Impacts of soil redistribution on the transport and fate of organic carbon in loess soils

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Chapter 3

Soil aggregation and the stabilization of organic carbon as affected by erosion and deposition

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Abstract

The importance of soil aggregation in determining the dynamics of soil organic carbon (SOC) during erosion, transportation and deposition is poorly understood. Particularly, we do not know how aggregation contributes to the often-observed accumulation of SOC at depositional sites. Our objective was to assess how aggregation affects SOC stabilization in comparison to interactions of SOC with minerals. We determined and compared aggregate size distributions, SOC distribution in density fractions, and lignin-derived phenols from aggregated soil samples at both eroding and depositional sites. The stabilization effect of aggregation was quantified by comparing mineralization from intact and crushed macro-aggregates. Deposition of eroded soil material resulted in carbon (C) enrichment throughout the soil profile. Both macro-aggregate associated SOC and C associated with minerals (heavy fraction) increased in their importance from the eroding to the depositional site. In the uppermost topsoil (0–5 cm), SOC mineralization from intact aggregates was larger at the depositional site than at the eroding site, reflecting the large input of labile organic matter (plant residues) promoting aggregation. Contrastingly, in the subsoil, mineralization rates were lower at the depositional site because of effective stabilization by interactions with soil minerals. Aggregate crushing increased SOC mineralization by 10 – 80% at the eroding site, but not at the depositional site. The content of lignin-derived phenols did not differ between eroding and depositional sites in the topsoil (24.6 – 30.9 mg per g C) but was larger in the subsoil of the eroding site, which was accompanied by higher lignin oxidation. Lignin data indicated minor effects of soil erosion and deposition on the composition of SOC. We conclude that SOC is better protected in aggregates at the eroding than at the depositional site. During transport disaggregation and consequently SOC mineralization took place, while at the depositional site re-aggregation occurred mainly in the form of macro-aggregates. However, this macro-aggregation did not result in a direct stabilization of SOC. We propose that the occlusion of C inside aggregates serves as a pathway for the eroded C to be later stabilized by organo-mineral interaction.
3.1 Introduction

Soil erosion influences terrestrial C dynamics through various processes (Berhe et al., 2007; Harden et al., 1999; Lal, 2003; van Oost et al., 2007). Soil erosion disturbs carbon-rich topsoils and preferentially removes soil organic carbon (SOC) from upslope sites, resulting in (partial) mineralization, as well as in (partial) redistribution and burial of SOC in depositional environments (Berhe et al., 2007; Lal, 2003). The calculation of carbon enrichment ratios (CER), i.e. the enrichment (or depletion) of carbon at depositional landforms relative to eroding sites has been used as an effective way to illustrate effects of soil erosion on carbon redistribution (Juarez et al., 2011; Wang et al., 2010).

Based on the mechanisms proposed by Sollins et al. (1996), von Lützow et al. (2006) and Schmidt et al. (2011), Berhe et al. (2012) summarized three key mechanisms governing SOC stabilization related to soil erosion and deposition: (i) physical isolation of SOC resulting in organic matter inaccessible to soil microorganisms and fauna (Six and Jastrow, 2002; Six et al., 2000b; Sollins et al., 1996); (ii) chemical interaction of organic matter with the soil mineral matrix (Torn et al., 1997) and (iii) molecular composition of organic matter influenced by environmental factors (Berhe et al., 2007; Schmidt et al., 2011).

Physical isolation of SOC, which governs its stabilization, is closely related to aggregation (Six et al., 2000b). Soil aggregation provides physical protection of SOC against rapid decomposition by soil microbes (Razafimbelo et al., 2008), and aggregate formation appears to be closely linked with soil C storage and stability (Barreto et al., 2009; Golchin et al., 1995; Salome et al., 2010; Thevenot et al., 2010). Generally, aggregation is perceived as an indicator of soil stability and erodibility (Bryan, 1971; Morgan, 2005). However, the role of soil aggregation and disaggregation in SOC dynamics during erosion, transportation and deposition is far less well understood (Kuhn et al., 2009; Nadeu et al., 2011).

Most process level investigations of SOC cycling focus on non-sloping sites that experience minimal soil erosion and deposition (Berhe et al., 2012; Schmidt et al., 2011; Six and Jastrow, 2002; Sollins et al., 1996). Soil erosion can promote breakdown of aggregates at the eroding landform positions leading to exposure of previously encapsulated SOC (Doetterl et al., 2012; Lal, 2003). This exposure of formerly physically protected SOC increased the potential availability of SOC to mineralization (Berhe et al., 2012) and release of soluble compounds. Goebel et al. (2009) found smaller SOC mineralization from intact aggregates in comparison to crushed ones demonstrating the stabilization effect of aggregation.

Interactions of SOC with mineral surfaces are considered as the most effective stabilization mechanisms in many soils (Schmidt et al., 2011; Torn et al., 1997; von Lützow et al., 2006). Stabilization occurs through sorption to pedogenic oxides, clay minerals or by co-precipitation with polyvalent cations. Effects of erosion and deposition on this chemical
stabilization of SOC are not well understood. Berhe et al. (2012; pp.13) concluded that “the potential to form new sorptive mineral-organic matter interactions is not always higher in depositional, compared to eroding, landform positions”. Stabilizing effects of organo-mineral interactions might be even more important than previously assumed because of the role of pedogenic oxides in aggregate formation and stabilization (Berhe et al., 2012), i.e. interactions between chemical and physical stabilization mechanisms.

