Soil-organic-matter stability in sandy cropland soils is related to land-use history

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Abstract
Sandy cropland soils in NW Europe were found to contain unusually high organic-carbon (OC) levels, and a link with their land-use history has been suggested. This study's aim was to assess the discriminating power of physical and chemical fractionation procedures to yield information on soil-organic-matter (OM) stability for these soils. In relict- and cultivated-heathland soils, much higher proportions of 6% NaOCl treatment–resistant but 10% HF–soluble OC (MOC) and N (32.2% and 29.9%) were measured compared to a set of "permanent"-cropland soils without a history of heathland land use (11.9% and 8.5%). Also, the proportions of 6% NaOCl– and 10% HF treatment–resistant OC and N in the relict and cultivated heathlands (19.2% and 12.0%) were higher than in the permanent-cropland soils (17.7% and 5.7%). Stepwise multiple linear-regression yielded a significant relationship between the annual mineralization (g C [100 g OC]–1), soil OC (g C kg–1) content, and %MOC: Annual mineralization = 4.347 – 0.087 soil OC – 0.032 %MOC (R2 = 0.65). Combinations of incubation experiments for quantification of the labile soil OM pool with chemical fractionation may thus yield meaningful data for development of soil-organic-matter models with measurable pools, but their applicability will be limited to specific combinations of former land use with soil, climate, and current management.

Key words: soil organic matter / sandy soils / chemical fractionation / NaOCl / HF / C mineralization

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1 Introduction
Large areas of sandy arable soils were found to have unusually high soil-organic-carbon (SOC) levels in NW Europe (Germany: Springob and Kirchmann, 2002; Belgium: Sleutel et al., 2006). Based on a very large SOC database (over 100,000 fields sampled between 1989 and 2000), Sleutel et al. (2006) found sandy soils in Flanders (the N part of Belgium) to have an average organic-carbon (OC) percentage of 1.9% OC compared to only 1.2% OC for example in silt soils. Previously, both regional-scaled OC modeling exercises (Sleutel et al., 2006) as well as OC balances (Sleutel et al., 2007a) indicated that differences in current management in terms of both crop rotation as well as fertilization are unable to explain these differences in SOC levels. The higher stability of the organic matter (OM) present in these soils may, however, be explained by specific types of earlier land use, e.g., heathland and the use of plaggen manure over centuries, which was practiced in NW Europe. Springob and Kirchmann (2002) found that OC in the OM fraction remaining after a 6M-HCl treatment accounted for 50% to 90% of the whole soil OC in 23 soil samples taken from the Ap horizon of sandy-textured soils in Germany with SOC levels ranging from 10 to 50 g OC kg–1. They concluded that old sandy Ap horizons may have high levels of very stable (refractory) constituents.

More recently, efforts have been directed towards measuring soil-organic-matter (SOM) fractions in which the OM is protected against decomposition by distinct stabilization mechanisms (e.g., Kögel-Knabner et al., 2008). Chemical methods which are based on either oxidation of labile SOM (e.g., by H2O2, NaOCl, or Na2S2O8) (Eusterhues et al., 2005; Kaiser and Guggenberger, 2003; Kleber et al., 2005; Zimmermann et al., 2007) or on extraction of mineral-bound SOM (e.g., by HF or dithionite citrate bicarbonate) have increasingly been applied recently (Mikutta et al., 2006; Schmidt and Gleixner, 2005), and numerous 14C-dating studies have confirmed the old age and longer turnover of SOM in such fractions (Trumbore and Zheng, 1996; Eusterhues et al., 2003; Kleber et al., 2005).

Today’s questions concerning the role of SOM in soil fertility, ecosystem functioning, and global change can only be addressed through knowledge of the controls on SOM stabilization and their interactions (Plante et al., 2009). This study wants to contribute to this goal and focuses on SOM composition and mechanisms for SOM stabilization in sandy cropland soils in NW Europe. In a previous study, we compared the chemical composition of SOM in a limited selection of native-heathland and cultivated-heathland sandy soils (Sleutel et al., 2008) in detail by means of pyrolysis–field ionization mass spectroscopy. It was concluded that a strong similarity in the SOM composition between the native and cultivated heathlands, even after years of cultivation, confirmed the relative stability of SOM in these soils. Specifically lipids, steroids, and to a lesser extent N-containing compounds were selectively retained. This paper builds on the latter study and on the study by Springob and Kirchmann (2003) and aims to
compare SOM stability and composition in a larger set of sandy cropland soils with and without historical heathland land use. The primary objective was to test the hypothesis that SOM composition and stability in sandy cropland soils are predominantly dependent on historical land use. Secondly, we aimed to directly assess the discriminating power of both physical and chemical fractionation procedures to yield information on SOM stability. To this end, the annual SOC mineralization, estimated from incubation studies, was here employed as a measure for SOC stability.

