MICROHETEROGENEITY OF SOIL ORGANIC MATTER INVESTIGATED BY C-1s XANES SPECTROSCOPY AND X-RAY MICROSCOPY

Dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
DOCTOR OF NATURAL SCIENCES

presented by

MARC SCHUMACHER
Dipl. phil. nat., University of Berne
born march 30, 1976
from Sennwald, SG

Prof. Dr. Ruben Kretzschmar, examiner
Dr. Andreas C. Scheinost, co-examiner
Dr. Iso D. Christl, co-examiner
Prof. Dr. Chris Jacobsen, co-examiner

2005
# Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of contents</td>
<td>I</td>
</tr>
<tr>
<td>Summary</td>
<td>V</td>
</tr>
<tr>
<td>Zusammenfassung</td>
<td>VII</td>
</tr>
<tr>
<td><strong>1  Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Natural organic matter in the environment</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Objectives of this study</td>
<td>5</td>
</tr>
<tr>
<td>Literature cited</td>
<td>7</td>
</tr>
<tr>
<td><strong>2  Fundamentals of X-ray absorption spectroscopy</strong></td>
<td>11</td>
</tr>
<tr>
<td>2.1 X-ray spectroscopy</td>
<td>11</td>
</tr>
<tr>
<td>2.1.1 Theoretical background</td>
<td>11</td>
</tr>
<tr>
<td>2.1.2 C-1s XANES spectroscopy</td>
<td>13</td>
</tr>
<tr>
<td>2.1.3 X-ray microscopy</td>
<td>15</td>
</tr>
<tr>
<td>2.2 The Stony Brook Scanning Transmission X-ray Microscope</td>
<td>16</td>
</tr>
<tr>
<td>2.2.1 Instrumentation</td>
<td>16</td>
</tr>
<tr>
<td>2.2.2 Sample preparation</td>
<td>18</td>
</tr>
<tr>
<td>2.2.3 Measuring modes</td>
<td>19</td>
</tr>
<tr>
<td>2.3 Literature cited</td>
<td>21</td>
</tr>
</tbody>
</table>
# Table of contents

## 3 C-1s XANES spectroscopy of humic substances:

**Comparison with $^{13}$C-NMR results**  
Summary  
3.1 Introduction  
3.2 Material and methods  
3.2.1 Selection of humic samples  
3.2.2 C-1s XANES spectroscopy  
3.2.3 Solid-state CP-MAS $^{13}$C-NMR spectroscopy  
3.3 Results and discussion  
3.3.1 C-1s XANES  
3.3.2 Solid-state CP-MAS $^{13}$C-NMR  
3.3.3 Correlations between C-1s XANES and $^{13}$C-NMR spectroscopy  
3.3.4 Correlation with O/C ratios  
3.4 Conclusions  
Acknowledgements  
Literature cited

## 4 Seasonal variation of the chemical composition of aquatic dissolved organic matter in boreal forest catchments

**Summary**  
4.1 Introduction  
4.2 Materials and methods  
4.2.1 Sampling sites  
4.2.2 Isolation of DOM  
4.2.3 Elemental analysis  
4.2.4 FT-IR spectroscopy  
4.2.5 Solid-state CP-MAS $^{13}$C-NMR spectroscopy
<table>
<thead>
<tr>
<th>4.2.6</th>
<th>C-1s NEXAFS spectroscopy</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>Results and Discussion</td>
<td>54</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Elemental composition of DOM</td>
<td>54</td>
</tr>
<tr>
<td>4.3.2</td>
<td>FT-IR spectroscopy</td>
<td>55</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Solid-state CP-MAS (^{13})C-NMR spectroscopy</td>
<td>57</td>
</tr>
<tr>
<td>4.3.4</td>
<td>C-1s NEXAFS spectroscopy</td>
<td>59</td>
</tr>
<tr>
<td>4.4</td>
<td>Conclusions</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Literature cited</td>
<td>65</td>
</tr>
</tbody>
</table>

5  Heterogeneity of water-dispersible soil colloids investigated by Scanning Transmission X-ray Microscopy and C-1s XANES micro-spectroscopy

| 5.1   | Introduction           | 71  |
| 5.2   | Materials and Methods  | 72  |
| 5.2.1 | Soil samples and isolation of water-dispersible colloids | 72  |
| 5.2.2 | X-ray microscopy and micro-spectroscopy           | 73  |
| 5.3   | Results and Discussion | 75  |
| 5.3.1 | Heterogeneity of colloids at the particle scale   | 75  |
| 5.3.2 | Inter-particle heterogeneity                        | 78  |
| 5.3.3 | Water-dispersible colloids from different soil horizons | 81  |
|       | Acknowledgements                           | 81  |
|       | Literature cited                           | 82  |
# Table of contents

6 Conclusions and outlook 85

Appendix A A-I
Appendix B B-I
Appendix C C-I
Acknowledgements
Curriculum vitae
Summary

The presence of natural organic matter (NOM) has a profound influence on almost all chemical reactions that occur in terrestrial and aquatic ecosystems. Adsorbed NOM on mineral particles can change the transport properties of nutrients or contaminants within the particular medium by several orders of magnitude. Organic matter adsorption depends first and foremost on the chemical characteristics of the particular carbon functional groups present in the organic structure from where it was originally derived. Depending on its origin, the chemistry of NOM may change significantly and at various scales.

This work focused on the investigation of the heterogeneity of natural organic matter and humic substances using synchrotron-based carbon-1s X-ray absorption near-edge structure (XANES) spectroscopy and Scanning Transmission X-ray Microscopy (STXM). The major goals of this dissertation were the qualitative and quantitative interpretation of C-1s XANES spectra of soil and aquatic environmental samples and its application for the research on the heterogeneity of NOM. Well characterized reference samples were analyzed in order to gain a deeper knowledge about the collection and interpretation of C-1s spectra of humic substances. The variability of NOM samples of aquatic origin was further investigated in terms of possible seasonal differences within their functional group distribution and elemental composition. In a next step, the chemical variations of the mobile colloidal fraction of NOM was analyzed by imaging and quantifying the organic carbon content of water-dispersible soil colloids extracted from different soil types.

In the first part of this project, the various functional groups of organic carbon in humic substances were successfully identified by C-1s XANES spectroscopy. A least-squares fitting scheme was applied in order to quantify the various features present in the absorption spectra of humic substances. The data was compared and correlated with results derived from published quantification schemes. In addition, the resulting data was compared with quantitative data derived from solid-state cross-polarization magic angle spinning (CP-MAS) ¹³C nuclear magnetic resonance (NMR) spectroscopy. Statistical analysis showed that the spectral quantification applied in this study provides reliable data for aromatic and phenolic as well as for carboxyl carbon groups. Further research showed that carboxyl functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic and phenolic bound carbon.
Summary

The variability of NOM of aquatic origin was investigated by analyzing samples from five different locations within Scandinavia, taken in autumn and summer, respectively. The samples were analyzed by Fourier-Transform infrared (FT-IR), CP-MAS $^{13}$C-NMR and C-1s XANES spectroscopy. Seasonal variations between the samples taken in spring and autumn in terms of their carbon group distribution could not be detected. The samples turned out to be remarkably similar without particular differences and all samples were manifested by strong spectral similarities to humic acid, implying their generation over decades or centuries.

The colloidal fraction of NOM derived from fresh soil material was investigated by analyzing the organic carbon content of water-dispersible soil colloids extracted from different soil types in Switzerland. Analysis was carried out at three different scales: The variations within regions of distinct particles were first investigated, followed by the analysis of differences between particles from the same soil horizons. Afterwards, variations between particles extracted from different soil types were analyzed and compared statistically. Spectral analysis was done using principal component and cluster analysis. The results demonstrated that NOM particles are chemically heterogeneous at the micron scale, especially in contents of phenolic and carboxylic carbon. The study revealed that differences in XANES spectra between regions of single particles were much smaller than differences between averaged spectra of different particles isolated from the same horizon. Moreover, it was shown that variations between particles derived from different soil types were smaller than the observed inter-particular variations within carbon functional group distribution.

In conclusion, this work demonstrated that heterogeneity within NOM and humic substances of terrestrial and aquatic origin occurs at various scales. The use of STXM and C-1s XANES spectroscopy allows the direct examination of aggregate structures of NOM and its association with minerals and permits to resolve these variations in functional group chemistry at high spatial resolution.
Zusammenfassung


Zusammenfassung


1 Introduction

1.1 Natural organic matter in the environment

The role of natural organic matter (NOM) in the environment is of major concern in environmental sciences. Its presence in soils, ground and surface waters strongly affects the chemical behaviour of heavy metals, nutrients and potential pollutants due to various sorption and complexation reactions (Stevenson, 1982). NOM can form strong complexes with metal ions like aluminum and iron and several trace elements like copper and lead, leading to either enhanced or decreased mobility of these heavy metals (Sparks, 1995). It bears great importance for mineral weathering in the upper soil horizons, where substantial quantities of trace metals such as Cr or Cd are leached out (McCarthy & Zachara, 1989). The mobility of these trace metals in soil and aquatic environments is controlled mainly by the distribution of NOM between immobile solid phases, mobile colloidal particles and dissolved species (Kretzschmar et al., 1999). After adsorption to stable colloidal phases, these trace metals can be transported over large distances and may infiltrate and contaminate groundwater resources over wide areas (McCarthy & Zachara, 1989).

Natural organic matter in soils, ground and surface waters exists as dissolved molecules, colloids and particles. These distinctions ought to be regarded as dynamic because NOM can be interconverted readily between these forms by dissolution and precipitation, sorption and aggregation processes (Sparks, 1995; Swift, 1996). Organic carbon in natural environments can be divided on the basis of particle size and volatility. Depending on the particular combination of filtration, acidification, purging and oxidation steps, a wide variety of fractions of carbon can be quantified. The classification into humic acids (HA), fulvic acids (FA) and humins, defined upon their solubility in acid and base, is the most referred one. The total amount of organic carbon present in a sample is commonly reported as TOC, whereas the term DOC is defined operationally as the fraction of organic matter in a sample that passes through a 0.45 µm filter (Swift, 1996). Due to the fact that the intensity of research on NOM has increased in various academic disciplines during the last decade, a rich and potentially confusing nomenclature has evolved for organic matter in soils and freshwaters. The terminology used in this thesis is referred to as natural organic matter, regardless of its origin from soils or water environments, except when it is necessary to be more specific.
Introduction

Plant litter and the microbial biomass are the major parent materials for the formation of NOM, whereas polysaccharides, lignin, aliphatic biopolymers and tannins are the major chemical constituents (Schwarzenbach et al., 1993). The composition and relative abundance of these components may vary widely among plant species and tissue type and reflect the large chemical heterogeneity that occurs in NOM (Spielvogel et al., 2004). Oades (1989) demonstrated that a number of factors are decisive for controlling the humification processes in soils and therefore for the formation of NOM. These factors are the amount of litter input, the proportion and distribution of plant parts, as well as the relative proportion of the different plant tissues and their initial chemical composition. As NOM is a mixture of different molecules with sizes varying between 0.5 to 400 nm and a molecular weight ranging from 200 to \( > 10^5 \) g mol\(^{-1}\), it is not easy to attribute a particular size to it (Clapp & Hayes, 1999). De Wit et al. (1993) stated that the diameter of high molecular weight humic molecules present in NOM can generally be estimated to be around 5 nm, whereas Kim et al. (1992) found humic colloids with diameters up to 400 nm in saline aquifer systems. Schulten et al. (2000) proposed a NOM model with an average elemental composition of \( \text{C}_{549} \text{H}_{401} \text{N}_{26} \text{O}_{173} \text{S} \), corresponding to an elemental analysis of 54.02\% C, 5.21\% H, 4.69\% N, 35.67\% O and 0.41\% S with a molecular weight of 7760.16 g mol\(^{-1}\).

For the characterization of the chemical composition of natural organic matter, several approaches using modern analytical techniques are available. The use of chemolytic techniques such as hydrolysis (Kögel-Knabner, 1995), solvent extraction (Dinel et al., 1990) or CuO oxidation (Goni & Hedges, 1990) have been described extensively to isolate amino acids, carbohydrates and lipids from soil material. However, only a part of the soil organic matter present in specific structures can be identified with these techniques. Another method for assessing the chemical composition of natural organic matter is the use of analytical pyrolysis as applied by Leinweber et al. (1998) and Saiz-Jimenez (1994). A disadvantage of this method is the fact that the interpretation of pyrolysis data requires detailed knowledge of the pyrolysis behaviour of the compounds under study (Schnitzer & Schulten, 1995). Furthermore, thermal secondary reactions can cause considerable modifications of the original compound (Schmiess et al., 1999). Alternative techniques for the chemical examination of NOM are non-destructive spectroscopic methods, which include infra-red (IR) spectroscopy, electron spin resonance (ESR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. IR spectroscopy provides rapid and reliable information on the gross chemical composition of NOM as described by Haberhauer et al. (1998), but the method suffers limitations in the quantification of particular carbon groups. The method of ESR spectroscopy gives information on species that contain unpaired electrons as in the case for free radicals within NOM as described by Cheshire.
et al. (1998). However, both methods are relatively insensitive and reveal low resolution, where specific organic compounds cannot be identified unambiguously. The use of cross-polarization magic angle spinning (CP-MAS) $^{13}$C-NMR spectroscopy has greatly increased in the past decade and the technique has become a standard tool for the structural investigation of NOM and humic substances (Cody & Saghi-Szabo, 1999). A wide variety of researchers applied $^{13}$C-NMR spectroscopy for the determination of the various carbon functional groups present in environmental samples (Christl et al., 2000; Kögel-Knabner, 2002; Spielvogel et al., 2004). Mahieu et al. (1999) conducted a statistical survey of the $^{13}$C-NMR spectra of several hundred whole soils and soil size fractions. The review by Perdue et al. (2003) provided as statistical summary of more than 82 reintegrated $^{13}$C-NMR spectra of humic substances and NOM samples derived from freshwaters. For the NOM samples, the mean percentages of carbonyl, carboxyl, aromatic, O-alkyl and alkyl were 5%, 19%, 27%, 25% and 24%, respectively. These results are in good correlation with data published by Mahieu et al. for samples of terrestrial origin, although aromatic and O-alkyl carbon groups were manifested by standard deviations of 13% and all other groups by values of 8%. These results demonstrate that NOM samples are manifested by large variations in their organic carbon content, depending on their origin and genesis from soil or aquatic environments. Mao et al. (2000) stated, that CP-MAS NMR spectroscopy techniques overestimate $sp^3$-hybridized carbon in alkyl and O-alkyl groups and underestimate $sp^2$-hybridized carbon in aromatic, carboxyl and carbonyl functional groups. Furthermore, the number of structural subunits and the chemical shift regions of each subunit may vary widely from one study to another. Conclusions regarding the quantitative interpretation of NMR data of NOM samples have therefore to be drawn with caution.

As mentioned earlier, the presence of NOM in natural systems affects the chemical behaviour of nutrients and potential contaminants to a large extent. Radionuclides and heavy metals have been found to be associated with anionic organic molecules comprising of both hydrophilic and hydrophobic acid fractions of natural organic matter (Champ et al., 1984). Kukkonen et al. (1990) showed that hydrophobic organic contaminants like polychlorinated biphenyl and benzo(a)pyrene bind preferentially to hydrophobic natural organic matter. Whether contaminant transport is enhanced (Dunnivant et al., 1992) or retarded (Totsche et al., 1997) depends on the mobility of the natural organic matter that acts as a carrier. The mobility of NOM in natural porous media is governed by its reactivity with the solid matrix and is further complicated by flocculation and precipitation reactions (Römkens & Dolfing, 1998). These reactions occur at low pH and with high concentrations of polyvalent and potentially bridging ions such as calcium, aluminum or iron. In soil and aquatic systems, the main components for adsorption of natural organic matter are iron and aluminum hydroxides as well as clay minerals (Sposito, 1984). The reactivity of
NOM further depends on hydrological properties of the system such as water saturation, porosity and flow rate. In soil and aquatic systems, these conditions may change over time and spatially along the flow path of transport. It is obvious, that functional group chemistry on one hand and molecular structure on the other governs the behaviour of NOM in the environment, whereas both properties are strongly interrelated and one influences the other (Schmidt et al., 1999). For instance, most studies suggest that the chemical composition varies with the size of the particular NOM fraction and that differences between size fractions may have a strong influence on the environmental behaviour of these molecules (Christl et al., 2000; Kukkonen et al., 1990). However, it is still unclear at which scale the occurring heterogeneity of natural organic matter becomes relevant and to which extent they further influence the chemical behaviour of possible contaminants within water and soil environments. Other studies have shown that the summarized chemical characteristics of organic-mineral particles have direct effects on properties such as soil aggregation and the resulting physical behaviour of soil material. It was clearly shown that the organic matter is a key factor for the physicochemical reactions at the organic-mineral surfaces (Cheshire & Senesi, 1998; Kaiser et al., 1997). Despite these studies, the role of NOM and its heterogeneity when adsorbed to mineral surfaces is still controversial and needs to be investigated at the particle level.