As one of the major components of plant biomass and SOC, lignin has been used as an indicator of the fate of plant-derived organic matter and the origin and state of degradation of soil organic matter (Guggenberger and Zech, 1994; Hedges et al., 1988; Klotzbücher et al., 2011). Variations in the relative proportions of lignin-derived phenols provide information about microbial degradation and adsorption and desorption processes (Hernes et al., 2007; Thevenot et al., 2010). These compositional changes of SOC affect stabilization and sequestration of SOC in soils (Schmidt et al., 2011). Soil erosion potentially affects lignin probably because it preferentially removes organic matter not associated with minerals or aggregates, which is easily transported (Berhe et al., 2012; Gregorich et al., 1998; Lal, 2003). Therefore, the assessment of amount and composition of lignin might improve our understanding of how erosion influences the origin, composition and fate of SOC from upslope to downslope positions.

Physical fractionation by density has been proven useful to identify meaningful soil fractions, which can be related to different stability and stabilization processes (von Lützow et al., 2006). The free light fraction (fLF) comprises relatively undecomposed labile organic matter, i.e. unprotected from physical or chemical processes. The occluded light fraction (oLF) comprises organic matter stabilized by aggregation, whereas in the heavy fraction (HF) C is strongly associated with soil minerals (Cerli et al., 2012; Golchin et al., 1994). Therefore, density fractionation of SOC is a useful tool to study soil organic matter stabilization in the context of soil erosion and deposition (e.g. Berhe et al., 2012; Doetterl et al., 2012; Gregorich et al., 1998).

To obtain a better understanding of SOC stabilization at sites that experience soil erosion and deposition we wanted to know how aggregation and disaggregation affect SOC stabilization in comparison to interactions of SOC with minerals. Particularly, we wanted to reveal the unknown role of aggregation for the often-observed SOC accumulation at depositional sites. Additionally, we used lignin to trace the fate of organic matter during erosion and deposition. We therefore studied topsoils and subsoils sampled from eroding and depositional sites, carried out aggregate and density fractionation and studied amounts, composition (lignin) and stability of SOC.
3.2 Material and methods

Study site and sampling

Soil samples were collected from a terraced hillslope in the Belgian Loess Belt near Leuven, Belgium, with an upslope eroding position (Haplic Cambisol) and a downslope depositional location (Colluvic Regosol), referred in the text as the eroding and depositional sites. The soils had a similar soil texture (silt loam according to FAO, 2006). The deposition rate in this area was estimated as 0.73 cm/year with $^{137}$Cs tracer techniques (Wang, 2011). Mean annual temperature is 9–10°C and annual precipitation is 750–800 mm in this region. In this study, five soil cores at 0–200 cm depths were taken at each site. These soil cores were firstly separated by depth to determine C contents. Then the five cores were mixed per depth and site for further analyses. Parts of the samples were air-dried and the other part was stored under field moist conditions at 4°C before analysis. More detailed information on the experimental area and the sampling methods was reported in Chapter 2.

Soil analysis

The C and N contents of bulk soils, aggregate size fractions and density fractions were determined using a C and N analyzer (Elementar Vario EL, Hanau, Germany; Standard deviation (SD) < 0.1% abs.). The samples were not pretreated with acid prior to C analysis. It was found that the soil was free of inorganic C because there was no reaction when 6 M HCl was added to the soil; hence total C was considered to be equivalent to SOC.

Isolation of soil fractions

Aggregate size distribution

Topsoil (0–5 and 5–10 cm) and subsoil (45–70 and 160–200 cm) samples from the eroding and depositional sites were subjected to aggregate size fractionation according to the dry sieving method (Cammeraat and Imeson, 1998). Briefly, 170–200 g of soil samples were air-dried at room temperature (20°C) and then fractionated using nine mesh sieves (16, 8, 5, 4, 2, 1, 0.5, 0.25 and 0.125 mm) to obtain ten size fractions. Sieves were shaken for 5 min at 30 Hz using a horizontal shaking machine (Retsch, AS 200 basic, Haan, Germany). Each fraction was weighed to determine the proportion of aggregates in each size fraction. For each fraction, the C and N content were determined. Macro-aggregates were defined as aggregates being >2 mm. The average recovery of the total soil mass and C was 99.4±3.7% (SD) and 127±22%, respectively. We used smaller depth increments for the topsoil than the subsoil.
samples because of the exponential decrease in organic C with depth, i.e. the large decrease in organic C particularly in the topsoil (Figure 2.2 in Chapter 2; Wang et al., 2013a).

**Soil density fractionation**

Density fractionation was applied to the topsoil (0–5 and 5–10 cm) and subsoil (45–70 and 160–200 cm) samples from eroding and depositional sites following the methods of Golchin et al. (1994) and Cerli et al. (2012). Twenty-five grams of air-dried material was weighted in a centrifugation tube and 125 ml of sodium polytungstate (SPT) solution with a density of 1.6 g cm\(^{-3}\) was added (Cerli et al., 2012). After one hour the suspension was centrifuged at 5100 g for 20 min at room temperature. The supernatant with floating particles was filtered (Whatman GF/F filter, 0.7 µm) and washed with deionized water till the conductivity of the washing water was <50 µS cm\(^{-1}\). The sediment was re-suspended into 125 ml of fresh SPT solution and sonicated at 300 J mL\(^{-1}\) (Sonopuls HD 3200 with VS70 probe, Bandelin electronic, Berlin, Germany, calibrated according to Schmidt et al. (1999) in an ice-bath to keep the temperature < 40°C). The amount of energy was pretested on a subset of samples according to Cerli et al. (2012) to ensure a proper separation between the organic matter enclosed into aggregates and associated with minerals. After letting the suspension stand for 30 min the samples were again centrifuged, filtered and rinsed with deionized water as described above for the fLF. This material constituted the occluded light fraction (oLF). The remaining sediment was re-suspended in deionized water and centrifuged several times till the conductivity of the washing water was <50 µS cm\(^{-1}\), resulting in the heavy fraction (HF). All fractions were freeze-dried, homogenized and used for the determination of C, N and lignin-derived phenols contents. The average recovery of the total soil mass and C was 95.6±2.3% (SD) and 84.8±15.4%.