2 Materials and methods

2.1 Study-area description and sampling

The study area comprised the sandy region located in the N of Belgium. Precise historical land-use maps are available for this region dating back to 1775 (map of Ferraris). Topsoil samples (0–30 cm) were collected from 8 relict-heathland soils and 8 cultivated-heathland soils, now cropland, at Beer nem (51°8′–3″20′), Wingene (51°4′–4″20′), Kalmthout (51°23′–4″28′), and Knesselare (51°8′–3″27′). Neither of these 16 sites has been under heathland permanently, but from the end of the 18th century, forestation periods alternated with heathland use. The eight croplands were put into cultivation ~ 60–100 y ago and have been under maize monoculture or maize temporary pasture since 3 to 4 decades. Podzols and “postpodzols” are the most prominent soil types. Cultivation of podzols created a 20–30 cm thick Ap horizon, which is composed of the former thin A and underlying E horizons of these soils. In some cases, past deeper rotating tillage operations (up to 60 cm depth) often have mixed parts of the Bh/s horizons into this Ap horizon leaving a fragmented Bh horizon (i.e., so-called postpodzols). Fifteen soil samples were taken at each plot by means of an auger (Ø 2.5 cm) within a 25 m x 40 m rectangle. Litter layers in the relict-heathland soils were removed. Bulked samples were then mixed and dried to the air. In addition, a group of 5 “permanent” sandy cropland soils under cultivation for hundreds of years, previously collected in the W of Flanders (Sleutel et al., 2006), was also included. Their historical land use as croplands was confirmed from the Ferraris maps at least until 1775 and immediately from the National Soil Survey dating back to the 1950s (Van Orshoven et al., 1988). Profile descriptions (Van Orshoven et al., 1988) confirm some more moderate profile development in these soils (i.e., Cambisols) with, however, the absence of spodic or plaggic horizons and a sandy texture till 120 cm depth. Sandy arable land in the study area, including both the sampled permanent croplands and cultivated heathlands, next to pasture, receives a considerable yearly N fertilization, mainly in the form of animal slurry (ranging at ≈ 240 kg N ha−1 y−1) and to a lesser extent through mineral fertilizer (ranging at ≈ 60 kg ha−1 y−1) (calculated from Seuntjens et al., 2008).

2.2 Characterization of the soil mineral matrix and particle-size fractionation

Total Fe and Al in pedogenic oxides were estimated by the dithionite citrate bicarbonate (DCB) method (Blakemore et al., 1987) (FeD and AlD). Pyrophosphate-extractable Fe (Fepp) and Al (Alpp) were determined at a soil-to-solution ratio of 1:100 (wt:v) in 0.1 M Na2HPO4 (Blakemore et al., 1987). Aluminum and Fe in <0.45 μm filtrates were measured with atomic-absorption spectroscopy (AAS). Soil texture was determined by the pipette-sedimentation method (Tab. 1).

Air-dried whole soil samples were separated into three particle-size fractions: clay (<2 μm), silt (2–53 μm), and sand (53–2000 μm). For this purpose, 30 g soil samples were dispersed in a two-step procedure according to Amelung (1997) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26, Sonics and Materials Inc., Newton, CT, USA). To preserve particulate OM from disruption, the soil suspension was first sonicated at 60 J mL−1 while the temperature was kept constant at 20°C. After this soft ultrasonication, the >53 μm fraction was removed by wet sieving, and a second ultrasonication at 440 J mL−1 was used to disperse the silt- and clay-sized fraction. The probe energy supply was calibrated from the heating of water inside a dewar vessel (Sleutel et al., 2007b). The resulting soil suspension was separated into the clay- and silt-particle-size fractions by gravity sedimentation in water following Stoke’s law. Sedimentation was repeated up to 10 times, and ≈ 4 L suspension containing the clay fraction was collected. A 1 L subsample of the clay suspension was centrifuged at 16,900 g. Clay and silt fractions were dried at 50°C after decanting the clear supernatant. Particle-size fractionations were carried out in duplicates.