While conventional laboratory spectroscopy and microscopy techniques suffer from lack of sensitivity, element-specific X-ray spectroscopy and spectromicroscopy methods using synchrotron radiation can allow the microscopic examination of NOM at the micron scale. The use of soft X-rays with wavelengths between the K absorption edges of oxygen and carbon (543 eV/2.3 nm and 284 eV/4.4 nm) is of particular interest for studies with environmental samples, for within this energy range water is highly transparent to X-rays compared to other substances. This “water-window” contrast arises from a difference in absorptivity for water and carbonaceous (or other dense inorganic) material (Kirz et al., 1991). The samples can easily be examined in a hydrated state, at atmospheric conditions and without any pre-treatment such as the removal of iron or oxygen (Jacobsen & Neuhäusler, 1999). Recent developments in soft X-ray optics and instrumental design have made it possible to obtain detailed information about the functional group chemistry of natural, environmental samples down to a spatial resolution of 30 nm (Medenwaldt & Uggerhøj, 1998). An addition to these techniques is synchrotron-based Scanning Transmission X-ray Microscopy (STXM), where the distribution of elements like carbon or oxygen can be mapped versus that of other, heavier elements. The addition of a tunable monochromator at energies from 280 eV to 320 eV allows the collection of X-ray absorption spectra close to the carbon 1s-absorption edge (C-1s XANES). In the
past decade, several studies were conducted on the research of coal samples (Cody et al., 1995), the investigation of biopolymers like peptides or amino acids (Boese et al., 1997) or the carbon chemistry of interplanetary dust particles (Chapman et al., 1995) by applying STXM and C-1s XANES spectroscopy. More recently, Scheinost et al. (2001) investigated the distribution of carbon within selected humic substances, whereas Jokic et al. (2003) and Schäfer et al. (2003) conducted X-ray absorption spectroscopy experiments on non-living organic matter in soils. However, only little research has been done focusing on the chemical heterogeneities of humic substances and aqueous NOM particles at the particle scale. Rothe et al. (2000) investigated interactions of humic acid with clay colloids whereas Thieme et al. (2003) mapped calcareous precipitates within soil samples of a few microns in scale. The use of STXM and C-1s XANES spectroscopy as a tool for the investigation of environmental samples opens up the possibility to speciate and quantify the major carbon groups present in NOM. Moreover, the method permits the in-situ analysis of nanoscale heterogeneity present in NOM with high spatial resolution and is able to provide direct data of organic-mineral interfaces.

1.2 Objectives of this study

The particular research objectives in this thesis addressed are (i) the quantitative interpretation of C-1s XANES spectra and its application to the research on the heterogeneity of NOM. A consistent quantification scheme is developed and applied on the spectral data of well characterized reference humic substances. The developed quantification scheme is then applied to a study, where (ii) the variability of NOM from different origins in terms of its carbon functional group distribution and elemental composition is investigated. For this, a set of ten well characterized NOM samples from five locations in Scandinavia is analyzed by C-1s XANES spectroscopy. Variations in terms of carbon chemistry and elemental content with respect to seasonal and geographic differences shall be investigated. In a next step, (iii) the variability of water-dispersible colloids derived from different soil types and origins shall be investigated and analyzed by statistical means.

The fundamentals of X-ray absorption spectroscopy and STXM are described in chapter 2. For studying the detection and quantification possibilities of C-1s XANES spectroscopy for specific carbon functional groups, standardized reference humic substances were investigated with STXM and solid-state CP-MAS $^{13}$C-NMR spectroscopy (chapter 3). In order to investigate the chemical variability of NOM of aquatic origin, ten samples were investigated by FT-IR, CP-MAS $^{13}$C-NMR and C-1s XANES spectroscopy (chapter 4) and analyzed with respect to seasonal and geographic differences. Finally, water-dispersible
Introduction

colloidal particles extracted from three soil types in Switzerland were analyzed by C-1s XANES spectroscopy and STXM. The aim of this study was to show if colloidal particles exhibit nominal changes in carbon functional group chemistry at different scales. Quantification of the main carbon groups was carried out with particular interest in variations at the level of particles, soil horizons and locations. (chapter 5).
**Literature cited**


Introduction


Introduction


2 Fundamentals of X-ray absorption spectroscopy

2.1 X-ray spectroscopy

2.1.1 Theoretical background

Electrons provide vital information about the bonding environment of the specific element they belong to. The number of electrons and their energy levels in a specific molecule govern its behaviour in the environment. X-ray spectroscopy methods can provide information on the status of these electrons in the molecules (Teo, 1986). These electron energy levels and their respective electronic spectra can be well understood by first examining the electronic states and spectra of simple mono- and diatomic molecules, as shown in Figure 2.1 (From Stöhr, 1996, with permission).

![Figure 2.1: Schematic potentials (bottom) and K-shell spectra (top) of atoms (left) and diatomic molecules (right). From Stöhr, with permission (1996).](image-url)
In single atoms as in the case for protons and noble gases, energy states of electrons can be determined from the Coulomb potential of the nucleus. Some of these states are empty (Rydberg states) and differences in their energy states become smaller with distance away from the nucleus. Finally, they converge to the vacuum level or continuum states, which correspond to the energy region above the ionization potential (IP) and have no relation to the atom. When electrons in single atoms are excited, they make transitions from occupied atomic orbitals to empty Rydberg orbitals. Such transitions occur when the incident energy is equal to the difference between the energy states of these orbitals. The electronic spectra show distinct, well developed peaks that correspond to the electronic transitions to these discrete levels. In the situation, where two atoms are brought closer together as in the case of diatomic molecules like O$_2$ or N$_2$, significant overlaps resulting from the atomic orbitals of these two atoms form molecular orbitals (MO). Electrons from these two atoms become delocalized and move into these molecular orbitals (Stöhr, 1996).

The linear combinations of atomic orbitals result in the formation of both bonding ($\pi, \sigma$) and antibonding ($\pi^*, \sigma^*$) molecular orbitals, which are below and above the energy levels of the atomic orbitals of the individual atoms. The energy levels of both bonding and antibonding orbitals as well as their energy differences are affected by the intensity of the overlap of the atomic orbitals. In addition, changes in the type of atom and its energy states also modify the energy levels of the particular molecular orbitals. The electronic spectra of these molecules provide information on orbital energy levels and therefore the strength of interactions between the atoms. Since the innermost (1s electrons) are localized and are not affected significantly by the overlap of the outermost (valence) orbitals, the transition of these 1s electrons can be used as a ruler to measure the bonding interactions between the different atoms (Stöhr et al., 1984). In the case of CO, the energy states of valence orbitals can be probed by exciting electrons either in carbon or in oxygen (Eustatiu et al., 2000).

The same approach can be used to understand the chemical bonding in polyatomic molecules and polymers. The electronic spectrum of a polymer can be described as the sum of the spectra of individual diatomic components (Kaznacheyev et al., 2002). Since the orbital composition and energy states of an atom of interest in a molecule are modified primarily by the energy states of its neighbours, the so-called building-block approach is valid for describing the electronic structure of polymers (Morra et al., 1996). However, not only interactions of diatomic molecules and their respective orbitals contribute to the overall spectrum of this central atom, but also the complete local coordination around it as well as their hybridized orbitals should be considered (Stöhr, 1996). Furthermore, in the case of molecules, which exhibit delocalized electrons such as those with conjugated...
Fundamentals of X-ray absorption spectroscopy

\(\pi\)-systems, the coordination environment beyond the first neighbours should be taken into account as well (Bianconi et al., 1988).

2.1.2 C-1s XANES spectroscopy

A typical X-ray absorption spectrum of a molecule exhibits intense peaks that correspond to the bound state transitions at low energies, followed by the scattering-related features a few eV above these transitions. The part of the X-ray absorption spectrum that corresponds to the bound state transitions is referred to as X-ray absorption near-edge structure (XANES) or near-edge X-ray absorption fine structure (NEXAFS), whereas the spectrum well above the bound state transitions is called extended X-ray absorption fine structure (EXAFS). Since the electronic transitions are sensitive to the local coordination environment, electronic spectra can be used to identify the functional groups of molecules and their local bonding environment. Therefore, X-ray absorption spectroscopy is described as an element specific spectroscopy (Teo, 1986).

When an element absorbs X-rays, electrons jump from a level in the atomic core, first to the lowest-lying unoccupied molecular orbitals and then at higher energies to the continuum, resulting in an ionization of the element. Such quantum jumps from core atomic levels to the empty orbitals require energies that are characteristic for each element. Quantum mechanical considerations, called selection rules, limit the type of electron transitions that are allowed, whereas each type of allowed transition represents a different edge designation and energy. The position of this ionization edge, meaning the energy difference between the initial electronic level and the continuum, is a direct expression of the ionization energy of the element in its chemical environment. That energy strongly depends on the valence (oxidation state) of the element. It becomes increasingly difficult to ionize an element, as its oxidation state changes from negative to neutral and then to positive values. The actual position of this absorption threshold depends first and foremost on the element absorbing the X-rays and second on its oxidation state. All X-ray absorption edges have fine structure at the edge, indicating allowed transitions into vacant electronic levels lying just below the continuum. These low-lying vacant states include anti-bonding orbitals whose energy and symmetry provide information about the chemical environment of the sample. In this study, the focus is laid on the spectral features of carbon, further referred to as C-1s XANES spectroscopy.

Molecules containing \(\pi\)-orbitals generally have their lowest energy inner-shell transition to the first unoccupied or antibonding \(\pi^*\)-molecular orbital (MO). This transition is typically observed as a sharp and well pronounced absorption band several eV below the ionization threshold for the specific molecule. Depending on the molecule, there are several numbers of \(\pi^*\) states observable. As the monochromator moves towards
higher energies, the energy of the incident photon exceeds the ionization threshold of the core electron. Due to the electronic environment surrounding the core electron, the ionization potential (IP) is chemically shifted towards higher energies. Typically, IP’s for carbon range from 290-296 eV, where low values correspond to aliphatic and aromatic carbon and highest values to carboxylic bound carbon (Stöhr, 1996). In addition to antibonding π-orbitals, there are also antibonding σ molecular orbitals. The 1s-σ* transitions to these molecular orbitals are found several eV above the ionization threshold. The more delocalized nature of the electron in the σ* state coupled with the multiplicity of several 1s-σ* transitions leads to a high overlap and make peak designations within this region of the XANES spectrum rather difficult. In order to illustrate the multiple features contributing to a C-1s XANES spectrum, spectra from common polymers as well as for graphite, calcite, and carbon dioxide are presented in Figure 2.2. Spectra for the six polymers were taken from published data with permission by Dhez at al. (2003). The spectra for graphite and calcite were taken with permission from Flynn et al., the spectrum for carbon dioxide was provided by Jacobsen et al. (1996). The spectra for ethylene propylene rubber (EPR) and polyethylene (PE) are dominated by saturated bonds, present in polyolefins and polyethers. The spectra exhibit features around 288.5 eV that are characteristic for 1s→π* transitions, present in carboxyl.
functional carbon groups (Cody et al., 1998). The main chemical differences between these both polymers are the addition of methyl groups to the CH₂-backbone, as manifested in small spectral differences. The C-1s spectra of the next four polymers, polyisoprene (PI), neoprene, polymethyl styrene (PMS), and polyethylene therephtalate (PET) are typical for molecules containing unsaturated functional groups such as phenyl rings and double bonds (Stöhr, 1996). They are dominated by low-energy 1s→π* transitions around 285 eV. The shape of these bands varies with the chemical and electronic structure of these polymers, especially in the presence of aromatic rings as in the case for PMS and PET (Urquhart et al., 2000). The peak at 286.5 eV, well developed in the spectrum of neoprene, corresponds to transitions that are due to electron-withdrawing bound atoms to the carbon structure, as in the case for phenols or aryl ether (Stöhr, 1996). In addition, all four spectra are manifested by transitions at 288.5 eV, characteristic for carboxyl functional groups as discussed previously. The spectrum for graphite is manifested by a broad band around 285 eV, indicating the presence of carbon double bonds. The spectra for calcite and carbon dioxide in contrast are uniquely dominated by bands at 291 eV, characteristic for transitions of C=O bonds within carbonaceous material. All these features make C-1s XANES spectroscopy a very sensitive tool to investigate the bonding environment of carbon present in organic rich samples.

2.1.3 X-ray microscopy

X-ray microscopy makes use of X-rays with an energy of 100 to 1000 eV or wavelengths of 1 to 10 nm. These X-rays are commonly referred to as soft X-rays. Their wavelengths are much smaller than those of visible light, giving the potential for high spatial resolution imaging. In Scanning Transmission X-ray Microscopy, the beam is focused on the sample and directly collected using a photodiode or a simple proportional counter. An illustration of a typical image scan is shown in Figure 2.3.
For imaging with X-rays, the beam needs to be focused on a small spot using either zone plates, Kirkpatrick-Baez mirrors, or tapered capillaries (Attwood, 2000). Except for photoemission electron microscopes, all soft X-ray microscopes are based on focusing with zone plates and use sample transmission to collect high resolution images (Ade et al., 1997). The spatial resolution of the incoming X-ray beam depends on the outer zone width of the zone plate. Up to now, a spatial resolution of 30 nm can be acquired (Feser, 2002). For data acquisition, a two-dimensional image is collected by scanning the sample stage at fixed photon energy, set by the grating and by keeping the zone plate at the appropriate focal distance from the sample. Absorption spectra at one specific point within the image or a sequence of images at different energies can be collected by changing the photon energy and by moving the zone plate accordingly. This stack of images provides the absorption contrast and a XANES spectrum in the third dimension at any selected location on the image.

### 2.2 The Stony Brook Scanning Transmission X-ray Microscope

All X-ray absorption experiments presented in this thesis were conducted at beamline X-1A at the National Synchrotron Light Source in Upton, N.Y., using the Scanning Transmission X-ray Microscope (STXM) developed by the group of J. Kirz and C. Jacobsen from State University of New York in Stony Brook.

#### 2.2.1 Instrumentation

A schematic setup of the X-1A beamline and the STXM is given in Figure 2.4.
In Scanning Transmission X-ray Microscopy, spatial coherence is crucially needed in order to get the highest possible spatial resolution, limited only by diffraction (Wang & Jacobsen, 1997). For this reason, the X1 undulator, a tunable, bright soft X-ray source with 35 periods of SmCo$_5$ hybrid magnets was built in. The undulator is working at photon energies between 200 to 800 eV, covering absorption thresholds for elements such as carbon, oxygen or chloride (Henke et al., 1982). A toroidal mirror deflects and focuses the generated synchrotron radiation from the undulator onto the monochromator entrance slit in the horizontal and onto the exit slits in the vertical plane. The monochromator itself consists of a spherical grating with 900 lines per mm. The wavelength is determined by the entrance slit size. The exit slit coming afterwards determines the spatial coherence of the X-ray beam. After having passed all beamline optics, the beam reaches the actual microscope setup. The X-rays exit the beamline vacuum and illuminate the zone plate. The order sorting aperture (OSA), located between zone plate and the sample, ensures unwanted diffraction orders, except for the first order used for imaging, entering the microscope. The OSA must be precisely aligned to the center stop of the zone plate, so that no other light from higher diffraction orders can reach the sample. The sample can be moved perpendicular to the beam both in x- and y-direction, using either stepper motors with step sizes typically between 1 to 10 µm for coarse, large image steps up to several mm, or piezoelectric actuators for high resolution scans of smaller image steps of 10 to 50 µm. The dwell time per image pixel ranges between 1 ms for quick overview scans to 120 ms for images with good photon statistics. The distance between exit window and counter can be up to 1 cm. A constant helium flow in the detector and sample region ensures higher transmission. During experiments, the microscope is covered with a plexiglas top in order to provide constant, undisturbed conditions. The radiation transmitted through the sample is detected by an improved multi-wire proportional counter, counting with low noise and linearly up to 1 Mhz count rate (Feser et al., 2001). A photograph of the microscope is presented in Figure 2.5. An extensive discussion on the microscope and its specifications is presented by Kirz et al. (1995).
2.2.2 Sample preparation

Dry films
For C-1s XANES measurements of freeze-dried sample material, thin films with 50 to 200 nm of thickness have to be prepared and deposited afterwards onto Si₃N₄ windows (Silson Ltd., UK) with 100 nm in thickness and air-dried. The high absorption coefficient of carbon at these wavelengths, $\gamma = 10^4 \text{ cm}^2 \text{ g}^{-1}$ requires the sample thickness to be less than 800 nm for imaging purposes and less than 400 nm for quantitative spectroscopy (Henke et al., 1982). Absorption spectra of samples thinner than 50 nm often show a poor signal-to-noise ratio, whereas the spectra of samples thicker than 200 nm can be distorted by absorption saturation (Urquhart et al., 1999). Typically, sections of ~ 100 nm in thickness are ideal for X-ray microscopy and C-1s XANES spectroscopy purposes.

Hydrated samples
In order to investigate colloidal particles in aqueous solutions, the sample mount has to be changed from a simple sample holder to a wet specimen chamber or wet cell. Figure 2.6 shows photographs of a mounted wet specimen chamber.

![Figure 2.6: Photographs of the wet specimen chamber. Adapted from Feser (2002).](image)

- a) The two halves of the wet specimen chamber, each with a silicon nitride membrane. After placing a droplet of sample material on one half of the sample holder, both parts are screwed together and adjusted in pressure with screws (backside).
- b) After the adjustment of the pressure within both silicon nitride windows and sealed the cell with tape in order to prevent the sample from drying out, the wet cell is ready for insertion into the microscope.

The wet cell is a double-sample mount with two opposite silicon nitride windows, whereas a thin water layer is formed between the surfaces within those windows. An O-ring seal is made between the metal parts of the chamber in order to retain the water and avoid evaporation. The thickness of the water layer is controlled by three screws that press the two parts together. Further details on the wet cell are presented by Neuhäusler et al. (2000).
2.2.3 Measuring modes

The STXM can be operated in two spectroscopic modes. The first mode is the conventional spectroscopy mode typically used for the collection of C-1s XANES spectra of dry films. The second operation mode is the spectromicroscopy mode, which is commonly used for the collection of XANES spectra of hydrated samples using the wet cell.

