

Clay-associated organic matter  
in kaolinitic and smectitic soils

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# Clay-associated organic matter in kaolinitic and smectitic soils

E.J.W. Wattel-Koekkoek

## **Proefschrift**

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## Stellingen

1. Hoe meer lading en oppervlak een kleimineraal heeft, des te groter de gemiddelde verblijftijd van de organische stof die ermee geassocieerd is.
2. Organic matter(s)!
3. Water is niet alleen H<sub>2</sub>O, het is hoogstens ook H<sub>2</sub>O.  
*A. van den Beukel. De dingen hebben hun geheim. Gedachten over God, natuurkunde en de mens.*
4. Als tolerantie tot onverschilligheid wordt, is dit bederf van het beste. Onverschilligheid wordt gekenmerkt door wanhoop aan waarheid en rede, door isolement van het zelf, door verwaarlozing van de ander, en leidt om al deze redenen tot uitholling en ondermijning van samenleving en staat.  
*naar: A.A. van Ruler. Theologisch werk I.*
5. De enorme populariteit van de Mattheüs Passion van J.S. Bach is in deze tijd van toenemende secularisering op z'n minst wonderlijk te noemen.
6. Een orkest zonder celli is als een boom zonder bodem.
7. Het meest leerzame onderdeel van congressen en symposia is de pauze tussen de lezingen.
8. Het succes van de romancyclus "Het Bureau" van J.J. Voskuil is hieruit verklaarbaar, dat het lezen ervan voor ambtenaren en wetenschappers één groot feest der herkenning is.

Stellingen behorend bij het proefschrift "Clay-associated organic matter in kaolinitic and smectitic soils"

Esther Wattel-Koekkoek

***“But we have this treasure in jars of clay”***  
*The Bible, 2 Corinthians 4:7*

*aan Koen*

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## **Chapter 1**

### **General introduction**

## Soil organic matter

The primary source of soil organic matter is plant debris of all kinds, such as roots, dead leaves and branches that enter into the soil and are then biologically decomposed at variable rates (Duchaufour, 1977). Soil organic matter consist of a number of organic components of which the main are polysaccharides, proteins, (poly)phenols, lignin, lipids, aliphatic polymers and the decomposition products thereof. Classical studies on soil organic matter differentiate between *humic* and *non-humic substances*. *Humic substances* or *humus* is defined as "decomposed plant material that has been transformed to dark-colored partly aromatic, acidic, hydrophilic, molecularly flexible polyelectrolyte materials" (Van Breemen and Buurman, 1998), or "considerably altered amorphous organic matter" (Duchaufour, 1977). However, in reality a continuum exists between non-humified and humified fractions and therefore I prefer to speak of *soil organic matter* as a whole.

## Functions of soil organic matter

The importance of organic matter research becomes clear when considering the different functions soil organic matter has on a global and local scale.

### *Global carbon cycle*

The earth contains about  $8 \times 10^{22}$  g of carbon. All but a small portion is buried in sedimentary rocks, where it is found in organic compounds (20%) and carbonate (80%). Of all organic carbon present, only  $40 \times 10^{18}$  g C actively participates in the carbon cycle. Active organic carbon can be divided into three pools: C in oceans, in terrestrial plants, and in soils (Table 1.1). Although soil organic matter (SOM) forms a negligible pool as a carbon reservoir, it does play an essential role in the global carbon cycle. Because of the large flux of carbon going into the atmosphere when soil organic matter is decomposed, SOM is major source/sink for atmospheric carbon.

Table 1.1 Source, pool-size, and annual flux to atmosphere (Schlesinger, 1997)

Active C Pools ( $10^{15}$ g)		Fluxes with respect to atmosphere ( $10^{15}$ g yr <sup>-1</sup> )	
<i>Organic C</i>		Plant to air	+ 60
Soils	1500	Air to plant	- 120
Land plants	560	Soil to air	+ 60
Ocean	38 000	Ocean to air	+ 90
		Air to ocean	- 92
		Vegetation destruction	+ 1
		Burning of fuels	+ 6
<i>Inorganic C</i>			
Atmosphere	750		

### *Local soil properties*

Soil organic matter is an important source of plant nutrients. When microbes mineralize organic matter, CO<sub>2</sub> and nutrients such as N, P, S, and Ca are released. Furthermore, SOM increases the capacity to adsorb water. It also increases the structural stability of a soil e.g. by forming aggregates with mineral components. Furthermore, it contains reactive carbonyl, carboxyl, and hydroxyl groups, which influence the total cation exchange capacity of a soil (Zech *et al.*, 1997).

### Organic matter content

The amount of organic matter in a soil is a function of production (litter input) and decomposition. By relating soil carbon stocks with selected parameters using correlation analysis (Jenny, 1930 and 1941; Loomis and Connor, 1992; Scott *et al.*, 1996; Sollins *et al.*, 1996; Zech *et al.*, 1997 and references cited therein), several factors have been identified that affect organic matter contents. The main factors are:

- *climate: temperature and precipitation.* SOM content increases in a diminishing-returns relationship with rainfall and declines in a negative exponential relationship with increasing temperature.
- *pH of the soil.* Under acidic circumstances microbial activity is relatively low, and thus the C stock increases with decreasing pH.
- *nutrient (N, P) status.* Within an ecosystem, total soil P and N stocks show a positive correlation with total C stock.
- *soil moisture content.* See oxygen availability.
- *oxygen availability.* In an anaerobic environment C accumulates because most microbes need oxygen while decomposing SOM.
- *amount and quality of litter.* Components such as aromatics and aliphatic biopolymers are relatively recalcitrant towards decomposition. Presence of plant species with high natural contents of these recalcitrant molecules may result in a relatively high amount of SOM.
- *texture/clay content.* In general, soils with a higher clay content also have a higher SOM content. Clay can help form aggregates in which SOM is protected from microbial decomposition, and clay can chemically bind SOM.

### Decomposition models

Several models have been constructed to predict organic matter dynamics (e.g. Parton *et al.*, 1987; Verberne *et al.*, 1990; Coleman and Jenkinson, 1996; Smith *et al.*, 1997; Falloon and Smith, 2000). They can be divided in empirical and mechanistic models. Most models are empirical in nature and contain a pool with a fast turnover and a pool with slow turnover. Factors that are used as input parameters in such models are water availability, temperature, pH, clay content/texture, soil N status, O<sub>2</sub> availability, biomass, tillage factors, crop cover/growth period, quality and quantity of the litter residue (Falloon and Smith, 2000). One of the major limitations of these models is that the pools are based on theoretical entities rather than physically or chemically separable SOM fractions (Christensen, 2000).

An example of a mechanistic model is the physical protection model by Hassink (1995), further elaborated by Hassink and Whitmore (1997). Hassink (1995) found that the physical capacity of a soil to preserve SOM is limited. Hassink and Whitmore (1997) developed a model in which the net rate of decomposition of SOM is related to the degree to which the (limited) protective capacity is already occupied.

Neither in the mentioned studies on factors that affect organic matter content, nor in modeling studies, the effect of clay *mineralogy* on organic matter decomposition was taken into account. Some models do use clay *content* as input variable, and mostly this parameter is used to calculate

the size of the passive pool, which size increases with the amount of clay in a soil. However, the effect of clay mineralogy so far remained an unexplored area.

### Objective

My objective is to study the long-term effect of different clay minerals on the dynamics of SOM in natural ecosystems. I chose kaolinite and smectite because 1) they have very different characteristics which will be discussed below, 2) they are characteristic for different major soil types in the world, and 3) soils can be found which clay-size fractions contain these minerals in almost pure form.

### Kaolinite and smectite

Smectites are expandible 2:1 layer silicate minerals (Figure 1.1). The individual layers of smectite crystallites are composed of two tetrahedral silicon-oxide sheets sandwiching one octahedral aluminum-hydroxide sheet. Smectites have a high permanent surface charge, a large surface area, and a high cation exchange capacity (CEC). Kaolinites (Figure 1.2) are 1:1 layer structured aluminosilicates with a low surface area and a low CEC (Dixon and Weed, 1989).

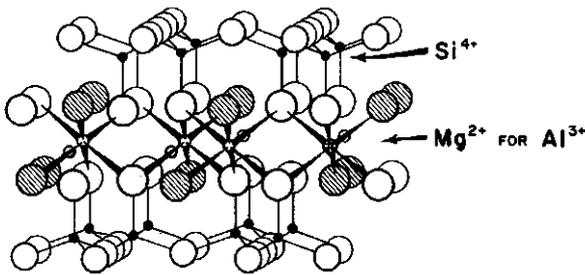
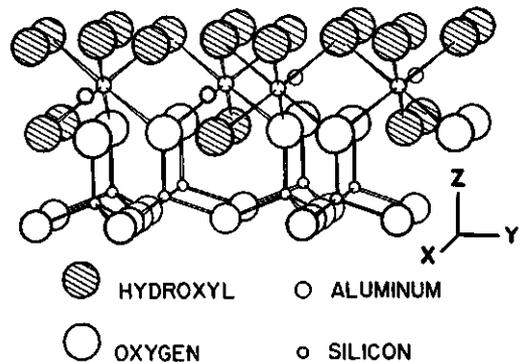


Figure 1.1 Crystal structure of smectite (Dixon and Weed, 1989).

Figure 1.2 Crystal structure of kaolinite (Dixon and Weed, 1989).



The reactivity of clay minerals can be described on the basis of their active sites. These active sites, or surface functional groups, are defined by the geometrical arrangement of surface atoms and by their chemical composition, and can participate in different types of binding mechanisms. They are, in a somewhat artificial categorization (Mortland, 1970; Tate and Theng, 1980; Sposito, 1984; Johnston, 1996):

1. **siloxane ditrigonal cavity.** The siloxane surface of a tetrahedral silica sheet consists of a layer of oxygen atoms arranged in a network of ditrigonal (hexagonal) cavities. The cavities have a diameter of about 0.26 nm. If there are no isomorphic cation substitutions to create deficits of positive charge (e.g. in kaolinite or in talc, not in smectites), the ditrigonal cavity functions as a very soft Lewis base (electron donor) and is likely to complex only neutral dipolar molecules, such as water molecules. These *hydrogen bonds* are not very stable, and the overall contribution of the neutral siloxane surface to SOM complexation is minimal due to the low specific surface area (SSA) of the minerals that have such a surface.
2. **isomorphic substitution sites.** Isomorphic substitutions result in a permanent negative charge on the basal surfaces of 2:1 layer silicates (e.g. smectites).
  - a. If isomorphic substitution of  $\text{Al}^{3+}$  by  $\text{Fe}^{2+}$  or  $\text{Mg}^{2+}$  occurs in the octahedral sheet, the resulting excess negative charge can distribute itself over the surface oxygen atoms of the silica tetrahedra. This distribution of negative charge enhances the Lewis base character of the ditrigonal cavity. The charge deficit is compensated for by the presence of exchangeable cations, in natural systems mainly Ca, Mg, Na and K. These mineral cations in turn can be replaced by organic cations such as quarternized nitrogen atoms in alkyl and aryl amines via *cation exchange*. The Lewis base character of the cavity also makes it possible to form complexes with dipolar molecules via *hydrogen bonds*.
  - b. If substitution of  $\text{Si}^{4+}$  by  $\text{Al}^{3+}$  occurs in the tetrahedral sheet, the excess negative charge can distribute itself primarily over just the three surface oxygen atoms of one tetrahedron, allowing similar, but much stronger complexes as under a) with cations (e.g.  $\text{K}^+$  in vermiculite) and dipolar molecules.
3. **exchangeable cations near the surface.** The exchangeable cations can form *cation bridges* between the negatively charged clay surface and an anionic or polar organic groups such as carboxylate, amines, carbonyl and alcoholic OH.
4. **polarized water molecules surrounding the exchangeable cations.** Water molecules near exchangeable (mainly polyvalent) cations can become polarized, resulting in the formation of a Lewis acid. Amino, carboxylate, carbonyl, and alcoholic OH functional groups of organic compounds can form bonds with the polarized hydration water through *water bridging*. Other possible binding mechanisms are *ligand exchange* and *protonation*. The last involves donation of protons by the mineral surface to organic basic molecules, so that these become cationic. A source of reactive protons may be the hydrolysis of water molecules associated with exchangeable cations.
5. **surface hydroxyl group.** The most abundant and most reactive surface functional group in soil clays is the hydroxyl group exposed on the outer periphery of a mineral (kaolinite and

smectite). Especially on the edges, exposed aluminol and silanol groups can become Lewis acid sites by coordinating to a water molecule. These groups can complex amines, heterocyclic N, carbonyl, and carboxyl through *protonation*, especially at low pH. Furthermore, this group can form an inner-sphere complex with a carboxylate group via *ligand exchange*. For clays with little or no isomorphous substitution (e.g. kaolinites), these pH-dependent sites are the principal source of reactivity.

6. **hydrophobic sites.** Sorption of organic molecules on clay surfaces can impart a hydrophobic nature to the clay surface. Perhaps the most common example is the exchange of alkyl ammonium cations for inorganic cations on montmorillonite. The presence of the organic cations creates a hydrophobic surface, to which other non-polar organic compounds can be bound through *Van der Waals forces*.
7. **microtopography** of the surface: broken edges, depressions, intercalation, grooves, defects, pores, etc.

Although the mechanisms described above are difficult to relate to a field situation as they cannot be measured directly, they are a useful tool in understanding the basic concepts of organo-mineral interactions. Here I use them to show possible differences between kaolinite and smectite in organic matter binding.

Kaolinite has one outwardly exposed aluminum-hydroxide sheet, while smectite does not. Furthermore, kaolinite-dominated soils nearly always contain poly-oxyhydrates of iron. In the pH range of most soils, aluminum and iron hydroxide layers are positively charged, enabling kaolinite-dominated soils to complex organic matter through protonation and ligand exchange (see no. 2 above).

Smectite has two outwardly exposed silicon-oxide sheets. Depending on the level and location of isomorphous substitution, the exposed siloxane sheets can participate in hydrogen bonding (no. 1). Parts of the siloxane sheets are inert. Because smectite has a large specific surface area, it can form many hydrophobic (Van der Waals) bonds with a-polar organic compounds, such as aromatic and alkyl carbon. Furthermore, smectite can form many cation- and water-bridges due to the presence of a large number of polyvalent exchangeable cations at the clay surface (no. 4 and 5). Finally, because smectite is an expandable clay mineral, some organic molecules can be intercalated in its interlayers (Theng *et al.*, 1986) (see no. 7).

Considering the above described differences in specific surface area and reactive sites, it is likely that kaolinite and smectite will influence organic matter binding and decomposition differently and therefore form interesting research objects.

To study differences in kaolinite- and smectite-associated organic matter, I focused on four aspects of organic matter: the **amount**, **extractability** (as a measure of the binding mechanism), **chemical composition**, and the **mean residence time** of SOM associated with/bound to kaolinite and smectite. In order to be able to study these aspects, the soils involved were first fractionated physically to separate clay minerals plus associated SOM. Below, I will first describe this physical fractionation process, and the soil samples that were used for this study.

Thereafter, I will for each of the four aspects, briefly discuss previous relevant research performed by other scientists, I will formulate a hypothesis based on the theoretical concepts discussed above and/or existing literature, and I will describe the methodology that I chose to test the hypothesis.

### **Physical fractionation**

Soil organic matter can be physically fractionated on basis of size and density. Size fractionation yields micro- and macro aggregates (composed of primary soil particles held together) and primary soil particles (clay, silt, sand). The basic structural units are considered to be micro-aggregates. They protect organic matter against microbial degradation (Christensen, 1996). To obtain primary particles, soils are usually dispersed ultrasonically (Elliott and Cambardella, 1991; Christensen 1992, 1996). Excessive sonication can however produce undesirable artifacts (Morra *et al.*, 1991).

Density fractionation yields a light and a heavy fraction; the light fraction consists largely of non- or partially decomposed plant residues that are not associated with soil minerals. The heavy fraction includes the mineral complexed SOM. During the last decade, aqueous solutions of inorganic salts such as sodium iodide, and sodium-polytungstate have been used progressively (Turchenek and Oades, 1979; Elliott and Cambardella, 1991; Christensen 1992, 1996).

As most clay minerals are present in the clay-size fraction, I first separated the clay-size fraction of each soil (chapters 2-5). All organic matter present in the clay-size fraction, is referred to in this thesis as *clay-associated* SOM

In addition, in the experiments described in chapters 4 and 5, sodium iodide was used to separate the free organic matter in the clay-size fractions from the mineral-complexed SOM, also referred to in this thesis as *clay-bound* SOM.

### **Soil samples**

For this study, two sets of soil samples were used. The first set was assembled from the International Soil Reference and Information Center (ISRIC, Wageningen, The Netherlands). It contained 12 soils from seven different countries: Brazil, Mali, Kenya, Mozambique, Nicaragua, Indonesia, and South Africa. Half of the soils had clay-size fractions dominated by kaolinite, the other half were dominated by smectite. The second set of samples was collected in April 1998 by Peter Buurman and myself west of Montepuez, Mozambique. It contained 10 soils, four of which clay-size fractions were dominated by smectite, and six by kaolinite. All soils used were under native savanna vegetation.

### **Amount**

Previous studies show interesting trends regarding the carbon content of clay-size fractions that support the importance of clay. In general, fine textured soils have a higher organic C and N content than coarse textured soils when supplied with similar input of organic material. The difference is assumed to result from the greater physical protection of soil organic matter in fine-textured soils (Christensen, 1992).

It has also been observed that the C content of clay and silt fractions are much higher in sandy soils than in loams and clays (Christensen, 1992; Hassink *et al.*, 1995). It has been

suggested that this is due to the fact that in sandy soils clay and silt particles are mainly present as individual particles, while in loams and clays the clay and silt particles are coagulated (Hassink *et al.*, 1995).

Schulten and Leinweber (2000) gathered from literature the carbon contents of clay-size fractions dominated by various clay minerals. They concluded that clay-size fractions rich in kaolinite often have small C contents (1-6%), while smectite-rich fractions contain C contents within a wide range (2-17%), with the highest contents in soils poor in clay. However, the history and land-use of the soils described is not stated and therefore it is difficult to generalize these conclusions.

As the literature on the amount of carbon associated with different clay minerals is very limited (and for that reason an interesting area to explore), I will formulate my hypothesis on the basis of the theoretical concepts described before. They indicate that smectites have a larger surface area available to bind organic matter and a larger variety of active sites and binding mechanisms than kaolinites. Therefore, *I hypothesize that smectite can bind a larger amount of carbon than kaolinite*. This hypothesis was tested by measurement of the carbon content of the clay-size fractions with a C/N analyzer.

### **Extractability**

In this study, chemical extractants have been used for two purposes. First, assuming that different chemical extractants dissolve different types of bonds, I used different extractants as a measure for the type of binding mechanisms between the clays and the SOM. Binding mechanisms cannot be measured directly, therefore I chose this indirect method. Second, in soils organic and inorganic constituents are often closely associated so that it is necessary to separate them before each can be examined in greater detail (see 'chemical composition'). This separation can be accomplished by extracting the SOM from the inorganic components (Schnitzer and Schuppli, 1989).

The classical and still most widely used approach to extract SOM, is based on the solubility of humus in water at varying pH's. Humin is insoluble in base (pH 13). The remaining soluble part is subjected to pH 2 to separate material insoluble in acid (humic acid) and material soluble in acid (fulvic acid) (Beyer, 1996; Nierop, 1999). Another widely employed extractant is pyrophosphate (at various pH's), which forms complexes with the polyvalent cations (which keep the humic molecules flocculated) and thus solubilizes humus through ligand exchange (Schnitzer and Schuppli, 1989; Piccolo, 1990). NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> yield high quantities of humic materials, but coextract a large amount of mineral contaminants that can be eliminated by a HCl/HF purification treatment. Other extractants frequently used are organic solvents such as *n*-hexane (Choudri and Stevenson, 1957; Schnitzer *et al.*, 1988; Schnitzer and Schuppli, 1989). These can be used to extract hydrophobic components such as lipids.

On basis of the different characteristics of the clays described before, I hypothesize that kaolinite will bind organic matter relatively weakly via its surface hydroxyl groups, and that smectite will bind organic matter relatively strongly via its exchangeable cations. This hypothesis was tested indirectly applying a sequential extraction method using NaOH and thereafter  $\text{Na}_4\text{P}_2\text{O}_7$  (Figure 1.3). I expected that smectite-bound organic matter would not dissolve in NaOH, but rather in  $\text{Na}_4\text{P}_2\text{O}_7$  as  $\text{Na}_4\text{P}_2\text{O}_7$  can attack SOM bound via exchangeable cations, and that kaolinite-bound SOM would dissolve in NaOH, which can deprotonate the Lewis acid sites of the hydroxyl groups (Choudri and Stevenson, 1957; Schnitzer and Schuppli, 1989).

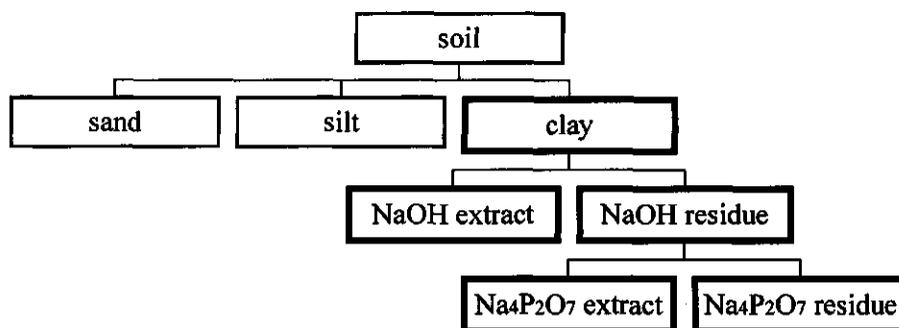


Figure 1.3 Fractionation scheme.

I also expected smectite to form relatively many hydrophobic bonds. The organic matter bound directly to the clay surface, can impart a hydrophobic nature to the clay surface (e.g. organic cations with non-polar 'tails'), enabling to complex non-polar organic compounds. However, I could not test this because all non-polar solvents are organic of nature and would therefore interfere with  $^{14}\text{C}$  measurements described below.

### Chemical composition

In the past thirty years, the possibilities to analyze SOM at a detailed molecular level have increased tremendously through the development of methods like  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$  NMR), pyrolysis gas chromatography mass spectrometry (Py-GC/MS) and Fourier Transform IR spectroscopy (FTIR) (Schnitzer and Schulten, 1995; Kögel-Knabner, 1997; Schulten and Leinweber, 2000). Consequently, the amount of literature on the chemical composition of SOM in natural soils has increased tremendously. However, most studies are not useful for this research as the authors fail to describe the mineralogy of the soil samples used. Studies that do mention the soil's clay mineralogy, mainly concern smectitic soils.

Theng *et al.* (1986) studied SOM with NMR in a micas-beidelite (smectite-type) spodosol and found that the clay-size fraction was very rich in polymethylene. They suggest 2:1 minerals can intercalate aliphatic chains. Arai *et al* (1996) analyzed the SOM of a combined NaOH/

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract of a Vertisol, and contrary to Theng *et al.*, they found that the NMR spectrum was dominated by aromatic carbon. Also, Leinweber *et al.* (1999) analyzed SOM in Vertisols with Py-GC/MS and found that the most abundant fragments were aromatics such as benzene, phenol, naphthalene. Furthermore, some pyridine, pyrroles, and aromatic nitriles were present. However, part of the aromatic carbon may also originate from charcoal (Skjemstad *et al.*, 1996). Altogether existing literature shows contrasting results that need further study.

The basic concepts on reactive sites mentioned earlier, indicate that although reaction mechanisms may vary between kaolinite and smectite, the functional groups of the organic molecules involved in the mechanisms are similar: both clays are likely to complex polar groups such as hydroxyl and carbonyl. These functional groups can occur on practically any organic compound, from proteins to polysaccharides. I therefore do not expect to find specific components on either one of the clays. *I hypothesize that the chemical composition of kaolinite and smectite-bound SOM will not be different.*

I used Py-GC/MS and solid state <sup>13</sup>C CPMAS NMR, to analyze the composition of the extracts, and where possible, the residues. The last depended on the ash content and the amount of paramagnetic ions present. Paramagnetics, such as Fe<sup>3+</sup>, can disturb the results as they influence the amount and type of carbon seen in <sup>13</sup>C solid state NMR spectra of clay fractions (Oades *et al.*, 1987).

### Mean residence time

Several investigations in natural field situations have been performed on the effect of non-crystalline minerals on SOM turnover. Torn *et al.* (1997) found that the abundance of non-crystalline minerals (e.g. allophane) accounted for more than 40% of the variation in organic C content and turnover ( $\Delta^{14}\text{C}$ ) in volcanic soils. Parfitt *et al.* (1997) simulated the turnover of C for Andisols and concluded that the pool of passive organic matter was very large, indicating that allophane has a stabilizing effect on a large part of the SOM in Andisols.

As far as I know the effect of crystalline clay minerals on the turnover speed of organic carbon in a field situation has not been studied systematically before, and most research concerns single <sup>14</sup>C datings of one soil profile. Theng *et al.* (1986) found that the carbon in a beidellite-containing clay-size fraction had a <sup>14</sup>C age of 5680 years. Arai *et al.* (1996) measured a <sup>14</sup>C age of 4650 years B.P. for the organic matter in a combined NaOH/Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract of a Vertisol. Hsieh (1992) measured <sup>14</sup>C of the total soil and calculated the stable fraction with a two-pool model. He found that the slow pool of montmorillonitic soils had a very long mean residence time of 850-3000 years.

I have not come across <sup>14</sup>C ages of the clay-size fraction of kaolinitic soils in literature. Bonde *et al.* (1992) used  $\delta^{13}\text{C}$  to estimate turnover in size fractions of Oxisols (strongly-weathered soils that contain kaolinite and iron(hydr)oxides) and found a mean residence time for the SOM in the clay-size fractions of about 59 years. Shang and Tiessen (1997) studied the stability of SOM in Oxisols using chemical oxidation, and found that the organic matter in a tropical Oxisol is quite labile, i.e. that there is no slow or residual C pool (Veldkamp, 1993).

Studies employing artificial mixtures of (crystalline) clay minerals and organic components, show strong evidence that organic matter decomposition is influenced by clay mineralogy. Organic fractions mixed with montmorillonite show a relatively slow decomposition, while organic fractions mixed with kaolinite show a relatively fast turnover (Allison, 1949; Lynch, 1956; Sorensen, 1975; Martin and Haider, 1986 and references cited therein; Quiquampoix, 1987; d'Acqui *et al.*, 1998). Sagggar *et al.* (1994) and Sagggar *et al.* (1996) used  $^{14}\text{C}$  labeled ryegrass to study short-term differences in SOM turnover between soils with different mineralogy, and found that SOM had a relatively large mean residence time in smectitic soils.

Considering the above and the expectation that smectite can bind SOM relatively strongly compared to kaolinite (see *extractability*), *I hypothesize that organic matter in smectitic soils has a relatively long mean residence time, and that organic matter in kaolinitic soils has a relatively short mean residence time.*

The main tools to measure and quantify organic matter decomposition are: respirometry, measuring changes in  $\delta^{13}\text{C}$  associated with shifts from C3 to C4 type vegetations (e.g. E. Veldkamp, 1993), labeled  $^{14}\text{C}$  (e.g. Sagggar *et al.*, 1996), and measuring natural  $^{14}\text{C}$  (e.g. Trumbore *et al.*, 1989). In this study, methods suitable for measuring short-term dynamics (respirometry or the use of labeled  $^{14}\text{C}$ ) were not an option, as we expected residence times over a hundred years. Measuring  $\delta^{13}\text{C}$  was also not an option, because to study the effect of clay mineralogy on SOM decomposition, factors like change in land use and vegetation had to be excluded and soils from natural systems were used only.

I therefore used  $^{14}\text{C}$  age as a measure for SOM turnover. I assumed that in systems under native vegetation, such as the savanna in large parts of Africa, over the past thousands of years, an equilibrium has developed where SOM inputs and outputs are equal. In such systems the  $^{14}\text{C}$  age of the organic carbon equals the mean residence time.

There are two ways to measure  $^{14}\text{C}$ : radiometric and by accelerated mass spectrometer (AMS). The last is the most expensive one, but has the advantage that small samples (of less than 1 mg carbon) can be measured.

## Outline

The outline of this thesis is as follows:

Chapters 2 and 3 contain studies on kaolinite- and smectite-associated organic matter in soil from 8 different countries. Chapter 2 describes the amount and chemical composition and Chapter 3 the mean residence time of the clay-associated organic matter.

Chapters 4 and 5 contain studies on kaolinite- and smectite-bound organic matter in soils from Mozambique. Parallel to chapters 2 and 3, chapter 4 describes the amount and chemical composition and chapter 5 the mean residence time of kaolinite and smectite-bound organic matter. Finally, in chapter 6, I have synthesized the results.

The individual chapters 2, 3, 4, and 5 have also been submitted as scientific papers. As a consequence, some parts are repeated among various sections.



## **Chapter 2**

### **Amount and composition of clay-associated soil organic matter in a range of kaolinitic and smectitic soils**

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### Abstract

In the global carbon cycle, soil organic matter (SOM) is a major source/sink of atmospheric carbon. Clay minerals stabilize part of the soil organic matter through mineral-organic matter binding. Stabilization of organic matter is essential for tropical soils. Since the climatic conditions of the tropics favor decomposition of organic matter, tropical soils would be very poor in organic matter without this stabilization process. This research aims at determining the effect of clay mineralogy on the amount and composition of organic matter that is bound to the mineral surface. We focused on organic matter that is associated with kaolinite and smectite. We characterized kaolinite- and smectite-associated SOM in soils from seven countries, employing  $^{13}\text{C}$  NMR spectroscopy and Py-GC/MS.

The content of carbon in the total clay-size fraction showed no significant difference between kaolinitic and smectitic soils. This suggests that the total amount of organic carbon in the clay-size fraction is independent of the clay mineralogy. We first extracted the clay fraction with NaOH and thereafter with  $\text{Na}_4\text{P}_2\text{O}_7$ . About half of the kaolinite-associated SOM was extractable by NaOH. In the smectitic soils, pyrophosphate extracted more organic carbon than did NaOH. The Py-GC/MS and NMR results indicate that kaolinite-associated SOM is enriched in polysaccharide products, while smectite-associated organic matter contains many aromatic compounds. We suggest that different clay minerals use different binding mechanisms to complex SOM. As a result, the composition of clay-associated organic matter would be influenced by the type of clay that is dominantly present in the soil.

### Introduction

Of the inorganic constituents in soil, clay minerals are particularly important in the stabilization of organic compounds (Greenland, 1971; Martin and Haider, 1986; Theng and Tate, 1989; Hassink, 1995). Clay minerals have a high specific surface area and carry a charge, enabling them to bind, and thereby chemically stabilize, organic matter. Clay aggregates also provide micropores for the physical protection of SOM.

The interactions between organic matter and clays have been reviewed by several authors (e.g. Mortland, 1986; Huang and Schnitzer, 1986; Oades *et al.*, 1989; Theng and Tate, 1989; Christensen, 1996; Schulten *et al.*, 1996). Various terms have been used to describe the resultant products, the most common being *organo-mineral complexes* (Christensen, 1996; Schulten *et al.*, 1996), and *associations* (Schnitzer *et al.*, 1988). These terms are frequently used interchangeably. For consistency the following definitions will be used here:

*Clay-associated organic matter* is all organic matter present in the "clay-size" separate, both free and bound.

*Clay-complexed organic matter* refers to organic matter bound to clay mineral surfaces, e.g. by Ca-bridging, or intercalation between clay layers (Theng and Tate, 1989). It is practically defined as the organic matter present in the heavy clay-size fraction after density fractionation of the clay-size fraction.

*Secondary organo-mineral associations*, often referred to as aggregates (Christensen, 1996), are involved in the physical protection of organic matter by occlusion. These associations are not further discussed in this paper.

Previous research has mainly been concerned with characterizing the amount and composition of the organic matter in the clay-size fraction relative to that present in the other size fractions (Christensen, 1992 and references cited therein). Recently, Leinweber *et al.*

(1999) studied the molecular composition of SOM in smectite-dominated soils (Vertisols). However, their study concerned the total SOM fraction. As far as we know, the amount and composition of clay-associated SOM of soils with different mineralogy has not been compared systematically. Here we focus on the amount and composition of organic matter that is associated with kaolinite and smectite, two of the most common clay minerals at the earth's surface.

Kaolinite and smectite have very different characteristics. Smectites are expandable 2:1 layer silicate minerals. The individual layers of smectite crystallites are composed of two tetrahedral silicon-oxide sheets sandwiching one octahedral aluminum-hydroxide sheet. Smectites have a high permanent surface charge, a large surface area, and a high cation exchange capacity (CEC). Kaolinites are 1:1 layer structured aluminosilicates with a low surface area and a low CEC.

To examine the composition of clay-associated organic matter it is necessary to separate it from the clay matrix, e.g. by extraction. Several extractants have been reported in the literature, such as H<sub>2</sub>O, NaOH, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and *n*-hexane. We applied sequential extraction using NaOH followed by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. We chose NaOH because we expect it to extract mainly 'free' (Choudri and Stevenson, 1957) and kaolinite-complexed organic components. NaOH deprotonates the aluminum-hydroxide edges of kaolinites, and part of the organic matter, thereby dissolving organic molecules. We chose Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> thereafter, because we expect it to form complexes with (exchangeable) polyvalent cations present at smectite surfaces, thereby breaking down the cation bridges between the exchangeable cations and organic matter (Choudri and Stevenson, 1957; Schnitzer and Schuppli, 1989).

The objective of our investigation was to compare the amount and composition of kaolinite- and smectite-associated soil organic matter in natural systems. We used six kaolinitic and six smectitic soils originating from various countries. <sup>13</sup>C CPMAS NMR spectroscopy and Py-GC/MS were applied to chemically analyze the associated SOM.