2.3 Chemical fractionation of soil organic matter

In search for a chemical fractionation procedure to isolate a stable SOM pool for process studies of SOM stabilization, Siregar et al. (2005) concluded that threefold sequential 6 h 6%-NaOCl treatments at pH 8 was most suitable for selective removal of OM, whilst minimally affecting the mineral soil constituents and hence mineral-associated OM. Mikutta et al. (2006) therefore further isolated a nonmineral bound fraction within the oxidation residue using HF acid extraction. HF reacts with silicates by forming soluble fluoride complexes. Dissolution of these minerals by HF treatment will lead to a release of mineral-bound OM into solution. We used this oxidation-extraction fractionation procedure to sequentially isolate (1) a stable SOM fraction composed of mineral-protected as well as biochemically recalcitrant presumed OM and (2) a nonbound biochemically recalcitrant presumed SOM fraction. Following Mikutta et al. (2006), the NaOCl+HF-res. OM can be assumed to constitute a recalcitrant SOM pool (NaOCl+HF-res. OC and NaOCl+HF-res. N) and the HF-extractable OM may coincide with a mineral-protected SOM pool (NaOCl-res.+HF-ext. OC and NaOCl-res.+HF-ext. N) (Fig. 1). A ball-milled 5 g soil sample was reacted three times for 6 h with 50 mL 6% NaOCl adjusted to pH 8.0 inside 65 mL nalgene centrifuge tubes. Samples were centrifuged and decanted in between oxidation cycles and were ultimately washed one time with 1M NaCl and three times with de-ionized H2O. After drying and weighing, a subsample was used for total C and N analysis. Next, 3 g of the oxidation residue were treated 4 times with 20 mL 10% HF in order to dissolve and remove mineral constituents and mineral-bound
Table 1: Selected properties of the heathland (H), cultivated-heathland (HC), and permanent-cropland (CC) soil groups. Below land-use group means ± standard error.

<table>
<thead>
<tr>
<th>Soil Location</th>
<th>Soil texture / %</th>
<th>OC / g kg⁻¹</th>
<th>N / g kg⁻¹</th>
<th>C : N</th>
<th>OCₘₑ</th>
<th>OCₐₚₑ</th>
<th>pH_KCl</th>
<th>Extractable Fe and Al / g kg⁻¹</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&gt;50 μm</td>
<td>2–50 μm</td>
<td>&lt;2 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fe₀</td>
</tr>
<tr>
<td>HC1 Beernem</td>
<td>92</td>
<td>3.2</td>
<td>4.8</td>
<td>17.3</td>
<td>1.3</td>
<td>14</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>HC2 Knesselare</td>
<td>81.9</td>
<td>11.6</td>
<td>6.5</td>
<td>20.6</td>
<td>1.6</td>
<td>13</td>
<td>0.15</td>
<td>0.26</td>
</tr>
<tr>
<td>CC1 Ledegem</td>
<td>81.2</td>
<td>10.3</td>
<td>8.5</td>
<td>8.3</td>
<td>0.9</td>
<td>9</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>CC2 Veurne</td>
<td>74.6</td>
<td>13.6</td>
<td>11.8</td>
<td>10.8</td>
<td>1.1</td>
<td>10</td>
<td>0.08</td>
<td>0.21</td>
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<td>ANOVA land use</td>
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<tr>
<td>H</td>
<td>88.9 ± 1.9</td>
<td>6.3 ± 1.3</td>
<td>4.8 ± 0.8a</td>
<td>22.8 ± 3.6a</td>
<td>1.0 ± 0.1a</td>
<td>22.6 ± 1.7a0.17 ± 0.03ab</td>
<td>0.49 ± 0.07</td>
<td>3.4 ± 0.1a</td>
</tr>
<tr>
<td>HC</td>
<td>88.7 ± 1.2</td>
<td>6.5 ± 0.9</td>
<td>4.9 ± 0.4a</td>
<td>22.7 ± 1.3a</td>
<td>1.6 ± 0.1b</td>
<td>14.5 ± 0.7b0.21 ± 0.03a</td>
<td>0.56 ± 0.13</td>
<td>5.0 ± 0.2b</td>
</tr>
<tr>
<td>CC</td>
<td>83.7 ± 2.9</td>
<td>8.5 ± 1.8</td>
<td>7.8 ± 1.3b</td>
<td>12.2 ± 1.2b</td>
<td>1.1 ± 0.1a</td>
<td>10.8 ± 0.7b0.09 ± 0.02b</td>
<td>0.24 ± 0.02</td>
<td>5.3 ± 0.4b</td>
</tr>
<tr>
<td>a Fe₀, Al₀ = Na dithionite citrate bicarbonate–extractable Fe and Al; Feᵤ, Alᵤ = Na pyrophosphate–extractable Fe and Al</td>
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<td>b * significantly different at the 5% significance level based on ANOVA F-test; N.S. not significantly different based on ANOVA F-test</td>
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<td>c a,b Means followed by a different letter are significantly different at 5% significance level according to Duncan’s multiple-range test.</td>
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</table>
OM. Extraction residues were washed five times with deionized H2O to remove salts and residual HF and were dried and weighed. Subsamples (200–800 mg) of the original samples, the NaOCl-oxidation, and the HF-extraction residues were analyzed for total C and N content by dry combustion with a Variomax CNS-analyzer (Elementar Analysysysteme, Germany). Chemical fractionations were carried out in duplicate.

