Aluminium stabilizes dissolved organic matter by precipitation

Dissertation

zur Erlangung des Doktorgrades (Dr. rer. nat.)
der Fakultät für Biologie, Chemie und Geowissenschaften der
Universität Bayreuth

vorgelegt von

Thorsten Scheel

Betreuer: **PD Dr. Karsten Kalbitz**

Lehrstuhl für Bodenökologie

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.).

Promotionsgesuch eingereicht am: 25. Januar 2008

Tag des wissenschaftlichen Kolloquiums: 13. November 2008

Prüfungsausschuss:

PD Dr. Karsten Kalbitz (Erstgutachter)

Prof. Dr. Stefan Peiffer (Zweitgutachter)

Prof. Dr. Bernd Huwe (Vorsitzender)

Prof. Dr. Egbert Matzner

Prof. Dr. Bernd Wrackmeyer

Die vorliegende Arbeit entstand im Zeitraum von Mai 2004 bis Januar 2008 und wurde am Lehrstuhl für Bodenökologie unter der Anleitung von PD Dr. Karsten Kalbitz angefertigt.

Content

List of Figures

List of Tables

List of Abbreviations

ABTS	2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonate)
AMC	7-amido-4-methylcoumarin hydrochloride
DAPI	4′-6-diamidino-2-phenylindole
DGT	Diffusive Gradients in Thin films
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EA	Enzyme activity
FTIR	Fourier transformed infrared spectra
HIX_{syn}	Humification index deduced from synchronous fluorescence spectra
ICP-OES	Inductively coupled plasma–optical emission spectrometer
LSM	Laser Scanning Microscopy
MUF	Methylumbelliferyl
NMR	Nuclear Magnetic Resonance spectra
OM	Organic matter
SE	Standard error
SI	Saturation index
SOM	Soil organic matter
UV	Ultraviolet

Summary

It is well known that carbon mineralization in acidic forest soils can be reduced by large Al concentrations. Precipitation of dissolved organic matter (DOM) by dissolved Al could contribute substantially to C retention in acidic forest soils. However, no information is available on the properties of precipitated organic matter (OM) and its stability against microbial decay although that might be an important mechanism for long-term carbon storage in soils. Therefore, I investigated the influence of (i) the DOM composition, (ii) the pH of precipitation, and (iii) the Al/C ratio in solution on the amount of OM precipitated, the associated changes in its composition, and the resulting stability of precipitated OM against microbial decay.

Between 13 and 84% of the C in solution was precipitated, depending on pH, Al/C ratio, and the composition of DOM. I observed preferential precipitation of aromatic compounds and enrichment of carboxylic C in precipitated OM, being associated by depletion in N and enrichment in P. The type of bonds between OM functional groups and the Al cations was independent of pH, Al/C ratios and solution composition. FTIR spectra indicated ligand exchange as possible binding mechanism. Furthermore, exoenzymes were present in a functional state in precipitated OM.

Carbon mineralization of DOM was up to 28 times larger than that of the respective precipitated OM. Only 0.5-7.7% of precipitated C was mineralized during 7 weeks of incubation. When precipitated OM remained in solution the reduction in C degradation by precipitation amounted up to 65%. This increase in stability by precipitation was significantly correlated with the amount of C precipitated. Thus, the bonds between Al and carboxylic groups of aromatic compounds with low N contents resulted in a reduced bioavailability of C. I found no indication for toxic effects of Al. The enzyme activity found in precipitates significantly increased the percentage of C mineralized of precipitated OM. However, after eight weeks of incubation the correlations between enzyme activity and C mineralization disappeared, despite substantial enzyme activity and C being still present. Thus, degradation of precipitated OM seems to be governed by enzyme activity during the first degradation phase, but the long term stability of precipitated OM is probably related to its chemical properties and structure, e.g. floc size.

The Al cations linked a large number of molecules of OM up to floc sizes of 110 µm in diameter, with smaller sizes on average at pH 3.8 (16.6 µm) than at pH 4.5 (27.6 µm). For a floc of 10 µm about 80 million molecules needed to be spatially aggregated. Thus, the spatial accessibility of OM was considerably reduced in these flocs leading to additional stabilization, besides the intrinsic stability of the organic compounds and the complexation by Al. The observation that only a certain fraction of precipitated OM is prone to degradation by enzymes supports this.

I conclude that precipitation of DOM is an important mechanism for the long-term carbon stabilization in mineral horizons of acidic forest soils. Changing environmental conditions can affect both the amount of OM precipitated and the degradability of the precipitate. Thus, more focus should be laid on the influence of OM precipitation on C retention and sequestration in acidic forest soils.

Zusammenfassung

Es ist bekannt, dass in sauren Waldböden die C Mineralisierung durch hohe Al Konzentrationen reduziert werden kann. Des Weiteren kann Al über die Ausfällung gelöster organischer Substanz (DOM) beträchtlich zur Festsetzung von C in sauren Waldböden beitragen. Es gibt jedoch keine Informationen zu den Eigenschaften ausgefällter organischer Substanz (OM) sowie ihrer Stabilität gegenüber mikrobiellem Abbau, obwohl die Ausfällung möglicherweise ein wichtiger Mechanismus für die langfristige Speicherung von C in Böden ist. Aus diesem Grund habe ich den Einfluss der (i) DOM Zusammensetzung, (ii) des pH-Wertes bei der Ausfällung, und (iii) des Al/C Verhältnisses in der Lösung auf die ausgefällte Menge an OM, die damit verbundenen Änderungen in der Zusammensetzung der OM sowie deren Stabilität gegenüber mikrobiellem Abbau untersucht.

In Abhängigkeit der oben genannten Faktoren wurden zwischen 13 und 84% des C der Lösung ausgefällt. Ich konnte feststellen, dass bevorzugt aromatische Bestandteile mit einem hohen Anteil an Carboxylgruppen ausgefällt wurden. Dies war mit niedrigeren N Gehalten und einer Anreicherung von P in der ausgefällten OM verbunden. Der Bindungstyp zwischen den funktionellen Gruppen der OM und den Al Kationen zeigte sich als unabhängig vom pH-Wert, dem Al/C Verhältnis und der Lösungszusammensetzung. Die FTIR Spektren deuteten als möglichen Mechanismus Ligandenaustausch an. Zudem befanden sich in der ausgefällten OM extrazelluläre Enzyme in funktionellem Zustand.

Die C Mineralisierung der DOM war bis zu 28-mal höher als die der entsprechenden ausgefällten OM. Während einer siebenwöchigen Inkubation wurden nur 0,5 bis 7,7% des ausgefällten C mineralisiert. Verblieb die ausgefällte OM in der Ausgangslösung, betrug der Rückgang des C Abbaus bis zu 65%. Diese erhöhte Stabilität der OM war signifikant mit der Menge des ausgefällten C korreliert. Die Bindung zwischen Al und den Carboxylgruppen der aromatischen Bestandteile, welche zudem niedrige N-Gehalte besaßen, führte somit zu einer reduzierten Bioverfügbarkeit des C. Indizien für toxische Effekte von Al konnte ich keine erkennen. Die Enzymaktivität der ausgefällten OM erhöhte signifikant den Anteil des mineralisierten C. Allerdings verschwand der Zusammenhang zwischen C Mineralisierung und Enzymaktivität nach 8 Wochen Inkubation, trotz beträchtlicher Mengen an C und vorhandener

Enzymaktivität. Der Abbau der OM wurde demzufolge nur in der ersten Abbauphase maßgeblich von der Aktivität der Enzyme beeinflusst. Die langfristige Stabilität wurde jedoch von den chemischen Eigenschaften und der Struktur, z.B. der Flockengröße, der ausgefällten OM bestimmt.

Der maximale Durchmesser der Flocken betrug 110 µm, wobei im Mittel kleinere Durchmessern bei pH 3,8 (16,6 µm) als bei pH 4,5 (27,6 µm) zu beobachten waren. Für eine Flocke von 10 µm mussten schätzungsweise um die 80 Millionen Moleküle durch Al Kationen miteinander räumlich verbunden werden. Die räumliche Angreifbarkeit der Moleküle in der Flocke dürfte folglich beträchtlich reduziert gewesen sein, was zu einer zusätzlichen Stabilisierung der OM führte, neben der eigenen Stabilität der organischen Substanz und der Komplexierung durch Al. Die Beobachtung, dass nur eine bestimmte Fraktion der ausgefällten OM durch Enzyme angreifbar war, unterstützt dies.

Ich schlussfolgere, dass die Ausfällung von DOM ein wichtiger Mechanismus der langfristigen C Speicherung in Mineralhorizonten saurer Waldböden ist. Sich verändernde Umweltbedingungen können sowohl die Menge der ausgefällten OM, als auch deren Abbaubarkeit bestimmen. Aus diesem Grund sollte ein stärkerer Fokus auf die Rolle der Ausfällung von OM bei der Rückhaltung und Stabilisierung von C in sauren Waldböden gelegt werden.

Chapter I

Synthesis:

Aluminium stabilizes dissolved organic matter by precipitation

Thorsten Scheel[a]

[a]Department of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany.

1. Introduction

1.1. General introduction

Forest soils are of special importance for C storage in the global carbon cycle, as they provide a significant sink for atmospheric CO_2. It is estimated that in the northern hemisphere 0.13 Pg C per year are stored in the forest floor and soil organic matter (Goodale et al., 2002). Dissolved organic matter (DOM) comprises only a small fraction of this carbon but it is significantly involved in many soil processes, due to its high mobility and bioavailability (Marschner and Kalbitz, 2003). Sources for DOM are recent litter, humus, root exudates and the microbial biomass (Kalbitz et al., 2000). Dissolved organic matter is considered as an easily degradable fraction when compared with solid soil organic matter (Burford and Bremner, 1975). Microorganisms take up most organic substrates and nutrients directly from the soil solution in dissolved form. Therefore, the transition of carbon from the liquid to the solid phase should affect microorganisms directly by decreasing the bioavailability. In forest soils the flux of dissolved organic carbon from the forest floor into the mineral horizon is considerable (115-500 kg C ha^{-1} $year^{-1}$), whereas the loss with seepage is much smaller (5-66 kg C ha^{-1} $year^{-1}$), indicating that a large portion is either mineralized or retained in the mineral soil (Guggenberger and Zech, 1993; Michalzik et al., 2001, Solinger et al., 2001). The portion to which the carbon is mineralized depends on the degree of stabilization against microbial decay. The most important mechanisms for the protection of OM against decomposition are (i) selective preservation of recalcitrance compounds, (ii) spatial inaccessibility of OM to microorganisms and their enzymes, (iii) and stabilization of OM by the interaction with mineral surfaces or polyvalent metal cations (von Lützow et al., 2006). Sorption of DOM to mineral surfaces is an important and well-documented process for organic C stabilization in soils (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005). Baldock and Skjemstad (2000) describe the precipitation of DOM by polyvalent cations as a process potentially involved in the stabilization of soil organic C against microbial decay. Nevertheless, little is known about stabilization of organic matter by formation of insoluble Al–OM complexes (Boudot, 1992), which could be an important pathway for the formation of stable soil OM.

1.2. Precipitation of dissolved organic matter

Dissolved organic matter can be precipitated from the liquid phase in acidic soil solutions by polyvalent cations, e.g. Al, Fe (Nierop et al., 2002). The fraction of DOM which is prone to precipitation by metal cations and their hydroxides can range up to 90% of DOM derived from organic horizons of acidic forest soils (Nierop et al., 2002). Plankey and Patterson (1987) observed that the formation of precipitates is a relatively rapid and temperature-independent process.

The extent of DOM precipitation by Fe is pH independent in the range of pH 3.5 to 4.5, whereas the extent of OM precipitated by Al depends strongly on pH (Nierop et al., 2002). The concentrations of dissolved organic carbon (DOC) in soil solutions are often largest at low pH (Kalbitz et al., 2000; Weng et al., 2002), which is in contrast to the larger solubility of DOM at higher pH. An explanation for this could be increased precipitation of DOM by Al with increasing pH. Further, considering that Al concentrations are generally significant in acidic forest soil solutions, the relevance of DOM precipitation by Al should be more important than that by Fe.

Increasing pH values in the range of 3.5 to 4.5 lead to clearly increasing precipitation of DOM by Al (Nierop et al., 2002). The formation of $Al(OH)_3$ controls the solubility of Al at a pH >4.2 (Gustafsson et al., 2001), whereas at lower pH, the proportion of Al^{3+} in solution is dominant. The solubility of the DOM itself is also affected by pH (Kalbitz et al., 2000). It increases with pH because of deprotonation of carboxylic groups. This and the changes in Al speciation with pH let us assume that the type and strength of bonds between Al and DOM depend on pH. This would directly affect the stability of the OM, as the binding mechanism can strongly influence its biodegradability (Mikutta et al., 2007). Dissolved organic matter tends to coprecipitate in the presence of Al hydroxides rather than forming complex bindings (Boudot, 1992). The term coprecipitation will be used for the immediate adsorption of DOM on freshly formed $Al(OH)_3(s)$ as well as for bonding between DOM and $Al(OH)_3$, leading to precipitation, because these processes can not be separated.

Furthermore, the extent of precipitate formation depends on the Al/C ratio in solution. At Al/C ratios <0.03 the major fraction of Al–OM complexes is soluble (Jansen et al., 2003a). Extensive precipitation of DOM occurs at Al/C ratios exceeding 0.03. With increasing Al/C ratios precipitation of OM increases (Nierop et al., 2002); however, only a certain fraction of DOM can be precipitated. Thus, after precipitation of this fraction, a further increase in Al/C ratios will not increase DOM precipitation. Soil solutions in acidic forest soils can have Al/C ratios of up to 0.5 (Schwesig et al., 2003; Lumsdon et al., 2005). Therefore, the formation of precipitates in acidic forest soils is very likely.

1.3. Effect of Al on microorganisms and enzymes

Large Al concentrations in solution can also lead to toxic effects which strongly depend on the species of microorganisms. Some species can be affected by Al concentrations as low as 50 µM (Pina and Cervantes, 1996) whereas others can survive concentrations of 100-200 mM Al (Kawai et al., 2000; Zhang et al., 2002). The toxicity of Al to microorganisms is mainly caused by monomeric Al-species (e.g. Al^{3+}), whereas organically complexed Al is generally assumed to be non-toxic (Illmer and Mutschlechner, 2004). At pH values below 5.0 the dominant aluminium species is Al^{3+} (Pina and Cervantes, 1996). Soil comprises an extremely diverse medium with as many as 13000 species of bacteria in a single gram of soil (Giller et al., 1998). Consequently, toxic effects on one species which performs a particular function in soil may be compensated by another species being more tolerant to this stress factor (Giller et al., 1998). Thus, the effects of Al on C mineralization can only reflect a summation of toxic effects on some microorganisms, being partially compensated by others.

Soil enzymes are the drivers for the turnover of carbon and all essential nutrient elements including nitrogen and phosphorus (Nannipieri et al., 2003). However, it is speculative whether also DOM comprises significant amounts of enzymes (McDowell, 2003). Further, we have no knowledge, whether enzymes are precipitated by metal cations, and if precipitation would affect their activity and consequently their influence on C mineralization. Tietjen and Wetzel (2003) reported that sorption of enzymes onto clay particles led in most cases to a reduction of enzyme activity, but they also found an enhancement of enzyme activity by sorption.

1.4. Composition and properties of precipitated organic matter

Precipitation of OM by Al could be of large importance for C cycling in soils, but little is known about the properties of precipitated OM. Kaiser et al. (1997) observed a preferential removal of aromatic and carboxylic C from the soil solution by sorption. Thus, it can be assumed that carboxylic groups play an essential role for the binding of OM to metal cations. But also phenolic groups are of importance for binding of organic matter to aluminium hydroxide surfaces (Guan et al., 2006a, b). Dissolved organic matter with a large number of functional groups and of high molecular weight, e.g., aromatic compounds, preferentially precipitates (Julien et al., 1994; Römkens and Dolfing, 1998; Blaser et al., 1999). These compounds already have a low solubility and offer a wide range of binding sites for Al. The negative charge of the functional groups is compensated by the positive charge of the Al species [e.g., Al^{3+}, $AlOH^{2+}$, $Al(OH)_2{}^{+}$], further reducing OM solubility (Weng et al., 2002), which leads to extensive coagulation; however, Ares and Ziechmann (1988) pointed out that the effect cannot be fully explained by charge neutralization. The precipitation is also enhanced by various structural changes induced by the binding of Al with functional groups (Ares and Ziechmann, 1988).

Besides the composition also the size of the precipitated OM flocs is of large importance e.g., for transport processes in soil profiles. Smaller flocs will be transported through smaller pores into greater depth with less microbial activity. We are not aware of any study which determined floc sizes of natural OM precipitated by Al in terrestrial systems. Flocs formed during sewage water treatment are well investigated, but often applied metal cation concentrations are hardly comparable to concentrations observed in forest soils. Jarvis et al. (2006) observed flocs of up to 1000 µm, but floc sizes were strongly influenced by inorganic precipitation of iron hydroxides. As the average organic molecule is much smaller, the metal cations must arrange a large number of organic molecules. To network the organic molecules, two or more functional groups are required, each forming monodentate bonds with an Al cation. If these groups are adjacent to each other then one metal cation possibly form bi- or tridentate bonds, which is disadvantageous for networking. Therefore, it is unlikely that low molecular weight organic acids are precipitated, despite a large content of functional groups. Whereas high molecular weight structures, with large contents of functional groups, e.g. deriving from lignin or tannin, seem to be likely candidates for precipitation. Aromatic structures in DOM solution are highly stable, whereas carbohydrates can be considered as easily biodegradable (Kalbitz et al., 2003a). Thus, precipitated OM should be more stable than the average DOM components. Addition of Al to DOM solutions resulted in a reduction of the total C mineralization (Schwesig et al., 2003). This stabilization might be a direct consequence of precipitate formation and a decreased bioavailability. Although there is broad discussion on the potential contribution of precipitated OM to podzolization (Gustafsson et al., 2001; Zysset and Berggren, 2001; Jansen et al., 2004), no information on the stability of such precipitates is available.

1.5. Objectives

The main aims of the study were (i) to elucidate the effects of precipitation of dissolved organic matter by aluminium on its stability against microbial degradation, and (ii) to identify factors determining the degree of stabilization. To achieve this we applied the following approach:

(i) **Factors influencing the amount of organic matter precipitated**

In the experiments Al was added to four solutions with different chemical composition of OM. The precipitation was initiated at controlled pH values of 3.8 and 4.5 and Al/C ratios ranging from 0.02 to 0.3 to achieve differences in the fraction of precipitated C and Al speciation. The chosen solutions and conditions were typical for soil solutions entering the mineral horizon, therefore being an appropriate experimental analogue for the processes occurring in situ.

(ii) **Characterisation of precipitated organic matter flocs**

The size and the surface structure of OM flocs are of large relevance for transportation and interaction with other components of the soil solution. Therefore, we investigated these parameters under a variety of conditions with laser scanning microscopy.

(iii) **Changes in composition from dissolved to precipitated organic matter**

The separation of DOM in a fraction which is precipitated and a fraction remaining in solution should result in a fractionation of OM with regard to its chemical properties. We evaluated characteristic changes from DOM to precipitated OM with a large variety of chemical and spectroscopic methods.

(iv) **Carbon mineralization of dissolved and precipitated organic matter**

The extent to which precipitated OM can be mineralized by microorganisms is unknown. However, this is of large importance for C storage in soil profiles, especially in the mineral horizon, where precipitation is supposed to occur. In two incubation experiments we quantified C mineralization of precipitated OM.

(v) **Factors influencing carbon mineralization of precipitated organic matter**
We investigated the most important factors affecting C mineralization of precipitated OM in the incubation experiments by relating the determined properties of precipitated OM to the observed degradability. Not only the climate is changing but also the anthropogenic input of compounds into soils, which will result in changing factors responsible for mineralization. Thus, knowledge about the effect of changing environmental conditions on C precipitation and mineralization is highly valuable.

2. Methods

2.1. Sites and samples

Samples of Oi and Oa horizons from a Norway spruce (Waldstein-Fichtelgebirge, Germany; Michalzik and Matzner, 1999) and a deciduous stand with European beech as the dominant tree species (Steinkreuz-Steigerwald, Germany; Solinger et al., 2001) were homogenized and stored frozen. Water extracts were prepared at 5°C with a soil to (ultrapure) water ratio of 1:10 (Chapter II). After one day, DOM solutions were filtered through a preconditioned ceramic filter plate and a 0.2-µm membrane filter at 5°C to exclude microorganisms. The DOM solutions were diluted with ultrapure water to 40 mg C l^{-1} to ensure comparability. The concentration was similar to dissolved organic C concentrations at the study sites (Solinger et al., 2001; Kalbitz et al., 2004).

2.2. Precipitation of dissolved organic matter

To precipitate DOM at conditions similar to those in acidic forest soils (Schwesig et al., 2003), Al was added as $AlCl_3 * 6H_2O$ solution (Al/C molar ratios: 0.02, 0.05, 0.1, 0.2 and 0.3) at pH values of 3.8 and 4.5. All samples were gently shaken for 24 h at 5°C to minimize mineralization. Each treatment was performed in triplicate. When needed, precipitates were separated by filtration over 0.4 µm from the solution.

2.3. Incubation

In a first experiment (Chapter II) the precipitated OM itself was incubated in an incubation solution, made up from ultrapure water, nutrients and inoculum. The precipitates were not removed from the filters because we considered them as an additional surface for microorganisms. All incubation solutions were adjusted to pH 4.5 by adding HCl. Samples were shaken manually each day and incubated at 20°C for 7 weeks.

For the second incubation experiment (Chapter IV) the formed precipitated OM was left in solution and incubated at the respective pH. Nutrients and an inoculum were added. The solutions were incubated at 22°C (± 2°C) for 34 days (Oi-beech) and 47 days (Oa-spruce) because the C mineralization of the Oa-spruce solution was smaller as already shown by Schwesig et al. (2003) and in chapter II.

2.4. CO_2 measurement

During incubation the CO_2 concentration in the headspace of each flask was measured by gas chromatography coupled to a thermal conductivity detector (HP 6890). We calculated the amount of CO_2 in the flasks by using the general gas equation, and added the amount of CO_2 dissolved in the liquid phase, estimated from the measured pH and the solubility constant. The amount of C mineralized from the precipitated OM was considered as the difference between the measured CO_2 of the sample and the blank without precipitates.

2.5. Elemental analysis

The DOM solutions and filtrates were analyzed for total organic C and total N (High TOC, Elementar), NO_3, PO_4, SO_4 (ion chromatography, Dionex DX 100), NH_4 (flow injection, photometric mLE– FIA LAB), and total Al, Ca, Cu, Fe, K, Mn, Na, S, Si (ICP–OES, GCP Electronics).

2.6. UV/Vis and fluorescence spectroscopy

Ultraviolet (UV) absorbance at 280 nm was recorded (UVIKON 930, Bio-Tek Instruments) from DOM solutions and the filtrates after removal of precipitated OM as an estimate of the aromaticity of DOM (Traina et al., 1990). Further, synchronous scan fluorescence spectra were recorded (SFM 25, BIO-TEK Instruments) followed by calculation of a humification index as a measure of the complexity of the organic molecules (Kalbitz et al. 1999). We calculated this index (HIX_{syn}) by dividing the intensity at bands or shoulders of a longer by a shorter wavelength (464 nm/352 nm).

2.7. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy

The ^{1}H and ^{13}C NMR spectra of freeze-dried samples of DOM, selected precipitates and filtrates (150 mg dissolved in 3 mL 0.5 M NaOD) were recorded on a DRX 500 NMR spectrometer (11.7 T, Bruker Analytische Messtechnik GmbH; chapter II+V).

2.8. Fourier transformed infrared (FTIR) spectroscopy

The FTIR spectra of freeze-dried dissolved and precipitated OM were recorded with a BioRad® FTS 135 in the range of wavenumbers between 3900 and 400 cm^{-1}. The sample material (0.5 mg) was mixed with 80 mg KBr and finely ground using an agate mortar. The resulting mixture was dried for 12 h over silica gel in a desiccator to standardize the water content. All spectra were recorded under the same conditions with a resolution of 1 cm^{-1} and 16 scans (Ellerbrock et al., 1999; chapter V).

2.9. Enzyme activity measurement

Enzyme activities of chitinase, 1,4-β-cellobiohydrolase, β-glucosidase, ß-D-glucuronidase, acid phosphatase, Xylan 1,4-β-xylosidase, leucine-aminopeptidase and laccase were measured using substrate concentrations as in the protocol of Courty et al. (2005) with prolonged incubation times. The measurements were done for the four DOM solutions, to determine the original enzyme activity. Further, we measured the enzyme activity of precipitated OM before and after incubation for 1, 4 and 8 weeks. For details, see chapter III.

2.10. Diffusive Gradients in Thin films (DGT)

To differentiate between the various soluble Al complexes and ‘free’ dissolved Al, we performed Diffusive Gradients in Thin films (DGT) analyses (Jansen et al., 2003b). In addition to the open pore gel that has a pore size of >5 nm, a more restrictive gel was used with a pore size of <1 nm (Zhang and Davison, 2000). It has been shown that the open pore gel allows smaller organo-metal complexes to penetrate the membrane, whereas they can hardly diffuse into the restrictive gel (Zhang and Davison, 2000). For details, see chapter IV.

2.11. Laser Scanning Microscopy (LSM)

To determine the size of precipitated OM the flocs were not removed from the solutions by filtration. The suspensions were shaken manually to ensure homogeneity before measurement. Then, the suspension containing flocs of precipitated OM was transferred and fixed between two glass slides. At least three micrographs were taken from each solution with a Laser Scanning Microscope (LSM 510 META, Zeiss; homonymous software Version 3.2 SP2). Afterwards, the micrographs were successfully analyzed with the software DAIME (Daims et al., 2006). For a particularly large floc we recorded a xyz-stack (98 slices) with a HeNe laser (λ = 633 nm, 5 mW); the thickness of the optical slices was 0.5 µm. The slices were afterwards reconstructed to a 3D image using the 3D module of the LSM 510 META software (Chapter V).