## **Materials and Methods**

### *Samples*

We selected two groups of six soils (Table 2.1) from the collection of the International Soil Research and Information Center (ISRIC), Wageningen. One group contained soils with clay-size fractions dominated by kaolinite clays, while the other group had clay-size fractions dominated by smectite clays. Clay mineralogy was determined by X-ray diffraction (XRD) of oriented samples of the clay-size fractions. The diffractograms were obtained on a Philips PW1820/PW1710 diffractometer, using a Co X-ray tube at 40 kV and 30 mA, with a focussing monochromator. The divergence slit was set at 1°, the receiving slit at 0.2 mm, and the anti-scatter slit at 1°. Peak areas of the clay minerals were measured to compare the (semi-quantitative) XRD diffractograms and reported in % of total peak area (Table 2.2). All samples contained small quantities of goethite and quartz.

The kaolinitic soils originated from Brasil (BR1 and BR2), Kenya (LABEX6), Mali (ML1 and ML8), and Mozambique (MOC4). The smectitic soils originated from Indonesia (ID25), Kenya (KE66 and LABEX17), Nicaragua (NI9), and South Africa (ZA8 and ZA9). All the soils have a high base saturation, and were under natural savanna vegetation. We only used the SOM-rich surface horizons.

Table 2.1 Site characteristics and soil analyses. \* International Soil Reference and Information Center, according to FAO (1990).

ISRIC key for sites <sup>a</sup>	Country, site	Latitude, Longitude	Parent Rock	Temp (°C)	Precip. (mm yr <sup>-1</sup> )	Vegetation	Soil Type <sup>b</sup>	Clay (g kg <sup>-1</sup> )	Org C (g kg <sup>-1</sup> )	pH H <sub>2</sub> O	pH KCl	CEC <sub>sat</sub> (cmol kg <sup>-1</sup> )	Base sat (%)
BR1	Brazil, Itaguai	22°45'S; 43°41'W	Residual from gneiss	22.9	1317	Grassland	Haplic Lixisol	150	10	4.5	4.1	3.7	49
BR2	Brazil, Aparibe	21°38'S; 42°7'W	Pre-Cambrian rocks	22.8	1234	Grassland	Haplic Lixisol	260	10	5.7	4.5	4.4	66
LABEX6	Kenya, Nairobi	1°15'N; 36°41'E	Residuals from intermediate igneous rocks	18	973	Grassland	Humic Nitisol	660	26	5.7	6.8	19	61
ML1	Mali, Koulikoro	12°5'N; 8°24'W	Alluvial clay	26.9	1087	Woodland	Ferric Acrisol	230	09	6.2	4.9	5.9	87
ML8	Mali, N'Tentou	11°25'N; 7°53'W	Colluvium from lateritic hill	27.3	1337	Grassland	Ferric Acrisol	130	09	6.5	5.6	3.5	97
MOC4	Mozambique, Montepuez	13°9'S; 38°37'E	Residual from basic igneous rock	24.2	932	Grassland ( <i>Oxytenanthera abyssinica</i> )	Ferric Acrisol	270	23	5.9	5.3	15.9	95
IN25	Indonesia, East Java	7°37'S; 112°50'E	Residual from tuff	26.8	1316	Grassland	Pellic Vertisol	860	11	7.6	6.4	92.5	100
KE66	Kenya, Kajiado	1°49'S; 36°49'E	Residual from tuff	17.5	504	Grassland	Pellic Vertisol	600	14	7.3	6.0	55.6	100
LABEX17	Kenya, Sultan Hamud	2°8'S; 37°28'E	Undifferentiated sediment	22.6	611	Grassland	Pellic Vertisol	860	18	7.9	6.6	86	100
NI9	Nicaragua, Timal	12°15'N; 86°2'W	Alluvium from tuff	27.3	1184	Grassland	Pellic Vertisol	790	11	6.4	4.7	77.2	100
ZA8	South Africa, north of Pietermaritzburg	29°22'S; 30°24'E	Residuals from intermediate igneous rocks	18.5	928	Shrub ( <i>Acacia</i> spp.)	Pellic Vertisol	460	24	6.3	5.0	42.0	63
ZA9	South Africa, north of Pietermaritzburg	29°22'S; 30°24'E	Residuals from intermediate igneous rocks	18.5	928	Shrub ( <i>Acacia</i> spp.)	Pellic Vertisol	570	16	6.8	5.3	45.2	87

The samples were air-dried and passed through a 2-mm sieve. The pH, organic carbon content, CEC<sub>soil</sub>, and particle size distribution were measured during previous studies by the ISRIC.

Table 2.2 Clay mineralogy measured by X-ray diffraction (relative peak area of diffractograms in %).

	Kaolinite	Mica	Smectite
<b>Kaolinite-dominated</b>			
BR1	92	8	0
BR2	98	2	0
LABEX6	95	5	0
ML1	96	4	0
ML8	99	0	1
MOC4	100	0	0
<b>Smectite-dominated</b>			
ID25	1	0	99
LABEX17	20	0	80
NI9	0	0	100
KE66	1	0	99
ZA8	21	0	79
ZA9	21	0	79

#### *Fractionation*

We used a combination of physical and chemical fractionation. Though clay minerals also occur in other size fractions, the largest part, with its associated organic matter, is in the clay-size fraction (Christensen, 1992). Therefore, we first collected the clay-size (< 2µm) fractions of all samples, using ultrasonic (full) dispersion and sedimentation. Secondly, the separated clay-size fraction was shaken in 0.5 M NaOH under nitrogen for 24 hours (0.5 L, soil : solution = 1 : 10). We centrifuged the solution, and shook the residue with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants to pH=1 with a solution of 0.1 M HCl and 0.3 M HF. Both the supernatant and the NaOH-residue were dialyzed to pH=6 against deionized water, and freeze-dried. Next, the NaOH-residue was shaken for 24 hours with 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> under nitrogen (soil : solution = 1 : 10). The solution was centrifuged, and the residue was shaken with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants again to pH=1 with a solution of 0.1 M HCl and 0.3 M HF. Both the supernatant and the pyrophosphate-residue were dialyzed and freeze-dried. We measured the amount of carbon in the clay-size fraction, the freeze-dried extracts, and the residue, using an Interscience elemental analyzer EA1108.

#### *<sup>13</sup>C NMR spectroscopy*

We used Cross Polarization-Magic Angle Spinning (CP-MAS) solid-state <sup>13</sup>C NMR spectroscopy to characterize the hydroxide- and pyrophosphate-extracts. The spectra were obtained on a Bruker AMX 400 spectrometer at a frequency of 100.628 MHz, with an acquisition time of 0.033 s. The residues were not measured because they contained large amounts of minerals that would have perturbed the spectra (Oades *et al.*, 1987). The subdivision of the spectra follows the commonly used scheme (Hatcher, 1987; Kögel-Knabner, 1992): aliphatic C (0-46 ppm), O-alkyl C (46-110 ppm), aromatic C (110-160 ppm), and carbonylic C (160-210 ppm). To obtain semi-quantitative data the spectra were integrated using the integration routine of the spectrometer.

*Py-GC/MS*

We used a Horizon Instruments Curie-Point pyrolyzer to pyrolyze the hydroxide- and pyrophosphate-extracts and residues. The samples were heated at 610 °C for 5 s in the instrument. The pyrolysis unit was connected to a Carlo Erba GC 8000 gas chromatograph and the products were separated by a silica column, using helium as carrier gas. The initial oven temperature (40 °C) was raised at a rate of 7 °C min<sup>-1</sup> to 320 °C and maintained at that temperature for 20 min. The end of the GC column was connected to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, ionization energy 70 eV).

**Results***Carbon distribution*

The distribution of organic carbon over the fractions is presented in Table 2.3. The content of carbon in the clay-size fraction varied between 0.9 and 4.2% (mass fraction) with no significant difference between kaolinite- and smectite-rich soils. Between 45 and 70% of that carbon was recovered in the combined hydroxide and pyrophosphate extracts. The amount of carbon (g) per 100g clay in the NaOH extracts is slightly larger for the kaolinitic soils than for the smectitic soils, while the opposite holds for the pyrophosphate extract.

Table 2.3 Carbon distribution in the clay-size fraction and its extracts. \* Std = standard deviation

	Clay-size fraction	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	Sum Extracted
	g C per 100 g clay			% fraction of C in clay		
<i>Kaolinite</i>						
BR1	3.8	1.9	0.4	50.8	10.6	61.4
BR2	2.3	1.0	0.2	43.4	9.5	52.9
LABEX6	1.4	0.9	0.1	64.0	7.0	71.0
ML1	2.1	0.7	0.3	34.7	15.0	49.7
ML8	3.0	1.0	0.4	32.1	13.4	45.5
MOC4	3.0	1.5	0.5	50.7	17.1	67.8
Average ± Std*	2.6 ± 0.8	1.2 ± 0.4	0.3 ± 0.1	46.0 ± 11.8	12.1 ± 3.7	58.1 ± 10.3
<i>Smectite</i>						
ID25	0.9	0.02	0.5	2.3	59.2	61.5
KE66	2.4	0.4	1.2	16.6	49.0	65.6
LABEX17	1.3	0.1	0.6	10.0	48.0	58.0
NI9	1.4	0.2	0.5	11.0	34.3	45.3
ZA8	4.2	0.9	1.2	20.4	29.5	49.9
ZA9	2.3	0.7	0.4	30.2	15.9	46.1
Average ± Std	2.1 ± 1.2	0.4 ± 0.4	0.7 ± 0.4	15.1 ± 9.6	39.3 ± 15.7	54.4 ± 8.5

NaOH extracted 46% and pyrophosphate 12% of the organic carbon from the clay-size fraction of kaolinitic soils, while in the case of smectitic soils pyrophosphate extracted more C than did hydroxide. ANOVA single factor analysis (Neter *et al.*, 1996) of the data of Table 2.3 indicates that hydroxide extracts significantly more organic C from the clay-size fractions of kaolinitic soil than of smectitic soils ( $p = 0.002$ ). By contrast, pyrophosphate extracts contain significantly more C in case of smectites than in case of kaolinites ( $p = 0.013$ ). One exception to this trend is the smectitic sample ZA9, for which we found more carbon in the NaOH extract than in the pyrophosphate extract.

The clay-size organic matter that was not extracted by either NaOH or  $\text{Na}_4\text{P}_2\text{O}_7$  may include both free organic matter that is not soluble in these reagents (e.g. plant remains, or charred material), and organic carbon that is bound very strongly to the clay.

### *<sup>13</sup>C NMR spectroscopy*

Figure 2.1 shows the <sup>13</sup>C NMR spectra of the NaOH extracts of five kaolinitic soils (LABEX6 was not analyzed). The spectra are similar, except for MOC4, which had an extra peak at 127 ppm. The most pronounced signals are in the O-alkyl region: near 62, 72, and 102 ppm. The 72 ppm signal is attributed to the ring carbons of carbohydrates while the signals near 102 and 62 ppm arise from the anomeric carbon and C-5 or C-6 carbon ( $\text{CH}_2$ ) of carbohydrate structures, respectively. In addition, the peak at 72 ppm shows a shoulder near 55 ppm, which probably originates from carbon bound to nitrogen. Table 2.4 lists the integrated signal areas corresponding to the four carbon groups (aliphatic C, O-alkyl C, aromatic C, and carbonylic C). It also illustrates the relatively large signals of O-alkyl C in kaolinite NaOH extracts. The peak areas in the <sup>13</sup>C NMR spectra do not reflect relative quantities, because the various functional groups have different relaxation times. The results in Table 2.4 can therefore be used only semi-quantitatively.

The alkyl peak near 23 ppm originates from  $-\text{CH}_3$  (Figure 2.2). Signals at 30-32 ppm can be ascribed to  $-\text{CH}_2$  alkyl carbon in long chain polymethylene structures (e.g. fatty acids, waxes, aliphatic biopolymers). Furthermore, we found signals for C- or H-substituted aromatic carbon near 127 ppm and for carboxylic, amide or ester carbon near 174 ppm. The spectrum of MOC4 shows a much larger signal for aromatic C than the other spectra.

The <sup>13</sup>C NMR spectra of the NaOH extracts from the smectitic soils are shown in Figure 2.2. ZA9 did not give a clear signal, and therefore its spectrum was not recorded. We did not have sufficient material to run an NMR spectrum of ID25 and NI9. The two samples that we did measure were ZA8 and KE66. The spectrum of ZA8 has similar features to those of kaolinitic soils showing a strong signal from O-alkyl carbon, while that of KE66 has strong signals for carbonyl, aromatic, and alkyl C relative to ZA8. It is therefore not possible to draw general conclusions about the composition of NaOH extractable SOM of smectitic soils.

Pyrophosphate appears to extract more carbonyl and aromatic C and less O-alkyl C from kaolinitic soils than does NaOH (Table 2.4 and Figure 2.3). Again, MOC4 shows a different behavior in that it gives a very strong aromatic signal.

The <sup>13</sup>C NMR spectra of the  $\text{Na}_4\text{P}_2\text{O}_7$  extracts from smectitic soils are shown in Figure 2.4. Compared to the NaOH extracts, the most noticeable features are a weak signal in the O-alkyl range and a strong signal in the aromatic and carbonyl range (Table 2.4), so the tendency is similar to kaolinite. There are, however, some variations among the samples.

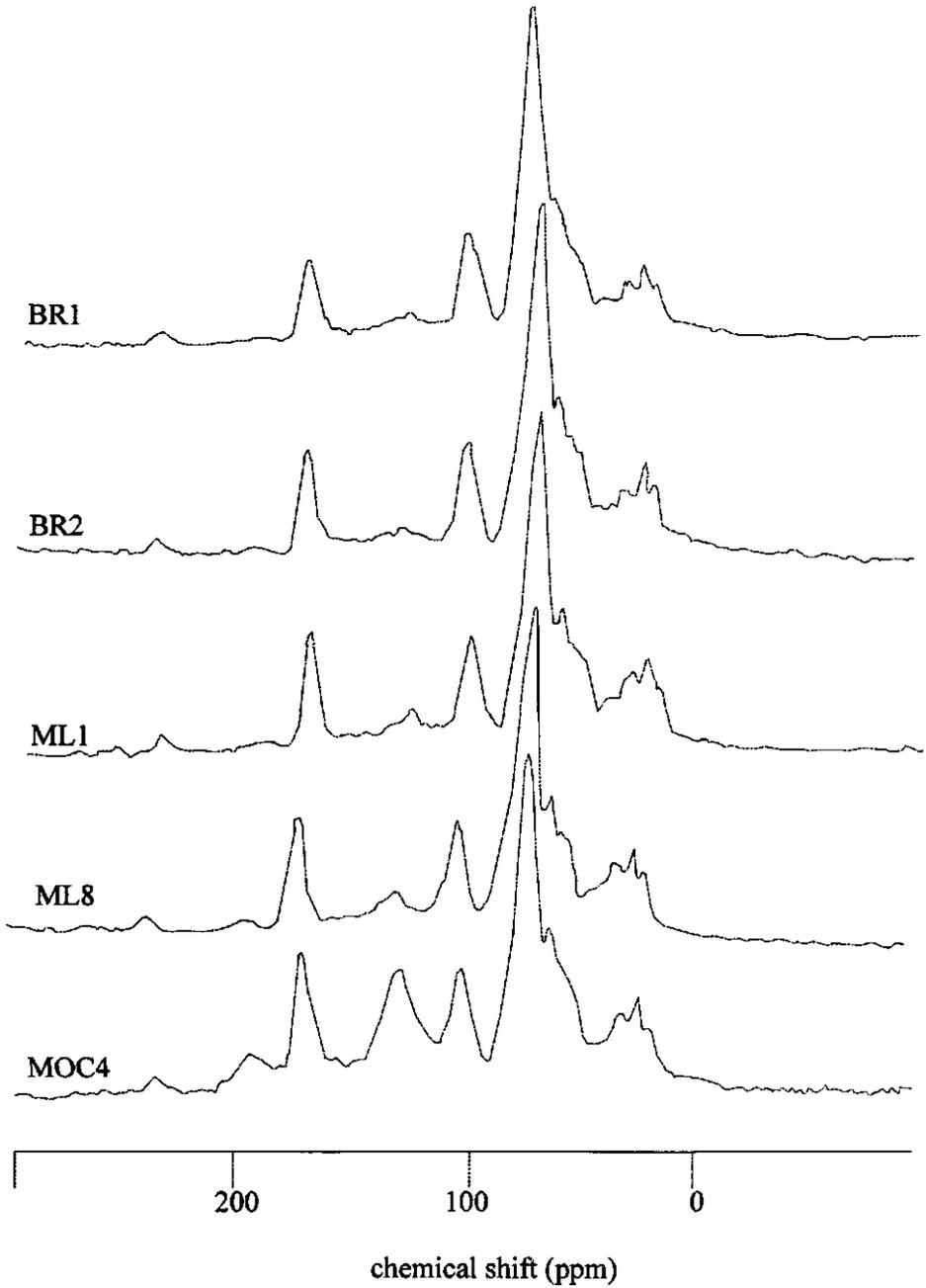


Figure 2.1  $^{13}\text{C}$  CPMAS-NMR spectra of NaOH extracts (kaolinitic soils).

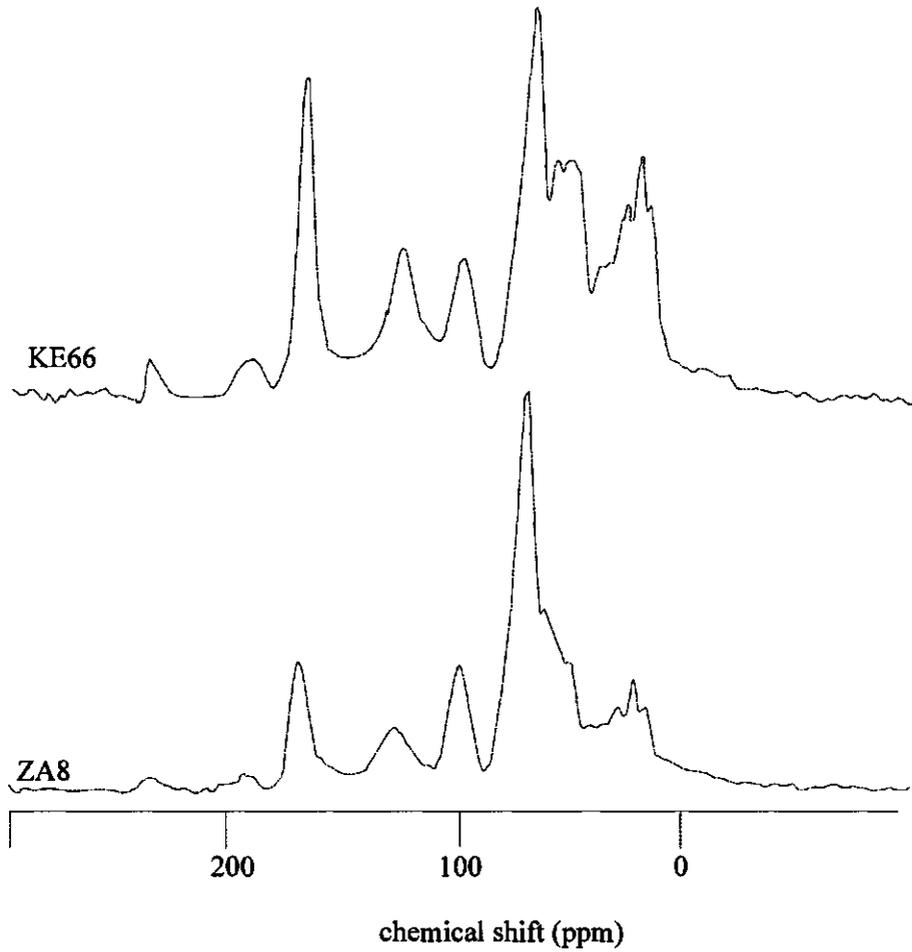


Figure 2.2  $^{13}\text{C}$  CPMAS-NMR spectra of NaOH extracts (smectitic soils).

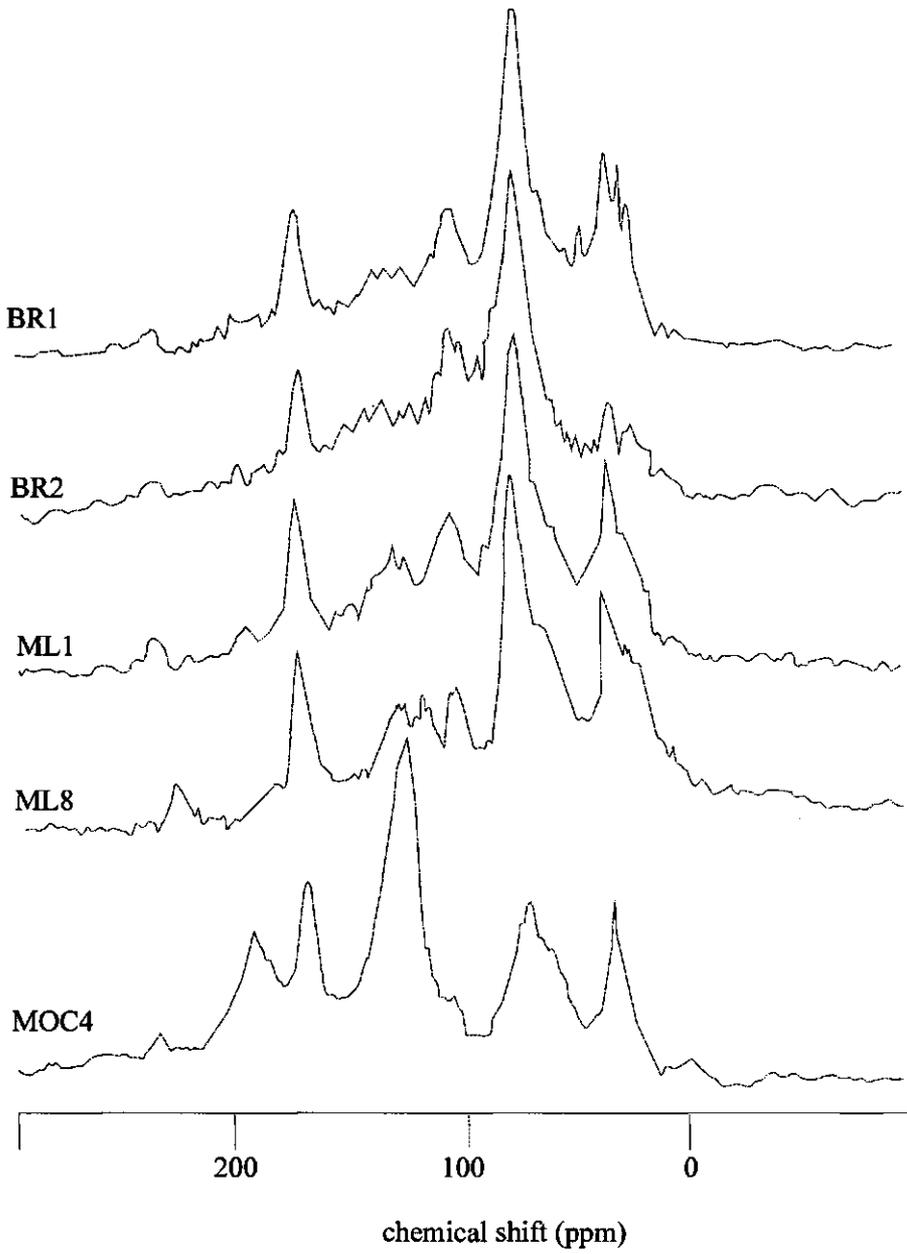


Figure 2.3  $^{13}\text{C}$  CPMAS-NMR spectra of  $\text{Na}_4\text{P}_2\text{O}_7$  extracts (kaolinitic soils).

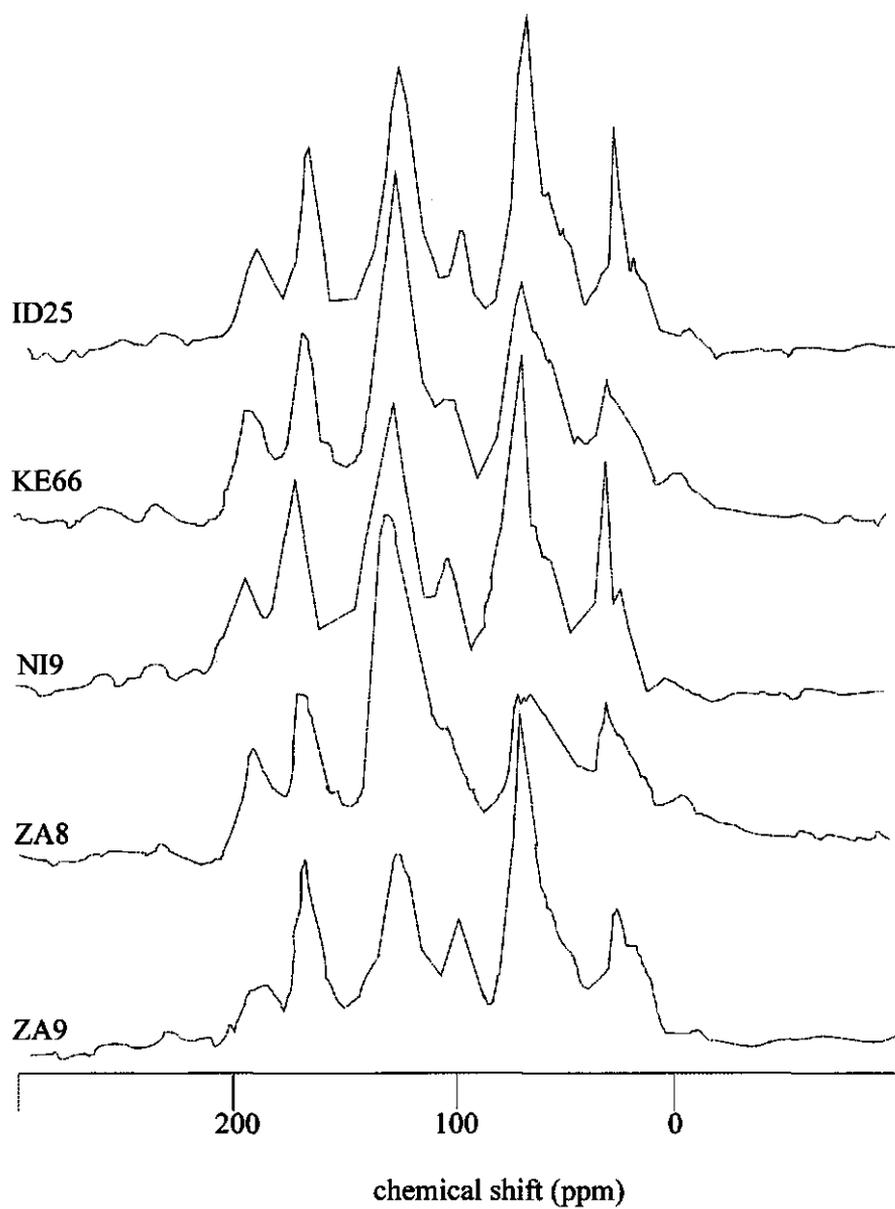


Figure 2.4 <sup>13</sup>C CPMAS-NMR spectra of Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts (smectitic soils).

Table 2.4 Integrals of areas of different carbon groups measured by  $^{13}\text{C}$ -NMR (%).

	NaOH extract				$\text{Na}_4\text{P}_2\text{O}_7$ extract			
	carbonyl	aromatic	O-alkyl	alkyl	carbonyl	aromatic	O-alkyl	alkyl
<i>Kaolinite</i>								
BR1	8.3	7.1	67.9	16.8	14.2	14.6	50.5	20.8
BR2	8.2	7.0	62.8	22.0	16.0	32.7	40.5	10.8
ML1	11.4	9.4	61.9	17.4	15.6	14.0	51.2	19.2
ML8	10.8	8.7	62.7	17.8	12.5	16.9	44.9	25.8
MOC4	16.7	18.0	51.1	14.2	27.9	36.9	25.1	10.2
Average $\pm$ Std	$11.1 \pm 3.5$	$10.0 \pm 4.6$	$61.3 \pm 6.2$	$17.6 \pm 2.8$	$17.2 \pm 6.1$	$23.0 \pm 10.9$	$42.4 \pm 10.6$	$17.3 \pm 6.7$
<i>Smectite</i>								
ID25					22.0	29.1	36.2	12.8
KE66	15.0	14.8	46.5	23.8	17.8	33.5	32.0	16.7
NI9					23.4	28.9	35.9	11.8
ZA8	11.0	8.8	61.7	18.5	19.1	44.1	22.5	19.1
ZA9					21.3	23.0	42.1	13.6
Average $\pm$ Std	$13.0 \pm 2.8$	$11.8 \pm 4.2$	$54.1 \pm 10.7$	$21.1 \pm 3.7$	$20.7 \pm 2.3$	$31.7 \pm 7.8$	$33.7 \pm 7.2$	$14.8 \pm 3.0$

*Py-GC/MS*

The 36 chromatograms were quite similar for a given combination of clay mineral and extractant/residue. So we will discuss only six pyrograms: the NaOH extract of the kaolinite-rich ML1 sample, the  $\text{Na}_4\text{P}_2\text{O}_7$  extract of the kaolinite-rich BR1 sample, the  $\text{Na}_4\text{P}_2\text{O}_7$ -residue of the kaolinite-rich LABEX6 sample, the NaOH extract of the smectitic-rich ZA8 sample, the  $\text{Na}_4\text{P}_2\text{O}_7$  extract of the smectite-rich NI9 sample, and the  $\text{Na}_4\text{P}_2\text{O}_7$ -residue of the smectite-rich LABEX17 sample. We summarized the assignments of peaks in Table 2.5 (page 36).

**NaOH extracts**

The pyrolysis results of the NaOH extracts of the kaolinitic and smectitic soils (Figure 2.5) are rather similar. In keeping with the NMR spectra, the extracts are dominated by polysaccharides (numbers 1 to 17 and 'Ps'). Polysaccharide-derived products identified in both pyrolysates are furans (2, 4, 5, 6, 11), anhydrosugars (13, 15), and dianhydrorhamnose (14) (Pouwels and Boon, 1990). Nitrogen-compounds in the pyrolysates of the NaOH extracts are identifiable with pyrroles (N1, N3, N4), pyridine (N2), indole (N6) and benzeneacetonitrile (N7). Pyrroles are derived from proline and hydroxyproline, and pyridines from alanine, polypeptides, and chitin. Benzeneacetonitrile is a degradation product of phenylalanine (Schulten and Schnitzer, 1998, and references cited therein).

Various aromatic moieties were detected, such as benzene (A1), toluene (A2), styrene (A3). Furthermore, phenol (P1), methylphenol (P2 and P3), dimethylphenol, (P4), 4-vinylphenol (P5), guaiacols (L1-L3) and syringols (L4-L6) are present. Alkylbenzenes and alkylphenols can originate from lignin (Saiz-Jimenez and de Leeuw, 1986; Van der Hage *et al.*, 1993) and/or from other polyphenolic macromolecules. Toluene and (methyl)phenol may also have a protein origin (Saiz-Jimenez, 1996). Guaiacols and syringols are typical pyrolysis products of lignin (Nierop, 1999, and references cited therein).

The aliphatic fraction of the pyrolysates consists of a homologous series of alkenes and alkanes ranging from  $\text{C}_{15}$  to  $\text{C}_{30}$ . Aliphatic compounds that had large peaks in the pyrolysates

of the NaOH extract of ML1 (Figure 2.5), were fatty acids (F1 and F2) and steroids (F3 and F4). These peaks weakened in the pyrolysates of other kaolinitic and smectitic NaOH extracts.

The NMR spectra of both extracts of MOC4 (kaolinite) indicated large amounts of aromatic C. Similarly, the corresponding chromatograms of the pyrolysis products had large peaks due to aromatic compounds such as benzene and toluene (not shown). The NMR spectrum of the NaOH extract of KE66 (smectite) gave large signals from carbonyl, aromatic, and aliphatic C compared to ZA8. The large peaks for toluene, phenol, and 2-methylphenol in the chromatogram of KE66 (not shown), are consistent with the NMR results. However, we did not find high contents of aliphatic and carbonyl carbon as the NMR spectrum indicated.

In short the pyrolysis results of the NaOH extracts of both clays are largely similar. They show a variety of products derived from polysaccharides, proteins, lignin, and lipids.

#### **Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts**

Figure 2.6 shows the chromatograms of the pyrolysates of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of kaolinite (top) and smectite (bottom). Peaks marked with an X are due to contaminants or compounds of unknown identity. The kaolinite extract clearly shows a larger number and stronger signals than its smectite counterpart, especially in the anhydrohexose-area ('S'), and among the peaks of other sugars (12, 14, and 15). The smectite extract has large peaks originating from 2-furaldehyde (7), 5-methyl-2-furaldehyde (11), and levoglucosenone (13) and medium signals originating from 2-methyl furan (2), acetic acid (3), and (2H)-furan-3-one (5).

We found few lignin-derived products: guaiacol (L1) (both clays) and a minor signal from syringol (L4) (smectite only). Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of both clays contained fewer phenolic compounds than the NaOH extracts, but more (alkyl-)aromatic compounds, especially in case of smectite. Compared to the NaOH extract, the pyrogram of the smectite Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract contained many aromatic peaks, such as styrene (A3), ethylbenzene (A4), naphthalene (A6), 1-methyl-naphthalene (A8), and 2-ethenyl-naphthalene (A9).

In both Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts, we identified homologous series of *n*-alkenes and *n*-alkanes, probably originating from aliphatic macromolecules (Nierop, 1998).