Figure 1: Schematic of the applied chemical fractionation procedure for the isolation of NaOCl-oxidizable OM (i.e., presumed labile) from NaOCl-resistant but HF-extractable OC and N (i.e., presumed mineral-protected OM) and NaOCl- and HF-resistant OC and N (i.e., presumed recalcitrant OM).

2.4 Soil respiration, microbial biomass, and hot water–extractable OM

An amount of 200 g air-dried bulk soil was weighed into PVC tubes (Ø 7.5 cm). The soil columns were slightly compacted in a vertical plane with a plastic cylinder to obtain a bulk density of 1.3 g cm⁻³. After compaction, the water content was adjusted to 50% water-filled pore space by adding distilled water. The soils were incubated in duplicate at (20.5 ± 1)°C in airtight closed jars in two batches: one for 113 d and another for 135 d. Amounts of evolved CO₂, captured in NaOH, were regularly measured by titration of the NaOH with 1 M HCl to pH 8.3 in the presence of BaCl₂ (Anderson, 1982). After removal of the vials containing NaOH, the glass jars were left open for 2 h to allow replenishment of oxygen. Soil moisture content was adjusted, fresh vials containing NaOH were added, and the jars were sealed again to continue the C-mineralization measurements. A parallel first- and zero-order kinetic model, which assumes that SOM can be divided into two components, an easily decomposable fast pool and a more stable slow pool, was considered here. The parallel first- and zero-order kinetic model:

\[ C(t) = C_f (1 - e^{-k_f t}) + k_l \]  

was fitted to the C-mineralization data, expressed as g C mineralized per 100 g SOC, with \( C_f(t) \), the cumulative amount of substrate (i.e., carbon) mineralized at time \( t \) (d), \( C_f \) the size of the fast pool [in g C (100 g SOC)⁻¹], \( k_f \) the first-order C-mineralization rate constant of the fast SOM pool (d⁻¹), and \( k_l \) the zero-order C-mineralization rate constant of the slow SOM pool [in g C (100 g SOC)⁻¹ d⁻¹]. By means of the temperature-dependence model of De Neve et al. (1996), it was determined that a 1 y mineralization at 9.8°C (the long-term average year temperature in the sandy region) corresponds to a 137 d incubation under the specific lab conditions (i.e., at 20.5°C) in this experiment. Using this model, the relative annual SOC mineralization was calculated. Soil microbial biomass OC (OC\(_{\text{mic}}\)) was determined at the end of the incubation period with the fumigation-extraction method (Vance et al., 1997). K₂SO₄ extracts before and after fumigation were filtered through paper filters (Whatman 5). The K₂SO₄ extracts were analyzed for dissolved OC (TOC analyser, Shimadzu, Japan). Extraction with hot aqueous solutions was used to measure labile OC (OC\(_{\text{RWE}}\)) according to Leinweber et al. (1995). An amount of 10 g of dry bulk soil was weighed into nalgene centrifuge tubes. A volume of 50 mL of distilled water was added to the tubes, and the tubes were capped and left for 60 min in a hot-water bath at 100°C. At the end of the incubation period, the tubes were centrifuged for 20 min at 3500 rpm, and the supernatants were filtered through Whatman (GF/C) glass microfiber filters and stored in vials (Ghani et al., 2003). The extracts were analyzed for OC concentration (TOC analyser, Shimadzu, Japan).

2.5 Statistical analysis

All statistical tests were conducted with the SPSS 15.0 package (SPSS Inc., Chicago). The parallel first- and zero-order kinetic model was fitted to the mineralization data points by nonlinear curve fitting using the Levenberg–Marquardt algorithm. ANOVA (\( p = 0.05 \)) with Duncan’s multiple-range post hoc test was used to test differences in the SOC and N distributions over physical and chemical fractions, mineralization parameters, OC\(_{\text{RWE}}\), and OC\(_{\text{mic}}\) between the different land-use groups. Multivariate linear regression was conducted with the parallel first- and zero-order kinetic-model parameters as dependents and SOC and N distributions over physical and chemical fractions as independents using SPSS’s “stepwise linear regression” function. The successive selection of independents in the final regression model herein takes the form of a sequence of F-tests in which the independent variables are successively entered (if \( F < P\text{-in} = 0.05 \)) or removed (if \( F > P\text{-out} = 0.10 \)).