**Soil aggregate-associated C mineralization**

The 8–16 mm aggregates were found to constitute the largest contributions to the SOC pool of the bulk soil among all aggregates size fractions (Figure 3.1). According to this result, the 8–16 mm aggregates of topsoils (0–5 and 5–10 cm) and subsoils (45–70 cm) from both the eroding and depositional sites were used to determine SOC mineralization in an incubation experiment. The use of this aggregate size fraction ensured that our results were representative for the effects of aggregation on C stabilization. In order to determine the stability of physically protected C within aggregates, intact (8–16 mm) and crushed (<0.125 mm) aggregates were incubated. The difference in C mineralization has been used as a
measure of C stabilization by aggregation. Some disturbance of the aggregates during fractionation could not be prevented. However, we consider these effects as small because the second largest fraction was used for incubation meaning a minimal effect of the sieving procedure. Crushed aggregates were obtained by grinding part of 8–16 mm aggregate material using a porcelain mortar and sieving the final powder through a 0.125 mm sieve. This approach particularly enables the determination of C stabilization by macro-aggregation whereas direct effects of micro-aggregation on C stabilization were not determined. Taking the aggregate size distribution into account (Figure 1) that seems a reasonable focus. Samples of both intact and crushed aggregates were rewetted to a water potential of −100 mbar (pF 2.0) and kept in darkness at 20°C for 7 days to activate microorganisms before starting the incubation. Ten grams (dry weight) of intact and crushed aggregate samples were placed inside 120 mL gas-tight glass jars fitted with rubber septa and sealed. Incubation was carried out in darkness for 52 days. The temperature was kept constant (20°C) throughout the experiment. The CO₂ concentration in the flask headspace never exceeded 3% by volume. The jars’ headspace was sampled on days 1, 3, 7, 10, 14, 21, 28, 52 of the incubation period. The CO₂ was determined by gas chromatography (Varian STAR 3600, California, USA). Air pressure in the headspace was measured by a tensiometer (TC 1085, Tensio Technik, Geisenheim, Germany).

Lignin analysis

The lignin content of soils and of different density fractions was analyzed by the CuO oxidation method developed by Hedges and Ertel (1982) and modified by Kögel and Bochter (1985) and Guggenberger and Zech (1994). Briefly, around 20 mg of organic and 800 mg mineral subsamples were weighed together with 100 mg Fe (NH₄)₂ (SO₄)₂·6H₂O, 500 mg CuO and 50 mg glucose. After the addition of ethylvanillin as internal standard and 15 ml of 2 M NaOH, the samples were heated for 2 h at 170 °C in a pressure digestor and allowed to cool down overnight. Solutions were separated by centrifugation (4100 g for 15 min) and the lignin-derived phenols were extracted after acidification (pH 1.8–2.2) and centrifugation (5400 g for 25 min) using C18 solid-phase columns (Mallinckrodt Baker Corp., Phillipsburg, NJ). Phenols were eluted from the column with 4 ml ethylacetate and derivatized with a mixture of 100 μl pyridine and 200 μl N,O-bis(trimethysilyl)-trifluoroacetamide. The derivatives were separated and quantified using a gas chromatograph equipped with a mass-sensitive detector (Trace Thermo Scientific, Rodano, Italy) and a DB-5 fused silica column (30m length, 0.25 μm i.d., 0.25 μm film; Agilent Technology, Colorado, USA). The instrument parameters were: injector temperature 250 °C; temperature programme: 100 °C for 3 min, 10 °C min⁻¹ to 250 °C, 250 °C for 10 min, 30 °C min⁻¹ to 300 °C, 300 °C for 5 min.
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Helium was the carrier gas, at constant pressure of 100 kPa. All samples were extracted in duplicate and the recovery of ethylvanillin averaged 81.0±17.9% (SD).

CuO oxidation yields a suite of phenolic oxidation products divided into three major groups: vanillyl, syringyl and p-coumaryl phenols (Kogel and Bochter, 1985). The concentration of V-type phenols (V) was calculated as the sum of vanillin, acetovanillone and vanillic acid. Syringyl type phenols (S) included syringaldehyde, acetosyringone and syringic acid. Cinnamyl compounds (C) include ferulic and p-coumaric acids. The sum of all those units (V+S+C) expressed as mg VSC g⁻¹ SOC was considered as an estimate of the total lignin amount. Additionally, the ratios of cinnary to vanillyl type phenols (C/V) and syringyl to vanillyl type phenols (S/V) were used as indicators of the source type of vascular plant materials (Thevenot et al., 2010). The ratios vanillic acid-to-vanillin (Ac/Al), and syringic acid-to-syringaldehyde (Ac/Al), were used as indicators for the side chain oxidation state of lignin e.g. (Thevenot et al., 2010).

Data and statistical analyses

The carbon enrichment ratios (CER) of SOC in bulk soils and three density fractions were calculated as: CER=[SOC] deposition / [SOC] erosion, where [SOC] deposition and [SOC] erosion are the SOC content (mg g⁻¹) at the depositional and at the eroding site, respectively. The lignin enrichment ratios (LER) in bulk soils were calculated as: LER=[Lignin] deposition / [Lignin] erosion, where [Lignin] deposition and [Lignin] erosion are the lignin content (mg VSC g⁻¹ SOC) at the depositional and eroding site respectively. Lignin enrichment ratios (LER) in the three density fractions were calculated as:

\[
LER = \frac{Lignin \times Mass}{SOC} \times \frac{fraction}{g soil} \div \frac{deposition}{erosion} \times \frac{fraction}{g soil} \div \frac{g soil}{g soil}
\]

