk concentration. Surface enrichment values of MOC and MN ranged between 1.3–16.8 and 1.2–8.4, respectively (Table 2). MOC and MN were always enriched at mineral surfaces with the strongest enrichment at the youngest (stage I) and oldest site (stage III) (Table 2). The surface C and N enrichment in LSAG endmember MOAs reflect a larger volume ratio of minerals to OM, or, in other words, that larger mineral grains are coated with OM. In contrast, in surface horizons of intermediate-aged sites, the smaller surface C and N enrichment as well as the larger volume contained in nanometer-sized pores (Table 2) imply that MOAs consist of a homogeneous array of PC minerals and OM. Volumetric ratios between sorbed OM (V_{OM}) and the mineral phase (V_{mineral}) were calculated to illustrate the potential mode of OM accumulation. Here, we utilized the pycnometric ρ data of MOAs (Table 1) and assumed ρ_{OM} to vary between 1.4 and 1.7 g/cm³, thus giving also credit to the potentially higher density of sorbed OM (Kaiser and Guggenberger, 2007b). Table 2 depicts the V_{OM}/V_{mineral} ratios, which range between 0.05 and 2.87 depending on the ρ_{OM} assumed. In endmember A-MOAs (0.3 and 4100 kyr), volumetric ratios were small (0.07–0.23) and consistent with the C surface enrichment data, thus favoring the ‘coating model’ of OM on particle surfaces, i.e., V_{mineral} >> V_{OM}. In contrast, in A-MOAs from intermediate-aged sites (stage II), V_{OM} was either close to or even larger than V_{mineral} (Table 2). The large volume fraction of sorbed OM means that adsorption processes alone are insufficient to explain the OM accumulation in A-MOAs during weathering stage II. More likely, the data imply that between 20 and 400 kyr, where the pH was lowest and the CuCl2-exchangeable Al was largest (Table 1), the precipitation of OM and the subsequent accretion of mineral particles or the precipitation of OM with multivalent metals (Al, Fe) or hydroxides play a significant role in the formation of MOAs (Chorover et al., 2004). Noteworthy, the V_{OM}/V_{mineral} ratios of MOAs were larger at lower soil pH (Fig. 3), possibly because charge neutralization by H⁻ or low-molecular Al or Fe species favors the precipitation of natural OM (Scheel et al., 2008). It is important to realize that the OM in stage-II A-MOAs is effectively stabilized despite of seemingly less direct mineral–organic bondings involved. This may, in addition to the stabilization derived from adsorption of OM to microporous PC minerals, also result from the fact that OM in precipitated flocs (i) is more recalcitrant due to its high aromatic C and low N content (Scheel et al., 2007) and (ii) is partly inaccessible for microorganisms due to the flocs’ small pore sizes and the high degree of metal-C cross-linkings (Scheel et al., 2008). In the respective B-MOAs, the volumetric ratios were generally smaller than in A-MOAs, with V_{mineral} always clearly exceeding V_{OM}, thus implying that adsorptive processes are more relevant in subsoil than in OM-rich surface soil horizons.

### 3.1.6. Transmission electron microscopy

The six A-MOA samples were imaged and analyzed by TEM and EDS to reveal potential differences in MOA structures across the LSG. For each MOA sample, a reasonable number of particles (10 < n < 15) of various dimensions were analyzed in order to investigate a representative fraction of MOAs for each site. At the

![Fig. 3. Plot of the volumetric ratio of sorbed OM (V_{OM}) versus mineral matter (V_{mineral}) in mineral–organic associations from A (A-MOA) and B (B-MOA) of the long substrate age gradient. Volumetric ratios were calculated based on the following assumptions: m_{OM} = 2 \times m_{OC}, \rho_{OM} = 1.4 \text{ g/cm}^3, V_{OM} = m_{OM}/\rho_{OM}, V_{mineral} = V_{MOA} - V_{OM}, where V_{MOA} = 1000 \text{ g/\rho_{MOA}}. The corresponding \rho_{MOA} values are given in Table 1.](image)
0.3-kyr site, we found massive aggregates (~10–20 μm) consisting of primary mineral particles that were partly coated with amorphous-like inorganic phases (Fig. 4A) enriched in Fe, Al and Si. Only small amounts of C were detected by EDS which confirms that OM is not present as uniform coating (not shown). Consistent with the largest Fe_{ox} concentration, at Laupahoehoe (20 kyr) we observed strong signals of Fe, also to a lesser extent of Ti, associated with PC Fe (hydr)oxides, likely ferrihydrite, that covered larger primary particles and aggregates (Fig. 4B). Interestingly, almost no carbon coexisted with these phases. At the 150-kyr site (Kohala), the imaged aggregates generally had a smaller size (1–2 μm), were more homogeneous, and contained more Al and Si relative to the 20-kyr site (Fig. 4C). At the 400-kyr site, where the concentration of MOC and the OM coverage was maximal, massive aggregates were observed (several tens of microns). Additionally, film-like amorphous structures rich in Fe, partly in Ti and clay minerals (Si, Al) were observed (Fig. 4D). Again, almost no carbon was detected in these PC structures, confirming the relatively small fractional OM coverage despite of the large OM content. However, as this MOA fraction likely contains precipitated OM, we cannot rule out that OM was partly lost during the ultrasonic pretreatment. At the 1400-kyr site, we observed more heterogenous aggregates, consisting of mixtures of crystalline aluminosilicates and oxides (Fig. 4E), as well as Si-depleted film-like structures. At the most weathered 4100-kyr site, again massive aggregates were present, whose surfaces appeared to be covered by aggregates of crystalline Fe (hydr)oxides (Fig. 4F). In summary, during weathering stage II, clearly PC phases dominate at the nanoscale. At weathering stage III, secondary clay minerals and crystalline Fe