The NMR spectrum of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract of BR2 (not shown) showed relatively large amounts of aromatic C and relatively low amounts of alkyl C. This was confirmed by the presence of large peaks of aromatic pyrolysis products like benzene and toluene. Furthermore, the chromatogram of BR2 showed hardly any signs of aliphatic components, such as homologous series of *n*-alkenes and *n*-alkanes.

The relatively large amounts of aromatic C indicated by NMR spectra of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract of ZA8 were confirmed by large peaks of aromatic pyrolysis products such as benzene, toluene, naphthalene, 2-methylnaphthalene, and 2-vinyl-naphthalene (GC trace not shown).

We could not identify the source of the relatively high contents of O-alkyl carbon in ZA9's Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract, because the chromatography failed for unknown reasons.

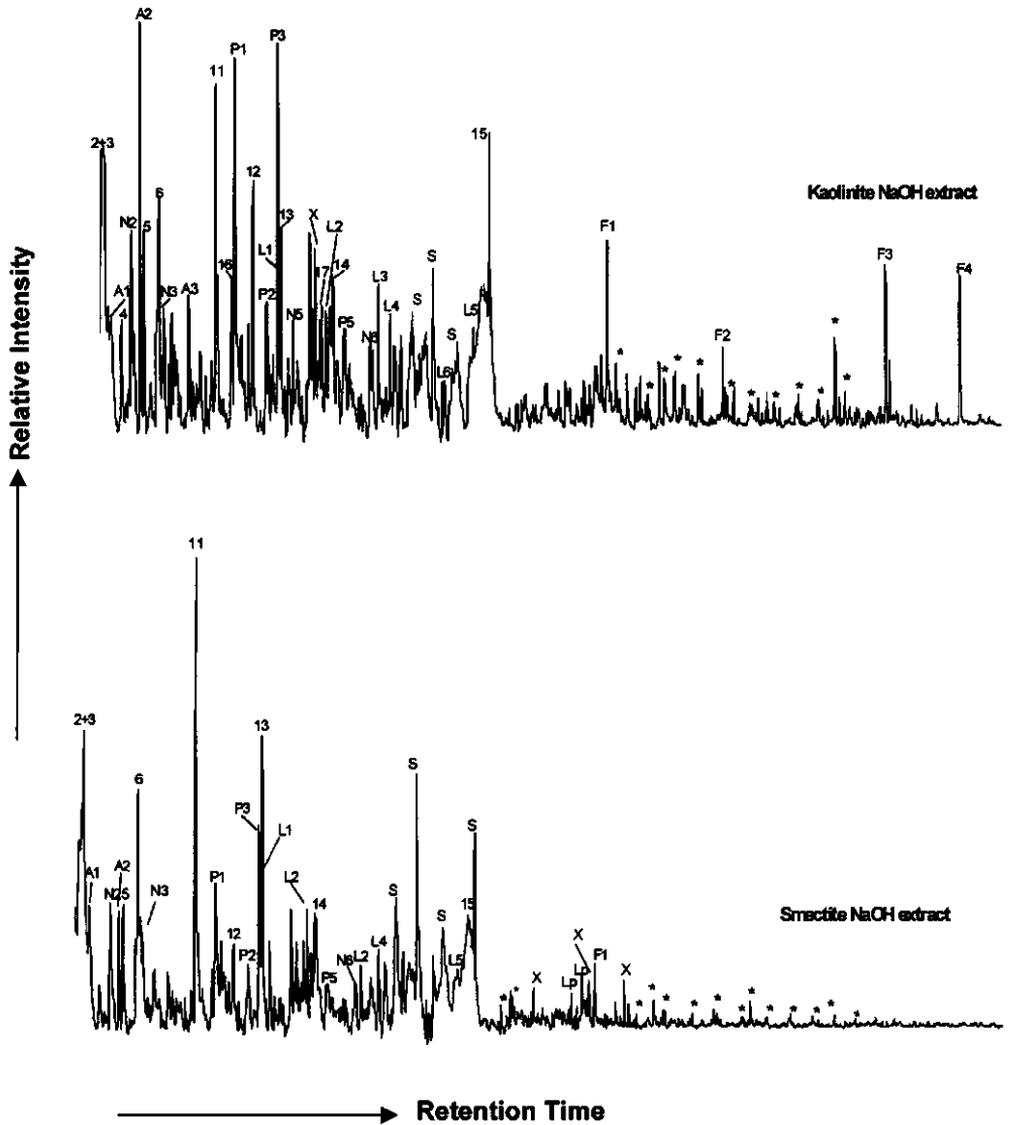


Figure 2.5 Pyrolysis-GC traces of the NaOH extracts of a kaolinitic and a smectitic soil. Numbers correspond to compounds listed in Table 2.5.

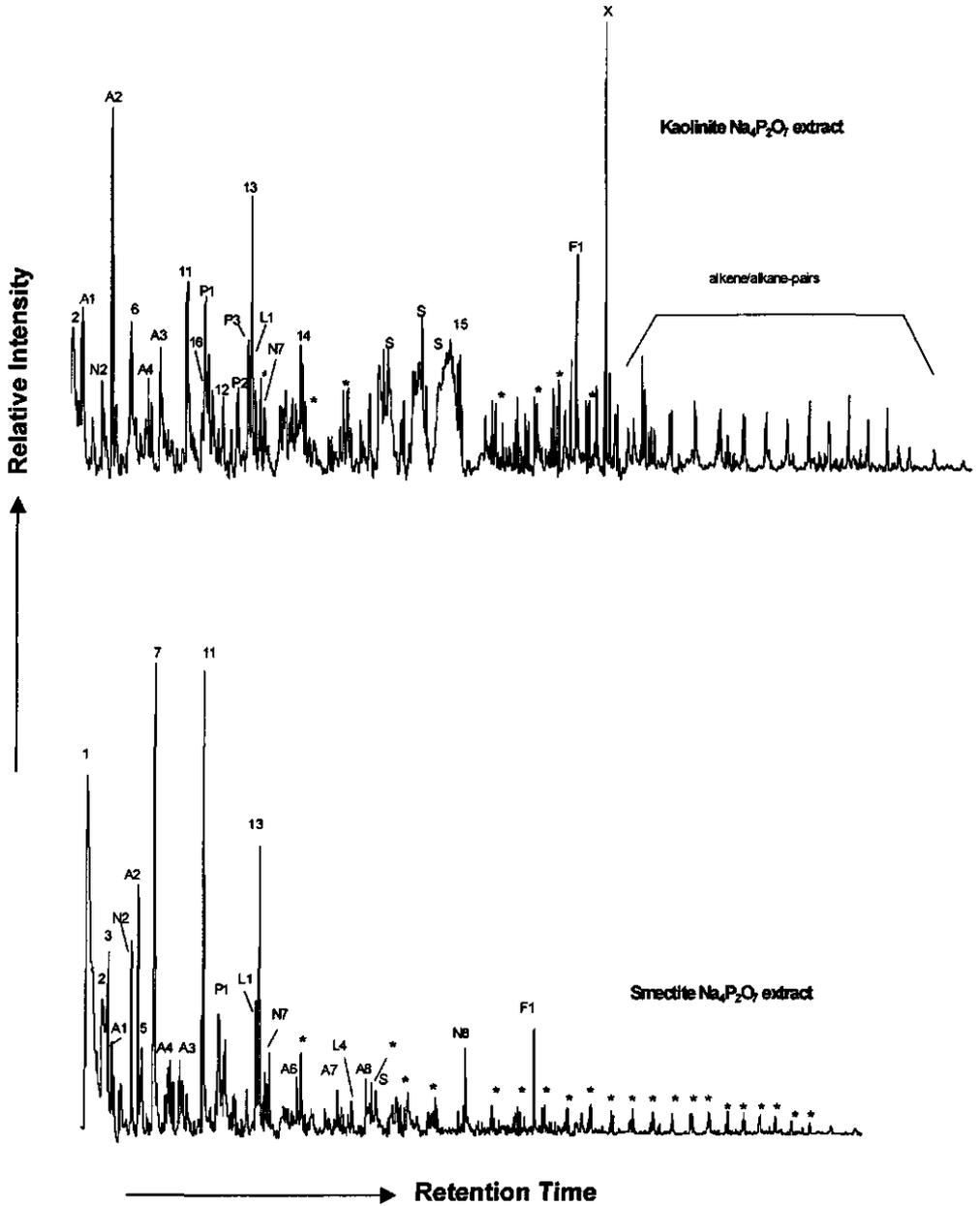


Figure 2.6 Pyrolysis-GC traces of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of a kaolinitic and a smectitic soil. Numbers correspond to compounds listed in Table 2.5.

Table 2.5 Main pyrolysis products. Abp, aliphatic biopolymers; Ar, aromatic compounds; Lg, lignin; Lp, lipids; N-Ar, Nitrogen containing aromatic compounds; Ph, phenols; Ps, polysaccharides.

No	Compound	Source	Mass
1	Furan	Ps	68
2	2-Methylfuran	Ps	82
3	Acetic acid	Ps	60
4	2,5-Dimethylfuran	Ps	96
5	(2H)-Furan-3-one	Ps	84
6	3-Furaldehyde	Ps	96
7	2-Furaldehyde	Ps	96
8	2-Acetylfuran	Ps	110
9	2,3-Dimethyl-5-furan-2-one	Ps	98
10	2-(5H)-5-Methylfuranone	Ps	98
11	5-Methyl-2-furaldehyde	Ps	110
12	Dianhydrorhamnose	Ps	128
13	Levogluconone	Ps	126
14	1,4:3,6-Dianhydro- $\alpha$ -D-glucopyranose	Ps	144
15	Anhydroglucosan (levoglucosan)	Ps	162
16	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	Ps	114
17	3,5-dihydroxy-2-methyl-(4H)-pyran-4-one	Ps	142
S	Polysaccharide-derived products	Ps	
A1	Benzene	Ar	78
A2	Toluene	Ar	92
A3	Styrene	Ar	104
A4	C <sub>2</sub> -benzene	Ar	106
A5	C <sub>4</sub> -benzene	Ar	134
A6	Naphtalene	Ar	128
A7	1-Methyl-naphtalene	Ar	142
A8	2-Vinyl-naphtalene	Ar	154
F1	Hexadecanoic acid	Lp	256
F2	Dioctyl ester hexanedioic acid	Lp	370
F3	3- $\beta$ -Cholesta-4,6-dien-3-ol	Lp	384
F4	Stigmasta-3,5-dien-7-one	Lp	410
F5	Tetradecanoic acid	Lp	228
F6	Pentadecanoic acid	Lp	242
F7	Octadecanoic acid	Lp	284
Lp	Lipid-derived product	Lp	
*	Alkene/alkane-pairs	Lp/Abp	
L1	Guaiacol	Lg	124
L2	4-Methylguaiacol	Lg	138
L3	4-Vinylguaiacol	Lg	150
L4	Syringol	Lg	154
L5	4-Vinylsyringol	Lg	180
L6	4-Acetylguaiacol	Lg	166
N1	Pyrrole	N-Ar	67
N2	Pyridine	N-Ar	79
N3	3-Methyl-1H-pyrrole	N-Ar	81
N4	2-Ethyl-1H-pyrrole	N-Ar	95
N5	1-Isocyano-2-methylbenzene	N-Ar	117
N6	Indole	N-Ar	117
N7	Benzeneacetonitrile	N-Ar	117
N8	Diketodipyrrole	N-Ar	

P1	Phenol	Ph	94
P2	2-Methylphenol	Ph	108
P3	3 and 4 Methylphenol	Ph	108
P4	Dimethylphenol	Ph	122
P5	4-Vinylphenol	Ph	120
X	ARTIFACT		

The pyrolysis results indicate the pyrophosphate extracts of kaolinite-associated SOM are relatively rich in polysaccharides and pyrophosphate extracts of smectite-associated SOM are relatively rich in aromatic compounds.

### $\text{Na}_4\text{P}_2\text{O}_7$ residues

The chromatograms of the pyrophosphate residues of both clay-types were completely dominated by aliphatic components, such as homologous series of *n*-alkenes and *n*-alkanes. All peaks occurred from 20 minutes (retention time) onwards, and therefore we omitted the first 20 minutes of the chromatogram (Figure 2.7).

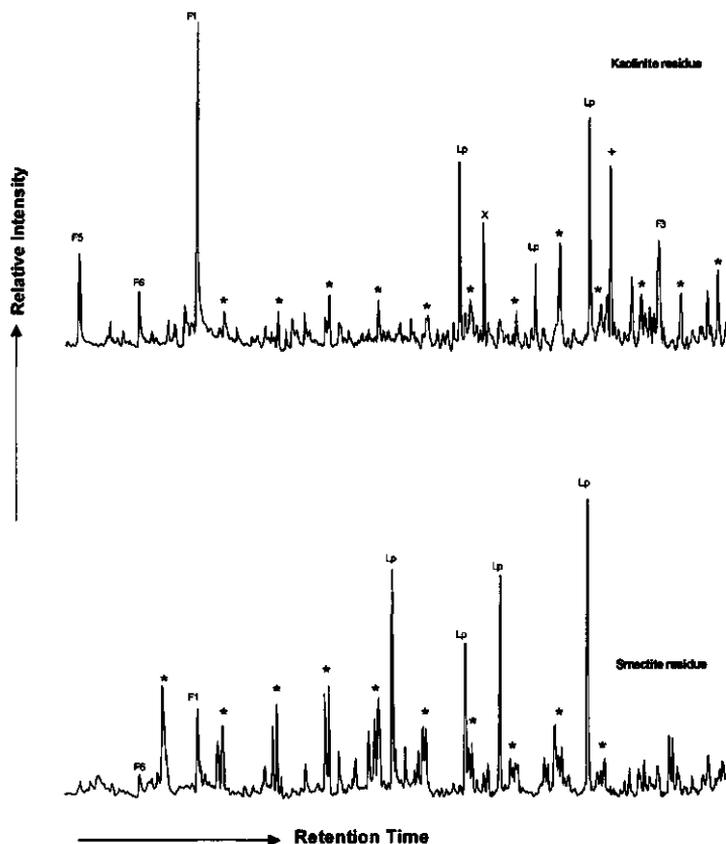


Figure 2.7 Pyrolysis-GC traces of the  $\text{Na}_4\text{P}_2\text{O}_7$  residues of a kaolinitic and a smectitic soil. Numbers correspond to compounds listed in Table 2.5.

## Discussion

### Carbon distribution

The content of carbon in the clay-size fraction showed no significant difference between kaolinitic and smectitic soils. This suggests that the total amount of organic carbon in the clay-size fraction is independent of the clay mineralogy. In kaolinitic soils, more SOM was extracted by hydroxide than pyrophosphate, while the reverse was true for smectitic soils. Though we cannot rule out that a proportion of the extracted organic matter was not bound to clay, the different extraction efficiencies suggest that different binding mechanisms are operative in kaolinite-organic matter interactions than in smectite-organic matter interactions. As pyrophosphate forms complexes with polyvalent cations present at a clay surface, the high extractability of smectite-associated SOM by  $\text{Na}_4\text{P}_2\text{O}_7$  suggests smectitic clay minerals preferentially bind organic matter through cationic bridges. We do not know the binding mechanism(s) between kaolinite and its associated organic matter.

We cannot explain why ZA9 gave more C in the hydroxide than in the pyrophosphate extract.

The clay-size organic matter that is not extracted by NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  may include both free organic matter (e.g. hydrophobic components, or plant remains), and organic carbon that is bound very strongly by the clay.

### $^{13}\text{C}$ NMR spectroscopy and Py-GC/MS

Table 2.6 presents the estimated relative importance of five groups of compounds for kaolinite- and smectite-associated SOM based on pyrolysis, NMR, and the carbon distribution. The associated SOM of both clays is dominated by aliphatic organic components such as alkanes and alkenes. These components were particularly visible in the pyrolysates of the residues. Alkenes and alkanes in pyrolysates are commonly attributed to insoluble, non-hydrolyzable aliphatic polymers, such as cutan (Nip *et al.*, 1986) and suberan (Tegelaar *et al.*, 1995) derived from above-ground plant tissues, such as cuticles and barks (de Leeuw and Largeau, 1993). They may also originate from suberan-like polymers in roots (Nierop, 1998). We could not determine whether the alkanes/alkenes were plant remains or humified organic compounds bound very strongly by the clay. Nor is it possible to say whether the preservation of these aliphatic compounds is strictly the effect of their "intrinsic recalcitrance" or due to interactions with clay minerals. For smectite the latter may be the case, and certainly when the aliphatic components are intercalated in the interlayers as Theng *et al.* (1986) have previously found.

Table 2.6. Composition of kaolinite- and smectite-associated soil organic matter.

	Polysacch.	Proteins	Lipids	Lignin	Aromatics/Phenols
Kaolinite	+++	+	++++	+	+
Smectite	++	+	++++	+	++

Furthermore, the results of both Py-GC/MS and NMR indicate that kaolinitic soils are relatively rich in polysaccharides, while smectitic soils contain relatively many aromatic and phenolic compounds. Our Py-GC/MS data of smectite-associated SOM agree with those of Leinweber *et al.* (1999). They found that the total SOM fraction of smectite-dominated soils contains relatively many aromatic products.

We suggest that different clay minerals preserve different kinds of organic components. This might be because different binding mechanisms are operative in the clay-organic interaction. However, the compositional differences may also be caused by a difference in

hydrology. Kaolinite-dominated soils are generally found in higher areas of a landscape, and are well aerated. Under these conditions, organic matter can be mineralized easily, and turnover will be fast. Smectite-dominated soils are frequently found in depressions where water can stagnate regularly, limiting aeration of the soil. Under these circumstances, microbial activity is restricted, the turnover of organic matter reduced, and humified components may accumulate. We did not have kaolinitic and smectitic samples at our disposal with a similar hydrology to test this postulate.

## **Conclusions**

We have analyzed the clay-associated organic matter of kaolinite- and smectite-rich soils originating from seven different countries. The results indicate that:

- the content of carbon in the clay-size fraction showed no significant difference between kaolinitic and smectitic soils. This suggests that the amount of organic carbon in the clay-size fraction is independent of the clay mineralogy.
- kaolinite-associated SOM was to a large extent extractable by NaOH. In the smectitic soils, pyrophosphate extracted more C than NaOH. It seems that smectitic clay minerals preferentially bind organic matter through cationic bridges.
- the associated organic matter of both clays is dominated by aliphatic components that are not extractable by NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$ .
- kaolinite-associated SOM is rich in polysaccharide products and smectite-associated SOM in aromatic compounds. We suggest that different clay minerals preserve different kinds of organic components. This might be because different binding mechanisms are operative in the clay-organic interaction although differences in soil hydrology and aeration may also contribute.

## **Acknowledgements**

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## **Chapter 3**

### **Mean residence time of kaolinite- and smectite-associated soil organic matter**

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### Abstract

We analyzed the  $^{14}\text{C}$  activity of clay-associated organic matter of kaolinite- and smectite-dominated soils from seven different countries. The soils originated from natural savanna systems. Assuming that carbon inputs and outputs are in equilibrium in such soils, we took the  $^{14}\text{C}$  age as mean residence time of the organic carbon. We corrected the  $^{14}\text{C}$  activity for the Suess effect, Bomb effect and difference between date of sampling and date of  $^{14}\text{C}$  measurement. Kaolinite-associated soil organic matter had a fast turnover (360 years on average). Smectite-associated soil organic matter had a relatively slow turnover, with an average mean residence time for the whole clay-size fraction of 1100 years. Differences in turnover times between organic matter associated to kaolinite and smectite were significant. Multiple linear regression indicates that clay mineralogy is the main factor explaining differences in the mean residence time of the extracted soil organic matter.

### Introduction

The past decades, the study of the carbon cycle has attracted great interest due to concern about global warming from the increasing atmospheric  $\text{CO}_2$  concentration. In the global C cycle, soil organic matter (SOM) is a major source/sink of atmospheric C. Soil organic matter is highly heterogeneous and consists of numerous components. These range from easily mineralizable sugars to recalcitrant aliphatics. Residence times of C in these soil organic compounds vary from a few minutes to thousands of years (Trumbore, 1993; Lichtfouse *et al.*, 1995; Torn *et al.*, 1997).

Models that describe the carbon cycle usually differentiate between at least two pools of SOM, e.g. a labile and a stable pool. There is no method available to physically separate and quantify labile and stable SOM. Turnover times are usually estimated on the basis of  $^{14}\text{C}$  dating of fractions (e.g. Buyanovsky *et al.*, 1994), modeling (e.g. Parton *et al.*, 1988), changes in  $^{13}\text{C}$  signatures (e.g. Balesdent *et al.*, 1988), or a combination of these methods. Labile SOM pools appear to have a mean residence time (MRT) of minutes to decades, and stable pools have a mean residence time of hundreds to thousands of years (Hsieh, 1992, and references cited therein).

The large variations in turnover times of the stable pool, which makes up 35 to 90% of all SOM, are partly related to climatic conditions. The MRT of the stable pool is estimated to be in the range of 250 to 380 years in tropical soils, and 850 to 3000 years in temperate soils (Hsieh, 1996). Furthermore, it is widely assumed that the variations in turnover times of stable SOM are related to interactions with mineral soil material, via physical and chemical stabilization (Greenland, 1971; Martin and Haider, 1986; Theng *et al.*, 1992; Hassink, 1995; Hsieh, 1996; Parfitt *et al.*, 1997; Torn *et al.*, 1997; Römkens *et al.*, 1998).

In an earlier paper we studied the effect of clay mineralogy on the chemical composition of clay-associated SOM (Wattel-Koekkoek *et al.*, 2001a). Here we will focus on the effect of mineralogy on the residence time of clay-associated SOM. We define *clay-associated organic matter* as all organic matter present in the clay-size separate, both free and bound (Wattel-Koekkoek *et al.*, 2001). We will restrict ourselves to the SOM associated with two of the most common clay minerals at the earth's surface: kaolinite and smectite.

Kaolinite and smectite have very different characteristics. Kaolinite clays are non-expandable aluminum-silicates, built of a sheet of aluminum-hydroxide octahedra, bound to a silicium-oxide tetrahedral sheet. They show little isomorphic substitution and therefore have a low permanent

surface charge. Because they also have a relatively low specific surface area, their cation exchange capacity (CEC) is low. Smectites are expandable clays, built of a sheet of metal (usually aluminum)-hydroxide octahedra, bound on both sides to silicium-oxide tetrahedra. Due to a fair amount of isomorphic substitution and a large specific surface area, smectites have a relatively high CEC.

We analyzed the clay-associated organic matter of kaolinite- and smectite-dominated soils from seven different countries (Wattel-Koekkoek *et al.*, 2001a). We sequentially treated soils samples with NaOH to extract free and aluminum-hydroxide-bound organic components, and with Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> to extract SOM bound by cation bridges. Kaolinite-associated SOM was to a large extent extractable by NaOH. In the smectitic soils, pyrophosphate extracted more C than NaOH. We suggested that smectitic clay minerals preferentially bind organic matter through cationic bridges, and that kaolinite-associated SOM is largely free or bound to the aluminum-hydroxide surface. Furthermore, we found the associated organic matter of both clays to be dominated by aliphatic components that are not extractable by NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. The main difference in composition was that kaolinite-associated SOM was relatively rich in polysaccharide products. Smectite-associated SOM contained relatively many aromatic compounds. The different behavior towards the extractants, and the difference in chemical composition suggest there is a difference in SOM dynamics between the two clay minerals.

The objective of this investigation was to compare the mean residence time of kaolinite- and smectite-associated soil organic matter in natural systems. We used six kaolinite and six smectite-dominated soils from old savanna systems. In these systems carbon inputs and outputs are assumed to be in equilibrium, and therefore the mean residence time of the organic carbon equals its <sup>14</sup>C age. We measured <sup>14</sup>C/<sup>12</sup>C isotope ratios of kaolinite- and smectite associated SOM by Accelerator Mass Spectrometry (AMS). From the isotope ratios we calculated the <sup>14</sup>C ages, after applying a correction for the bomb effect (Harkness *et al.*, 1986). Furthermore, we estimated the importance of climate (temperature), clay mineralogy (specific surface area (SSA) and effective cation exchange capacity (ECEC)) and the chemical composition of the organic matter (NMR analyses) on the mean residence time of the SOM in the clay-size fraction using multiple linear regression.

## **Materials and methods**

### *Samples*

We selected six kaolinite-dominated and six smectite-dominated soils (Tables 3.1 and 3.2) from the collection of the International Soil Research and Information Center (ISRIC, Wageningen). The kaolinitic soils originated from Brasil (BR1 and BR2), Kenya (LABEX6), Mali (ML1 and ML8), and Mozambique (MOC4). The smectitic soils originated from Indonesia (IN25), Kenya (KE66 and LABEX17), Nicaragua (NI9), and South Africa (ZA8 and ZA9). All soils have a high base saturation, and were under natural savanna vegetation. We used the surface horizons only as these have a relatively high carbon content enabling high carbon yields during the extraction. The samples were air-dried and passed through a 2-mm sieve. The pH, organic carbon content, and particle size distribution were measured previously by the ISRIC.

Table 3.1 Site characteristics and soil analyses. <sup>a</sup> International Soil Reference and Information Center. <sup>b</sup> according to FAO (1990)

ISRIC key for sites <sup>a</sup>	Country, site	Latitude, Longitude	Parent Rock	Temp (°C)	Precip. (mm yr <sup>-1</sup> )	Vegetation	Soil Type <sup>b</sup>	Clay		pH H <sub>2</sub> O	pH KCl	CEC <sub>soil</sub> (cmol kg <sup>-1</sup> )	Base sat. (%)
								Org. C (g kg <sup>-1</sup> )	g kg <sup>-1</sup>				
BR1	Brasil, Itaguaí	22°45'S; 43°41'W	Residual from gneiss	22.9	1317	Grassland	Haplic Lixisol	150	10	4.5	4.1	3.7	49
BR2	Brasil, Aparibé	21°38'S; 42°7'W	Pre-Cambrian rocks	22.8	1234	Grassland	Haplic Lixisol	260	10	5.7	4.5	4.4	66
LABEX6	Kenya, Nairobi	1°15'N; 36°41'E	Residuals from intermediate igneous rocks	18	973	Grassland	Hamic Nitisol	660	26	5.7	6.8	19	61
ML1	Mali, Koulikoro	12°5'N; 8°24'W	Alluvial clay	26.9	1087	Woodland	Ferric Acrisol	230	09	6.2	4.9	5.9	87
ML8	Mali, N'Tentou	11°25'N; 7°53'W	Colluvium from lateritic hill	27.3	1337	Grassland	Ferric Acrisol	130	09	6.5	5.6	3.5	97
MOC4	Mozambique, Montepuez	13°9'S; 38°37'E	Residual from basic igneous rock	24.2	932	Grassland ( <i>Oxytenanthera abyssinica</i> )	Ferric Acrisol	270	23	5.9	5.3	15.9	95
IN25	Indonesia, East Java	7°37'S; 112°50'E	Residual from tuff	26.8	1316	Grassland	Pellic Vertisol	860	11	7.6	6.4	92.5	100
KE66	Kenya, Kajjido	1°49'S; 36°49'E	Residual from tuff	17.5	504	Grassland	Pellic Vertisol	600	14	7.3	6.0	55.6	100
LABEX17	Kenya, Sultan Hamud	2°8'S; 37°28'E	Undifferentiated sediment	22.6	611	Grassland	Pellic Vertisol	860	18	7.9	6.6	86	100
NI9	Nicaragua, Timal	12°15'N; 86°2'W	Alluvium from tuff	27.3	1184	Grassland	Pellic Vertisol	790	11	6.4	4.7	77.2	100
ZA8	South Africa, north of Pietermaritzburg	29°22'S; 30°24'E	Residuals from intermediate igneous rocks	18.5	928	Shrub ( <i>Accacia spp.</i> )	Pellic Vertisol	460	24	6.3	5.0	42.0	63
ZA9	South Africa, north of Pietermaritzburg	29°22'S; 30°24'E	Residuals from intermediate igneous rocks	18.5	928	Shrub ( <i>Accacia spp.</i> )	Pellic Vertisol	570	16	6.8	5.3	45.2	87

The specific surface area (SSA) of the clay-size fraction after removal of organic carbon by H<sub>2</sub>O<sub>2</sub> was measured using ethylene glycol monoethyl ether (EGME) according to Van Rееuwijk (1992).

We did not use a dispersing agent such as NaOH while separating the (whole) clay-size fractions, so that the ECEC of the fractions could be determined (Table 3.2). The ECEC (BaCl<sub>2</sub> method) of the clay-size fractions were measured for each whole clay-size fraction, using three replicates according to Van Lagen (1996). The sum of exchangeable bases was taken as ECEC, because exchangeable Al<sup>3+</sup> was expected to be negligible at the soil pH (6.2-6.4). In order to get the ECEC of the mineral phase only (ECEC<sub>min</sub>), the ECEC of the whole clay-size fraction was corrected for the ECEC of the organic matter, according to the formula

$$\text{ECEC}_{\text{min}} = \text{ECEC} - 2 \times \%C \times 2 \quad (\text{cmol/kg clay}) \quad (1)$$

assuming the ECEC of organic matter is 200 cmol/kg organic matter, and that

$$\% \text{SOM} = 2 \times \%C \quad (2)$$

where %SOM is the organic matter content of the clay-size fraction and %C is the carbon content of the clay-size fraction, assuming that organic matter is for 50% carbon (Table 3.2).

Table 3.2 Mineralogical analyses of the clay-size fraction. The clay mineralogy was measured by X-ray diffraction (relative peak area of diffractograms in %), the specific surface area (SSA) by ethylene glycol monoethyl ether (Van Rееuwijk *et al.*, 1992), the effective cation exchange capacity (ECEC) by the BaCl<sub>2</sub> method (in cmol kg<sup>-1</sup>) (Buurman *et al.*, 1996), and the effective cation exchange capacity of the mineral phase (ECEC<sub>min</sub>). n.d.=not determined

Sample	Kao- linite	Mica	Smec- -tite	SSA (m <sup>2</sup> /g)	Exchangeable cations (cmol kg <sup>-1</sup> )				ECEC (cmol kg <sup>-1</sup> )	ECEC <sub>min</sub> (cmol kg <sup>-1</sup> )
					Ca	Mg	Na	K		
<i>Kaolinitic</i>										
BR1	92	8	0	169.4 ± 3.3	3.8	2.4	0.0	0.1	6.3	0
BR2	98	2	0	170.0 ± 3.2	3.3	0.0	0.0	0.0	3.4	0
LABEX6	95	5	0	n.d.	5.9	1.7	0.0	0.3	8.0	2.4
ML1	96	4	0	245.2 ± 5.5	6.7	7.5	0.0	0.1	14.5	6.1
ML8	99	0	1	154.4 ± 4.8	7.1	4.8	0.0	0.1	12.0	0
MOC4	100	0	0	177.0 ± 2.4	7.3	5.8	0.0	0.0	13.1	1.1
<i>Smectitic</i>										
IN25	1	0	99	801.7 ± 11.9	57.1	16.0	2.0	0.0	75.2	70.6
LABEX17	20	0	80	n.d.	52.5	32.5	0.1	0.1	85.3	80.1
NI9	0	0	100	721.5 ± 3.7	44.6	29.5	0.1	0.0	74.2	68.6
KE66	1	0	99	727.8 ± 8.6	71.2	23.5	13.2	0.1	107.9	98.3
ZA8	21	0	79	498.7 ± 5.4	32.1	23.3	0.1	0.0	55.5	38.7
ZA9	21	0	79	582.1 ± 0.6	26.4	29.5	0.1	0.0	56.1	46.9

*Fractionation*

We used a combination of physical and chemical fractionation (Figure 3.1). Though clay minerals also occur in other size fractions, the largest part, with its associated organic matter, is in the clay-size ( $< 2 \mu\text{m}$ ) fraction (Christensen, 1992). We collected the clay-size fractions of all samples, using ultrasonic (full) dispersion and sedimentation. The ultrasonically dispersed clay-size fraction was shaken in 0.5 M NaOH under  $\text{N}_2$  for 24 hours (0.5 L, soil : solution = 1 : 10).

Table 3.3 Carbon distribution in the clay-size fraction its extracts, and residues. \* Std = standard deviation.

	Clay-size fraction	NaOH extract	$\text{Na}_4\text{P}_2\text{O}_7$ extract
	g C per 100 g clay		
<i>Kaolinite</i>			
BR1	3.8	1.9	0.4
BR2	2.3	1.0	0.2
LABEX6	2.1	0.9	0.1
ML1	2.1	0.7	0.3
ML8	3.0	1.0	0.4
MOC4	3.0	1.5	0.5
Average $\pm$ Std*	$2.7 \pm 0.7$	$1.2 \pm 0.5$	$0.3 \pm 0.1$
<i>Smectite</i>			
IN25	0.9	0.02	0.5
KE66	2.4	0.4	1.2
LABEX17	1.6	0.1	0.7
NI9	1.4	0.2	0.5
ZA8	4.2	0.9	1.2
ZA9	2.3	0.7	0.4
Average $\pm$ Std	$2.1 \pm 1.2$	$0.4 \pm 0.3$	$0.7 \pm 0.4$

We centrifuged the solution, and shook the residue with demineralized water for 2 hours. After centrifugation, we acidified the combined supernatants to  $\text{pH}=1$  with 0.1 M HCl and 0.3 M HF. In order to remove salts, the supernatants were dialyzed to  $\text{pH}=6$  against demineralized water. Thereafter the supernatants were freeze-dried. Next, the NaOH-residue was shaken for 24 hours with 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  under nitrogen (soil : solution = 1 : 10). The solution was centrifuged, and the residue was shaken with demineralized water for 2 hours. After centrifugation, we acidified the combined supernatants again to  $\text{pH}=1$  with a solution of 0.1 M HCl and 0.3 M HF. Both the supernatant and the pyrophosphate-residue were dialyzed and freeze-dried. The amount of

carbon in the total clay-size fractions, the freeze-dried extracts, and the residues, were measured using an Interscience elemental analyzer EA1108 (Table 3.3).