The values obtained were normally distributed. One-way ANOVA and the Duncan post hoc test were used to test the effect of aggregate crushing on cumulative C mineralization, SOC content in aggregate size fractions, SOC content in density fractions and also lignin content in soils and density fractions between different depths from eroding and depositional sites (P<0.05). We could not compare these results with data from undisturbed reference sites of the studied hillslope area, i.e. sites neither affected by erosion nor by deposition. All results are expressed as the mean of three lab replications, except for lignin data (duplicates). All data and statistical analyses were performed using SPSS 17 for Windows, SAS software or Microsoft Office.
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3.3 Results

Soil organic carbon in aggregate size fractions

The relative distribution of C in aggregates was calculated from the SOC content per size fraction multiplied by the mass of aggregates per fraction. The macro-aggregates (>2 mm) contained more than 60% of the total SOC at both sites. The relative contribution of macro-aggregates to the total SOC was significantly higher at the depositional site than at the eroding site and increased with depth at both sites. The size fraction 8–16 mm was the fraction that contained by far the most SOC at both sites because of its large abundance in bulk soils. In terms of carbon contents per g soil micro-aggregates contained more SOC in topsoil samples than the 8–16 mm fraction did.

Soil OC contents in the bulk soil and in most aggregates of the same size were significantly higher at the depositional site than at the eroding site (Figure 3.1, Table 3.1), except for the 5–10 cm depth. This was mainly due to the < 4 mm aggregates, which had higher SOC contents at the eroding than at the depositional site. At both sites, the 0.125–0.25 mm aggregates of the 0–5 cm depth had the highest SOC contents among all aggregate size classes.

![Figure 3.1 Soil organic carbon (SOC) in the ten different aggregate size fractions from the eroding and the depositional site. The values were calculated by multiplying the C content in aggregate size fractions (Table 3.1) with the corresponding mass of the fractions. Numbers in the figure refer to the contribution of macro-aggregates (> 2 mm) to SOC (total SOC = 100%) in the respective soil layer. The bars with the slashes represent micro-aggregates (< 2 mm); the bars without the slashes represent macro-aggregates.](image)

Figure 3.1 Soil organic carbon (SOC) in the ten different aggregate size fractions from the eroding and the depositional site. The values were calculated by multiplying the C content in aggregate size fractions (Table 3.1) with the corresponding mass of the fractions. Numbers in the figure refer to the contribution of macro-aggregates (> 2 mm) to SOC (total SOC = 100%) in the respective soil layer. The bars with the slashes represent micro-aggregates (< 2 mm); the bars without the slashes represent macro-aggregates.
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Table 3.1 Carbon content (mg C g\(^{-1}\) fraction) of ten different aggregate size fractions (three replications except 160-200 cm depth). ‘n.p.’ means not present.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Depth (cm)</th>
<th>&gt;16</th>
<th>16–8</th>
<th>8–5</th>
<th>5–4</th>
<th>4–2</th>
<th>2–1</th>
<th>1–0.5</th>
<th>0.5–0.25</th>
<th>0.25–0.125</th>
<th>&lt;0.125</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eroding site</td>
<td>0–5</td>
<td>15.3 ± 6.9</td>
<td>13.3 ± 5.7</td>
<td>12.8 ± 2.4</td>
<td>13.7 ± 3.8</td>
<td>14.6 ± 5.1</td>
<td>15.9 ± 4.5</td>
<td>17.3 ± 4.9</td>
<td>19.4 ± 7.0</td>
<td>23.9 ± 8.4</td>
<td>13.0 ± 3.1</td>
<td>15.9 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>5–10</td>
<td>14.5 ± 6.5</td>
<td>8.7 ± 1.1</td>
<td>11.0 ± 3.6</td>
<td>11.3 ± 4.3</td>
<td>12.1 ± 6.5</td>
<td>14.4 ± 6.6</td>
<td>15.2 ± 7.1</td>
<td>17.2 ± 8.9</td>
<td>20.8 ± 7.7</td>
<td>12.2 ± 2.7</td>
<td>13.7 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>45–70</td>
<td>2.5 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>160–200</td>
<td>n.p.</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Depositional</td>
<td>0–5</td>
<td>14.9 ± 1.4</td>
<td>17.7 ± 0.9</td>
<td>18.0 ± 2.0</td>
<td>18.2 ± 4.3</td>
<td>20.2 ± 5.6</td>
<td>20.8 ± 4.7</td>
<td>21.9 ± 4.1</td>
<td>26.2 ± 6.6</td>
<td>26.9 ± 5.7</td>
<td>16.2 ± 0.8</td>
<td>20.1 ± 4.0</td>
</tr>
<tr>
<td>site</td>
<td>5–10</td>
<td>12.1 ± 1.9</td>
<td>13.1 ± 1.4</td>
<td>12.2 ± 0.9</td>
<td>11.7 ± 0.4</td>
<td>11.8 ± 0.5</td>
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<td>12.8 ± 1.9</td>
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<tr>
<td></td>
<td>45–70</td>
<td>7.4 ± 1.5</td>
<td>6.9 ± 1.2</td>
<td>6.8 ± 0.2</td>
<td>7.1 ± 0.7</td>
<td>7.3 ± 0.8</td>
<td>7.3 ± 0.4</td>
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<tr>
<td></td>
<td>160–200</td>
<td>n.p.</td>
<td>2.7</td>
<td>2.7</td>
<td>2.6</td>
<td>3.1</td>
<td>3.8</td>
<td>3.0</td>
<td>3.2</td>
<td>3.5</td>
<td>3.0</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>
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Soil organic carbon in density fractions

In all samples both light fractions (free light fraction (fLF), occluded light fraction (oLF)) had a C content exceeding 200 mg g\(^{-1}\) fraction indicating the separation of mainly organic material. Contrastingly, the heavy fraction (HF) contained only a small percentage of OC (1.7–17.0 mg g\(^{-1}\) fraction – data not presented).