3 Results

3.1 General soil properties

Amongst the three land-use groups, there were no significant differences in physical soil properties, except for a slightly higher clay percentage of the permanent-cropland soils (Tab. 1). The Duncan multiple-range post-hoc test revealed significantly lower OC contents in the permanent-cropland soil group. Significantly higher contents of N and microbial biomass OC were measured in the cultivated-heathland soils than in the other two groups. Significantly higher C : N ratios and a lower pH were measured in the relict-heathland soils. Contents of Fe\(_{\text{p}}\), Fe\(_{\text{d}}\), Al\(_{\text{p}}\), and Al\(_{\text{d}}\) were low in all soils, and in general, the permanent-cropland soils contained more Fe...
and less Al than the relict and cultivated-heathland soils. The amounts of OC$_{\text{HWE}}$ and OC$_{\text{mic}}$ extracted from the investigated soils constituted on average 2%–3% and 0.7%–0.9% of the bulk SOC, respectively (Tab. 1). Both were significantly higher in the relict-heathland compared to the permanent-cropland soils, but when recalculated as a relative fraction of the bulk SOC they were equal.

### 3.2 Soil respiration

The observed CO$_2$ respiration from the incubated soils was recalculated into a relative OC mineralization expressed in g C (100 g SOC)$^{-1}$ (Fig. 2). The fitted parameters of the parallel first- and zero-order model are given in Tab. 2. An excellent model fit to the data was obtained ($R^2 \geq 0.99$) and, hence, the fitted models could be used to extrapolate the SOC mineralization to 137 d, i.e., equivalent to 1 y under field conditions.

ANOVA with Duncan’s multiple-range post-hoc test was used to compare the model parameters between the different land-use groups. Only small differences were observed in the mineralization rate of the fast OC pool (parameter $k_f$). The proportion of the labile or fast SOC pool $C_f$ followed the order permanent-cropland soils [average ± standard error: (0.28 ± 0.06) g C (100 g SOC)$^{-1}$] > cultivated-heathland soils [(0.14 ± 0.05) g C (100 g SOC)$^{-1}$] > relict-heathland [(0.09 ± 0.02) g C (100 g SOC)$^{-1}$] soils and was significantly different between the permanent-cropland and relict-heathland soil groups. There was a large and significant difference between $k_s$, the mineralization rate of the slow SOC pool, of the relict-heathland [(0.0089 ± 0.0023) g C (100 g SOC)$^{-1}$ d$^{-1}$] and cultivated-heathland [(0.0091 ± 0.0024) g C (100 g SOC)$^{-1}$ d$^{-1}$] soils on the one hand and the permanent-cropland soils [(0.0196 ± 0.0032) g C (100 g SOC)$^{-1}$ d$^{-1}$] on the other. The calculated annual mineralization [in g C (100 g SOC)$^{-1}$] followed the same course and was on average 2.2 times higher in the permanent-cropland soils compared to the heathland and cultivated-heathland soils.

### 3.3 Distribution of OC and N over isolated chemical fractions

The distribution of the OC and N over the obtained chemical fractions, expressed on a bulk soil OC and N basis [g OC (100 g SOC)$^{-1}$ and g N (100 g N)$^{-1}$], is given in Fig. 3. Briefly, the NaOCl oxidation removed (52 ± 4)% and (64 ± 4)% of the whole soil OC and N, respectively. This result matches the 49% NaOCl-resistant OC and 55% NaOCl-resistant N, measured by Kleber et al. (2005). Subsequent mineral dissolution by 10% HF reduced the sample dry matter by (40 ± 6) wt% (data not shown) and released (55 ± 3)% of the NaOCl-resistant OC, which is higher than the 38%–57% range of Helfrich et al. (2007). HF treatment released (67 ± 3)% of the NaOCl-resistant N. This potentially mineral-protected NaOCl-res.+HF-ext. OM pool held a larger proportion of the soil OC [(27 ± 7)%] and N [(25 ± 5)%] than the recalcitrant presumed NaOCl+HF-res. OC [(19 ± 39)%] and N [(11 ± 29)%].

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According to the ANOVA (Tab. 3), the permanent-cropland group of soils contained significantly more NaOCl-oxidizable OC and N than the heathland and cultivated-heathland soils. Differences in relative proportions of NaOCl-res.+HF-ext. OC and N and NaOCl+HF-res. OC and N between the relict-heathland and cultivated-heathland soils were relatively small and insignificant (Fig. 3). In contrast, significantly lower proportions of these fractions were measured in the permanent-cropland soils group than in the heathland and cultivated-heathland soils.