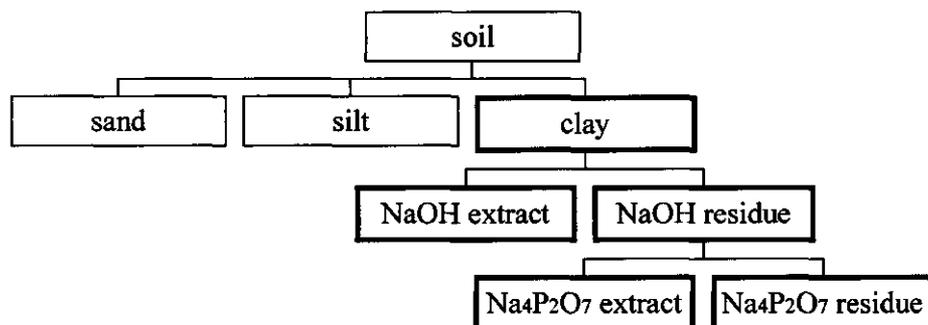


Figure 3.1 Fractionation scheme.

#### <sup>14</sup>C analysis

Hydrolysis of soil samples with 6 N HCl prior to isotope measurements is part of the standard pretreatment procedure in the Center for Isotope Research (Groningen, The Netherlands) for isotope analysis. The purpose of this procedure is to obtain the oldest fraction. The acid is assumed to hydrolyze proteins, amino sugars and carbohydrates, which are presumably compounds readily utilized by microbes. So, acid hydrolysis removes the potentially most labile, rapidly cycling carbon into solution, leaving a more refractory, slower cycling residue (Scharpenseel and Schiffmann, 1977; Anderson and Paul, 1984; Trumbore *et al.*, 1989). Since we were not interested in the fraction with the lowest possible <sup>14</sup>C concentration, but in the “true” mean age of the total SOM in the extracts and the residues, we omitted hydrolysis. This also allowed us to directly link the <sup>14</sup>C results to chemical analyses performed earlier (Wattel-Koekkoek *et al.*, 2001a). One kaolinite and one smectite sample, LABEX6 and LABEX17, were measured with and without hydrolysis.

The clay-size fractions, NaOH extracts, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts and residues were combusted at 850 °C under continuous flow of oxygen. The CO<sub>2</sub> was purified by CuO and Ag using standard methods. <sup>13</sup>C values were measured by Mass Spectrometry at the Center for Isotope Research, Groningen. Following <sup>13</sup>C analysis, the CO<sub>2</sub> was trapped cryogenically and converted to graphite for subsequent AMS analysis. The <sup>14</sup>C activity was measured by the Groningen AMS facility (Van der Plicht *et al.*, 2000). The amount of <sup>14</sup>C in soil organic matter relative to the <sup>14</sup>C concentration in the atmosphere can be used to calculate the age of the material. The measured relative <sup>14</sup>C activity (<sup>14</sup>a<sub>m</sub>) is reported in % and corrected for isotopic fractionation with <sup>13</sup>C according to Mook and Van der Plicht (1999).

$${}^{14}a_m = \frac{{}^{14}A}{{}^{14}A_0} \quad (\%) \quad (1)$$

where  $A_0$  is the original specific  $^{14}\text{C}$  activity defined by the 1950 standard and  $A$  is the measured specific  $^{14}\text{C}$  activity of the sample.

#### Correction of $^{14}\text{C}$ analyses

As the  $^{14}\text{C}$  measurements took place years after the soil samples had been taken from the field (Table 3.4),  $^{14}\text{C}$  decay had taken place. To correct the analyses, the  $^{14}\text{C}$  activity in the year of sampling ( $^{14}a_s$ ) was calculated according to:

$$^{14}a_s = \frac{^{14}a_m}{e^{-\lambda\Delta t}} \quad (\%) \quad (2)$$

where  $^{14}a_m$  is the measured  $^{14}\text{C}$  activity and  $\Delta t$  is the time (years) between the year of soil sampling and the year of the measurements and  $\lambda$  is the decay constant ( $1.210 \times 10^{-4} \text{ yr}^{-1}$ , based on the half life of 5730 years).

Table 3.4 Year of sampling and year of  $^{14}\text{C}$  analysis.

Sample	Year of sampling	Year of analysis	$\Delta t$ (yr)
<i>Kaolinite</i>			
BR1	1984, 1985	1998	14
BR2	1984, 1985	1998	14
LABEX6	1976	1997	21
ML1	1988	1998	10
MOC4	1982, 1983	1998	16
<i>Smectite</i>			
KE66	1985	1998	13
LABEX17	1985	1997	12
NI9	1992	1998	6
ZA8	1975	1998	23
ZA9	1975	1998	23

The soil samples were taken after the 1960's, and therefore influenced by both the "Suess-effect" (decrease in atmospheric  $^{14}\text{C}$  activity due to the burning of fossil fuels starting around 1850) and the "Bomb-effect" (increase in atmospheric  $^{14}\text{C}$  activity due to nuclear bomb tests in the 1960's).

Before 1850 anthropogenic effects on the atmospheric  $^{14}\text{C}$  activity can be neglected, and the  $^{14}\text{C}$  activity of carbon in a uniformly mixed pool at year  $t$  can be expressed as

$$^{14}a_t = ^{14}a_{atm} e^{-\lambda T} \quad (\%) \quad (3)$$

where  $^{14}a_{atm}$  is the natural  $^{14}\text{C}$  activity in the atmosphere (using the "single year curve" from Stuiver and Braziunas (1993)) and  $T$  is the mean  $^{14}\text{C}$  age of the carbon pool. We realize that the assumption that soil is a uniformly mixed pool is a simplification (Harkness *et al.*, 1986), but this model is our best guess. A model with multiple pools (e.g. a 'labile' and a 'stable' pool) would

require assumptions about the distribution of the carbon over the slow and fast pools, for which data is lacking.

After 1850, assuming a steady state SOM pool, the carbon decomposed is replaced by depleted (Suess) or enriched (bomb) carbon from the atmosphere. If at  $t-1$  the activity of the total carbon pool is  $^{14}a_{t-1}$ , then at time  $t$ , the activity equals

$$^{14}a_t = ^{14}a_{t-1} + ^{14}a_{in} - ^{14}a_{out} \quad (\%) \quad (4)$$

where  $^{14}a_{in}$  is the  $^{14}C$  activity 'gained' between  $t-1$  and  $t$ , and  $^{14}a_{out}$  is the  $^{14}C$  activity lost by soil organic matter decomposition and  $^{14}C$  radioactive decay. As the soils originate from old savanna systems in which carbon inputs and outputs are in equilibrium we can assume steady state with organic matter turnover at a decomposition rate  $k$  ( $yr^{-1}$ ). Then

$$^{14}a_{in} = ^{14}a_{atm,p} (1 - e^{-k}) \quad (\%) \quad (5)$$

where  $^{14}a_{atm,p}$  is the (input)  $^{14}C$  activity of the atmospheric  $CO_2$  during the preceding year  $p$ , and

$$^{14}a_{out} = ^{14}a_{t-1} (1 - e^{-(k+\lambda)}) \quad (\%) \quad (6)$$

so that

$$^{14}a_t = ^{14}a_{t-1} e^{-(k+\lambda)} + ^{14}a_{atm,p} (1 - e^{-k}) \quad (\%) \quad (7)$$

Because the samples originated from close to the equator and from the Southern Hemisphere to  $29^\circ S$ , we used the  $^{14}C$  activity in the atmosphere,  $^{14}a_{atm,p}$ , of the Southern Hemisphere measured by Manning *et al* (1992). Knowing the sampling year and  $^{14}a_s$ , the model was used to calculate  $k$  from equation 7 and the mean residence time (*MRT*) from equation 8. In Figure 3.2 the distribution of atmospheric  $^{14}C$  activity measured for the past 150 years is compared with the theoretical curves calculated for specific *MRT*'s.

$$MRT = \frac{1}{k} \quad (8)$$

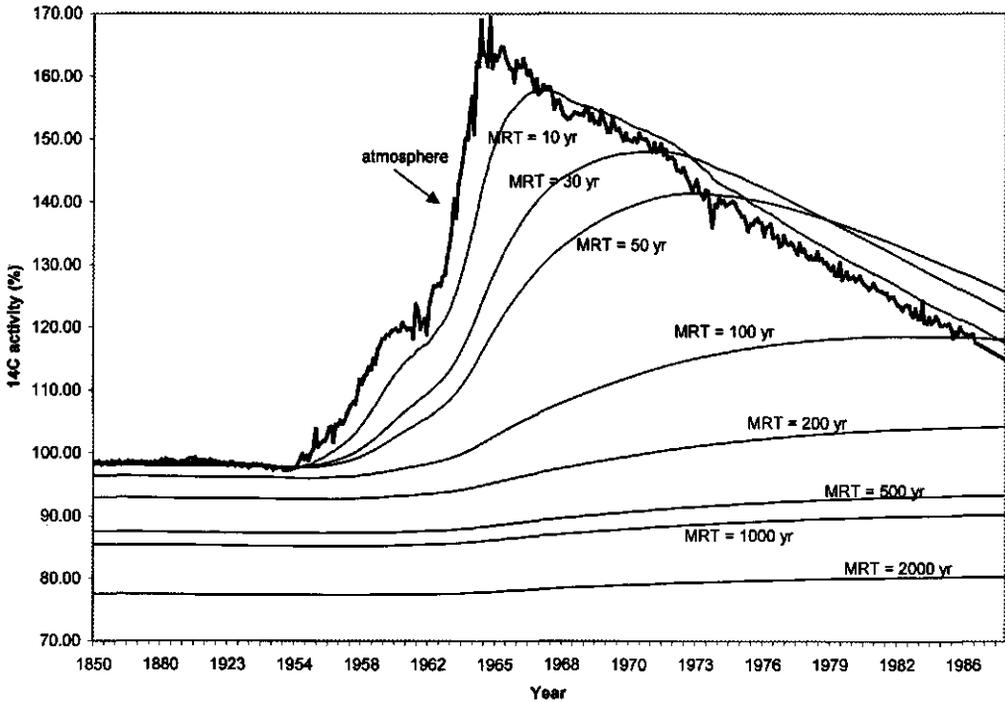


Figure 3.2 Distribution of atmospheric  $^{14}\text{C}$  activity measured on the Southern hemisphere for the past 150 years, compared with the theoretical curves calculated for specific MRT's.

We used the MRT to calculate the  $^{14}\text{C}$  activity of the sample it would have had had there been no changes in atmospheric  $^{14}\text{C}$  according to

$$^{14}a_{\text{corr}} = e^{-\lambda \text{MRT}} \quad (9)$$

where  $^{14}a_{\text{corr}}$  is the  $^{14}\text{C}$  activity corrected for the Bomb effect, Suess effect and the time difference between sampling year and measurement year.

#### Scanning electron microscopy

To check for the presence of unhumified, recognizable cell-fragments, Scanning Electron Microscope (SEM) analyses were performed on the pyrophosphate extracts and residues of BR2, ML1 (both kaolinitic), KE66 and ZA8 (both smectitic), using a Phillips 515 SEM at ATO-DLO in Wageningen.

## **Results**

### *MRTs of non-hydrolyzed clay separates*

Table 3.5 shows the measured  $^{14}\text{C}$  activity, the calculated MRT (using equations 7 and 8) and corrected  $^{14}\text{C}$  activity (using equation 9) for the whole clay-size fractions, NaOH extracts,  $\text{Na}_4\text{P}_2\text{O}_7$  extracts and residues. The averages and standard deviations concern the non-hydrolyzed fractions only. In order to calculate the average MRT, first we calculated the average activity, and from that value we calculated the average MRT (in years).

The average MRT of kaolinite-associated organic matter was  $357 \pm 242$  year. T-tests (Sokal and Rohlf, 1995) indicate the four fractions of the kaolinitic soils (clay-size fraction, NaOH extract,  $\text{Na}_4\text{P}_2\text{O}_7$  extract, residues) were not significantly different from each other. Of all kaolinitic clay-size fractions, ML1 has the fastest turnover, and LABEX6 and MOC4 the slowest.

The average MRT of smectite-associated organic matter is  $1089 \pm 315$  year. According to the t-test the pyrophosphate extracts are significantly older than the hydroxide extracts ( $p = 0.027$ ). Of all smectite-dominated samples, NI9 has a relatively short MRT and LABEX17 a relatively long MRT.

Overall, clay-size SOM of smectite-dominated soils is significantly older than clay-size SOM of kaolinite-dominated soils ( $p = 0.011$ ). Also, NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted SOM of smectite-dominated soils is significantly older than NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracted SOM of kaolinite-dominated soils ( $p=0.039$  and  $p=0.005$  respectively). The residues of the kaolinite- and smectite clays were not significantly different ( $p = 0.120$ ).

As the t-test can only be used in case of a normal distribution, and we have too few data determine whether this is the case, we repeated all calculations using the distribution-free Mann-Whitney test. Also according to this test, the clay-size SOM of smectite-dominated soils is significantly older than clay-size SOM of kaolinite-dominated soils ( $p = 0.029$ ). Also, NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted SOM of smectite-dominated soils is significantly older than NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracted SOM of kaolinite-dominated soils ( $p=0.016$  and  $p=0.008$  respectively). The residues of the kaolinite- and smectite clays were not significantly different ( $p = 0.151$ ).

To indicate the level of accuracy of this experiment, we calculated the weighted  $^{14}\text{C}$  activity of the clay-size fraction based on the carbon distribution (Table 3.3) and the measured (corrected)  $^{14}\text{C}$  activity of the NaOH extracts,  $\text{Na}_4\text{P}_2\text{O}_7$  extracts and residues (Table 3.5). Furthermore, we calculated the difference ( $\Delta$ ) between the corrected  $^{14}\text{C}$  activity of the whole clay-size fraction and the calculated weighted  $^{14}\text{C}$  activity (Table 3.6). The large  $\Delta$  for KE66 and ZA9 suggests that the mass balance and/or the  $^{14}\text{C}$  data are inaccurate. The  $\Delta$ 's of the other samples fall within the indicated standard deviation of the measurements.

Table 3.5 Measured and corrected  $^{14}\text{C}$  activity and mean residence time (MRT, yr) of the clay size fractions, extracts, and residues. LABEX6H and LABEX17H are the hydrolyzed fractions of LABEX6 and LABEX17 respectively. n.d. = not determined

	Clay (< 2 $\mu\text{m}$ )				NaOH extract				$\text{Na}_2\text{P}_2\text{O}_7$ extract				Residue				
	MRT		$t_{\text{R,cor}}$		MRT		$t_{\text{R,cor}}$		MRT		$t_{\text{R,cor}}$		MRT		$t_{\text{R,cor}}$		
	$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		$^{14}\text{a}_{\text{m}}$		
<b>Kaolinite</b>																	
BR1	116.40 $\pm$ 0.60	225	97.2	122.45 $\pm$ 0.63	153	98.1	119.30 $\pm$ 0.62	188	97.7	115.87 $\pm$ 0.60	232	97.2					
BR2	109.58 $\pm$ 0.59	341	95.8	117.57 $\pm$ 0.61	209	97.4	114.23 $\pm$ 0.60	256	96.9	96.57 $\pm$ 0.54	782	90.7					
LABEX6H	96.17 $\pm$ 0.71	739	91.2	99.21 $\pm$ 0.50	600	92.8	96.86 $\pm$ 0.65	705	91.6	89.30 $\pm$ 0.90	1153	86.6					
LABEX6	n.d.			106.09 $\pm$ 0.37	378	95.4	101.39 $\pm$ 0.37	516	93.8	94.90 $\pm$ 0.37	805	90.5					
ML1	121.49 $\pm$ 0.62	158	98.1	123.40 $\pm$ 0.63	136	98.3	119.06 $\pm$ 0.62	188	97.7	120.25 $\pm$ 0.62	173	97.9					
MOC4	98.88 $\pm$ 0.55	712	91.5	103.80 $\pm$ 0.56	485	94.1	98.79 $\pm$ 0.54	671	92.0	101.83 $\pm$ 0.55	551	93.4					
Average		357	95.7		273	96.7		360	95.6		502	93.9					
St.dev.		242	2.9		151	1.8		214	2.6		294	3.5					
<b>Smectite</b>																	
KE66	90.69 $\pm$ 0.53	1124	86.9	103.64 $\pm$ 0.56	498	94.0	77.89 $\pm$ 0.50	2162	76.4	96.93 $\pm$ 0.54	767	90.9					
LABEX17H	58.56 $\pm$ 0.63	4409	57.8	89.54 $\pm$ 0.58	1202	86.1	55.75 $\pm$ 0.62	4779	55.2	52.23 $\pm$ 0.57	5306	51.7					
LABEX17	n.d.			86.33 $\pm$ 0.32	1419	83.8	62.61 $\pm$ 0.25	3848	61.9	72.69 $\pm$ 0.29	2679	71.6					
NI9	96.45 $\pm$ 0.54	803	90.5	106.54 $\pm$ 0.57	421	94.9	90.96 $\pm$ 0.53	1120	87.0	107.75 $\pm$ 0.58	390	95.3					
ZA8	92.83 $\pm$ 0.53	906	89.3	100.04 $\pm$ 0.56	550	93.4	88.30 $\pm$ 0.51	1208	86.0	93.31 $\pm$ 0.53	877	89.7					
ZA9	84.14 $\pm$ 0.51	1532	82.6	94.29 $\pm$ 0.56	821	90.3	79.43 $\pm$ 0.50	1946	78.5	85.80 $\pm$ 0.51	1397	84.0					
Average		1089	87.3		733	91.3		2000	78.0		1184	86.3					
St.dev.		315	3.5		389	4.5		977	10.1		810	9.2					

*Mean residence time of clay-associated organic matter*

Table 3.6 Comparison of corrected and weighted MRT of the total clay-size fraction.

	Corrected		Weighted average		$\Delta$ (Corrected - Weighted)
	$^{14}\text{a}$ (%)	MRT (yr)	$^{14}\text{a}$ (%)	MRT (yr)	$^{14}\text{a}$ (%)
<i>Kaolinite</i>					
BR1	97.2 ± 0.60	225	97.7 ± 1.85	187	-0.5
BR2	95.8 ± 0.59	341	94.1 ± 1.75	487	1.7
ML1	98.1 ± 0.62	158	98.0 ± 1.87	162	0.1
MOC4	91.5 ± 0.55	712	93.4 ± 1.65	545	-1.9
<i>Smectite</i>					
KE66	86.9 ± 0.53	1124	84.3 ± 1.60	1368	2.6
NI9	90.5 ± 0.54	803	92.5 ± 1.68	629	-2.0
ZA8	89.3 ± 0.53	906	89.4 ± 1.60	903	-0.1
ZA9	82.6 ± 0.51	1532	85.0 ± 1.57	1302	-2.4

*MRTs of hydrolyzed clay separates*

Hydrolysis generally removed relatively young carbon, resulting in a decreased the  $^{14}\text{C}$  activity. Hydrolyzed fractions of LABEX6 and LABEX17 were invariably older than the non-hydrolyzed fractions, except for the NaOH extract of smectite (1202 years BP with hydrolysis and 1419 years BP without hydrolysis). The age difference between the hydrolyzed and the non-hydrolyzed samples was especially large for the residues. The organic matter in the residue of LABEX6 (kaolinite) had an MRT of 1153 years BP with hydrolysis and 805 years BP without hydrolysis. The SOM in the residue of LABEX17 (smectite) had an MRT of 5306 years BP with hydrolysis and 2679 years BP without hydrolysis. As a consequence, the residues contain the oldest fraction, followed by the pyrophosphate extracts and the hydroxide extracts respectively (Table 3.5).

*SEM*

SEM images (not shown) were used to determine whether the measured isotopic data reflect the  $^{14}\text{C}$  activity of recognizable plant remnants or of completely humified, i.e. unrecognizable amorphous material. The images of  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted SOM of smectitic samples KE66 and ZA8 are dominated by 'amorphous' organic components, i.e. without recognizable cell structures. The pyrophosphate extract of kaolinitic samples BR2 and ML1 contained plant tissue fragments. Both showed some amorphous material, though less than in the  $\text{Na}_4\text{P}_2\text{O}_7$ -extracts from smectites. The pyrophosphate-residues of smectitic samples KE66, ZA8, and kaolinitic samples BR2 and ML1 are dominated by plant fragments and hollow spheres (approximately 2  $\mu\text{m}$  in diameter) probably originating from microbes. We did not obtain SEM images from the NaOH extracts.

## Discussion

### *MRTs of non-hydrolyzed clay separates*

The fast turnover of kaolinite-associated organic matter ( $357 \pm 242$  yr) suggests that the bulk of the organic matter is not, or only weakly bound to the mineral surfaces in kaolinite-dominated soils (such as iron-oxides or the edges of the octahedral sheets of kaolinites).

Our findings agree with those of Shang and Tiessen (1997), who studied the stability of organic matter in an Oxisol. Oxisols are strongly weathered soils that contain kaolinite and iron-oxides. The most stable organic fraction they found in the Oxisol had a  $^{14}\text{C}$  age of only 100 years. Overall the organic matter in this soil was labile i.e. there is no slow pool (as defined by Parton *et al.*, 1987).

Previously Wattel-Koekkoek *et al.* (2001a) showed that NaOH and pyrophosphate-extracted SOM from kaolinite is rich in sugars. Sugars are relatively easy to decompose, so their presence suggests that kaolinite-associated SOM has a relatively fast turnover. This is confirmed by the short mean residence time of the NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts of kaolinite.

The residues mainly consist of aliphatic components (Wattel-Koekkoek *et al.*, 2001a), which are relatively resistant to decomposition (De Leeuw and Largeau, 1993; Lichtfouse *et al.*, 1995). Our  $^{14}\text{C}$  data however, indicate that despite their inherent recalcitrance, the aliphatic residues associated to kaolinite do not have a significantly slower turnover time than the NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts.

Of all kaolinitic soils, ML1 had the fastest turnover. This could be related to the mean annual air temperature of the profile, which is relatively high and possibly enhancing the decomposition process. The clay-associated SOM of both LABEX6 and MOC4 had a relatively slow turnover rate. For LABEX6 this could also be related to the temperature, which is relatively low.

The slow turnover of MOC4 could be related to its high content of aromatic C (Wattel-Koekkoek *et al.*, 2001a), which is resistant to decomposition.

Smectite-associated SOM had a relatively slow turnover ( $1089 \pm 315$  yr). A large residence time of smectite-associated SOM agrees with earlier studies on 2:1 clay minerals. Buyanovski *et al.* (1994) used data from Balesdent *et al.* (1988) on montmorillonitic soils, to calculate the turnover of various size fractions. They found MRTs for the clay-size fraction in the range of 1000 to 1600 years. Slower turnover was observed by Theng *et al.* (1992), who reported a  $^{14}\text{C}$  age of 5680 years for the C in the clay-size fraction of a mica-beidellite from a Spodosol.

The pyrophosphate extracts of the smectitic clays had a long MRT. This agrees with previous results that indicated this fraction is bound by cation-bridges and contains relatively many aromatic components and is therefore difficult to decompose (Wattel-Koekkoek *et al.*, 2001a).

The SEM images of the pyrophosphate extracts of kaolinite and smectite also indicated a difference in type of organic matter. Smectites showed mostly amorphous organic matter, indicating a high stage of humification, and thus slow turnover. The pyrophosphate extracts of kaolinites contained relatively many recognizable plant remnants, suggesting incomplete humification, and a fast turnover.

There are large variations in residence time of the pyrophosphate-residues of the smectite-dominated soils (from 250 to 2560 years). These variations may be related to the amount of debris from plant and microbial cells present in the residues, as shown by the SEM. Due to its slow sedimentation, non-complexed organic matter will end up in the clay-size fraction (even when larger than 2  $\mu\text{m}$ ) during the separation procedure. As these plant remnants hardly dissolve in NaOH or  $\text{Na}_4\text{P}_2\text{O}_7$ , they remain behind in the residues. The large variation in turnover times for the residues may be related to the amount of debris: the more debris, the younger the  $^{14}\text{C}$  age. However, we were not able to test this hypothesis by SEM for lack of sufficient residual material and the limited number of samples studied.

Of all smectite-dominated samples, NI9 had a relatively short turnover time. NI9 originates from an area with a relatively high mean annual temperature, which may increase decomposition. LABEX17 had a relatively large MRT. We do not know what causes the slow turnover.

We calculated (multiple) linear regressions ( $n = 14$ ), using  $^{14}a_{\text{corr}}$  of the extracts as dependent variable and the following independent variables:

- effective cation exchange capacity of the mineral phase of the clay-size fraction ( $ECEC_{\text{min}}$ ), as a parameter for clay mineralogy
- specific surface area (SSA), also as a parameter of clay mineralogy
- *temperature* (mean annual), as a parameter for the climate
- *type of extract* (1 for NaOH extracts and 0 for  $\text{Na}_4\text{P}_2\text{O}_7$  extracts), as a measure for the binding mechanisms assuming that NaOH loosens different bonds than  $\text{Na}_4\text{P}_2\text{O}_7$
- percentage of *alkyl C*, *O-alkyl C*, *aromatic C* and *carbonyl C* (based on NMR analyses) in the extracts as a characteristic for the chemical composition of the organic matter.

The independent variables were partly determined earlier (Wattel-Koekkoek *et al.*, 2001a). We did not use the values of the  $^{14}a_{\text{corr}}$  of the whole clay-size fractions for the regressions because we did not have NMR data on those fractions.

When using only one of the NMR components as independent variable (*O-alkyl*, *alkyl*, *carbonyl*, or *alkyl C*), *O-alkyl* is the best explaining factor for the variance in  $^{14}\text{C}$  activity, explaining 34.1% (Table 3.7). Furthermore, we see a negative correlation between *aromatic C* and  $^{14}\text{C}$  activity. It seems plausible that aromatic C gives a lowering in  $^{14}\text{C}$  activity and thus a slower turnover because it is relatively resistant to decomposition. *Carbonyl C* also shows a negative correlation. Carbonyl groups may cause a lowering in decomposition speed because they can form bonds with exchangeable cations at the clay surface, thereby restricting decomposition.

The *type of extract* (the number "1" was assigned to NaOH extracts and "0" to pyrophosphate extracts) shows a positive correlation, indicating that the NaOH extract contains young, easily decomposable SOM. As suggested previously, *temperature* shows a positive correlation with  $^{14}\text{C}$  activity of the extracts, explaining 22.5% of the variance.

The best explaining factors for the variance in  $^{14}\text{C}$  activity were the SSA (49.9%) and the  $ECEC_{\text{min}}$  (42.8%), both giving a negative correlation with the  $^{14}\text{C}$  activity. This supports our hypothesis that clay mineralogy effects organic matter decomposition. Clay minerals with a high  $ECEC_{\text{min}}$ , like smectites, have relatively slow organic matter turnover as the exchangeable

cations enable the clays to bind organic matter, while clay minerals with a low ECEC, like kaolinites, have relatively fast organic matter turnover, as the ability to bind SOM is much less. Clay minerals with a large surface area have plenty of space to bind SOM e.g. by Van der Waals forces, while clays with little SSA have less space to bind SOM.

Table 3.7 Percentage of variance explained (%), correlation coefficients and coefficient of determination ( $R^2$ ) after (multi) linear regression analyses using different combinations of independent variables to predict the corrected  $^{14}\text{C}$  activity of the NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts.

Independent variables	% of variance explained	Correlation coefficient	$R^2$
O-alkyl C (%)	34.1	0.32	0.39
Aromatic C (%)	29.6	-0.35	0.35
Alkyl C (%)	0	0.46	0.07
Carbonyl C (%)	24.6	-0.72	0.30
ECEC <sub>min</sub> (cmol/kg)	42.8	-0.14	0.47
SSA (m <sup>2</sup> /g)	49.9	-0.02	0.54
Temperature (°C)	22.5	1.09	0.28
Extract (1/0)*	16.9	6.85	0.23
O-alkyl (%)	21.3	O-alkyl 0.42	0.39
Aromatic C (%)		Aromatic C 0.14	
Alkyl C (%)		Alkyl C 0.07	
Alkyl C (%)	70.4	Alkyl C 0.57	0.80
SSA (m <sup>2</sup> /g)		SSA -0.02	
Temperature (°C)		Temperature 0.76	
Extract (1/0)		Extract 3.68	
Alkyl C (%)	66.9	Alkyl C 0.62	0.77
ECEC <sub>min</sub> (cmol/kg)		ECEC -0.10	
Temperature (°C)		Temperature 0.74	
Extract (1/0)		Extract 3.98	

The NMR data are not completely independent: they are relative peak areas and therefore together always 100%. We therefore made several regressions omitting one of the NMR components at the time, using the other 3 components as independent variables. We found that the combination of three components that gave the best explanation, was *O-alkyl*, *aromatic C*, *alkyl C* (21.3%). The main explaining factor again was *O-alkyl*. *O-alkyl* carbon is mainly present in polysaccharides, and easy to decompose, probably therefore resulting in a high  $^{14}\text{C}$  activity.

When using all independent variables available, 48.2% of the variance in  $^{14}\text{C}$  activity of the extracts can be explained. We tried all possible combinations of the independent variables and found that the optimal explanation was reached when using *SSA*, *alkyl %*, *temperature*, and *extract type* as input variables (70.4% of the variance explained), of which *SSA* is by far the most important factor explaining  $^{14}\text{C}$  variance. The second best set of variables was *ECEC<sub>min</sub>*, *alkyl-C %*, *temperature*, and *extract type*, explaining 66.9% of the variance (Table 3.7).

When *SSA* or *ECEC<sub>min</sub>* are used as independent variables, the percentage of *alkyl-C*, and not *O-alkyl*, is the best predicting factor of the chemical data available. Addition of any other NMR-based variable lowers the percentage of variance explained. This suggests there is a relation between clay mineralogy and % alkyl associated to the clay. Teng *et al.* (1986) found that

aliphatic components are intercalated in the interlayers of smectites. However, it seems improbable that the aliphatic components that we found in the extracts were intercalated, as intercalation is an extremely strong type of bond that is unlikely to be dissolved by NaOH or  $\text{Na}_4\text{P}_2\text{O}_7$ .

It is remarkable that the percentage of alkyl-C shows a positive correlation with  $^{14}\text{C}$  activity. Aliphatic components are relatively resistant to decomposition (De Leeuw and Largeau, 1993; Lichtfouse *et al.*, 1995), and therefore we expected a negative correlation with  $^{14}\text{C}$  activity. A possible explanation for the positive correlation is that the extracts contain 'free' plant remains. These remains may mainly exist of cuticles and root-remains, which are known to contain aliphatic polymers like cutan and suberan (Nip *et al.*, 1986; Nierop, 1998; de Leeuw and Largeau, 1993).

#### *MRTs of hydrolyzed clay separates*

In general, hydrolyzed samples had a lower  $^{14}\text{C}$  activity than non-hydrolyzed samples. This agrees with previous research that indicated hydrolysis removes labile, rapidly cycling carbon, leaving behind a more refractory, slower cycling residue (Trumbore *et al.*, 1989; Trumbore *et al.*, 1996). We cannot explain why hydrolysis apparently rejuvenated the hydroxide extract of LABEX17.

#### **Conclusion**

By comparing the  $^{14}\text{C}$  activity of soil organic matter present in the NaOH extract,  $\text{Na}_4\text{P}_2\text{O}_7$  extract and residue of the clay-size fractions ( $<2 \mu\text{m}$ ) of kaolinite- and smectite-dominated soils originating from seven countries, we showed that

- kaolinite and smectite-associated SOM have significantly different turnover times
- clay mineralogy is the most important factor to explain variance in the mean residence time of the NaOH- and  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted organic matter

#### **Acknowledgements**

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## **Chapter 4**

### **Physical and chemical fractionation and characterization of clay-bound organic matter in kaolinitic and smectitic soils from Mozambique**

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submitted to *Geoderma*

## Abstract

Two kaolinite- and two smectite-dominated soils from a native savannah in Mozambique were studied in order to determine the difference in amount and molecular composition of kaolinite- and smectite-bound organic matter. The amount of soil organic matter (SOM) bound was independent of clay mineralogy. The extractability by NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> (sequentially) of the clay-bound organic matter also showed no significant difference between the clays: 50% of the clay-bound SOM was extracted by NaOH and thereafter about 15% by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. The SOM of all four soils was dominated by polysaccharides. The smectitic soils seem to contain slightly more aliphatic components than the kaolinitic soils. Aromaticity varied among the four soils. Results disagree with previously found differences, suggesting there is probably no relationship between amount and composition of clay-bound SOM and clay mineralogy.

## Introduction

Soil organic matter (SOM) is a major source/sink of atmospheric carbon, and therefore an essential pool of the carbon cycle. Part of the soil organic matter is stabilized through mineral-organic matter binding (Chesire *et al.*, 2000; Christensen, 1992; Christensen, 1996; d'Acqui *et al.*, 1998; Schulten and Leinweber, 2000). This research aims at determining the effect of clay mineralogy on the amount and composition of organic matter that is bound to the mineral surface. We focused on organic matter that is bound to kaolinite and smectite. Smectite is particularly common in Vertisols. Kaolinitic minerals occur in a wide range of weathered tropical soil, such as Oxisols, Ferralsols, Nitisols, Acrisols (FAO, 1990).

Previously we characterized kaolinite- and smectite-associated SOM (= all SOM present in the 'clay-size' fraction) in soils from seven countries, employing <sup>13</sup>C NMR spectroscopy and Py-GC/MS (Wattel-Koekkoek *et al.*, 2001a, from now on also referred to as 'the ISRIC experiment'). The content of carbon in the total clay-size fraction showed no significant difference between kaolinitic and smectitic soils, suggesting that the total amount of organic carbon in the clay-size fraction is independent of clay mineralogy.