2.12. Modelling, calculations and statistics

The elemental speciation of the soil solution was modelled with VisualMinteq v2.51. Statistical tests were performed with SPSS and linear regressions calculated with SigmaPlot.

3. Results and Discussion

3.1. Factors influencing the amount of organic matter precipitated

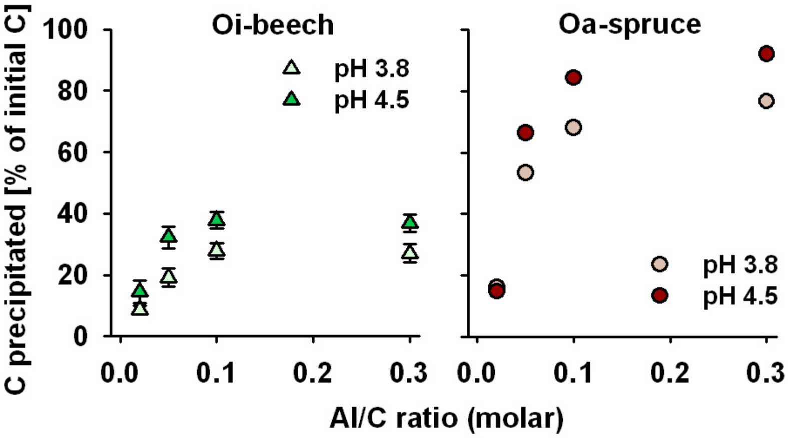

Fig. I.1: Fraction of C precipitated in dependence on the Al/C ratio and pH for two solutions.

Dissolved organic matter precipitated within minutes after adding Al to the solution, as already reported by Plankey and Patterson (1987). The amount of carbon precipitated by Al increased strongly up to an initial Al/C ratio in solution of 0.1 (Fig. I.1, Fig. IV.2) whereas a further increase of the Al/C ratio to 0.3 did not strongly change the amount of precipitated C. Similar results were reported by Nierop et al. (2002). We consistently observed a larger fraction of DOM precipitating at pH 4.5 than at pH 3.8 (Fig. I.1, Table II.4), which confirms results of Nierop et al. (2002). The amount of precipitated C increased over the time period of an incubation experiment (Fig. IV.2), despite of C degradation in the meantime. This increase in precipitation was slightly larger for precipitates formed at pH 3.8 (Fig. IV.2). The maximum proportions of DOM that precipitated from the four solutions increased in the order of Oi-beech < Oi-spruce < Oa-beech < Oa-spruce and ranged from 29 to 84%.

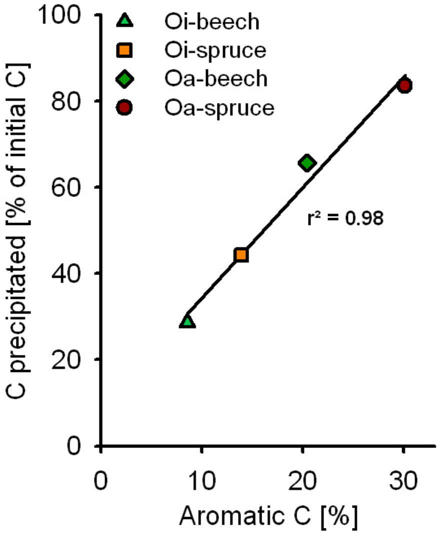

Fig. I.2: Relationship between the aromatic C content of the four solutions and the maximum percentage of dissolved organic C which could be precipitated.

The content of aromatic C in solution (Table II.5) determined the maximum portion of DOM that could be precipitated (Fig. I.2, Fig. II.2). A linear regression between these two factors resulted in a coefficient of determination of $r^2 = 0.98$. Generally, DOM solutions from Oa horizons contain larger proportions of aromatic compounds (Kalbitz et al., 2003b) and more carboxylic groups (Guggenberger et al., 1994) than the Oi solutions. The larger number of possible binding sites in DOM solutions from Oa horizons resulted in larger amounts of precipitates.

3.2. Characterisation of precipitated organic matter flocs

The flocs of precipitated OM had diameters ranging up to 110 µm (Fig. I.3, Fig. V.11, Table V.3). The shape of the flocs differed largely and had a cloud-like structure which was highly irregular (Fig. I.3, Fig. V.11). We consistently observed a larger number of flocs when OM was precipitated at pH 3.8 than at pH 4.5 (Table V.3). However, the average diameters of the flocs were larger at pH 4.5 (~27.6 µm) than at pH 3.8 (~16.6 µm). Thus, the floc size seems to reflect the larger amount of OM precipitated at larger pH. At pH 4.5 we did not find a significant number of flocs with diameters below 5µm, but a considerable fraction was still larger than 50 µm (Table V.3). In opposite, flocs formed at pH 3.8 had a much larger fraction of flocs

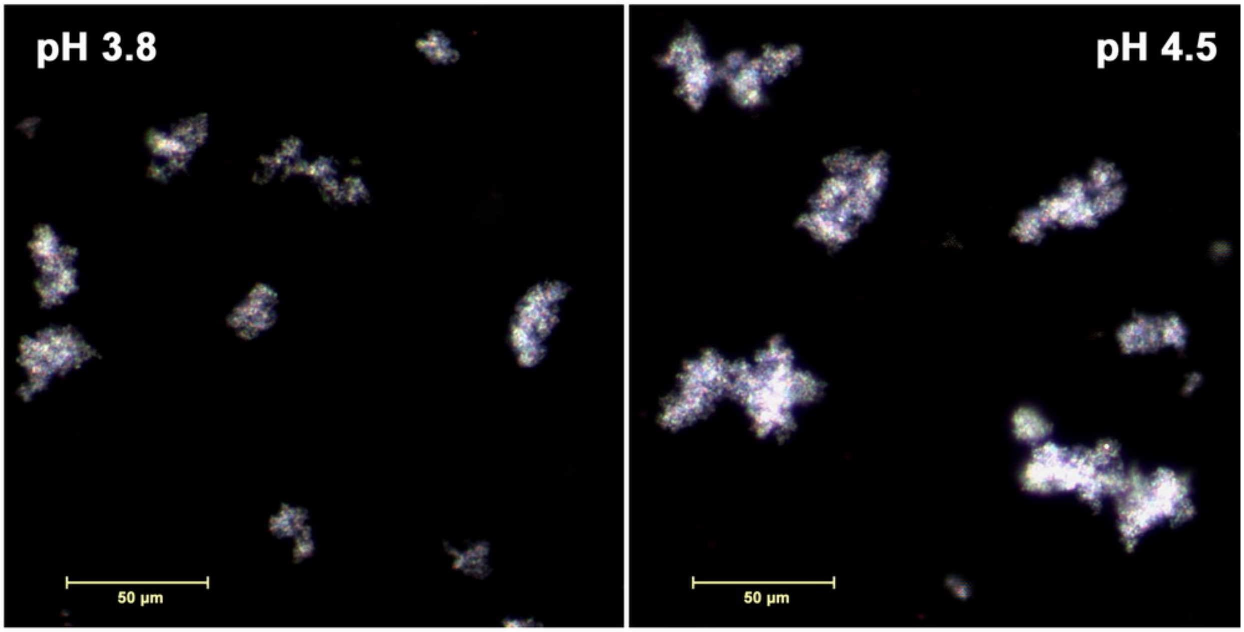

Fig. I.3: Micrographs of precipitated organic matter flocs (magnification: 400 fold) formed at pH 3.8 (left) and pH 4.5 (right) from the Oa-spruce solution at an Al/C ratio of 0.1. The average circular diameter of flocs in this solution was 16.6 µm at pH 3.8 and 27.6 µm at pH 4.5.

smaller than 5 µm, and less large flocs. Further, we observed a larger number of flocs in the Oa-spruce solutions when compared with the Oi-beech solutions, reflecting the larger amount of OM precipitated from solution (Chapter II+IV). Surprisingly, the average sizes of the flocs and the distribution over the size classes in the Oa-spruce and Oi-beech solution was almost similar for both pH values (Table V.3).

The observed pH dependence of the floc sizes is of large importance for transport processes in soil profiles. For podzols an increase in pH with depth can be observed which would also lead to an increase in floc size. This could decrease the mobility of the precipitated OM or even completely disable the movement into smaller pores. Furthermore, the smaller flocs formed at lower pH could enter smaller pores. Strong et al. (2004) reported that OM in pores from 15-60 µm is readily decomposed, whereas OM in smaller pores is protected against biodegradation.

By laser scanning microscopy a three-dimensional micrograph was recorded from a large floc. With this method we demonstrated the very porous structure of the flocs (Fig. V.12) giving them a large surface area which was at least 10 times larger than the comparable spherical surface.

For an average floc of 10 µm diameter we estimated that 80 million molecules needed to be linked by Al cations. Each Al cation would need to perform at least two bonds to achieve this kind of network between the organic molecules. We estimated the number of the remaining hydroxide or water molecules in the hydrate sphere of the precipitated Al to 1.9 (Oa-spruce), 4.0 (Oa-beech), 3.5 (Oi-spruce) and 4.4 (Oi-beech). This implied that per Al cation on average 1.6 to 4.1 bonds were formed, with an overall average number of 2.5 (Chapter V). The bonds between Al and the functional groups were probably formed by ligand exchange reactions of carboxylic and phenolic groups with the hydrate sphere of the Al cation. The larger number and the type of bonds should result in larger stability of the OM (see section 3.5).

3.3. Changes in composition from dissolved to precipitated organic matter

The specific UV absorption at 280 nm decreased in the DOM solutions by 7 to 77% after precipitation (Fig. II.5). This indicates that aromatic compounds precipitated preferentially. The decrease in specific UV absorption by precipitation became more pronounced with increasing Al/C ratios. Precipitation at pH 4.5 reduced the specific UV absorption to a larger extent (on average, 24–54%) than at pH 3.8 (on average, 9–30%), reflecting also the larger portion of precipitates formed at pH 4.5. Also, humification indices deduced from synchronous fluorescence spectra (HIX_{syn}) of the filtered DOM solutions decreased with increasing Al/C ratios (Fig. IV.6). Again, this indicated the removal of more aromatic and complex structures possibly deriving from lignin in the solutions (Kalbitz et al., 2004). We related the percentage of C precipitated to the reduction of HIX_{syn} (Fig. IV.7, top). This demonstrated that the precipitation process was clearly associated with the preferential removal of the more complex and aromatic structures from solution. Furthermore, it can be seen that the reduction in HIX_{syn} per mg precipitated C was larger for small amounts of precipitation (Fig. IV.7, bottom). After removal of about 30% of C from solutions the reduction in HIX_{syn} per mg precipitated C became almost constant. That means, compounds having a particularly large humification index will be precipitated first. Such compounds should be of utmost stability against microbial decay (Kalbitz et al., 2003a).

These findings were supported by the results of the ^{13}C and ^{1}H NMR data. They indicated a selective precipitation of a certain group of compounds, having large aromatic contents and associated carboxylic groups (Table V.2). Kaiser et al (1997) made a similar observation for the removal of OM from solution by sorption. The results of the UV absorption measurements also indicated a removal of aromatic compounds and showed a good correlation with the results of the NMR analysis (Fig. V.7). The correlation between UV absorption and carboxylic C was less strong ($r^2 = 0.44$) than for aromatic C ($r^2 = 0.85$; Fig. V.7). This seems to be contradictory as the Al cations are supposed to bind to the carboxylic groups and not to aromatic C atoms. But, probably only carboxylic groups attached to aromatic rings were precipitated after complexation, whereas carboxylic groups in low

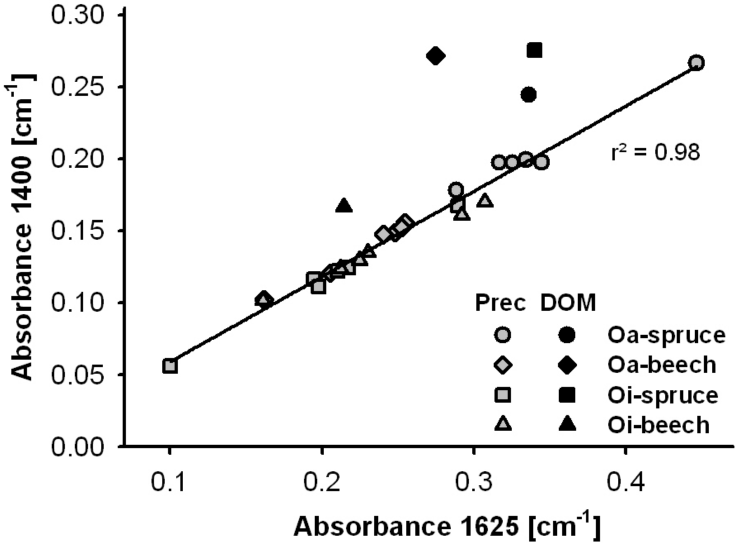

Fig. I.4: Fourier transformed infrared spectra were recorded of the four dissolved organic matter (DOM) solutions and the precipitated organic matter (Prec) formed at pH values of 3.8 and 4.5 (Al/C ratios: 0.05, 0.1, 0.3). The height of the absorbance peak at 1625 cm^{-1} was related to the absorbance peak at 1400 cm^{-1}.

molecular weight organic acids remained as colloidal organo-metal complexes in solution (Chapter IV). Therefore, the correlations were stronger to the aromatic C contents.

This selective precipitation was also supported by the large similarity of the precipitated OM in the FTIR spectra (Fig. I.4, Fig. V.9). The, significant differences in composition of DOM were equalized in the precipitates. The relative increase of the carboxylate band in precipitated OM when compared with DOM hinted towards ligand exchange as an important mechanism of precipitate formation. In turn, the relative decrease of the complex band indicated that chelation might was of minor importance. This further indicated that carboxylic groups must have been involved in the precipitation process. Furthermore, the relative decrease in the wavenumber 1080 cm^{-1} (Fig. V.8B) of the FTIR spectra could be seen as a first indication that polysaccharides were not preferentially precipitated, being in contrast to the observations made by Schwertmann et al. (2005).

The precipitated OM was in nearly all cases enriched in P when compared to the respective DOM (Fig. V.1, Table V.1). Consequently the P concentrations in the solution were considerably reduced (Fig. IV.5). Further, we observed depletion of organic N in the precipitated OM. This, indicated that a large proportion of DON did not precipitate with Al. Kaiser et al. (2000) and Kaiser and Zech (2000) observed a preferential adsorption of DOM with low contents of organic N to the mineral soil and attributed this to diminished adsorption of hydrophilic compounds, enriched in DON. Therefore, the depletion of organic N in the precipitates can be explained by preferential precipitation of aromatic and hydrophobic compounds, possibly derived from lignin or tannin with low N contents. Nitrogen rich compounds like peptides, proteins, or amino acids only tend to precipitate when bound to high-molecular-weight or aromatic structures (Yu et al., 2002).

For enzymes mainly involved in the degradation of carbon sources, the fraction of enzyme activity found in the precipitates was 17–41% on average of the original activity in DOM (Fig. III.1). In contrast, precipitated OM displayed on average only 4% and 7% of leucin-aminopeptidase and acid-phosphatase activity, enzymes mainly involved in the transformation of N and P (Fig. III.1). However, there was no significant correlation between the amount of C precipitated and the fraction of enzyme activity found in the precipitated OM for all investigated enzymes. Thus, also larger pH values and Al/C ratios, basically influencing the amount of C precipitating, had no significant influence on the fraction of enzyme activity found in the precipitates.

The proportion of enzyme activity found in the precipitates was in nearly all cases smaller than the fraction of precipitated carbon, which could be the result of a lower tendency to precipitate due to chemical properties. Additionally, aluminium could inhibit enzymes (Pina and Cervantes, 1996) resulting in decreasing enzyme activity. But, taking into account the addition of Al, the low pH values and the subsequent precipitation, enzyme activity found in precipitated OM was still substantial. While direct protein detection methods in soil water DOM failed to detect enzymes involved in degradation of biological materials (Schulze et al., 2005) the indirect enzyme detection via their activity showed that extracellular enzymes were present in a functional state in 0.2 µm filtered DOM and precipitated OM.

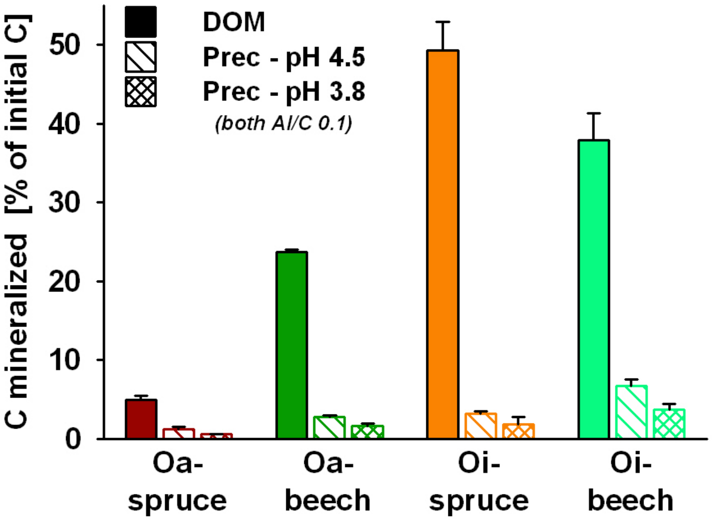

Fig. I.5: Carbon mineralization of dissolved and precipitated organic matter (Al/C 0.1) after 7 weeks of incubation of four solutions.

3.4. Carbon mineralization of dissolved and precipitated organic matter

The extent of C mineralization was significantly ($p < 0.01$) less for precipitates from all DOM solutions, at all Al/C ratios and pH values, than for the corresponding untreated DOM solutions (Fig. I.5). During 7 weeks of incubation, only 0.5 to 7.7% of the precipitated C was mineralized (Fig. II.8), whereas the corresponding DOM solutions showed a much larger mineralization of 5% (Oa-spruce), 24% (Oa-beech), 38% (Oi-beech), and 49% (Oi-spruce), respectively (Fig. I.5).

The type of DOM, the pH at precipitation, and the Al/C ratio had all a significant effect on the mineralizable C of the precipitates (Chapter II). The percentage of C mineralized was significantly smaller for precipitates from Oa solutions than for those from Oi solutions. We further found that the precipitates from beech DOM showed a significantly larger mineralization than those from spruce DOM. These findings reflected the mineralizability of the original DOM solutions.

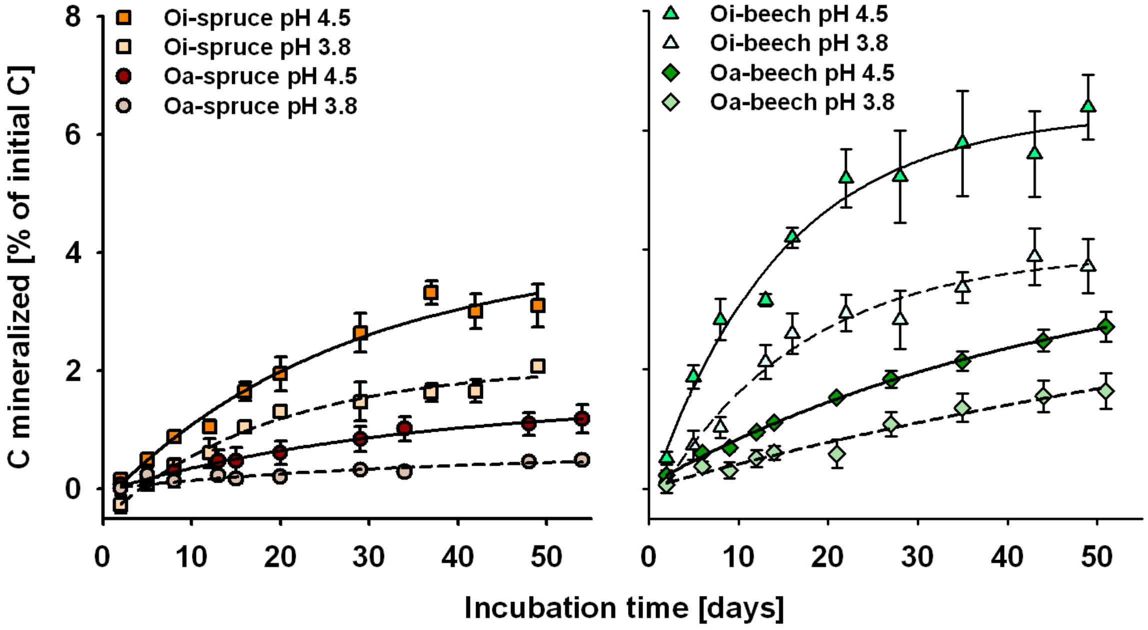

Fig. I.6: Dynamics of C mineralization of precipitated organic matter (Al/C 0.1) of four solutions and two pH values.

Mineralization of precipitates formed at pH 4.5 was in nearly all cases increased by approximately 50 to 75% when compared with the corresponding precipitates formed at pH 3.8 (Fig. I.6, Fig. II.8). Surprisingly, we found a significant increase in mineralization with increasing Al/C ratios up to an Al/C ratio of 0.2. The influence of the Al/C ratio on mineralization of precipitated OM was more pronounced at pH 4.5 than at pH 3.8.

In a second incubation experiment precipitated OM was not removed from the solutions, so that the overall stabilization effect could be determined (Chapter IV). There, carbon degradation comprised the dissolved and precipitated OM together. The C degradation without Al addition in the Oi-beech solution (66%) was much larger than in the Oa-spruce solution (16%), despite a 13 days shorter incubation for the Oi-beech solution (Fig. IV.1). A large fraction of carbon was already degraded during the first three days of incubation (Fig. IV.1). These observations are in accordance with results presented in chapter II. The addition of Al to the solutions resulted in significantly decreasing C degradation of dissolved and precipitated OM by up to 65%. Larger

Al/C ratios led to a larger reduction in C degradation when compared to the respective solutions without Al addition. However, again Al/C ratios exceeding 0.1 resulted only in a small additional decrease in C degradation at the end of the experiment (Fig. IV.1). The used concentrations were in the range of acidic forest soils. Therefore, it can be assumed that the decrease in C degradation caused by Al might be representative for field conditions as well.

3.5. Factors influencing carbon mineralization of precipitated organic matter

Amount of C precipitated

When precipitated OM was not removed from solution, the amount of stabilized C correlated significantly with the amount of precipitated C (Fig. I.7, Fig. IV.8). Larger Al additions resulting in increased precipitation led to an increased stabilization for both solutions and pH values (Fig. I.7, Fig. IV.8). The stabilization by Al was consistently larger at pH 4.5 than at pH 3.8, as a result of the larger amount of precipitates formed. The precipitated OM itself was less stable at pH 4.5 (Chapter II). But, the larger amount of OM precipitated had more impact. As a consequence, one of the main factors for the observed total stabilization were the amounts of C and Al precipitated (Fig. I.7, Fig. IV.4, Fig. IV.8). Therefore, pH governs C degradation indirectly by determining the amount of C and Al precipitating. For equal amounts of C precipitated, the stabilizing effect was larger in the Oi-beech solution, being a consequence of the better degradability of the solution itself. The extent of stabilization seemed to depend much larger on the amounts of precipitated OM than on preferential precipitation of certain compounds.

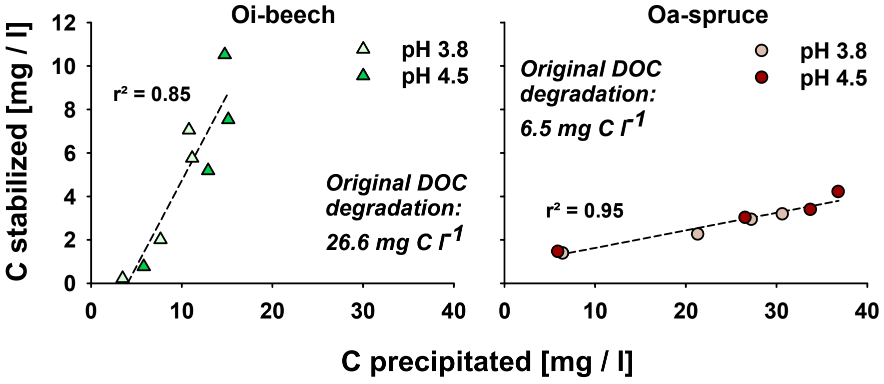

Fig. I.7: Amounts of stabilized C (reduction in C degradation) in dependence on the amounts of C precipitated by Al for both solutions and pH values. Please note, the initial concentrations of dissolved organic C were 40 mg C/l and zero stabilization is equal to stabilization without addition of Al.