Conventional spectroscopy mode
In conventional spectroscopy mode, the sample is positioned in a way that the X-ray focus spot hits the region of interest within the sample. While the sample stays in place, absorption data are taken by changing the photon energy and re-focusing the zone plate accordingly and detecting the signal in transmission. Although strongly depending on the chosen energy resolution, a common absorption spectrum consists of 512 data points and covers an energy range of about 30 eV in order to cover pre- and post-edge signals. A normalization spectrum I₀ without sample material has to be taken in order to take variations, resulting from non-constant efficiencies of the undulator, the beamline and the zone plate optics into account (Jacobsen & Kirz, 1998).

Spectromicroscopy mode
The second possibility of collecting X-ray absorption data is the spectromicroscopy mode or alternatively the “image-stack” acquisition mode as implemented by Jacobsen et al. (2000). An automated routine takes a data set of images at closely spaced photon energies within a defined range of energies. The set of images obtained can be aligned afterwards using an autocorrelation routine whereas variations in X-ray intensity or offsets of the sample can be eliminated. The region from which a spectrum is obtained is limited by the microscope resolution, allowing the collection of spectral information from very small sample regions that would otherwise not be accessible with the conventional spectroscopy technique. Special attention has to be paid in considering regions in the image without sample material (I₀) for spectra normalization. These regions have to be in practice at least sample regions that do not contain the particular absorption edge element. For compounds that do not contain the edge element, the X-ray absorption can be assumed to be constant over a small energy interval (Winn et al., 2000). A disadvantage of this measuring mode is that it is very time-consuming. Even if the quality of the images is chosen to be of lower quality, acquisition of a stack data set takes between 3 and 6 hours. The advantage of such a set is that it contains spectra of all image points within the investigated sample. Some sample regions might not attract the attention of the user during the experiment, but might turn out to be interesting when analyzing the
data set, for example with principal component or cluster analysis (Lerotic et al., 2004). Further details on the collection of image stacks are given in Jacobsen et al. (2000).
2.3 Literature cited


Chapter 3 was prepared for publication as:

Marc Schumacher, Iso Christl, Andreas C. Scheinost, Chris Jacobsen and Ruben Kretzschmar

C-1s XANES spectroscopy of humic substances: Comparison with $^{13}$C-NMR results

*European Journal of Soil Science*
3 C-1s XANES spectroscopy of humic substances: Comparison with $^{13}$C-NMR results

Summary

Synchrotron-based C-1s X-ray absorption near-edge structure (XANES) spectroscopy and solid-state $^{13}$C nuclear magnetic resonance (NMR) spectroscopy were used to characterize organic carbon structures in selected humic acid, fulvic acid and NOM samples. C-1s spectra were quantitatively evaluated using a new fitting method which considers K-shell 1s-transitions for aromatic carbon (285 eV), phenolic carbon (286.5 eV), carboxyl carbon (288.5 eV) and carbonyl carbon (290.5 eV), two 1s-transitions (295 eV and 300 eV) and the ionization step (290 eV). In addition, C-1s spectra were quantified using the fitting method recently reported by Solomon et al. (2005). Results obtained with both methods were compared to $^{13}$C-NMR results and O/C elemental ratios.

The results showed that our new XANES fitting method gave reasonable correlations with NMR results for aromatic plus phenolic carbon ($R^2 = 0.85$) as well as for carboxyl carbon ($R^2 = 0.85$). In addition, O/C ratios correlated with C-1s XANES peak areas for aromatic plus phenolic carbon ($R^2 = 0.71$) and for carboxyl carbon ($R^2 = 0.80$). Using the method from Solomon et al. (2005), a reasonable correlation with NMR results was only obtained for aromatic, phenolic and quinone carbon ($R^2 = 0.85$) while the correlation for carboxyl carbon was poor ($R^2 = 0.28$). We conclude that using our fitting method, quantitative information on aromatic and phenolic as well as carboxyl functional groups of humic samples can be obtained from C-1s XANES spectroscopy, which is in agreement with $^{13}$C-NMR results.
3.1 Introduction

Soil organic matter (SOM) is a fundamental component of in the global carbon cycle (Sparks, 1995). SOM is considered to be a heterogeneous mixture of naturally occurring compounds of plant and microbial origin in various stages of decomposition (Stevenson, 1982). These biomass residues can be transformed by degradation reactions into a series of complex organic compounds (Oades, 1989). Their chemical composition consist of carbohydrates (10%), lipids (10%), nitrogen-components (10%) and humic substances (70%) (Schnitzer, 1991), but may vary considerably with specific environments. As a major portion of the total organic carbon pool in soils, humic substances (HS) play an important role in many significant biogeochemical and environmental processes due to their protolyzing and complexing characteristics (McCarthy & Zachara, 1989; Schlesinger, 1991). Based upon their solubility in acid and base, HS can be chemically fractionated into fulvic acids, humic acids and humins (Clapp & Hayes, 1999), containing unknown and highly variable amounts of reactive carbon functional groups. The heterogeneity within various scales has made the structural and functional characterization of HS extremely challenging.

CP-MAS $^{13}$C nuclear magnetic resonance (NMR) spectroscopy has been used for several decades to identify and characterize the chemical properties of HS. However, questions arise about the quantitativeness of the various chemical functional groups present in HS (Mahieu et al., 1999). The use of $^{13}$C-NMR spectroscopy permits the investigation of the chemical bulk properties of HS and imparts information that is the net result of all the chemical structures present in the sample. In this regard, advances in the characterization of HS further benefited from the progresses made in non-destructive micro-scale X-ray spectroscopy techniques. Hereby, synchrotron-based Scanning Transmission X-ray microscopy (STXM) combined with X-ray absorption near-edge structure (XANES) spectroscopy has been shown to be a powerful method to investigate the carbon chemistry of HS at high spatial resolution (Schäfer et al., 2003). The application of X-ray microscopy probes the local bonding environment of carbon in the presence of water without substantial attenuation of the sample material (Jacobsen, 1999). The imaging possibilities provide further insight into the functional group chemistry of carbon-rich areas within the scale of one micron (Jacobsen et al., 2000).

In the past, C-1s XANES spectroscopy has been effectively employed to study coal (Cody et al., 1995; Cody et al., 1996), humic substances (Rothe et al., 2000) and biopolymers (Ade & Urquhart, 2000; Kikuma & Tonner, 1996). In these studies, the carbon functional group chemistry of samples with unknown composition has been inferred from spectra of reference samples by comparing band heights and shapes. Other studies have
demonstrated the potential of this technique for characterizing single colloidal particles in soils (Jokic et al., 2003) and for the semi-quantification of organic functional groups in humic and fulvic acids (Scheinost et al., 2001). Despite these studies on the investigation of environmental samples with C-1s XANES and STXM, the method suffers limitations in terms of the detection and quantification possibilities of the different carbon groups present in organic rich samples. Due to the complexity of the spectra of environmental samples, signals for specific carbon groups are difficult to assign and make the use of ab-initio calculations rather difficult or even impossible (Kaznacheyev et al., 2002). The current work presents the application of C-1s XANES and solid-state CP-MAS 13C-NMR spectroscopy on standardized IHSS reference samples from different origins. The objectives of this study are to characterize and quantify the spectroscopic features of the reference samples by C-1s XANES and solid-state CP-MAS 13C-NMR spectroscopies. Results from both techniques as well as information about elemental ratios are compared with data derived from the quantification scheme recently reported by Solomon et al. (2005). The information obtained provides new insight into the understanding of C-1s XANES spectra of HS and the establishment of a consistent quantification scheme for C-1s spectra.

3.2 Material and methods

3.2.1 Selection of humic samples

Ten well characterized humic samples were selected for this study. First, a purified humic and fulvic acid sample isolated from a hemic Gleysol in Northern Switzerland (Christl et al., 2000) were selected for analysis. In addition, eight reference samples from the International Humic Substances Society (IHSS) were selected and used without further purification (McCarthy & Malcolm, 1978). The samples were chosen in order to cover a wide range of different origins such as aquatic, soil and peat environments as well as coal. A list of the IHSS reference samples and the Swiss samples with their corresponding abbreviations and reference numbers is given as follows: Purified Unterrickenzopfen humic acid (PUHA), purified Unterrickenzopfen fulvic acid (PUFA), Suwannee river humic acid (SR-HA; 1S101H), Suwannee river fulvic acid (SR-FA; 1S101F) and Suwannee river natural organic matter (SR-NOM; 1R101N), Elliott soil humic acid (Soil-HA; 1S102H) and Elliott soil fulvic acid (Soil-FA; 1S102F), Pahokee peat humic acid (Peat-HA; 1S103H); and Pahokee peat fulvic acid (Peat-FA; 1S103F) and Leonardite humic acid (Leonardite-HA; 1S104H).
3.2.2 C-1s XANES spectroscopy

Sample preparation
For C-1s XANES measurements, thin films of 50 to 200 nm of sample thickness were prepared. Absorption spectra of samples thinner than 50 nm often show a poor signal-to-noise ratio, whereas the spectra of samples thicker than 200 nm can be distorted by absorption saturation (Urquhart et al., 1999). Typically, sections of 100 nm in thickness are ideal for C-1s XANES spectroscopy. For each sample, 2 mg of freeze dried material was dissolved in 500 mL high purity deionized water at room temperature. A droplet of the prepared solution was deposited onto 100 nm in thickness Si₃N₄ windows (Silson Ltd., UK) and air-dried. The high absorption coefficient of carbon, \( \mu = 10^4 \text{ cm}^2 \text{ g}^{-1} \), and the high concentration of carbon present in HS require the sample thickness to be less than 800 nm for imaging purposes and less than 400 nm for quantitative spectroscopy (Henke et al., 1982). These limitations require the concentration of the sample material in the aqueous solution to be 4 mg L⁻¹ in order to obtain high quality spectra with good signal-to-noise ratios.

Collection of C-1s XANES spectra
C-1s XANES spectra were collected on beamline X-1A at the National Synchrotron Light Source (NSLS) in Upton, N.Y. The Scanning Transmission X-ray Microscope (STXM) was operated inside a He purge enclosure at room temperature and atmospheric pressure. The precise composition of the He purge atmosphere can not be measured, but the flow rate was kept constant for all experiments. For calibration purposes, CO₂ gas was added to the He purge in the microscope and normalized to -8.01 eV relative to the C-1s → 3s transition (292.8 eV). Details on the instruments specifications have been reported elsewhere (Jacobsen et al., 1991).

After mounting the sample on the sample holder, the X-ray beam was focused on the sample surface. For this, a first overview image was taken in order to optimize the focal spot size. A region with high contrast between 280 eV and 290 eV was chosen for high resolution imaging. The sample was scanned under computer control with capacitance-controlled piezo-transducers in order to acquire images as well as point-spectra. Alternatively, X-Y positions were held fixed, while the photon energy and the Z-position of the zone plate were scanned in synchrony to obtain an energy scan from a small spot. Spatial variations in intensity in the energy region of 280 eV to 315 eV relate to variations in sample thickness or sample density. Images acquired at energies above the absorption edge of carbon at 290 eV reveal absorption due to the number of carbon atoms interacting with the X-ray beam. This number can vary, depending on changes in the sample density.
or thickness. In each case, spatial variations in X-ray transmission at the low and high energies can be compared with variations in intensity in the XANES region. As a result, artefacts from sample preparation such as fibres or dry cracks on the film can be clearly distinguished from contrast based on chemically distinct micro-domains. Reduction in transmission at energies below the absorption edge is related to residual absorption e.g. due to the chlorine L-edge at nearly 270 eV. In addition to imaging, X-ray absorption spectroscopy was applied by leaving the focused beam on one particular spot, while the photon energy was scanned within the energy range from 280 to 310 eV.

Figure 3.1 shows a STXM image of a dry film of the PUFA sample, acquired at a photon energy of 290 eV. Strong absorption on the outer rim is due to variations in thickness. Spectra (I) were recorded at 25 different spots within the strongly absorbing regions of the sample and normalized to energy scans recorded at areas without sample material (I₀).

Figure 3.1: STXM image of the sample PUFA acquired at a photon energy of 290 eV. Strong absorption on the outer rim is due to variations in thickness. Spectra (I) were recorded at 25 different spots within the strongly absorbing regions of the sample and normalized to energy scans recorded at areas without sample material (I₀).
**Interpretation of spectra**

X-ray absorption spectroscopy measures the photoabsorption cross-section for the excitation or photoionization of tightly bound core electrons of a specific element. The details of a characteristic spectrum reveal the unoccupied electronic structure of the system in the presence of a core hole. These spectra are element-specific, as each element has a characteristic core binding energy (i.e. C-1s: 290 eV, N-1s: 400 eV, O-1s: 530 eV). The spectral features correspond to transitions from the ground state to a core excited state. This spectral signature is referred to as X-ray absorption near edge structure (XANES). The various spectral features contributing to a C-1s XANES spectrum shall be discussed in more detail.

Figure 3.2 shows a deconvoluted C-1s XANES spectrum, derived from the sample PUFA with Gaussian line shapes for various carbon functional groups. Typically, the lowest excitation energy corresponding to double carbon-carbon bonds without heteroatoms as nearest neighbours is near 285.0 eV. This feature can be assigned to the first C(1s)→π* transition, originating from aromatic C=C bonds (Stöhr, 1996). This well pronounced peak is followed by a transition taking place at an energy of 286.5 eV and is related to permitted transitions C(1s)→π*, induced by the presence of oxygen or other electron withdrawing substituents. This spectral feature is assigned to resonances of the aromatic carbon bonded to oxygen, as in the case of phenol or aryl ether (Ishii & Hitchcock, 1988; Thieme et al., 2000). The third peak at 288.5 eV can be assigned to the second C(1s)→π*
transition, sometimes referred to as a $\text{C}(1s)\rightarrow2\pi^*$ transition, present in carboxyl carbon (Urquhart et al., 1999). Note that the ~3 eV increase in energy in the respective transition is due to the electron withdrawing nature of oxygen. An important contributor to this feature is absorption due to a 1s transition to a mixed Rydberg/C-H* state, centered near 288.0 eV (Cody et al., 1996; Thieme et al., 2000). A feature present, but in this array of samples not readily observable, is the transition taking place around 290.5 eV. This peak is assigned to the $\text{C}(1s)\rightarrow\pi^*$ transition, present in carbonyl carbon (Urquhart & Ade, 2002). While absorption due to this fourth transition is not observed directly, its presence is inferred from other samples (Vogt et al., 2001). The following transitions higher than 292 eV are corresponding to $\sigma^*_{\text{C-C}}$ of aromatic and $\sigma^*_{\text{C-C}}$ aliphatic bound carbon (Stöhr, 1996). These 1s-$\sigma^*$ transitions are found several eV above the ionization threshold. The more delocalized nature of the electron in the $\sigma^*$ state, coupled with the multiplicity of several 1s-$\sigma^*$ transitions, leads to a high overlap between the signals and makes a deconvolution of this region of the XANES spectrum rather difficult. An extensive discussion about peak assignments, related quantum mechanics and the numerous factors contributing to a XANES spectrum can be found in Kaznacheyev et al. (2002).

In order to compare the chemistry of the different samples quantitatively, a least-squares fitting scheme was used to extract relative absorption-band intensities. The $\pi^*$ resonances were fitted with Gaussian bands at 285.0 eV (aromatic-C), 286.6 eV (phenolic-C), 288.5 eV (carboxyl-C) and 290.5 eV (carbonyl-C), whereas positions varied within the range of $\leq 0.2$ eV. Furthermore, the spectra were deconvoluted with two Gaussian functions for the $\sigma^*$ transitions above the ionization threshold and an arctangent function for the ionization step around 290 eV. Note that in the case where a number of chemically different bound carbon atoms are present, several ionization steps are required to faithfully fit the absorption edge of each carbon atom. In practice, one cannot accurately simulate the intensity and positions of the continuum steps without detailed knowledge of the carbon chemistry, which is a problem as in the case of complex natural compounds such as humic substances. For the present purpose, the position and the intensity of the continuum steps of each sample were selected using simplifying assumptions regarding the localized carbon chemistry. The simplest approach is to use a step function, however an arctangent or step-error function may provide a more realistic fit to the data (Lenardi et al., 1999; Stöhr, 1996). In the present work, an arctangent function was used. We have considered the step width as a free parameter in the fit in order to include many different ionization potentials within a single broad step. For the best fit, a step width of about 0.9 eV was obtained. Since the fine structure in the region above 290 eV transitions tends to be very broad and overlap, only main $\text{C}(1s)\rightarrow\pi^*$ transition were used for subsequent quantification and interpretation of the results in the present investigation. Therefore
the first four peaks were taken into account for quantification. For each sample, single peak areas of 1s-π* transitions were referred to the sum of areas obtained for all four 1s-π* transitions in order to obtain relative peak areas. In order to compare the quantitativeness of the fitting procedure presented in this study, the samples were fitted according a deconvolution procedure as described by Solomon et al. (2005), where six carbon functional groups between 280 to 290 eV were taken into account for quantification. The π* resonances were fitted with Gaussian bands at 284.3 eV (Quinone type C), 285.0 eV (Aromatic C), 286.5 eV (Phenolic C), 287.3 eV (Aliphatic C), 288.4 eV (Carboxyl C) and 289.3 eV (O-Alkyl C), whereas positions were held fixed. The fitting parameters were in exact analogy to published data, except for the step width, where a value of 0.9 eV was used. An arctangent function for the ionization step at 290 eV with a full width at half maximum (FWHM) of 0.8 eV was further used in order to simulate the absorption at the ionization threshold.