### 3.4 Distribution of OC and N over isolated size fractions

The distribution of OC and N over the obtained size fractions, expressed on a bulk-SOC and soil-N basis [g OC (100 g SOC)$^{-1}$ and g N (100 g N)$^{-1}$], is given in Fig. 4. In general, most of the SOC and N was present in the silt and clay soil fractions in spite of the sandy texture of these soils (average sand percentage is 88%). According to ANOVA, the proportion of OC in the clay-size fraction was significantly higher in the cultivated-heathland soils [(52 ± 3)% clay OC (means ± standard error)] compared to the heathland [(37 ± 4)% clay OC] and permanent-cropland [(38 ± 2)% clay OC] soils. For clay-size N, there was only a significant difference between the cultivated-heathland [(57 ± 3)% clay N] and heathland [(43 ± 39)% clay N] soils. No significant differences were found in the proportions of the silt-size fraction OC and N between the different land-use groups. Consequently on the other hand, significantly more OC and N was present in the sand-size fraction of the heathland soils [(38 ± 4)% sand OC, (36 ± 4)% sand N] compared to the cultivated-heathland [(21 ± 1)% sand OC, (19 ± 1)% sand N] and permanent-cropland [(30 ± 4)% sand OC, (22 ± 5)% sand N] soils.

### 4 Discussion

The sampled cropland soils from the sandy region in N Belgium had unexpectedly very high SOC contents [on average (22.7 ± 3.7) g OC kg$^{-1}$]. Soil-organic-carbon levels of $\approx$ 10 g OC kg$^{-1}$ or less may be expected for sandy cropland soils in NW Germany under steady state (Körschens et al., 1998; Springob and Kirchmann, 2002), which is very similar to OC

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**Table 2**: Mean C-mineralization parameters (± standard error) of a parallel first- and zero-order kinetic model and the calculated annual relative C mineralization of the heathland (H), cultivated-heathland (HC), and permanent-cropland (CC) soil groups.

<table>
<thead>
<tr>
<th>Land-use group</th>
<th>Fitted kinetic-model parameters</th>
<th>Annual SOC mineralization</th>
<th>ANOVA land use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{f}$/ g C (100 g OC)$^{-1}$</td>
<td>$k_{f}$/ d$^{-1}$</td>
<td>$k_{s}$/ g C (100 g OC)$^{-1}$ d$^{-1}$</td>
</tr>
<tr>
<td>Heathland (H)</td>
<td>0.094 ± 0.020a $^b$</td>
<td>0.043 ± 0.011</td>
<td>0.009 ± 0.002a $^a$</td>
</tr>
<tr>
<td>Cultivated heathland (HC)</td>
<td>0.142 ± 0.045a$^a$</td>
<td>0.079 ± 0.023</td>
<td>0.009 ± 0.002a</td>
</tr>
<tr>
<td>Permanent cropland (CC)</td>
<td>0.279 ± 0.058b$^b$</td>
<td>0.068 ± 0.009</td>
<td>0.020 ± 0.003b</td>
</tr>
</tbody>
</table>

$^a$ *significantly different at the 5% significance level based on ANOVA F-test; N.S. not significantly different based on ANOVA F-test

$^b$ a,b Means followed by a different letter are significantly different at 5% significance level according to Duncan’s multiple-range test.
contents in the group of permanent cropland soils [(12.2 ± 2.7) g OC kg⁻¹]. The small C : N ratios (close to 10) of the permanent-cropland group were similar to C : N ratios of cropland soils in long-term field experiments (Helfrich et al., 2007; Springob and Kirchmann, 2002; Sleutel et al., 2006) under similar management. The C : N ratios of the soils in the heathland land-use group, however, was much larger. The N content of the cultivated-heathland soils was found to be significantly higher than in the heathland soils. Abundant additions of mineral-N fertilizers and animal slurries may well explain the higher proportions of N in these cropland soils. Still, several of the cultivated-heathland soils had maintained rather large C : N ratios, even after six decades of cultivation and N fertilization. Springob and Kirchmann (2003) as well reported that former heathland sites conserved such wide C : N ratios and high OC levels over decades after cultivation. Heumann et al. (2003) found that land-use history had a determinant impact on C : N ratio and N mineralization in sandy soils in Germany.

Our results suggest that the SOC formed during the historical period of heathland land use was not mineralized to a substantial extent and also points to accumulation of N during several decades of intensive agricultural production. The high OC contents of soils in the heathland and cultivated-heathland land-use groups compared to the permanent-cropland soils group may be explained by different mechanisms.

A first pathway would be enrichment in unstable, decomposable OM as a result of continued large input of fresh OM. This would, however, contradict the significantly slower C mineralization (i.e., parameter $k_e$) in the heathland and cultivated-heathland soils when compared to the permanent-cropland soils. Hence, as indicated by the significantly higher $C_i$ parameter in the permanent-cropland soil group, the latter held a larger share of labile SOM to the total SOM. While these parameters may not be realistic estimates of truly biologic quantities, the results clearly demonstrate the cultivated heathland and heathland soils to contain a biologically less labile SOM than the permanent-cropland soils. Furthermore, there is no reason to assume a larger input of fresh OM from crop residues or organic fertilization in the heathland and cultivated-heathland soils than in the set of permanent-cropland soils. This is confirmed by a regional study by Sleutel et al. (2007a) which shows that the average application of N from animal manure is equal in sandy areas with and without historical heathland land use.