Fig. 4. Representative transmission electron micrographs and X-ray electron dispersive spectra of mineral–organic associations from A horizons of the long substrate age gradient. Primary mineral particles at the 0.3-kyr site are partly covered with phases of poor crystallinity (A) while such poorly crystalline phases dominate the 20-kyr (B), 150-kyr (C) and 400-kyr site (D). Note, the presence of film-like structures at the 400-kyr site (black arrow). At the 1400-kyr (E) and 4100-kyr (F) site, aggregates are composed to a much larger extent by secondary crystalline aluminosilicates and Fe (hydr)oxides, the latter particularly covering larger mineral particles at the 4100-kyr site.
(hydr)oxides were abundant, the latter particularly covering larger aggregates.

3.2. Time-dependent chemical alteration of sorbed organic matter

3.2.1. X-ray photoelectron spectroscopy

XPS was used as a non-destructive surface-probing tool that provides in-situ information about the chemical state of OM at particle surfaces. The chemical composition of MOM was revealed by studying the effect of O, C, and N on XPS C 1s binding energies. Peak deconvolution was performed similar to Monteil-Rivera (2000) because this fitting routine allows distinction between (i) amide C and carboxyl C (not possible by 13C NMR) and (ii) aromatic and aliphatic C, and (iii) yielded comparable quantities of carboxyl groups in humic acids as the wet chemical analysis. Spectral shifts in core level C 1s binding energy were assigned to different chemical environments of C: (1) unsubstituted aromatic carbon (C=C; 284.4 eV), (2) aliphatic carbon (C=C=C=C=H; 285 eV), (3) α-carbon (C=C(O)O; 285.6 eV), (4) ether or alcohol carbon (C=O; 286.4 eV), (5) ketonic or aldehyde carbon (C=O; 287.7 eV), (6) amide carbon (C(O)N; 288.5 eV), (7) carboxylic carbon (C(O)O; 289.3 eV) and (8) π-π* shake-up satellite from the aromatic structure (290.8 eV). Examples of high-resolution XPS C 1s spectra across the LSAG MOAs are shown in Fig. 5. Despite the major mineral transformations during weathering stages I–III there were only moderate differences in the average chemical composition of MOM in A horizons (Table 3). All MOAs were rich in aromatic C (29–34%) and contained relatively small amounts of amide C and carboxylic C; the two latter being comparably abundant (3.2–6.3% versus 4.7–7.1%). The average speciation of MOC in A horizons followed the sequence: aromatic C > ether/alcohol C > α-C > aliphatic C > ketonic/aldehyde C > amide C ∼ carboxylic C > amide C, suggesting the prevalence of phenolic substances (e.g., lignin, polyphenols, tannins) and carbohydrates. This structural analogy was interrupted at the 20-kyr site, where aromatic C was significantly depleted in favor of α-C and aliphatic C (Table 3). The only moderate impact of minerals on the chemical variability of A-horizon MOM derives from the large input of fresh C from a similar source (M. polymorpha). In contrast, the chemical composition of OM at particle surfaces in B horizons was more variable than in A horizons, in particular with respect to aliphatic and aromatic C moieties but followed no clear trend through weathering stages I–III (Table 3). The order of abundance was: aromatic C > α-C = ether/alcohol C > aliphatic C > carboxylic C > ketonic/aldehyde C > amide C > π-π*. Whereas the average fraction of amide C in A- and B-MOAs was similar, the B-MOAs were slightly enriched in carboxylic C. This suggests a larger degree of oxidative alteration of subsoil MOM and/or a stronger retention of acidic OM components.

To test for a mineralogical effect on MOM’s chemical composition, we related the spectral abundance of each carbon type (in atom%) to the XPS-based inorganic surface composition of MOAs (n = 12; normalized based on the sum of Fe, Al, Si and Ti). We found that aliphatic C correlated positively (r² = 0.72; p < 0.001) with surficial Fe concentrations whereas carboxylic C correlated positively with surficial Al concentrations (r² = 0.78; p < 0.001). Alcoholic C, mainly originating from carbohydrates, correlated...
positively with Si concentrations ($r^2 = 0.69$; $p < 0.001$). These correlations suggest an impact of the bulk inorganic composition of MOAs on the chemical composition of MOM but, due to the source inspecificity of XPS, they do not indicate causation. Therefore, we transformed the atom% concentrations of C types into their absolute concentrations (mg C type/g MOA) and used the more specific selective chemical extraction data for regressions, being aware that the elemental composition of the bulk sample and its surfaces can differ. When evaluating A- and B-MOAs independently, we observed that in both A and B horizons aliphatic and amide C correlated positively with Feox concentrations (A horizons: $r^2 = 0.94$; 0.67; B horizons: $r^2 = 0.90$; 0.94; $n = 6$; Fig. 6). This surprising correlation probably points to a preferential association of PC Fe phases with microbial remnants and/or to the biogenic origin of PC Fe phases in these moist LSAG soils but this hypothesis still needs to be ascertained. Due to the only moderate differences in OM composition in A-MOAs no further relationship between the concentration of other C types and mineralogical properties was observed. In contrast, in the B-MOAs, aromatic C was more abundant in samples rich in PC aluminosilicates and Al-organic complexes as revealed by positive relationships with Al$_{ox}$, Al$_{ois}$, and Si$_{ox}$ ($r^2 = 0.92$; 0.96; 0.83; Fig. 6). The same holds true for carboxylic C ($r^2 = 0.98$; 0.88; 0.85; Fig. 6). Although only revealed for MOAs from the B horizons, the data suggest that, on a mass-basis, particularly PC aluminosilicates and Al phases favor the retention of acidic aromatic compounds. Guan et al. (2006) and Scheel et al. (2007) observed that acidic, UV-absorbing aromatic components of natural OM were preferentially adsorbed to PC Al(OH)$_3$ or precipitated with Al and, once adsorbed/precipitated, possessed a remarkable resistance against biodegradation (Scheel et al., 2007). This stability arose from two factors: the strong chemical binding of OM to the inorganic phase (thus limiting desorption) and the a priori recalcitrant structure of aromatic molecules. Analogously, we rationalize that beside the association of OM with microporous, high-SS:4 minerals or with monomeric/polymeric metal species also the inherent stability of such acidic aromatic compounds assists in the stabilization of OM.