For the ISRIC experiment, we first extracted the clay fraction with NaOH and thereafter with Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. About half of the kaolinite-associated SOM was extractable by NaOH. In the smectitic soils, pyrophosphate extracted more organic carbon than did NaOH. The results of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and nuclear magnetic resonance (<sup>13</sup>C CPMAS NMR) indicated that kaolinite-associated SOM was enriched in polysaccharide products, while smectite-associated organic matter contained many aromatic compounds. It was suggested that different clay minerals use different binding mechanisms to complex SOM. As a result, the composition of clay-associated organic matter would be influenced by the type of clay that is dominantly present in the soil.

However, in that experiment the variation between the different samples of one group was large. We think the variation is related to two factors. First, the samples originated from 7 different countries. Second, the whole clay-size fraction was studied, including its 'free' organic matter. In order to get a better understanding of the effect of clay mineralogy on organic matter composition, it is necessary to focus on clay-bound organic matter.

To eliminate the geographical effect, we will use samples of one region only. We chose an undulating landscape near Montepuez, Mozambique, which holds soils that are dominated by

smectite (in the depressions) and soils that are dominated by kaolinite (in the higher areas). To eliminate the possible effect of particulate organic matter, we will study clay-bound instead of clay-size organic matter.

## Methods

### *Samples*

We sampled six Nitisols and four Vertisols, from which we selected two sets of two soils due to limited laboratory capacity (Table 4.1), from an area west of Montepuez, near the village N'Ropa. One set contained soils with clay-size fractions dominated by kaolinite clays (RED3 and RED5), while the other set had clay-size fractions dominated by smectite clays (VER3A and VER3B).

Table 4.1 Site characteristics.

	RED3	RED5	VER3A	VER3B
Latitude S	38°40'71"	38°41'26"	38°38'91"	38°38'81"
Longitude E	13°11'04"	13°10'52"	13°12'26"	13°11'71"
T mean, annual (° C)	24.2	24.2	24.2	24.2
Precipitation, annual (mm)	932	932	932	932
Parent Rock	Residue from basic igneous rock		Alluvium	
Vegetation	Bambooforest ( <i>Oxythenantera</i> sp.)		Digitaria, Laudetia, Hyperrennia; poaceae <80%	
Drainage	well	well	imperfectly drained	imperfectly drained
Soil Type	Rhodic Nitisol	Rhodic Nitisol	Eutric Vertisol	Eutric Vertisol
Clay (%)	39.5	35	23.9	43.4
Organic C (g /100 g soil)	4.2	2.7	1.6	2.2
pH H <sub>2</sub> O	6.2	6.2	6.3	6.4
pH KCl	5.3	5.4	4.8	4.4
CECsoil (cmol/kg)	23.9	18.1	16.8	30.3
Base Sat. (%)	82.8	86.7	83.3	83.8
Remarks	Signs of burning (charcoal) in all profiles			

Clay mineralogy was determined by X-ray diffraction (XRD) of oriented samples of the clay-size fractions. The diffractograms were obtained on a Philips PW1820/PW1710 diffractometer, using a Co X-ray tube at 40 kV and 30 mA, with a focussing monochromator. The divergence slit was set at 1°, the receiving slit at 0.2 mm, and the anti-scatter slit at 1°. Peak areas of the clay minerals were measured to compare the (semi-quantitative) XRD diffractograms and are reported in % of total peak area (Table 4.2). The two sets of two samples were selected because they had the highest % peak area from kaolinite or smectite. All samples contained small quantities of quartz.

All the soils have a high base saturation. We only used the SOM-rich surface horizons.

Table 4.2 Mineralogical analyses of the clay-size fraction. The clay mineralogy was measured by XRD (relative peak area of diffractograms in %). The effective cation exchange capacity of the whole clay-size fraction (ECEC) was determined by the BaCl<sub>2</sub> method (in cmol kg<sup>-1</sup>) (Van Lagen, 1996). The effective cation exchange capacity of the mineral phase of the clay-size fraction only (ECEC<sub>min</sub>) was calculated according to Wattel-Koekkoek *et al.* (2001b).

Sample	Kaolinite	Smectite	Exchangeable cations (cmol kg <sup>-1</sup> )				ECEC (cmol kg <sup>-1</sup> ) SUM	ECEC <sub>min</sub> (cmol kg <sup>-1</sup> )
			Ca	Mg	Na	K		
<i>Kaolinitic</i>								
RED3	93	7	13.5	6.7	0.4	1.6	22.2	5.8
RED5	96	4	15.1	7.5	0.7	1.6	24.9	11.3
<i>Smectitic</i>								
VER3A	18	82	19.5	15.7	1.4	0.4	37.0	24.6
VER3B	13	87	17.2	24.6	0.8	0.3	42.9	34.1

The samples were air-dried and passed through a 2-mm sieve. The pH, organic carbon content, CEC<sub>soil</sub>, and particle size distribution were measured according to Buurman *et al.* (1996). The ECEC (BaCl<sub>2</sub> method) of the clay-size fractions were measured for each whole clay-size fraction, using three replicates according to Van Lagen (1996). The sum of exchangeable bases was taken as ECEC, because exchangeable Al<sup>3+</sup> was expected to be negligible at the soil pH (6.2-6.4). In order to get the ECEC of the mineral phase only (ECEC<sub>min</sub>), the ECEC of the whole clay-size fraction was corrected for the ECEC of the organic matter, according to Wattel-Koekkoek *et al.* (2001b).

#### Fractionation

We used a combination of physical and chemical fractionation (Figure 4.1). Though clay minerals also occur in other size fractions, the largest part, with its associated organic matter, is in the clay-size fraction (Christensen, 1992). Therefore, we first collected the clay-size (< 2 μm) fractions of all samples, using ultrasonic (full) dispersion and sedimentation in water. To ensure complete ultrasonic disruption, we measured mass recovery curves using different values of applied energy against the amount of clay using the method described by Roscoe *et al.* (2000). To reach maximum recovery of the clay-size fraction, we measured that 430 J/ml was needed for RED3 and RED5, and 365 J/ml was needed for VER3A and VER3B, using a soil:solution ratio of 1:10.

After sedimentation, the clay-size fractions were freeze-dried. For all four samples 100 g of clay-size fraction was accumulated. The dried clay-size fractions were separated into a light and a heavy fraction using a NaI solution with a density of 1.7 g cm<sup>-3</sup> (Roscoe *et al.*, 2001). Of each clay-size fraction, 50 gram was placed in a 500 ml centrifuge tube with 500 ml NaI solution, shaken end-over-end for 60 minutes, and left standing at room temperature for 15 minutes. After centrifugation (15 minutes, 3500 rpm), the supernatant was filtered through a membrane filter (Whatman, GF/A) into a millipore vacuum unit. The fraction recovered on the filter was washed with 0.01 M CaCl<sub>2</sub> solution (100 ml) and distilled water (200 ml). This fraction was called the "light clay-size fraction", or "free" organic matter. The sediment was resuspended in NaI and centrifuged two more times as described above. The three subfractions were joined, oven-dried at 50 °C and stored for analyses. The heavy fraction was flocculated one time with 0.01 M CaCl<sub>2</sub>

and about 10 times with distilled water until the clay fraction remained in suspension after 24 hours. The heavy clay fraction was freeze-dried. This procedure was repeated twice for each soil, to separate all 100 g of clay per soil sample.

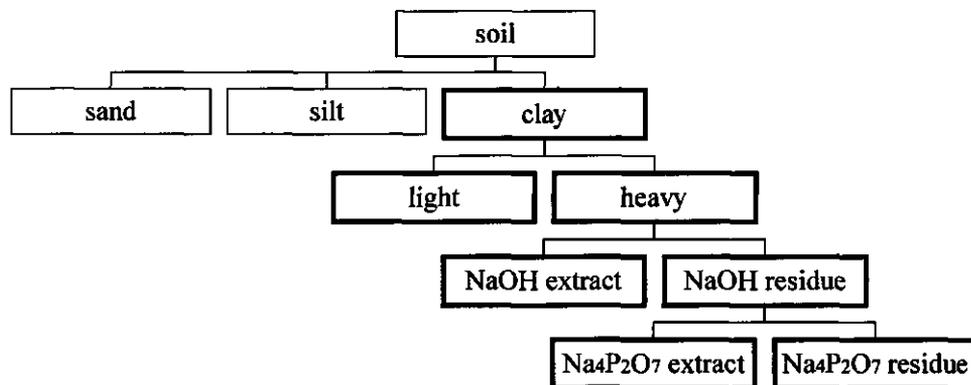


Figure 4.1. Fractionation scheme.

The heavy clay-size fraction was shaken in 0.5 M NaOH under  $N_2$  for 24 hours (soil : solution = 1 : 10). We centrifuged the solution, and shook the residue with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants (=NaOH extract) to pH=1 with concentrated HCl. Concentrated HF was added until a concentration of 0.3 M HF was reached. The supernatant was dialyzed to pH=6 against deionized water, and freeze-dried. Next, the NaOH-residue was shaken overnight with 0.1 M  $Na_4P_2O_7$  under nitrogen (soil : solution = 1 : 10). The solution was centrifuged, and the residue was shaken with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants (=pyrophosphate extract) again to pH=1 with concentrated M HCl. Thereafter concentrated HF was added until a concentration of 0.3 M HF was reached. Both the supernatant and the pyrophosphate-residue were dialyzed and freeze-dried.

We measured the amount of carbon in the total and heavy clay-size fraction, the freeze-dried extracts, and the residue, using an Interscience elemental analyzer EA1108.

#### <sup>13</sup>C NMR spectroscopy

We used Cross Polarization-Magic Angle Spinning (CP-MAS) solid-state <sup>13</sup>C NMR spectroscopy to characterize the hydroxide- and pyrophosphate-extracts. The spectra were obtained on a Bruker AMX 300 spectrometer at a frequency of 75.478 MHz, with an acquisition time of 0.033 s and a spinning rate of 54 Hz. The residues were not measured because they contained large amounts of minerals that would have perturbed the NMR spectra (Oades *et al.*, 1987). The subdivision of the spectra follows the commonly used scheme (Hatcher, 1987; Kögel-Knabner, 1992): aliphatic C (0-46 ppm), O-alkyl C (46-110 ppm), aromatic C (110-160

ppm), and carbonylic C (160-210 ppm). To obtain semi-quantitative data, signal intensities were measured at different contact times as described by Van Lagen and De Jager (2001).

### Py-GC/MS

We used a Horizon Instruments Curie-Point pyrolyzer to pyrolyze the hydroxide- and pyrophosphate-extracts and residues. The samples were heated at 610 °C for 5 s in the instrument. The pyrolysis unit was connected to a Carlo Erba GC 8000 gas chromatograph. The compounds were separated by a silica column, using helium as carrier gas. The initial oven temperature (40 °C) was raised at a rate of 7 °C min<sup>-1</sup> to 320 °C and maintained at that temperature for 20 min. The end of the GC column was connected to a Fisons MD 800 mass spectrometer (mass range *m/z* 45-650, ionization energy 70 eV).

## Results

### Carbon distribution

The distribution of organic carbon over the fractions is presented in Table 4.3. Three replicates were taken of each soil to determine the carbon content of the total and the heavy clay-size fractions. Due to lack of material, the carbon content of the NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts was determined in duplicate. Therefore, no standard deviations were calculated for the extracts. The content of carbon in the total clay-size fraction varied between 2 and 4 % (mass fraction).

The yield of the light fraction, both in terms of total mass (about 2 mg light fraction out of 100 g clay-size fraction) as in terms of carbon content (on average 0.04 mg carbon in 2 mg light fraction), was negligible compared to the total mass and carbon content of the whole clay-size fraction. After NaI treatment, all the carbon present in the clay-size fraction ended up in the heavy clay fraction.

Between 62 and 67% of the carbon was recovered by hydroxide and pyrophosphate extracts together. For all four soils the amount of carbon (g) per 100g clay in the NaOH extracts is larger than in the subsequent pyrophosphate extracts. NaOH extracted about 50% and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> about 15% of the organic carbon from the clay-size fraction of the soils.

Table 4.3 Carbon distribution in the total and heavy clay-size fraction and the extracts, with density fractionation.  
\* = 3 repetitions

sample	Total clay*	Heavy clay*	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	residue
	g C per 100 g clay		g C per 100 g heavy clay		% fraction C in heavy clay		
RED3	4.1 ± 0.1	3.6 ± 0.3	2.0	0.5	54	13	33
RED5	3.4 ± 0.3	3.0 ± 0.2	1.6	0.4	53	14	33
VER3A	3.1 ± 0.1	3.1 ± 0.5	1.6	0.5	50	15	35
VER3B	2.2 ± 0.3	2.5 ± 0.3	1.2	0.4	47	15	38

<sup>13</sup>C NMR spectroscopy

Table 4.4 lists the integrated signal areas of the <sup>13</sup>C NMR spectra corresponding to the four carbon groups (aliphatic C, O-alkyl C, aromatic C, and carbonylic C). The NaOH extracts of all four heavy clay-size fractions are dominated by O-alkyl C (39-48% of the surface area). The carbonyl signals in the NaOH extracts are similar for all four soils, around 20% of the surface area. The signal of aromatic C has a value 14% for RED3 26% for RED5, 21% for VER3A and 13% for VER3B. Alkyl C seems to have a slightly stronger signal in de extracts from VER3A and VER3B than RED3 and RED5. Overall the NaOH extracts of the heavy clay fractions of kaolinite- and smectite-dominated soils show similar NMR spectra.

The spectra of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of the kaolinite-dominated heavy clay fractions are dominated by aromatic C and show much less signal for O-alkyl carbon compared to the NaOH extracts of these soils and compared to the pyrophosphate extracts of the smectitic soils. The pyrophosphate extracts of RED3 and RED5 have a much higher hydrophobicity than the NaOH extracts, using the ratio (aromatic C + alkyl C)/(O-alkyl C + carbonyl C) as an indicator.

The spectra of the pyrophosphate extracts of the smectite-dominated heavy clay fractions also show a decrease in O-alkyl carbon compared to the NaOH extracts, but O-alkyl C still dominates the spectra. For both VER3A and VER3B there is a slight increase in alkyl-C. VER3B shows an increase in the signal from aromatic-C in the pyrophosphate extract compared to its NaOH extract.

Table 4.4 Integrals of areas of different carbon groups measured by <sup>13</sup>C-NMR (%) and hydrophobicity index.

	NaOH extract				Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract				(Aromatic C + Alkyl-C) (O-alkyl C + Carbonyl C)	
	Carbonyl	Aromatic	O-alkyl	Alkyl	Carbonyl	Aromatic	O-alkyl	Alkyl	NaOH	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub>
<i>Kaolinite</i>										
RED3	20	26	40	15	20	41	18	21	0.7	1.6
RED5	22	14	48	16	22	39	24	15	0.4	1.2
<i>Smectite</i>										
VER3A	20	21	39	20	21	20	35	24	0.7	0.8
VER3B	21	13	48	18	22	23	33	23	0.5	0.8

Py-GC/MS

We summarized the assignments of peaks in Table 4.5.

NaOH extracts

The pyrolysis results of the NaOH extracts of RED3 and RED5 (Figure 4.2) are rather similar. As expected from the NMR spectra, the most pronounced signals are pyrolysis products derived from polysaccharides, e.g. no.3 (acetic acid), 11 (2-furaldehyde), 18 (5-methyl-2-furaldehyde), 28 (levoglucosenone), and 48 (levoglucosan) (Ralph and Hatfield, 1991). Several nitrogen-containing compounds probably originating from proteins were identified: pyrroles (8, 12, 13, 51), pyridines (7, 15), piperazines (54), indoles (38, 46) and benzeneacetonitrile (30) (van Bergen *et al.*, 1998; Schulten and Schnitzer, 1998, and references cited therein).

Table 4.5 Main pyrolysis products. Abp, aliphatic biopolymers; Ar, aromatic compounds; Lg, lignin; Lp, lipids; N-Ar, nitrogen-containing aromatic compounds; Ph, phenols; Ps, polysaccharides.

No	Pyrolysis product	Mass	Origin
1.	Furan	68	Ps
2.	2-Methylfuran	82	Ps
3.	Acetic Acid	60	Ps
4.	Benzene	78	Ar
5.	2,5-Dimethylfuran	96	Ps
6.	N-methylpyrrole	81	N-Ar
7.	Pyridine	79	N-Ar
8.	Pyrrole	67	N-Ar
9.	Toluene	92	Ar
10.	(2H)-furan-3-one	84	Ps
11.	2-Furaldehyde	96	Ps
12.	2-Methylpyrrole	81	N-Ar
13.	3-Methylpyrrole	81	N-Ar
14.	2-Propylfuran	110	Ps
15.	C1-Methylpyridine	93	N-Ar
16.	C2-benzenes (multiple)	106	Ar
17.	Vinylbenzene (styreen)	104	Ar
18.	5-methyl-2-furaldehyde	110	Ps
19.	3-hydroxy-2-penteno-1,5-lactone	114	Ps
20.	Phenol	94	Ph
21.	1-H-pyrrole-2-carboxaldehyde	95	N-Ar
22.	Benzenepropanol	122	Ar
23.	Dianhydrorhamnose	128	Ps
24.	Benzeneacetaldehyde	120	Ar
25.	2-Methylphenol	108	Ph
26.	Guaiacol	124	Lg
27.	3- en 4-Methylphenol	108	Ph
28.	Levogluosenone	126	Ps
29.	2-furoic acid methyl ester	126	Ps
30.	Benzeneacetonitrile	117	N-Ar
31.	Dimethylphenol	122	Ar
32.	4-Ethylphenol	122	Ph
33.	4-Methylguaiacol	138	Lg
34.	Naphtalene	128	Ar
35.	1,4:3,6-Dianhydro- $\alpha$ ,D-glucofuranose	144	Ps
36.	4-Vinylphenol	120	Ph
37.	4-Ethylguaiacol	152	Lg
38.	Indole	117	N-Ar
39.	2-Methylnaphtalene	142	Ar
40.	1,4-Dideoxy-D-glucero-hex-1-enopyranos-3-ulose	144	Ps?
41.	4-Vinylguaiacol	150	Lg
42.	Syringol	154	Lg
43.	Biphenyl	154	Ar
44.	Methyl-2-O-methyl- $\beta$ -D-xylopyranoside	178	Ps
45.	Eugenol	164	Lg
46.	1H-isoindole-1,3(2H)-dione	147	N-Ar
47.	4-Acetylguaiacol	166	Lg
48.	Levogluosan	162	Ps
49.	4-Vinylsyringol	180	Lg

50.	3 (4-methoxyphenyl)-2-propenoic acid (methyl ester)	192	Lg
51.	Diketodipyrrole	186	N-ar
52.	C14-fatty acid	228	Lp
53.	C15-fatty acid	242	Lp
54.	2,5 Diketopiperazine (+ derivatives)	194	N-Ar
55.	C16-fatty acid	256	Lp
56.	C18-fatty acid	284	Lp
*	Alkane/alkene		Lp/Abp
PS	Polysaccharide-derived products		
X	Phtalates (contaminants)		

Several aromatic compounds were present, such as benzenes (4, 9, 16, 17), phenols (20, 27, 31, 32, 36), guaiacols (26, 41) and syringol (42). Alkylbenzenes, alkylphenols, guaiacols and syringols may originate from lignin (Nierop, 1999, and references cited therein). Furthermore, we found some aliphatics such as fatty acids (52, 53, 55) and small signals of homologous series of alkenes and alkanes ranging from C<sub>20</sub> to C<sub>30</sub>.

The NaOH extracts of the smectite-dominated heavy clays (Figure 4.3) had smaller signals of 2-furaldehyde (11) and 5-methyl-2-furaldehyde (18) and a relatively large signal of the polysaccharide-derived compound levoglucosenone (28) compared to the NaOH extracts of RED3 and RED5. The organic matter in the hydroxide extracts of the smectitic clays seemed enriched in aliphatic components such as C-16 fatty acid and alkenes/alkanes compared to the NaOH extracted organic matter from the kaolinitic clays.

#### **Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts**

The pyrolysis results of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of RED3 and RED5 (Figure 4.4) are less dominated by products derived from polysaccharides than their NaOH extracts. The most pronounced polysaccharide-derived peaks for RED3 are 3 (acetic acid), 11 (2-furaldehyde), 18 (5-methyl-2-furaldehyde), and 28 (levoglucosenone).

The pyrogram of RED3 indicates that relatively many aromatic compounds are present, such as such as benzenes (4, 9, 16, 17, 22, 43), phenols (20, 25, 27), guaiacols (26, 33) and naphtalene (34, 39). Alkylphenols and guaiacols may originate from lignin (Nierop, 1999, and references cited therein). Alkylbenzenes and naphtalenes may be derived from charcoal.

Furthermore, we identified nitrogen-containing compounds probably originating from proteins: pyrroles (8, 51), pyridine (7) and benzeneacetonitrile (30). We also found signals from aliphatics such as fatty acids (52, 53, 55, 56) and homologous series of alkenes and alkanes. The pyrophosphate extract of RED5 was quite similar to RED3.

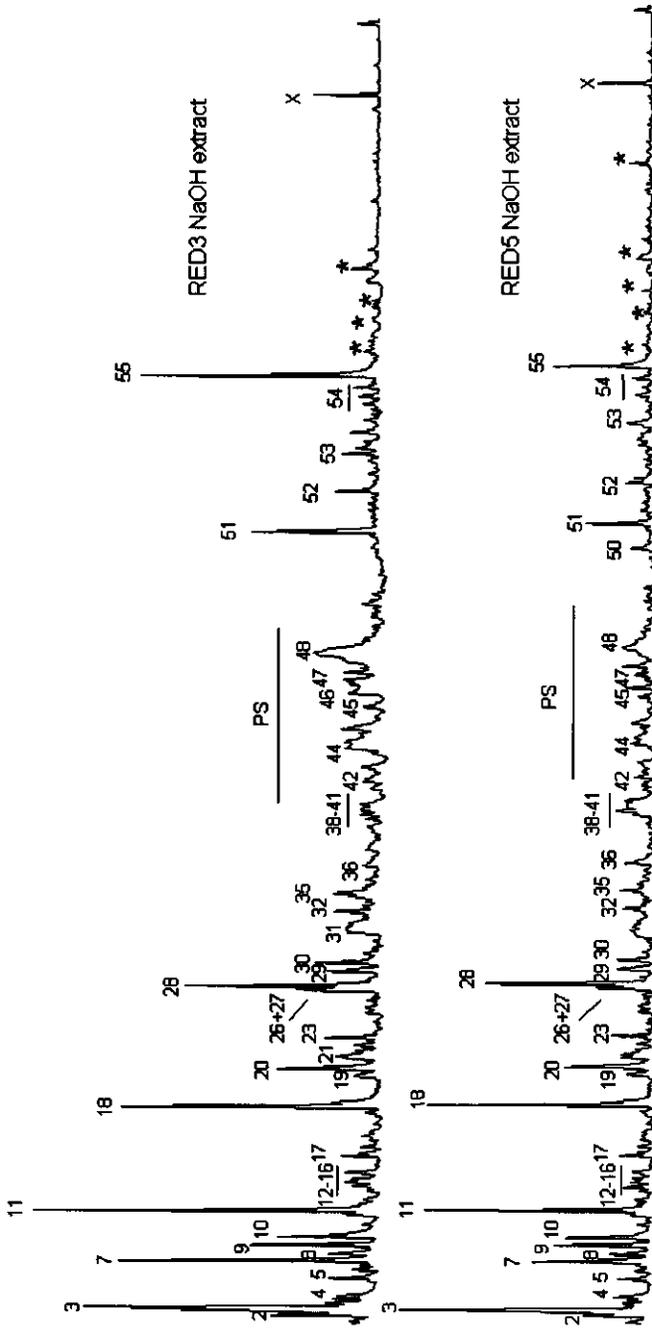


Figure 4.2 Pyrolysis-GC/MS traces of the NaOH extracts of two kaolinitic soils. Numbers correspond to compounds listed in Table 4.5.

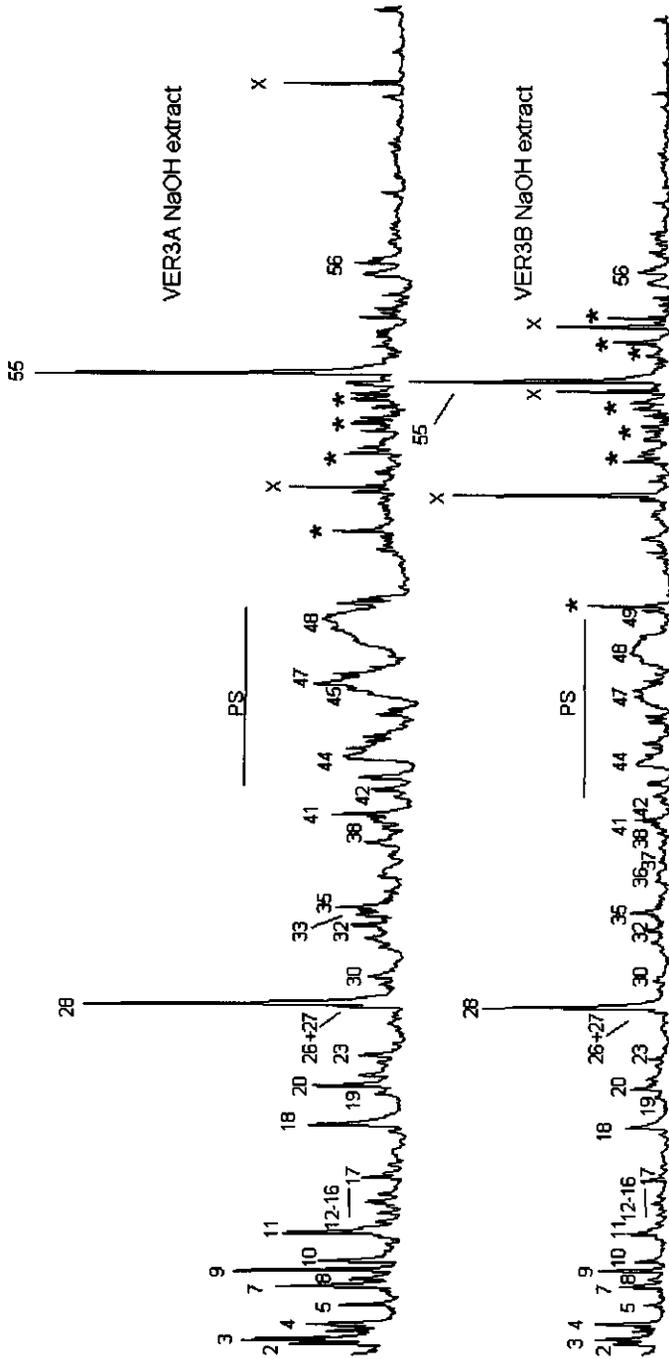


Figure 4.3 Pyrolysis-GC/MS traces of the NaOH extracts of two smectitic soils. Numbers correspond to compounds listed in Table 4.5.

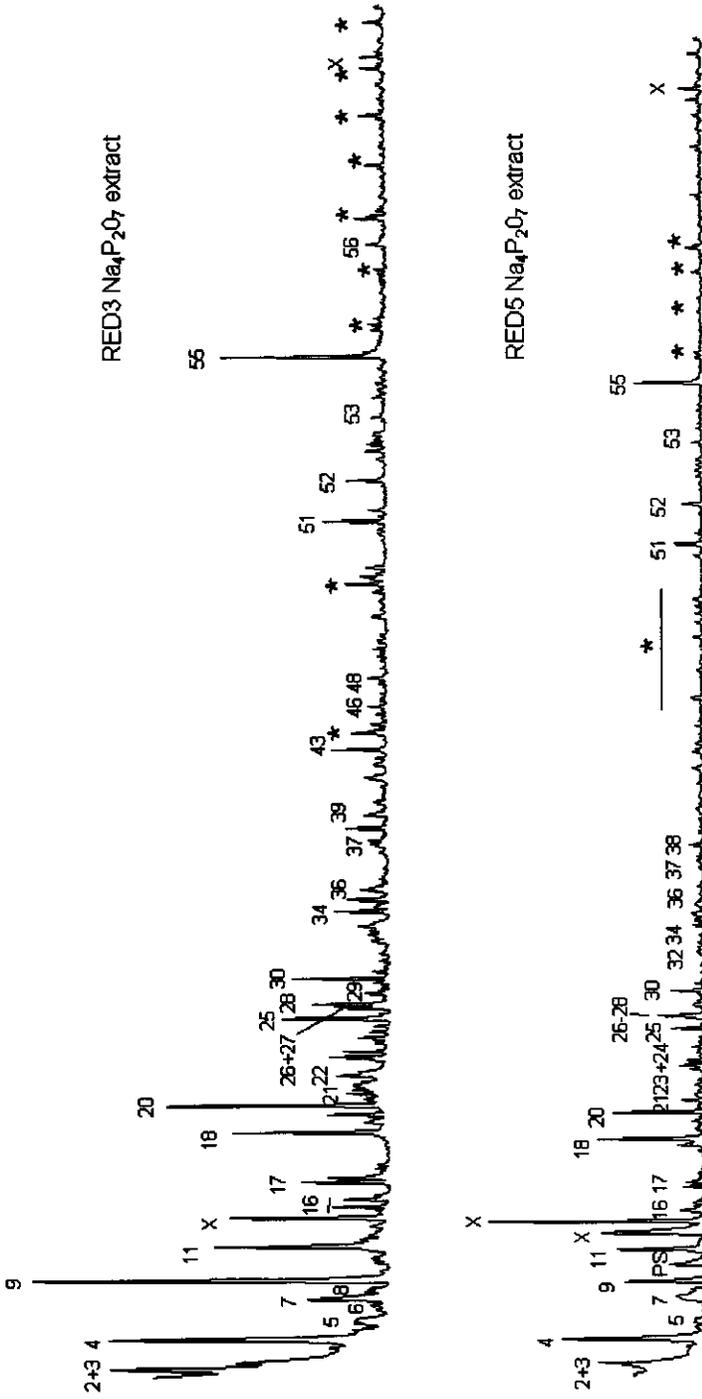


Figure 4.4 Pyrolysis-GC/MS traces of the  $\text{Na}_4\text{P}_2\text{O}_7$  extracts of two kaolinitic soils. Numbers correspond to compounds listed in Table 4.5.

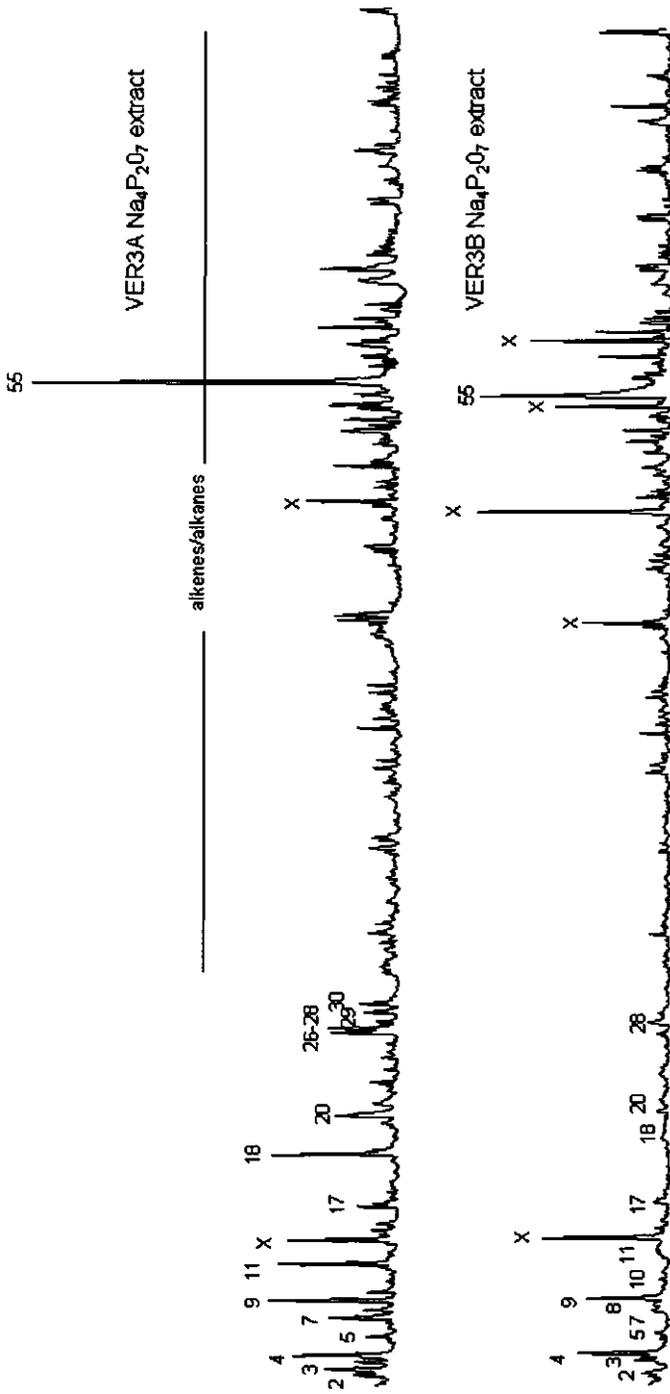


Figure 4.5 Pyrolysis-GC/MS traces of the Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of two smectitic soils. Numbers correspond to compounds listed in Table 4.5.