Hence, the relatively larger OC content of sandy cropland soils appears to be related to the quality of SOM in these soils itself or to a stabilization of mineral-protected OM. Further insight in SOM quality was derived from the chemical fractionation. A first observation is that the proportions of NaOCl-res.+HF-ext. OC and N were very similar between the heathland and cultivated-heathland soils in spite of differences in their bulk soil OC and N contents (Tab. 3). In contrast, these proportions were three times smaller in the permanent-cropland soils. Next, there were also strong similarities in the proportion of NaOCl+HF-res. OC and N between the cultivated-heathland and heathland soils. The proportion of NaOCl+HF-res. OC was equal amongst all land-use groups and constituted on average 19% of the SOC. Körschens et al. (1998) considered the OC content of unfertilized control plots of 21 long-term European field experiments as a criterion for inert OC. These authors found a strong positive relation between the topsoil OC content in such control plots and the clay percentage. If we apply this relation to the soils in this study, with an average clay percentage of 5.6%, they would contain $\approx 7$ g inert OC kg⁻¹. In the heathland and cultivated-heathland soils $\approx 4.6$ g NaOCl-res.+HF-res. OC kg⁻¹ was measured, which, in spite of methodological differences, compares to this estimate.

As discussed by Sleutel et al. (2008), because of the low content of both crystalline and poorly crystalline Fe and Al (Tab. 1), binding between the latter and SOM plays only a limited role in SOM stabilization in these soils. Indeed, there was no significant correlation between the relative proportion of NaOCl-res.+HF-ext. OC or N and the clay%, $Fe\text{,} Al\text{,} Fe\text{DCB,}$ or $Al\text{DCB}$ for any of the land-use groups. Rather, the SOM in the HF-extractable pool might therefore be interpreted to simply be SOM with a low solubility and reactivity in NaOCl. This would suggest that mineral binding does not contribute significantly to SOM stabilization in the cultivated-heathland soils. Indeed, unexpected high silt+clay OC loadings were observed in these soils, as demonstrated by comparison to the formula for texture-preserved OC after Six et al. (2002),

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Table 3: Mean relative content (± standard error) of NaOCl-oxidizable OC and N, NaOCl-res.+HF-ext. OC and N, and NaOCl+HF-res. OC and N of the heathland, cultivated-heathland, and permanent-cropland soil groups.

<table>
<thead>
<tr>
<th>Land-use group</th>
<th>NaOCl-oxidizable OM</th>
<th>NaOCl-resistant OM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OC (% of SOC)</td>
<td>N (% of N)</td>
</tr>
<tr>
<td>Heathland (H)</td>
<td>49.5 ± 7.2a b</td>
<td>55.7 ± 6.1a</td>
</tr>
<tr>
<td>Cultivated heathland (HC)</td>
<td>46.7 ± 4.0a</td>
<td>59.3 ± 5.1a</td>
</tr>
<tr>
<td>Permanent cropland (CC)</td>
<td>71.2 ± 4.6b</td>
<td>86.25 ± 3.1b</td>
</tr>
<tr>
<td>ANOVA land use</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

a *significantly different at the 5% significance level based on ANOVA F-test; N.S. not significantly different based on ANOVA F-test
b a,b Means followed by a different letter in each SOM fraction are significantly different at 5% significance level according to Duncan’s multiple-range test.
namely silt and clay OC (in g C kg\(^{-1}\)) = 7.18 + 0.2 (% sum of particles < 50 \(\mu m\)) (Fig. 5). However, there was a significant correlation \((p = 0.05)\) between the relative proportion of NaOCl-res.+HF-ext. OC and the clay% for the heathland \((R = 0.785)\) and permanent-cropland \((R = 0.993)\) groups. Since treatment by 10% HF is assumed to extract mineral-bound OC, any correlation of the proportion of residual non-bound OM fraction with parameters indicative of reactive mineral surface such as content of clay and pedogenic oxides seems contradictory. Possibly, this would suggest that the removal of clay minerals and clay-associated OM by the 10%-HF treatment was incomplete. This would also suggest that this chemical fractionation was unable to exclusively isolate mineral-bound OM from recalcitrant OM as also indicated by significant positive correlations between the amounts of NaOCl-res.+HF-ext. OC and N and NaOCl+HF-res. OC and N. Possibly, the large loading of SOM on the clay particles may have impeded contact between HF and the mineral surface, as was also suggested before by Eusterhues et al. (2007). As such, the applied chemical fractionation procedure may be less suitable for these OC-rich sandy soils. Nonetheless, despite the limited success of the applied chemical fractionation for separating mineral-protected from recalcitrant OM, clearly the very large differences in the proportions of NaOCl-res.+HF-ext. OC and N and NaOCl+HF-res. N still reflect a considerable difference in SOM quality between the heathland and cultivated-heathland soils on the one hand and the permanent-cropland soils on the other. In a stepwise linear regression between the relative annual OC mineralization \([in \text{ g C (100 g SOC)}^{-1}\]) (Tab. 2) and the proportions of OC of all isolated fractions, the sand, silt, and clay percentages and Fe and Al contents, the proportion NaOCl-res.+HF-ext. OC \([in \text{ g C (100 g OC)}^{-1}]\) was the only retained predictor along with a significant intercept and the SOC content (in g OC kg\(^{-1}\)):