### 3.2.2. XPS ‘depth profiles’

To examine the composition of deeper MOA layers and the binding state of carbons, a representative area (≈500 μm$^2$) of A-MOAs was analyzed after Ar$^+$ sputtering. Note that the XPS elemental composition of powdered MOA samples following Ar$^+$ sputtering represents an integral over all exposed, rough particle surfaces, which inevitably includes variable erosion effects for different kinds of materials. Fig. 7 shows that signals from Si and Al, predominantly originating from aluminosilicates, emerged most markedly during sputtering in almost all A-MOAs. In contrast, at the 20-kyr and 4100-kyr site, Fe signals from Fe phases increased most substantially with depth, consistent with the larger Fe (hydr)oxide content of these MOAs (Table 1). At $t_{km}$, surficial OC concentrations were still >13 atom% (≥ 64 mg OC/g MOA), suggesting the presence of thick organic coatings/agglomerations. At the 400-kyr site

### Evolution of mineral–organic associations upon substrate aging 2047

**Table 3**

<table>
<thead>
<tr>
<th>Site</th>
<th>Ar–C–C</th>
<th>Ar–C–H</th>
<th>C–C–(O)O</th>
<th>C–O</th>
<th>C=O</th>
<th>C(O)N</th>
<th>C(O)O</th>
<th>π–π$^*$-shake up</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>7.3</td>
<td>13.1</td>
<td>26.0</td>
<td>9.6</td>
<td>3.4</td>
<td>7.1</td>
</tr>
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<td>8.5</td>
<td>16.7</td>
<td>22.8</td>
<td>9.1</td>
<td>4.3</td>
<td>5.9</td>
</tr>
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<td>8.5</td>
<td>16.7</td>
<td>22.8</td>
<td>9.1</td>
<td>4.3</td>
<td>5.9</td>
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<td>9.1</td>
<td>4.3</td>
<td>5.9</td>
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<td>22.8</td>
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<td>10.6</td>
<td>15.6</td>
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<tr>
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<td>11.0</td>
<td>17.9</td>
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<td>Kokee</td>
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<td>11.0</td>
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<tr>
<td>Mean</td>
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<td>10.8</td>
<td>18.3</td>
<td>17.5</td>
<td>15.7</td>
<td>4.8</td>
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</tr>
<tr>
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<td>48.0</td>
<td>17.1</td>
<td>10.9</td>
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<td>48.1</td>
<td>17.8</td>
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</tr>
</tbody>
</table>

*a* Binding energy.
Fig. 6. (A–C) Regression plots of XPS-based carbon types with oxalate-extractable Fe, Al, and Si concentrations in mineral–organic associations from A (A-MOA) and B horizons (B-MOA). Note that the oxalate-extractable metal concentrations refer to bulk MOA samples whereas XPS data reveal the average composition of OM at particle surfaces.

Fig. 7. Change of the inorganic surface composition (Al, Si, Ti and Fe) and of C and N concentrations in mineral–organic associations from A horizons upon Ar⁺ sputtering. The data for C and N represent the percentage loss of C and N concentrations after 30 min of etching.
where the MOC concentration and the volumetric OM–
mineral ratio were largest, the XPS C 1s signal decline was
much less intense, again revealing that MOM exists in a
particularly homogeneous mineral–organic microfabric. In
contrast, at the two oldest sites (stage III), thinner organic
coatings/agglomerations are indicated by a more pro-
nounced decline of the C concentration (Fig. 7).

While the sample number is limited, the XPS data reveal
consistent depth trends in all examined MOAs. These
changes can be followed in Fig. 8, showing the alteration
of C functionalities in the 20-kyr A-MOA upon sputtering.
Ar + etching generally caused (i) a roughly parallel decrease
of surface C and N concentrations (Fig. 7), (ii) a relative in-
crease of the signal at 284.4 eV reflecting aromatic C, and
(iii) a concomitant relative decrease of signals in the binding
energy range 287.7–289.3 eV, connoting mainly ketonic/
aldehyde, amide, and carboxylic C, respectively (Table 4).
Also alcoholic C, mainly comprising carbohydrates, tended
to decrease with sputter duration (except from the 1400-kyr
sample). Since abrasion is not likely to destroy the microag-
gregate structure, the observed shifts in C functional groups
with sputter time reflects differences in the molecular struc-
ture of MOM. It is known that Ar + gassing under ultra-
high vacuum conditions can induce chemical modifications
of synthetic polymers such as the selective removal of pheno-
yl rings (Zaporojtchenko et al., 2005, 2007). Also, poly-
mers exposed to Ar plasma revealed a decrease in
aromatic C in favor of aliphatic C (France and Short,
1998). Beside polymer scission and the formation of
cross-linkings, ion treatment can also cause the removal
of oxygen-functional groups depending on the polymer’s
structure (Zaporojtchenko et al., 2007). We therefore exam-
ine OM extracted from the 0.3-kyr MOA by means of
NaOH–NaF in order to test for the effect of Ar + irradiation
on the chemical composition of LSAG-specific natural OM.
In line with previous work, we found that Ar + sputtering
for 5 min reduced the atom% abundance of aromatic C
by 4.5% while increasing the concentration of aliphatic C
by 6.2% (Fig. 7). A general decrease of aromatic C in
Ar + -treated polymers and natural OM, however, was not
observed in our MOA samples, but rather an increase (Ta-
ble 4). This discrepancy, therefore, points to vertical differ-
ces in OM composition at mineral particles rather than to
Ar + -induced chemical modifications. The increasing aro-
matic C content with depth agrees well with the idea of aro-
matic compounds possessing a high affinity to oxide-type
minerals (Kaiser and Guggenberger, 2000; Chorover and
Amistadi, 2001; Mikutta et al., 2007; Scheel et al., 2008),
while with their larger propensity to accumulate more di-
rectly at the mineral–organic interface. Of note, carboxylic
C declined slightly with depth (Table 4), possibly indicating
their preferential removal upon Ar + treatment (Zap-
orojtchenko et al., 2007).