Al toxicity

The smaller C mineralization of OM precipitated at pH 3.8 than pH 4.5 can not be caused by toxic effects, as all samples were incubated at pH 4.5 and Al concentrations were far below toxic levels (Chapter II). In the second incubation experiment (Chapter IV) the precipitated OM was left in the solutions and was incubated at the respective pH of precipitation. Thus, the decrease in C degradation of the OM solutions after Al addition could also be caused by toxic effects of 'free' Al on microorganisms. We consistently observed a larger fraction of 'free' Al at pH 3.8 than at pH 4.5 comparing the individual Al/C ratios (Fig. I.8, Fig. IV.4). Assuming a toxic effect of Al one would expect smaller C degradation at lower pH with larger amounts of 'free' Al. In contrast, we always found increased C degradation at lower pH and larger amounts of 'free' Al for the respective Al/C ratios (Fig. IV.4). Furthermore, we observed the largest

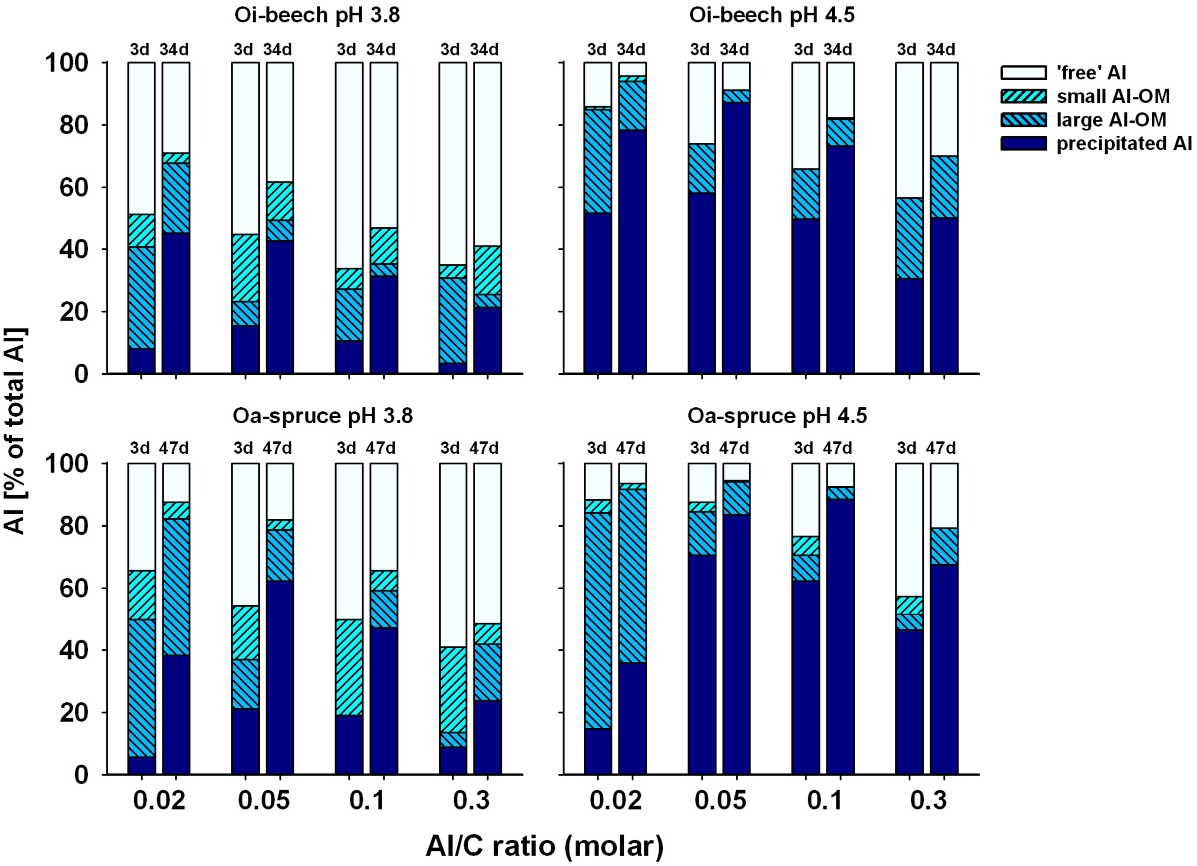

Fig. I.8: Portions of aluminium not bound to organic matter ('free' Al), Al bound in small and large soluble organo Al complexes (small and large Al-OM) and Al bound to precipitated organic matter (> 0.4µm). Distribution of the Al fractions are shown at the start (after 3 days) and at the end of the experiment (Oi-beech: 34 days; Oa-spruce: 47 days). Al was added to two different organic matter solutions at 2 pH values and 4 Al/C ratios.

reduction in C degradation for the three smallest Al/C ratios (0.02–0.1) with maximal ‘free’ Al concentrations of 4 mg Al l^{-1} (0.15 mM), being in the lower part of the range given for Al toxicity by Pina and Cervantes (1996). At the same time, the largest Al/C ratio with maximal ‘free’ Al concentrations of 12 mg Al l^{-1} (0.45 mM) resulted only in a comparably small additional reduction of C degradation. This could be taken as further indication that the decrease in C degradation was not caused by toxic effects, but by the extent of C precipitation (Fig. IV.4).

Composition of precipitated organic matter

Furthermore, preferential precipitation of inherently stable compounds can contribute to the observed stabilization. Aromatic and complex compounds were preferentially removed from the soil solution by precipitation and comprise stable fractions of DOM (Kalbitz et al., 2003a). The different DOM used varied in chemical composition, especially in aromatic C content, and showed large differences in C mineralization. Therefore, it was not surprising that the precipitates from the four solutions differed in stability too. The most likely reason for the greater stability of precipitates from Oa than from Oi solutions was the larger aromatic C content of Oa DOM solutions (Table II.5). Spruce DOM solutions contained more aromatic C than the respective beech DOM solutions; the precipitates reflected this.

We further observed that besides the aromatic C content also the C/N ratio of the precipitates significantly influenced the stability of precipitated OM (Chapter II). However, these two factors did not fully explain the differences caused by the variation in pH and Al/C ratio. A likely explanation for the observed differences would be changes in binding mechanisms. But, the FTIR spectra gave no indication for different binding mechanisms between OM and Al at the different pH values or Al/C ratios (Chapter V). Bonds were probably formed by ligand exchange reactions of functional groups of OM with the hydrate sphere of the Al cation.

We observed clear differences in the Al/C ratio of precipitated OM with larger Al/C ratios at pH 4.5 than at pH 3.8. The Al/C ratios of OM precipitated at pH 3.8 showed only a slight increase with increasing Al/C ratios (0.05-0.3) in solution (Chapter V; Table V.1). In contrast, the Al/C ratio of OM precipitated at pH 4.5 did strongly increase with larger Al/C ratios in solution (Chapter V; Table V.1). This general trend was also observed for C mineralization of precipitated OM (Chapter II). Also there, the Al/C ratio influenced C mineralization stronger at pH 4.5 than at 3.8. Further, larger Al contents of the precipitated OM led mostly to larger C mineralization. This was unexpected as larger Al contents would imply a larger degree of OM complexation by Al, which should be unfavourable for C mineralization.

Precipitation of amorphous Al-hydroxide primary particles

The Al/C ratios in precipitated OM correlated with the fraction of Al being present as $Al(OH)^{2+}$ in solution (Chapter V). This may be an explanation for the strong increase in the Al/C ratios of OM precipitated at pH 4.5, due to increasing precipitation of amorphous Al-hydroxide primary particles, as indicated also by saturation indices for Al-hydroxide (Chapter II). Additionally, we observed in the FTIR spectra of precipitated OM also indication for precipitation of Al-hydroxide (Chapter V). Dissolved organic matter can be sorbed on the surface of these particles and be co-precipitated.

The sorption of only a small fraction of DOM (5-10%) onto amorphous Al-hydroxide surfaces resulted in surprisingly large degradability of these OM (Fig. I.9; Schneider, 2006). The sorbed OM derived from Oa solutions was even better degradable than the bulk DOM itself (Fig. I.9; Schneider, 2006). We observed that the compounds which sorbed first were rich in N and showed little UV absorption. As these compounds needed to be present in the soil solution in the range of several percent, we considered proteins, peptides and amino acids as the most likely candidates. If these compounds would be weakly bound to the mineral surface by phosphate this would explain their large degradability (Schneider, 2006).

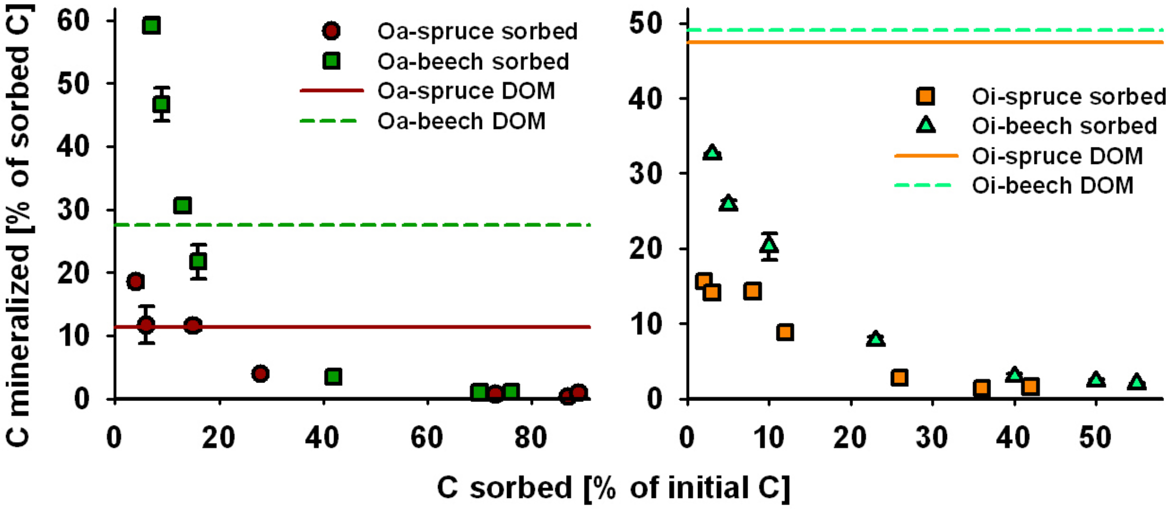

Fig. I.9: Amount of sorbed organic matter from four DOM solutions and the influence on the percentage of C mineralized. The C mineralization of the bulk DOM is given for comparison. (data according to Schneider, 2006).

Phosphate possesses a stronger affinity for the sorption on Al-hydroxide than humic substances (Boorgard et al., 2005). Thus, we can suppose that on the Al-hydroxide primary particles phosphate was sorbed, which is supported by the observed decrease in P concentrations in the solutions after precipitation (Chapter IV+V). How the removal of phosphate from solution affects the microorganisms and thus C mineralization is unclear. The transition to the solid phase could have a negative effect on the microbial community. But, the spatial vicinity of OM and phosphate enriched surfaces may even be beneficial for microorganisms. He and Zhu (1998) demonstrated that microorganisms can readily use also phosphate sorbed to mineral surfaces.

If precipitated OM contained Al-hydroxide primary particles with OM sorbed on their surface this would increase the mineralizability of the bulk precipitated OM. This could be an explanation for the better degradability of OM precipitated at pH 4.5 than OM precipitated at pH 3.8. Further, this would also explain why C mineralization increased only slightly with the Al/C ratio at pH 3.8 as at this pH the formation of Al-hydroxide is unlikely. At larger pH the precipitation of Al-hydroxide became more important, which was reflected in the more pronounced dependency of C mineralization on the Al/C ratio.

Soluble Al-OM complexes

In the incubation experiment described in chapter II the precipitated OM was separated from the solution. Thus, soluble Al-OM complexes were not incubated. The fraction of Al bound in soluble complexes was clearly larger at low pH and small Al/C ratios (Fig. I.8). As we observed a larger fraction of soluble Al-OM complexes at lower pH we assume that this fraction represents true complexes between Al and OM and not Al-hydroxide primary particles. The decrease of the soluble Al-OM fraction after incubation also supports this (Chapter IV). This decrease indicates that the OM of this fraction was either mineralized or complexes grew in size during incubation and were precipitated. At the end of the incubation experiment there was still a considerable fraction of Al bound in soluble Al-OM complexes. Thus, the OM of that fraction must be either hard to degrade or the OM to which the Al was bound was degraded and the Al complexed other organic molecules.

For the comparison of the two incubation experiments this is important, as in the first experiment only the precipitated OM itself was incubated, whereas in the second experiment the total stabilization was measured. There, also the dissolved complexes possibly contributed to the reduced C degradation, as particularly at small Al/C ratios the majority of Al was bound in soluble Al-OM complexes.

The stability of the precipitated OM itself depended on pH which probably was related to the formation of Al-hydroxide primary particles at pH 4.5 with OM sorbed on their surface (see above). At pH 3.8 the formation of these particles was unlikely. There the OM which sorbed on the particles at pH 4.5 was probably complexed by Al and remained soluble. Thus, after filtration the complexes remained in solution and the primary particles together with the sorbed OM were filtered out as precipitated OM, explaining the different findings in chapter II and chapter IV. The stability of precipitated OM did not depend on pH when the flocs where left in solution (Chapter IV). In this case it made no difference if these compounds were sorbed on primary particles or complexed by Al cations as their degradability was probably comparable.

Enzyme activity

The activity of all eight enzymes measured in precipitated OM before incubation significantly correlated with the fraction of C mineralized (Fig. III.2), indicating an influence of precipitated enzymes on C mineralization. The coefficients of determination for acid-phosphatase and leucine-aminopeptidase were smaller than for the other enzymes, probably because they do not directly affect C degradation. A further explanation might be the smaller proportion of enzyme activity of these two enzymes found in precipitated OM (Fig. III.1).

The C mineralization rates after one and 4 weeks were still strongly correlated logarithmically with the activity of enzymes involved

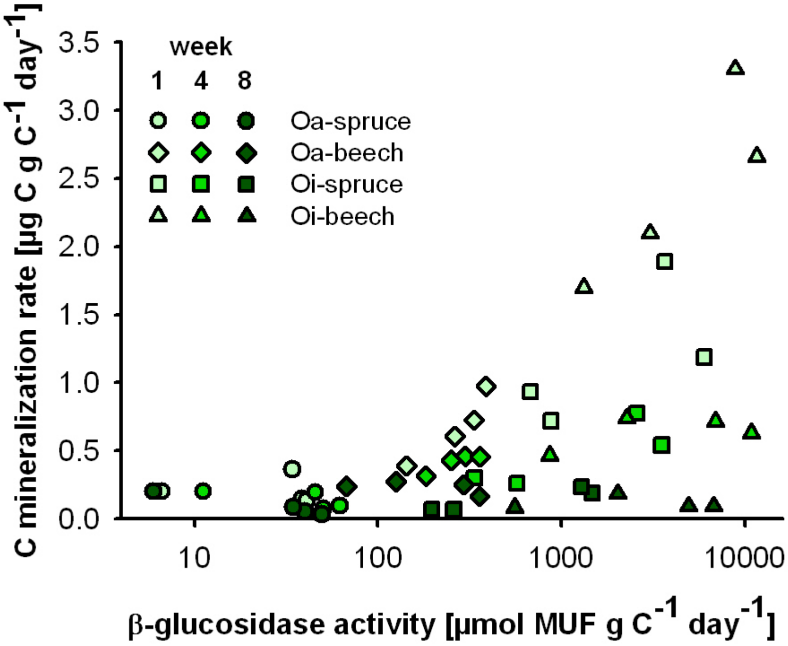

Fig. I.10: Relation between β-glucosidase activity and the C mineralization rate of precipitated organic matter from four solution during incubation.

in C degradation (Fig. III.3). The correlations were again weaker for acid-phosphatase and leucine-aminopeptidase (Fig. III.4). The logarithmic relationship between the enzyme activity and the C mineralization rate implies that the C mineralization did not increase directly proportional to the increase in enzyme activity. This indicates that with increasing enzyme activity the availability of substrate became limited. After 8 weeks of incubation no correlations between C mineralization rate and enzyme activity were visible (Fig. I.10, Fig. III.3, Fig. III.4). As C mineralization only amounted up to 0.5-7.7% of precipitated OM and EA was still substantial, we suppose that the more labile fraction was degraded first and that enzymes were probably hindered to degrade the stable fraction in an analogous manner. This indicates that chemical properties, like C/N ratio and aromatic C content (Chapter II), could be the main factors for the large stability of precipitated OM.

Spatial inaccessibility

Precipitated OM contained substantial amounts of aromatic C with carboxylic and phenolic groups being bridged by Al cations. But, the large number of linked organic molecules should not only lead to a stabilization of OM because of organo-metal complexes or preferential precipitation of aromatic compounds but also lead to spatial inaccessibility of the linked molecules in the flocs. Enzymes like laccase, β-glucosidase or phosphatase are important for the degradation of OM. The molecular weights of these enzymes range from 31-360 kDa (Yan et al., 1998; Xie et al., 2004; Jaouani et al., 2005; Turowski et al., 1997). So that an average diameter for enzymes of about 5-10 nm can be assumed. Thus, the inner part of precipitated OM is probably not easily accessible for enzymes, which explains the finding, that only a certain fraction of precipitated OM is prone to degradation by enzymes (Chapter III). Henriques et al. (2005) showed that the availability of substances in larger flocs clearly decreased. Therefore, precipitated OM should be also additionally stabilized by spatial inaccessibility of OM for microorganisms and their enzymes.

4. Conclusions

In this study it was clearly demonstrated that a large fraction of DOM can be precipitated by Al and is thereby substantially stabilized against microbial decay. The C stabilization upon Al addition did not result from toxic effects, but was caused by reduced bioavailability of OM after its precipitation. Carbon mineralization of DOM was up to 28 times larger than that of the respective precipitated OM. This was related to the preferential precipitation of aromatic compounds with low N contents and a large number of functional groups being complexed by Al.

When precipitated OM remained in solution C degradation was reduced by up to 65%. This overall stabilization was strongly dependent on the amount of OM precipitated. It can not be explained by the precipitation of recalcitrant OM, but was clearly related to interactions between OM with metal cations and mineral surfaces which were possibly associated with spatial inaccessibility of OM. Thus, also the large amount of non-precipitated, colloidal Al-OM complexes which remained in solution could contribute to OM stabilization. An important question which needs to be resolved is, to which compounds do metal cations bind first. We observed some evidence that Al binds preferentially to low molecular weight compounds (Chapter IV) which was reflected in large amounts of soluble Al-OM complexes in comparison to precipitated OM at small Al/C ratios. There is no information available on the stabilization of low molecular weight DOM components against microbial decay after complexation by Al. Also the involved low molecular weight compounds and their properties after complexation are unclear. Furthermore, the mobility of these colloids in the soil profile should be considerably larger than for the precipitated OM flocs with sizes of up to 110 µm. Thus, they are subjected more rapidly to changing conditions in the soil profile which makes them a very dynamic fraction.

Precipitation and complexation of OM could also be of large influence on surface waters to which OM might be transported. Increasing pH values accompanied by decreasing Al concentrations can lead to a reduced formation of precipitated OM or Al-OM complexes, which would not only result in less C stabilization in the soil, but also in larger amounts of DOM being exported to surface waters. Thus, the additional DOM in many surface waters should be mainly comprised of microbiologically stable aromatic compounds, as these were preferentially precipitated.

Furthermore, precipitation can also be initiated in acidic soils by Fe and at neutral pH by Ca cations. However, there is only very sparse information available about the properties and the stability of such precipitates. We would assume that the mechanisms involved in precipitation and the properties of precipitated OM might be similar to the ones observed for Al, but this needs to be shown.

For the application of the results to the field, there is great need for the differentiation between sorption and precipitation of OM. Both processes substantially stabilize OM, but to a different degree, with a larger reduction in C mineralization for precipitation by Al than for sorption to phyllosilicates and goethite (Kalbitz and Kaiser, 2008). Thus, differences in stabilization mechanisms lead to different long term stability of OM. Unfortunately; there is no appropriate method available to separate these mechanisms, which would allow the linkage of potential in situ stabilization to the mechanisms.

In soil profiles the Al-OM complexes and precipitates are also subjected to droughts, which would destroy the floc structure of OM as it dries up in soil pores. This may be beneficial for aggregate formation, but also lead to strongly hydrophobic surfaces due to the large aromatic C content in precipitated OM. We assume that droughts not only change the structure of complexed and precipitated OM, but also influence its stability against degradation. Therefore, changing environmental conditions as climate change or the anthropogenic input of substances will strongly influence precipitation of OM and consequently C stabilization.

The in situ investigation of precipitated OM would be highly desirable, but research was never focused on observing precipitated OM in soil profiles. It is probably hard to detect, as precipitated OM can be sorbed at surfaces or be incorporated into microaggregates or even form such. Consequently, precipitated OM is masked and not recognized as such. Also the separation from particulate OM derived from plant residues will cause experimental difficulties. However, it would be desirable to establish methods to detect precipitated OM in situ as this will considerably improve our understanding of C cycling and stabilization in soils.

5. References

Ares, J., Ziechmann, W. (1988). Interactions of organic matter and aluminum ions in acid forest soil solutions: Metal complexation, flocculation, and precipitation. Soil Science 145, 437–447.

Baldock, J.A., Skjemstad, J.O. (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31, 697–710.

Blaser, P., Heim, A., Luster., J. (1999). Total luminescence spectroscopy of NOM-typing samples and their aluminium complexes. Environment International 25, 285–293.

Borggaard, O.K., Raben-Lange, B., Gimsing, A.L., Strobel, B.W. (2005). Influence of humic substances on phosphate adsorption by aluminium and iron oxides. Geoderma 127, 270-279.

Boudot, J.-P. (1992). Relative efficiency of complexed aluminum, noncrystalline Al hydroxide, allophane and imogolite in retarding the biodegradation of citric acid. Geoderma 52, 29–39.

Burford, J.R., Bremner, J.M. (1975). Relation between denitrification capacities of soils and total water soluble and readily decomposable soil organic matter. Soil Biology and Biochemistry 7, 389-394.

Courty, P. E., Pritsch, K., Schloter, M., Hartmann, A., Garbaye, J. (2005). Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytologist 167, 309-319.

Daims, H., Lücker, S., Wagner, M. (2006). daime, a novel image analysis program for microbial ecology and biofilm research. Environmental Microbiology 8, 200-213.

Ellerbrock, R.H., Höhn, A., Rogasik, J. (1999). Functional analysis of soil organic matter as affected by long-term manurial treatment. European Journal of Soil Science 50, 65-71.

Giller, K.E., Witter, E., McGrath, S.P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil Biology and Biochemistry 30, 1389-1414.

Goodale, C.L., Apps, M.J., Birdsey, R.A., Field, C.B., Heath, L.S., Houghton, R.A., Jenkins, J.C., Kohlmaier, G.H., Kurz, W., Liu, S.R., Nabuurs, G.J., Nilsson, S., Shvidenko, A.Z. (2002). Forest carbon sinks in the Northern Hemisphere. Ecological Applications 12, 891-899.

Guan, X.H., Chen, C.-H., Shang, C. (2006a). Combining kinetic investigation with surface spectroscopic examination to study the role of aromatic carboxyl groups in NOM adsorption by aluminum hydroxide. Journal of Colloid and Interface Science 301, 419-427.

Guan, X.H., Shang, C., Chen, C.-H. (2006b). ATR-FTIR investigation of the role of phenolic groups in the interaction of some NOM model compounds with aluminium hydroxide. Chemosphere 65, 2074-2081.

Guggenberger, G., Zech, W. (1993). Dissolved organic carbon control in acid forest soils of the Fichtelgebirge (FRG) as revealed by distribution patterns and structural composition analysis. Geoderma 59, 109-129.

Guggenberger, G., Zech, W., Schulten, H.-R. (1994). Formation and mobilization pathways of dissolved organic-matter: Evidence from chemical structural studies of organic-matter fractions in acid forest floor solutions. Organic Geochemistry 21, 51–66.

Gustafsson, J.P., Berggren, D., Simonsson, M., Zysset, M., Mulder, J. (2001). Aluminium solubility mechanisms in moderately acid Bs horizons of podzolized soils. European Journal of Soil Science 52, 655–665.

He, Z., Zhu, J. (1998). Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. Soil Biology and Biochemistry 30, 917-923.

Henriques, I.D.S., Holbrook, R.D., Kelly II, R.T., Love, N.G. (2005). The impact of floc size on respiration inhibition by soluble toxicants – a comparative investigation. Water Research 39, 2559-2568.

Illmer, P., Mutschlechner, W. (2004). Effect of temperature and pH on the toxicity of aluminium towards two new, soil born species of Arthrobacter sp. Journal of Basic Microbiology 44, 98-105.

Jansen, B., Nierop, K.G.J., Verstraten, J.M. (2003a). Mobility of Fe(II), Fe(III) and Al in acidic forest soils mediated by dissolved organic matter: Influence of solution pH and metal/organic carbon ratios. Geoderma 113, 323–340.

Jansen, B., Mulder, J., Verstraten, J.M. (2003b). Organic complexation of Al and Fe in acidic soil solutions. Comparison of diffusive gradients in thin films analyses with Models V and VI predictions. Analytica Chimica Acta 498, 105-117.

Jansen, B., Nierop, K.G.J., Verstraten, J.M. (2004). Mobilization of dissolved organic matter, aluminium and iron in podzol eluvial horizons as affected by formation of metal–organic complexes and interactions with solid soil material. European Journal of Soil Science 55, 287–297.

Jaouani, A., Guillén, F., Penninckx, M.J., Martínez, A.T., Martínez, M.J. (2005). Role of Pycnoporus coccineus laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzyme and Microbial Technology 36, 478-486.

Jarvis, P., Jefferson, B., Parsons, S.A. (2006). Floc structural characteristics using conventional coagulation for a high doc, low alkalinity surface water source. Water Research 40, 2727-2737.

Julien, F., Gueroux, B., Mazet, M. (1994). Comparison of organic-compounds removal by coagulation–flocculation and by adsorption onto preformed hydroxide flocs. Water Research 28, 2567–2574.

Kaiser, K., Guggenberger, G. (2000). The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry 31, 711–725.

Kaiser, K., Zech, W. (2000). Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. European Journal of Soil Science 51, 403–411.

Kaiser, K., Guggenberger, G., Haumaier, L., Zech, W. (1997). Dissolved organic matter sorption on subsoils and minerals studied by 13C-NMR and DRIFT spectroscopy. European Journal of Soil Science 48, 301-310.

Kaiser, K., Guggenberger, G., Zech., W. (2000). Organically bound nutrients in dissolved organic matter fractions in seepage and pore water of weakly developed forest soils. Acta Hydrochim. Hydrobiol. 28, 411–419.

Kalbitz, K., Kaiser, K. (2008). Contribution of dissolved organic matter to carbon storage in forest mineral soils. Journal of Plant Nutrition and Soil Science, in press.

Kalbitz, K., Geyer, W., Geyer, S. (1999). Spectroscopic properties of dissolved humic substances – a reflection of land use history in a fen area. Biochemistry 47, 219-238.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E. (2000). Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 165, 277–304.

Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E. (2003a). Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113, 273–291.

Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumaier, L., Glaser, B., Ellerbrock, R., Leinweber, P. (2003b). Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biology and Biochemistry 35, 1129-1142.

Kalbitz, K., Glaser, B., Bol, R. (2004). Clear-cutting of a Norway spruce stand: Implications for controls on the dynamics of dissolved organic matter in the forest floor. European Journal of Soil Science 55, 401–413.

Kalbitz, K., Schwesig, D., Rethemeyer, J., Matzner., E. (2005). Stabilization of dissolved organic matter by sorption to the mineral soil. Soil Biology and Biochemistry 37, 1319–1331.

Kawai, F., Zhang, D., Sugimoto, M. (2000). Isolation and characterization of acid- and Al-tolerant microorganisms. FEMS Microbiology Letters 189, 143-147.