The quality of the pyrograms of the pyrophosphate extracts, especially of VER3A and VER3B, was quite poor, probably due to the presence of iodide traces. The main difference between the NaOH and the  $\text{Na}_4\text{P}_2\text{O}_7$  extract of VER3A is that the pyrophosphate extract shows a less large signal of levoglucosenone (28) compared to the NaOH extract. The pyrophosphate extract of VER3B also shows a much less large signal of levoglucosenone compared to its NaOH extract. Furthermore, the  $\text{Na}_4\text{P}_2\text{O}_7$  extract of VER3B has relatively large peaks of 4 (benzene) and 9 (toluene) compared to its NaOH extract. It also shows a relatively large number of homologous series of alkenes and alkanes: C10-C30.

#### **$\text{Na}_4\text{P}_2\text{O}_7$ residues**

Due to a high mineral content, we did not succeed in getting meaningful pyrograms of the residues.

### **Discussion**

The discussion below must be read as a series of indications rather than statistically significant trends because of the lack of replicates (we only analyzed two kaolinite- and two smectite-dominated soils).

#### *Carbon distribution*

Both in the total and in the heavy clay fraction, the content of carbon varied between 2 and 4 % (mass fraction). Due to lack of replicates we cannot say whether the carbon content of the clay-size fraction of kaolinite and smectite-dominated soils is significantly different.

The mass of light organic matter separated by NaI was negligible. Previously, we found 'free' plant remains in the clay-size fraction using a scanning electron microscope, (Wattel-Koekkoek *et al.*, 2001a). Surprisingly, in the samples of this experiment, the light fraction was negligible. Apparently, all organic matter present in the clay-size fraction of these samples is complexed or the NaI-method did not succeed in separating such fine plant residues.

Both for the kaolinite- and the smectite-dominated soils about 50% of all organic carbon present in the heavy clay-size fraction was extracted by NaOH. Previously, Wattel-Koekkoek *et al.* (2001a) found that organic matter in the clay-size fraction of smectite-dominated soils was only for a small part (15%) extractable by NaOH, and that the extractability by subsequent pyrophosphate was much higher. Pyrophosphate is known to form complexes with polyvalent cations, thereby breaking down cationic bridges between SOM and clay. It was therefore suggested that smectitic clay minerals preferentially bind organic matter through cationic bridging, as smectitic clays have a much higher CEC than kaolinites and have more (polyvalent) cations at the surface. The results of this study seem to contradict the importance of polyvalent cationic bridging, as 50% of the smectite-complexed organic matter was extractable by NaOH. The difference between the two experiments in extractability of smectite-bound SOM may be related to the ECEC of the clay-size fraction and clay content of the soils. The smectitic soils used in the ISRIC experiment had an average clay content of  $69 \pm 17$  % and a  $\text{ECEC}_{\min}$  of  $67 \pm 22$  cmol/kg (Wattel-Koekkoek *et al.*, 2001), while the smectitic soil from Mozambique have an average clay content of 34% and an average  $\text{ECEC}_{\min}$  of 29 cmol/kg. The relatively low ECEC of the Mozambican soils may have enhanced extractability by NaOH.

Another reason for the difference in extractability of smectite-bound SOM by NaOH between the experiments may be in the use of NaI. To check this possibility, we repeated the fractionation as used in the ISRIC experiment, excluding the separation between light and heavy matter (Table 4.6). Again, a much larger part of the smectite-associated SOM in the total clay-size fraction was extractable with NaOH than during the first experiment, although NaOH extraction yielded less organic carbon without than with the use of NaI. These results indicate cation bridges occur in a much less dominant way in smectitic soils than we suggested earlier (Wattel-Koekkoek *et al.*, 2001).

Table 4.6 Carbon distribution in the total clay-size fraction and the extracts, without density fractionation

sample	Total clay	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	residue
	g C per 100 g clay	g C per 100 g clay		% fraction C in clay		
RED3	4.1 ± 0.1	2.0	0.4	49	10	41
RED5	3.4 ± 0.3	1.7	0.4	50	11	40
VER3A	3.1 ± 0.1	1.2	0.3	37	11	52
VER3B	2.2 ± 0.3	0.6	0.3	26	15	59

The decrease in amount of carbon extracted from smectitic clay by NaOH with or without usage of NaI, may also indicate that Na<sup>+</sup> ions replaced part of the polyvalent cations at the surface of the clay thereby increasing the solubility of the organic matter.

#### <sup>13</sup>C NMR spectroscopy and Py-GC/MS

Both Py-GC/MS and <sup>13</sup>C NMR indicate that the NaOH extracts of kaolinitic soils from Mozambique are dominated by polysaccharides. This agrees with the results of the ISRIC-experiment. The signal of aromatic C of RED3 and RED5 is 14 and 26% of the signal area respectively. These values are relatively high, compared to the average value of 10% for the hydroxide extracts of kaolinitic soils in the ISRIC-experiment. However, the ISRIC experiment had one exception in the group of kaolinitic clays, which was a soil from Mozambique (originating from an adjacent area from RED3 and RED5), coded MOC4 (Wattel-Koekkoek *et al.*, 2001). MOC4 had, similar to RED3 and RED5, a high content of aromatic C in its NaOH extract (18%).

Py-GC/MS and <sup>13</sup>C NMR indicate that the pyrophosphate extracts of the kaolinite-dominated soils in this study were relatively rich in aromatic carbon (about 40%) and less dominated by polysaccharides and O-alkyl carbon (about 20%) compared to the NaOH extracts of RED3 and RED5. These findings again disagree with the general trend found in our previous study where we found that pyrophosphate extracts from kaolinitic clays were relatively rich in polysaccharides (about 40%) and relatively poor in aromatic C (about 20%), but agree with the composition of the pyrophosphate extract of MOC4 (37% of aromatic C, 25% of O-alkyl-C).

The NaOH extracts of smectitic soils VER3A and VER3B have a composition similar to those of the kaolinitic soils: they are dominated by polysaccharide-derived products and O-alkyl C, and the aromatic C content varies between 13 and 21%. The only difference is that the amount of aliphatics (Py-GC/MS) and alkyl-C (<sup>13</sup>C NMR) is relatively high in the smectite-extracts

compared to the kaolinite-extracts. This may be related to intercalation (Theng *et al.*, 1986) due to the swelling and shrinking properties of the smectites.

The pyrophosphate extract of VER3A contained relatively many polysaccharides, according to Py-GC/MS and NMR, and had a similar composition as its NaOH extract. These findings disagree with Wattel-Koekkoek *et al.* (2001a) who found that smectite-dominated SOM was relatively rich in aromatic C.

For the pyrophosphate extract of VER3B, the results of pyrolysis and NMR do not coincide. The NMR results indicate a slight dominance of O-alkyl carbon, while the Py-GC/MS data indicate dominance of aliphatic compounds.

Table 4.7 Weighted average of different carbon groups measured by <sup>13</sup>C NMR (%) of extracted carbon and hydrophobicity index (%).

Sample	Carbonyl C	Aromatic C	O-alkyl C	Alkyl C	(aromatic C + alkyl-C)/ (O-alkyl C + carbonyl C)
RED3	19.9	28.7	35.4	16.1	0.81
RED5	21.9	19.0	43.3	15.8	0.53
VER3A	20.6	20.5	38.5	20.6	0.70
VER3B	21.5	15.1	43.8	19.5	0.53

In Table 4.7 we calculated the weighted average composition of the total extracted organic matter based on the carbon distribution (Table 4.3) and the NMR data (Table 4.4). The main striking difference between the four soils is that RED3 has the highest aromaticity and VER3B the least. The profile of RED3 was under bamboo vegetation (*Oxythenantera sp.*), while the VER3B was under *Digitaria*, *Hyperrenia*, and *Laudetia*. (Bamboo is known for its sturdiness, and in the region of Montepuez frequently used as building material.) As the composition of soil organic matter is related to the original composition of the input, the high content of aromatic carbon in the extracts of RED3 may be related to the chemical composition of bamboo. We therefore pyrolyzed all the mentioned different Poaceae species. The pyrograms indicated however, that bamboo was not enriched in aromatic C compared to *Digitaria*, *Hyperrenia*, and *Laudetia* and therefore the difference in vegetation did not explain the difference in composition of pyrophosphate extracted SOM.

The savannah vegetation was frequently burned, a.o. for hunting purposes. This may have resulted in the accumulation of black carbon. Charred plant materials are relatively rich in aromatic carbon. Burning may therefore explain the relatively high aromaticity of RED3 and the pyrophosphate extract of RED5. It remains unclear, however, why the effect would vary between the four soil profiles as they originate from the same area.

The organic matter extracted from VER3A and VER3B seems to be enriched in alkyl-C. As mentioned before, the swelling and shrinking of smectites may enable intercalation of alkyl-chains. The long chains may bind parallel to the clay surfaces using Van der Waals bonds.

## Conclusions

We have analysed the clay-associated organic matter of two kaolinite- and two smectite-rich soils originating from Mozambique. The results indicate that:

- the content of carbon in the clay-size fraction showed no significant difference between kaolinitic and smectitic soils. This suggests that the amount of organic carbon in the clay-size fraction is independent of the clay mineralogy.
- the clay-size fractions of both soils do not contain significant amounts of 'free' or 'light' organic matter (density < 1.7 g cm<sup>-3</sup>)
- the extractability by NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> of the clay-bound organic matter does not differ between kaolinite and smectite, suggesting that there are no clay-specific binding mechanisms
- the organic matter bound to both clays is dominated by polysaccharides. SOM extracted from smectite-dominated clay-size fraction contain slightly more aliphatic (alkyl) carbon than SOM from kaolinite-dominated clay-size fractions.
- the amount of aromatic carbon shows a large variation between the samples

### **Acknowledgements**

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## **Chapter 5**

### **Mean residence time of kaolinite- and smectite-bound organic matter in soils from Mozambique**

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### Abstract

We analyzed the  $^{14}\text{C}$  activity of organic matter in the whole and heavy clay-size fraction of kaolinite- and smectite-dominated soils from N'Ropa, in northern Mozambique. The soils originated from natural savanna systems and bamboo forest. We assumed that carbon inputs and outputs are in equilibrium in such soils, so that the  $^{14}\text{C}$  age equals the mean residence time of the organic carbon. For both kaolinite- and smectite-dominated soils, the organic matter in the whole and heavy clay-size fraction and extracts had a fast turnover (400 to 500 years on average). The mean residence time of kaolinite-bound organic matter did not differ significantly from that of smectite-bound organic matter. Multiple linear regression indicates that the Effective CEC is the main factor explaining differences in the mean residence time of the extracted soil organic matter. These results agree with previously found trends in organic matter turnover of kaolinite and smectite-associated clay.

### Introduction

In order to gain understanding about the process of global warming, it is essential to study the global carbon cycle. In the global C cycle, soil organic matter (SOM) is a major source/sink of atmospheric C. Soil organic matter consists of a variety of components, such as sugars, proteins, lignin, lipids, and aliphatic polymers. Turnover times of C in these soil organic compounds vary from hours to thousands of years (Trumbore, 1993; Lichtfouse *et al.*, 1995; Torn *et al.*, 1997; Huang *et al.*, 1999).

Minerals can stabilize soil organic matter through the formation of organo-mineral bonds (Cheshire *et al.*, 2000; Christensen, 1992; Christensen, 2000; d'Acqui *et al.*, 1998; Schulten and Leinweber, 2000). The aim of this research is to determine the effect of clay mineralogy on the mean residence time of organic matter that is bound to the mineral surface. We especially studied organic matter that is bound to kaolinite or smectite. Kaolinite is present in a range of weathered tropical soil, such as Acrisols, Nitisols and Oxisols (FAO, 1990). Smectite is particularly abundant in Vertisols.

Previously we determined the mean residence time of kaolinite- and smectite-associated SOM (= all SOM present in the 'clay-size' fraction) in soils taken from the ISRIC collection, sampled from seven countries, using  $^{14}\text{C}$  (Wattel-Koekkoek *et al.*, 2001b; from now on also referred to as 'the ISRIC experiment'). Kaolinite-associated soil organic matter had a fast turnover (360 years on average). Smectite-associated soil organic matter had a relatively slow turnover, with an average mean residence time for the whole clay-size fraction of 1100 years. Differences in turnover times between organic matter associated to kaolinite and smectite were significant. Multiple linear regression indicated that clay mineralogy, described by the effective cation exchange capacity (ECEC) or specific surface area, was the main factor explaining differences in the mean residence time of the extracted soil organic matter.

However, in the ISRIC experiment the variation within each group was large. There are two possible factors that may cause for this variation. First, the samples originated from 7 different countries, implying differences in local climate, vegetation, etc. Second, for the ISRIC experiment we studied the whole clay-size fraction, including its 'free' organic matter. In order

to better understand the effect of clay mineralogy on the mean residence time of organic matter, in this paper we focus on *clay-bound* organic matter and exclude the free organic fraction.

We decided to take samples of one region only, to eliminate the geographical effect. We chose an undulating landscape in N'Ropa, near Montepuez in northern Mozambique, with soils that are either dominated by kaolinite (in the higher areas) or by smectite (in the depressions). We applied density fractionation to eliminate the possible effect of particulate organic matter. We measured  $^{14}\text{C}/^{12}\text{C}$  isotope ratios of kaolinite- and smectite bound SOM by Accelerator Mass Spectrometry (AMS).

## Materials and methods

### Samples

We sampled ten soils, from an area west of Montepuez, near the village N'Ropa in Mozambique, using only the SOM-rich surface (Ah) horizons. Clay mineralogy was determined by X-ray diffraction (XRD) of oriented samples of the clay-size fractions. The diffractograms were obtained with a Philips PW1820/PW1710 diffractometer, using Co-K $\alpha$ -radiation at 40 kV and 30 mA, with a focussing monochromator. The divergence slit was set at 1°, the receiving slit at 0.2 mm, and the anti-scatter slit at 1°. Peak areas of the clay minerals were measured to compare the (semi-quantitative) XRD diffractograms and reported in % of total peak area (Table 5.1). The clay fractions did not contain phyllosilicates other than kaolinite and smectite.

All ten soils were physically fractionated as described under *physical fractionation*, and the  $^{14}\text{C}$  age of the whole and heavy clay-size fractions was determined according to the procedure described under  *$^{14}\text{C}$  analysis*.

Table 5.1 Mineralogy analyses of the clay-size fraction. Clay mineralogy measured by XRD (relative peak area of diffractograms in %).

Sample	Kaolinite	Smectite
RED1	56	44
RED1A	68	32
RED2	85	15
RED3	93	7
RED4	79	21
RED5	96	4
VER2	23	77
VER3A	18	82
VER3B	13	87
PHAE3	23	77

From the ten soils, two sets of two samples with the highest content of kaolinite and smectite were selected for further analysis. We selected only two soils per clay type due to limited analytical capacity: RED3 and RED5 (kaolinite clays) and VER3A and VER3B (smectite clays). All clay-size fractions contained small quantities of quartz.

The four samples were air-dried and passed through a 2-mm sieve. The pH, organic carbon content,  $\text{CEC}_{\text{soil}}$ , and particle size distribution were measured according to Buurman *et al.* (1996) (Table 5.2). Furthermore, the four soils were fractionated not only physically, but also

chemically (see *chemical fractionation*). The carbon distribution over the fractions was determined, and the  $^{14}\text{C}$  age of the NaOH-extracted,  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted and residual organic material was measured

Table 5.2 General characteristics of the samples.

	RED3	RED5	VER3A	VER3B
Latitude S	38°40'71"	38°41'26"	38°38'91"	38°38'81"
Longitude E	13°11'04"	13°10'52"	13°12'26"	13°11'71"
T mean, annual (° C)	24.2	24.2	24.2	24.2
Precipitation, annual (mm)	932	932	932	932
Parent Rock	Residue from basic igneous rock		Alluvium	
Vegetation	Bambooforest ( <i>Oxythenantera</i> sp.)		Digitaria, Laudetia, Hyperrennia; Poacea <80%	
Drainage	well	well	imperfectly drained	imperfectly drained
Soil Type	Rhodic Nitisol	Rhodic Nitisol	Eutric Vertisol	Eutric Vertisol
Clay (%)	39.5	35	23.9	43.4
Organic C (g /100 g soil)	4.2	2.7	1.6	2.2
pH H <sub>2</sub> O	6.2	6.2	6.3	6.4
pH KCl	5.3	5.4	4.8	4.4
CECsoil (cmol/kg)	23.9	18.1	16.8	30.3
Base Sat. (%)	82.8	86.7	83.3	83.8
Remarks	Signs of burning (charcoal) in all profiles			

### Physical fractionation

The fractionation procedure has been described by Wattel-Koekkoek *et al.* (2001c). The procedure is as follows:

We collected the clay-size (< 2 $\mu\text{m}$ ) fractions of all samples, using ultrasonic (full) dispersion and sedimentation in water. To ensure complete ultrasonic disruption, we measured mass recovery of clay using different values of applied energy, using the method described by Roscoe *et al.* (2000). To reach maximum recovery of the clay-size fraction, 430 J/ml was needed for RED1, RED1A, RED2, RED3, RED4 and RED5, and 365 J/ml for VER2, VER3A, VER3B and PHAE3, using a soil:solution ratio of 1:10.

The clay-size fractions were siphoned off after a certain sedimentation time, and freeze-dried. In total 100 g of clay-size fraction was accumulated for all four samples. The dried clay-size fractions were separated into a light and a heavy fraction using a NaI solution with a density of 1.7 g cm<sup>-3</sup> (Roscoe *et al.*, 2001). Of each clay-size fraction, 50 gram was placed in a 500 ml centrifuge tube with 500 ml NaI solution, shaken end-over-end for 60 minutes, and left standing at room temperature for 15 minutes. After centrifugation (15 minutes, 3500 rpm), the supernatant was filtered through a membrane filter (Whatman, GF/A) into a millipore vacuum unit. The fraction recovered on the filter was washed with 0.01 M CaCl<sub>2</sub> solution (100 ml) and distilled water (200 ml). The sediment was resuspended in NaI and centrifuged two more times as

described above. The three subfractions recovered from the filter were joined, oven-dried at 50 °C and stored for analysis. This fraction was called the “light clay-size fraction”, or “free” organic matter. The heavy fraction was flocculated once with 0.01 M CaCl<sub>2</sub> and washed about 10 times with distilled water until the clay fraction remained in suspension after 24 hours, after which it was freeze-dried. This procedure was repeated twice for each soil, to separate all 100 g of clay per soil sample (Figure 5.1).

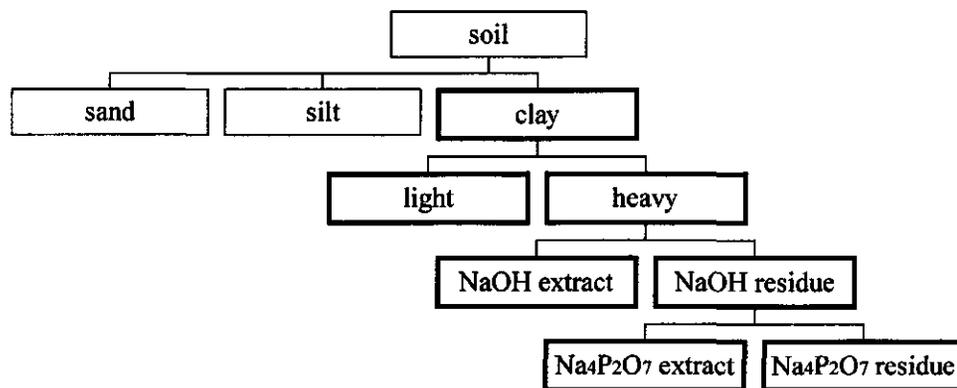


Figure 5.1 Fractionation scheme.

#### *Chemical fractionation*

The heavy clay-size fractions of RED3, RED5, VER3A and VER3B were shaken in 0.5 M NaOH under N<sub>2</sub> for 24 hours (0.5 L, soil : solution = 1 : 10). We centrifuged the solution, decanted the supernatant, and shook the residue with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants (=NaOH extract) to pH=1 with a solution of 0.1 M HCl and 0.3 M HF. Both the supernatant and the NaOH-residue were dialyzed to pH=6 against deionized water, and freeze-dried. Next, the NaOH-residue was shaken for 24 hours with 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> under nitrogen (soil : solution = 1 : 10). The solution was centrifuged and decanted, and the residue was shaken with deionized water for 2 hours. After centrifugation, we acidified the combined supernatants (=pyrophosphate extract) to pH=1 with a solution of 0.1 M HCl and 0.3 M HF. Both the supernatant (= pyrophosphate extract) and the pyrophosphate-residue were dialyzed and freeze-dried (Figure 5.1).

We chose to extract first with NaOH because we expect it to extract mainly ‘free’ (Choudri and Stevenson, 1957) and kaolinite-complexed organic components. NaOH deprotonates the aluminum-hydroxide edges of kaolinites, and part of the organic matter, thereby liberating organic molecules. We chose Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> for subsequent extraction, because we expect it to form complexes with (exchangeable) polyvalent cations present at smectite surfaces, thereby breaking down the cation bridges between the exchangeable cations and organic matter (Choudri and Stevenson, 1957; Schnitzer and Schuppli, 1989).

We measured the amount of carbon in the total and heavy clay-size fraction, the freeze-dried extracts, and the residue, using an Interscience elemental analyzer EA1108.

*ECEC*

In order to get the ECEC of the mineral phase only ( $ECEC_{min}$ ), the ECEC of the whole clay-size fraction was corrected for the ECEC of the organic matter, according to Wattel-Koekkoek *et al.* (2001b). For your convenience we repeat the formula below:

$$ECEC_{min} = ECEC - 2 \times \% C \times 2 \quad (\text{cmol/kg clay}) \quad (1)$$

assuming the ECEC of organic matter is 200 cmol/kg organic matter, and that

$$\%SOM = 2 \times \%C \quad (2)$$

where %SOM is the organic matter content of the clay-size fraction and %C is the carbon content of the clay-size fraction, assuming that organic matter is for 50% carbon (Table 5.3).

Table 5.3 Effective cation exchange capacity of the whole clay-size fractions (ECEC, in cmol kg<sup>-1</sup>), effective cation exchange capacity of the minerals phase in the clay-size fractions only ( $ECEC_{min}$ , in cmol kg<sup>-1</sup>), and dithionite and oxalate-extractable iron (% Fe) of the clay-size fractions.

Sample	ECEC (cmol kg <sup>-1</sup> )					$ECEC_{min}$	extractable Fe (%)	
	Ca	Mg	Na	K	SUM		oxalate	dithionite
<i>Kaolinitic</i>								
RED3	13.5	6.7	0.4	1.6	22.2 ± 0.3	5.8	0.21 ± 0.01	10.8 ± 0.1
RED5	15.1	7.5	0.7	1.6	24.9 ± 0.1	11.3	0.21 ± 0.01	10.6 ± 0.1
<i>Smectitic</i>								
VER3A	19.5	15.7	1.4	0.4	37.0 ± 0.7	24.6	0.63 ± 0.03	2.4 ± 0.1
VER3B	17.2	24.6	0.8	0.3	42.9 ± 1.2	34.1	0.78 ± 0.02	2.4 ± 0.1

<sup>14</sup>C analysis

The clay-size fractions, heavy clay-size fractions, NaOH extracts, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts and residues were combusted at 850 °C under continuous flow of oxygen. The CO<sub>2</sub> was purified by CuO and Ag using standard methods. <sup>13</sup>C values were measured by mass spectrometry at the Center for Isotope Research, Groningen. After <sup>13</sup>C analysis, the CO<sub>2</sub> was trapped cryogenically and converted to graphite for subsequent AMS analysis. The <sup>14</sup>C activity was measured by the Groningen AMS facility (Van der Plicht *et al.*, 2000). The amount of <sup>14</sup>C in soil organic matter relative to the <sup>14</sup>C concentration in the atmosphere can be used to calculate the age of the material. The measured relative <sup>14</sup>C activity (<sup>14</sup>a<sub>m</sub>) is reported in % and corrected for isotopic fractionation with <sup>13</sup>C.

$${}^{14}a_m = \frac{{}^{14}A}{{}^{14}A_0} \quad (\%) \quad (3)$$

where  $A_0$  is the original specific <sup>14</sup>C activity defined by the 1950 standard,  $A$  is the measured specific <sup>14</sup>C activity of the sample, and  $t$  is the time elapsed since the activity was  $A_0$ . The mean residence time (MRT) was calculated using <sup>12</sup>a<sub>m</sub>, to correct for Suess and Bomb effect, according

to Wattel-Koekkoek *et al.* (2001b). The MRT was used to calculate the  $^{14}\text{C}$  activity the sample would have had, had there been no changes in atmospheric  $\text{CO}_2$  ( $^{14}\text{a}_{\text{corr}}$ ).

**Results**

*Effective cation exchange capacity and iron content*

The kaolinitic whole clay-size fractions of RED3 and RED5 had an effective cation exchange capacity of 22 and 25  $\text{cmol kg}^{-1}$  respectively, mostly due to organic matter. They had a high amount of dithionite extractable iron (10.8 and 10.7 % Fe for RED3 and RED5) (Table 5.3). The percentage of oxalate-extractable iron of both clay-size fractions was 0.21.

The smectitic whole clay-size fractions of VER3A and VER3B had an ECEC of 37 and 43  $\text{cmol kg}^{-1}$  respectively, mostly due to the mineral phase, and 2.4% of dithionite extractable Fe.

*Carbon distribution*

Three replicates of each soil were taken to determine the carbon content of the total and the heavy clay-size fractions. The carbon content of the NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts was determined only in duplicate, because of lack of material. Standard deviations were not calculated for these extracts.

The total clay-size fractions had carbon contents varying between 2 and 4 % (mass fraction) (Table 5.4). Compared to the total mass and carbon content of the whole clay-size fraction, the yield of the light fraction was negligible. After NaI treatment, all the carbon present in the clay-size fraction ended up in the heavy clay-size fraction. Therefore, the C content of heavy and total clay-size fractions were not significantly different.

NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  together extracted 62 to 67% of the carbon present in the heavy clay-size fraction. For all four soils the amount of carbon (g) per 100g clay extracted by NaOH is larger than that sequentially extracted by pyrophosphate. NaOH extracted about 50% and  $\text{Na}_4\text{P}_2\text{O}_7$  about 15% of the organic carbon from the heavy clay-size fraction of the soils.

Table 5.4 Carbon distribution in the total and heavy clay-size fractions and extracts. \* = 3 repetitions

sample	Total clay*	Heavy clay*	NaOH extract	$\text{Na}_4\text{P}_2\text{O}_7$ extract	NaOH extract	$\text{Na}_4\text{P}_2\text{O}_7$ extract	residue
	g C per 100 g clay		g C per 100 g heavy clay		% fraction C in heavy clay		
RED3	4.1 ± 0.1	3.6 ± 0.3	2.0	0.5	54	13	33
RED5	3.4 ± 0.3	3.0 ± 0.2	1.6	0.4	53	14	33
VER3A	3.1 ± 0.1	3.1 ± 0.5	1.6	0.5	50	15	35
VER3B	2.2 ± 0.3	2.5 ± 0.3	1.2	0.4	47	15	38

<sup>14</sup>C age

Table 5.5 shows the measured <sup>14</sup>C activity, the calculated MRT and corrected <sup>14</sup>C activity for the whole and the heavy clay-size fractions. In the kaolinitic soils, the average MRT of organic matter was  $523 \pm 137$  years in the clay-size fractions and  $536 \pm 136$  in the heavy clay-size fractions. The average MRT of organic matter in smectite-dominated soils was  $438 \pm 227$  years in the clay-size fractions and  $456 \pm 256$  in the heavy clay-size fractions. T-tests (Sokal and Rohlf, 1995) indicate that in both kaolinitic and smectitic soils, the <sup>14</sup>C ages of whole and heavy clay-size fraction were not significantly different from each other. Also the MRT of the organic matter in the whole and heavy clay-size fraction did not differ between kaolinite-dominated and smectite-dominated soils.

Table 5.5 Measured and corrected <sup>14</sup>C activity and mean residence time (MRT, yr) of the whole and heavy clay-size fractions.

	Clay (< 2 μm)			Heavy clay		
	<sup>14</sup> a <sub>m</sub> %	MRT yrs	<sup>14</sup> a <sub>corr</sub> %	<sup>14</sup> a <sub>m</sub> %	MRT yrs	<sup>14</sup> a <sub>corr</sub> %
<i>Kaolinite-dominated</i>						
RED1	101.22 ± 0.62	605 ± 55	92.9	101.77 ± 0.61	584 ± 54	93.2
RED1A	97.97 ± 0.63	747 ± 57	91.4	97.40 ± 0.60	774 ± 54	91.1
RED2	102.82 ± 0.66	545 ± 58	93.6	102.38 ± 0.62	561 ± 55	93.4
RED3	107.15 ± 0.68	410 ± 59	95.2	107.05 ± 0.65	414 ± 56	95.1
RED4	108.23 ± 0.68	384 ± 59	95.5	107.26 ± 0.65	409 ± 56	95.2
RED5	105.62 ± 0.63	454 ± 55	94.7	104.80 ± 0.63	479 ± 55	94.4
<i>Average</i>		523	93.9		536	93.7
<i>St. dev.</i>		137	1.6		136	1.5
<i>Smectite-dominated</i>						
VER2	97.29 ± 0.63	780 ± 57	91.0	96.03 ± 0.59	845 ± 54	90.3
VER3A	112.04 ± 0.67	300 ± 57	96.4	112.10 ± 0.67	299 ± 57	96.4
VER3B	108.29 ± 0.69	382 ± 60	95.5	108.20 ± 0.66	384 ± 57	95.5
PHAE3	111.97 ± 0.67	301 ± 57	96.4	111.64 ± 0.67	308 ± 57	96.3
<i>Average</i>		438	94.8		456	94.6
<i>St. dev.</i>		227	2.6		256	2.9

The organic matter extracted by NaOH had a MRT of  $443 \pm 57$  years for RED3,  $503 \pm 55$  years for RED5,  $393 \pm 61$  years for VER3A and  $392 \pm 58$  years for VER3B, suggesting that turnover time of SOM in NaOH extracts does not differ between kaolinitic and smectitic soils (Table 5.6). However, as we only extracted two soils dominated by kaolinite and two dominated by smectite, we cannot statistically analyze these data and therefore the results should only be read as indications.

Table 5.6 Measured and corrected  $^{14}\text{C}$  activity and mean residence time (MRT, yr) of the heavy clay- size fractions, extracts, and residues.

	Heavy clay (< 2 $\mu\text{m}$ )			NaOH extract			$\text{Na}_2\text{P}_2\text{O}_7$ extract			Residue		
	$^{14}\text{C}_{\text{th}}$	MRT	$^{14}\text{C}_{\text{corr}}$	$^{14}\text{C}_{\text{th}}$	MRT	$^{14}\text{C}_{\text{corr}}$	$^{14}\text{C}_{\text{th}}$	MRT	$^{14}\text{C}_{\text{corr}}$	$^{14}\text{C}_{\text{th}}$	MRT	$^{14}\text{C}_{\text{corr}}$
<i>Kaolinite</i>												
RED3	107.05 $\pm$ 0.65	414 $\pm$ 56	95.1	106.00 $\pm$ 0.65	443 $\pm$ 57	94.8	104.94 $\pm$ 0.63	475 $\pm$ 55	94.4	108.35 $\pm$ 0.69	381 $\pm$ 60	95.5
RED5	104.80 $\pm$ 0.63	479 $\pm$ 55	94.4	104.07 $\pm$ 0.63	503 $\pm$ 55	94.1	100.65 $\pm$ 0.63	628 $\pm$ 56	92.7	105.70 $\pm$ 0.67	452 $\pm$ 58	94.7
<i>Smectite</i>												
VER3A	112.10 $\pm$ 0.67	299 $\pm$ 57	96.4	107.85 $\pm$ 0.70	393 $\pm$ 61	95.4	104.32 $\pm$ 0.62	495 $\pm$ 54	94.2	110.68 $\pm$ 0.71	328 $\pm$ 61	96.1
VER3B	108.20 $\pm$ 0.66	384 $\pm$ 57	95.5	107.90 $\pm$ 0.67	392 $\pm$ 58	95.4	100.80 $\pm$ 0.61	622 $\pm$ 54	92.8	107.12 $\pm$ 0.69	412 $\pm$ 60	95.1

Pyrophosphate-extracted SOM had a MRT of  $475 \pm 55$  years for RED3,  $628 \pm 56$  years for RED5,  $495 \pm 54$  years for VER3A, and  $622 \pm 54$  years for VER3B, suggesting that turnover time of SOM in pyrophosphate extracts does not differ between kaolinitic and smectitic soils.

The organic matter in de pyrophosphate-residues had a MRT of  $381 \pm 60$  years for RED3,  $452 \pm 58$  years for RED5,  $328 \pm 61$  years for VER3A, and  $412 \pm 60$  years for VER3B. The results seem to indicate that the turnover time of SOM in residues also does not differ between kaolinite- and smectite-dominated soils.

The measurements suggest that the four fractions of the RED3, RED5, and VER3A, and VER3B (heavy clay-size fraction, NaOH extract,  $\text{Na}_4\text{P}_2\text{O}_7$  extract, residues) were not significantly different from each other. However, of the four fractions, the pyrophosphate extracts seem to be slightly older than the other ones.

## Discussion

The carbon content of the total and heavy clay-size fractions varied between 2 and 4 % (mass fraction). We cannot say whether the carbon content of the clay-size fraction of kaolinite- and smectite-dominated soils is significantly different, because of lack of replicates.

During the ISRIC experiment, we did find 'free' plant remains in the clay-size fraction using a scanning electron microscope (Wattel-Koekkoek *et al.*, 2001a). In the present case, the mass of light organic matter separated by NaI was negligible (Table 5.4). The  $^{14}\text{C}$  measurements support these findings, as the whole and the heavy clay-size fractions are not significantly different in  $^{14}\text{C}$  activity (Table 5.5). This may indicate that all organic matter in the clay-size fraction is bound or that density fractionation is not effective to separate fine organic materials. Previous studies also show very low yields of the light fraction of the clay-size fraction and suggest that in clay-size fractions a continuum exists between density extremes of organic and mineral properties (Christensen, 1992).