At both the depositional and the eroding site, the HF represented the most important part of the total SOC at all depths, constituting > 80% of SOC (Figure 3.2). The contribution of the HF to SOC was slightly lower at the depositional site than at the eroding site at all depths, indicating the larger contribution of fLF and oLF to SOC at the depositional site. The relative contribution of fLF and oLF to SOC decreased with depth at both sites. No free and occluded light fractions were present at 160–200 cm depth at the eroding site.

Carbon to nitrogen ratios of the HF tended to decrease with depth at both sites whereas an opposite pattern was observed for the fLF and the oLF at the eroding site (Table 3.2). The increase in C/N ratios of the fLF was larger than that of the oLF.

At the depositional site 1.4 to 2.1 times more C was found in the fLF than at the eroding site (Table 3.3). The C enrichment at the depositional site was even larger in the oLF (CER: 1.8 – 4.1) and the HF (CER: 1.2 – 3.3). Carbon enrichment in these fractions of the depositional site was stronger in subsoil than in topsoil.

Figure 3.2 Soil organic carbon (SOC) in three density fractions (free light fraction (fLF), occluded light fraction (oLF) and heavy fraction (HF)) from the eroding and depositional site. Numbers in the figure refer to the contribution of the heavy fraction to SOC (total SOC = 100%) in the respective soil layer.
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Table 3.2 C/N ratios of free light faction (fLF), occluded light fraction (oLF) and heavy fraction (HF) and soil microbial biomass C (mg C g⁻¹ soil C) from topsoils and subsoils at the eroding and depositional site. ‘n.p.’ means not present. ‘n.d.’ means not detectable, i.e. smaller than 1 mg C g⁻¹ soil C.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Depth (cm)</th>
<th>C/N ratio</th>
<th>Soil microbial biomass C (mg C g⁻¹ soil C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fLF</td>
<td>oLF</td>
<td>HF</td>
</tr>
<tr>
<td>Eroding site</td>
<td>0–5</td>
<td>19.9 ±2.5</td>
<td>16.4 ±0.5</td>
</tr>
<tr>
<td></td>
<td>5–10</td>
<td>28.0 ±5.7</td>
<td>20.9 ±0.5</td>
</tr>
<tr>
<td></td>
<td>45–70</td>
<td>59.3 ±40.9</td>
<td>33.6 ±3.6</td>
</tr>
<tr>
<td></td>
<td>160–200</td>
<td>n.p.</td>
<td>n.p.</td>
</tr>
<tr>
<td>Depositional site</td>
<td>0–5</td>
<td>21.2 ±0.8</td>
<td>16.4 ±0.6</td>
</tr>
<tr>
<td></td>
<td>5–10</td>
<td>18.5 ±4.7</td>
<td>15.1 ±0.1</td>
</tr>
<tr>
<td></td>
<td>45–70</td>
<td>33.3 ±8.0</td>
<td>22.3 ±0.5</td>
</tr>
<tr>
<td></td>
<td>160–200</td>
<td>10.5 ±3.7</td>
<td>36.4 ±4.0</td>
</tr>
</tbody>
</table>
Table 3.3 Carbon and lignin enrichment ratios in bulk soils, free light fraction (fLF), occluded light fraction (oLF) and heavy fraction (HF) from topsoils and subsoils at the depositional site with respect to the eroding sites (mean ± standard error). ‘n.p.’ means not present.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>C enrichment ratio (CER)</th>
<th>Lignin enrichment ratio (LER)</th>
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<tr>
<td></td>
<td>Bulk soils</td>
<td>fLF</td>
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<tr>
<td>0–5</td>
<td>1.30 ± 0.13</td>
<td>1.50 ± 0.39</td>
</tr>
<tr>
<td>5–10</td>
<td>1.27 ± 0.09</td>
<td>1.38 ± 0.32</td>
</tr>
<tr>
<td>45–70</td>
<td>3.12 ± 0.11</td>
<td>2.11 ± 1.41</td>
</tr>
<tr>
<td>160–200</td>
<td>3.59 ± 0.44</td>
<td>n.p.</td>
</tr>
</tbody>
</table>
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**SOC mineralization from aggregates**

There was an opposing pattern of C mineralization from intact aggregates comparing topsoils and subsoils of the eroding and depositional site (Figure 3.3). Cumulative CO₂ emission was larger at the depositional site than at the eroding site for the 0–5 cm depth. There was no significant difference for the 5–10 cm depth. In the subsoil (45–70 cm) cumulative CO₂ emission was larger at the eroding site than at the depositional site and smaller than in topsoil layers.

Aggregate crushing caused an increase in cumulative C mineralization at the eroding site (Figure 3.3). This effect increased with depth and was significant in 45–70 cm depth. In contrast, aggregate crushing had almost no effect on C mineralization at the depositional site.

![Figure 3.3 Cumulative CO₂-C emissions (mg g⁻¹ SOC) during 52 days of incubation of intact aggregates and crushed ones from topsoils and subsoils at the eroding (ERO) and depositional site (DEP; mean and standard error of three replications).](image)
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Lignin

*Lignin in bulk soils*

The content of lignin-derived phenols (VSC) ranged from 11.7 to 30.9 mg g\(^{-1}\) SOC at the eroding site and from 4.3 to 25.6 mg g\(^{-1}\) SOC at the depositional site. At the depositional site, the content of lignin derived phenols decreased with depth, while at the eroding site a less constant trend was observed. The content of lignin-derived phenols tended to be larger at the eroding site than at the depositional site in all depths (Figure 3.4). However, only the difference in the subsoil at 160–200 cm depth was statistically significant.