Lumsdon, D.G., Stutter, M.I., Cooper, R.J., Manson, J.R. (2005). Model assessment of biogeochemical controls on dissolved organic carbon partitioning in an acid organic soil. Environmental Science and Technology 39, 8057–8063.

Marschner, B., Kalbitz, K. (2003). Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113, 211-235.

McDowell, W. H. (2003). Dissolved organic matter in soils – future directions and unanswered questions. Geoderma 113, 179-186.

Michalzik, B., Matzner, E. (1999). Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. European Journal of Soil Science 50, 579–590.

Michalzik. B., Kalbitz, K., Park, J.-H., Solinger, S., Matzner, E. (2001). Fluxes and concentrations of dissolved organic carbon and nitrogen – a synthesis for temperate forests. Biogeochemistry 52, 173-205.

Mikutta, R., Mikutta, C., Kalbitz, K., Scheel, T., Kaiser, K., Jahn, R. (2007). Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica et Cosmochimica Acta 71, 2569-2590.

Nannipieri, P., Ascher, J., Ceccherini, L., Landi, L., Pietramellara, G., Renella, G. (2003). Microbial diversity and soil functions. European Journal of Soil Science 54, 655-670.

Nierop, K.G.J., Jansen, B., Verstraten, J.M. (2002). Dissolved organic matter, aluminium and iron interactions: Precipitation induced by metal/carbon ratio, pH and competition. Science of the Total Environment 300, 201–211.

Pina, R.G. and Cervantes, C. (1996). Microbial interactions with aluminium. BioMetals 9, 311-316.

Plankey, B.J., Patterson, H.H. (1987). Kinetics of aluminum–fulvic acid complexation in acidic waters. Environmental Science and Technology 21, 595–601.

Römkens, P.F.A.M., Dolfing, J. (1998). Effect of Ca on the solubility and molecular size distribution of DOC and Cu binding in soil solution samples. Environmental Science and Technology 32, 363–369.

Schneider, M. (2006). Stabilisierung gelöster organischer Substanz durch Sorption an amorphe Aluminiumhydroxide. Diplomarbeit.

Schulze, W. X., Gleixner, G., Kaiser, K., Guggenberger, G., Mann, M., Schulze, E. D. (2005). A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia 142, 335-343.

Schwertmann, U., Wagner, F., Knicker, H. (2005). Ferrihydrite-humic associations: Magnetic hyperfine interactions. Soil Science Society of America Journal 69, 1009-1015.

Schwesig, D., Kalbitz, K., Matzner, E. (2003). Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. European Journal of Soil Science 54, 311–322.

Solinger, S., Kalbitz, K., Matzner, E. (2001). Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55, 327–349.

Strong, D.T., de Wever, H., Merckx, R., Recous, S. (2004). Spatial location of carbon decomposition in the soil pore system. European Journal of Soil Science 55, 739–750.

Tietjen, T., Wetzel, R. G. (2003). Extracellular enzyme-clay mineral complexes: Enzyme adsorption, alteration of enzyme activity, and protection from photodegradation. Aquatic Ecology 37, 331-339.

Traina, S., Novak, J., Smeck, N.E. (1990). An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. Journal of Environmental Quality 19, 151–153.

Turowski, P., Favre, B., Campbell, K.S., Lamb, N.J.C., Hemmings, B.A. (1997). Modulation of the enzymatic properties of protein phosphatase 2A catalytic subunit by the recombinant 65-kDa regulatory subunit PR65α. European Journal of Biochemistry 248, 200-208.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. (2006). Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions. European Journal of Soil Science 57, 426-445.

Weng, L., Temminghoff, E.J.M., van Riemsdijk, W.H. (2002). Interpretation of humic acid coagulation and soluble soil organic matter using a calculated electrostatic potential. European Journal of Soil Science 53, 575–587.

Xie, Y., Gao, Y., Chen, Z. (2004). Purification and characterization of an extracellular β-glucosidase with high transglucosylation activity and stability from Aspergillus niger No. 5.1. Applied Biochemistry and Biotechnology 119, 229-240.

Yan, T.-R., Lin, Y.-H., Lin, C.-L. (1998). Purification and characterization of an extracellular β-glucosidase II with high hydrolysis and transglucosylation activites from Aspergillus niger. Journal of Agricultural and Food Chemistry 46, 431-437.

Yu, Z., Zhang, Q., Kraus, T.E.C., Dahlgren, R.A., Anastasio, C., Zasoski, R.J. (2002). Contribution of amino compounds to dissolved organic nitrogen in forest soils. Biogeochemistry 61, 173–198.

Zhang, D., Duine, J.A., Kawai, F. (2002). The extremely high Al resistance of Penicillium janthineleum F-13 is not caused by internal or external sequestration of Al. BioMetals 15, 167-174.

Zhang, H., Davison, W. (2000). Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using Diffusive Gradients in Thin Films. Analytical Chemistry 72, 4447-4457.

Zysset, M., Berggren, D. (2001). Retention and release of dissolved organic matter in Podzol B horizons. European Journal of Soil Science 52, 409–421.

Chapter II

Precipitation of dissolved organic matter by aluminum stabilizes carbon in acidic forest soils

Thorsten Scheel[a]

Catharina Dörfler[a], and Karsten Kalbitz[a]

[a]Department of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany.

Soil Science Society of America Journal 71 (2007), 64-74

0. Abstract

Dissolved organic matter (DOM) is often neglected as a factor in the formation of stable soil organic matter (OM). Precipitation of DOM by dissolved Al could contribute substantially to C retention in acidic forest soils; however, no information is available on the stability of precipitated OM against microbial decay. We investigated the stability of Al–OM precipitates against microbial decay as related to (i) DOM composition, (ii) Al speciation, and (iii) the dissolved Al/C ratio. We produced Al–OM precipitates by adding $AlCl_3$ (molar Al/C ratios: 0.05–0.3) at pH values of 3.8 and 4.5 to DOM solutions derived from Oi and Oa horizons, from either beech (*Fagus sylvatica* L.) or spruce [*Picea abies* (L.) Karst.] litter. Between 13 and 84% of the C was precipitated, depending on pH, Al/C ratio, and the type of DOM. Precipitates were found to be enriched in aromatic C and mostly depleted in N when compared with DOM. Only 0.5 to 7.7% of precipitated C was mineralized during 7 wk of incubation. Mineralization of Al–OM precipitates was up to 28 times less than that of the respective DOM solutions. The extent of mineralization of Al–OM precipitates formed at pH 3.8 was reduced by 50 to 75% when compared with those formed at pH 4.5. The stability of precipitates against microbial decay increased with larger aromatic C contents and larger C/N ratios. Our study clearly demonstrated that a large fraction of DOM can be precipitated and is thereby substantially stabilized against microbial decay.

1. Introduction

Sorption of DOM to mineral surfaces is an important and well-documented process for organic C stabilization in soils (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005). Another process potentially involved in the stabilization of soil organic C against microbial decay is precipitation of DOM by polyvalent cations, such as Fe and Al (Baldock and Skjemstad, 2000). Nevertheless, little is known about stabilization of organic matter by formation of insoluble Al–OM complexes (Boudot, 1992), which could be an important pathway for the formation of stable soil OM.

The amount of precipitated OM may comprise up to 90% (Nierop et al., 2002) of the initial dissolved organic carbon (DOC). Plankey and Patterson (1987) observed that the formation of precipitates is a relatively rapid and temperature-independent process. The extent of DOM precipitation by Al depends strongly on the prevailing pH in the soil solution and the interrelated speciation of Al (Nierop et al., 2002). Larger pH values lead to an increase in precipitation of DOM by Al (Nierop et al., 2002). The formation of $Al(OH)_3$ controls the solubility of Al at a pH >4.2 (Gustafsson et al., 2001), whereas at lower pH, the proportion of Al^{3+} in solution is dominant. Dissolved organic matter tends to coprecipitate in the presence of Al hydroxides rather than forming complex bindings (Boudot, 1992). The term *coprecipitation* will be used for the immediate adsorption of DOM on freshly formed $Al(OH)_3(s)$ as well as for bonding between DOM and $Al(OH)_3$, leading to precipitation, because these processes are not separable. Also tridecameric Al (Al_{13}), $AlO_4Al_{12}(OH)_{24}(OH_2)_{12}{}^{7+}$, could be of importance for DOM precipitation. The Al_{13} forms at pH values >4.2 (Furrer et al., 1992; Hu et al., 2006) with Al concentrations $>10^{-5}$ mol L^{-1}. Yamaguchi et al. (2004) observed the depletion of Al_{13} in solutions after adding humic acid and interpreted this as an aggregation–precipitation reaction of Al_{13}–humic acid complexes.

The solubility of the DOM itself is also affected by pH (Kalbitz et al., 2000). It increases with pH because of deprotonation of carboxylic groups. This and the changes in Al speciation with pH let us assume that the form and strength of bondings between Al and DOM depend on pH.

Furthermore, the extent of precipitate formation depends on the Al/C ratio in solution. At Al/C ratios <0.03 the major fraction of Al–OM complexes is soluble (Jansen et al., 2003). Extensive precipitation of DOM occurs at Al/C ratios exceeding 0.03. With increasing Al/C ratios also DOM precipitation increases (Nierop et al., 2002); however, only a certain fraction of DOM can be precipitated. Thus, after precipitation of this fraction, a further increase in Al/C ratios will not increase DOM precipitation. Soil solutions in acidic forest soils can have Al/C ratios of up to 0.5 (Schwesig et al., 2003; Lumsdon et al., 2005). Thus, the formation of precipitates in acidic forest soils is very likely.

The precipitation of DOM cannot be induced only by Al, but also by Fe(III) and, to a minor extent, by Fe(II) (Nierop et al., 2002). The extent of DOM precipitation by Fe is pH independent, whereas the extent of Al–OM precipitate formation depends strongly on pH (Nierop et al., 2002). The concentrations of DOC in soil solutions are often largest at low pH (Kalbitz et al., 2000; Weng et al., 2002), which is in contrast to the larger solubility of DOM at higher pH. An explanation for this could be the increase in DOM precipitation by Al with increasing pH. Further, considering that Al concentrations are generally significant in acidic forest soil solutions, the relevance of DOM precipitation by Al should be more important than that by Fe.

Dissolved organic matter compounds with a large number of functional groups (e.g., -OOH and -OH) and of high molecular weight, e.g., aromatic compounds, preferentially precipitate from DOM solutions (Julien et al., 1994; Römkens and Dolfing, 1998; Blaser et al., 1999). These compounds already have a low solubility and offer a wide range of binding sites for Al. The negative charge of the functional groups is compensated by the positive charge of the Al species [e.g., Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$], further reducing solubility. Aromatic structures in DOM solution are highly stable, whereas carbohydrates can be considered as easily biodegradable (Kalbitz et al., 2003a). Thus, we expected Al–OM precipitates to be more stable. So, precipitation of citric acid by Al led to greater stability against microbial decay (Boudot, 1992). This effect increased with increasing Al/C ratios. Although there is broad discussion on the potential contribution of Al–OM precipitates to podzolization (Gustafsson et al., 2001; Zysset and Berggren, 2001; Jansen et al., 2004), no information on the stability of such precipitates is available.

The objective of our study was to determine the stability of Al–OM precipitates as related to (i) the composition of the DOM solution, (ii) the Al speciation, affected by pH, and (iii) the influence of the Al/C ratio. Further goals were the identification of the intrinsic properties of the precipitates that govern their stability.

2. Materials and Methods

2.1. Samples

We used soil samples from a Norway spruce site (Waldstein-Fichtelgebirge, Germany; Michalzik and Matzner, 1999) and a deciduous stand with European beech as the dominant tree species (Steinkreuz-Steigerwald, Germany; Solinger et al., 2001). Samples were taken at both sites from the litter horizon (Oi-spruce, Oi-beech; thickness: 1 cm) and the humified horizon at the spruce site (Oa-spruce; sampling depth: 5–9 cm) and a mixture of the fermented and humified horizon at the beech site (Oa-beech; sampling depth: 1–3 cm). Samples were homogenized and stored frozen (Kalbitz et al., 2003).

2.2. Preparation of Dissolved Organic Matter Solutions

Water extracts of the soil samples were prepared at 5°C with a soil to (ultrapure) water ratio of 1:10 and stirred three times manually. After 1 d, solutions were filtered through a preconditioned ceramic filter plate and filtered through a 0.2-µm membrane filter at 5°C (OE 66, Schleicher and Schuell, Dassel, Germany) to exclude microorganisms. The obtained solutions varied in DOC concentrations (Table II.1). Therefore, all solutions were diluted with ultrapure water to 40 mg C L^{-1} to ensure comparability. We chose that specific concentration because preliminary tests showed that sufficient amounts of precipitates would be produced. The concentration was also similar to DOC concentrations at the study sites (Solinger et al., 2001; Kalbitz et al., 2004). The concentrations of Al and Fe in the DOM solutions were fairly small (Table II.2), so an initial presence of precipitates could be excluded.

Table II.1: Total organic carbon (TOC) content of the soils used and dissolved organic carbon (DOC) content and pH of the water extracts.

Site or sample	TOC†	DOC	pH
	g kg^{-1}	mg L^{-1}	
Beech forest (Dystric Cambisol)			
Litter horizon (Oi, beech forest)	456	924	5.25
Fermented or humified horizon (Oa, beech forest)	295	87.9	5.05
Spruce forest (Haplic Podzol)			
Litter horizon (Oi, spruce forest)	474	193	5.10
Humified horizon (Oa, spruce forest)	312	56.7	4.05

† Data from Kalbitz et al. (2003).

2.3. Production of Precipitates

We added $AlCl_3 \cdot 6H_2O$ (analytical grade, Merck, Darmstadt, Germany) from a stock solution to 200 mL of the diluted DOM solutions to achieve molar Al/C ratios of 0.05, 0.1, 0.2, and 0.3. As our intention was to conduct the experiments under conditions similar to those in the field, we limited the maximum Al/C ratio to 0.3. Immediately after the addition of $AlCl_3$, the pH was adjusted with NaOH or HCl to either 3.8 or 4.5. These two pH values were chosen to include different types of interaction of Al with OM. At pH 4.5, the importance of hydrous Al species for precipitation should be larger than at pH 3.8, where Al^{3+} is the dominant species (Gustafsson et al., 2001). Each treatment was performed in triplicate. All bottles were gently shaken for 24 h at 5°C to minimize mineralization before incubation. Then precipitates were separated by passing the solution through a prewashed quartz fiber filter (QF20, Schleicher and Schuell, Dassel, Germany). The properties of the filtrates were analyzed as described below.

Table II.2: Chemical compositions of the dissolved organic matter solutions used, after adjustment to 40 mg C L^{-1} (standard error of three replicates in parentheses).

Site and sample	Al	N	NH_4–N	NO_3–N	S	SO_4	PO_4	Ca	Fe	Si	K	Na	Mn
	mg L^{-1}												
Beech													
Oi horizon	<0.15	3.29 (0.06)	0.25 (0.01)	<0.08	0.53 (0.01)	0.58 (0.01)	2.42 (0.07)	1.41 (0.01)	<0.05	2.44 (0.01)	3.50 (0.04)	<0.15	0.66 (0.01)
Oa horizon	0.23 (0.01)	5.75 (0.03)	2.68 (0.01)	0.97 (0.01)	0.93 (0.01)	1.51 (0.01)	5.49 (0.16)	1.83 (0.02)	0.07 (0.01)	2.85 (0.01)	4.10 (0.01)	0.32 (0.04)	0.62 (0.08)
Spruce													
Oi horizon	<0.15	4.4 (0.06)	1.21 (0.01)	0.17 (0.01)	0.49 (0.01)	0.84 (0.02)	3.01 (0.13)	0.93 (0.01)	<0.05	4.28 (0.03)	5.15 (0.02)	<0.15	0.08 (0.01)
Oa horizon	0.53 (0.08)	2.21 (0.02)	0.73 (0.04)	0.23 (0.01)	0.8 (0.02)	1.32 (0.01)	1.89 (0.14)	0.38 (0.01)	0.24 (0.02)	1.77 (0.11)	0.84 (0.06)	0.41 (0.01)	<0.02

2.4. Inoculation

Air-dried samples of Oa-spruce and Oa-beech material were used to obtain a mixed inoculum, which contained microorganisms from both sites and ensured the presence of a broad diversity of degraders. Before extraction, the soils were rewetted to a water capacity of 60% and incubated for 2 wk at 20°C to reactivate the microorganisms. The soils were shaken separately for 30 min with a 4 m*M* $CaCl_2$ solution (1:2 soil/solution ratio) and filtered through 5-µm filters (SMWP 4700, Millipore, Bedford, MA). We counted the total cell number for each solution by DAPI (4′-6-diamidino-2-phenylindole) staining (Zarda et al., 1997). The solutions were combined so that the inoculum contained a similar number of cells from each solution.

2.5. Incubation

We placed the quartz fiber filter with the Al–OM precipitates into incubation flasks (120 mL) and added 60 mL of incubation solution, made up from ultrapure water, NH_4NO_3, and K_2HPO_4 (analytical grade, Merck, Darmstadt, Germany). The precipitates were not removed from the filters because we considered them as an additional surface for microorganisms. All incubation solutions were adjusted to pH 4.5 by adding HCl. The C/N and C/P ratios of the incubation solution were set to 20 (mean load of 2 mg precipitated C per filter). We added 0.5 to 1 mL of inoculum per flask, giving a total cell count of 5×10^6 per flask. Further, we incubated quartz fiber filters without precipitates under identical conditions as blank values, to measure mineralization of the added inoculum. Samples were shaken manually each day and incubated at 20°C for 7 wk, as preliminary tests had shown that this time period was sufficient to cover the largest part of C mineralization.

2.6. Analytical Methods

The CO_2 concentration in the headspace of each incubation flask was measured at increasing intervals ranging from 2 d at the beginning to 9 d at the end of the experiment (gas chromatograph HP 6890, Hewlett-Packard, thermal conductivity detector, Böblingen, Germany). To ensure proper sampling of the headspace, we applied an overpressure of about 30 kPa to each flask. Ambient air pressure and the pressure in the incubation flasks were measured before each CO_2 analysis. We calculated the amount of CO_2 in the flasks by using the general gas equation, and added the amount of CO_2 dissolved in the liquid phase, estimated from the measured pH and the respective solubility constant. The amount of C mineralized from the Al–OM precipitates was considered as the difference between the measured CO_2 of the sample and the blank without precipitates.

The DOM solutions and filtrates were analyzed for total organic C and total N (High TOC, Elementar, Hanau, Germany), NO_3 (ion chromatography, Dionex DX 100, Idstein, Germany),

NH_4 (flow injection, photometric mLE– FIA LAB, Dresden, Germany), total Al (inductively coupled plasma–optical emission spectrometer [ICP–OES, GCP Electronics, Melbourne, Australia), pH (pH 323, WTW, Weilheim, Germany), and ultraviolet (UV) absorbance at 280 nm (UVIKON 930, Bio-Tek Instruments, Bad Friedrichshall, Germany) as an estimate of the aromaticity of DOM (Chin et al., 1994; McKnight et al., 1997).

The DOM solutions were further analyzed for PO_4, SO_4 (ion chromatography, Dionex DX 100), and Ca, Cu, Fe, K, Mn, Na, S, Si (ICP–OES, GCP Electronics). Also ^{1}H-NMR and ^{13}C-nuclear magnetic resonance (NMR) spectra of freeze-dried samples of DOM (150 mg) dissolved in 3 mL 0.5 *M* NaOD solution were recorded on a DRX 500 NMR spectrometer (11.7 T, Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) at a temperature of 290 K under the following conditions: ^{13}C NMR–10-mm sample tubes, spectrometer frequency–125 MHz, inverse-gated decoupling, acquisition time–0.16 s, delay time–1.84 s, line-broadening factor–100 Hz; ^{1}H NMR: 5-mm sample tubes, spectrometer frequency–500 MHz, homonuclear presaturation for solvent suppression, acquisition time–1.16 s, delay time–1 s, line-broadening factor–2 Hz. Intensities of signals were determined by electronic integration.

Incubation solutions were analyzed at the end of the experiment for Al^{3+} (capillary electrophoresis, HP 3D CE, Waldbronn, Germany).

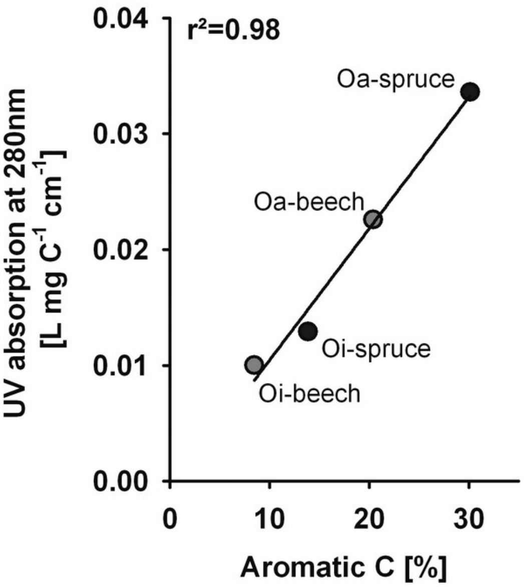

Fig. II.1: Specific ultraviolet (UV) absorption at 280 nm of the extracted dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest in relation to their aromatic C content, determined by solution ^{13}C nuclear magnetic resonance.

2.7. Calculation of Aluminum, Carbon, Aromatic Carbon, and Nitrogen Content of Precipitates

The Al, C, and N contents of the precipitates were calculated from the differences in Al, organic C, and organic N concentrations in solution before and after precipitation. The specific UV absorption of the DOM solutions correlated very well with the aromatic C content ($r^2 = 0.98$)

as determined by ^{13}C NMR (Fig. II.1). The regression was used to calculate the aromatic C content in the filtrates. The aromatic C content of the precipitates was estimated by difference, taking into account the amount of DOM precipitated.

2.8. Statistics and Modeling

We used a general linear model with repeated measurements to test for differences in the extent of mineralization between precipitates obtained (i) from different DOM solutions, (ii) at different Al/C ratios, and (iii) at different pH values. The analysis was performed with the statistical package SPSS (SPSS, 2003). This analysis required a normal distribution of the data. To meet this criterion, our data set was transformed by extracting the root of the amount of C mineralized. The variances of the transformed values were homogenous. To test the significance of the differences, Fisher's LSD posthoc test was applied. A three-factorial variance analysis was performed with the residuals of the multiple linear regression between the amount of C mineralized and the aromatic C content and the C/N ratio of the precipitates. The residuals were tested on differences originating from different (i) DOM solutions, (ii) Al/C ratios, and (iii) pH values. The significance of the differences was again tested with Fisher's LSD posthoc test.

The mineralization data were best described using an exponential model (Table II.3). Fitting was done using SigmaPlot (SPSS, 2002), with the following equation:

$$\text{mineralized C [\% of initial C]} = A[1 - \exp(-kt)] + c$$

where

k is the mineralization rate constant, A is the mineralizable C (% of initial C),
t is time (d), and c is a correction factor (axis intercept).

The correction factor was implemented to include axis intercepts below zero at the beginning of the incubation, which originated from the subtraction of the blank values. Fitting a double exponential model to the data was successful only in few cases.

For calculation of saturation indices of $Al(OH)_3$, the program Visual Minteq (Gustafsson, 2005) was used.

Table II.3: Fitting parameters of an exponential model ($C_{\text{mineralized}}$ [%] = A[1 – exp(–kt)] + c, where k is the mineralization rate constant, t is time [d], A is mineralizable dissolved organic C [%], and c is a correction factor) for the mineralization of Al–organic matter precipitates.

Sample	pH	Al/C	A	k	c	r^2
Oi horizon, beech	3.8	0.05	4.76	7.28×10^{-2}	–0.71	0.97
		0.1	4.38	6.14×10^{-2}	–0.41	0.98
		0.2	9.50	2.18×10^{-2}	–0.04	0.98
		0.3	6.14	4.96×10^{-2}	–0.68	0.96
	4.5	0.05	6.23	6.57×10^{-2}	–0.34	0.97
		0.1	6.49	6.86×10^{-2}	–0.17	0.98
		0.2	6.71	6.29×10^{-2}	–0.17	0.97
		0.3	7.83	7.28×10^{-2}	–0.29	0.97
Oa horizon, beech	3.8	0.05	1.67	4.22×10^{-2}	–0.37	0.98
		0.1	4.26	9.86×10^{-3}	0.01	0.97
		0.2	2.94	2.24×10^{-2}	–0.08	0.99
		0.3	4.27	1.60×10^{-2}	–0.23	0.99
	4.5	0.05	3.64	1.52×10^{-2}	–0.09	0.99
		0.1	3.95	2.17×10^{-2}	0.06	1.00
		0.2	4.32	2.80×10^{-2}	0.03	1.00
		0.3	3.55	3.60×10^{-2}	0.19	0.99
Oi horizon, spruce	3.8	0.05	2.14	7.27×10^{-2}	–0.53	0.98
		0.1	2.61	5.34×10^{-2}	–0.52	0.98
		0.2	3.05	3.33×10^{-2}	–0.47	0.98
		0.3	2.14	4.82×10^{-2}	–0.40	0.97
	4.5	0.05	1.81	3.72×10^{-2}	0.20	0.97
		0.1	4.21	3.73×10^{-2}	–0.23	0.98
		0.2	5.15	4.64×10^{-2}	–0.19	0.99
		0.3	6.12	4.25×10^{-2}	0.04	0.99
Oa horizon, spruce	3.8	0.05		no fit possible		
		0.1	0.60	2.74 × 10–2	0.01	0.98
		0.2	0.95	2.01×10^{-2}	0.06	0.89
		0.3	0.82	2.15×10^{-2}	0.21	0.92
	4.5	0.05	1.29	2.33×10^{-2}	–0.09	0.97
		0.1	1.54	2.93×10^{-2}	–0.04	0.99
		0.2	3.24	8.74×10^{-3}	–0.03	0.99
		0.3		no fit possible		

3. Results and Discussion

3.1. Formation and Properties of Aluminum–Organic Matter Precipitates

Dissolved organic matter precipitated within minutes after adding Al to the solution, as already reported by Plankey and Patterson (1987). We found that increasing Al/C ratios led mostly to increased amounts of precipitated OM (Table II.4). Aluminum/C ratios beyond ~0.1 led to only slightly increasing DOM precipitation. We consistently observed a larger fraction of DOM precipitating at pH 4.5 than at pH 3.8 (Table II.4), which confirms the results of Nierop et al. (2002). The maximum proportions of DOM that precipitated from the four solutions increased in the order of Oi-beech < Oi-spruce < Oa-beech < Oa-spruce and ranged from 29 to 84%. The content of aromatic C in solution (Table II.5) determined the maximum portion of DOM that could be precipitated (Fig. II.2). A linear regression between these two factors resulted in a coefficient of determination of $r^2 = 0.98$ ($p < 0.01$, $n = 4$). Generally, DOM

Table II.4: Percentage of dissolved organic carbon (DOC) precipitated at different Al/C ratios and two pH values (standard error of three replicates in parentheses).