3.2.3 Solid-state CP-MAS 13C-NMR spectroscopy

All samples were analyzed by solid-state cross-polarization magic-angle spinning 13C nuclear magnetic resonance (CP-MAS 13C-NMR) spectroscopy. Although all IHSS samples have been characterized previously by solution-state 13C-NMR and 1H-NMR spectroscopy in 1989, we decided to re-analyze all samples with solid-state 13C-NMR spectroscopy. Solid-state 13C-NMR spectroscopy provides a better signal-to-noise ratio as well as a higher spectral resolution, resulting in lower detection limits and spectra of higher quality (Malcolm, 1989; Peersen et al., 1993).

Freeze-dried samples were analyzed on a NMR spectrometer (DSX 200, Bruker, Germany) at a resonance frequency of 50.3 MHz. A magic-angle spinning speed of 6.8 kHz was used, with a contact time of 1 ms and a pulse delay of 400 ms, respectively. In order to avoid spin modulation of Hartmann-Hahn conditions, a ramped 1H pulse decreasing was used (Peersen et al., 1993). 18’000 to 20’000 single scans were collected for each sample. Line broadening between 50 and 150 Hz was applied prior to Fourier transformation. The chemical shift was referenced to tetramethylsilane (0 ppm) and adjusted using glycine (176.04 ppm) as an external standard. For data analysis, all spectra were divided into chemical shift regions assigned to the chemical group classes alkyl (0-45 ppm), O-alkyl C (45-110 ppm), aromatic C (110-160 ppm), carboxyl C (160-185 ppm) and carbonyl C (185-220 ppm), respectively. The relative intensity of these regions was determined by means of integration. Details on the investigation of soil and environmental samples by CP-MAS 13C-NMR spectroscopy and their quantification are given in Kögel-Knabner et al. (1988).
3.3 Results and discussion

3.3.1 C-1s XANES

C-1s XANES spectra of all samples are presented in Figure 3.3. The experimental data is shown as dots, whereas solid lines show the fitted spectrum. The fitted areas of the six carbon groups are reported in Table 3.1.

Figure 3.3: C-1s XANES spectra of fulvic acid (FA), humic acid (HA) and natural organic matter (NOM) reference samples. Open circles correspond to the experimental data. Solid lines represent fits.
Table 3.1:
Integrated spectral regions for C-1s XANES data with corresponding quantifications for fulvic acid (FA), humic acid (HA), and natural organic matter (NOM) samples. Values correspond to raw data of the fitted spectra.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Integrated peak areas within indicated regions [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>285.0 ± 0.1</td>
</tr>
<tr>
<td>SR HA</td>
<td>0.744</td>
</tr>
<tr>
<td>Soil HA</td>
<td>0.750</td>
</tr>
<tr>
<td>Peat HA</td>
<td>0.742</td>
</tr>
<tr>
<td>PUHA</td>
<td>0.771</td>
</tr>
<tr>
<td>Leonardite HA</td>
<td>0.847</td>
</tr>
<tr>
<td>SR NOM</td>
<td>0.540</td>
</tr>
<tr>
<td>SR FA</td>
<td>0.390</td>
</tr>
<tr>
<td>Soil FA</td>
<td>0.577</td>
</tr>
<tr>
<td>Peat FA</td>
<td>0.777</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.696</td>
</tr>
</tbody>
</table>

Values represent raw data and correspond to integrated spectral regions of the C-1s XANES spectra. For quantification, only the first four bands were taken into account and set as 100%. For comparison, each peak was divided through 100% area.

Table 3.2:
Integrated spectral regions for C-1s XANES data for fulvic acid (FA), humic acid (HA), and natural organic matter (NOM) samples, derived according to the fitting procedure by Solomon et al. (2005). Values correspond to raw data of the fitted spectra.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Integrated peak areas within indicated regions [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>284.3</td>
</tr>
<tr>
<td>SR HA</td>
<td>0.032</td>
</tr>
<tr>
<td>Soil HA</td>
<td>0.301</td>
</tr>
<tr>
<td>Peat HA</td>
<td>0.050</td>
</tr>
<tr>
<td>PUHA</td>
<td>0.112</td>
</tr>
<tr>
<td>Leonardite HA</td>
<td>0.076</td>
</tr>
<tr>
<td>SR NOM</td>
<td>0.057</td>
</tr>
<tr>
<td>SR FA</td>
<td>0.011</td>
</tr>
<tr>
<td>Soil FA</td>
<td>0.032</td>
</tr>
<tr>
<td>Peat FA</td>
<td>0.036</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.085</td>
</tr>
</tbody>
</table>

In Table 3.2, the values derived from the fitting procedure described by Solomon et al. (2005) are listed. Reported values are raw data. For quantification, all six bands were counted as for 100%. Each peak area was divided by the sum of areas of all six peaks for comparison. Note that only main C(1s) → π* transitions were used for quantification.
3.3.2 Solid-state CP-MAS $^{13}$C-NMR

Solid-state CP-MAS $^{13}$C-NMR spectra of all samples are presented in Figure 3.4.

Figure 3.4: Solid-state CP-MAS $^{13}$C-NMR spectra of fulvic acid (FA), humic acid (HA), and natural organic matter (NOM) reference samples.
The relative intensities of the chemical shift regions for $^{13}\text{C}$-NMR spectroscopy are summarized in Table 3.3. Comparison with solution-state CP-MAS $^{13}\text{C}$-NMR studies conducted by Thorn et al. (1989) showed that the relative intensities differed by less than 8% for amounts of phenolic, carboxyl and carbonyl carbon, whereas the differences for alkyl and aromatic carbon were up to 10%, compared to the results obtained in this study.

Table 3.3:
Integrated, spectral regions of solid-state CP-MAS $^{13}\text{C}$-NMR data for fulvic acid (FA), humic acid (HA), and natural organic matter (NOM) samples. Values correspond to percentages of the total area.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SR HA</td>
<td>26</td>
<td>30</td>
<td>17</td>
<td>8</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Soil HA</td>
<td>23</td>
<td>26</td>
<td>27</td>
<td>7</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Peat HA</td>
<td>27</td>
<td>25</td>
<td>22</td>
<td>8</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>PUHA</td>
<td>28</td>
<td>31</td>
<td>16</td>
<td>6</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Leonardite HA</td>
<td>34</td>
<td>11</td>
<td>31</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>SR NOM</td>
<td>29</td>
<td>39</td>
<td>11</td>
<td>5</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>SR FA</td>
<td>33</td>
<td>31</td>
<td>11</td>
<td>4</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Soil FA</td>
<td>25</td>
<td>36</td>
<td>13</td>
<td>5</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Peat FA</td>
<td>26</td>
<td>29</td>
<td>14</td>
<td>6</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>PUFA</td>
<td>24</td>
<td>29</td>
<td>17</td>
<td>6</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

Alkyl carbon groups are equally distributed within all samples. The distribution of O-alkyl groups shows that highest amounts can be observed for the NOM sample, followed by the FA samples and the HA samples. Amounts determined for aromatic bound carbon showed that quantities were highest for the HA samples, followed by moderate amount for the NOM and the FA samples. The same distribution can be observed for phenolic carbon. Carboxyl functional groups are distributed differently, whereas highest amounts can be observed within the FA samples, followed by the NOM sample and the FA samples. The carbonyl carbon groups are most abundant within the FA samples, followed by the NOM and the HA samples. An exception in terms of functional group distribution is the Leonardite HA sample, where very high amounts for aromatic and phenolic bound carbon on one hand and very low amounts of O-alkyl functional groups on the other are observed. This can be explained due to the fact that this sample was derived from coal with a high degree of aromaticity and condensed structures and very low amounts of carbon-oxygen components such as carboxylic acids. A detailed chemical characterization of the sample was recently reported by Olivella et al. (2002).
All three groups of HS showed the same amounts of functional group distribution, wherein several trends between HA, FA and NOM samples could be observed: HA samples are dominated by high amounts of aromatic and phenolic carbon groups, whereas the FA samples exhibit a high amount of carboxyl and O-alkyl functional groups. The NOM sample shows a slightly different distribution pattern, with similar percentages for alkyl and O-alkyl carbon like the FA-samples, but with less carboxyl functional groups. These results for NMR analysis are in good agreement with data published by Mahieu et al (1999) and Malcolm (1989), who investigated a large number of humic substances and NOM samples by means of $^{13}$C-NMR spectroscopy.

### 3.3.3 Correlations between C-1s XANES and $^{13}$C-NMR spectroscopy

In Figure 3.5, relative peak areas derived from C-1s XANES spectroscopy, using the fitting scheme of this study, are plotted against peak areas of $^{13}$C-NMR spectra. The plots report correlations for the sum of aromatic and phenolic bound carbon (Figure 3.5 a) as well as for carboxyl carbon groups (Figure 3.5 b).

![Figure 3.5: $^{13}$C NMR peak areas and C-1s XANES peak areas for aromatic plus phenolic carbon (a) and carboxyl carbon (b). Values for C-1s XANES were calculated using the fitting procedure of this study. The calculated linear regression is shown as dotted line. The corresponding equations with correlation coefficients are shown on the left.](image)

In order to obtain comparable results for XANES and NMR, the NMR spectra were integrated from 110 ppm to 220 ppm and counted as 100 %. The areas for aromatic, phenolic and carboxyl bound carbon were determined by means of integration and divided through the total area from 110 ppm to 220 ppm. These relative values were correlated with results obtained from XANES spectroscopy by calculating the corresponding correlation coefficient. The samples are grouped into fractions of humic acid (squares), fulvic acid
(triangles) and natural organic matter (dots). The dotted lines correspond to the calculated regression lines for each correlation, with the corresponding equations and correlation coefficients ($R^2$) shown on the left side of each plot.

As can be seen on the plot for aromatic and phenolic bound carbon, the values derived from both NMR and XANES spectroscopy correlate well ($R^2 = 0.85$, slope 0.75). Data for the humic acid and NOM samples tend to be distributed along the regression line whereas the fulvic acid samples are manifested by larger scattering. The correlation plot for carboxyl carbon also shows good correlations ($R^2 = 0.85$, slope 0.95). The samples for humic acid and NOM are very close to the calculated regression line. Fulvic acid samples exhibit more scattering than the other samples. Correlations for the carbon groups as well as for aromatic/phenolic data are reported in Table 3.4. The results indicate that differences in amounts of aromatic/phenolic and carboxyl bound carbon within HA, FA and NOM samples can be resolved using the fitting scheme of this study for the analysis of C1s XANES spectra. Relative peak areas of carboxyl carbon and aromatic plus phenolic carbon obtained from C1s XANES spectra follow the trends in NMR data. These findings are in good agreement with results obtained by Scheinost et al. (2001). Amounts detected for pure aromatic carbon as well as for carbonyl bound carbon appear to be less reliable. This may be due to signal varying contributions of unknown carbon groups to particular absorption bands at the low energy region of the spectrum on one hand and overlaps in signal intensity near the carbon absorption threshold on the other.

**Figure 3.6:**
$^{13}$C NMR peak areas and C1s XANES peak areas for aromatic, phenolic, plus quinone carbon (a) and carboxyl carbon (b). Values for C1s XANES were calculated using the fitting procedure reported by Solomon et al. (2005). The linear regression is shown as dotted line. The corresponding equations with correlation coefficients are shown on the left.
In Figure 3.6, relative peak areas derived from C-1s XANES spectroscopy using the fitting scheme and quantification described by Solomon et al. (2005) are plotted against peak areas of $^{13}$C-NMR spectra. In analogy to this scheme, amounts for quinone-type carbon were counted within the combined amounts for aromatic and phenolic bound carbon (Figure 3.6 a). The correlation with data derived from NMR spectroscopy is good ($R^2 = 0.85$, slope 0.84). Data for the fulvic acid and NOM samples are distributed along the calculated regression line, whereas the humic acid samples exhibit higher scattering. The plot for carboxyl bound carbon (Figure 3.6 b) appears to be less good. The high scattering is reflected in the low value of the correlation coefficient ($R^2 = 0.28$).

Table 3.4:
Correlations between results from $^{13}$C-NMR and C-1s XANES spectroscopies. The left side reports correlations with C-1s XANES quantifications derived from the quantification scheme presented in this study. The right side reports C-1s XANES quantifications derived from the scheme reported by Solomon et al. (2005).

<table>
<thead>
<tr>
<th>CP-MAS $^{13}$C-NMR</th>
<th>C-1s XANES †</th>
<th>$R^2$</th>
<th>CP-MAS $^{13}$C-NMR</th>
<th>C-1s XANES ‡</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic C</td>
<td>Aromatic C</td>
<td>0.00</td>
<td>Aromatic C</td>
<td>Aromatic C</td>
<td>0.20</td>
</tr>
<tr>
<td>Phenolic C</td>
<td>Phenolic C</td>
<td>0.44</td>
<td>Phenolic C</td>
<td>Phenolic C</td>
<td>0.33</td>
</tr>
<tr>
<td>Carboxyl C</td>
<td>Carboxyl C</td>
<td>0.85</td>
<td>Carboxyl C</td>
<td>Carboxyl C</td>
<td>0.28</td>
</tr>
<tr>
<td>Carboxyl C</td>
<td>Carboxyl C</td>
<td>0.06</td>
<td>O-Alkyl C</td>
<td>O-Alkyl C</td>
<td>0.27</td>
</tr>
<tr>
<td>Arom. &amp; Phen. C</td>
<td>Arom. &amp; Phen. C</td>
<td>0.85</td>
<td>Arom. &amp; Phen. C</td>
<td>Arom./Phen./Quin. C</td>
<td>0.85</td>
</tr>
<tr>
<td>Carbox. &amp; Carbon. C</td>
<td>Carbox. &amp; Carbon. C</td>
<td>0.85</td>
<td>Arom. &amp; Quinone C</td>
<td>Arom. &amp; Quinone C</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carbox. &amp; O-Alk. C</td>
<td>Carbox. &amp; O-Alk. C</td>
<td>0.58</td>
</tr>
</tbody>
</table>

† Results derived from this study
‡ Results derived from scheme by Solomon et al. (2005)

The left part of Table 3.4 reports correlations between $^{13}$C-NMR and C-1s XANES results derived from the quantification scheme presented in this study. For aromatic and carbonyl carbon groups, NMR and XANES data were not correlated ($R^2 = 0.0$ and $R^2 = 0.06$, respectively). The correlation for phenolic bound C ($R^2 = 0.44$) is moderate. Good correlation coefficients of $R^2 = 0.85$ are obtained for carboxyl bound carbon as well as the combined data of aromatic/phenolic and carboxyl/carbonyl bound C. The right part of Table 3.4 reports correlations coefficients for quantification results of C-1s XANES spectroscopy data derived after the scheme by Solomon et al. (2005). Correlations for aromatic ($R^2 = 0.20$ ), phenolic ($R^2 = 0.33$ ), carboxyl ($R^2 = 0.28$ ) and O-alkyl ($R^2 = 0.27$ ) carbon are poor. Combined results for aromatic and quinone-type carbon ($R^2 = 0.47$) and carboxyl and O-alkyl carbon ($R^2 = 0.58$) result in fair correlations, whereas the correlation of aromatic, phenolic and quinone type carbon derived by XANES with amounts for aromatic and phenolic C results in a good correlation with $R^2 = 0.85$. 
It appears that the applied fitting procedure is strongly dependent on the investigated sample material. For Solomon et al. (2005) reported good correlations between XANES and NMR results for all carbon groups of humic material except O-alkyl carbon.

3.3.4 Correlation with O/C ratios

Figure 3.7 shows correlations between O/C ratios and C-1s XANES data quantified with the fitting scheme of this study. Values for O/C ratios were taken from published data (IHSS, 2000). The plot for combined values of aromatic and phenolic carbon (Figure 3.7 a) exhibits a good correlation ($R^2 = 0.71$) and is manifested by a negative slope of -54.9. The humic acid and NOM samples show a well-developed distribution along the calculated regression line, whereas the fulvic acid samples are manifested by scattered distributions. Furthermore, humic acid samples exhibit an O/C ratio of about 0.5, whereas the NOM and FA samples have O/C ratios of 0.8 and lower contents of aromatic and phenolic carbon groups than the HA samples. The plot for carboxyl carbon groups (Figure 3.7 b) exhibits a slightly different pattern, whereas the correlation for data derived from XANES spectroscopy shows a well-developed correlation ($R^2 = 0.80$) and a positive slope of 61.4. Again, the HA and NOM samples are distributed along the calculated regression line, whereas data on fulvic acid samples exhibit more scattering.
Table 3.5 reports calculated correlation coefficients obtained for correlations between values for O/C ratios and relative peak areas of aromatic C, phenolic C, carboxyl C and carbonyl bound carbon, derived from C-1s XANES and $^{13}$C-NMR spectra. Furthermore, correlations for combined data of aromatic and phenolic carbon on one hand as well as carboxyl and carbonyl carbon groups are reported.

**Table 3.5:**
Correlations for C-1s XANES and $^{13}$C-NMR results and O/C ratios. Results for C-1s XANES were derived from the quantification scheme of this study.