In line with the strong MOC–MN correlation in MOAs,
the parallel decrease in C and N concentration confirms
that OM bound to minerals is the major source of organic
N (Yuan et al., 1998). Moreover this result demonstrates
that in LSAG A-MOAs, organic N does not become en-
riched towards the mineral–organic interface relative to
OC. Hence, this finding does not support the recently pro-
posed conceptual model of mineral–organic associations
which states that amphiphilic nitrogenous compounds are

Fig. 8. Depth-dependent change of the chemical composition of mineral-associated OM (20-kyr site; A horizon) upon Ar + etching for 15 and
30 min. Numbers of deconvoluted sub-peaks refer to (1) unsubstituted aromatic carbon (Ar–C–C/Ar–C–H), (2) aliphatic carbon (C–C/
C–H), (3) each carbon (C–O), (4) ether or alcohol carbon (C–O), (5) ketonic or aldehyde carbon (C=O), (6) amide carbon (C=O), (7)
carboxylic carbon (C=O), and (8) π–π*-shake-up satellite from the aromatic structure.

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preferentially sorbed to mineral surfaces to initiate a vertical differentiation (multilayering) of organic coatings (Kleber et al., 2007).

Table 4
Changes of the C binding environments in mineral–organic associations from A horizons in the assigned spectral regions (see Table 3) upon Ar⁺ sputtering for 15 min \((t_{15})\) and 30 min \((t_{30})\). The analyzed area corresponded to \(\sim500\ \mu\text{m}^2\). The \(\Delta\) notation corresponds to percent changes between \(t_0\) and \(t_{30}\).

<table>
<thead>
<tr>
<th>(Atom%)</th>
<th>Thurston</th>
<th>Laupahoehoe</th>
<th>Kohala</th>
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<td>(t_{15})</td>
<td>(t_{30})</td>
</tr>
<tr>
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<tr>
<td>(\text{C–C, C–H})</td>
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<td>3</td>
</tr>
<tr>
<td>(\text{C–C(O)O})</td>
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<tr>
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Fig. 9. Effect of Ar⁺ sputtering (5 min) on the chemical composition of freeze-dried OM extracted from the 0.3-kyr MOA (A horizon) by means of NaOH–NaF. Numbers of deconvoluted sub-peaks refer to (1) unsubstituted aromatic carbon (Ar–C–C/Ar–C–H), (2) aliphatic carbon (C–C/C–H), (3) \(\pi\)-carbon (C–C(O)O), (4) ether or alcohol carbon (C–O), (5) ketonic or aldehyde carbon (C=O), (6) amide carbon (C(O)N), (7) carboxylic carbon (C(O)O), and (8) \(\pi–\pi^*\)-shake-up satellite from the aromatic structure.

3.2.3. Absolute concentrations of lignin-derived phenols

Beside cellulose and hemicelluloses, lignin is the most important biopolymer added to soil by plants and the ma-
jor source of phenolic compounds in soil OM. The contribution of lignin-derived phenols to organic soil horizons and MOAs across the LSAG was analyzed by alkaline CuO oxidation (Table EA2). Even though the organic layers (L and O) at the six sites contained similar amounts of lignin-derived phenols (A8), the concentration of A8 in MOAs was highly variable and closely matched the accumulation pattern of MOC as shown by a positive correlation between A8 and MOC ($r^2 = 0.81$; $p < 0.001$; $n = 12$). No relationship with mineral phase variables was observed, indicating no source-specific retention of lignin residues. In A-MOAs, A8 increased steadily during weathering stage I and II, reaching a maximum at Pololu (400 kyr), and decreasing thereafter into stage III (Fig. 10A). The more favorable accumulation of lignin-derived phenols in A-MOAs during stage I and II is revealed by the increase in A8 concentrations towards the next older site, which exceeded the increase in MOC concentrations (Table 5). In contrast, A8 contents in B-MOAs were about one order of magnitude smaller than in A-MOAs and remained at a low level through all weathering stages (Fig. 10A). Beside by adsorption of lignin-rich OM to minerals, the pronounced increase of A8 towards the 400-kyr site may mirror to some extent the precipitation of forest floor leachates rich in aromatic C moieties. This would accord with the high $V_{OM}/V_{mineral}$ ratios of stage-II A-MOAs (Fig. 3) as well as with the finding that carboxyl-rich, UV-absorbing aromatic compounds of dissolved OM have a high propensity to precipitate with Al (Scheel et al., 2007, 2008).