The S/V and C/V ratios ranged from 0.57 to 1.02 and from 0.22 to 1.72, respectively, for the eroding site, and from 0.72 to 1.28 and 0.21 to 1.26, respectively, for the depositional site (Figure 3.5). The S/V ratios decreased at the depositional site whereas the C/V ratios increased with depth at both sites. The S/V ratios were mostly higher at the depositional site than at the eroding site, except for the deep subsoil (160–200 cm). In contrast, the C/V ratios were higher at the eroding site than the depositional site. The (Ac/Al)\(_v\) and (Ac/Al)\(_s\) ratios increased with depth at both sites. No significant differences were observed between topsoils from the two sites, but the (Ac/Al)\(_v\) ratios of subsoils were higher at the eroding site than the depositional site.

![Figure 3.4](image.png)

Figure 3.4 Contribution of the free light fraction (fLF), occluded light fraction (oLF) and heavy fraction (HF) to lignin-derived phenols (VSC) in topsoils and subsoils at the eroding and depositional site. Different capital letters indicate significant differences of VSC content of the bulk soil between depths at two sites; different small letters indicate significant differences between the eroding and depositional site.
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**Lignin in density fractions**

Most of the lignin was found in the HF, irrespective of soil depth and site (Figure 3.4). The contents of lignin-derived phenols of the HF were higher at the eroding site than at the depositional site. Contrastingly, in the topsoil, lignin-derived phenols in the fLF and oLF were higher at the depositional site compared with the eroding site. Correspondingly to the bulk soil lignin-derived phenols decreased with depth in all fractions at the depositional site, while at the eroding site a less constant trend with depth were observed. The contribution of the fLF to lignin-derived phenols was significantly higher in the uppermost soil layer (0–5 cm) than in any other depths at both sites, which was simply caused by the larger abundance of the fLF in this layer.

The oLF of the depositional site was 1.6 – 2.7 times enriched in lignin than that of the eroding site irrespective of depth (Table 3.3). In contrast, the HF of the two topsoil layers (0–5 and 5–10 cm) of the depositional site was depleted in lignin in comparison to the eroding site. Plotting the C/V versus S/V ratios of bulk soils and the HF did not reveal contrasting patterns between the eroding and depositional sites (Figure 3.6). The C/V ratios were higher for subsoils whereas the S/V ratios tended to be higher for topsoils. However, the 45–70 cm depth of the depositional site resembled topsoils and not subsoils indicating the redistribution of topsoil material. No clear patterns in these ratios could be found for the fLF and oLF.
Figure 3.5 Ratio of cinnamyl to vanillyl type phenols (C/V), ratio of syringyl to vanillyl type phenols (S/V), ratios of vanillic acid to vanillin (Ac/Al)$_v$ and syringic acid to syringaldehyde (Ac/Al)$_s$ of lignin derived phenols in topsoils and subsoils at the eroding and the depositional site (mean and standard error). ‘ND’ means not detectable. Different capital letters indicate significant difference between depths at each site; different small letters indicate significant differences between the eroding and the depositional site.
Figure 3.6 Plot of the ratios cinnamyl to vanillyl type phenols (C/V) and syringyl to vanillyl type phenols (S/V); plots of the bulk soil (A) and the heavy fraction (B).

### 3.4 Discussion

**Effects of aggregation on SOC stability**

Our findings suggest that SOC and aggregation are strongly influenced by erosion and deposition. The results of Wang et al. (2013a) (cf. Chapter 2) argued that higher carbon contents of the bulk soil at depositional sites in comparison to eroding sites should be the result of translocation of organic rich topsoil material followed by the decreased mineralization after deposition. The generally higher C contents of aggregates at the depositional site than at the eroding site as found in our study should be just a reflection of the differences in the bulk soil, i.e. higher C contents at the depositional site. However, some of the micro-aggregate size fractions contained more SOC at the eroding site than at the depositional site in 5–10 cm soil depth. This rather surprising although not significant phenomenon of increased micro-aggregation and increased C concentrations in aggregates
smaller than 4 mm at the eroding site could be a topic of further research and has not been further investigated here.

The already large contribution of macro-aggregates to SOC at the eroding site (more than 67%) further increased after erosion, transport and deposition of this soil material to more than 88% at the depositional site (Figure 3.1). Three processes should be responsible for this observation: (i) a preferential breakdown of macro-aggregates under eroding conditions at the eroding site (Denef et al., 2001a; Kuhn et al., 2009; Lal, 2003), (ii) increased macro-aggregation after deposition of organic C rich soil at the depositional site and (iii) a higher net primary production (NPP) which is generally assumed for depositional sites in comparison to eroding sites (Berhe et al., 2008) resulting in increased C input as well. These processes and the translocation of organic C rich topsoil material contributes to an additional input of fresh organic matter (e.g., plant residues), only weakly protected against mineralization at the depositional site. This is reflected in the largest (relative and absolute) contribution of the free light fraction to SOC in the uppermost layer of the depositional site (1.5 – 2.1 more C present as fLF at the depositional site than at the eroding site; Table 3.3). Therefore, mineralization from intact aggregates and bulk soil was largest from the uppermost soil layer (0–5 cm) of the depositional site. Obviously, large input of organic C rich topsoil material is the most important reason for high C contents of the uppermost soil layer of the depositional site. Decreased mineralization contributes to C accumulation after deposition just in the deep topsoil (5–10 cm) and in subsoil as also shown by Wang et al. (2013a) (Chapter 2).