<table>
<thead>
<tr>
<th>C-1s XANES</th>
<th>Elemental ratio</th>
<th>$R^2$</th>
<th>CP-MAS $^{13}$C-NMR</th>
<th>Elemental ratio</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic C</td>
<td>O/C</td>
<td><strong>0.04</strong></td>
<td>Aromatic C</td>
<td>O/C</td>
<td><strong>0.85</strong></td>
</tr>
<tr>
<td>Phenolic C</td>
<td>O/C</td>
<td><strong>0.65</strong></td>
<td>Phenolic C</td>
<td>O/C</td>
<td><strong>0.19</strong></td>
</tr>
<tr>
<td>Carboxyl C</td>
<td>O/C</td>
<td><strong>0.80</strong></td>
<td>Carboxyl C</td>
<td>O/C</td>
<td><strong>0.79</strong></td>
</tr>
<tr>
<td>Carboxyl C</td>
<td>O/C</td>
<td><strong>0.03</strong></td>
<td>Carboxyl C</td>
<td>O/C</td>
<td><strong>0.45</strong></td>
</tr>
<tr>
<td>Arom. &amp; Phen. C</td>
<td>O/C</td>
<td><strong>0.71</strong></td>
<td>Arom. &amp; Phen. C</td>
<td>O/C</td>
<td><strong>0.79</strong></td>
</tr>
<tr>
<td>Carbox. &amp; Carbon. C</td>
<td>O/C</td>
<td><strong>0.71</strong></td>
<td>Carbox. &amp; Carbon. C</td>
<td>O/C</td>
<td><strong>0.79</strong></td>
</tr>
</tbody>
</table>

The left part of Table 3.5 reports correlation coefficients between C-1s XANES results and calculated O/C ratios. For aromatic and carbonyl carbon groups, no correlation with O/C ratios was found. Phenolic bound carbon as quantified by C-1s XANES spectroscopy tends to correlate with the O/C ratio ($R^2 = 0.65$). As for the correlation of NMR and XANES data, the best correlation is obtained for values of carboxyl bound carbon ($R^2 = 0.80$). Furthermore, combined data of aromatic and phenolic as well as of carboxyl and carbonyl bound carbon exhibit reasonable correlations with $R^2 = 0.71$. For comparison, correlations between calculated O/C ratios and results derived from $^{13}$C-NMR spectroscopy are shown on the right side of Table 3.5. As in the case for results derived from C-1s XANES spectroscopy, the correlation for carbonyl ($R^2 = 0.45$) and phenolic ($R^2 = 0.19$) bound carbon does not appear to be good. In contrast to correlations with XANES results, data for aromatic bound carbon correlate very well with O/C ratios ($R^2 = 0.85$). Moreover, correlations for carboxyl carbon and for combined data of aromatic and phenolic as well as of carboxyl and carbonyl bound carbon exhibit correlation coefficients of $R^2 = 0.79$. The comparison of C-1s XANES results with calculated O/C ratios shows that the method provides valuable quantitative information on carboxyl carbon groups as well as on total aromatic carbon (unsubstituted and substituted).
3.4 Conclusions

The investigation of selected samples of humic acid (HA), fulvic acid (FA) and natural organic matter (NOM) with C-1s XANES spectroscopy showed that carboxyl functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic and phenolic bound carbon. Carbonyl carbon was present to a lesser extend within all three sample groups. Furthermore, we were able to show that HA samples were dominated by elevated amounts of aromatic and phenolic bound carbon, whereas FA and NOM samples exhibit higher amounts of carboxyl carbon. Amounts for carbonyl functional groups appeared to be equally distributed for all three fractions. The NOM sample was found to be very close to the chemical composition of the FA samples.

Solid-state CP-MAS $^{13}$C-NMR spectroscopy provided good information about the quantitative distribution of carbon functional groups present in HS. The dominant forms of organic C were identified as alkyl and O-alkyl carbon groups, followed by aromatic and carboxyl bound C. Phenolic as well as carbonyl bound carbon groups were present in minor amounts. HA samples were dominated by higher amounts of aromatic and phenolic bound carbon, compared to the FA and NOM samples. The FA and NOM samples in contrast were dominated by elevated amounts of O-alkyl carbon groups and higher amounts for carboxyl and carbonyl bound C, compared to the HA samples. All results were in good agreement with data derived from XANES spectroscopy, although alkyl as well as O-alkyl carbon groups could not be detected with this technique.

Quantitative correlations were performed for aromatic, phenolic, carboxyl and carbonyl functional groups, where data derived from C-1s XANES spectroscopy was correlated with results from $^{13}$C-NMR spectroscopy. In addition, the quantification scheme proposed by Solomon et al. (2005) was applied for the same dataset and compared with results obtained for this study by calculating correlation coefficients for results from both methods. The deconvolution applied for this study has shown to provide results for aromatic and carboxyl bound carbon, that are in good correlation ($R^2 > 0.8$) with NMR-spectroscopy. The correlation for phenolic bound C was moderate, whereas the correlation for carbonyl bound carbon appeared to be less reliable. The correlations for results derived from the fitting scheme after Solomon et al. (2005) were poor ($R^2 < 0.58$), except for combined data of aromatic, phenolic and quinone type carbon ($R^2 = 0.85$). The quantification of the C-1s XANES features of HS proved to be a difficult task due to the highly variable spectral features present in XANES spectra on one hand and due to the lack of an established fitting scheme for this kind of sample material on the other. Moreover, it appeared that the applied quantification scheme is strongly dependent on the investigated sample material, as could be observed by using a quantification procedure that has been used.
for HS from a different origin. Nevertheless, the method is able to provide qualitative information about the chemical environment of carbon present in HS samples as well as reliable quantitative information for aromatic and phenolic as well as carboxyl carbon. The quantification of aromatic and carbonyl bound carbon does not appear to be very good. The development of a consistent fitting scheme for all carbon functional groups present in humic substances is crucially needed in order to further develop this technique as a substantial investigation tool for environmental samples.

Acknowledgements

This project has been financially supported by the Swiss Federal Institute of Technology Zurich (Grant No. 01753). All XANES analyses were performed at beamline X-1A at the National Synchrotron Light Source at the Brookhaven National Laboratory in Upton, N.Y., which is supported by the Department of Energy. Operation of the Stony Brook STXM is supported by National Science Foundation grants CHE-0221934 and OCE-0221029. The NMR analyses were kindly performed by Dr. H. Knicker at the Technical University Munich. Furthermore, the authors would like to gratefully acknowledge S. Wirick of the Department of Physics, SUNY Stony Brook and K. Barmettler from ETH Zurich for their contribution and support to this work.
C-1s XANES spectroscopy of humic substances: Comparison with 13C-NMR results

Literature cited


Kikuma, J. & Tonner, B.P. 1996. XANES spectra of a variety of widely used organic polymers at the C K-edge. J. Electron Spectrosc. 82: 53-60.


C-1s XANES spectroscopy of humic substances: Comparison with $^{13}$C-NMR results


Chapter 4 was prepared for publication as:

Marc Schumacher, Iso Christl, Rolf D. Vogt, Andreas C. Scheinost, Chris Jacobsen and Ruben Kretzschmar

Seasonal variation of the chemical composition of aquatic dissolved organic matter in boreal forest catchments

Biogeochemistry
4 Seasonal variation of the chemical composition of aquatic dissolved organic matter in boreal forest catchments

Summary

Ten samples of dissolved organic matter derived from five freshwater sample sites in Scandinavia were investigated by elemental analysis, FT-IR, solid-state CP-MAS $^{13}$C-NMR and C-1s XANES spectroscopy. The samples were extracted by reverse osmosis during the spring and fall seasons and analyzed, to see if seasonal or geographical factors led to chemical variations within their carbon functional group distribution. Results of elemental analysis and FT-IR spectroscopy showed that the samples did not exhibit significant seasonal differences. However, three samples showed spectral differences within their FT-IR spectra due to inorganic contributions of sodium sulphate and silicon dioxide. Further analysis with solid-state $^{13}$C-NMR spectroscopy proved that the samples were not influenced by seasonal variations. All samples showed carbon functional group distribution, which corresponded well with values reported in the literature (Perdue & Ritchie, 2003). Analysis with C-1s XANES spectroscopy further confirmed the results obtained by FT-IR and CP-MAS $^{13}$C-NMR spectroscopy. Comparison to results from IHSS references showed, that all samples exhibited remarkable spectral similarities to humic acid with slightly elevated amounts of O-alkyl carbon.

Overall, the results of this study imply that climate change will have only a minor influence on the chemical composition of aquatic DOM.
Dissolved organic matter (DOM) is present in nearly all aquatic environments including lakes, rivers, oceans, soil water and groundwater. Average concentrations, expressed as dissolved organic carbon (DOC), range from about 0.1 mg L\(^{-1}\) in groundwater to 33 mg L\(^{-1}\) in peat bogs (Perdue & Ritchie, 2003). The transport of dissolved organic matter in rivers is an important flux component within the global carbon cycle and it also has a profound influence on the cycling and bioavailability of metals (e.g., Al, Fe, Cu), which can form stable complexes with organic functional groups of DOM (Buffle et al., 1987; Tipping et al., 2002).

The composition of DOM in freshwater environments has been studied intensively and has been recently reviewed by Perdue and Ritchie (2003). On average, freshwater DOM has an elemental mass composition of 49.5 ±3.3 % C, 5.0 ±1.0 % H, 43.0 ±4.1 % O, 1.7 ±1.0 % N, and 2.0 ±1.3 % S. The average molecular weight is variable with typical values ranging from < 1 kDa to more than 100 kDa (Leenheer & Croue, 2003). Values given in the literature strongly depend on the method used. For size exclusion chromatography, Perdue and Ritchie (2003) report a median value of 1700 Da for weight-averaged molecular weight of 37 NOM samples. Typically, more than 80 % of aquatic DOM can be isolated by resins either as hydrophobic or hydrophilic acid fractions, which are often present in a ratio of about 2:1. The hydrophobic acid fraction consists of fulvic acids and smaller amounts of humic acids. Less than 20 % of DOC consists of hydrophilic bases and neutral compounds. Amounts of identifiable biomolecules of terrestrial or aquatic origin, such as amino acids (~1.8 %), sugars (~3.0 %) and lignin-derived phenols (~0.6 %) are typically small (Perdue & Ritchie, 2003).

Aquatic DOM in rivers is derived more from terrestrial soil and plant material rather than from aquatic phytoplankton (Hedges & Oades, 1997), thus reflecting the properties of the surrounding soils, which on their part are strongly influenced by the vegetation and climate of the region (Lydersen, 1995). Rivers in the subarctic and cold continental climatic zones in the northern hemisphere are often particularly rich in DOM. Under boreal forest and bog vegetation, the soil cover is often dominated by Podzols and Histosols. The acidic pH values, the low Ca and Mg concentrations (soft water) and the low temperatures of these soils favour leaching of DOM into the rivers (Lobbes et al., 2000; van Hees, 1998). Gjessing et al. (1999) speculated that increased temperatures during summer season may accelerate the degradation of organic material in soil, generating larger amounts of DOM to be washed out by increased precipitation than during the winter season. Moran & Zepp (1997) have shown that exposure to solar radiation plays an important role in the degradation of DOM in lakes, increasing its bioavailability significantly. Consequently,
decreased retention times in lake waters cause decreased photochemical reactions and will therefore lead to enhanced colour and DOM concentrations (Tranvik, 2003). Skjelvåle et al. (2001) reported that fluctuations in temperature are the main reason for the observed changes in surface water colour and changes in the chemistry of DOM in Scandinavia. Heikkinen (1994) reported seasonal differences of DOC extracted from a boreal river drainage basin in the ability to bind iron, which result from changes in temperature-dependent microbiological processes. The author further stated the importance of these processes during the summer season due to active microbial breakdown of plant litter and intensive growth of *Sphagnum* as a primary source of DOC.

These studies are in contrast to findings of Solinger et al. (2001), who investigated the dynamics of DOC in forest soils in central Europe. He showed that concentrations of DOC in the forest floor and mineral soil exhibited no seasonal variability. Moreover, Yano et al. (2004) was able to show that the net production of DOC in a coniferous forest soil was greater in the shallow mineral soil than in the O horizon. They stated that the influence of seasonal variability of DOC decreased with soil depth, resulting in the generation of DOC of uniform chemistry which is not affected by seasonal changes. These findings go along with results published by Porcal et al. (2004) who found DOM concentrations within an acidified lake in eastern Europe to differ largely in terms of concentrations during summer and winter. However, the chemical composition did not show significant differences between these surface and subsurface tributaries (P < 0.01). Abbt-Braun & Frimmel (1999) pronounced that reliable conclusions on the seasonal variation of NOM and DOC can only be drawn by means of long-term observations.

Despite the somewhat contradictory findings of these studies about the seasonal variability of NOM, possible changes within the carbon chemistry of NOM during annual season are likely to have a deep influence on various reactions. These reactions may affect trace metal speciation, the removal of NOM during water treatment (Gjessing *et al.*, 1999) and the biological activity in boreal aquatic environments, as reported by Heikkinen *et al.* (1994) and need to be investigated more intensively and at larger scales.

In this study, we have characterized a set of ten aquatic DOM samples isolated by reverse osmosis during the spring and fall seasons in five river catchments in Scandinavia. The DOM samples were analyzed by elemental analysis, FT-IR spectroscopy, CP-MAS 13C-NMR spectroscopy, and synchrotron-based C-1s NEXAFS spectroscopy. Our objectives were (i) to study the composition of the DOM in five river catchments differing in climate, soil types, and vegetation, and (ii) to explore whether there are significant seasonal variations in the aquatic DOM composition by comparing spring and fall samples collected at each site.
4.2 Materials and methods

4.2.1 Sampling sites

Five river catchments in Sweden, Norway and Finland were selected within the framework of a larger project (NOMiNiC, http://www.kjemi.uio.no/envir/nominic/) on DOM in Nordic countries (Vogt et al., 2001) The location of the five sampling sites is depicted in Figure 4.1.

Figure 4.1: Map of Scandinavia with the sample sites marked with numbers. (1) Valkea-Kotinen, SF, (2) Svartberget, S, (3) Skjervatjern, N, (4) Hietajärvi, SF, (5) Birkenes, N.
Table 4.1 summarizes some key properties of the sites, which differ in climate, dominating soil types, and vegetation (Bergström, 1995; Gjessing et al., 1999; Vogt et al., 2001).

**Table 4.1:**
*Main climatic data and site descriptions of the five selected sample sites after Bergström et al. (1995). Climate data, mean temperature and precipitation have been recorded by weather stations from 1990-1992. Air temperature, precipitation volume, discharge and evapotranspiration have been recorded during the period from 1991-1993.*

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Valkea-Kotinen #1</th>
<th>Svartberget #2</th>
<th>Skjervatjern #3</th>
<th>Hietajärvi #4</th>
<th>Birkenes #5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>Finland</td>
<td>Sweden</td>
<td>Norway</td>
<td>Finland</td>
<td>Norway</td>
</tr>
<tr>
<td>Phytogeographic zone</td>
<td>Boreal</td>
<td>Boreal</td>
<td>Boreal-coastal</td>
<td>Middle Boreal</td>
<td>Boreal-nemoral</td>
</tr>
<tr>
<td>Forest type</td>
<td>Spruce</td>
<td>Spruce</td>
<td>Pine</td>
<td>Pine/Spruce</td>
<td>Spruce</td>
</tr>
<tr>
<td>Elevation [m a.s.l.]</td>
<td>150</td>
<td>235</td>
<td>136</td>
<td>165</td>
<td>190</td>
</tr>
<tr>
<td>Sulphur deposition [g m⁻² yr⁻¹]</td>
<td>0.32</td>
<td>0.19</td>
<td>0.77</td>
<td>0.27</td>
<td>1.03</td>
</tr>
<tr>
<td>Precipitation [mm yr⁻¹]</td>
<td>618</td>
<td>720</td>
<td>2560</td>
<td>592</td>
<td>1500</td>
</tr>
<tr>
<td>Annual mean temperature [ºC]</td>
<td>3.1</td>
<td>0.0</td>
<td>5.5</td>
<td>2.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Discharge [mm]</td>
<td>186</td>
<td>325</td>
<td>NA</td>
<td>416</td>
<td>1200</td>
</tr>
<tr>
<td>Evapotranspiration [mm]</td>
<td>432</td>
<td>395</td>
<td>NA</td>
<td>176</td>
<td>300</td>
</tr>
<tr>
<td>Bedrock</td>
<td>Gneiss/Granodiorite</td>
<td>Gneiss/Schist</td>
<td>Gneiss</td>
<td>Gneiss/Granodiorite</td>
<td>Granite</td>
</tr>
<tr>
<td>Dominant soil type</td>
<td>Dystric Cambisol</td>
<td>Ferric Podzol</td>
<td>Gleyic Podzol</td>
<td>Haplic Podzol</td>
<td>Humic Gleysol</td>
</tr>
<tr>
<td>Histosol coverage [%] *</td>
<td>25</td>
<td>16</td>
<td>42</td>
<td>45</td>
<td>13</td>
</tr>
</tbody>
</table>

*NA denotes not available
*Values are given as percentages of the total area

The annual average temperature ranges from 0 to 7 ºC and the average annual precipitation from 590 to 2500 mm. All sampling sites are forested by pine (*Pinus strobus*) and spruce (*Picea abies*) trees on soils developed on glacial till. The dominant soil types are dystric Cambisols at the site Valkea-Kotinen, humic Gleysols at Birkenes, and Podzols...
Seasonal variation of aquatic DOC composition in boreal forest catchments

at Svartberget, Skjervatjern, and Hietajärvi, respectively. In addition to these soil types, a large area of peatlands with well developed histosols are present at each sample site. The coverage of these soil types with respect to the total area of each catchment ranges from 45 % for the Hietajärvi site to 13 % of the total area for the Birkenes site.

4.2.2 Isolation of DOM

Dissolved organic matter was isolated from surface water collected at all five sites during fall 1999 and spring 2000, respectively. The autumn and spring sample campaigns were conducted September 26 to October 12 (1999) and April 22 to Mai 26 (2000), respectively. At each site, 500 to 1100 L of surface water was processed through a mobile reverse osmosis (RO) unit (PROS/2S, RealSoft, USA) as described by Serkiz and Perdue (1990). Briefly, the surface water was passed through a pre-filter into a reservoir, from where it was pumped through a sodium saturated cation exchange resin (Dowex 50) to prevent precipitation of insoluble salts. The water was then passed through the RO membranes with pores of about 150 Å. In the RO membranes, the water was separated into a permeate solution, containing virtually no solutes and a retentate solution that contained nearly all of the dissolved inorganic and organic material. The permeate solution was discharged and the retentate solution was recycled back into the reservoir. At the end of the RO treatment, 25 L of concentrated solution was further concentrated by a rotary evaporator at 30 ºC to a volume of about 5 L and freeze dried (Vogt et al., 2001).