\[
\text{Annual mineralization} = 4.347 - 0.087 \text{SOC} - 0.032\%	ext{NaOCl-res.+HF-ext}. \text{OC} \quad (R^2 = 0.65) \quad (2)
\]

A similar result was obtained with \(k_s\) \([in \text{ g C (100 g SOC)}^{-1} \text{d}^{-1}]\) as the dependent:

\[
k_s = 0.02935 - 0.00058 \text{SOC} - 0.00022\%	ext{NaOCl-res.+HF-ext}. \text{OC} \quad (R^2 = 0.60) \quad (3)
\]
Hence, as a direct measure for SOM stability, the relative amount of OC that is mineralized in 1 y under field conditions or the mineralization rate of a slow SOC pool, can be estimated from the SOC content and the proportion of the NaOCl-res.+HF-ext. OC pool through chemical fractionation. The negative relation between SOC content and relative C mineralization may be explained by a relative lower OM degradability of the SOC-rich heathlands and cultivated-heathland soils compared to the reference soils. The fact that SOC content was retained rather than other variables out of this stepwise regression, Designates them inadequate for predicting OC degradability of the considered soil set. In contrast, only poor relationships were established between $k_f$ and $C_t$ and the proportions of the isolated OC fractions, pH, and textural data. It thus seems that none of these variables, including $OC_{mic} - OC_{PWE}$ and sand-sized OC, may be useful to predict the labile SOC fraction in these soils. This outcome contrasts with other researches (Janzen et al., 1992; Kadono et al., 2008; Paul et al., 2004) and seems to be specific for these OC-rich sandy soils in NW Europe. Incubation experiments seem therefore the only reliable means for quantifying the labile OC pool here.

Even more so than for OC, Differences in N fractions between the land-use groups were very pronounced with a much smaller share of N attributed to the stable NaOCl-res.+HF-ext. N and NaOCl+HF-res. N pools for the permanent-cropland group. From a series of lab incubations on sandy arable soils in central Germany, Heumann et al. (2003) as well found that the pool size of slowly mineralizable soil N depended very strongly on former land use. A higher N content of the cultivated-heathland soils compared to the permanent heathlands can be readily explained by the long-term N input from animal slurry and mineral-N fertilizer after cultivation. However, a similar management for the permanent-cropland group did not result in the relative accumulation of stable N but instead a very large proportion of the soil N was oxidizable by NaOCl. At this stage, we can only speculate on possible pathways for stabilization of N in soils with a heathland history, but one could involve the active immobilization of N under heather vegetation. The horizons of forest and heathland soils in which mycorrhizal roots proliferate typically contain large quantities of polyphenolic materials (tannins, humic and fulvic acids, melamins and quinones). These have the potential to bind organic N including protein, peptides, amino acids, chitin, and nucleic acids, in recalcitrant complexes (Bending and Read, 1996). Kristensen and Mc Cartney (1999) stated that the ability to rapidly immobilize large amounts of N could play a major role in the competition between Calluna and grass species because ericaceous vegetation would be favored by the maintenance of low N availability. This pool of organically bound N appears to still be present in the cropland soils even after several decades of intense cropland management.

5 Conclusions

A series of lab incubations and chemical fractionation confirmed both the similarity in SOM stability of relict and cultivated heathlands on the one hand and the lower stability of SOM in cropland soils without this historical land use on the other. In accordance with the few previous studies on this issue, these results stress the importance of knowledge of and accounting for the often neglected historical land use in research on SOM stability. To date, no single biological, physical, or chemical fractionation technique has been developed which adequately describes the stabilization of SOM. In this study, however, the proportion of a presumed mineral-protected pool isolated through subsequent oxidation in NaOCl and HF extraction was negatively related to the “mineralizability” of OC and by extension possibly to SOM stability for a range of sandy soils with differing historical land use. Such a mathematical relation between a particular SOC
fraction and “mineralizability” is most likely applicable only to specific combinations of soil, current land use and climate. Still, this outcome demonstrates a modest potential for development of SOC models that comprise combined biologically and chemically measurable OC pools, but only for specific combinations of soil with current land use and climate.

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