Aggregation started after deposition as indicated by the larger contribution of macro-aggregates and of the occluded light fraction to SOC in the topsoil of the depositional site than to the topsoil of the eroding site. According to our previous discussion, aggregation was stimulated by the high carbon contents and the large contribution of fresh plant residues (free light fraction) (Denef et al., 2001a; Six et al., 2000b). Furthermore, the occluded light fraction contained 1.8 to 4.1 times more carbon at the depositional site than at the eroding site and this C enrichment after deposition increased with depth (Table 3.3). Hence aggregation increased with time after deposition as indicated by a higher proportion of the oLF and a larger contribution of macro-aggregates to SOC with depth at the depositional site. However, we are not able to differentiate between the different additional C sources at the depositional site, i.e. C deposited from upslope positions and C from increased NPP at the depositional site. Our results from aggregate size distribution and density fractionation would suggest aggregation as an important mechanism promoting carbon stability after deposition. That would also explain the often-found smaller carbon mineralization from depositional sites than from eroding sites (subsoils of our study, Chapter 2; Doetterl et al., 2012). Doetterl et al. (2012) also suggested that physical protection played a key role in stabilizing SOC after burial. However, carbon mineralization between intact and crushed aggregates from any depth did not differ significantly at the depositional site (Figure 3.3) although macro-aggregation did
further proceed with time, i.e. depth (Figure 3.1). The incubated macro-aggregates probably did not contain any more easily degradable C that could be released by crushing. Therefore, macro-aggregation at the depositional site did not result in direct C stabilization. In contrast to this observation, aggregation did contribute to C stabilization at the eroding site, at least in larger depths as indicated by the difference in C mineralization between intact and crushed aggregates.

We speculate that at the eroding site besides macro-aggregation also micro-aggregation takes place to a larger extent as the next step according to the concept of aggregate hierarchy (Six et al., 2000b). This assumed larger importance of micro-aggregation at the eroding site than at the depositional site is supported by larger SOC contents of micro-aggregate size fractions in the 5–10 cm soil depth (Table 3.1). Angers et al. (1997) and Six (2002) demonstrated the stronger stabilization of organic C by micro-aggregation in comparison to macro-aggregation. The reasons why micro-aggregation might be less important at the depositional site warrant further investigation.

The higher C/N ratios of fLF and oLF at the eroding site than at the depositional site particularly in 45–70 cm depths (Table 3.2) might indicate the presence of charcoal (high C/N ratio) at the eroding site (Rumpel et al., 2006; Schmidt et al., 2011). Another possible explanation is the contemporaneous enrichment of microbial products (low C/N ratio) in the occluded light fraction of the depositional site (e.g. Chapter 2; Dungait et al., 2013).

**Effects of mineral-associated C on SOC stability – interactions with aggregation**

Mineral-associated organic C (i.e. HF) was by far the dominant density fraction at both sites (Figure 3.2). It was 1.2 – 3.3 times enriched in C at the depositional site in comparison to the eroding site, and exhibited an increasing larger proportion of SOC with depth (Figure 3.2, Table 3.3). The larger potential for the formation of mineral-associated organic C at the depositional site in comparison to the eroding site is reflected by about 50% higher contents of pedogenic oxides (dithionite extractable; Chapter 2). Therefore, we assume that SOC was particularly stabilized by interaction with soil minerals as time proceeded and burial continued. This stabilization may also explain the smaller C mineralization rates in 45 – 70 cm at the depositional site from both aggregates (this study) and bulk soils (Chapter 2) despite a larger microbial biomass at the depositional site (Table 2.2, Chapter 2). This small metabolic quotient and the often-related high substrate use efficiency (Insam and Haselwandter, 1989; Steinweg et al., 2008) at the depositional site indicate changes in the microbial community contributing to SOC stabilization (Chapter 2). Our results supported the
observation of Berhe et al. (2012) who suggested that burial of eroded carbon at depositional sites could promote C accumulation and extend its residence time (Doetterl et al., 2012).

The high importance of mineral-associated organic matter for C stabilization at the depositional site might appear to be in contrast with the increased aggregation after deposition. This apparent contradiction can be resolved if both processes, i.e. aggregation and interactions of organic matter with mineral surfaces are considered together as stabilization mechanisms dependent on each other and working at different time scales. Aggregation is important for the stabilization of organic matter at an intermediate time scale (decennia) whereas the formation of mineral-associated organic matter stabilizes C at the time scale of centuries to millennia (von Lützow et al., 2008).

The first step of SOC stabilization after deposition is the formation of macro-aggregates promoted by large inputs of organic C rich topsoil with a high contribution of labile organic matter (free light fraction). However, our incubation experiment did not show evidence that macro-aggregation contributes to C stabilization at the depositional site although aggregate size distribution (dry sieving) and density fractionation indicated SOC accumulation in aggregates (cf. first part of the discussion). The expected second step of SOC stabilization, i.e. the enhanced sequestration of C in micro-aggregates at the depositional site, could only partly be observed by larger SOC contents of micro-aggregates in 0–5 cm depth. Contrarily, the opposite was observed in 5–10 cm depth. Furthermore, the relative contribution of micro-aggregates to SOC was rather small in comparison to macro-aggregates and decreased from the eroding site to the depositional site (Figure 3.1). However, we did not determine micro-aggregates within the macro-aggregates and did not apply any other methods to further study micro-aggregation. Therefore, the potential impact of micro-aggregation on C stabilization at depositional sites has to be assessed in future studies.

We propose that the large input of relatively fresh and labile C and its fast turnover (largest C mineralization in the uppermost layer of the depositional site) favored the contact between organic compounds and the minerals resulting in the direct formation of mineral-associated SOC within the macro-aggregates. This pathway to stable SOC formation benefits from the high availability of water at depositional sites enabling efficient diffusion within the macro-aggregates (Denef et al., 2001a). Furthermore, the high content of microbial biomass and a high substrate use efficiency of labile C input should promote the formation of stable, mineral-associated SOC (Cotrufo et al., 2013). Our hypothesis of a direct formation of mineral-associated SOC within macro-aggregation and the role of micro-aggregation have to be tested in future studies.
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Changes in lignin upon erosion and deposition

The determined content of lignin-derived phenols (VSC; Figure 4) was in the range of values reported in the literature (De Baets et al., 2012; Thevenot et al., 2010). Our results did not support the observation of Juarez et al. (2011) who found that eroded sediments were 3.7 times enriched in lignin compared with control soils. Total lignin content and lignin oxidation in the topsoil did not significantly differ between the two sites (Figure 3.4 and 3.5), indicating that lignin was hardly affected by soil erosion. This result underpins the conclusions by De Baets et al. (2012) and Berhe et al. (2012). They suggested soil erosion was not likely to significantly change the chemistry of SOC during transport. Furthermore the very large values of S/V and C/V clearly indicate angiosperm, non-lignified species as the dominant source of lignin at both sides with no major changes (Hedges, 1979).