3.2.4. Lignin-derived phenols in mineral-associated organic matter and the degree of alteration

The contribution of lignin-derived phenols to MOC in A horizons also increased steadily towards the 1400-kyr site

Fig. 10. (A–F) Time-dependent change of lignin-derived phenol concentrations (A8) in organic layers and mineral–organic associations from A (A-MOA) and B horizons (B-MOA) (A); acid-to-aldehyde ratio of vanillyl (B) and syringyl units (C). The grey bands in B and C connote the acid-to-aldehydes ratios in collected plant source materials. Non-cellulosic carbohydrate concentrations (D); the GM/AX ratio (E) and RF/AX ratio (F). The GM/AX ratio refers to the mass quotient of (galactose + mannose)/(arabinose + xylose) and that of the RF/AX ratio to (rhamnose + fucose)/(arabinose + xylose). Roman numbers indicate the different weathering stages (see text).
and then dropped off at the oldest site (Fig. 11A). This means that during weathering stage I and II not only did the total concentration of lignin-derived phenols increase over time but also the relative contribution of lignin-derived phenols to MOM increased. This finding corroborates that in weathering stage II (20–400 kyr) lignin-derived phenols are preferentially stabilized in associations with PC minerals or monomeric/polymeric metal species. The minor contribution of lignin phenols to total MOC in A-MOAs at the 0.3-kyr site can be explained by the larger abundance of low-S/A primary minerals (olivine, pyroxene and feldspar), which do not favor the selective retention of lignin-derived phenols in comparison to other OM components like carbohydrates (see Section 3.2.7). Except from the 0.3-kyr site, the MOC-normalized A8 yields of B-MOAs were 87–92% smaller than in A-MOAs, comprising only between 0.9 and 1.6 mg/g of MOC (Table EA2). We do not think that the smaller subsoil A8 yields are caused by a less efficient extractability in NaOH, because subsoil MOC was generally more extractable under alkaline conditions (Table 1). Given the large XPS-based concentration of aromatic C in A- and B-MOAs (~30%) and the small amounts of CuO-digestible lignin monomers particularly in B-MOAs, this implies either (i) an underestimation of lignin phenols due to the CuO technique, (ii) major lignin transformations (thus escaping the analytical window), or (iii) the presence of other, non-lignin-derived phenolic compounds. The occurrence of non-lignin-derived phenols appears realistic since, besides coming from lignin, soluble polyphenols (92 ± 3 mg/g) and tannin (65 ± 4 mg/g) are major aromatic components of Metrosideros litter (Rothstein et al., 2004).

Fig. 12A plots the S/V versus C/V ratios of lignin in source leaf materials, organic layers, and MOAs. The litter layer reflects the predominant angiosperm (M. polymorpha) origin (S/V 0.7–1.1), which becomes progressively altered in the O layers as shown by decreasing S/V and increasing acid-to-aldehyde ratios of vanillyl (ac/alu) and syringyl (ac/als) units (Fig. 10B and C; Table EA2). This trend results from the preferential removal of syringyl moieties (Miltner and Zech, 1998; Möller et al., 2002; Otto and Simpson, 2006). Compared with the organic layers, lignin phenols in A- and B-MOAs exhibit a much higher degree of side chain oxidation as evidenced by markedly increased (ac/alu) and (ac/als) ratios (Fig. 10B and C). In addition to microbial alteration, the high acid-to-aldehyde ratios in MOAs can derive from the selective retention of acidic lignin compounds by charged mineral surfaces (Grünewald et al., 2006; Hernes et al., 2007) or from the preferential precipitation of acidic aromatic substances (Scheel et al., 2007, 2008). The latter aspects can explain the larger absolute lignin-derived phenol concentrations as well as the larger contribution of lignin phenols to MOM during weathering stage II. However, the generally high degree of lignin side chain oxidation persisting through time suggests that in particular acidic lignin components are stabilized by interaction with soil minerals and hydrolyzable metals. Hence, the long-term mineralogical shift primarily affects the absolute concentration of mineral-associated lignin phenols rather than their ‘diagenetic’ state.

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>MOC</th>
<th>Lignin phenols (A8)</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A horizons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 → 20 kyr</td>
<td>9.5</td>
<td>12.8</td>
<td>8.6</td>
</tr>
<tr>
<td>20 → 150 kyr</td>
<td>1.1</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>150 → 400 kyr</td>
<td>1.5</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>400 → 1400 kyr</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>1400 → 4100 kyr</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>B horizons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 → 20 kyr</td>
<td>4.9</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>20 → 150 kyr</td>
<td>1.1</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>150 → 400 kyr</td>
<td>0.7</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>400 → 1400 kyr</td>
<td>0.2</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>1400 → 4100 kyr</td>
<td>0.6</td>
<td>0.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

3.2.5. **Contribution of hydroxybenzenecarboxylic acids**

Beside the lignin phenol monomers, a suite of hydroxybenzenecarboxylic acids (BA) was detected as CuO oxidation products (Table EA2; Fig. 11A and B). These compounds comprise between 23–39% and 50–255% of MOC-normalized A8 in A- and B-MOAs, respectively. However, the origin of most of these compounds in soils is not specific. Dihydroxybenzoic acid (3,5di-H BA) may derive from polyhydroxyaromatic tannins or other flavonoids (Goni and Hedges, 1995; Louchoaurm et al., 1999; Dickens et al., 2007) but is also considered as a degradation product of lignin (Prahl et al., 1994). All detected BAs were already liberated from source vegetation leaves and are present in both A- and B-MOAs (Table EA2). The BAs extracted from live leaf tissues of M. polymorpha upon CuO oxidation likely originate from its polyphenol/tannin components (Hättenschwiler et al., 2003). The BA/A8 ratio, including 3,5di-H BA, increased exponentially from source vegetation leaves, O and L layers to the MOAs from A and B horizons (Fig. 12B). The facts that BAs (i) are released from source vegetation leaves, organic soil layers, and MOAs and (ii) become progressively enriched in MOAs relative to lignin-derived phenols suggests a high stability and/or a strong retention of respective source compounds (likely polyphenols/tannins). However, we cannot exclude that some BAs are lignin degradation products.