To check our methodology, we performed an extra experiment using glass filters. Results are discussed below.

### *Did density fractionation work?*

To test the density fractionation method, we filtered the NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  extracts using a glass filter (Schleicher and Schuell, GF 55,  $\varnothing$  1  $\mu\text{m}$ ). If the NaI method failed, the filters should catch 'free' organic matter  $> 1 \mu\text{m}$  such as plant remains, and the filtrate should be older of age than the non-filtered extracts, assuming that relatively large plant remains have a young  $^{14}\text{C}$  age.

The results (Table 5.7), however, show that there is no significant difference between filtered and non-filtered extracts. With the naked eye, we only saw a small amount of mineral material on the filter. Both indicate that the density fractionation did work. However, we cannot be completely certain because it also possible that the free organic fraction was smaller than 1  $\mu\text{m}$ .

Table 5.7 Measured and corrected  $^{14}\text{C}$  activity and mean residence time (MRT, yr) of the filtered and non-filtered extracts of the heavy clay-size fractions.

	NaOH extract filtered			NaOH extract			$\text{Na}_2\text{P}_2\text{O}_7$ extract filtered			$\text{Na}_2\text{P}_2\text{O}_7$ extract		
	$^{14}\text{C}_{\text{act}}$ %	MRT yrs	$^{14}\text{C}_{\text{corr}}$ %	$^{14}\text{C}_{\text{act}}$ %	MRT yrs	$^{14}\text{C}_{\text{corr}}$ %	$^{14}\text{C}_{\text{act}}$ %	MRT yrs	$^{14}\text{C}_{\text{corr}}$ %	$^{14}\text{C}_{\text{act}}$ %	MRT yrs	$^{14}\text{C}_{\text{corr}}$ %
<i>Kaolinite</i>												
RED3	105.65 ± 0.67	454 ± 58	94.7	106.00 ± 0.65	443 ± 57	94.8	100.38 ± 0.63	639 ± 56	92.6	104.94 ± 0.63	475 ± 55	94.4
RED5	104.62 ± 0.68	485 ± 60	94.3	104.07 ± 0.63	503 ± 55	94.1	101.55 ± 0.55	592 ± 49	93.1	100.65 ± 0.63	628 ± 56	92.7
<i>Smectite</i>												
VER3A	108.00 ± 0.69	389 ± 60	95.4	107.85 ± 0.70	393 ± 61	95.4	101.52 ± 0.59	594 ± 52	93.1	104.32 ± 0.62	495 ± 54	94.2
VER3B	108.73 ± 0.69	372 ± 60	95.6	107.90 ± 0.67	392 ± 58	95.4	100.17 ± 0.56	648 ± 50	92.5	100.80 ± 0.61	622 ± 54	92.8

#### *Kaolinite-dominated clay-size fractions*

RED3 and RED5 have a high content of dithionite-extractable iron and little oxalate-extractable iron, which indicates that their clay-size fractions contain a lot of crystalline iron oxides. Furthermore, both soils had a red colour, suggesting that the crystalline iron is hematite.

The SOM in the whole and heavy clay-size fractions of all six kaolinite-dominated soils had a fast turnover (Table 5.5). Apparently the kaolinite and the iron-oxides present, bind organic matter only weakly, hardly affecting SOM turnover. These results agree with our findings in the ISRIC experiment (Wattel-Koekkoek *et al.*, 2001b), and with those of Shang and Tiessen (1997).

Of all organic carbon present in the extracts of the heavy clay-size fractions of RED3 and RED5, 50% was extractable by NaOH and 15% by  $\text{Na}_4\text{P}_2\text{O}_7$ . Similar results of extractability were found by Wattel-Koekkoek *et al.* (2001a).

Wattel-Koekkoek *et al.* (2001a and 2001c) showed that NaOH-extracted SOM from kaolinite is rich in polysaccharides. Polysaccharides are relatively easy to decompose, so their presence suggests that kaolinite-bound SOM has a relatively fast turnover. This is confirmed by the short mean residence time of the NaOH extracts of RED3 and RED5 (Table 5.6).

The SOM in the pyrophosphate extracts of RED3 and especially RED5 has a slightly longer MRT compared to the other fractions of these soils and compared to the pyrophosphate extracted SOM of the kaolinitic soils of the ISRIC experiment. This could be related to the chemical composition of the pyrophosphate-extracted SOM. According to Wattel-Koekkoek *et al.* (2001c), the pyrophosphate extracts of RED3 and RED5 contain relatively many aromatic compounds. These compounds have a larger recalcitrance towards decomposition than polysaccharides (Baldock *et al.*, 1992).

The organic carbon in the pyrophosphate-residues of RED3 and RED5, which represents about 35% of the carbon in the heavy clay-size fraction (Table 5.4), had a relatively fast turnover (Table 5.6). This suggests that the organic matter in the residues is only weakly bound to the mineral surfaces.

#### *Smectite-dominated clay-size fractions*

The organic matter in the whole and heavy clay-size fractions of the four smectite-dominated soils had a relatively fast turnover: 438 years for the whole clay-size fraction and 456 years for the heavy clay-size fraction on average (Table 5.5). These results contrast with those of the ISRIC experiment, where smectite-associated soil organic matter had a relatively slow turnover (average mean residence time for the whole clay-size fraction of 1100 years). Theng *et al.* (1992) even found a MRT of 5680 years for the organic matter in a smectitic clay-size fraction from New Zealand.

About 50% of all organic carbon present in the heavy clay-size fraction of the smectite-dominated soils was extractable by NaOH (Table 5.4). This contrasts with Wattel-Koekkoek *et al.* (2001a), who found that only a small part (15%) of the organic matter in the clay-size fraction of smectite-dominated soils was extractable by NaOH, and that the extractability by subsequent pyrophosphate was much higher.

The SOM in the NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts of VER3A and VER3B have a relatively short mean residence time of about 400-600 years. This agrees with Wattel-Koekkoek *et al.* (2001b), who found that the NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts from the heavy clays of VER3A and VER3B, were dominated by easily decomposable O-alkyl (polysaccharide) carbon, suggesting fast turnover. The fast turnover, however, seems to disagree with the ISRIC experiment, where we found that the organic matter extracted by NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> had an average MRT of 730 years and 2000 years respectively. Also Arai *et al.* (1996) analyzed the <sup>14</sup>C activity of the carbon in a combined NaOH/ Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extract of a Vertisol from India and found a relatively long MRT of 4650 years.

The difference between the two experiments in extractability by NaOH and mean residence time of smectite-bound SOM will be further discussed under "*factors that determine the MRT*".

Pyrophosphate-extracted organic matter of smectitic soils VER3A and VER3B seems to have the longest mean residence time compared to the NaOH extract and residue. This agrees with the ISRIC experiment, where pyrophosphate extracted SOM of smectitic clays was significantly older than the other fractions. It was then suggested that smectitic clays can retain SOM by forming cationic bridges with the SOM, thereby restricting decomposition.

The organic carbon in the pyrophosphate-residues of VER3A and VER3B had a relatively fast turnover (Table 5.6). This indicates that the organic matter in the residues was only weakly bound to the mineral surfaces, or that density fractionation did not work and the residue contained particulate organic matter (< 1µm).

In summary, the organic matter bound to the smectite-dominated clays from Mozambique was largely extractable by NaOH, was probably rich in polysaccharides (Wattel-Koekkoek *et al.*, 2001c), and had a relatively fast turnover. The organic matter of the smectitic soils from the ISRIC experiment was extractable by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> rather than NaOH, relatively rich in aromatic carbon, and had a long mean residence time. How can we reconcile these seemingly contrasting results?

#### *Factors that determine the Mean Residence Time*

Multi-linear regression of the data of the ISRIC experiment (Wattel-Koekkoek *et al.* (2001b) revealed that the main factors explaining differences in the <sup>14</sup>C activity of the organic matter in the different fractions, were the ECEC<sub>min</sub> and the specific surface area of the clay-size fraction, each on their own explaining 43% and 50% of the variance. This showed that charge and the amount of mineral surface available play a crucial role in the retention of organic matter. Furthermore, we then found a negative correlation between the ECEC<sub>min</sub> and the <sup>14</sup>C activity of a fraction, and suggested that clays with a high ECEC can stabilize SOM via e.g. cationic bridges, thereby limiting the decomposability, resulting in a high MRT, and thus a low <sup>14</sup>C activity.

The smectitic soils used during the ISRIC experiment had an average ECEC<sub>min</sub> of 67 ± 22 cmol/kg. The smectitic soil from Mozambique however, only had an ECEC<sub>min</sub> of about 29 cmol/kg (Table 5.3). In the ISRIC-experiment, SOM from smectite-dominated samples was hardly extractable by NaOH and largely extractable by pyrophosphate, supposedly because

pyrophosphate can dissolve SOM that is bound via exchangeable cations. The relatively low number of polyvalent cations in the smectitic soils from Mozambique may explain why a large part was extractable by NaOH. Furthermore, the low number of polyvalent cations in smectitic soils from Mozambique may have limited its ability to bind SOM via cation bridges, resulting in a short MRT and thus a high  $^{14}\text{C}$  activity. In short, we hypothesise that the seemingly contrasting behaviour by the smectitic soils from Mozambique is caused by their low ECEC and that in actuality they do behave similar.

To check the role of the  $\text{ECEC}_{\min}$ , and other possible explaining factors for variation in  $^{14}\text{C}$  age, we calculated (multiple) linear regressions ( $n = 22$ ; 14 from the ISRIC experiment and 8 from Mozambique), using  $^{14}a_{\text{corr}}$  of the extracts as dependent variable and a selection of independent variables. The selection was made based on literature and the available data.

Previous authors, using correlation analysis, have identified several factors that control SOM stocks (Jenny, 1930 and 1941; Scott et al., 1996; Sollins, 1996; Zech, 1997 and references cited therein). The most commonly mentioned factors are climate, nutrient status, oxygen availability, soil moisture content, amount and quality of the litter, and texture. Here, we have no data available on the nutrient status, oxygen availability, soil moisture content, and amount of the litter. We did not use texture as a variable, as we studied the clay-size fraction only. We did use

- mean annual *temperature*, as a parameter for the climate
- percentage of *alkyl C*, *O-alkyl C*, *aromatic C* and *carbonyl C* (based on NMR analyses) in the extracts as a characteristic for the chemical composition of the organic matter.
- effective cation exchange capacity of the mineral phase of the clay-size fraction ( $\text{ECEC}_{\min}$ )
- *type of dominating clay mineral* (1 for smectite and 0 for kaolinite) or percentage of *smectite* or *kaolinite* in a clay-size fraction, as calculated from the XRD
- *type of extract* (1 for NaOH extracts and 0 for  $\text{Na}_4\text{P}_2\text{O}_7$  extracts), as a parameter for the binding mechanisms, assuming that NaOH disrupts different bonds from  $\text{Na}_4\text{P}_2\text{O}_7$  (Wattel-Koekkoek et al., 2001a)

The independent variables were partly measured earlier (Wattel-Koekkoek et al., 2001a, 2001c).

The single factor best explaining the variance in  $^{14}\text{C}$  activity was the  $\text{ECEC}_{\min}$  (43%) (Table 5.8), which correlated negatively. This agrees with results from the multiple regression from the ISRIC experiment (Wattel-Koekkoek et al., 2001b). Furthermore, this finding supports our hypothesis that the apparently deviating behaviour by the smectitic soils from Mozambique is caused by their low ECEC, and that in fact they behave similar to the soils from the ISRIC experiment.

The percentage of *smectite* or *kaolinite* were the next best explaining factors, contributing close to 30% to the variance. *Smectite* content showed a negative correlation with  $^{14}\text{C}$  activity. Smectite usually has a relatively high specific surface area and relatively many cations at its surface that can form cationic bridges with organic matter, thereby decreasing its decomposability. The percentage of *kaolinite* (%) shows a positive correlation with  $^{14}\text{C}$  activity. Kaolinite has relatively little charge and surface, which limits its ability to bind organic matter.

Table 5.8 Percentage of variance of the corrected  $^{14}\text{C}$  activity explained (%), correlation coefficients and coefficient of determination ( $R^2$ ) after (multi) linear regression analyses using different combinations of independent variables to predict the corrected  $^{14}\text{C}$  activity of the NaOH and  $\text{Na}_2\text{P}_2\text{O}_7$  extracts.

Independent variables	% of variance explained	Correlation coefficient	$R^2$
O-alkyl (%)	15.7	0.19	0.20
Aromatic-C (%)	18.8	-0.24	0.23
Alkyl-C (%)	2.1	0.38	0.07
Carbonyl-C (%)	11.0	-0.45	0.15
Smectite (%)	29.5	-0.07	0.33
Kaolinite (%)	28.2	0.08	0.32
ECEC <sub>min</sub> (cmol/kg)	42.6	-0.13	0.45
Temperature (°C)	27.2	1.10	0.31
Extract (1/0)*	14.5	5.0	0.19
Alkyl-C (%)	69.5	Alkyl-C 0.51	0.75
ECEC <sub>clay</sub> (cmol/kg)		ECEC -0.10	
Temperature (°C)		Temperature 0.62	
Extract (1/0)		Extract 3.82	
Alkyl-C (%)	68.3	Alkyl-C 0.61	0.74
Smectite (%)		Smectite -0.06	
Temperature (°C)		Temperature 0.80	
Extract (1/0)		Extract 3.74	

When using only one of the NMR components as independent variable (*O-alkyl*, *alkyl*, *carbonyl*, or *alkyl-C*), *Aromatic C* best explains factor for the variance in  $^{14}\text{C}$  activity, explaining 18.8%. *Aromatic C* correlates negatively, presumably because aromatic C is relatively resistant to decomposition. *Carbonyl C* also shows a negative correlation. Carbonyl groups may lower decomposition rates by forming bonds with exchangeable cations at the clay surface. *Alkyl C* did not explain variance in  $^{14}\text{C}$  activity.

The *type of extract* (1 for hydroxide extracts and 0 for pyrophosphate extracts) is positively correlated with  $^{14}\text{C}$  activity, indicating that the NaOH extract contains young, easily decomposable SOM and the pyrophosphate extract contains old, recalcitrant SOM. This suggests that pyrophosphate-extracted SOM was relatively strongly bound (e.g. via exchangeable cations) and hydroxide-extracted SOM rather loosely bound to the mineral surface. *Temperature* shows a positive correlation with  $^{14}\text{C}$  activity of the extracts, explaining 27.2% of the variance. The positive correlation was expected because microbial activity and thus decomposition increases with temperature.

The optimal fit, explaining 69.5% of the variance, was reached when using *ECEC<sub>min</sub>*, *alkyl-C* %, *temperature*, and *extract type* as input variables. The coefficient of determination of this fit was 0.75. This agrees with the ISRIC experiment, where the same factors explained 66.9% of the variance with an  $R^2$  of 0.77, indicating again that the soils from Mozambique behave similar to the soils from the ISRIC. The second best set of variables was *smectite* %, *alkyl-C* %, *temperature*, and *extract type*, explaining 68.3% of the variance (Table 5.8).

When *ECEC* or % *smectite* are used as independent variables, the percentage of *alkyl-C*, and not *O-alkyl*, is the best predicting factor of the chemical data available. Addition of any other NMR-

based variable lowers the percentage of variance explained. This suggests a relationship between clay mineralogy and % alkyl associated to the clay. Theng *et al.* (1992) found that aliphatic components are intercalated in the interlayers of smectites of a Spodosol. However, it seems improbable that the aliphatic components that we found in the extracts were intercalated, because intercalated matter is bound very strongly and is unlikely to be dissolved by NaOH or Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>.

It is remarkable that the percentage of alkyl-C shows a positive correlation with <sup>14</sup>C activity. We expected a negative correlation with <sup>14</sup>C activity as aliphatic components are relatively resistant to decomposition (Lichtfouse *et al.*, 1995; Huang *et al.*, 1999). Our results however agree with Meredith (1997), who analyzed soils of several ages with solid state <sup>13</sup>C NMR, and found that an old stagnopodzol soil had a lower proportion of alkyl C than younger brown earth soils. Although his study concerns completely different soils than ours, it is remarkable in both studies that a high content of alkyl-C is related to young organic matter. Possibly the alkyl-C represents relatively easily decomposable lipids.

### Conclusion

We compared the <sup>14</sup>C activity of soil organic matter present in the whole and heavy clay-size fractions, the NaOH extracts, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts and residues of kaolinite- and smectite-dominated soils originating from Mozambique. We showed that

- All organic matter in the clay-size fraction seems bound to the mineral surfaces; the amount of carbon in the light clay-size fraction separated by density fractionation was negligible.
- The mean residence time of kaolinite bound organic matter did not differ significantly from that of smectite-bound organic matter. This seemingly contrasts with findings from a previous experiment (the ISRIC).
- However, with multiple regression it was shown that the soils from Mozambique behave similar to the soils from the ISRIC experiment. The optimal explanation, having close to 70% of the variance in <sup>14</sup>C activity explained, was reached when using *ECEC<sub>min</sub>*, *alkyl C %*, *temperature*, and *extract type* as input variables.
- The best single factor to explain variance in <sup>14</sup>C activity, was the *ECEC<sub>min</sub>*, which explained 43%, and correlated negatively with <sup>14</sup>C activity. This indicates that the charge of clay minerals has a large influence on SOM turnover.

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## **Chapter 6**

### **General discussion**

The objective of this thesis was to study the long-term effect of the clay minerals kaolinite and smectite on the dynamics of soil organic matter in natural ecosystems. I focused on four aspects: the amount, extractability (as a measure of the binding mechanisms), chemical composition, and mean residence time of soil organic matter in kaolinite and smectite-dominated soils originating from savanna systems in various countries. This was done by employing a C/N analyzer to measure the amount of carbon the clay-size fraction, a sequential extraction method with NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> to test the extractability, CPMAS <sup>13</sup>C NMR and Pyrolysis-GC/MS to characterize the chemical composition, and by measuring the natural <sup>14</sup>C activity to determine the mean residence time. For this study, two sets of soil samples were used. The first set was selected from the collection of the International Soil Reference and Information Center (ISRIC, Wageningen, The Netherlands). It contained 12 soils from seven different countries. Half of the soils had clay-size fractions dominated by kaolinite, the other half was dominated by smectite. The second set of samples was collected in April 1998 by Peter Buurman and myself west of Montepuez, Northern Mozambique. It contained 10 soils, four of which had clay-size fractions dominated by smectite, and six were kaolinitic. All soils used were under native savanna vegetation. In this chapter, the hypotheses formulated in the introductory chapter are discussed in the light of the findings of this thesis and the methodology is evaluated.

### Physical fractionation

To collect the clay minerals, I first separated the clay-size fraction of each soil. All organic matter present in the clay-size fraction was defined as *clay-associated* SOM. In the ISRIC-soils (chapters 2 and 3), I only studied this clay-associated SOM. Scanning electron microscopy indicated that this fraction also included 'free' plant remains. Therefore, in the experiments in chapters 4 and 5 in which I studied soils from Mozambique, sodium iodide was used additionally to separate the 'free' (light) organic matter in the clay-size fractions from the mineral-complexed (heavy) SOM, defined as *clay-bound* SOM. Surprisingly, the light fraction in these samples from Mozambique was negligible. This could indicate that all organic matter present in the clay-size fraction of these samples was complexed or that NaI did not separate very fine plant material. To check whether the density fractionation method was effective, the NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> extracts were filtered (glass filter, 1 μm Ø). If the NaI method failed to do so, the filters should catch 'free' organic matter > 1 μm such as plant remains, and the <sup>14</sup>C age of the filtrate should be older than that of the unfiltered extracts, assuming that relatively large plant remains have a young <sup>14</sup>C age. However, there was no significant difference between filtered and non-filtered extracts. With the naked eye, I only saw a small amount of mineral material on the filter. This suggests that the density fractionation did work. However, free organic matter smaller than 1 μm might be present.

As in the soils from Mozambique the light fraction was negligible, the results on the amount, extractability, chemical composition, and mean residence time of organic matter in the heavy clay-size fraction will be discussed below as if they are representative for the whole clay-size fraction. An advantage of this approach is that the results of the ISRIC-series and of Mozambique can be combined for statistical analysis.

### Amount

I hypothesized that smectite binds a larger amount of carbon than kaolinite. This hypothesis was rejected. Neither the amount of clay-associated organic matter, nor the amount of clay-bound organic matter varied between kaolinite and smectite (chapters 2 and 4). From these findings the question arises why the carbon stock in the clay-size fraction of smectite-dominated soils is not higher than in kaolinite-dominated soils. In my opinion, this can be explained by the difficult physical conditions under which the vegetation grows in smectite-dominated soils. The swelling and shrinking of the smectitic soils impedes root growth. Vertisols are less well drained than kaolinite-dominated soils, which also limits plant-growth. Altogether, it seems likely that the input of organic matter through litterfall and from roots in smectite-dominated soils is relatively small and that it is this limited input of dead organic matter that limits the amount of carbon present in the clay-size fraction. Unfortunately, neither from the ISRIC-soils nor from the soils from Mozambique carbon input data were available to check the explanation. However, a difference in the input of organic matter between kaolinite- and smectite dominated soils could readily be seen in the field in Mozambique. Kaolinite-dominated soils were covered by thick bamboo forests, while the coverage of the smectite-dominated soils was much less dense and by much smaller grass-species.

### Extractability

I hypothesized that kaolinite will bind organic matter relatively weakly via its surface hydroxyl groups, and that smectite will bind organic matter relatively strongly via its exchangeable cations. This hypothesis was tested indirectly by applying a sequential extraction method using NaOH followed by  $\text{Na}_4\text{P}_2\text{O}_7$ . I hypothesized that smectite-bound organic matter would not dissolve in NaOH, but rather in  $\text{Na}_4\text{P}_2\text{O}_7$  as  $\text{Na}_4\text{P}_2\text{O}_7$  can attack SOM bound via exchangeable cations, and that kaolinite-bound SOM would dissolve in NaOH, which can deprotonate the Lewis acid sites of the hydroxyl groups.

In chapter 2 I found that for the ISRIC-series indeed smectite-associated SOM was hardly soluble in NaOH, but was for a large part soluble in  $\text{Na}_4\text{P}_2\text{O}_7$ , while kaolinite-associated SOM was for a large part directly extractable by NaOH (Table 6.1). The fraction % of carbon in the clay-size fraction extracted by NaOH was significantly higher for kaolinite than for smectite (t-test,  $p < 0.05$ ), while the fraction % of carbon extracted thereafter by pyrophosphate was significantly larger for smectite than for kaolinite (t-test,  $p < 0.05$ ). These findings support the hypothesis.

The soils from Mozambique, however, (RED3, RED5, VER3A, VER3B, chapter 4), did not show differences in extractability between kaolinite and smectite-dominated soils (Table 6.1). The smectitic soils behaved similar to the kaolinitic soils: for both soil types the organic matter in the clay-size fraction was largely extractable by NaOH.

Table 6.1 Distribution of C in the extracts and the effective cation exchange capacity of the mineral phase of the clay-size fraction.

soils	set	NaOH extract	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	ECEC <sub>min</sub>
<i>Kaolinite</i>		% fraction of C in clay		cmol per kg
BR1	ISRIC	50.8	10.6	0.0
BR2	ISRIC	43.4	9.5	0.0
LABEX6	ISRIC	64.0	7.0	2.4
ML1	ISRIC	34.7	15	6.1
ML8	ISRIC	21.1	13.4	0.0
MOC4	ISRIC	50.7	17.1	1.1
RED3	MOZ	54	13	5.8
RED5	MOZ	53	14	11.3
<i>Smectite</i>				
IN25	ISRIC	2.3	59.2	70.6
KE66	ISRIC	16.6	49	98.3
LABEX17	ISRIC	10.0	48.0	80.1
NI9	ISRIC	11	31.3	68.6
ZA8	ISRIC	20.4	29.5	38.7
ZA9	ISRIC	30.2	15.9	46.9
VER3A	MOZ	50	15	24.6
VER3B	MOZ	47	15	34.1

The difference between the two experiments in extractability of smectite-bound SOM was shown to be related to the ECEC of the clay minerals. The smectitic soils used during the ISRIC experiment had an average ECEC<sub>min</sub> of 67 cmol/kg, while the smectitic soils from Mozambique have an average ECEC<sub>min</sub> of 29 cmol/kg (Table 6.1). The relatively low ECEC<sub>min</sub> of the Mozambican smectitic soils probably enabled extraction by NaOH. Single factor linear regression of the data from Table 6.1 supports this explanation (Table 6.2). The ECEC<sub>min</sub> explains 76% of the variation in fraction of carbon extracted from the clay-size fraction (%) by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> (correlation coefficient: 0.43), while the percentage of smectite only explains 47% of the variation in fraction (%) of carbon in the clay-size fraction extracted by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. This indicates that the charge of the mineral phase influences the extractability much more than the mineralogy as such. The percentage of C in the clay-size fraction extracted by NaOH is less explained by the ECEC or mineralogy as this fraction is not or less bound via polyvalent cations.

Table 6.2 Percentage of variance explained (%), correlation coefficients and coefficient of determination ( $R^2$ ) after linear regression analyses using different input variables to predict the extractability of clay-size organic carbon by NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>.

output variable	input variable	% variance explained	correlation coefficient	R <sup>2</sup>
% of clay-size C in NaOH extract	ECEC <sub>min</sub>	56.7	-0.45	0.60
	% smectite	38.6	-0.27	0.43
% of clay-size C in Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract	ECEC <sub>min</sub>	76.2	0.43	0.78
	% smectite	46.7	0.25	0.50

When the results of the ISRIC-experiment and Mozambique are combined for statistical analysis, the fraction % of carbon in the clay-size fraction extracted by NaOH is still significantly higher

for kaolinite than for smectite (Student's t-test,  $p < 0.05$ ). Also the fraction % of carbon extracted thereafter by pyrophosphate is significantly larger for smectite than for kaolinite (t-test,  $p < 0.05$ ). This again supports the hypothesis.

A large part of the organic matter was not extractable by NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  (30 to 50%). This was partly expected, because SOM partly consists of aliphatic biopolymers and lipids, which because of their hydrophobicity are not or only partly extractable by polar solutions such as NaOH/  $\text{Na}_4\text{P}_2\text{O}_7$ . Particularly SOM in the clay-size fraction is often dominated by alkyl C (Schulten and Leinweber, 2000). Nevertheless, I choose not to use any non-polar solvent, because they are per definition organic and therefore their usage would contaminate the fractions for measurement of natural  $^{14}\text{C}$ .

### Chemical composition

I hypothesized that the chemical composition of kaolinite and smectite-bound SOM would not be different. It turned out to be difficult to test this hypothesis. Firstly, the pyrophosphate residue (containing 30 to 50% of the carbon present in the clay-size fraction) was difficult to analyze due to its high mineral content. Secondly, the methodologies used (pyrolysis-GC/MS and CPMAS  $^{13}\text{C}$  NMR), were not quite suitable for quantitative analyses. The extracts were analyzed semi-quantitatively using NMR, but these results should be interpreted with care as quantification of NMR data is still under lively discussion (Van Lagen en de Jager, 2001; see also *evaluation of methods*).

Table 6.3 shows the weighted average of the chemical composition of all samples. The calculation was based on the carbon distribution over the extracts and the NMR data (chapters 2 and 4). The percentage of carbonyl C, aromatic C, and alkyl C are not significantly different in the extracted SOM of kaolinite and smectite (t-test,  $p > 0.05$ ). This supports the hypothesis. The percentages of O-alkyl C however, is significantly higher in kaolinite-extracted SOM than in smectite-extracted SOM (t-test,  $p < 0.05$ ). A possible explanation for this difference in composition is that bonds formed via the hydroxyl groups of kaolinite are probably relatively weak. This limits the 'SOM retention capacity' of kaolinite and enables relative fast decomposition. This results in a relatively short mean residence time and thus the relatively large presence of 'fresh' organic matter containing relatively many components still in an early stage of decomposition, such as sugars (see also *mean residence time*).

In general, the extracted organic matter was remarkably rich in O-alkyl C compared to other clay-size fractions studied with NMR in literature (Schulten and Leinweber, 2000), which are frequently dominated by alkyl C. An explanation for this, is that the statistical analysis above only concerns extracted SOM and not the pyrophosphate residues, which contain 30 to 50% of the clay-size organic carbon. I did not succeed in analyzing the residues by NMR due to a high mineral content. I did however analyze the residues by pyrolysis-GC/MS (chapter 2). The pyrograms of the pyrophosphate-residues of both kaolinite and smectite were indeed dominated by peaks of aliphatic components.

Table 6.3 Weighted average chemical composition of the extracted SOM as measured with  $^{13}\text{C}$  NMR.

	Carbonyl C	Aromatic C	O-alkyl C	Alkyl C
<i>Kaolinite</i>				
BR1	9.3	8.4	64.9	17.5
BR2	9.6	11.6	58.8	20.0
ML1	12.7	10.8	58.6	17.9
ML8	11.3	11.1	57.5	20.1
MOZ4	19.5	22.8	44.6	13.2
RED3	19.9	28.7	35.4	16.1
RED5	21.9	19.0	43.3	15.8
<i>Smectite</i>				
KE66	17.1	28.7	35.7	18.5
ZA8	15.8	29.6	38.5	18.8
VER3A	20.6	20.5	38.5	20.6
VER3B	21.5	15.1	43.8	19.5

### Mean residence time

I hypothesized that organic matter in smectitic soils has a relatively long mean residence time, and that organic matter in kaolinitic soils has a relatively short mean residence time. To test this hypothesis, I used soils that were under native savanna systems. In these systems carbon inputs and outputs are assumed to be in equilibrium, and therefore the mean residence time of the organic carbon equals its  $^{14}\text{C}$  age. The  $^{14}\text{C}/^{12}\text{C}$  isotope ratios of the SOM in the clay-size fractions, extracts and residues were measured by Accelerator Mass Spectrometry (AMS) at the Center for Isotope Research in Groningen. From the isotope ratios I calculated the  $^{14}\text{C}$  ages, after applying a correction for the bomb and Suess effect (chapter 2).

The hypothesis was shown to be true for the ISRIC experiment (Table 6.4). The clay-size SOM of smectite-dominated soils is significantly older than clay-size SOM of kaolinite-dominated soils ( $p = 0.011$ ). Also,  $\text{NaOH}$  and  $\text{Na}_4\text{P}_2\text{O}_7$ - extracted SOM of smectite-dominated soils is significantly older than  $\text{NaOH}$  and  $\text{Na}_4\text{P}_2\text{O}_7$ - extracted SOM of kaolinite-dominated soils ( $t$ -test,  $p = 0.039$  and  $p = 0.005$  respectively). The residues were not significantly different from each other ( $p = 0.12$ ).

Multi-linear regression of the data of the ISRIC experiment (chapter 3) was performed using the following input variables:

- effective cation exchange capacity of the mineral phase of the clay-size fraction ( $ECEC_{min}$ ), as a parameter for clay mineralogy
- specific surface area ( $SSA$ ), also as a parameter of clay mineralogy
- temperature (mean annual), as a parameter for the climate
- type of extract (1 for  $\text{NaOH}$  extracts and 0 for  $\text{Na}_4\text{P}_2\text{O}_7$  extracts), as a measure for the binding mechanisms assuming that  $\text{NaOH}$  loosens different bonds than  $\text{Na}_4\text{P}_2\text{O}_7$
- percentage of *alkyl C*, *O-alkyl C*, *aromatic C* and *carbonyl C* (based on NMR analyses) in the extracts as a characteristic for the chemical composition of the organic matter.

## General discussion

Table 6.4 Corrected  $^{14}\text{C}$  activity of the (heavy) clay-size fractions, extracts, and residues

Sample	Set	clay-size fraction	NaOH extract	$\text{Na}_4\text{P}_2\text{O}_7$ extract	residue
<i>kaolinite</i>					
BR1	ISRIC	97.2	98.1	97.7	97.2
BR2	ISRIC	95.8	97.4	96.9	90.7
LABEX6	ISRIC	n.d.	95.4	93.8	90.5
ML1	ISRIC	98.1	98.3	97.7	97.9
MOC4	ISRIC	91.5	94.1	92.0	93.4
RED1	MOZ	93.2	n.d.	n.d.	n.d.
RED1A	MOZ	91.1	n.d.	n.d.	n.d.
RED2	MOZ	93.4	n.d.	n.d.	n.d.
RED3	MOZ	95.1	94.8	94.4	95.5
RED4	MOZ	95.2	n.d.	n.d.	n.d.
RED5	MOZ	94.4	94.1	92.7	94.7
<i>smectite</i>					
KE66	ISRIC	86.9	94.0	76.4	90.9
LABEX17	ISRIC	n.d.	83.8	61.9	71.6
NI9	ISRIC	90.5	94.9	87.0	95.3
ZA8	ISRIC	89.3	93.4	86.0	89.7
ZA9	ISRIC	82.6	90.3	78.5	84.0
PHAE 3	MOZ	96.3	n.d.	n.d.	n.d.
VER2	MOZ	90.3	n.d.	n.d.	n.d.
VER3A	MOZ	96.4	95.4	94.2	96.1
VER3B	MOZ	95.5	95.4	92.8	95.1

The regressions revealed that the main factors explaining differences in the  $^{14}\text{C}$  activity of the organic matter in the different extracts, were the  $\text{ECEC}_{\text{min}}$  and the specific surface area of the mineral phase of the clay-size fraction, each on their own explaining 43% and 50% of the variance. This showed that charge and the amount of mineral surface available play a crucial role in the retention of organic matter. Furthermore, a negative correlation was found between the  $\text{ECEC}_{\text{min}}$  and the  $^{14}\text{C}$  activity of a fraction, which suggested that clays with a high  $\text{ECEC}_{\text{min}}$  can stabilise SOM via e.g. cationic bridges, thereby limiting the decomposability, resulting in a high MRT, and thus a low  $^{14}\text{C}$  activity. The best explanation of the variance was reached when using *SSA*, *alkyl %*, *temperature*, and *extract type* as input variables (70.4% of the variance explained). The second best set of variables was *ECEC<sub>min</sub>*, *alkyl-C %*, *temperature*, and *extract type*, explaining 67.7% of the variance.