Site and sample	DOC precipitated							
	Al/C = 0.05		Al/C = 0.1		Al/C = 0.2		Al/C = 0.3	
	pH 3.8	pH 4.5	pH 3.8	pH 4.5	pH 3.8	pH 4.5	pH 3.8	pH 4.5
	% of initial C							
Beech								
Oi horizon	15.0 (1.0)	25.3 (0.8)	15.2 (0.3)	28.5 (0.4)	14.4 (0.2)	28.4 (0.4)	12.2 (0.1)	28.6 (0.3)
Oa horizon	26.2 (0.4)	35.0 (1.2)	31.9 (0.6)	61.0 (0.6)	34.0 (0.4)	64.3 (0.4)	36.7 (0.6)	65.7 (0.3)
Spruce								
Oi horizon	27.0 (1.3)	44.0 (0.6)	26.5 (0.6)	44.3 (0.4)	24.3 (0.1)	41.8 (0.6)	24.3 (1.6)	37.2 (0.9)
Oa horizon	23.9 (1.1)	58.5 (1.3)	36.4 (1.6)	74.3 (0.4)	43.1 (1.2)	79.0 (0.9)	47.7 (3.0)	83.6 (0.7)

Table II.5: Carbon and H moieties (as a percentage of the total) of the dissolved organic matter solutions, as determined by solution ^{13}C and ^{1}H nuclear magnetic resonance.

Sample and site	C moieties				H moieties		
	Alkyl C (0–60 ppm)	O-Alkyl C (60–105 ppm)	Aromatic C (105–160 ppm)	Carboxyl C (160–200 ppm)	Alkyl H (0.8–3 ppm)	Aromatic H (5.5–10 ppm)	Carbohydrate H (3.0–4.8 ppm)
	%				%		
Beech							
Oi horizon	27.6	46.3	8.5	17.7	33.4	2.9	44.6
Oa horizon	30.2	26.7	20.4	22.7	35.4	8.3	29.9
Spruce							
Oi horizon	26.1	45.5	13.9	14.6	30.9	6.2	42.8
Oa horizon	22.8	22.5	30.1	24.6	25.2	14.8	32.9

solutions from Oa horizons contain larger proportions of aromatic compounds (Kalbitz et al., 2003) and more carboxylic groups (Guggenberger et al., 1994) than the Oi solutions. The larger number of possible binding sites in Oa DOM solutions resulted in larger amounts of precipitates.

One reason for precipitation of DOM is charge neutralization by Al, resulting in a reduced solubility of Al–OM complexes. At pH 4.5, the DOM is less protonated than at pH 3.8, allowing more Al to bind and, thus, more precipitates to form. The binding of cations reduces the solubility of DOM strongly, as the electrical charge of DOM becomes more neutral (Weng et al., 2002), which leads to extensive coagulation; however, Ares and Ziechmann (1988) pointed out that the effect cannot be fully explained by charge neutralization. Aluminum may also cause formation of *n*-dentate complexes with organic groups, probably inducing various structural changes, such as folding or formation of macrocycles, that enhance precipitation (Ares and Ziechmann, 1988).

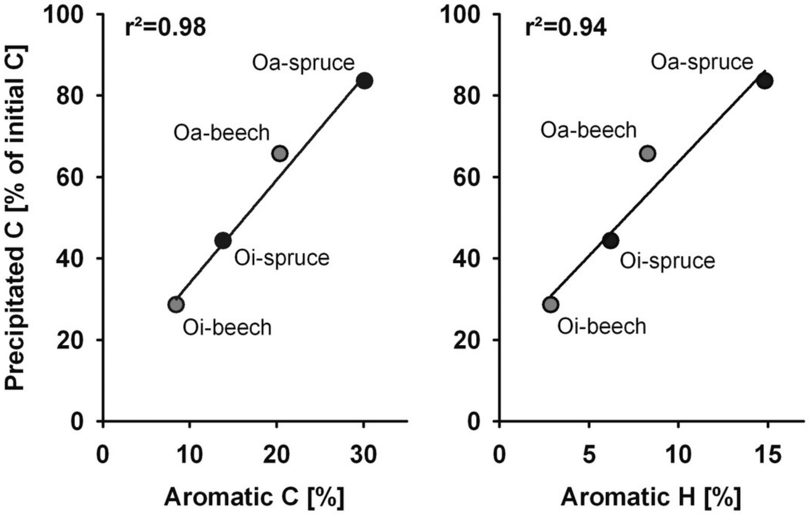

Fig. II.2: The maximum percentage of dissolved organic C precipitated from the dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest (at pH 4.5 and Al/C ratio of 0.3, except for the Oi-spruce solution, which had an Al/C ratio of 0.1) after Al addition in relation to the content of aromatic C and aromatic H.

Coprecipitation of organic macromolecules after adsorption onto colloidal Al (hydr)oxides is a further mechanism of DOM precipitation (Boudot, 1992; Dolfing et al., 1999), which explains the increased precipitation at pH 4.5.

At an Al/C ratio of 0.05, less DOM precipitated than at larger Al/C ratios. This agrees well with the results of Jansen et al. (2002), who found that the fraction of Al bound in soluble Al–OM complexes strongly decreased with increasing Al/C ratios. The Al/C ratios >0.1 led to only minor changes in DOM precipitation. Therefore a fraction of DOM must exist that does not precipitate with Al. This has been observed before by Schwesig et al. (2003).

3.2. Changes in Aluminum/Carbon Ratio from Solution to Precipitate

Generally, the Al/C ratios of the precipitates reflected the initial Al/C ratios in the solutions. The precipitates produced at pH 3.8 had, on average, 55 to 75% smaller Al/C ratios than the Al/C ratios in solution (Fig. II.3). Also, precipitates produced at pH 4.5 and Al/C ratios in solution of 0.1 or larger had, on average, 10 to 40% smaller Al/C ratios than the Al/C ratios in solution. In contrast, precipitation at pH 4.5 and Al/C ratios in solution smaller than 0.1 led to an increase of the Al/C ratios of up to 40% in comparison to the Al/C ratios in solution. In all cases, the largest decrease of the Al/C ratios from solution to the precipitates was found for large Al/C ratios (0.3).

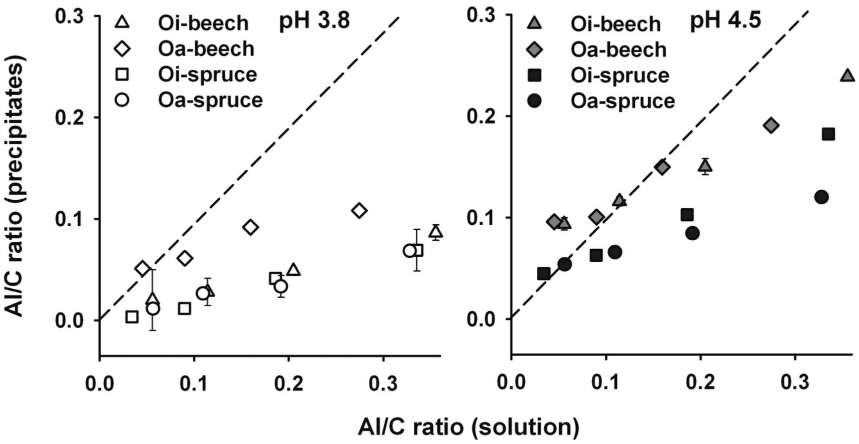

Fig. II.3: The Al/C ratios in precipitates (solid phase) of the dissolved organic matter (DOM) solutions from the Oa and Oi horizons of a beech and a spruce forest in relation to the initially adjusted Al/C ratios in the DOM solutions. Precipitation was initiated at two different pH values: pH 3.8 (left) and pH 4.5 (right). Mean values and standard error of three replicates.

At pH 4.5, the formation of $Al(OH)_3$ (Lofts et al., 2001) and possibly also Al_{13} (Furrer et al., 1992; Hu et al., 2006) is likely, leading to subsequent coprecipitation of DOM with Al hydroxides. Because hydrous oxide Al forms have a less positive charge than Al^{3+}, more Al was needed to achieve the same extent of charge neutralization. This could explain the larger Al/C ratios in the Al–OM precipitates at pH 4.5 than at pH 3.8. Another reason for smaller Al/C ratios at pH 3.8 may be that the solubility of DOM at pH 3.8 was less than at pH 4.5 (Kalbitz et al., 2000) because of increased protonation. Thus, less Al was needed to achieve the same extent of charge neutralization, consequently resulting in more efficient precipitation of DOM by Al at lower pH values.

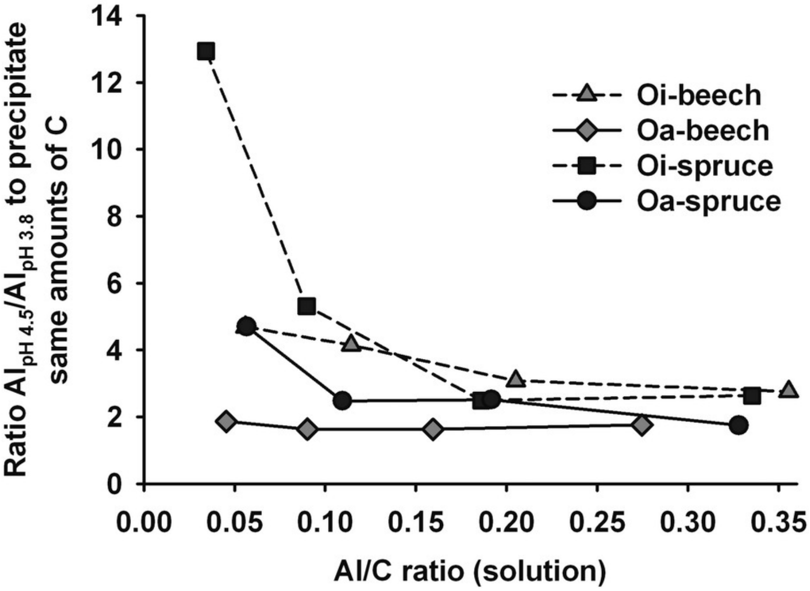

Fig. II.4: Ratio of Al needed to precipitate similar amounts of dissolved organic C at pH 4.5 and 3.8 (dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest, different Al/C ratios). Ratios >1 mean that more Al was necessary at pH 4.5 to precipitate the same amount of C as at pH 3.8.

We observed that precipitation of equal amounts of DOM required two to 13 times more Al at pH 4.5 than at pH 3.8, depending on whether the Al/C ratio was large or small, respectively (Fig. II.4). Thus, larger precipitation of DOM at pH 4.5, as observed in our experiments (Table II.4), can be expected only if Al is present in excess. At limiting Al concentrations, precipitation of DOM should be considerably greater at lower pH values.

3.3. Changes in Organic Matter Composition by Precipitation

The specific UV absorption at 280 nm decreased in the DOM solutions by 7 to 77% after precipitation (Fig. II.5). Because specific UV absorption and aromatic C content correlated well (Fig. II.1), this indicates that aromatic compounds precipitated preferentially. The decrease in specific UV absorption by precipitation became more pronounced with increasing Al/C ratios. Precipitation at pH 4.5 reduced the specific UV absorption to a larger extent (on average, 24–54%) than at pH 3.8 (on average, 9–30%), reflecting also the larger portion of precipitates formed at pH 4.5.

The calculated content of aromatic C of the precipitates increased in comparison with the original DOM solution by 13 to 270% (Fig. II.6). In most cases, the aromatic C content was largest in precipitates formed at Al/C ratios of 0.05. It decreased with increasing solution Al/C ratios. Furthermore, precipitates formed at pH 3.8 contained more aromatic C than those formed at pH 4.5. The pH effect was most pronounced for the Oa solutions.

The enrichment of aromatic compounds in all precipitates (Fig. II.6) supported the idea that aromatic compounds are preferentially removed from solution, as already observed for DOM adsorption on soils (Kalbitz et al., 2005). Aromatic C was most enriched in precipitates formed at small Al/C ratios. This indicated that aromatic compounds must be the first components precipitating after Al binding. We observed less aromatic C in precipitates formed at pH 4.5 than at pH 3.8. This indicated a less selective precipitation of aromatic compounds by Al hydroxides. Also, it should be considered that the DOM solutions contained limited amounts of aromatic compounds. Therefore, increased precipitation, as observed for large Al/C ratios and at higher pH, may

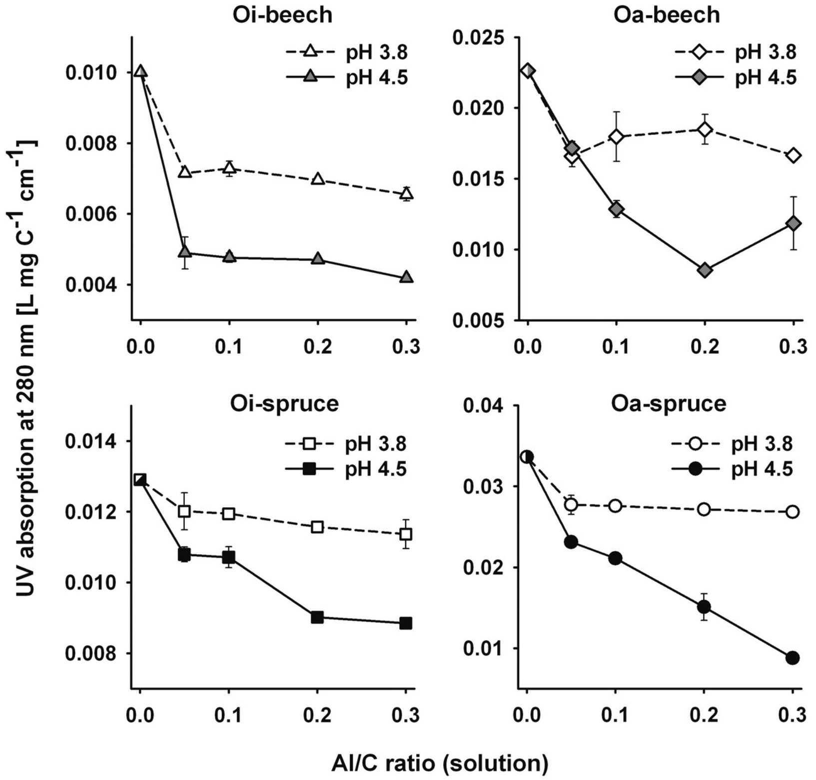

Fig. II.5: Specific ultraviolet (UV) absorption (280 nm) of initial dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest (Al/C ratio 0) and after removal of precipitates by filtration. Precipitation was initiated at two pH values and four different Al/C ratios. Mean values and standard error of three replicates.

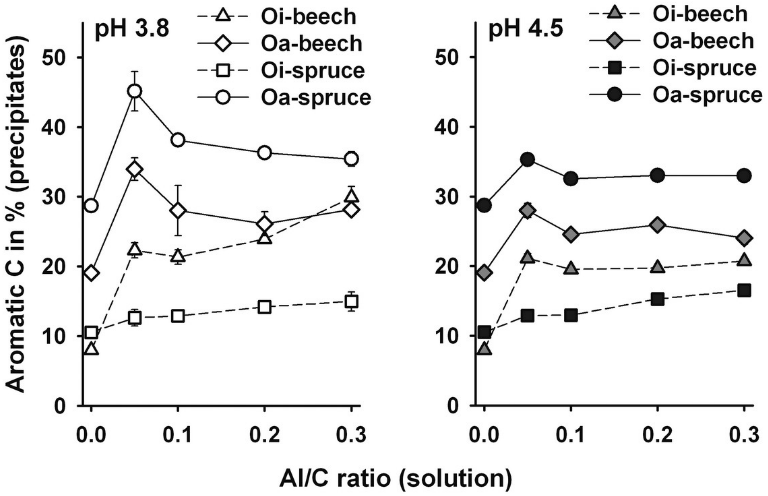

Fig. II.6: Aromatic C content of initial dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest (Al/C ratio 0) and of precipitates for two pH values and four Al/C ratios. Mean values and standard error of three replicates.

have resulted in less aromatic C in the precipitates.

In the initial DOM solutions, a large proportion of total N was bound organically (portion of dissolved organic nitrogen [DON] to total N: Oa-beech, 37%; Oa-spruce, 57%; Oi-spruce, 69%; Oi-beech, 89%). Precipitation resulted in most cases in larger organic C/organic N ratios of the precipitates than in the original DOM solutions (Fig. II.7). In some cases, we found an enrichment of N in precipitates, mostly for those from the Oi-beech solution. Precipitates produced at pH 3.8 had larger C/N ratios than precipitates formed at pH 4.5, except for precipitates from the Oa-spruce solution.

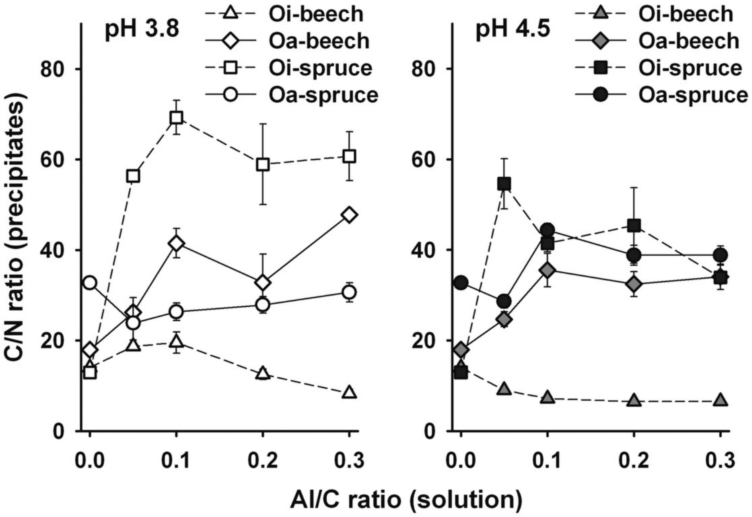

Fig. II.7: Organic C/organic N ratios of initial dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest (Al/C ratio 0) and of precipitates for two pH values and four Al/C ratios. Mean values and standard error of three replicates.

The observed depletion of organic N in the Al–OM precipitates indicated that a large proportion of DON did not precipitate with Al. Kaiser et al. (2000) and Kaiser and Zech (2000) observed a preferential adsorption of DOM with low contents of organic N to the mineral soil and attributed this to diminished adsorption of hydrophilic compounds, enriched in DON. Therefore, the depletion of organic N in the precipitates can be explained by preferential precipitation of aromatic and hydrophobic compounds, possibly derived from lignin or tannin and therefore being poor in N. Only peptides, proteins, or amino acids bound to high-molecular-weight or aromatic structures tend to precipitate (Yu et al., 2002). In precipitates of the Oi-beech solution, enrichment of organic N was observed. Thus, a larger portion of DON must have been bound in organic structures that formed insoluble complexes with Al than in the other DOM solutions. We cannot present a convincing explanation why only a major part of the organic N in the Oi-beech solution was able to form insoluble complexes with Al.

3.4. Mineralization of Aluminum–Organic Matter Precipitates

The extent of C mineralization was significantly ($p < 0.01$) less for precipitates from all DOM solutions, at all Al/C ratios and pH values, than for the corresponding untreated DOM solutions. During 7 wk of incubation, only 0.5 to 7.7% of the precipitated C was mineralized (Fig. II.8), whereas the corresponding DOM solutions showed a much larger mineralization of 5% (Oa-spruce), 24% (Oa-beech), 38% (Oi-beech), and 49% (Oi-spruce), respectively.

Three-factorial variance analysis indicated that the different DOM solutions, the pH at precipitate formation, and the Al/C ratio as well as all cross-interactions of the factors had a highly significant ($p < 0.001$) effect on the mineralizable C of the precipitates. The percentage of C mineralized was significantly ($p < 0.01$) smaller for precipitates from Oa solutions (spruce: 0.5–1.2%; beech: 1.0–3.3%) than for those from Oi solutions (spruce: 1.8–4.8%; beech 3.7–7.7%). We further found that the precipitates from beech DOM showed a significantly ($p < 0.01$) larger mineralization than those from spruce DOM. These findings reflect the mineralizability of the original DOM solutions.

Mineralization of precipitates formed at pH 4.5 was in nearly all cases increased by approximately 50 to 75% when compared with the corresponding precipitates formed at pH 3.8 (Fig. II.8, II.9). Surprisingly, we found a significant ($p < 0.01$) increase in mineralization with increasing Al/C ratios up to an Al/C ratio of 0.2. The influence of the Al/C ratio on mineralization of Al–OM precipitates was more pronounced at pH 4.5 than at pH 3.8. From Al/C ratios of 0.05 to 0.2, the mineralization increased on average by 33% for precipitates formed at pH 4.5 and by 19% for precipitates formed at pH 3.8.

Reduced mineralization of precipitates compared with the original DOM solutions suggested C stabilization. It might have been the result of spatial rearrangements and inaccessibility of organic matter for microorganisms and enzymes due to complexation and precipitation by Al. Furthermore, preferential precipitation of inherently stable compounds can contribute to the stabilization. Aromatic and complex compounds are preferentially removed from the soil solution by sorption (Kalbitz et al., 2005) and comprise stable fractions of DOM (Kalbitz et al., 2003). An analogous effect can be expected for precipitation by Al. The different DOM used varied in chemical composition, especially in aromatic C content, and showed large differences in mineralization. Therefore, it was not surprising that the precipitates from the four

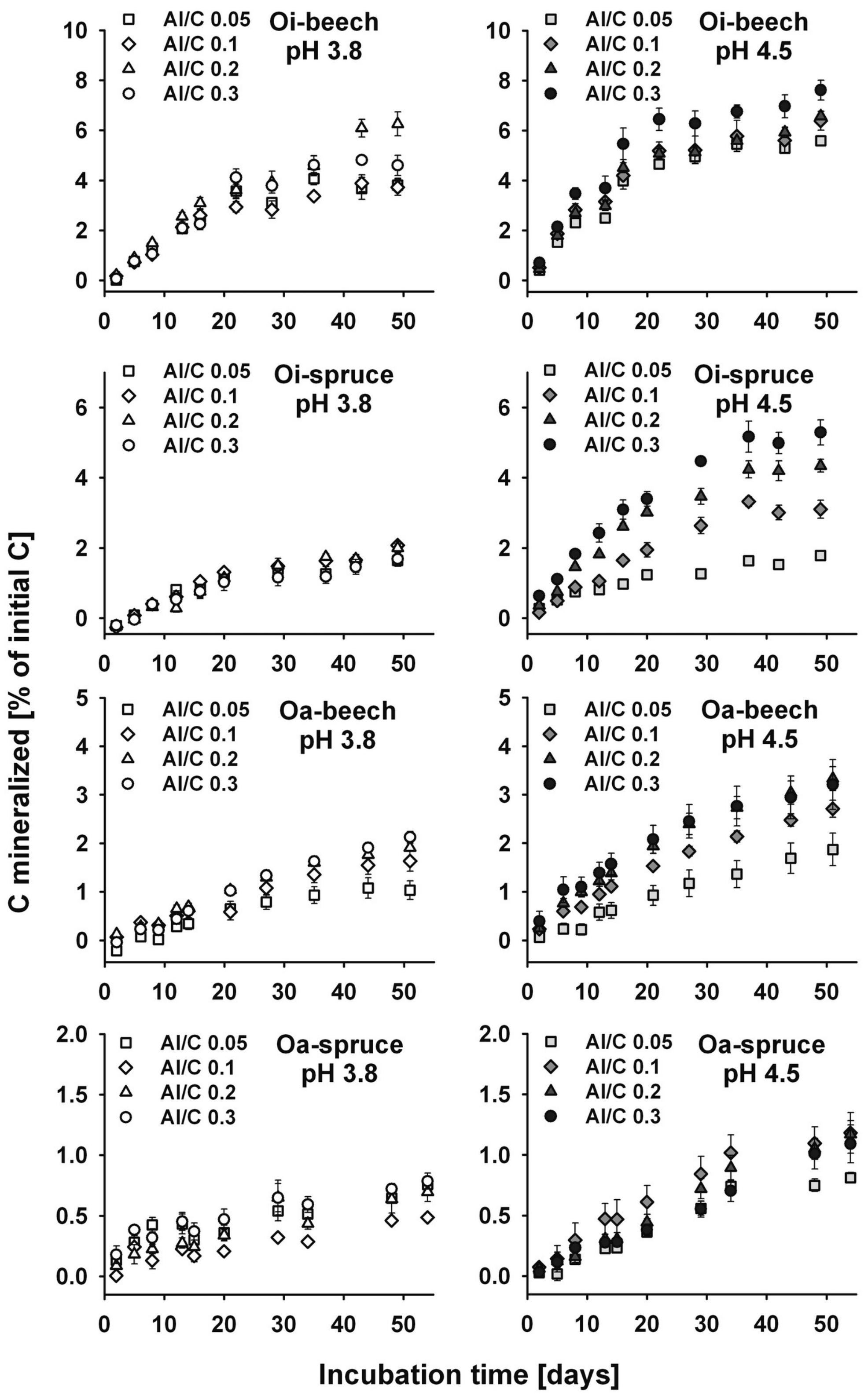

Fig. II.8: Dynamics of C mineralization of Al–organic matter (OM) precipitates (as a percentage of initial C) during 7 wk of incubation at pH 4.5 and 20°C. The Al–OM precipitates were produced from dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest at two pH values and four Al/C ratios. Mean values and standard error of three replicates.

solutions differed in stability also. The most likely reason for the greater stability of precipitates from Oa than from Oi solutions was the larger aromatic C content of Oa DOM solutions (Table II.5). Spruce DOM solutions contained more aromatic C than the respective beech DOM solutions; the precipitates reflected this.