4.2.3 Elemental analysis

The freeze-dried DOM samples were analyzed for total C, H, N, and S contents using a micro-CHNS analyzer (CHNS-932, Leco, USA). All samples were analyzed with five replicates, each using 2 mg of DOM. Inorganic cations and anions were analyzed after dissolving 1 mg of the freeze-dried DOM in 10 mL deionized water. The concentrations of Na, Ca, Mg, K, Al, Fe, Mn, and Si were analyzed by ICP-OES (Vista-MPX, Varian, Germany) after acidifying the solutions with ultra-pure nitric acid. The concentrations of SO\textsubscript{4}^2-, NO\textsubscript{3}-, and Cl were measured by ion chromatography (761 Compact IC, Metrohm, Switzerland) with autosampler (813 Compact Autosampler, Metrohm, Switzerland). All elemental concentrations were corrected for the moisture content of the DOM, which was determined gravimetrically after drying the samples at 105°C during 24 h.
4.2.4 FT-IR spectroscopy

Transmission mode Fourier-transform infrared (FT-IR) spectra were collected in ambient air at room temperature using KBr pellets containing 0.5 mg of freeze-dried DOM (Spectrum One, Perkin Elmer, USA). For each sample, 20 scans were collected from 500 to 4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$, averaged and background corrected.

4.2.5 Solid-state CP-MAS $^{13}$C-NMR spectroscopy

The freeze-dried DOM samples were analyzed by solid-state cross polarization magic-angle spinning $^{13}$C nuclear magnetic resonance (CP-MAS $^{13}$C-NMR) spectroscopy (DSX 200, Bruker, Germany). The NMR spectra were collected at a resonance frequency of 50.3 MHz, using a magic-angle spinning speed of 6.8 kHz and a contact time of 1 ms. A pulse delay of 400 ms was used. A ramped $^1$H pulse decreasing was used in order to avoid spin modulations of Hartmann-Hahn conditions (Peersen et al., 1993). The number of scans for each sample varied from 18’000 to 20’000. For quantitative analysis, each spectrum was divided into six chemical shift regions which were assigned to alkyl-C (0-45 ppm), O-alkyl-C (45-110 ppm), aromatic-C (110-160 ppm), phenolic-C (140-160 ppm), carboxyl-C (160-185 ppm), and carbonyl-C (185-20 ppm), respectively. The relative intensity of these peaks was determined by numerical integration of the spectral regions. Further details on the quantitative interpretation of NMR-spectra are given in Knicker & Kögel-Knabner (1998).

4.2.6 C-1s NEXAFS spectroscopy

Near edge X-ray absorption fine structure (NEXAFS) spectra at the C-1s edge were measured using the Stony Brook Scanning Transmission X-ray Microscope (STXM) at the NSLS beamline X-1A (National Synchrotron Light Source, Upton, N.Y.). The STXM was operated inside a He purge enclosure at room temperature and atmospheric pressure. A detailed description of the instrument can be found in Jacobsen et al. (1996). For specimen preparation, 2 mg of freeze-dried DOM were dissolved in 500 mL deionized water. A droplet of this solution was deposited onto a Si$_3$N$_4$ window (Silson Ltd., UK) and air-dried, resulting in a film of organic carbon (50-200 nm) on the X-ray transparent Si$_3$N$_4$ window. On each specimen, absorbance spectra were collected from 280 to 310 eV at 25 different spots on the dry film and averaged. At least four spectra of the clean Si$_3$N$_4$ window were collected, averaged, and used for background correction of the DOM spectra. For comparison of the spectra of different films, which can vary in thickness, the averaged spectra were normalized relative to their absorbance at 310 eV. The normalized NEXAFS spectra were
deconvoluted by fitting the spectral region between 280 and 310 eV with six Gaussian line shapes representing 1s-\(\pi^*\) and 1s-\(\sigma^*\) transitions and one arctangent function for the ionization step around 292 eV. For quantification, only the main 1s-\(\pi^*\) transitions from 280 eV to 290.5 eV were taken into account.

# 4.3 Results and Discussion

## 4.3.1 Elemental composition of DOM

The elemental composition of the DOM samples isolated by reverse osmosis (RO) is reported in Table 4.2.

| Table 4.2: Elemental composition and elemental ratio for the NOM sample array. Values are based on ash-free DOC. The mean distributions for the carbon functional groups of autumn and spring samples are given with their standard deviation (n=5). |
|---|---|---|---|---|---|---|
| Elemental content | C [g kg\(^{-1}\)] | H [g kg\(^{-1}\)] | N [g kg\(^{-1}\)] | S [g kg\(^{-1}\)] | TOC* [mg L\(^{-1}\)] | C/N |
| Birkenes | 1999 | 511 | 38 | 16 | 9 | 5 | 33 |
| | 2000 | 524 | 71 | 18 | 12 | 4 | 30 |
| Hietajärvi | 1999 | 520 | 37 | 9 | 9 | 6 | 59 |
| | 2000 | 546 | 46 | 12 | 10 | 5 | 44 |
| Skjervatjern | 1999 | 516 | 39 | 8 | 6 | 10 | 61 |
| | 2000 | 526 | 42 | 7 | 5 | 5 | 76 |
| Svartberget | 1999 | 569 | 32 | 7 | 7 | 11 | 82 |
| | 2000 | 520 | 41 | 4 | 2 | 19 | 119 |
| Valkea-Kotinen | 1999 | 551 | 40 | 13 | 7 | 9 | 41 |
| | 2000 | 565 | 40 | 10 | 6 | 11 | 57 |
| Autumn | 533 ± 25 | 37 ± 3 | 11 ± 4 | 7 ± 1 | 8 ± 3 | - |
| Spring | 536 ± 17 | 48 ± 13 | 10 ± 5 | 7 ± 4 | 9 ± 6 | - |
| Total | 535 ± 21 | 43 ± 11 | 11 ± 4 | 7 ± 3 | 9 ± 5 | - |

* Concentration in the original water (Vogt et al., 2001)

Elemental values are given in g per kg DOC, whereas values for TOC are given in mg L\(^{-1}\), corresponding to concentrations in the original water (Vogt et al., 2001). The freeze-dried DOM samples contained between 29 to 66 % inorganic material (ash), which is typical for RO isolates (Gjessing et al., 1999; Serkiz & Perdue, 1990). The ash consisted mainly of sodium sulphates and chlorides and was further manifested by moderate amounts in contents of calcium, magnesium and silicon dioxide. A remarkable point was the distribution of sodium sulphate in the samples from Skjervatjern and in the spring sample.
from Svartberget, which were low in comparison to their carbon content. Furthermore, the same samples were manifested by the highest contents of silicon dioxide compared to the other samples. The carbon content of the organic fraction of the DOM isolates was rather constant (median = 52.5 % C, standard deviation = 2.1 % C) and the median value was in good agreement with the carbon contents (median = 49.6 % C) of freshwater DOM compiled by Perdue and Ritchie (2003). The differences between the carbon contents of the DOM samples isolated in spring and fall were remarkably small and statistically not significant (P = 0.09). Furthermore, the contents for hydrogen and nitrogen were in good agreement with values published by Alberts & Takács. (1999), who carried out elemental analysis of eight Norwegian NOM samples also extracted by RO. Egeberg et al. (1999) stated that NOM from water sources surrounded by large catchments contained relatively more nitrogen than the NOM samples from small catchments, which could not be verified in this study.

4.3.2 FT-IR spectroscopy

The normalized FT-IR spectra of the DOM isolates are presented in Figure 4.2. All spectra exhibit the typical absorption bands of natural organic matter and humic substances (Clapp et al., 1994; Stevenson, 1982). The broad absorption band around 3400 to 3300 cm\(^{-1}\) corresponds to stretching vibrations of H-bonded hydroxyl groups. The very weak bands at 2980 to 2900 cm\(^{-1}\) represent aliphatic C-H stretching vibrations. The band at 1720 cm\(^{-1}\) is attributed mainly to C=O stretching of carboxylic groups and ketones. The band at 1630 cm\(^{-1}\) is due to C=O stretching of H-bonded carboxylic groups, quinones, or conjugated ketones and to aromatic C=C vibrations (Stevenson, 1982). The bands at 1390 cm\(^{-1}\) may arise from O-H deformation and C-O stretching of phenolic OH, C-H deformation of CH\(_2\) and CH\(_3\) groups, and COO\(^{-}\) antisymmetric stretching, respectively. The bands around 1270 cm\(^{-1}\) can be assigned to C-O stretching of aryl ethers and phenols, and to C-O stretching as well as O-H deformation of carboxyl groups and polysaccharides. The bands around 780 cm\(^{-1}\) may be due to out-of-plane bending of aromatic C-H (Senesi, 1990). However, also inorganic constituents of the DOM isolates may contribute to the FT-IR spectra. For example, Si-O bonds in silicates may contribute to a small extent to the absorption bands at 1270 cm\(^{-1}\) and 780 cm\(^{-1}\), respectively (Haberhauer & Gerzabek, 1998; Orlov, 1992). Furthermore, there is strong evidence that sulphate contributes to the absorption band at 1270 cm\(^{-1}\), as sulphate has several absorption bands between 1110 cm\(^{-1}\) and 1280 cm\(^{-1}\) (Nakamoto, 1997; Smidt et al., 2002). The influence of sulphate may be responsible for the small bands of both samples from Skjervatjern and the difference in intensity between both samples from the Svartberget site. As mentioned in the previous section, these three samples are manifested by low sulphate contents compared to their amounts of
carbon as well as elevated amounts of silicon dioxide. The most striking feature of the FT-IR spectra is the similarity between the spring and fall samples. For the Valkea-Kotinen site, the FT-IR spectra of spring and fall samples were virtually identical. For the Skjervatjern, Hietajärvi, and Birkenes sites only minor spectral differences are observed and the relative intensities of most peaks remained constant. Larger differences between spring and fall samples were only observed for the Svartberget site. Overall, the FT-IR spectra of the DOM samples suggest that the seasonal variations in DOM composition are minor. Also, the differences between the five sampling sites appear to be relatively small. The major part of the variations in the FT-IR spectra are caused by the presence of inorganic components, thus complicating the interpretation of the spectra with respect to the organic matter composition.
4.3.3 Solid-state CP-MAS $^{13}$C-NMR spectroscopy

The CP-MAS $^{13}$C-NMR spectra of the DOM isolates are presented in Figure 4.3.

![Solid-state CP-MAS $^{13}$C-NMR spectra](image)

*Figure 4.3:* Solid-state CP-MAS $^{13}$C-NMR spectra of the DOC samples. Spectra from samples taken in autumn 1999 and spring 2000 are plotted with dotted and solid lines, respectively.

The spectral differences between the spring and fall samples from each site were again minor. Note, that this was also true for the Svartberget site, which exhibited the
largest differences in FT-IR spectra. $^{13}$C-NMR spectroscopy probes the chemical bonding environment of carbon and therefore the inorganic components in the samples do not interfere with the characterization of the organic matter. The quantitative evaluation of the $^{13}$C-NMR spectra is summarized in Table 4.3.

**Table 4.3:**
Percentage of carbon distribution as derived from integrated solid-state CP-MAS $^{13}$C-NMR spectra. The mean distributions for the carbon functional groups of autumn and spring samples as well as for all samples are given with their standard deviation (n=5).

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Alkyl C</th>
<th>O-Alkyl C</th>
<th>Aromatic C</th>
<th>Phenolic C</th>
<th>Carboxyl C</th>
<th>Carbonyl C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectral region [ppm]</td>
<td>0 - 45</td>
<td>45 - 110</td>
<td>110 - 160</td>
<td>140 - 160</td>
<td>160 - 185</td>
<td>185 - 220</td>
</tr>
<tr>
<td>Birkenes 1999</td>
<td>23</td>
<td>37</td>
<td>17</td>
<td>6</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>2000</td>
<td>27</td>
<td>39</td>
<td>13</td>
<td>4</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Hietajärvi 1999</td>
<td>27</td>
<td>45</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2000</td>
<td>30</td>
<td>43</td>
<td>12</td>
<td>3</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Skjervatjern 1999</td>
<td>22</td>
<td>42</td>
<td>16</td>
<td>5</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>2000</td>
<td>22</td>
<td>44</td>
<td>17</td>
<td>5</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Svartberget 1999</td>
<td>24</td>
<td>40</td>
<td>21</td>
<td>7</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2000</td>
<td>23</td>
<td>40</td>
<td>19</td>
<td>6</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Valkea-Kotinen 1999</td>
<td>26</td>
<td>45</td>
<td>13</td>
<td>4</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>2000</td>
<td>31</td>
<td>44</td>
<td>11</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Autumn</td>
<td>24 ± 2</td>
<td>42 ± 3</td>
<td>16 ± 3</td>
<td>5 ± 2</td>
<td>12 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Spring</td>
<td>27 ± 4</td>
<td>42 ± 2</td>
<td>14 ± 3</td>
<td>4 ± 1</td>
<td>11 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>26 ± 3</td>
<td>42 ± 3</td>
<td>15 ± 3</td>
<td>5 ± 1</td>
<td>11 ± 2</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

All ten spectra exhibited strong peaks in the chemical shift regions 45-110 ppm and 160-185 ppm, respectively. The chemical shift region 45-110 ppm is assigned to O-alkyl-C, including alcohols, ethers and hemiacetals. On average, the DOM contained 42% O-alkyl-C, which is considerably higher than the median O-alkyl-C content of freshwater DOM reported in the literature (Perdue & Ritchie, 2003). The peak in the chemical shift region 160-185 ppm is attributed mainly to carboxyl-C, although esters and amides may also contribute to this spectral region. The DOM samples had an average carboxyl-C content of 11.3%, which is relatively low. Perdue and Ritchie (2003) reported that the median carboxyl-C content of freshwater DOM is 19% of the total carbon. The spectral region 110-160 ppm is commonly assigned to aromatic-C (unsubstituted and substituted), including the phenolic-C region (140-160 ppm). The average content of aromatic-C in the DOM samples was 15%, which is lower than the median C-contents of freshwater DOM reported in the literature (Abbt-Braun & Frimmel, 1999). The average contents of carbonyl-C (6%) and alkyl-C (26%) were very similar to literature median values (Abbt-Braun & Frimmel, 1999; Perdue & Ritchie, 2003).
When comparing the composition of DOM from the five sampling sites, some small differences between sites can be observed. The Birkenes and Svarterget DOM contained slightly less O-alkyl-C and slightly higher contents of carbonyl-C than the DOM from the other three sites. The DOM from Svarterget had the highest content of aromatic-C, while the Hietajärvi and Valkea-Kotinen samples had the lowest aromatic-C contents. However, these differences are relatively small, particularly when comparing the values with the average composition of DOM reported in the literature (Perdue & Ritchie, 2003).

Concerning seasonal variation within the samples, no significant differences could be observed. Table 4.3 also reports the average values for all samples taken in autumn and spring as well as the total average with their corresponding standard deviations. These observations are in contrast to studies by Kaiser et al. (2001), who investigated changes in the chemical composition of DOC in forest floor layer leachates and found 13C-NMR results to exhibit seasonal variations for alkyl, O-alkyl and aromatic carbon. Kaiser et al. examined DOC samples from seepage waters from beech and pine forests over a 27-month period. According to liquid-state 13C-NMR spectroscopy, the summer samples had larger abundances of aromatic and aliphatic structures as well as higher proportions of carboxyl groups, whereas the winter and spring samples were dominated by resonances indicating carbohydrates. These results were explained by the fact that the winter samples were mainly controlled by leaching of fresh biomass debris with large contributions of bacterial and fungal-derived carbohydrates, whereas the summer samples were controlled by decomposition processes in the forest floor. Despite these results, the trends in temporal variations were found to be remarkably similar for both sites, which is consistent with results from this study.

4.3.4 C-1s NEXAFS spectroscopy

Another element specific technique which probes the chemical bonding environment of carbon is C-1s NEXAFS spectroscopy (Stöhr, 1996). Figure 4.4 shows the averaged and normalized C-1s NEXAFS spectrum of the Svarterget DOM. The spectrum exhibits four well-developed peaks which arise from the excitation of core 1s-electrons up to low-energy unoccupied molecular orbitals, e.g., antibonding π* orbitals. At higher X-ray energies near and above the ionization energy, 1s-σ* transitions give rise to broad absorption peaks. The first peak at 285.5 eV corresponds to C=C 1s-π* transitions of aromatic carbon (protonated and alkylated to carbonyl-substituted), and may also include quinone-C and possibly olefinic-C (Boyce et al., 2002; Cody et al., 1996; Kong et al., 1998). The second peak at 286.5 eV is typical for the C-O 1s-π* transition of phenolic-C, including O-substituted aryl-C and other substituted aromatic rings with electron-withdrawing substituents groups (Hitchcock et al., 1997; Urquhart et al., 1999). The sharp peak at 288.0 eV is assigned to the
C=O 1s-π* transition of carboxyl-C (Ishii & Hitchcock, 1988; Urquhart et al., 1999). The peak at 290.5 eV corresponds to C=O 1s-π* transitions in carbonyl-C and C-O 1s-σ* transitions in O-alkyl-C, including ketones, polysaccharides, alcohols, and ethers (Boyce et al., 2002; Urquhart & Ade, 2002).