3.2.6. **Absolute concentrations of non-cellulosic carbohydrates**

Table EA3 provides the MOA- and OC-based concentrations of hydrolyzable neutral and acidic (glucuronol acid, galacturonol acid) monosaccharides across the LSAG. On a mol% basis, glucose, mannose, and galactose were the dominant hexoses across the LSAG, accounting on average for 62 ± 2% and 59 ± 2% of the total carbohydrate pool in A- and B-MOAs, respectively (not shown). Plant-derived pentoses (xylose and arabinose) were less abundant, comprising on average only ~25 mol% in both top- and subsoil MOAs. Uronic acid concentrations were generally <5 mol% (2.7 ± 0.3 mol%; mean ± standard error; n = 12). As with lignin phenol monomers, the concentration of neutral and acidic carbohydrates paralleled the MOC accumula-
tion, culminating during weathering stage II and declining thereafter (Fig. 10D). Thus, neutral and acidic carbohydrate concentrations correlated positively and significantly with MOC concentrations ($r^2 = 0.76$ and $r^2 = 0.78$, respectively; $p < 0.001$). In contrast to the lignin phenols, the fractional change of carbohydrate concentrations in A-MOAs towards the next older site was roughly congruent to that of MOC concentrations (Table 5). This reveals that carbohydrate components respond similarly to mineralogical shifts (in terms of quantity) as bulk MOC. Although absolute carbohydrate concentrations in A-MOAs (mg/g MOA) increase towards the 400-kyr site, the respective concentrations in B-MOAs decrease continuously from weathering stage I to III (Fig. 10D). This inverse trend in A- and B-MOAs suggests an input control for carbohydrates into deeper soil horizons, i.e., carbohydrates appear already effectively retained in A horizons. The large absolute carbohydrate concentrations in A-MOAs of weathering stage II suggest that

Fig. 11. (A–D) Time-dependent change of phenol concentrations (A = A horizon; B = B horizon) and non-cellulosic carbohydrate concentrations (C = A horizon; D = B horizon) normalized to mineral-associated OC (MOC). Abbreviations: A8, lignin phenols; BA, benzoic acid; 2H BA, 2-hydroxybenzoic acid; 3H BA, 3-hydroxybenzoic acid; 4H BA, 4-hydroxybenzoic acid; 3,5 diH BA, 3,5-dihydroxybenzoic acid; Xyl, xylose; Ara, arabinose; Rib, ribose; Rha, rhamnose; Fuc, fucose; Man, mannose; Gal, galactose; Glu, glucose; GluA, glucuronic acid; GalA, galacturonic acid.
non-cellulosic carbohydrates significantly contribute to stabilized OM in MOAs, despite being generally considered an easily available C source.

3.2.7. Concentration and source of non-cellulosic carbohydrates in mineral-associated organic matter

Fig. 11C and D show that the MOC-normalized concentrations of carbohydrates decline from the 0.3-kyr to the 4100-kyr site, with the 1400-kyr site being an exception. The large contribution of carbohydrates in the A-MOA of the youngest site, with lignin phenols contributing least, suggests that sugar components are more relevant in MOAs formed with less reactive primary minerals. Similarly, the \( K_8 \) /carbohydrate ratio in A-MOAs tended to increase with time (Fig. 12C), indicating that, relative to lignin phenols, carbohydrates are more strongly retained at the youngest site whereas in mineralogical settings where reactive secondary minerals prevail, the amount of lignin phenols is relatively larger. Hence, at least for weathering stage II, we infer that carbohydrates are discriminated against other OM components, including lignin phenols, either by adsorption and/or precipitation processes. At the 1400-kyr site, MOC-normalized carbohydrate and lignin-derived phenol concentrations were largest, suggesting that the mineral assemblage at Kolekole is particularly suitable in stabilizing both, carbohydrate and lignin components (Figs. 10A and D and 11A and C). Although the \( A8 \)/carbohydrate ratio slightly declined with respect to the 400-kyr site, the ratio was still larger than at the youngest site (Fig. 12C). At the oldest site, carbohydrate concentrations were smallest among all sites, both absolute and when normalized to OC (Figs. 10D and 11C). The relatively minor occurrence of carbohydrates (and lignin phenols) at the 4100-kyr site is consistent with the limited surface reactivity of the most mature mineral assemblage (Table 1). The large \( A8 \)/carbohydrate ratio, however, indicates that at the oldest site lignin-derived phenols were again preferentially associated with minerals (Fig. 12C). This can be ascribed to the presence of minerals which still offer more variable surface charge than those at the youngest site (Chorover et al., 1999), thus favoring the sorption of lignin-derived phenols over that of carbohydrates.