Toxic effects of Al on microorganisms during incubation were unlikely, as no appreciable redissolution of Al from Al–OM precipitates occurred. Incubation solutions had average Al^{3+} concentrations of 0.2 mg L^{-1} (maximum 0.5 mg L^{-1}) at the end of the experiment. According to the literature, such concentrations are at least 10 times less than those reported to have toxic effects (De Wit et al., 2001; Illmer et al., 2003).

The observed differences in the stability of precipitates formed at the two pH values clearly indicated that the binding form is crucial for stabilization of DOM. Aluminum hydroxides preferentially form mono- or bidentate bonds, in contrast to Al^{3+}, which can form *n*-dentate bonds (Ares and Ziechmann, 1988), resulting in more bonds between Al and DOM, and probably more complex spatial structures at lower pH. The fraction of Al present in solution as Al^{3+} is much larger at low pH (Jansen et al., 2002), which could cause stronger binding to OM in the precipitates and consequently less mineralization of precipitates. Differences in mineralization of precipitates formed at the two pH values studied were much smaller at small Al/C ratios. This could be attributed to the equally reduced formation of Al hydroxide species at small Al/C ratios at both pH values.

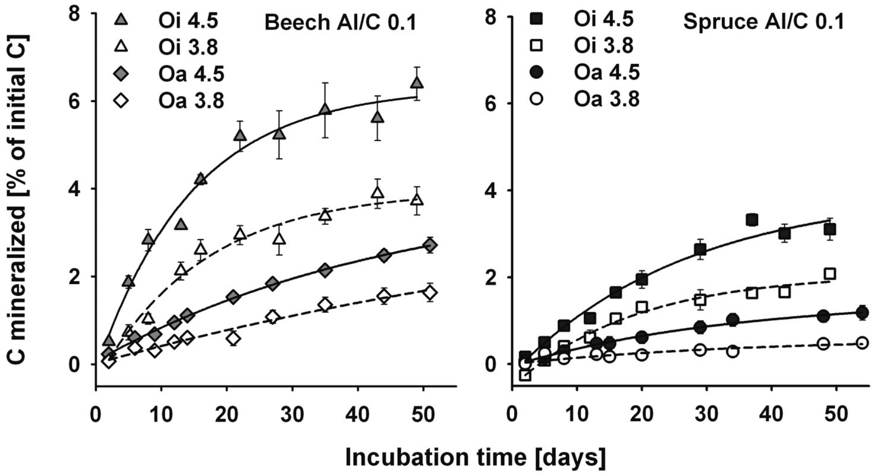

Fig. II.9: Mineralization of Al–organic matter precipitates (Al/C ratio 0.1) produced from dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest at pH 3.8 and 4.5 (incubation at pH 4.5 and 20°C for 7 wk). Mean values and standard error of three replicates.

The reduced mineralization of precipitates produced at smaller Al/C ratios is in contrast to the findings of Boudot et al. (1989) and Boudot (1992). They observed less mineralization with increasing Al/C ratios, testing citric acid and fungal melanins, which are by far not as complex as DOM from soil. One explanation for the decreased mineralization at smaller Al/C ratios could be preferential precipitation of aromatic (Fig. II.6) and high-molecular-weight structures, leading to small but stable precipitates. With increasing amounts of Al added, also less complex and aromatic compounds precipitate, resulting in precipitates more prone to mineralization. A further explanation could be that first bidentate bonds were formed, which are more stable (Simonsson, 2000).

The influence of the Al/C ratio on mineralization was more pronounced at pH 4.5 than at pH 3.8. With increasing Al concentrations, more hydrous Al forms were present in solution, allowing for formation of coprecipitates. Thus, at higher pH and larger Al/C ratios, the fraction of Al–OM precipitates formed by coprecipitation was larger, which was reflected in the larger mineralization of these precipitates. The assumption that more $Al(OH)_3$ was present at higher pH values and larger Al/C ratios was tested by using the speciation program Visual Minteq (Gustafsson, 2005). Input parameters were the chemical characterizations of the initial DOM solutions (Table II.2) and the added amount of Al at the respective pH. By including the Stockholm humic model, organic complexation was taken into account. Positive saturation indices (SI) indicate oversaturation, negative SI undersaturation of a phase. The results of the modeling (Table II.6) indicated oversaturation for $Al(OH)_3$ only at pH 4.5. Further, it can be seen that the SI increased with increasing Al/C ratios, supporting the idea that at small Al/C ratios,

Table II.6: Saturation indices [SI = log(IAP/K_S), where IAP is the ion activity product and K_s is the saturated hydraulic conductivity] for $Al(OH)_3$ in the dissolved organic matter solutions at 20°C and different pH and Al/C ratios, calculated with Visual Minteq (SI > 0 = oversaturation, SI < 0 = undersaturation).

Sample and site	pH 3.8				pH 4.5			
	Al/C 0.05	Al/C 0.1	Al/C 0.2	Al/C 0.3	Al/C 0.05	Al/C 0.1	Al/C 0.2	Al/C 0.3
Beech								
Oi horizon	−1.40	−1.04	−0.74	−0.59	0.39	0.92	1.27	1.44
Oa horizon	−1.48	−1.09	−0.77	−0.62	0.23	0.83	1.23	1.40
Spruce								
Oi horizon	−1.42	−1.05	−0.75	−0.60	0.35	0.90	1.26	1.43
Oa horizon	−1.39	−1.04	−0.75	−0.62	0.42	0.93	1.27	1.41

formation of Al hydroxide was less important, and thus, differences in mineralization of precipitates between the pH values were less at small Al/C ratios.

The Al_{13} species might also influence the formation and stability of Al–OM precipitates. At pH values <4.2, no formation of Al_{13} can be expected, whereas the prevailing Al concentrations (0.17–1 m*M*) at pH 4.5 favor the formation of Al_{13}. On the other hand, organic ligands can depolymerize Al_{13} (Masion et al., 1994). Disappearance of Al_{13} from DOM solutions has been observed by Yamaguchi et al. (2004) and Thomas et al. (1993), and attributed by them to the formation of Al_{13}–organic complexes. This effect is of increasing influence with increasing Al/C ratios (Furrer et al., 1992; Thomas et al., 1993). Here, the influence of Al_{13} species on precipitation and stabilization could not be separated from the effect of Al hydroxides, but may be important.

3.5. Factors Governing the Stability of Aluminum–Organic Matter Precipitates

Although we found preferential precipitation of aromatic compounds, which should be the most stable DOM components, the correlation between aromatic C content in the precipitates and mineralizable C was poor ($r^2 = 0.21$, $p < 0.001$, $n = 94$). The C/N ratio of the precipitates and mineralizable C were equally poorly correlated ($r^2 = 0.30$, $p < 0.001$, $n = 94$). Multiple linear regression with both variables explains 76% of the variability ($p < 0.001$; $n = 94$) in mineralizable C (Fig. II.10). A collinearity analysis confirmed (variance inflation factor = 1.1,

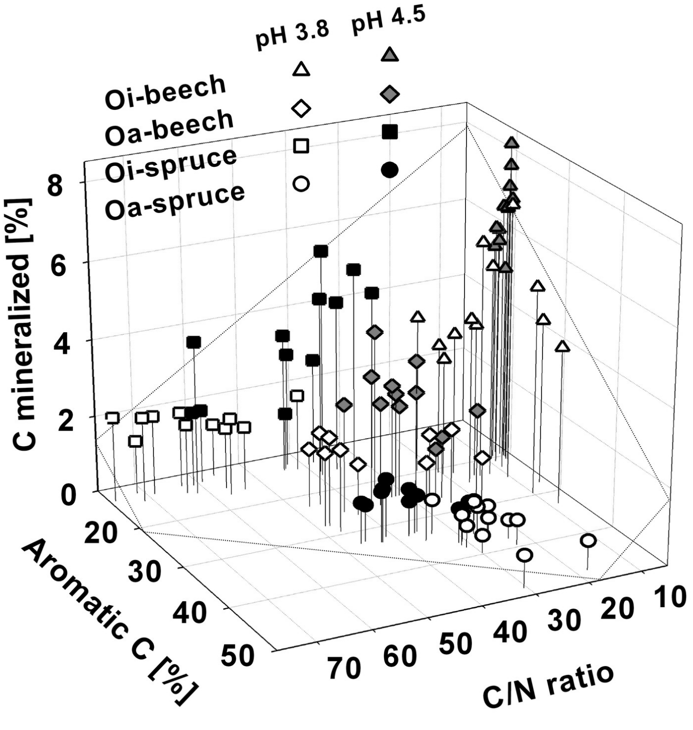

Fig. II.10: Mineralization of Al–organic matter precipitates (from dissolved organic matter solutions from the Oa and Oi horizons of a beech and a spruce forest) in dependence on the aromatic C content and the organic C/organic N ratio of the precipitates (multiple linear regression: $R^2 = 0.76$; dissolved organic carbon [DOC] mineralized [as a percentage of initial DOC] = 9.992 – 0.091(C/N ratio) – 0.161(aromatic C) [%]).

condition index = 8.8) that the two parameters C/N ratio and aromatic C were independent. Therefore, the aromatic C content and the C/N ratio of the precipitates were the two main factors controlling the stability of Al–OM precipitates. Further, we tested whether the type of DOM, pH, and Al/C ratio influenced only these two factors or directly affected C mineralization. A three-factorial variance analysis was performed with the residuals from the multiple linear regression. The type of DOM did not significantly ($p > 0.1$) influence C mineralization after removing the effect on aromatic C and the C/N ratio; however, the pH ($p < 0.05$) and the Al/C ratio ($p < 0.01$) had an additional effect on C mineralization, besides their effect on aromatic C content and C/N ratio of the precipitates. This means that differences in mineralization between precipitates from the four solutions could be satisfactorily explained by changes in aromatic C content and by the C/N ratio of the precipitates; however, these two factors did not fully explain the differences caused by pH variation and by variations in the amount of Al added. This supported the idea that Al speciation and the functional groups involved, as discussed above, influenced the degree of stabilization.

3.6. Implications of Aluminum–Organic Matter Precipitation on Acidic Forest Soils and Surface Waters

The Al concentrations and pH values used in this study were typical of acidic forest soils. Therefore, the observed stability of precipitates should be important for C stabilization in the field. Soil solutions from the organic horizon that percolate downward into the mineral soil are gradually enriched in dissolved Al (Schwesig et al., 2003), similar to the artificial Al addition in our experiments, resulting in subsequent precipitation. In mineral soils, decreasing DOC concentrations have been observed with depth (Schwesig et al., 2003). Besides adsorption on Al and Fe oxides, this decrease may be explained by the formation of Al–OM precipitates. Differentiation in the field between sorptive preservation and stabilization by precipitation is difficult because the mechanisms would occur at the same time and the observable effects are rather similar. Nevertheless, precipitation of DOM by Al should result in a substantial accumulation of C and Al in the soil with time, as in podzols, because mineralization of precipitates proved to be very small.

Our results may also help to understand other recent observations, such as the reported increase in DOC concentrations of surface waters (Freeman et al., 2001; Worrall et al., 2004;

Evans et al., 2005). It is assumed that soils are the main source of the additional C. A huge fraction of Al was leached from the upper soil until the mid-1980s, resulting in a smaller Al reservoir in the soil (Mulder et al., 1989; Berggren et al., 1998). Reduction of acid deposition in recent years led to increasing pH values and decreasing Al concentrations in soil solutions (Matzner et al., 2004). Therefore, Al might be the limiting factor for the formation of Al–OM precipitates in the field. We have shown that, at larger pH values, more Al was needed to precipitate the same amount of DOM (Fig. II.4), especially at small Al concentrations. If we assume that larger pH values and smaller Al concentrations lead to reduced precipitate formation, this would not only result in less C stabilization in the soil, but also in larger amounts of DOM being exported to surface waters. The assumed reduction of precipitation should further lead to a change in the chemical composition of the transported DOM because precipitation removes preferentially stable compounds from the soil solution. Thus, the additional DOM in many surface waters should be mainly comprised of microbiologically stable compounds.

4. Conclusions

The precipitation of DOM by Al resulted in significant C stabilization against microbial decay. We observed preferential precipitation of compounds with large contents of aromatic C and little N. This was one reason for the large stability of Al–OM precipitates against microbial decay when compared with the original DOM. Precipitates formed at pH 3.8 and small dissolved Al/C ratios (0.05) had a greater stability against microbial decay than those formed at pH 4.5 and large dissolved Al/C ratios (>0.2). Thus, also the amount of Al bound as Al hydroxides in the precipitates had an influence on the stability of Al–OM precipitates against microbial decay.

The amounts of DOM precipitated by Al and the subsequent increase in stability against microbial decay were very sensitive to changes in environmental parameters, such as pH or the concentration of dissolved Al. Therefore, knowledge about the dynamics of this mechanism significantly improves our understanding of environmental processes such as podzolisation or the recently observed increase of DOC in surface waters. Thus, the stabilization of OM by the formation of Al–OM precipitates from DOM seems to be fundamental and should be incorporated into existing models and theories of C cycling.

5. References

Ares, J., and W. Ziechmann. 1988. Interactions of organic matter and aluminum ions in acid forest soil solutions: Metal complexation, flocculation, and precipitation. Soil Sci. 145:437–447.

Baldock, J.A., and J.O. Skjemstad. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org. Geochem. 31:697–710.

Berggren, D., J. Mulder, and R. Westerhof. 1998. Prolonged leaching of mineral forest soils with dilute HCl solutions: The solubility of Al and soil organic matter. Eur. J. Soil Sci. 49:305–316.

Blaser, P., A. Heim, and J. Luster. 1999. Total luminescence spectroscopy of NOM-typing samples and their aluminium complexes. Environ. Int. 25:285–293.

Boudot, J.-P. 1992. Relative efficiency of complexed aluminum, noncrystalline Al hydroxide, allophane and imogolite in retarding the biodegradation of citric acid. Geoderma 52:29–39.

Boudot, J.-P., A.B.H. Brahim, R. Steiman, and F. Seigle-Murandi. 1989. Biodegradation of synthetic organo–metallic complexes of iron and aluminium with selected metal to carbon ratios. Soil Biol. Biochem. 21:961–966.

Chin, Y.P., G. Aiken, and E. O’Loughlin. 1994. Molecular-weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28:1853–1858.

De Wit, H.A., T. Groseth, and J. Mulder. 2001. Predicting aluminum and soil organic matter solubility using the mechanistic equilibrium model WHAM. Soil Sci. Soc. Am. J. 65:1089–1100.

Dolfing, J., W.J. Chardon, and J. Japenga. 1999. Association between colloidal iron, aluminum, phosphorus, and humic acids. Soil Sci. 164:171–179.

Evans, C.D., D.T. Monteith, and D.M. Cooper. 2005. Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. Environ. Pollut. 137:55–71.

Freeman, C., C.D. Evans, D.T. Monteith, B. Reynolds, and N. Fenner. 2001. Export of organic carbon from peat soils. Nature 412:785.

Furrer, G., B. Trusch, and C. Müller. 1992. The formation of polynuclear Al_{13} under simulated natural conditions. Geochim. Cosmochim. Acta 56:3831–3838.

Guggenberger, G., W. Zech, and H.-R. Schulten. 1994. Formation and mobilization pathways of dissolved organic-matter: Evidence from chemical structural studies of organic-matter fractions in acid forest floor solutions. Org. Geochem. 21:51–66.

Gustafsson, J.P. 2005. Visual Minteq ver 2.50. Available at http://www.lwr.kth.se/english/OurSoftware/Vminteq (verified 28 Aug. 2006). Dep of Land and Water Resour. Eng., R. Inst. Technol., Stockholm.

Gustafsson, J.P., D. Berggren, M. Simonsson, M. Zysset, and J. Mulder. 2001. Aluminium solubility mechanisms in moderately acid Bs horizons of podzolized soils. Eur. J. Soil Sci. 52:655–665.

Hu, C.Z., H.J. Liu, J.H. Qu, D.S. Wang, and J. Ru. 2006. Coagulation behavior of aluminum salts in eutrophic water: Significance of Al_{13} species and pH control. Environ. Sci. Technol. 40:325–331.

Illmer, P., U. Obertegger, and F. Schinner. 2003. Microbiological properties in acidic forest soils with special consideration of KCl extractable Al. Water Air Soil Pollut. 148:3–14.

Jansen, B., K.G.J. Nierop, and J.M. Verstraten. 2002. Influence of pH and metal/carbon ratios on soluble organic complexation of Fe(II), Fe(III) and Al(III) in soil solutions determined by diffusive gradients in thin films. Anal. Chim. Acta 454:259–270.

Jansen, B., K.G.J. Nierop, and J.M. Verstraten. 2003. Mobility of Fe(II), Fe(III) and Al in acidic forest soils mediated by dissolved organic matter: Influence of solution pH and metal/organic carbon ratios. Geoderma 113:323–340.

Jansen, B., K.G.J. Nierop, and J.M. Verstraten. 2004. Mobilization of dissolved organic matter, aluminium and iron in podzol eluvial horizons as affected by formation of metal–organic complexes and interactions with solid soil material. Eur. J. Soil Sci. 55:287–297.

Julien, F., B. Gueroux, and M. Mazet. 1994. Comparison of organic-compounds removal by coagulation–flocculation and by adsorption onto preformed hydroxide flocs. Water Res. 28:2567–2574.

Kaiser, K., and G. Guggenberger. 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Org. Geochem. 31:711–725.

Kaiser, K., G. Guggenberger, and W. Zech. 2000. Organically bound nutrients in dissolved organic matter fractions in seepage and pore water of weakly developed forest soils. Acta Hydrochim. Hydrobiol. 28:411–419.

Kaiser, K., and W. Zech. 2000. Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. Eur. J. Soil Sci. 51:403–411.

Kalbitz, K., B. Glaser, and R. Bol. 2004. Clear-cutting of a Norway spruce stand: Implications for controls on the dynamics of dissolved organic matter in the forest floor. Eur. J. Soil Sci. 55:401–413.

Kalbitz, K., J. Schmerwitz, D. Schwesig, and E. Matzner. 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113:273–291.

Kalbitz, K., D. Schwesig, J. Rethemeyer, and E. Matzner. 2005. Stabilization of dissolved organic matter by sorption to the mineral soil. Soil Biol. Biochem. 37:1319–1331.

Kalbitz, K., S. Solinger, J.H. Park, B. Michalzik, and E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Sci. 165:277–304.

Lofts, S., C. Woof, E. Tipping, N. Clarke, and J. Mulder. 2001. Modelling pH buffering and aluminium solubility in European forest soils. Eur. J. Soil Sci. 52:189–204.

Lumsdon, D.G., M.I. Stutter, R.J. Cooper, and J.R. Manson. 2005. Model assessment of biogeochemical controls on dissolved organic carbon partitioning in an acid organic soil. Environ. Sci. Technol. 39:8057–8063.

Masion, A., F. Thomas, D. Tchoubar, J.Y. Bottero, and P. Tekely. 1994. Chemistry and structure of Al(OH)/organic precipitates—a small-angle x-ray scattering study. 3. Depolymerization of the Al_{13} polycation by organic ligands. Langmuir 10:4353–4356.

Matzner, E., T. Zuber, and G. Lischeid. 2004. Response of soil solution chemistry and solute fluxes to changing deposition rates. p. 339–360. In E. Matzner (ed.) Biogeochemistry of forested catchments in a changing environment: A German case study. Ecol. Stud. 172. Springer, Berlin.

McKnight, D.M., R. Harnish, R.L. Wershaw, J.S. Baron, and S. Schiff. 1997. Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. Biogeochemistry 36:99–124.

Michalzik, B., and E. Matzner. 1999. Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. Eur. J. Soil Sci. 50:579–590.

Mulder, J., N. van Breemen, and H.C. Eijck. 1989. Depletion of soil aluminium by acid deposition and implications for acid neutralization. Nature 337:247–249.

Nierop, K.G.J., B. Jansen, and J.M. Verstraten. 2002. Dissolved organic matter, aluminium and iron interactions: Precipitation induced by metal/carbon ratio, pH and competition. Sci. Total Environ. 300:201–211.

Plankey, B.J., and H.H. Patterson. 1987. Kinetics of aluminum–fulvic acid complexation in acidic waters. Environ. Sci. Technol. 21:595–601.

Römkens, P.F.A.M., and J. Dolfing. 1998. Effect of Ca on the solubility and molecular size distribution of DOC and Cu binding in soil solution samples. Environ. Sci. Technol. 32:363–369.

Schwesig, D., K. Kalbitz, and E. Matzner. 2003. Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. Eur. J. Soil Sci. 54:311–322.

Simonsson, M. 2000. Interactions of aluminium and fulvic acid in moderately acid solutions: Stoichiometry of the H^+/Al^{3+} exchange. Eur. J. Soil Sci. 51:665–666.

Solinger, S., K. Kalbitz, and E. Matzner. 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55:327–349.

SPSS. 2002. SigmaPlot. Release 8.0. SPSS Inc., Chicago.

SPSS. 2003. SPSS for Windows. Release 12.0.1, SPSS Inc., Chicago.

Thomas, F., A. Masion, J.Y. Bottero, J. Rouiller, F. Montigny, and F. Genévrier. 1993. Aluminum(III) speciation with hydroxy carboxylic acids. ^{27}Al NMR study. Environ. Sci. Technol. 27:2511–2516.

Weng, L., E.J.M. Temminghoff, and W.H. van Riemsdijk. 2002. Interpretation of humic acid coagulation and soluble soil organic matter using a calculated electrostatic potential. Eur. J. Soil Sci. 53:575–587.

Worrall, F., R. Harriman, C.D. Evans, C.D. Watts, J. Adamson, C. Neal, E. Tipping, T. Burt, I. Grieve, D. Monteith, P.S. Naden, T. Nisbet, B. Reynolds, and P. Stevens. 2004. Trends in dissolved organic carbon in UK rivers and lakes. Biogeochemistry 70:369–402.

Yamaguchi, N., S. Hiradate, M. Mizoguchi, and T. Miyazaki. 2004. Disappearance of aluminum tridecamer from hydroxyaluminum solution in the presence of humic acid. Soil Sci. Soc. Am. J. 68:1838–1843.

Yu, Z., Q. Zhang, T.E.C. Kraus, R.A. Dahlgren, C. Anastasio, and R.J. Zasoski. 2002. Contribution of amino compounds to dissolved organic nitrogen in forest soils. Biogeochemistry 61:173–198.

Zarda, B., D. Hahn, A. Chatzinotas, W. Schönhuber, A. Neef, R.I. Amann, and J. Zeyer. 1997. Analysis of bacterial community structure in bulk soil by in situ hybridization. Arch. Microbiol. 168:185–192.

Zysset, M., and D. Berggren. 2001. Retention and release of dissolved organic matter in Podzol B horizons. Eur. J. Soil Sci. 52:409–421.

Chapter III

Precipitation of enzymes and organic matter by aluminium – impacts on carbon mineralization

Thorsten Scheel[a]

Karin Pritsch[b], Michael Schloter[c] and Karsten Kalbitz[a]

[a]Department of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany.

[b]Chair for Soil Ecology, Technical University of Munich, 85764 Neuherberg, Germany

[c]Institute of Soil Ecology, GSF-National Research Center for Environment and Health, 85764 Neuherberg, Germany

Journal of Plant Nutrition and Soil Science 171 (2008), 900-907

0. Abstract

The precipitation of dissolved organic matter (DOM) by Al results in a stable soil organic matter (OM) fraction. Extracellular enzymes can also be removed from soil solution by sorption or precipitation, but whether this affects their activity and their importance for C mineralization is largely unknown. We studied the activity of eight extracellular enzymes, precipitated by Al together with DOM, in relation to C mineralization of the precipitated OM. Dissolved organic matter was obtained from the Oi and Oa horizon of two forest soils and precipitated at different Al/C ratios and pH values to achieve a large variation in composition and C mineralization of precipitated OM. All eight enzymes were present in a functional state in precipitated OM. On average 53% of DOM was precipitated, containing on average 17–41% of the enzyme activity (EA) involved in C degradation (chitinase, cellobiohydrolase, β-glucosidase, glucuronidase, lacasse and xylosidase) previously present in soil solution. In contrast, on average only 4–7% of leucine-aminopeptidase and acid-phosphatase activity was found in precipitated OM. The EA found in precipitates significantly increased the percentage of C mineralized of precipitated OM, with a stronger influence of C degrading enzymes than enzymes involved in N and P cycling. However, after eight weeks of incubation the correlations between EA and C mineralization disappeared, despite substantial EA being still present and only 0.5-7.7% of C mineralized. Thus, degradation of precipitated OM seems to be governed by EA during the first degradation phase, but the long term stability of precipitated OM is probably related to its chemical properties.

1. Introduction

Soil enzymes are the drivers for the turnover of carbon and all essential nutrient elements including nitrogen and phosphorus, which are of central importance for plant nutrition and health (Nannipieri et al., 2003). Soil enzymes are not only associated with proliferating cells but also associated as extracellular enzymes with organic colloids and clay minerals (Kandeler, 2007). Measurements of the activity of extracellular soil enzymes give valuable information about effects of changing environmental conditions on soil organic matter decomposition (Sinsabaugh et al., 2005; Allison and Vitousek, 2004). Activities of extracellular enzymes in soils and hence their significance for C mineralization show distinct differences depending on the particle-size fraction in which they are measured (Marx et al., 2005; Allison and Jastrow, 2006). However, it is speculative whether also dissolved organic matter (DOM) comprises significant amounts of enzymes (McDowell, 2003). A large fraction (80-90%) of DOM derived from organic horizons of acidic forest soils can be precipitated by metal cations (Fe, Al) and their hydroxides (Nierop et al., 2002; Scheel et al., 2007) at concentrations also found at the respective field sites. However, it is unknown, to which extent enzymes are precipitated by metal cations, e.g. Al, or co-precipitated with DOM. Further, we have no knowledge, whether enzymes are present in DOM in significant amounts, and if precipitation affects their activity and consequently their influence on C mineralization. Tietjen and Wetzel (2003) reported that sorption of enzymes onto clay particles led in most cases to a reduction of enzyme activity, but they also found an enhancement of enzyme activity by sorption.