In this study, the NEXFAS spectra were deconvoluted by fitting four Gaussian peaks representing the main transitions discussed above, two broad Gaussian peaks representing 1s-σ* transitions above the ionization threshold, and one arctangent function for the ionization step around 292 eV. The example in Figure 4.4 demonstrates that this fitting scheme resulted in a good representation of the overall NEXAFS spectrum. Because the molar absorption coefficient differs between organic functional groups, NEXAFS spectra can only be quantitatively interpreted in terms of carbon distribution to different functional groups if the peak intensities are normalized individually to known reference spectra (Boyce et al., 2002). Here, we normalized the integrated peak areas to the sum of all fitted 1s-π* transition peaks of the same spectrum. This yields relative peak areas which do not represent the percentages of the total carbon, but they only serve to identify chemical differences between the samples.
The C1s-NEXAFS spectra of all DOM samples are depicted in Figure 4.5. The relative peaks areas for aromatic-C (285.5 eV), phenolic-C (286.5 eV), carboxyl-C (288.0 eV), and carbonyl + O-alkyl-C (290.5 eV) are given in Table 4.4.

![C-1s XANES spectra of the DOC sample array. Spectra of samples taken in autumn 1999 and spring 2000 are plotted with dotted and solid lines, respectively. Spectra of IHSS peat fulvic (FA) and humic acid (HA) are plotted for comparison purposes.](image)
The spectral differences between the fall and spring samples were again extremely small and statistically not significant (P=0.09). Slightly larger differences were again observed between the DOM from the different sampling sites. The relative peak areas for aromatic-C were largest for the Svartberget and Skjervatjern sites. The Birkenes and Hietajärvi DOM exhibited the largest relative peak areas for carboxylic-C. Overall, the spectral differences between the DOM samples from the five sampling sites were small, however, which is in good agreement with the FT-IR and $^{13}$C-NMR results and the elemental composition of the samples. In Figure 4.5 we also show two C1s-NEXAFS spectra of a reference peat fulvic acid (IHSS 1R103F) and a peat humic acid (IHSS 1R103H), respectively. Comparing the spectra of the DOM samples with the fulvic and humic acid spectra, some differences can be observed. Firstly, the relative intensities between the 1s-π* transition peaks at 285.5 eV and 286.5 eV were higher for fulvic and humic acids than for the DOM samples. This may indicate that the fulvic and humic acid samples contained more unsubstituted aromatic carbon relative to phenolic and O-aryl carbon. Secondly, the DOM samples had a lower peak intensity at the 1s-π* transition for carboxylic carbon (288.0 eV) than the fulvic acid. This is in agreement with the relatively low carboxylic-C content detected by $^{13}$C-NMR spectroscopy. Finally, the DOM samples exhibited a peak at 290.5 eV, which was not visible in the spectra of the fulvic and humic acids. This may be explained by

### Table 4.4:
Integrated, spectral regions for C-1s XANES data in eV with corresponding quantifications of four carbon functional groups. Values represent peak areas and are given as percentage of the sum of peak areas for aromatic, phenolic, carboxyl, and carbonyl carbon. The mean distributions for the carbon functional groups of autumn and spring samples are given with their standard deviation (n=5).

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Aromatic C</th>
<th>Phenolic C</th>
<th>Carboxyl C</th>
<th>O-Alkyl &amp; Carbonyl C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectral region [eV]</td>
<td>285.0</td>
<td>286.5</td>
<td>288.0</td>
<td>290.5</td>
</tr>
<tr>
<td>Birkenes 1999</td>
<td>16</td>
<td>28</td>
<td>52</td>
<td>4</td>
</tr>
<tr>
<td>2000</td>
<td>14</td>
<td>25</td>
<td>56</td>
<td>5</td>
</tr>
<tr>
<td>Hietajärvi 1999</td>
<td>16</td>
<td>28</td>
<td>51</td>
<td>5</td>
</tr>
<tr>
<td>2000</td>
<td>16</td>
<td>26</td>
<td>54</td>
<td>4</td>
</tr>
<tr>
<td>Skjervatjern 1999</td>
<td>18</td>
<td>30</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>2000</td>
<td>19</td>
<td>29</td>
<td>49</td>
<td>3</td>
</tr>
<tr>
<td>Svartberget 1999</td>
<td>20</td>
<td>31</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>2000</td>
<td>18</td>
<td>28</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>Valkea-Kotinen 1999</td>
<td>17</td>
<td>28</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>2000</td>
<td>17</td>
<td>27</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Autumn</td>
<td>17 ± 2</td>
<td>29 ± 2</td>
<td>50 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Spring</td>
<td>17 ± 2</td>
<td>27 ± 2</td>
<td>52 ± 3</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Total</td>
<td>17 ± 2</td>
<td>28 ± 2</td>
<td>51 ± 3</td>
<td>4 ± 1</td>
</tr>
</tbody>
</table>
the high contents of O-alkyl-C in the DOM samples, which may arise for example from
the presence of polysaccharides in the DOM, which would be consistent with the high
O-alkyl-C contents evidenced by $^{13}$C-NMR spectroscopy.

In summary, the seasonal variations observed with C-1s XANES spectroscopy were minor
and statistically not significant ($P = 0.08$). These results are consistent with published
results by Yano et al. (2004) and Solinger et al. (2001) as discussed in the previous section.
According to Abbt-Braun and Frimmel (1999), seasonal variations of NOM have to be
investigated by long-term studies in order to obtain conclusive results. The comparison
of the NOM samples from different sample sites showed remarkable similarities as well,
which is consistent with results published by Kaiser et al. (2001) who found samples from
different sites to exhibit remarkably similar variations. The results obtained in this study
suggest that litter decomposition does not seem to be very selective. These findings are in
analog to studies by Almendros et al (2000) who found in $^{13}$C NMR analysis of decaying
forest litter the degradation to occur similarly for all carbon types.

The comparison of the C-1s XANES data of the samples with the spectra of the IHSS
reference samples of peat humic and fulvic acid shows, that the NOM samples contain
higher concentrations of O-alkyl-C, possibly present as polysaccharides, and lower
concentrations of aromatic-C and phenolic-C. Further comparison shows that all samples
exhibit remarkable spectral similarities of humic acid. These observations lead to the
assumption that the generation of NOM investigated in this study is of older age than
previously supposed. These assumptions go along with the fact, that a large percentage
of the area of each catchment consists of peatland histols, which may serve as the main
input source of “old” NOM, compared to the recent material, which is generated within
the surrounding freshwaters. A further proof by $^{14}$C-dating will hopefully verify this
hypothesis and explain the occurring uniformity of these samples.
4.4 Conclusions

Ten aquatic DOM samples isolated by reverse osmosis in spring and fall seasons at five different boreal forest catchments in Scandinavia were analyzed using a combination of elemental analysis, FT-IR spectroscopy, CP-MAS $^{13}$C-NMR spectroscopy and C-1s NEXAFS spectroscopy. The results clearly demonstrate that there are no chemical differences between DOM samples collected in fall and spring, respectively. Further, our results show that the differences between the sites with respect to climate, dominating soil type, and vegetation had little influence on aquatic DOM composition. Our results disprove previous assumptions, stating that seasonal variations in DOM composition may be larger than variations between different catchments (Vogt et al., 1999; Vogt et al., 2001). Compared to peat humic and fulvic acids, the DOM samples contained higher concentrations of O-alkyl-C, possibly present as polysaccharides, and lower concentrations of aromatic-C and phenolic-C. The aromatic components in DOM may stem from the leaching of humic and fulvic acids from terrestrial soils. $^{13}$C-NMR studies on the chemical composition of soil organic matter investigated as a function of soil depth showed that the degradation of organic material with different initial composition leads to the formation of humic materials with very similar functional group distribution (Dignac et al., 2002; Mahieu et al., 1999). Thus, differences in vegetation may be minor if well-humified organic carbon is leached into the rivers. The high content of O-alkyl-C may be related to aquatic organisms producing e.g. polysaccharides. Overall, the results of this study imply that climate change will have only a minor influence on the chemical composition of aquatic DOM.

Acknowledgements

Data for this study was obtained by using the X-1A STXM at the NSLS at the Brookhaven National Laboratory, Upton, N.Y.. The STXM was developed by the group of J. Kirz and C. Jacobsen at SUNY Stony Brook (Jacobsen et al., 1991; Zhang et al., 1994) with support from the Office of Biological and Environmental Research, U.S. DoE under contract DE-FG02-89ER60858, and the NSF under grant DBI-9605045. The zone plates were developed by S. Spector and C. Jacobsen of Stony Brook and D. Tennant of Lucent Technologies Bell Labs (Spector et al., 1997) with support from the NSF under grant ECS-9510499. Furthermore, the authors would like to thank Dr. H. Knicker from TU Munich, S. Wirick from the Brookhaven National Laboratory and K. Barmettler from ETH Zurich for their contributions to this work.
**Literature cited**


Seasonal variation of aquatic DOC composition in boreal forest catchments


Chapter 5 was submitted for publication as:

Marc Schumacher, Iso Christl, Andreas C. Scheinost, Chris Jacobsen and Ruben Kretzschmar

Heterogeneity of water-dispersible soil colloids investigated by Scanning Transmission X-ray Microscopy and C-1s XANES micro-spectroscopy

*Environmental Science & Technology*
Summary

The kinetics of colloid release and deposition in natural porous media and the sorption of inorganic and organic pollutants to mobile colloids are strongly influenced by the composition and heterogeneity of the colloidal particles. In this study, we have applied synchrotron X-ray microscopy and micro-spectroscopy to characterize the chemical heterogeneity of water-dispersible soil colloids isolated from three forest soils. The heterogeneity of the soil colloids is discussed at three different scales: (i) differences between distinct regions within single colloidal particles (intra-particle heterogeneity), (ii) differences between colloidal particles isolated from the same soil horizon (inter-particle heterogeneity), and (iii) differences between colloidal particles isolated from different soils or soil horizons. In total, 61 single particles were imaged using scanning transmission X-ray microscopy and 130 images of each particle were recorded at X-ray energies ranging from 280 to 310 eV. From the image arrays, C-1s XANES spectra were obtained and analyzed by principle component analysis and cluster analysis to characterize the intra-particle heterogeneity of the organic components. The results demonstrate for the first time that the organic matter associated with water-dispersible soil colloids is heterogeneous at the single particle scale. However, the differences in XANES spectra obtained for different regions within single particles were much smaller than the differences between average spectra of particles isolated from the same soil horizon (inter-particle heterogeneity). At the soil profile scale, no significant differences in the average composition of water-dispersible colloids from H and Ah horizons were detected due to the high inter-particle heterogeneity.
Heterogeneity of soil colloids investigated by STXM and C-1s XANES spectroscopy

5.1 Introduction

Mobile colloidal particles can serve as carriers for strongly sorbing contaminants and thereby facilitate contaminant transport in soils, groundwater aquifers, and fractured rocks (Grolimund et al., 1996; Kretzschmar et al., 1999; McCarthy & Zachara, 1989; Saiers & Hornberger, 1996). In soils and surface-near aquifers, mobile colloids usually consist of mixtures or complexes of phyllosilicates, hydrous oxides of Si, Fe and Al, and natural organic matter. The mobility of colloids, and therefore the importance of colloid-facilitated transport of contaminants, strongly depends on colloid chemistry and colloidal stability in the geochemical environment (Kretzschmar et al., 1999; Ryan & Elimelech, 1996). The colloidal stability of clay mineral and oxide particles is often greatly enhanced by the presence of adsorbed natural organic matter (Gibbs, 1983; Kretzschmar et al., 1998; Tiller & O'Melia, 1993). It has also been shown that the stability, transport, and deposition of colloids in porous media are strongly affected by the chemical heterogeneity of the colloidal particle and matrix surfaces (Johnson et al., 1996; Song & Elimelech, 1994). However, little is known about the nature of natural organic matter associated with mobile soil colloids and the heterogeneity of the colloids at the particle scale.

At the soil profile scale, the chemical composition of soil organic matter (SOM) is often quite variable. For example, Spielvogel et al. (2004) demonstrated that the functional group distribution of SOM, determined by $^{13}$C-NMR spectroscopy, exhibited large variations for 42 top soil horizons from different soil types located in Germany. This heterogeneity of SOM was explained by the variable organic input materials as well as differences in moisture and temperature conditions favoring different decomposition processes (Kögel-Knabner et al., 1988; Lydersen, 1995). On the other hand, $^{13}$C-NMR studies on chemical composition of SOM investigated as a function of soil depth showed that the degradation of organic materials with different initial composition led to the formation of soil organic matter with remarkably similar functional group distribution (Dignac et al., 2002; Mahieu et al., 1999). Although $^{13}$C-NMR spectroscopy is a very powerful tool to investigate the chemical composition of soil organic matter at the profile scale, no information can be obtained about the heterogeneity of soil colloids at the particle scale.

In recent years, Synchrotron Scanning Transmission X-ray Microscopy (STXM) combined with X-ray absorption near-edge structure (XANES) spectroscopy has been developed for combined imaging and spectroscopic characterization of thin organic specimen. For example, this technique has been applied to quantify carbon bound in major functional groups in a variety of environmental samples, ranging from petroleum (Hitchcock et al., 1986; Waldo et al., 1991) and coal (Cody et al., 1995; Spiro et al., 1984) to atmospheric and interplanetary dust particles (Flynn et al., 2003; Russell et al., 2002). First studies on the
chemistry of soil organic matter using STXM and XANES spectroscopy are also available. For example, Morra et al. (1996) and Xia et al. (1998) used S-XANES spectroscopy to determine sulfur oxidation states in humic and fulvic acid samples. Thieme et al. (2003) investigated the structures of microorganisms and carbonate precipitates in soils using STXM and C-1s XANES spectroscopy. Some studies have also demonstrated the potential of this technique for characterizing single colloidal particles (Schäfer et al., 2003) and for the quantification of organic functional groups in humic substances (Scheinost et al., 2001).

Here, we present a study on the chemical heterogeneity of water-dispersible soil colloids using synchrotron scanning transmission X-ray microscopy (STXM) and C-1s XANES microspectroscopy. Our objectives were (i) to investigate the chemical heterogeneity of natural organic matter associated with water-dispersible soil colloids at the single particle scale, (ii) to assess the inter-particle heterogeneity of water-dispersible soil colloids within soil horizons, and (iii) to identify variations in soil colloid composition between three different sampling locations.

5.2 Materials and Methods

5.2.1 Soil samples and isolation of water-dispersible colloids

Soil samples were taken along a toposequence located on a lateral moraine near Langenthal, northern Switzerland. The first site (Riedhof, RH, 525 m elevation) is situated on top of the lateral moraine and the soil is classified as a dystric Luvisol (ISSS-ISRIC-FAO, 1998). The second site (Oberrickenzopfen, ORZ, 505 m elevation) is located on the mid-slope of the moraine and the soil is a humic Gleysol. The third site (Unterrickenzopfen, URZ, 490 m elevation) is located at the bottom of the moraine and the soil is also classified as a humic Gleysol. All three sites are forested, with beech trees (Fagus sylvatica) dominating at the Riedhof site and spruce trees (Picea abies) dominating at the other two sites, respectively. The region has a humid-temperate climate, with an annual mean temperature of 8.7 °C and 1154 mm mean annual precipitation. At all three sites, we sampled the well-humified organic surface horizons (H) and the top mineral horizons (Ah). After sampling, the field-moist soil material was passed through a 5-mm sieve and stored in the dark at -17 °C. Water-dispersible soil colloids were isolated from frozen soil samples using the following procedure. Two hundred grams of moist soil was suspended in 1 L of deionized water, shaken for 8 h at 25 ± 1 °C, and centrifuged for 6 min at 2500 g. Aliquots of the supernatant suspensions containing the water-dispersible soil colloids were transferred into 50-mL glass vials and analyzed within a few days.
In addition to water-dispersible colloids, we also isolated the humic acid and fulvic acid fractions of each soil horizon using standard IHSS procedures (Thurman & Malcolm, 1981) and a water-soluble dissolved organic carbon (DOC) fraction. The DOC was obtained by suspending two hundred grams of most soil in 1 L of deionized water, shaking it for 12 h at 25 ± 1 °C and centrifuging at for 20 min at 900 g. The supernatant suspension was passed through a 0.45 µm membrane filter (Schleicher & Schüll, Fluka), dialysed for one week against deionized water (SpectraPor 2, Spectrum) and freeze-dried.

5.2.2 X-ray microscopy and micro-spectroscopy

In total, 61 colloidal particles from the six different soil horizons were imaged using STXM and further characterized by C-1s XANES micro-spectroscopy on beamline X-1A at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY. For specimen preparation, one drop of soil colloid suspension was deposited onto a Si3N4 window (Silson Ltd., UK) with 100 nm thickness, which was previously glued onto the wet-cell of the microscope. The wet-cell was then closed by covering it with another Si3N4 window and screwing both parts together (Neuhäusler et al., 2000). A mean sample thickness of about 100 nm was obtained with three adjustable screws in the wet-cell. The sample thickness is important in order to obtain a good signal-to-noise ratio in XANES spectroscopy (Urquhart et al., 1999a).