The percentage of *alkyl C*, and not *O-alkyl*, was the best predicting factor of the chemical data when *SSA* or *ECEC* were used as independent variables (chapter 3). Addition of any other NMR-based variable lowered the percentage of variance explained. This suggested there is a relationship between clay mineralogy and % *alkyl C* associated to the clay. Theng *et al.* (1986) found that aliphatic components are intercalated in the interlayers of smectites. However, it seems improbable that the aliphatic components that I found in the extracts were intercalated, as intercalation is a very strong type of bond that is unlikely to be dissolved by NaOH or  $\text{Na}_4\text{P}_2\text{O}_7$ .

The percentage of alkyl C correlates positively with  $^{14}\text{C}$  activity. This contradicted my expectation of a negative correlation with  $^{14}\text{C}$  activity, which I based on studies by De Leeuw and Largeau (1993) and Lichtfouse *et al.* (1995) saying that aliphatic components are relatively resistant to decomposition. However, my results do agree with Meredith (1997), who analyzed soils of several ages with solid state  $^{13}\text{C}$  NMR and found that an old stagnopodzol soil had a lower proportion of alkyl C than younger brown earth soils. Although his study concerns completely different soils than ours, it is remarkable in both studies that a high content of alkyl-C is related to young organic matter. Possibly the alkyl C is derived from relatively easily decomposable lipids.

From the soils from Mozambique, both the whole and heavy clay-size fraction, and the NaOH extracts, pyrophosphate extracts, and residues were analyzed (chapter 5). I expected the heavy clay-size fraction to be older than the whole clay-size fraction as the heavy fraction should not contain any free organic matter, but only bound organic matter. However, as mentioned before, the amount of carbon in light fraction was negligible, so that all organic carbon of the whole clay-size fraction also belonged to the heavy clay-size fraction. This was also reflected in the  $^{14}\text{C}$  activities of the fractions: for both the kaolinitic and smectitic soils, the whole and heavy clay-size fraction did not differ significantly.

Furthermore, kaolinite- and smectite-dominated soils from Mozambique did not show differences in  $^{14}\text{C}$  activity, indicating that the hypothesis that organic matter in smectitic soils has a relatively long mean residence time, and that organic matter in kaolinitic soils has a relatively short mean residence time, is not true for these samples. The smectitic soils behaved similar to the kaolinitic soils; for both soil types the organic matter in the clay-size fraction had a short mean residence time. Also when the data of the two experiments are combined (Table 6.4), the  $^{14}\text{C}$  activity of the organic matter in the clay-size fraction of kaolinite-dominated soils does not differ significantly from the organic matter in the clay-size fraction of smectite-dominated soils (t-test,  $\nu = 16$ ,  $p = 0.06$ ), rejecting the hypothesis. The factor best explaining the variance in  $^{14}\text{C}$  activity for the combined data was the  $ECEC_{\min}$  (42.6%), which correlated negatively. This agrees with results from the multiple regression from the ISRIC experiment. There were no data available on the specific surface area from the clay-size fraction from Mozambique, and therefore that correlation was not calculated.

The optimal fit, explaining 69.5% of the variance, was reached when using  $ECEC_{\text{clay}}$ , *alkyl %*, *temperature*, and *extract type* as input variables. The coefficient of determination of this fit was 0.75. This agrees with the ISRIC experiment, where the same factors explained 66.9% of the variance with an  $R^2$  of 0.77. This indicates that the soils from Mozambique behave similarly as the soils from the ISRIC.

It needs to be noted that the input variables used are not all completely independent. For example, the  $ECEC$  and *type of extract* are probably related (see *extractability*). As a consequence, it is not possible, to give weights to the importance of the various input variables used in the multi-linear regression.

## Evaluation of materials and methods

### *Soil samples*

I chose to use soil samples originating from natural savanna systems only, with a clay-size fraction either dominated by kaolinite or by smectite. The first set, as I mentioned before, was assembled from the ISRIC collection. An advantage of this was that I had access to soils from a wide variety of countries, which would have been impossible to collect myself due to high costs in terms of money and time. A disadvantage of this set was that some of these samples had been 'on the shelves' for up to 23 years. Storage possibly affected the organic matter.

The clay mineralogy of a soil is a 'product' of environmental conditions such as 1) the composition of the parent material, 2) intensity and, 3) duration of weathering (Dixon and Weed, 1989). Kaolinite and smectite naturally occur under different environmental conditions. Kaolinite-dominated soils are generally found in well-drained areas of a landscape, are strongly leached, and are well aerated. Under these conditions, organic matter can be mineralized easily, and turnover will be fast. Smectite-dominated soils are frequently found in depressions where water stagnates regularly, limiting aeration of the soil. Under these circumstances, microbial activity is restricted, the turnover of organic matter slowed down, and humified components may accumulate. It is, however, not possible to separate between the effect of clay mineralogy/ECEC and the effect of the environmental conditions as I did not have kaolinitic and smectitic samples originating from similar environmental conditions (e.g. a similar hydrology and position in the landscape), if they exist at all.

### *Physical fractionation*

As my research concerned the effect of clay-mineralogy, I first collected the clay-size fraction of each soil sample by ultrasonic disruption of the sediment, and decantation and freeze-drying of the clay-size fraction. Especially the freeze-drying was very time-consuming; it took several months before 100 g of clay of each sample was collected.

In chapters 4 and 5 density fractionation with NaI was used to study clay-bound organic matter only. As mentioned before, it remains unclear how effective this method was in removing 'free' organic matter from the clay-size fraction.

### *Chemical extraction*

The binding mechanisms between organic matter and the clay minerals were studied indirectly, i.e. by looking at the extractability. This method is an extreme simplification, and better methods to study binding mechanisms between organic matter and clay minerals need to be developed.

I used a relatively high concentration of NaOH (0.5 M) to extract organic matter. This is the concentration used in classical humus studies, which should give results comparable with other studies. One of the questions that it raises, is if such a high dose of sodium does or does not remove all (polyvalent) cations at the clay-surface. If so, than it is questionable whether the organic matter extracted subsequently by pyrophosphate was actually bound via polyvalent cations.

### *Chemical analyses*

Both NMR and pyrolysis-GC/MS, although very valuable in increasing our insight into organic matter chemistry, have important shortcomings. CPMAAS  $^{13}\text{C}$  NMR gives information about the most basic building blocks. Some of the drawbacks of this method are that it is sensitive to paramagnetic ions, such as Fe, and that methods to quantify the results are still under discussion (Van Lagen and de Jager, 2001, in preparation).

Pyrolysis-GC/MS provides information on the chemical composition of the pyrolysis products of the organic matter analyzed, which implicates that the products released, are not the same as the organic entities present in the sample. Although the origin of some components is reasonably certain (e.g. methoxyphenols usually originate from lignin), many pyrolysis products cannot be attributed to a specific category of primary organic components (e.g. benzene).

I only chemically analyzed the hydroxide- and pyrophosphate-extractable organic matter. The pyrophosphate residue, which contains 30-50% of the organic matter, may contain very strongly bound organic matter and therefore forms an important subject for future research.

### *$^{14}\text{C}$ activity*

One of the underlying assumptions for using the  $^{14}\text{C}$  age as the mean residence time of the organic matter, is in that the soils that were used, organic matter input and output were in equilibrium, a so called steady state condition. It is debatable whether this assumption holds for any situation. In my opinion, the usage here however is justified, as the soils originate from native savanna systems, which have been there probably for thousands of years, without having been affected by changes in land-use, etc. The mean residence time of the organic matter that I found in the various soils is relatively low (< 1500 years) compared with the age of the ecosystem.

### **Relevance**

The importance of studying soil organic matter becomes clear when looking at the many functions it has on a global and local scale. Soil organic matter is an important source of plant nutrients. It increases the capacity to adsorb water. It also increases the structural stability of a soil e.g. by forming aggregates. One of the factors which influences organic matter decomposition and so far remained unexplored, is clay mineralogy. I showed that the charge (ECEC) and surface (SSA) of clay minerals explain a large part of the variance in the turnover of organic matter.

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## Summary

The primary source of soil organic matter is plant debris of all kinds, such as dead roots, leaves and branches that enter into the soil and are then biologically decomposed at variable rates. Organic matter has many different important functions on a local and global scale. Soil organic matter is an important source of plant nutrients: when microbes mineralize organic matter, CO<sub>2</sub> and nutrients such as N, P, S, and Ca are released. SOM increases the capacity to adsorb water, and it increases the structural stability of a soil e.g. by forming aggregates with mineral components. Furthermore, forms a major source/sink for atmospheric carbon.

The effect of clay *mineralogy* on organic matter dynamics has not been studied before. My objective was to study the long-term effect of the structurally different minerals kaolinite and smectite on the decomposition of SOM in natural ecosystems.

Smectites are expandible 2:1 layer silicate minerals. The crystal structure of smectite is composed of two tetrahedral silicon-oxide sheets sandwiching one octahedral aluminum-hydroxide sheet. Smectites have a high permanent surface charge, a large surface area, and a high cation exchange capacity (CEC). Kaolinites are 1:1 layer structured alumino-silicates with a low surface area and a low CEC.

I studied four aspects: the amount, extractability (as a measure of the binding mechanisms), chemical composition, and mean residence time of soil organic matter in kaolinite and smectite-dominated soils originating from savanna systems in various countries. We employed a C/N analyzer to measure the amount of carbon the clay-size fraction, a sequential extraction method with NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> to test extractability, solid state CPMAS <sup>13</sup>C NMR and Pyrolysis-GC/MS to characterize the chemical composition, and by measuring the natural <sup>14</sup>C activity to determine the mean residence time.

For this study, two sets of soil samples were used. The first set was selected from the collection of the International Soil Reference and Information Center (ISRIC, Wageningen, The Netherlands). It contained 12 soils from seven different countries. Half of the soils had clay-size fractions dominated by kaolinite, the other half were dominated by smectite. The second set of samples was collected in April 1998 by Peter Buurman and myself, west of Montepuez in northern Mozambique. It contained 10 soils, four of which had clay-size fractions dominated by smectite, and six of which were kaolinitic. All soils used were under native savanna vegetation.

As most clay minerals are present in the clay-size fraction, I first separated the clay-size fraction of each soil. All organic matter present in the clay-size fraction was defined as *clay-associated* SOM. In the ISRIC-soils (chapters 2 and 3), only this clay-associated SOM was studied. A study by scanning electron microscopy indicated that this fraction also included 'free' plant remains. Therefore, in the experiments in chapters 4 and 5, in which I studied soils from Mozambique, sodium iodide was used before the extraction to separate the 'free' (light) organic matter in the clay-size fractions from the mineral-complexed (heavy) SOM, defined as *clay-bound* SOM.

The research in chapter 2 aimed at determining the effect of clay mineralogy on the amount and composition of organic matter that is associated with the mineral surface. <sup>13</sup>C NMR and Py-GC/MS were used to chemically characterize the SOM associated with kaolinite and smectite in clay-size fractions from the ISRIC-soils. The total content of carbon in the clay-size fraction showed no significant difference between kaolinitic and smectitic soils. This suggested that the total amount of organic carbon in the clay-size fraction is independent of the clay mineralogy.

The organic matter in the clay-size fraction was first extracted by NaOH and thereafter by  $\text{Na}_4\text{P}_2\text{O}_7$ . About half of the kaolinite-associated SOM was extractable by NaOH. In the smectitic soils, pyrophosphate extracted more organic carbon than did NaOH. The Py-GC/MS and NMR results indicated that kaolinite-associated SOM is relatively rich in polysaccharide products, while smectite-associated organic matter contains many aromatic compounds. The results suggest that different clay minerals use different mechanisms to bind SOM. As a result, the composition of clay-associated organic matter would be influenced by the type of clay that is dominant in the soil.

In chapter 3, the  $^{14}\text{C}$  activity of clay-associated organic matter of the ISRIC soils was analyzed. The soils originated from natural savanna systems. Assuming that carbon inputs and outputs are in equilibrium in such soils, the  $^{14}\text{C}$  age was taken as mean residence time of the organic carbon. The  $^{14}\text{C}$  activity was corrected for the Suess effect, the Bomb effect and difference between date of sampling and date of  $^{14}\text{C}$  measurement. Kaolinite-associated soil organic matter had a fast turnover (360 years on average). Smectite-associated soil organic matter had a relatively slow turnover, with an average mean residence time for the whole clay-size fraction of 1100 years. Differences in turnover times between organic matter associated to kaolinite and smectite were significant. Multiple linear regression indicated that clay mineralogy, parameterized by specific surface area and effective cation exchange capacity of the mineral phase of the clay-size fraction ( $\text{ECEC}_{\text{min}}$ ), are the main factors explaining differences in the mean residence time of the extracted soil organic matter.

In chapter 4, two kaolinite and two smectite-dominated soils from a native savannah in Mozambique were studied in order to determine the difference in amount and molecular composition of kaolinite- and smectite-bound organic matter in one climatic area. The amount of soil organic matter (SOM) bound was independent of clay mineralogy. Furthermore, the amount of carbon in the light fraction was negligible. The extractability by NaOH and  $\text{Na}_4\text{P}_2\text{O}_7$  (sequentially) of the clay-bound organic matter showed no significant difference between the clays: 50% of the clay-bound SOM was extracted by NaOH and thereafter about 15% by  $\text{Na}_4\text{P}_2\text{O}_7$ . The extracted SOM of all four soils was dominated by polysaccharides. The smectitic soils seem to contain slightly more aliphatic components than the kaolinitic soils. Aromaticity varied among the four soils.

The seemingly contrasting results regarding the extractability of smectite-associated SOM between chapters 2 and 4 may be related to the  $\text{ECEC}_{\text{min}}$  of the clay-size fraction. The smectitic soils used from the ISRIC had an average  $\text{ECEC}_{\text{min}}$  of  $67 \pm 22$  cmol/kg, while the smectitic soil from Mozambique have an average  $\text{ECEC}_{\text{min}}$  of 29 cmol/kg. The relatively low ECEC of the Mozambican soils may have enhanced extractability by NaOH.

In chapter 5, the  $^{14}\text{C}$  activity of organic matter was analyzed in the whole and heavy clay-size fraction of six kaolinite- and four smectite-dominated soils from N'Ropa, in northern Mozambique. For both kaolinite- and smectite-dominated soils, the organic matter in the whole and heavy clay-size fraction and extracts had a fast turnover (400 to 500 years on average). The mean residence time of kaolinite-bound organic matter did not differ significantly from that of smectite-bound organic matter. These results seem to disagree with those of chapter 3. Multiple linear regression of the data from Mozambique and the ISRIC samples, however, agree with the trends found in chapter 3 and indicate that the variation in  $^{14}\text{C}$  activity is not governed by mineralogy directly, but by the  $\text{ECEC}_{\text{min}}$ , which is related to mineralogy. Relatively low

ECEC<sub>min</sub>'s of the investigated smectitic soils from Mozambique bridge the gap in <sup>14</sup>C activity between the two minerals found in chapter 2.

In short, when combining all data, neither the amount of clay-associated organic matter, nor the amount of clay-bound organic matter differs between kaolinite and smectite (chapters 2 and 4). The extractability of the organic matter is different for the two clay minerals: smectite-associated SOM is less extractable by NaOH than kaolinite-associated SOM, but it is relatively well extractable by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. Multiple regressions of the combined data of the soils from the ISRIC and from Mozambique show that the surface charge of the clay minerals, parameterized by the ECEC<sub>min</sub>, explains 76% of the variation in fraction of carbon extracted from the clay-size fraction by Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. This indicates that the charge of the mineral phase influences the extractability much more than the clay mineralogy as such. The chemical composition of the organic matter associated with the two clay minerals varies slightly: kaolinite-associated SOM seems to have more O-alkyl carbon (from polysaccharides) than smectite-associated SOM. A possible explanation for this difference in composition is that bonds formed via the hydroxyl groups of kaolinite are probably relatively weak. This limits the 'SOM retention capacity' of kaolinite and enables relative fast decomposition. This results in a relatively short mean residence time and thus the relatively large presence of 'fresh' organic matter containing relatively many components still in an early stage of decomposition, such as sugars. The <sup>14</sup>C activity of the organic matter in the clay-size fraction of kaolinite-dominated soils does not differ significantly from the organic matter in the clay-size fraction of smectite-dominated soils. The factor best explaining the variance in <sup>14</sup>C activity for the combined data was the ECEC<sub>min</sub> (42.6%). The optimal fit, explaining 69.5% of the variance, was reached when using ECEC<sub>min</sub>, alkyl %, temperature, and extract type as input variables. The coefficient of determination of this fit was 0.75. Of these four input variables, the ECEC<sub>min</sub> gave a negative correlation with the <sup>14</sup>C activity. Clay minerals with a high ECEC<sub>min</sub> have relatively slow organic matter turnover as the exchangeable cations enable the clays to bind organic matter, while clay minerals with a low ECEC<sub>min</sub>, like kaolinites, have relatively fast organic matter turnover, as the ability to bind SOM is much less. Surprisingly, the percentage of alkyl-C showed a positive correlation with <sup>14</sup>C activity. Possibly the alkyl-C represents relatively easily decomposable lipids. Temperature also showed a positive correlation with <sup>14</sup>C activity of the extracts: microbial activity and thus decomposition increases with temperature. The type of extract (1 for hydroxide extracts and 0 for pyrophosphate extracts) was also positively correlated with <sup>14</sup>C activity, indicating that the NaOH extract contains young, easily decomposable SOM and the pyrophosphate extract contains old, recalcitrant SOM. This suggests that pyrophosphate-extracted SOM was relatively strongly bound (e.g. via exchangeable cations) and hydroxide-extracted SOM rather loosely bound to the mineral surface.

The results of the regressions agree with the ISRIC experiment, where the same factors explained 66.9% of the variance with an R<sup>2</sup> of 0.77. This indicates that the soils from Mozambique and from the wide spectrum of locations of the ISRIC set behave similarly in terms of effect of clay mineralogy on organic matter turnover.

## Samenvatting

De belangrijkste bron voor organische stof in de bodem (hierna 'organische stof') wordt gevormd door diverse soorten plantenafval zoals dode wortels, bladeren en takken, welke eenmaal in de bodem met verschillende snelheden door biota worden afgebroken. Op wereldschaal en lokale schaal, heeft organische stof vele belangrijke functies: wanneer microben organische stof mineraliseren, komt er CO<sub>2</sub> vrij en nutriënten zoals N, P, S en Ca. Organische stof zorgt voor toename van de adsorptiecapaciteit van water en voor toename van structuurstabiliteit, bijv. door de vorming van aggregaten met minerale bestanddelen. Daarnaast in organische stof een belangrijke bron voor atmosferische CO<sub>2</sub>.

Tot op heden is de invloed van klei mineralogie op de dynamiek van organische stof nauwelijks bestudeerd. Het doel van mijn onderzoek was om het lange-termijn effect van twee sterk verschillende mineralen, kaolinit en smectiet, op de afbraak van bodem organische stof te bestuderen.

Smectieten zijn 2:1 mineralen die kunnen zwellen. De kristalstructuur van smectiet bestaat uit twee siliciumoxide tetraëder lagen, met daartussen een aluminiumhydroxide octaëder laag. Smectieten hebben een hoge permanente lading, een groot specifiek oppervlak, en een grote kation uitwisselingscapaciteit (CEC). Kaoliniten zijn 1:1 gelaagde aluminium silicaten met een klein specifiek oppervlak en een lage CEC.

Ik heb vier aspecten bestudeerd: hoeveelheid, extraheerbaarheid (als maat voor de bindingsmechanismen), chemische samenstelling, en gemiddelde verblijftijd van organische stof. Dit deed ik aan door kaolinit- en smectiet-gedomineerde gronden van savanne systemen uit diverse landen. Om de hoeveelheid organische koolstof in de kleifractie te bepalen, is gebruik gemaakt van een C/N analyzer; om de extraheerbaarheid te testen is gebruik gemaakt van een sequentiële extractiemethode met NaOH en Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>; om de chemische samenstelling te bepalen is gebruik gemaakt van Curie Point Magic Angle Spinning <sup>13</sup>C Nucleaire Magnetische Resonantie (CPMAS <sup>13</sup>C NMR) en Pyrolyse-Gas Chromatografie/Massa Spectrometrie (Py-GC/MS) en om de gemiddelde verblijftijd vast te stellen is het natuurlijke gehalte aan <sup>14</sup>C gemeten.

Voor deze studie is gebruikt gemaakt van twee sets van bodemmonsters. De eerste set werd geselecteerd uit de verzameling van het International Soil Reference and Information Center (ISRIC, Wageningen). Deze set bestond uit 12 gronden afkomstig uit zeven verschillende landen. Van de ene helft van de gronden werd de klei fractie gedomineerd door kaolinit, van de andere helft door smectiet. De tweede set bodemmonsters is in April 1998 door Peter Buurman en mijzelf verzameld ten westen van Montepuez, in noordelijk Mozambique. Deze set bestaat uit 10 gronden, waarvan er vier door smectiet gedomineerde klei fracties hebben en zes door kaolinit gedomineerde klei fracties. Alle gronden stonden onder een savanne vegetatie.

Aangezien het grootste deel van de kleimineralen zich in de kleifractie bevindt, heb ik eerst van iedere grond de kleifractie afgescheiden. Alle organische stof aanwezig in de klei fractie heb ik gedefinieerd als *klei-geassocieerde* organische stof. Van de ISRIC monsters (hoofdstukken 2 en 3), is alleen deze klei-geassocieerde organische stof bestudeerd. Scanning Electronen Microscopie gaf aan dat deze fractie ook 'vrije' plantenfragmenten bevat. Daarom is in de experimenten van hoofdstukken 4 en 5, waarin ik de gronden uit Mozambique heb onderzocht, natriumjodide gebruikt om voorafgaand aan de extractie 'vrije' (lichte) organische stof in de klei

fractie te scheiden van door mineralen-gecomplexeerde (zware) organische stof. Deze laatste fractie is gedefinieerd als *klei-gebonden organische stof*.

Het onderzoek richtte zich in hoofdstuk 2 op het bepalen van het effect van klei-mineralogie op de hoeveelheid en samenstelling van organische stof die geassocieerd is met het minerale oppervlak. Om de organische stof die geassocieerd was met kaolinit en smectiet in de klei fracties van de ISRIC-gronden chemisch te karakteriseren, werd gebruik gemaakt van CPMAS  $^{13}\text{C}$  NMR en Py-GC/MS. Er was geen verschil tussen kaolinitische en smectitische gronden in het totale koolstofgehalte in de kleifracties. Dit suggereert dat de totale hoeveelheid organische koolstof in de kleifractie onafhankelijk is van de klei mineralogie. De organische stof in de kleifractie werd eerste geëxtraheerd met NaOH en vervolgens met  $\text{Na}_4\text{P}_2\text{O}_7$ . Ongeveer de helft van de kaolinit-geassocieerde bodem organische stof was extraheerbaar met NaOH. In de smectiet-gronden extraheerde pyrofosfaat meer dan NaOH. De Py-GC/MS en NMR resultaten gaven aan dat kaolinit-geassocieerde organische stof relatief rijk was aan polysacchariden, terwijl smectiet-geassocieerde organische stof relatief veel aromatische componenten bevatte. De bevindingen suggereren dat verschillende kleimineralen verschillende mechanismen gebruiken om bodem organische stof te binden. Ten gevolge hiervan, zou de samenstelling van de klei-geassocieerde organische stof beïnvloed kunnen worden door het type klei dat dominant aanwezig is in de bodem.

In hoofdstuk 3 is de  $^{14}\text{C}$  activiteit van de klei-geassocieerde organische stof van de ISRIC gronden geanalyseerd. De gronden waren afkomstig van natuurlijke savanne systemen. Aangenomen dat de koolstof toevoer en afvoer in dergelijke gronden in evenwicht zijn, kon als gemiddelde verblijftijd de  $^{14}\text{C}$  leeftijd genomen worden. De  $^{14}\text{C}$  activiteit is gecorrigeerd voor het Suess-effect, het Bom-effect en het verschil tussen datum van monsternamen en datum waarop de  $^{14}\text{C}$  activiteit werd gemeten. Kaolinit-geassocieerde organische stof had een snelle omzettingstijd (in gemiddeld 360 jaar). Smectiet-geassocieerde organische stof had een relatief langzame omzettingstijd, met een gemiddelde verblijftijd voor de hele klei fractie van 1100 jaar. De verschillen in omzettingstijd tussen organische stof geassocieerd met kaolinit en smectiet waren significant. Multiple lineaire regressie gaf aan dat klei mineralogie, weergegeven door het specifiek oppervlak en de effectieve uitwisselcapaciteit van de minerale fase van de kleifractie ( $\text{ECEC}_{\text{min}}$ ), de belangrijkste factor is om verschillen in de gemiddelde verblijftijd van de geëxtraheerde organische stof te verklaren.

In hoofdstuk 4 zijn twee kaolinit- en twee smectiet-gedomineerde gronden afkomstig van een oorspronkelijke savanne in Mozambique bestudeerd, met het doel om het verschil in hoeveelheid en moleculaire samenstelling van kaolinit- en smectiet-gebonden organische stof te bepalen binnen één klimatologisch gebied. De hoeveelheid organische stof bleek onafhankelijk van de klei mineralogie. Daarnaast was de hoeveelheid lichte fractie verwaarloosbaar. De extraheerbaarheid van klei-gebonden organische stof door (achtereenvolgens) NaOH en  $\text{Na}_4\text{P}_2\text{O}_7$ , vertoonde ook geen significante verschillen tussen de twee kleien: 50% van de klei-gebonden bodem organische stof werd door NaOH geëxtraheerd en 15% door  $\text{Na}_4\text{P}_2\text{O}_7$ . De geëxtraheerde bodem organische stof van alle vier de gronden werd gedomineerd door polysacchariden. De smectitische gronden leken iets meer aliphatische componenten te bevatten

dan de kaolinitische gronden. Het gehalte aan aromatische koolstof varieerde tussen de vier gronden.

Een verklaring voor de schijnbaar tegenstrijdige resultaten uit hoofdstukken 2 en 4 met betrekking tot de extraheerbaarheid van smectiet-geassocieerde organische stof, is mogelijk te vinden in de  $ECEC_{min}$  van de kleifractie. De smectitische gronden van het ISRIC hadden een gemiddelde  $ECEC_{min}$  van  $67 \pm 22$  cmol/kg, terwijl de smectitische gronden uit Mozambique een gemiddelde  $ECEC_{min}$  van 29 cmol/kg hadden. De relatief lage  $ECEC_{min}$  van de gronden uit Mozambique kan de extraheerbaarheid door NaOH verbeterd hebben.

In hoofdstuk 5 is de  $^{14}C$  activiteit van de organische stof bepaald in de gehele en zware klei fractie van zes door kaolinit gedomineerde gronden en vier door smectiet gedomineerde gronden uit N'Ropa, in noordelijk Mozambique. In beide populaties gronden, bleek de organische stof in de gehele en zware klei fractie, en in de extracten een korte omzettingstijd te hebben (gemiddeld 400 tot 500 jaar). De gemiddelde verblijftijd van kaolinit-gebonden organische stof was niet significant verschillend van die van smectiet-gebonden organische stof. De resultaten lijken in strijd met die uit hoofdstuk drie. Multiple lineaire regressies van de gegevens van Mozambique en de ISRIC gronden, komen echter overeen met de trends die eerder gevonden waren in hoofdstuk 3, en geven aan dat de variatie in  $^{14}C$  activiteit niet direct door mineralogie gestuurd wordt, maar door de  $ECEC_{min}$ , welke gerelateerd is aan mineralogie. De relatief lage  $ECEC_{min}$ 's van de onderzochte smectitische gronden uit Mozambique overbruggen het in hoofdstuk 3 gevonden verschil in  $^{14}C$  activiteit tussen de twee kleimineralen.

Kortom, wanneer alle data gecombineerd wordt, blijkt dat noch de hoeveelheid klei-geassocieerde organische stof, nog de hoeveelheid klei-gebonden organische stof verschilt tussen kaolinit en smectiet (hoofdstukken 2 en 4). De extraheerbaarheid verschilt tussen de twee klei mineralen: smectiet-geassocieerde organische stof is minder goed extraheerbaar met NaOH dan kaolinit-geassocieerde organische stof, en is daarentegen relatief goed extraheerbaar met  $Na_4P_2O_7$ . Multiple regressie van de gecombineerde data van de gronden van het ISRIC en uit Mozambique laten zien dat de oppervlakte lading van de klei mineralen, weergegeven door de  $ECEC_{min}$ , 76% van de variatie in de fractie koolstof geëxtraheerd van de klei fractie door  $Na_4P_2O_7$  verklaart. Dit geeft aan dat de lading van de minerale fase de extraheerbaarheid meer beïnvloedt dan de kleimineralogie op zich. De chemische samenstelling van de organische stof geassocieerd met de twee klei mineralen toont een lichte variatie: kaolinit-geassocieerde organische stof lijkt iets meer O-alkyl C te bevatten (afkomstig van polysacchariden) dan smectiet-geassocieerde organische stof. Een mogelijke verklaring voor dit verschil in samenstelling is dat de bindingen die gevormd worden via de hydroxyl groepen van kaolinit waarschijnlijk relatief zwak zijn. Dit beperkt de capaciteit van kaolinit om bodem organische stof vast te houden en dit maakt een relatief snelle afbraak mogelijk. Een en ander resulteert in een relatief korte verblijftijd en de aanwezigheid van relatief veel 'vers' organische stof dat relatief veel componenten bevat die zich nog in een vroeg stadium bevinden in het afbraakproces, zoals suikers. De  $^{14}C$  activiteit van de organische stof in de kleifractie van door kaolinit-geassocieerde gronden verschilt niet significant van de organische stof in de kleifractie van door smectiet-geassocieerde gronden. De  $ECEC_{min}$  bleek de beste factor te zijn om variantie in  $^{14}C$  activiteit van de gecombineerde data te verklaren (42.6%). Het hoogste percentage

verklaarde variantie werd bereikt bij het gebruik van  $ECEC_{min}$ ,  $alkyl-C\%$ ,  $temperatuur$ , en  $type\ extract$ , als invoer variabelen. De  $R^2$  hierbij was 0,75. Van de vier variabelen, vertoonde  $ECEC_{min}$  een negatieve correlatie met  $^{14}C$  activiteit. Gronden met kleimineralen met een hoge  $ECEC_{min}$ , hebben een relatief langzame omzetting van de organische stof, omdat de uitwisselbare kationen het de klei mogelijk maken organische stof te binden. Gronden met kleimineralen met een lage  $ECEC_{min}$ , zoals kaolinit, hebben een relatief snelle omzetting van de organische stof, omdat de mogelijkheid om organische te binden, beperkt is.

Het percentage  $alkyl-C$  laat een verrassende positieve correlatie zien met de  $^{14}C$  activiteit. Wellicht vertegenwoordigt  $alkyl-C$  relatief eenvoudig afbreekbare vetten.

De factor  $temperatuur$  vertoonde ook een positieve correlatie met de  $^{14}C$  activiteit: microbiële activiteit, en daarmee afbraak, nemen toe bij stijging van temperatuur.

Het  $type\ extract$  (1 voor de hydroxide-extracten en 0 voor de pyrofosfaat-extracten) correleerde ook positief met de  $^{14}C$  activiteit. Hieruit blijkt dat de NaOH-extracten jong, relatief eenvoudig afbreekbaar materiaal bevatten, terwijl de pyrofosfaat extracten relatief oude, recalcitrante organische stof bevatten. Dit suggereert dat de pyrofosfaat-geëxtraheerde organische stof relatief sterk gebonden zat (bijv. via uitwisselbare kationen), terwijl de met hydroxide-geëxtraheerde organische stof relatief los gebonden zat aan het mineraaloppervlak.

De resultaten van deze regressies komen overeen met die van het ISRIC-experiment, waarin dezelfde vier factoren 66,9% van de variantie in  $^{14}C$  activiteit verklaarden, met een  $R^2$  van 0,77. Dit geeft aan dat de gronden uit Mozambique en van de diverse locaties van het ISRIC zich vergelijkbaar gedragen ten aanzien van het effect van kleimineralogie op de omzetting van organische stof.

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Ede 2001,

Esther Wattel-Koekkoek

## **Curriculum vitae**

Esther Johanna Wilhelmina Wattel-Koekkoek was born in Tilburg on July 22<sup>nd</sup>, 1974. She completed secondary school (VWO-gymnasium) at St. Pauluslyceum in Tilburg. In 1992, she started the study 'Tropical Landuse' at Wageningen Agricultural University, with erosion and soil- and water-conservation as specialization. This study included an internship in 1995 at the soil physics group of the Texas Agricultural Experiment Station in Lubbock, Texas. In 1995, she started the study 'Soil, Water, Atmosphere', specializing in land evaluation, GIS and Remote Sensing. For her MSc thesis, she developed Neural Network models to predict soil water retention. In 1996 she graduated cum laude in 'Soil, Water, Atmosphere'. From January 1997 until January 2002 she worked on this PhD thesis at the Laboratory of Soil Science and Geology of Wageningen Agricultural University. During part of that time, from May 2000 until March 2001, she worked at the Section Soil Quality on the development of an internet-version of the Introductory Course in Soil Science.