It has been shown that precipitation of DOM by Al results in a distinct reduction of C mineralization (Scheel et al., 2007) when compared with bulk DOM. The resulting carbon mineralization of precipitated OM ranged from 0.5 to 7.7% of initial C after 8 weeks of incubation, whereas the respective DOM solutions showed with 5 to 49% a much larger C mineralization (Scheel et al., 2007). Thus, precipitated OM comprises a very stable carbon pool and C mineralization depends mainly on organic matter properties. The smallest mineralization rates were observed for precipitated OM with high contents of aromatic C and large C/N ratios (Scheel et al., 2007). It remained unknown whether a reduced activity of extracellular enzymes additionally contributes to the observed stability of precipitated OM or if it is only determined by their chemical properties.

In the present paper, we studied if selected enzymes are co-precipitated with DOM through use of Al and if precipitation of these enzymes affects their activity and the carbon mineralization of the precipitated OM. For this experiment samples were selected that showed the largest possible differences in C mineralization and chemical properties of precipitated OM (Scheel et al., 2007). The chosen solutions and conditions were typical for soil solutions entering the mineral horizon, therefore being an appropriate experimental analogue for the processes occurring in situ. We hypothesized that precipitated OM will contain a significant fraction of co-precipitated enzymes and that they will influence the amount of C mineralized. For this purpose, we investigated the activity of eight different extracellular enzymes in OM precipitated by Al and the relation of their enzymatic activity (EA) to C mineralization of precipitated OM.

2. Materials and Methods

2.1. Samples

Samples of Oi and Oa horizons from a Norway spruce (Waldstein-Fichtelgebirge, Germany; Michalzik and Matzner, 1999) and a European beech (Steinkreuz-Steigerwald, Germany; Solinger et al., 2001) forest site were homogenized and stored frozen. Water extracts were prepared at 5°C with a soil to (ultrapure) water ratio of 1:10 (Scheel et al., 2007). After one day, DOM solutions were filtered through a preconditioned ceramic filter plate and filtered through a 0.2-µm membrane filter (OE 66, Schleicher and Schuell) at 5°C to exclude microorganisms. The DOM solutions were diluted with ultrapure water to 40 mg C L^{-1} to ensure comparability.

2.2. Precipitation of dissolved organic matter and enzymes

To precipitate the DOM and enzymes at conditions similar to those in acidic forest soils, Al was added as $AlCl_3 * 6H_2O$ solution (Al/C ratios: 0.1 and 0.3) at pH values of 3.8 and 4.5 to the 4 different DOM solutions. The resulting 16 treatments were shaken separately for 24 h and precipitates loaded onto individual quartz fibre filters (QF20, Schleicher & Schuell) which were subsequently cut into small pieces of identical surface (7 mm²). Filter pieces without precipitates were treated similar and used as control (see below).

2.3. Incubation

Microbial inoculum was prepared from 4 m*M* $CaCl_2$ extracts (soil/solution ratio 1:2, 30 min of extraction) of Oa material of both investigated sites (Scheel et al., 2007). After filtration through 5-µm filters (SMWP 4700, Millipore) and enumeration of cells both extracts were combined and used for inoculation (Scheel et al., 2007). Filter pieces loaded with precipitates and unloaded filter pieces were incubated in 60 ml nutrient solution (NH_4NO_3 and K_2HPO_4, C/N and C/P ~ 20) after addition of 1 ml inoculum (5×10^6 cells per flask) at pH 4.5 and 20 °C for up to 8 weeks (Scheel et al., 2007).

2.4. Carbon mineralization

Data on carbon mineralization were taken from an parallel performed experiment (Scheel et al., 2007; Table III.1) with identical sources of soil for water extracts and subsequent precipitation of OM and identical sources of microbial inoculum. In the current study, we correlated the corresponding C mineralization rates to enzyme activities.

Table III.1: Carbon precipitated from dissolved organic matter (DOM) solutions in % and carbon mineralization of precipitated organic matter (OM) in % of initial carbon after 8 weeks of incubation. Precipitated OM was produced from 4 DOM solutions at 2 pH and 2 Al/C ratios. Data of C mineralization was taken from Scheel et al. (2007). Mean values of 3 replicates.

	C precipitated [%]				C mineralized [%]			
	Oa-spruce	Oa-beech	Oi-spruce	Oi-beech	Oa-spruce	Oa-beech	Oi-spruce	Oi-beech
pH 3.8 – Al/C 0.1	56.8	54.0	38.3	38.2	0.5	1.6	2.1	3.7
pH 3.8 – Al/C 0.3	71.8	58.6	40.9	26.9	0.8	2.1	1.7	4.6
pH 4.5 – Al/C 0.1	79.5	62.6	51.0	40.0	1.2	2.7	3.1	6.7
pH 4.5 – Al/C 0.3	87.0	71.1	48.9	29.1	1.1	3.2	5.3	7.7

2.5. Analyses and measurement of enzyme activity

The DOM solutions and filtrates were analyzed for total organic C and total N (High TOC, Elementar), NO_3 (ion chromatography, Dionex DX 100), NH_4 (flow injection, photometric mLE–FIA LAB), total Al (inductively coupled plasma–optical emission spectrometer [ICP–OES], GCP

Electronics) and pH (pH 323, WTW). The amount of precipitated OM was considered as the difference in concentrations before and after precipitation.

Enzyme activities (see Table III.2 for the analyzed enzymes) were measured of i) the four DOM solutions, to determine the original activity; ii) filter pieces without precipitated OM, inoculated and incubated for 1, 4, and 8 weeks as control for substrates and enzymes added along with the inoculum; iii) filter pieces loaded with precipitates before incubation and after incubation for 1, 4 and 8 weeks.

Table III.2: Enzyme activities (in µmol MUF/AMC/ABTS day^{-1} g C^{-1}) of the four dissolved organic matter solutions before precipitation of organic matter. Mean values of 6 replicates (SE in brackets).

	Oa-spruce		Oa-beech		Oi-spruce		Oi-beech	
chitinase	537	(15)	1315	(30)	12377	(151)	6035	(44)
1,4-β-cellobiohydrolase	1066	(54)	1054	(41)	2921	(57)	1662	(37)
β-glucosidase	321	(10)	540	(13)	5335	(53)	9599	(194)
β-D-glucuronidase	121	(6)	189	(11)	412	(6)	440	(10)
laccase	2730	(136)	6840	(196)	29102	(161)	74672	(423)
1,4-β-xylosidase	148	(11)	425	(10)	1282	(23)	2532	(38)
leucine-aminopeptidase	555	(10)	651	(24)	328	(16)	679	(29)
acid-phosphatase	18496	(241)	23724	(732)	47771	(1550)	52809	(581)

Enzyme assays were performed at pH 4.5 that was the optimum for most enzymes except leucine-aminopeptidase with optimal pH 6.5 using substrate concentrations as in the protocol of Courty et al. (2005) with prolonged incubation times: Methylumbelliferyl (MU)-β-1,4-N-acetylglucosaminide 500 µM / chitinase (EC 3.2.1.14) 40 min, MU-cellobiohydrofurane 400 µM / 1,4-β-cellobiohydrolase (EC 3.2.1.91) 80 min, MU-β-1,4-glucopyranoside 500 µM / β-glucosidase (EC 3.2.1.21) 40 min, MU-ß-D-glucuronide 500 µM / ß-D-glucuronidase (EC 3.2.1.31) 120 min, MU-phosphate 800 µM / acid phosphatase (EC 3.1.3.2) 20 min, MU-ß-D-xyloside 500 µM / Xylan 1,4-β-xylosidase (EC 3.2.1.37) 120 min, L-Leucin-7-amido-4-methylcoumarin hydrochloride (Leu-AMC) 400 µM / leucine-aminopeptidase (EC 3.4.11.1) 120 min, and 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) 2 mM / laccase (EC 1.10.3.2) 120 min. Filter pieces were directly placed in the incubation wells of a microtiter plate, that was supplied with 50 µL of ultrapure H_2O, and 50 µL of buffer (as a mixture of 100 mM maleic acid and 100 mM Tris to the desired pH). In each well 50 µL of substrate solution were added and the reaction was stopped with 100 µL of 2.5 M Tris. For determining EA in DOM 50 µL H_2O were replaced by 50 µL DOM solution. All EA measurements were performed in 6 replicates. The small EA of the control filter pieces without precipitated OM (Table III.3) was subtracted from that of precipitated organic matter. Further, we corrected the measured EA for the autofluorescence of the sample, the quenching of the fluorescence signal by OM and the fluorescence added with the enzyme substrate itself. Finally, enzyme activities were related to the carbon content of the measured samples.

Table III.3: Enzyme activities (in pmol MUF/AMC/ABTS min^{-1} cm^{-2}) of the inoculated incubation solutions without precipitated organic matter (control) and with precipitated organic matter (OM) after 1 week of incubation. Mean values of all 16 treatments (4 DOM solutions, 2 pH and 2 Al/C ratios) of 6 replicates.

	control	Precipitated OM		
		Min	Median	Max
chitinase	2	15	162	1615
1,4-β-cellobiohydrolase	1	31	65	2410
β-glucosidase	5	8	162	2821
β-D-glucuronidase	1	3	14	101
laccase	142	282	1287	13011
1,4-β-xylosidase	4	11	50	639
leucine-aminopeptidase	8	20	40	111
acid-phosphatase	108	400	899	4843

2.6. Statistics and Calculations

The measured EA of non-incubated precipitates was used to calculate the proportion of EA found in the precipitates relative to the EA of the original DOM. For this purpose, the EA measured in the non-incubated precipitated OM was expressed as fraction of the EA determined in the respective DOM solutions (set 100%) from which it was precipitated. Between the logarithmic values of the initial EA of precipitated OM and the C mineralization after incubation linear regressions were performed (significance: $p<0.05$). The regressions between EA and C mineralization rates after 1, 4, and 8 weeks of incubation were calculated similarly (significance: $p<0.05$).

3. Results and Discussion

3.1. Enzyme activity in dissolved and precipitated organic matter

The amount of C precipitated ranged from 27 to 87% (on average 53%; Table III.1; Fig. III.1) of initial C of the DOM solutions. The four DOM solutions contained significant amounts of enzyme activity (Table III.2) with significantly larger EA for solutions from Oi horizons compared to solutions from Oa horizons. Only leucine-aminopeptidase had similar activities in all solutions. For enzymes mainly involved in the degradation of carbon sources, the fraction of EA found in the precipitates was 17–41% on average of the original activity in DOM (Fig. III.1). In contrast, precipitated OM displayed on average only 4% and 7% of leucin-aminopeptidase and acid-phosphatase activity, enzymes mainly involved in the transformation of N and P (Fig. III.1). However, there was no significant correlation between the amount of C precipitated and the fraction of EA found in the precipitated OM for all investigated enzymes. Thus, also larger pH values and Al/C ratios, basically influencing the amount of C precipitating, had no significant influence on the fraction of EA found in the precipitates. Consequently, we will not include these factors in the discussion.

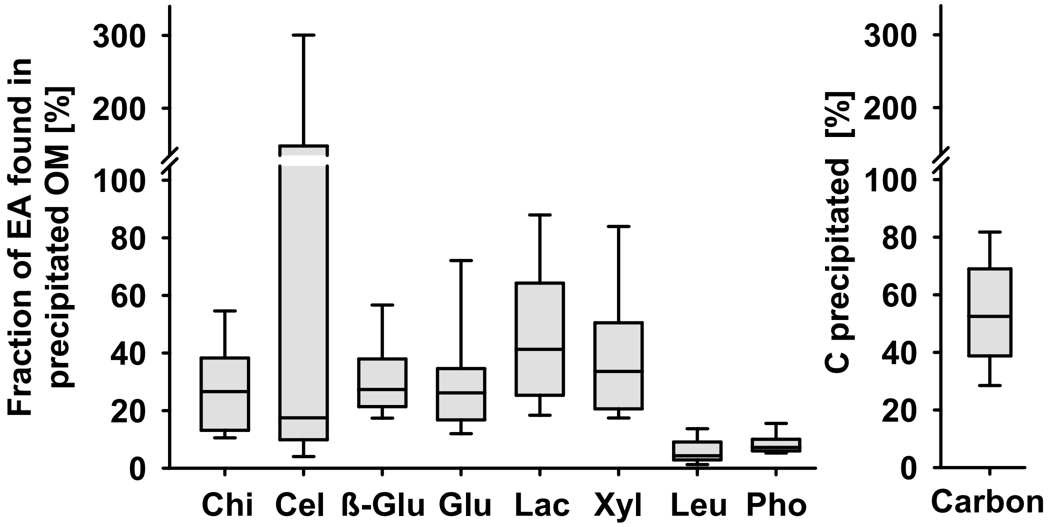

Fig. III.1: Fraction of enzyme activity (EA) in the precipitated organic matter (OM) related to the EA in the respective DOM solution (left). Carbon precipitated from DOM solutions (right). The box plots display the median of all 16 treatments (4 DOM solutions, 2 pH values, 2 Al/C ratios), the upper and lower quartile and the 5% and 95% percentiles.

The proportion of EA found in the precipitates was in nearly all cases smaller than the fraction of precipitated carbon, which could be the result of a lower tendency to precipitate due to chemical properties, e.g. low C/N ratio, of some enzymes. Scheel et al. (2007) reported a preferential precipitation of C compared to N for three of the four DOM solutions. Additionally, aluminium could bind to substrates preventing their degradation or inhibit enzymes (Garciduenas Pina and Cervantes, 1996) thus decreasing apparent enzyme activity. Also Tietjen and Wetzel (2003) observed that sorption of alkaline-phosphatase and leucine-aminopeptidase onto clay surfaces led to a strong decrease in activity, whereas the activity of β-glucosidase and xylosidase did increase. The activity of cellobiohydrolase was in some precipitated OM even larger than in the original DOM solution (Fig. III.1). This may be explained by the larger spatial concentration of substrate in precipitated OM or the activation of enzymes (Tietjen and Wetzel, 2003). Taking into account the addition of Al, the low pH values and the subsequent precipitation, EA found in precipitated OM was still substantial. While direct protein

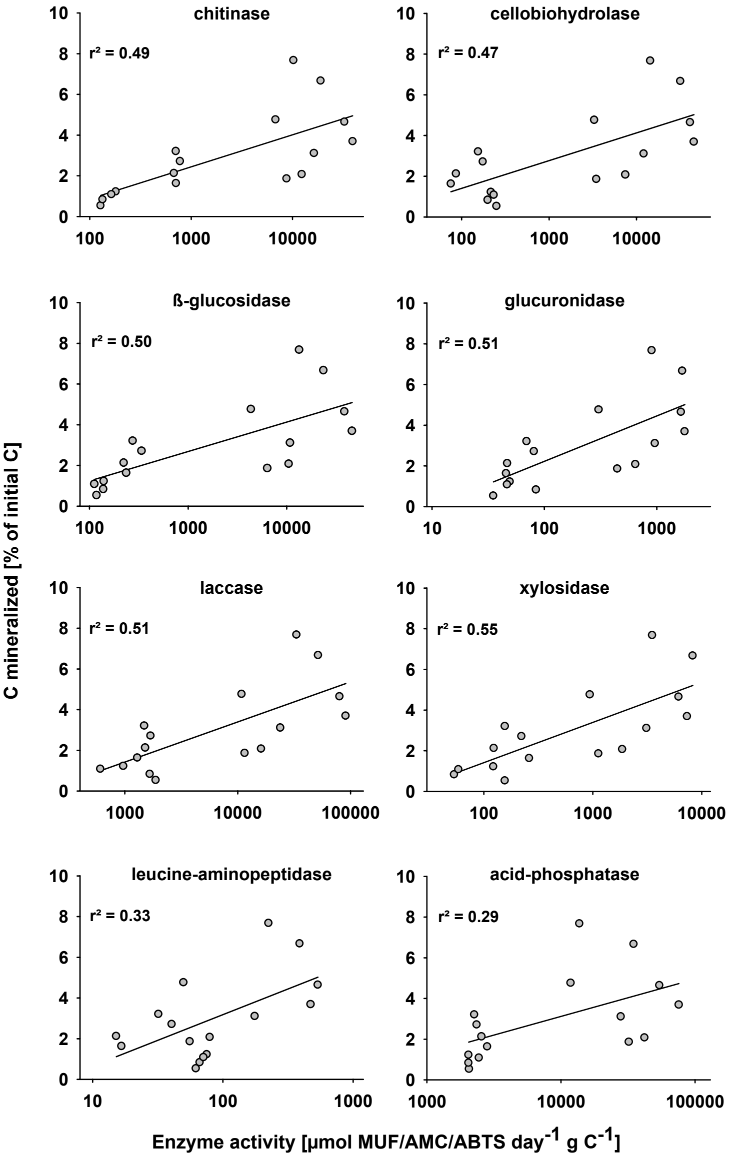

Fig. III.2: Influence of initial enzyme activity (EA) of precipitated organic matter (OM) before incubation on the amount of C mineralized after 8 weeks of incubation of precipitated OM (significance: $p<0.05$). Mean values of 6 replicates (4 DOM solutions, 2 pH values, 2 Al/C ratios).

detection methods in soil water DOM failed to detect enzymes involved in degradation of biological materials (Schulze et al., 2005) the indirect enzyme detection via their activity showed that extracellular enzymes were present in a functional state in 0.2 µm filtered DOM and precipitated OM.

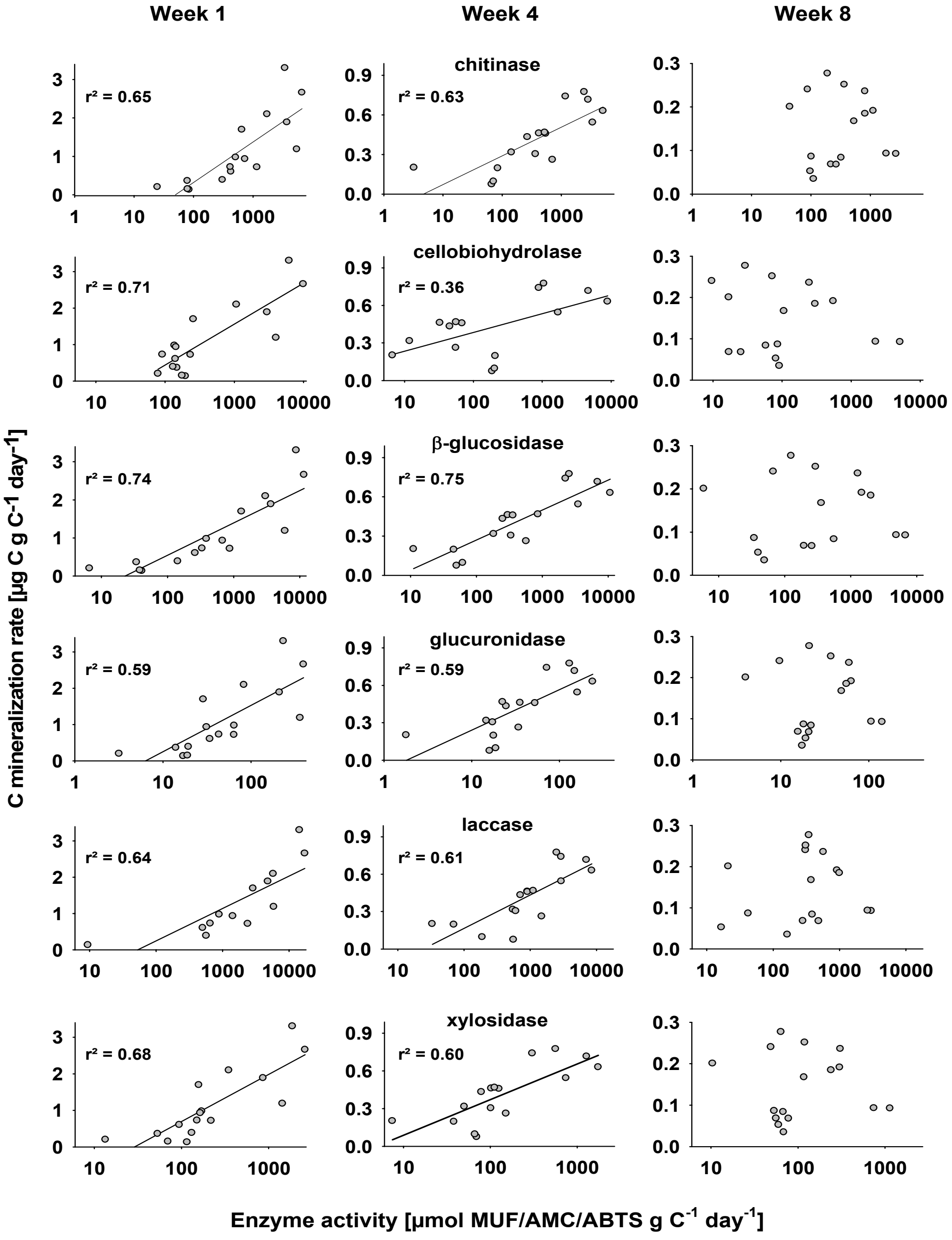

Fig. III.3: Relationship between 6 different enzyme activities (EA) involved in C degradation and the C mineralization rate (Cmin) of precipitated organic matter (4 DOM solutions, 2 pH values, 2 Al/C ratios) after 1, 4 and 8 weeks of incubation (significance: $p<0.05$). Mean values of 6 replicates.

3.2. Effect of enzyme activity on C mineralization

The activity of all eight enzymes measured in precipitated OM before incubation significantly correlated with the fraction of C mineralized (Fig. III.2), indicating an influence of precipitated enzymes on C mineralization. The coefficients of determination for acid-phosphatase and leucine-aminopeptidase were smaller than for the other enzymes, probably because they do not directly affect C degradation. A further explanation might be the smaller proportion of EA of these two enzymes found in precipitated OM (Fig. III.1).

The C mineralization rates after one week (r^2=0.59–0.74) and 4 weeks (r^2=0.36–0.75) are still strongly correlated logarithmically with the activity of enzymes involved in C degradation (Fig. III.3). The correlations were again weaker for acid-phosphatase and leucine-aminopeptidase (Fig. III.4). The logarithmic relation between the EA and the C mineralization rate implies that the C mineralization did not increase directly proportional to the increase in EA. This indicates that with increasing EA the availability of substrate became limited. After 8 weeks of incubation no correlations between C mineralization rate and EA were visible (Fig. III.3 and III.4). As C mineralization only amounted up to 0.5-7.7% of precipitated OM at this time and EA was still substantial, we suppose that the more labile fraction was degraded first and that enzymes were probably hindered to degrade the stable fraction in an analogous manner. This indicates that

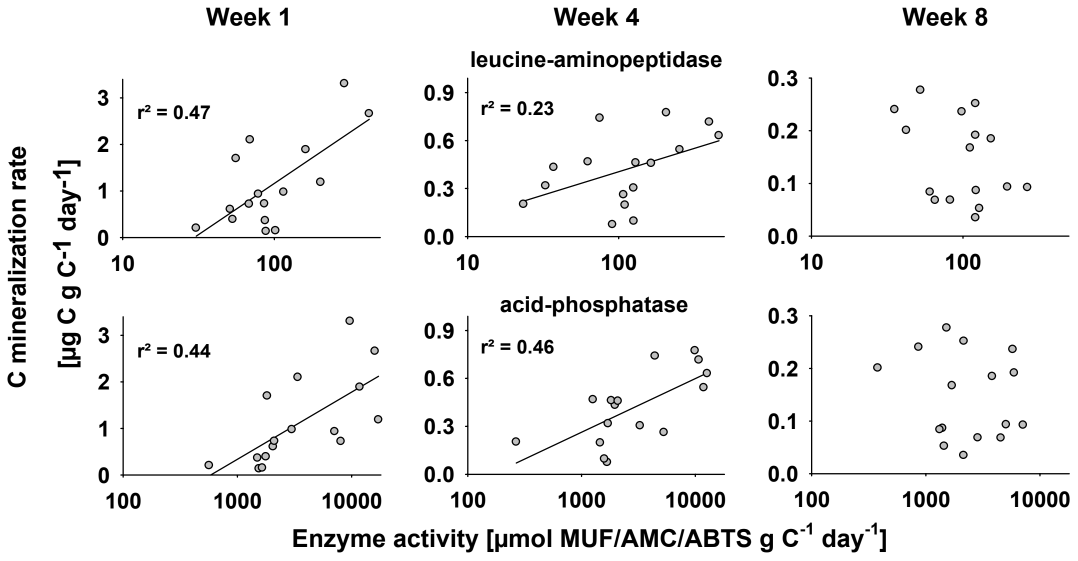

Fig. III.4: Relationship between the activity of two enzymes involved in P and N cycling and the C mineralization rate (Cmin) of precipitated organic matter (4 DOM solutions, 2 pH values, 2 Al/C ratios) after 1, 4 and 8 weeks of incubation (significance: $p<0.05$). Mean values of 6 replicates.

chemical properties, like C/N ratio and aromatic C content (Scheel et al., 2007), could be the main factors for the large stability of precipitated OM.

We did not observe an increasing activity of leucine-aminopeptidase and acid-phosphatase in samples with large C/N and C/P ratios (Fig. III.5). In contrast, acid-phosphatase activity decreased with increasing C/P ratios may reflected the microbial activity in these samples before filtration and precipitation (Fig. III.5, bottom). Thus, an increasing activity of leucine-aminopeptidase or acid-phosphatase in order to acquire the necessary nutrients could be ruled out. This effect should have become particularly evident with increasing duration of incubation. However, we did not find such relationships in our samples (Fig. III.5) probably because of the addition of nutrients with the incubation solution. Also for enzymes involved in C degradation, there was no response of microorganisms to wide C to nutrient ratios of precipitated OM, e.g. laccase activity (Fig. III.5).