In order to assess the origin of sugar components, we calculated the ratios of microbial-derived hexoses/deoxyhexoses to plant-derived pentoses in MOAs (Murayama, 1984; Oades, 1984) according to \( GM/AX = (\text{galactose} + \text{mannose})/(\text{arabinose} + \text{xylose}) \) and \( RF/AX = (\text{rhamnose} + \text{fucose})/(\text{arabinose} + \text{xylose}) \) (Fig. 10E and F). GM/AX values for plant sources are typically <0.5 and those of microorganisms >2 (Oades, 1984). Increasing RF/AX values from 0 to 0.38 during the biodegradation of straw has been ascribed to the microbial synthesis of deoxysugars (Murayama, 1984). At each site, the GM/AX ratio increased steadily from organic layers (litter: \( 0.53 \pm 0.05 \); O layer: \( 0.91 \pm 0.09 \)) to MOAs (\( 1.69 \pm 0.10 \); \( n = 12 \)) (Fig. 10E). The high GM/AX values suggest a predominant, though not exclusive, microbial origin of hydrolyzable non-cellulosic carbohydrates in MOAs. This finding clearly highlights the importance of microbial carbohydrate resynthesis in the formation of MOAs. Although absolute carbohydrate concentrations in A-MOAs increased during weathering stage I and II, the proportion of microbial hexoses tended to decrease with time, as reflected by slightly declining GM/AX and RF/AX values (Fig. 10E and F). This suggests a somewhat larger availability of OM for sustaining microbial activity at the younger sites with mineral–organic interactions being less strong, which translates into a larger microbial necromass, hence more mineral-bound...
microbial hexoses/deoxyhexoses. As the mineral–organic associations at later weathering stages become more stable, microbial utilization of OM and production of microbial sugars is likely more restricted.

3.3. Time-dependent physical alteration of mineral-associated organic matter

The binding of OM to minerals that differ in grain size and surface reactivity should influence the mobility of organic side chains to different extents, thus reducing their degree of freedom to alter their three-dimensional structure upon hydration or to interact with solutes. In order to investigate the potential change in OM rigidity across the LSAG, differential scanning calorimetry (DSC) was applied to the A- and B-MOAs of the six sites (Fig. 13). All A-MOAs exhibit a clear step transition in the temperature range between 332 and 348 K. Except from the oldest sample, all B-MOAs revealed one significant and reproducible transition located between 366 and 382 K. Fig. 14 depicts the step transition temperature ($T^*$) of MOM at the different sites. Except from the 20-kyr site, the $T^*$ values in the A-MOAs were comparable across all weathering stages and were always significantly smaller than in the respective B-MOAs (Fig. 14). Generally, the $T^*$ in the A-MOAs are in line with those reported in the literature (Hurrass and Schaumann, 2005; Schaumann and LeBoeuf, 2005) while in the B horizons the $T^*$ values exceed the range of data published for A and O horizons. The larger $T^*$ values of MOM in the B horizons than in A horizons indicate a larger rigidity of MOM in the B horizon. However, the roughly similar $T^*$ values across the different weathering regimes suggest that the mineral composition as well as the spatial arrangement of the mineral–organic fabric have no

![Fig. 13. Representative DSC thermograms of mineral–organic associations isolated from A (left) and B horizons (right). The asterisk (*) indicates samples where not all thermograms revealed a significant transition. The sample marked with (**) showed a weak reversing transition, which has been eliminated by the baseline correction.](image)

![Fig. 14. Time-dependent change of the DSC step transition temperature ($T^*$) of organic matter in mineral–organic associations from A (A-MOA) and B horizons (B-MOA). Marked samples did not show transitions in every replicate (*) or showed a weak, but reversing transition only (**).](image)
major effect on the rigidity of MOM. The observed difference in rigidity between A- and B-horizon MOM is probably due to a more intimate association of OM with minerals in the B horizon, i.e., a larger extent of direct attachment to the minerals, which in line with the smaller $V_{\text{OM}}/V_{\text{mineral}}$ ratios in B-MOAs (Table 2). A larger extent of mineral–organic interaction is generally favored at smaller OM fractional coverages (as indicated by the larger $C_{\text{BET}}$ constants of B-MOAs; Table 2), because OM can bind in an ‘uncoiled formation’ with a maximum number of ligands involved (Kaiser and Guggenberger, 2003). Thus, complexation with minerals and metals can reduce the mobility of the structural parts of OM. Although not present in all samples and in all measurement repetitions, a second step transition was measured at $T = 353–373$ K in many A-MOAs samples showing the presence of the more rigid mineral–organic associations in the A horizons as well. The detection of this transition, however, is difficult due to the overlap with the strong lower-temperature transition. We assume that this high-temperature transition probably reflects the property of OM in close proximity to the mineral interface. Consequently, the lower-temperature transition prevailing in A-MOAs might be more indicative of the bulk OM structure where the mineral–organic binding has less impact on matrix rigidity. This idea is supported by the fact that only in A-MOAs, $T^*$ correlated positively with structural properties of MOM that is the XPS-based aromatic Caliphatic C ratio ($r^2 = 0.76$; $p < 0.05$). The stronger rigidity of OM in B-MOAs might, due to a larger extent of mineral–organic binding and the formation of multiple bonds, limit OM desorption and/or microbial utilization and can therefore help explaining the longer mean residence time of OM in subsoil compared to topsoil horizons.