In our soils, lignin contents tended to be lower in the subsoil of the depositional sites and not at the eroding sites. These lower contents of lignin-derived phenols were accompanied by less oxidation as indicated by smaller (Ac/Al)\text{v} and by the disappearing of (Al)s. These two parameters clearly indicate that less lignin in subsoil of the depositional site cannot be related to increased lignin degradation because that would be reflected by larger oxidation (Thevenot et al., 2010). At the depositional site the continuous input of fresh material and burial of the existing one together with the rapid turnover and formation of HF could be responsible for the anomalously large increase in C/V ratio with depth as well as for the better preservation and slightly lower side chain oxidation of S and V units when compared with the eroding site. The expected higher water content in subsoils of the depositional site might be another reason for lower lignin oxidation (De Baets et al., 2012; Schmidt et al., 2011).

On the contrary, the higher oxidation of lignin in the subsoil of the eroding site fits well with the higher C mineralization (Figure 2.3, Chapter 2). Contents of dissolved organic carbon (DOC) in the subsoil of the eroding site were also larger than those at the depositional site (Chapter 2). The aggregate breakdown and the disturbance of the topsoil at the eroding site also promoted the release of soluble compounds and their leaching along the profile and their interaction with the mineral phases, as indicated by the larger proportion of C and lignin associated with the HF at the eroding site (Figure 3.2 and 3.4). Hernes et al. (2007) and Kramer et al. (2012) argued that the distribution pattern of lignin in mineral subsoils could be affected by preferential adsorption of soluble aromatic acids. The larger content of C vs V phenols of the source material (plant residues, e.g. grass) could be the reason of the unusual contribution of the C-phenols with depth in such conditions. Furthermore, along the profile S/V ratio remained rather constant, and only the most resistant V acidic phenols were found in the deepest soil layers. The degree of side chain lignin oxidation increases with soil depth as indicated by increasing (Ac/Al)\text{v} ratios (Figure 3.5).
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Generally, the content of lignin-derived phenols in the different density fractions reflects the C distribution in these fractions in topsoils. The occluded fraction in the topsoil horizons of the depositional site was enriched in lignin in comparison to the eroding site (Table 3.3, Figure 3.4). This lignin enrichment could reflect the contribution of lignin to (macro-) aggregation after deposition of carbon rich topsoil material with high contents of plant residues. It also highlights the shorter time scale of carbon stabilization by aggregation since in subsoil horizons, where turnover of organic matter takes longer, whereby almost all of the lignin (and carbon) is stabilized by interactions with soil minerals.

3.5 Conclusions

Our study reveals that the interplay between aggregation and the formation of mineral-associated SOC is the key to understanding changes in amounts and stability of SOC upon soil erosion and deposition. We developed a conceptual model as presented in Figure 3.7 to integrate the various findings of our study.

At the eroding site subsoils will be exposed after removal of the topsoils. Erosion-induced breakdown of aggregates, for which we used crushing as an analog, exposed previously protected C within aggregates at the eroding site, enabling SOC readily to be decomposed (Figure 3.7). The readily soluble components will be released and partially leached along the profile but larger parts of this C rich soil and solutes will be transported and deposited in lower areas of the landscape leading to C enrichment once the material is deposited.

Soil erosion and deposition facilitated the modification of aggregate size distribution and associated carbon in the aggregate fractions. After the erosion-induced breakdown of aggregates, the redistribution of aggregate associated SOC led to increased macro-aggregation and macro-aggregate associated C content at the depositional site. However, macro-aggregation does not result directly in smaller C mineralization. Stabilization occurs only by the interplay of aggregation with the formation of mineral-associated SOC. Optimal conditions for macro-aggregation as the deposition of organic C rich soil with a high proportion of plant residues are the pre-condition for the formation of mineral-associated SOC. A high water content and enough mineral surfaces are important abiotic conditions promoting mineral-associated SOC. Our results support the recently proposed conceptual “Microbial Efficiency-Matrix Stabilization (MEMS)” framework (Cotrufo et al., 2013) i.e. that a high microbial biomass and a microbial community with a high metabolic efficiency are necessary for optimal conditions for macro-aggregation (e.g. large input of readily available C), which results in formation of mineral-associated SOC and subsequent stabilization. This stabilization is underpinned by the increasing C enrichment of the HF at the depositional site. More research is needed to further determine the relationships between aggregation and the
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formation of mineral-associated SOC as affected by soil erosion and to specify optimal conditions for SOC stabilization. Follow-up studies should include undisturbed reference sites without any effect of erosion and deposition and should try to reveal the role of micro-aggregation for C stabilization.

We propose that the occlusion of C inside macro-aggregates is an important pathway for eroded C to be later stabilized by organo-mineral interaction (Figure 3.7). Stabilization does not mean SOC will not change with time. Changes in lignin composition with soil depth clearly indicated continuous transformations of SOC independently from soil erosion and deposition.

Figure 3.7 Conceptual model of the interplay between physical and chemical stabilization of soil organic carbon during erosion and deposition. Physical stabilization means mainly macro-aggregation and chemical stabilization the formation of mineral-associated organic matter. The model is explained in detail in section conclusions (acronyms: OC=organic carbon, fLF=free light fraction, HF=heavy fraction).