The STXM was operated inside a He purge enclosure at room temperature and atmospheric pressure. For calibration purposes, CO2 gas was added to the He purge in the microscope and normalized to -8.01 eV relative to the C-1s→3s transition (292.8 eV). Details on the instruments specifications have been reported elsewhere (Jacobsen et al., 1991; Kirz et al., 1991). After mounting the wet-cell sample on the sample holder, spectra were recorded using an automated imaging routine, which collects sets of images at X-ray energies ranging from 280 eV to 310 eV in energy steps of 0.5 eV, except near the absorption edge (282 to 290 eV), where the step size was reduced to 0.1 eV. In total, 130 images were recorded from each particle and aligned afterwards using an autocorrelation routine in order to remove possible offsets in the image array. Particular care was taken of the background correction of the spectra. Image regions which did not contain detectable amounts of carbon were chosen and used as the background I0. Absorbance (A) spectra were then derived from the transmitted X-ray intensity (I), assuming validity of Beer’s law with A = μρt = −ln (I/I0), where μ is the energy dependent mass adsorption coefficient, ρ is the sample density and t is the sample thickness (Kirz et al., 1991). To minimize the effects of spectral distortions, we included only spectra with maximum absorbance values below 2 in our data analysis. In order to allow quantitative comparisons, all collected spectra were normalized to the absorbance at 310 eV (Stöhr, 1996).
The spectral data was extracted from the image stacks by using the IDL routines “stack_analyze” and “spectra_gui”, written by Jacobsen et al. (2000). Principal component analysis (PCA) and cluster analysis (CA) were carried out on the same image stacks, using an IDL program routine written by Lerotic et al. (2004). A semi-quantitative analysis of XANES spectra was carried out by peak deconvolution using the program WinXAS 3.1.

The following peak assignments were considered (Table 5.1): the peak at energies around 285 eV was assigned to the photoinduced transition of an electron from the 1s orbital to an aromatic π* orbital (Urquhart et al., 2000) and will herein be referred to as aromatic C. The second peak at around 286.5 eV was assigned to transitions taking place for O-substituted aromatic carbon, as in the case of phenol or aryl ether (Cody et al., 1995), and will be referred to as phenolic C. The most intense peak near 288.5 eV was assigned to carboxylic carbon (Urquhart et al., 1999b). The last spectral feature at 289.9 eV was assigned to transitions of carbonyl carbon (Urquhart & Ade, 2002). For all assignments listed above, Gaussian peaks with a fixed full width at half maximum (FWHM) of 0.5 eV were fitted to the data. The ionization threshold was described with an arctangent function at 290.3 eV with a FWHM of 1. In addition, σ* transitions were simulated by two Gaussian functions with maxima at 295 eV and 303 eV and FWHMs of 8 eV and 6 eV, respectively. For our semi-quantitative analysis, the sum of all four π*-transitions (aromatic, phenolic, carboxylic, and carbonyl C) was set to 1. Peak areas for aromatic, phenolic, carboxylic, and carbonyl C are reported as values relative to the summed peak area of all four π*-transitions.
5.3 Results and Discussion

5.3.1 Heterogeneity of colloids at the particle scale

The heterogeneity of organic matter associated with water-dispersible soil colloids was first investigated at the particle scale.

Figure 5.1 shows an example of a colloidal particle isolated from the H horizon of the Unterrickenzopfen (URZ) soil. The first image (Figure 5.1 a) was recorded at a X-ray energy of 280 eV, that is, below the C-1s edge energy of carbon (283 eV). The image contrast is produced mainly by absorption of X-ray energy by inorganic components within the particle. The second image (Figure 5.1 b) was recorded at an X-ray energy of 290 eV, i.e., above the C-1s absorption threshold. The two lower plots show the areas of the three chemically different regions as evaluated by cluster analysis and the respective average C-1s spectra in corresponding colours.

Figure 5.1: Intra-particular heterogeneities of a colloidal particle from the H-horizon at Unterrickenzopfen. The upper two images were taken at 280 eV and 290 eV, i.e., below and above the C-1s absorption threshold. The two lower plots show the areas of the three chemically different regions as evaluated by cluster analysis and the respective average C-1s spectra in corresponding colours.
image contrast and organic-rich regions within the colloidal particle also become visible. Further characterization of the heterogeneity of natural organic matter associated with the colloidal particles was achieved by principle component and cluster analysis (PCA-CA) of C-1s XANES spectra. Figures 5.1 c and 5.1 d show the results, in this case yielding three distinct regions. The blue color represents the background without detectable amounts of carbon. The inner region (orange) appeared denser and was slightly richer in aromatic carbon, while the outer region (red) appeared to be less dense and richer in carboxylic carbon. Possibly, the components rich in carboxylic carbon were more negatively charged and more hydrophilic, while the dense inner region was more aromatic and therefore more hydrophobic.

Figure 5.2: Intra-particular heterogeneity of a colloidal particle from the Ah-horizon at Unterrickenzopfen. The upper two images were taken at 280 eV and 290 eV, i.e., below and above the C-1s absorption threshold. The two lower plots show the areas of the three chemically different regions as evaluated by cluster analysis and the respective average C-1s spectra in corresponding colours.
Figure 5.2 shows corresponding results for a water-dispersible colloidal particle isolated from the organic Ah horizon of the Unterrickenzopfen soil. The two images recorded below and above the C-1s absorption edge demonstrate that the particle (or aggregate), which is about 2 µm in diameter, is composed of both mineral and organic components. Again, PCA-CA analysis yielded two distinct regions within the colloidal particle. The C-1s spectra of the two distinct regions within the particle show remarkable differences (Figure 5.2 d). The orange color represents regions within the particle that contain mineral components in addition to organic carbon, while the red color represents regions that contain mainly organic substances. The peak intensities for aromatic (285.0 eV) and carboxylic (288.5 eV) carbon in both spectra are very similar. However, the highly organic regions (red) appear richer in phenolic carbon, as indicated by a stronger peak at 286.6 eV. The strong peaks at 297 and 300 eV in the spectrum for the mineral-rich regions (orange) are due to the potassium L$_{II}$ and L$_{III}$ absorption edges, respectively. This suggests that the mineral components may consist of K-bearing phyllosilicates, such as illite.

The two examples, which served to illustrate characteristic features observed for 61 particles, demonstrate for the first time that the organic matter associated with water-dispersible soil colloids is chemically heterogeneous at the particle scale. In the H horizons, the greatest differences tended to be observed in the contents of carboxylic carbon, while the peaks for aromatic and phenolic carbon were rather invariable. In Ah horizons, we observed the greatest differences in the contents of phenolic carbon, while the peak intensities assigned to aromatic, carboxylic, and carbonyl carbon tended to be rather constant. Although clearly detectable, the differences in XANES spectra between regions of single particles (intra-particle heterogeneity) were much smaller than differences between averaged spectra of different particles isolated from the same horizon (inter-particle heterogeneity). This inter-particle heterogeneity is discussed in the next section.
5.3.2 Inter-particle heterogeneity

The inter-particle chemical heterogeneity of the water-dispersible soil colloids was analyzed on the basis of the C-1s XANES spectra of all particles analyzed from each horizon (n=5 to 18). The single particle spectra were obtained by averaging normalized XANES spectra for all pixels within the entire particle region, e.g., the red and orange regions in Figures 5.1 and 5.2. The average of all single particle spectra for each horizon then gave an average spectrum for the water-dispersible colloids of that soil horizon. Selected spectra for the Unterrickenzopfen H horizon are presented in Figure 5.3. The first spectrum represents the average spectrum representing the entire population of water-dispersible colloids. The following four spectra are examples for single particle spectra within that population of particles, illustrating the variability of particles within this soil horizon. The bottom three spectra are from the humic acid (HA), fulvic acid (FA), and dissolved organic carbon (DOC) isolated from the same soil for comparison. The single particle spectra of the H horizon exhibited a rather large variability in the peak intensities for aromatic and phenolic carbon, while the peak intensities for carboxylic and carbonyl carbon were less variable. One of total eighteen particles showed clear potassium absorption peaks at 297 eV and 300 eV, suggesting that this particle was an association of clay and organic matter. In order to compare the composition of organic components associated with water-dispersible soil colloids with more traditional fractions.

Figure 5.3: C-1s XANES spectra of four colloidal particles (C1 – C4) extracted from the H-horizon at Unterrickenzopfen. The average spectrum (Av) represents the averaged spectra for all investigated particles of the respective soil horizon. The number of n corresponds to the total number of particles evaluated. At the bottom, spectra for extracted humic acid (HA), fulvic acid (FA) and DOC are shown.
of soil organic matter, we also analyzed the humic acid (HA), fulvic acid (FA) and watersoluble organic carbon (DOC) fractions of the H horizon (Figure 5.3). The spectrum for HA and FA had similar peak intensities for aromatic, phenolic, and carbonyl carbon, but the FA exhibited a much stronger peak for carboxylic carbon. The DOC spectrum showed a somewhat different pattern: Peak intensities for aromatic and phenolic carbon were lower for DOC than for humic and fulvic acid. In addition, very pronounced peaks appeared in the DOC spectrum for carboxylic and carbonyl carbon. Comparison of the spectra suggests that the organic matter associated with water-dispersible soil colloids was most similar to the humic acid fraction. The average spectrum of water-dispersible colloids from the Unterrickenzopfen Ah horizon and selected single particle spectra of that population are depicted in Figure 5.4. The particle spectra showed distinct differences in peak intensities for aromatic and carboxylic carbon, while peak intensities for phenolic and carbonyl carbon remained fairly stable. Five of total 11 particles showed potassium absorption features in their spectra, again suggesting the presence of K-bearing clay minerals. This is expected, since the Ah horizon is a mineral soil horizon containing large quantities of clays.

**Figure 5.4:**
C-1s XANES spectra of four colloidal particles (C1 – C4) extracted from the Ah-horizon at Unterrickenzopfen. The average spectrum (Av) represents the averaged spectra for all investigated particles of the respective soil horizon. The number of n corresponds to the total number of particles evaluated.
Heterogeneity of soil colloids investigated by STXM and C-1s XANES spectroscopy

The relative peak areas for aromatic, phenolic, carboxylic, and carbonyl carbon of all 61 single particle spectra from six soil horizons are summarized in Figure 5.5. The box plots show the minimum and maximum (stars), 25 and 75 percentiles (upper and lower corners of open polygons), median (dashed line) and mean value (square symbol). Overall, the intra-particle heterogeneity of organic functional group composition was somewhat larger in the H horizons than in the Ah horizons. This may be explained by increasing humification of organic matter components with increasing soil depth. Mahieu et al. (1999) reported that $^{13}$C-NMR spectra of soil organic matter from various soils were increasingly similar to each other with increasing soil depth, which was also explained by the degree of humification.

Figure 5.5: Distribution of carbon functional groups as determined by C-1s XANES spectroscopy for water-dispersible colloids extracted from organic soil horizons (H) and top mineral soil horizons (Ah) of the locations Riedhof, Oberrickenzopfen and Unterrickenzopfen. Note the legend on top for description.
5.3.3 Water-dispersible colloids from different soil horizons

The average functional group composition of organic matter associated with water-dispersible soil colloids isolated from the H and Ah horizons of the three forest soils can be compared in Figure 5.5. Due to the large inter-particle variability discussed in the previous section, significant differences between the H and Ah horizons could not be detected. However, several trends can be observed. The median values of relative peak areas for aromatic carbon in all soil horizons ranged between 0.10 and 0.16. No significant differences between H and Ah horizons were observed. In contrast, the relative peak areas for phenolic carbon were much more variable and the median values ranged from 0.09 in the Riedhof Ah to 0.37 in the Oberrickenzopfen H horizon. The largest relative peak areas were measured for carboxylic carbon in all soil horizons, with median values ranging from 0.35 in the Oberrickenzopfen H to 0.71 in the Riedhof Ah horizons. The variability of peak areas for carboxylic carbon was also large for most soil horizons. Finally, carbonyl carbon gave median relative peak areas between 0.05 in the Oberrickenzopfen Ah and 0.25 in the Riedhof H horizons.

In summary, evaluation of C-1s XANES spectra of 61 water-dispersible soil colloids extracted from six different soil horizons revealed a high inter-particle variability in organic functional group composition, especially in the abundances of phenolic and carboxylic carbon. Chemical heterogeneities within single colloidal particles (intra-particle heterogeneity) were clearly detectable, but the differences were relatively small compared to the very large inter-particle heterogeneity.

Acknowledgements

This project was financially supported by the ETH Zurich (TH project Nr. 01753). All XANES analyses were performed at beamline X-1A at the National Synchrotron Light Source at the Brookhaven National Laboratory in Upton, N.Y., which is supported by the U.S. Department of Energy. Operation of the Stony Brook STXM is supported by National Science Foundation grants CHE-0221934 and OCE-0221029. Furthermore, the authors would like to gratefully acknowledge S. Wirick, Dr. T. Beetz and Dr. M. Feser of the Department of Physics, SUNY Stony Brook, and K. Barmettler from ETH Zurich for their contribution and support to this work.
Heterogeneity of soil colloids investigated by STXM and C-1s XANES spectroscopy

Literature cited


Heterogeneity of soil colloids investigated by STXM and C-1s XANES spectroscopy


Heterogeneity of soil colloids investigated by STXM and C-1s XANES spectroscopy


6 Conclusions and outlook

This work focused on the investigation of the chemical heterogeneity occurring in natural organic matter and humic substances by the use of STXM and C-1s XANES spectroscopy. In order to quantify the various features occurring in a typical C-1s spectrum, a least-squares fitting scheme was applied in the first study and compared with results derived from CP-MAS $^{13}$C-NMR spectroscopy and elemental analysis. All experiments were conducted on well characterized humic substances reference samples. The results were further compared with data derived from previous quantification procedures and statistically correlated, whereas carboxyl carbon turned out to be the major fraction of organic C, followed by moderate amounts of aromatic and phenolic bound carbon. The quantification scheme was further applied on spectra of ten natural organic matter samples of aquatic origin, sampled in autumn and spring at five locations in Scandinavia. The samples were analyzed with FT-IR, CP-MAS $^{13}$C-NMR and C-1s XANES spectroscopy in order to resolve possible seasonal variations within their carbon functional group distribution. Results showed that particular seasonal variations were not detectable with any of the three techniques and that all samples in contrast exhibited remarkable spectral similarities. In order to investigate the chemical properties of the mobile fraction of natural organic matter in soils, the carbon functional group characteristics of 61 water-dispersible colloids extracted from three different soils was investigated by C-1s XANES spectroscopy and STXM. Investigations were carried out at three different scales using principal component and cluster analysis. Results showed that single particles, extracted from the same horizon and soil type, showed much higher differences in carbon functional group distribution than particles extracted from different soil types. Due to this high inter-particle heterogeneity, no significant variations between horizons or soil types were observed either.

The study of complex, natural samples such as natural organic matter and humic substances is of crucial interest in environmental sciences. The use of STXM and C-1s XANES spectroscopy techniques does not only permit the detection and quantification of specific carbon groups present in environmental samples but also visualizes their spatial distribution at the micron scale. The possibility to analyze these samples in aqueous solutions as well as under atmospheric conditions makes the technique a valuable tool for their investigation, especially at the colloidal particle scale. In situ investigation on hydrated environmental samples with high spatial resolution in combination with the
Conclusions and outlook

capability of highlighting elements naturally by their absorbance is currently not possible by any other technique than STXM and is one of its intriguing advantages. Information about the chemical structure and the association of components in hydrated aggregates can be revealed and can help to further develop theoretical models about the interaction of these components.
Spectral assignments and quantitative interpretation for the functional group chemistry of organic macromolecules are still in their early stages. The establishment of a XANES spectroscopy database, similar to the spectroscopy database available for infrared spectroscopy, would greatly facilitate the identification and analysis of materials for which prior knowledge is very limited. In parallel to the development of such a database, theoretical modelling together with calculated electronic spectra, as performed by Kaznacheyev et al. (2002) for a wide range of amino acids, would help in the interpretation of XANES spectra and the chemical structural information they provide. The collection of XANES spectra at the oxygen absorption edge can provide further valuable information about the chemical bonding environment between carbon and oxygen. Correlation of C-1s with O-1s XANES data would help in the development of a consistent quantification scheme that provides reliable data for at least all oxygen-containing carbon groups such as phenolic, carboxyl or carbonyl C. Details on other complementary techniques such as small and wide angle X-ray scattering or X-ray emission can provide complementary information on the macromolecular structure and functional group chemistry of environmental samples.
This work contributed to the development of a deeper knowledge of the physico-chemical properties of natural organic matter particles and their interaction with other chemical compounds in soil and aquatic systems. The potential and limits of XANES spectroscopy and STXM on the study of humic substances, natural organic matter and colloidal soil samples as a novel tool for research in agriculture, forestry and soil science have been described and offer the chance to make significant contributions to the understanding of the complex chemistry of natural organic matter and its role in environmental processes.
I am very grateful to Prof. Ruben Kretzschmar who gave me the opportunity to carry out this dissertation and supervised this work with sustained and constructive support. I am indebted to Prof. Chris Jacobsen for his support, the review of this manuscript and for taking on the co-examiners position.

I would like to thank Dr. Iso Christl for the fast reviewing of the manuscripts, all the helpful and constructive advices and the motivating working atmosphere he created. I am indebted to Dr. Andreas C. Scheinost for the start of this project, his support and for giving me the possibility to work autonomously.

I would like to thank Prof. Rolf Vogt for providing the NOM samples and experimental data. I am very grateful to Kurt Barmettler for helping with numerous experiments and for hints regarding laboratory practice. I want to thank Sue Wirick for providing information and help during my beamtime at the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratory (BNL), as well as her professional and personal support. I want to thank all the members of the X-ray optics group from Stony Brook University for their support, especially Dr. Tobi Beetz, Dr. Michael Feser, Holger Fleckenstein and Mirna Lerotic.

Furthermore, I would like to acknowledge all the colleagues of the Soil Chemistry group and other members of ITO, especially Andreas Birkefeld, Olivier Jacquat, Charlotte Wüstholz and Petra Gulz. I want to thank my family for their support and all my friends outside the Institute, especially Pascal Gerner and Raphael Köchli, with whom I shared good and bad times.

Last but not least I would like to thank my girlfriend Nadine for supporting my visions, her patience and her love. Without her support, this work would have never been possible.