4. SUMMARY AND IMPLICATIONS

(1) Composition of mineral–organic associations and implications for organic matter stabilization. The application of density fractionation in combination with multiple analytical techniques provided detailed insight into the time-dependent evolution of MOAs across a long-term mineralogical soil gradient. Mineral transformations along this 0.3–4.100 kyr soil chronosequence caused a differential storage of OM in MOAs that is primarily driven by the weathering-induced formation of metastable PC Fe, Al, Si phases and Al-organic complexes (stage II). Both, the surface C and N enrichment data as well as OM–mineral volumetric ratios suggest that OM at the youngest and oldest site is bound to larger mineral grains on average, likely to edge faces of primary/secondary silicates, leaving much mineral surface area uncovered by OM. In contrast, the MOAs at the 20–400 kyr old sites are composed of a ‘well mixed’ homogeneous array of OM and nanometer-sized mineral phases, with the OM volume partly exceeding the mineral volume. Such volumetric ratios indicate that in OM-rich surface soil horizons, precipitation in addition to adsorption reactions is involved in the formation of MOAs. In these stage-II MOAs, particularly in the subsurface ones, OM is more effectively protected against biodegradation than at the youngest and older sites (Torn et al., 1997). This likely is a combined result of the following factors: (i) the strong chemical binding of OM to the PC minerals via ligand exchange reactions at low pH, (ii) the sterically favorable association of OM with mineral micropores, (iii) the effective ‘embedding’ of OM in the mineral matrix due to the saturation of OM functional groups with small mineral colloids or monomeric/polymeric metals, thus also physically protecting OM, and (iv) the large intrinsic stability of bound aromatic compounds (Kalbitz et al., 2005; Scheel et al., 2007). Factors i–iii all hamper the desorption of OM and thus its microbial utilization.

(2) Accessibility and reactivity of mineral surfaces towards organic matter. Despite about one-third to one-half of the mass of A-MOAs in stage-II soils being attributable to OM, the mineral surfaces appear only moderately covered by OM. This suggests that even under productive wet forest ecosystems that constantly deliver organic detritus and dissolved OM to the soil (Neff et al., 2000), mineral surfaces are not completely covered by OM. In the youngest and oldest soil, mineral surfaces are less favorable for OM sorption because of their small reactivity, i.e., less SSA, microporosity and variable surface charge (Chorover et al., 2004). In contrast, in the intermediate-aged soils, significant surface area as probed by N$_2$ may be located in small agglomerations/aggregates that cannot be penetrated by OM. It is also possible that OM units are simply too large to effectively coat agglomerations of nanometer-sized, high-SSA minerals.

(3) Mineral control on the chemical composition of mineral-associated organic matter. The attempt to reveal definite mineralogical controls on the composition of MOM using XPS yielded ambiguous results. The MOM in A horizons showed little compositional variation despite of the pronounced mineralogical changes, suggesting that the chemical signature of topsoil MOM predominantly reflects the uniform source vegetation. In contrast, in subsoil MOAs containing PC aluminosilicates and Al-organic complexes, a larger abundance of acidic and aromatic OM components is consistent with the high affinity of acidic aromatic compounds for variable-charged minerals. Of note, in both A- and B-MOAs, we found that PC Fe minerals significantly explained the concentrations of aliphatic and amide C, but we have no unequivocal explanation for this result. This finding suggests more complex pathways in the formation of MOAs, possibly involving microbial activity in connection with precipitation reactions during redox-oscillations (Thompson et al., 2006).

Lignin and carbohydrate biomarker analyses proved more suitable in revealing distinct mineral–organic patterns across the mineralogical soil gradient. Lignin-derived phenols accumulated preferentially in MOAs rich in high-SSA PC minerals and metal–organic complexes during weathering stage II (20–400 kyr), but were, in absolute numbers, less important at the youngest and oldest site. Independent from the mineralogical regime, acidic lignin phenols were selectively associated with minerals. However, given that only a minor fraction of aromatic components (as shown by XPS) is attributable to lignin-derived phenols, additional aromatic sources like tannins/polyphenols...
should contribute and/or the lignin in MOAs might already be highly altered and thus be invisible for the CuO technique. The analysis of the carbohydrate fraction showed that sugars are an integral part of MOAs and can become effectively stabilized particularly in presence of PC mineral phases and hydrolyzable metal species during weathering stage II. In contrast to lignin-derived phenols, non-cellulosic carbohydrates were relatively more important in the formation of MOAs at the youngest site where primary minerals prevail. Consequently, the fractionation of OM during adsorption or precipitation reactions is an important pathway that imposes differences in the chemical composition of MOM.

(4) Chemical fractionation within mineral-associated organic matter. Depth profiling of A-MOAs by means of Ar+ sputtering revealed a parallel loss of C and N intensity, thus questioning the recently proposed importance of proteins in the initial formation of MOAs at least for these acidic tropical soils. This result more supports the view that N is an integral component of mineral-associated OM. While Ar+ sputtering bears the risk of chemical modifications in OM such as the removal of aromatic structures, the fact that aromatic C increased with sputter time towards the mineral–organic interface whereas aliphatic/amide C declined suggests a zonal differentiation within mineral-attached OM especially with regards to aromatic moieties. Such a zonal differentiation within organic coatings/agglomerations is intriguing and certainly warrants further research.

(5) Matrix rigidity of mineral-associated organic matter. The DSC measurements revealed that in spite of the mineralogical changes and different properties of MOAs, the rigidity of sorbed OM did not differ appreciably over time. However, the transition temperature, $T^*$, of subsoil MOM was significantly higher than for MOM in A horizons, suggesting a more rigid structure of subsoil MOM. This likely arises from a larger degree of direct mineral–organic bindings that restricts the side chain mobility of OM. We speculate that such limited flexibility may increase the residence time of OM in subsoil MOAs, because desorption as well as enzymatic reactions with sorbed OM are less likely to occur as the degree of chain movements within OM becomes restricted by complexation with metals and minerals.

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APPENDIX A. SUPPLEMENTARY DATA


REFERENCES


Evolution of mineral–organic associations upon substrate aging

2059


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