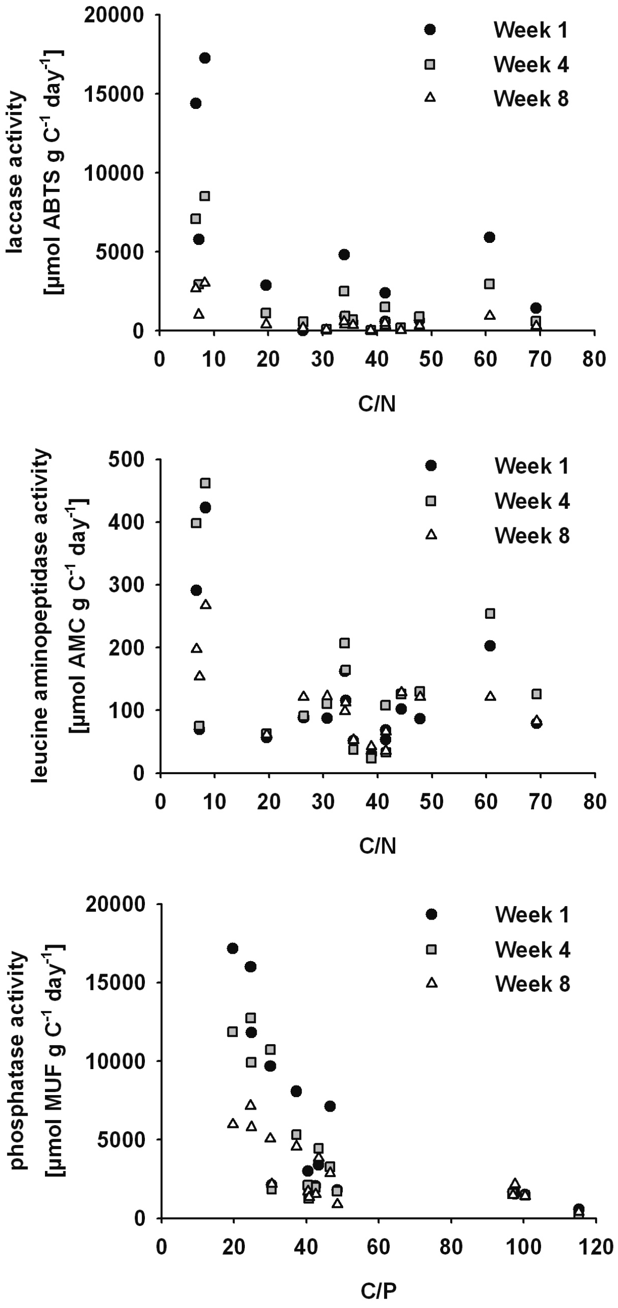

Fig. III.5: Relationship between the C/N ratio of precipitated organic matter (OM) and laccase activity (top), leucine aminopeptidase activity (middle), each after 1, 4 and 8 weeks of incubation. Further, the C/P ratio of precipitated OM was related to phosphatase activity (bottom), after 1, 4 and 8 weeks of incubation. Mean values of 6 replicates of all 16 treatments (4 DOM solutions, 2 pH values, 2 Al/C ratios).

3.3. Changes in enzyme activity during incubation

After one week of incubation, all enzymes involved in C degradation showed a decrease in activity when compared with EA of precipitated OM before incubation (Table III.4). The decrease in EA became even more pronounced after 8 weeks of incubation, with the largest reduction for laccase with only 5% of initial EA left. The strong decrease in laccase activity with time indicates that this enzyme was rapidly inactivated, e.g. by degradation of the enzyme. Formation of new laccase enzymes was supposedly low since we could not observe fungal components in the inoculum by microscope, the main producers of laccases. The majority of fungi are located in the organic horizon of a soil profile, whereas precipitation of OM is supposed to occur in the mineral horizon. Therefore, the small contribution of fungi to C mineralization and enzyme formation may even reflect the situation in situ. The large decrease of laccase activity could be an explanation for one mechanism of organic matter stabilization, the selective enrichment of aromatic compounds (von Lützow et al., 2006). This was observed after incubation of precipitated OM (Scheel et al., 2007) and in other incubation studies (Kalbitz et al., 2003). Thus, a stable subsequent delivery of new enzymes by bacteria and fungi is of great importance for continuous C mineralization.

The decrease in activity was less pronounced for acid-phosphatase and only for leucine-aminopeptidase an increase in EA after incubation was found. The increase in activity of leucine-

Table III.4: Proportion of EA found in the precipitates after 1 week, 4 weeks and 8 weeks of incubation when compared with the EA of precipitates before incubation, which was set 100%. Values are medians of all 16 treatments (4 DOM solutions, 2 pH values, 2 Al/C ratios; lower and upper quartile in brackets), where each n represents a mean of 6 analytical replicates.

	Proportion of EA after incubation for					
	1 week [%]		4 weeks [%]		8 weeks [%]	
chitinase	39	(14; 54)	27	(5; 48)	16	(6; 49)
cellobiohydrolase	48	(24; 89)	28	(8; 49)	14	(4; 37)
β-glucosidase	42	(10; 75)	44	(9; 64)	28	(5; 38)
glucuronidase	27	(9; 48)	19	(5; 43)	16	(6; 39)
laccase	20	(3; 42)	14	(7; 33)	5	(3; 11)
xylosidase	40	(17; 60)	28	(12; 50)	20	(9; 46)
leucine-aminopeptidase	135	(103; 266)	162	(131; 230)	155	(69; 201)
acid-phosphatase	67	(23; 81)	66	(12; 78)	34	(14; 69)

aminopeptidase could be attributable to the very low starting value, because of the small amount of EA found in precipitated OM, when compared with all other enzymes, so that the microbial contribution originating from the inoculum became observable.

4. Conclusions

The use of highly sensitive assays enabled us to observe that enzymes are significant components in the extracted DOM and possess a large variation in the susceptibility for precipitation by Al. From our results we can conclude that precipitated extracellular enzymes were in a functional state and played a significant role in the turnover of a stable soil organic matter fraction like precipitated OM during the initial degradation phase. As there was no correlation between enzyme activity and C mineralization rate at the end of the experiment we conclude that the stability of precipitated OM is mainly governed by chemical properties and not by lack of enzymes. Nevertheless, we can not rule out that C mineralization was also limited especially by the strong decrease in laccase activity over time. During incubation also new enzymes might have been formed. Although our data indicate large effects of enzymes previously present in DOM and a declining influence over time, our experimental design did not allow to differentiate between precipitated and newly formed enzymes. Thus, a distinction of these two sources would enable us to elucidate the conditions for stabilization and partial inhibition of extracellular enzymes in soils and the delivery of extracellular enzymes by bacteria and fungi, and consequently their importance for SOM mineralization.

5. References

Allison, S. D., Vitousek, P. M. (2004): Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36, 285-296.

Allison, S. D., Jastrow, K. D. (2006): Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem. 38, 3245-3256.

Courty, P. E., Pritsch, K., Schloter, M., Hartmann, A., Garbaye, J. (2005): Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytol. 167, 309-319.

Garciduenas Pina, R., Cervantes, C. (1996): Microbial interactions with aluminium. BioMetals 9, 311-316.

Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumaier, L., Glaser, B., Ellerbrock, R., Leinweber, P. (2003): Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biol. Biochem. 35, 1129-1142.

Kandeler, E. (2007): Physiological and biochemical methods for studying soil biota and their function, in Paul, E. A.: Soil microbiology, ecology, and biochemistry. Third edition, Academic Press, Oxford, UK, pp. 53-83.

Marx, M. C., Kandeler, E., Wood, M., Wermbter, C., Jarvis, S. C. (2005): Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biol. Biochem. 37, 35-48.

McDowell, W. H. (2003): Dissolved organic matter in soils – future directions and unanswered questions. Geoderma 113, 179-186.

Michalzik, B., Matzner, E. (1999): Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. Eur. J. Soil Sci. 50, 579–590.

Nannipieri, P., Ascher, J., Ceccherini, L., Landi, L., Pietramellara, G., Renella, G. (2003): Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655-670.

Nierop, K. G. J., Jansen, B., Verstraten, J. M. (2002): Dissolved organic matter, aluminium and iron interactions: Precipitation induced by metal/carbon ratio, pH and competition. Sci. Total Environ. 300, 201–211.

Scheel, T., Dörfler, C., Kalbitz, K. (2007): Precipitation of dissolved organic matter by Al stabilizes C in acidic forest soils. Soil Sci. Soc. Am. J. 71, 64-74.

Schulze, W. X., Gleixner, G., Kaiser, K., Guggenberger, G., Mann, M., Schulze, E. D. (2005): A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia 142, 335-343.

Sinsabaugh, R. L., Gallo, M. E., Lauber, C., Waldrop, M. P., Zak, D. R. (2005): Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochem. 75, 201-215.

Solinger, S., Kalbitz, K., Matzner, E. (2001): Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochem. 55, 327–349.

Tietjen, T., Wetzel, R. G. (2003): Extracellular enzyme-clay mineral complexes: Enzyme adsorption, alteration of enzyme activity, and protection from photodegradation. Aquat. Ecol. 37, 331-339.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. (2006): Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions. Eur. J. Soil Sci. 57, 426-445.

Chapter IV

Stabilization of dissolved organic matter by Aluminium – A toxic effect or stabilization through precipitation?

Thorsten Scheel[a]

Boris Jansen[b], Antonius J van Wijk[b], Jacobus M Verstraten[b] and Karsten Kalbitz[a]

[a]Department of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany.

[b]IBED-Earth Surface Processes and Materials, Universiteit van Amsterdam, NL-1018WV Amsterdam, The Netherlands

European Journal of Soil Science 59 (2008), 1122-1132

0. Abstract

It is well known that carbon mineralization in acidic forest soils can be reduced by large Al concentrations. However, it is still unclear whether Al reduces C mineralization by direct toxicity to microorganisms or by decreased bioavailability of organic matter (OM) as dissolved organic matter (DOM) is precipitated by Al. We performed an incubation experiment (6 weeks) with two DOM solutions (40 mg C l^{-1}) derived from two acidic forest sites and possessing large differences in composition. Aluminium was added to the solutions in realistic ranges for acidic soils (1.6-24 mg Al l^{-1}) at pH values of 3.8 and 4.5, to achieve differences in Al speciation. We determined different Al species including the potentially toxic Al^{3+}, by Diffusive Gradients in Thin Films (DGT) in order to evaluate toxic effects on microorganisms. Precipitation of OM increased with larger amounts of added Al and higher pH and we measured a larger fraction of dissolved ‘free’ Al at pH 3.8 than at pH 4.5. Carbon degradation significantly decreased with Al addition and surprisingly we found larger C degradation at pH 3.8 than at pH 4.5 for the respective Al additions. Consequently, the observed reduction in C degradation (i.e. stabilization) cannot be explained by toxic effects of ‘free’ Al. However, C stabilization correlated significantly with C precipitation. The pH did not influence C stabilization directly, but determined the amount of C being precipitated. Phosphorous was removed along with OM by precipitation which possibly also affected C stabilization. We conclude that C stabilization upon Al addition did not result from toxic effects, but was caused by reduced bioavailability of OM after its precipitation. Considering that the C degradation was reduced by up to 65%, this is of large relevance for the overall C stabilization in acidic forest soils. Increasing pH and decreasing Al concentrations upon recovery from acidic deposition should therefore not result in decreased stabilization of OM by this mechanism.

1. Introduction

Dissolved organic matter (DOM) is considered as an easily degradable fraction when compared with solid soil organic matter (Burford & Bremner, 1975). Microorganisms take up most substrates and nutrients directly from the soil solution in dissolved form. Therefore, the transition of carbon from the liquid to the solid phase should affect microorganisms directly by decreasing the bioavailability. In the context of organic matter (OM), the term bioavailability describes the potential of microorganisms to interact with organic compounds (Marschner & Kalbitz, 2003). Precipitation of DOM by polyvalent metal cations can remove up to 90% of C from solution (Nierop et al., 2002; Scheel et al., 2007), with clearly smaller precipitation of C by Al at pH 3.5 when compared with pH 4.5. Soil solutions with a larger amount of aromatic and complex structures also exhibit larger fractions of C being precipitated (Scheel et al., 2007).

The precipitated OM is very stable against microbial decay. In a previous study, carbon mineralization of precipitated OM ranged from 0.5 to 7.7% after 7 weeks of incubation, whereas with 5 to 49% the respective DOM solutions were mineralized to a much larger extent (Scheel et al., 2007). Precipitation of OM by Al also resulted in a reduction of the total C mineralization, when precipitated OM was not separated from solution (Schwesig et al., 2003). This stabilization might be a direct consequence of the decreased bioavailability. Microbial growth and hence C mineralization can also be inhibited when essential nutrients such as phosphate are removed from soil solution by sorption or precipitation (Khare et al., 2005; Borggaard et al., 2005).

Aluminium concentrations leading to toxic effects strongly depend on the species of microorganisms and can be as low as 50 µM (Pina & Cervantes, 1996) whereas other species can survive concentrations of 100-200 mM Al (Kawai et al., 2000; Zhang et al., 2002). The toxicity of Al to microorganisms is mainly caused by monomeric Al-species (e.g. Al^{3+}), whereas organically complexed Al is generally assumed to be non-toxic (Illmer & Mutschlechner, 2004). At pH values below 5.0 the dominant aluminium species is Al^{3+} (Pina & Cervantes, 1996). The exudation of organic acids to complex Al^{3+} is a typical protection reaction of roots (Jones, 1998). Microorganisms can excrete organic acids, e.g. citric acid (Zhang et al., 2002), to reduce Al toxicity as well. However, mechanisms of aluminium toxicity (Pina & Cervantes, 1996) as well as the mechanisms by which microorganisms develop tolerance to such toxicity are still unclear

(Zhang et al., 2002). Soil comprises an extremely diverse medium with as many as 13000 species of bacteria in a single gram of soil (Giller et al., 1998). Consequently, toxic effects on one species which performs a particular function in soil may be compensated by another species being more tolerant to this stress factor (Giller et al., 1998). Thus, the effects of Al on C mineralization can only reflect a summation of toxic effects on some microorganisms, being partially compensated by others.

The differentiation between substrate availability and toxicity is of special interest for the interpretation and prediction of stabilization processes in acidic forest soils like podzols. These soils are characterized by large C stocks (Batjes, 1996) when compared with other soil types and substantial stabilization of organic C by sorption and precipitation processes (Kalbitz & Kaiser, 2008). In a changing environment, e.g. decreased acidic deposition, it is crucial to know if decreased availability of C by increased precipitation after rising pH values as observed by Nierop et al. (2002) and Scheel et al. (2007) will result in increased stabilization against microbial decay. However, also decreased stabilization can be assumed as rising pH results in declining concentrations of free Al^{3+} and consequently less toxic effects.

The aim of our investigation was to evaluate whether Al reduces C mineralization because of toxicity to microorganisms or by reducing the bioavailability of OM after its precipitation. We did not study direct effects of Al on specific microorganisms, but focused our study on the influence of different Al species including the potentially toxic free Al^{3+} on C degradation. By applying a novel technique called Diffusive Gradients in Thin Films (DGT) we were able to differentiate between (i) Al bound in precipitated OM, (ii) Al bound in dissolved complexes and (iii) dissolved not organically complexed, ‘free’ Al. In previous studies precipitated OM proved to be very stable against microbial decay, thus, we hypothesized that the observed reduction in C mineralization after Al addition is not caused by toxic effects of ‘free’ Al, but correlates with the extent of C precipitation and therefore with reduced bioavailability. In our experiment we added Al in varying concentrations to two solutions with different chemical composition of OM. The precipitation was initiated at controlled pH values of 3.8 and 4.5 to achieve differences in the fraction of C precipitating and Al speciation.

2. Materials and Methods

2.1. Samples

Samples of the Oa horizon from a Norway spruce forest site (Waldstein, Fichtelgebirge, Germany; Michalzik & Matzner, 1999) and the Oi horizon from a European beech forest site (Steinkreuz, Steigerwald, Germany; Solinger *et al.*, 2001) were homogenized separately and stored frozen. In a previous study water extracts of these two horizons were found to differ largely in respect to the amount of C mineralized, C precipitated and also their chemical composition (Scheel *et al.*, 2007). Water extracts were prepared at 5°C with a soil to (ultrapure) water ratio of 1:10 (Scheel *et al.*, 2007). After one day, solutions were filtered through a ceramic filter plate, preconditioned by the respective solution, and filtered through a 0.2-µm membrane filter (OE 66, Schleicher and Schuell) at 5°C to exclude microorganisms. The DOM solutions were diluted with ultrapure water to 40 mg C l^{-1} to ensure comparability (Table IV.1). The concentration was also similar to dissolved organic C concentrations in soil solutions at the study sites (Solinger *et al.*, 2001; Kalbitz *et al.*, 2004).

Table IV.1: Chemical compositions of the DOM solutions used, after adjustment to 40 mg C l^{-1} (Standard error of 3 replicates in brackets).

	Oi-beech		Oa-spruce	
	/ mg l^{-1}			
Al	0.02	(0.01)	0.44	(0.02)
P	1.08	(0.02)	0.86	(0.01)
PO_4-P	0.96	(0.05)	0.68	(0.04)
N [a]	3.29	(0.06)	2.21	(0.02)
NH_4-N [a]	0.25	(0.01)	0.73	(0.04)
NO_3-N [a]	<0.08		0.23	(0.01)
S [a]	0.53	(0.01)	0.80	(0.02)
SO_4 [a]	0.58	(0.01)	1.32	(0.01)
Ca [a]	1.41	(0.01)	0.38	(0.01)
Fe [a]	<0.05		0.24	(0.02)
Si [a]	2.44	(0.01)	1.77	(0.11)
K [a]	3.50	(0.04)	0.84	(0.06)
Na [a]	<0.15		0.41	(0.01)
Mn [a]	0.66	(0.01)	<0.02	
	C moieties / %			
Alkyl C [a]	27.6		22.8	
O-Alkyl C [a]	46.3		22.5	
Aromatic C [a]	8.5		30.1	
Carboxyl C [a]	17.7		24.6	

[a] taken from Scheel et al. (2007)

2.2. Precipitation and incubation of dissolved organic matter

To precipitate dissolved organic matter at conditions similar to those in acidic forest soils (Schwesig *et al.*, 2003), Al was added as $AlCl_3 \cdot 6H_2O$ solution (Al/C molar ratios: 0.02, 0.05, 0.1 and 0.3; resulting in Al concentrations of 1.6-24 mg l^{-1}) at pH values of 3.8 and 4.5 to 500 ml of the two different DOM solutions in 1 litre glass bottles. As control treatments both DOM solutions were incubated at both pH values without Al addition. Each treatment was performed in triplicate. The resulting 60 samples were shaken for 24 h at 22 °C. The formed Al-OM precipitates were left in the DOM solutions at the respective pH and incubated together.

Microbial inoculum was prepared from 4 mM $CaCl_2$ extracts (soil/solution ratio 1:2 (w/v), 30 min of extraction) of Oa material of both investigated sites (Scheel et al., 2007). After filtration through 5-µm filters (SMWP 4700, Millipore) both extracts were combined and 5ml was added as inoculum to the 500 ml solution (Scheel *et al.*, 2007). The solutions were incubated at 22°C (± 2°C) for 34 days (Oi-beech) and 47 days (Oa-spruce) because the C mineralization of the Oa-spruce solution was smaller as already shown by Schwesig *et al.* (2003) and Scheel *et al.* (2007). During incubation, samples were shaken manually each day to ensure aeration.

2.3. Analyses

After 3 days and at the end of the experiment, aliquots of 50 ml were taken from the incubated solutions. Precipitated OM was removed by filtration over 0.4 µm membrane filters (HTTP 04700, Millipore) from 30 ml of the taken samples. In the other 20 ml precipitated OM was redissolved by addition of 1 ml 1 M HCl. All samples were analyzed for dissolved organic carbon (Shimadzu TOC-5050), total Al and total P (ICP-OES, Perkin Elmer Optima 3000XL) and pH (pH 323, WTW). Synchronous scan fluorescence spectra were recorded (SFM 25, BIO-TEK Instruments) followed by calculation of a humification index (HIX_{syn}) as a measure of the complexity of the organic molecules (Kalbitz *et al.* 1999). We calculated HIX_{syn} by dividing the intensity at bands or shoulders of a longer by a shorter wavelength (464 nm/352 nm). All of these analyses were also performed on the original solutions before starting the experiment with an additional measurement of ortho-phosphate concentration by colorimetric determination on a Skalar continuous-flow autoanalyzer. The ortho-phosphate forms in acid solutions a blue complex with ammoniummolybdate and potassiumantimonyltartrate which was measured after reduction with ascorbic acid at 880 nm in a 5 cm cuvet (Boltz & Mellon, 1948). To ensure a constant pH throughout the experiments, it was regularly checked and readjusted if necessary by adding minute quantities of HCl.

To differentiate between the various soluble Al complexes and ‘free’ dissolved Al, we performed Diffusive Gradients in Thin films (DGT) analyses after 3 days and at the end of the experiment (Jansen *et al.*, 2003). The pH was additionally checked before and after each measurement. As DGT does not work well for solutions with low ionic strength we adjusted the ionic strength of our incubation solutions to 0.01 M by addition of $NaNO_3$ at the beginning of the experiment. All DGT equipment was ordered from DGT Research Ltd. Lancaster. In addition to the conventional APA open pore gel (polyacrylamid gel cross-linked with an agarose derivative) that has a pore size of >5nm, a more restrictive gel was used with a pore size of <1nm (Zhang & Davison, 2000). It has been shown that the open pore gel allows smaller organo-metal complexes to penetrate the membrane, whereas they can hardly diffuse into the restrictive gel (Zhang & Davison, 2000). Model calculations by Jansen *et al.* (2003) indicated that the measured Al fraction with the open pore gel contained a significant portion of small dissolved organic Al complexes. In that study acidic soil solutions were used which were similar to the ones in the present study. To get more direct information about the size fractionation of dissolved organic Al complexes, we decided to perform the DGT measurements with the open pore gel in parallel with measurements using the restrictive gel. Measurements were performed directly in the incubated 500 ml DOM solutions. The open pore and restrictive DGT devices were deployed in parallel for 8 to 20 hours depending on the Al concentration present. During this time Al which diffused through the gels, was fixed on a Chelex 100 chelating resin in the Na^+ form. As there was no information available on diffusion constants of Al for the restrictive gel, we determined the diffusion constants experimentally for both gel types and pH values (Table IV.2). The diffusion constants were determined in six repetitions for the open pore gel and in five repetitions for the restrictive gel in 0.33 mM $AlCl_3 \cdot 6H_20$ solutions with an ionic strength of 0.01 M, adjusted by $NaNO_3$. The Al concentrations were determined by ICP-OES before and after the measurement. The deployment of the DGT units did not lead to a measurable decrease in total Al concentrations in the DOM solutions. We calculated the diffusion constants according to Jansen *et al.* (2002). The reported diffusion constants (Table IV.2) apply to all dissolved inorganic species of Al at 20 °C, and were corrected with the Stokes-Einstein

Table IV.2: Determined diffusion constants (D) in cm² s^{-1} for Al in the open pore and restrictive gel for both investigated pH values at 20°C. Mean values of 5 to 6 replicates and standard error in percent.

	open pore		**restrictive**	
pH 3.8	3.82×10^{-6}	± 4.1%	2.90×10^{-6}	± 6.1%
pH 4.5	4.80×10^{-6}	± 3.3%	3.12×10^{-6}	± 4.0%

equation to the actual temperature which was 22 °C. We observed different diffusion constants for both pH values (Table IV.2). This is in agreement with results from Gimpel *et al.* (2001) and Pesavento & Biesuz (1997) who found smaller uptake efficiencies of DGT devices for Cd, Mn and Zn at lower pH values.

The determined diffusion constants for Al for the restrictive gels were 65% smaller at pH 4.5 and 76% smaller at pH 3.8 when compared with the open pore gel (Table IV.2). The observed smaller diffusion constants correspond well with the results for other metals by Zhang & Davison (2000), who found a 72% decrease in the diffusion constants for Cu and Cd for the restrictive gel. Scally *et al.* (2006) observed clearly smaller diffusion coefficients for the restrictive gels in comparison to the open pore gels.

Aluminium bound on the chelex gel was eluted by 1M HNO_3 and concentrations measured by ICP-OES. The Al concentrations in the sample solutions were calculated from these values (Jansen *et al.*, 2003) taking into account the thickness of the diffusion gel layer, the deployment time and the area of exposure to the sample solution. Repeatability of the DGT measurements was found to be good (standard error <10%), while in some samples the DGT Al fraction was somewhat larger (<25%) than the dissolved Al concentration measured by ICP-OES. To compensate for the difference a correction factor was applied to all measured DGT values. The differences may be caused by the elution of Al from the chelex gel as the elution factor by the manufacturer assumes 20% of metal not to be eluted upon extraction of the gel. Furthermore, Warnken *et al.* (2007) demonstrated that dissociation rate constants measured in situ, with fulvic acids present, provide clearly larger values than those previously measured in the laboratory in absence of fulvic acids.

2.4. Statistics, Calculations & Modelling

We calculated linear regressions between the amount of organic C precipitated and the P concentration of the filtrate and the amount of organic C stabilized with SPSS (significance $p<0.001$). Further, differences between the organic C degradation of the different treatments were tested with ANOVA (SPSS) at a significance level of $p<0.05$.

We calculated the distribution of Al into different size classes using total Al concentrations (Al_{total}) of the unfiltered samples, the concentrations in the filtrate ($Al_{filtrate}$), and Al concentration determined with the open pore ($Al_{DGT\text{-}open\ pore}$) and restrictive ($Al_{DGT\text{-}restrictive}$) DGT gels:

Dissolved Al not bound to organic matter:

‘free’ Al = $Al_{DGT\text{-}restritive}$

Dissolved Al bound in small organo-Al complexes:

small Al-OM = $Al_{DGT\text{-}open\ pore} - Al_{DGT\text{-}restrictive}$

Dissolved Al bound in large organo-Al complexes:

large Al-OM = $Al_{filtrate} - Al_{DGT\text{-}open\ pore}$

Al bound in precipitated organic matter:

precipitated Al = $Al_{total} - Al_{filtrate}$

The P speciation of the used solutions was modelled with VisualMinteq v2.51. The solution compositions were taken from Table IV.1. The modelling was performed at the pH of precipitation and the added amount of Al for the respective Al/C ratio (t = 22°C).

3. Results and discussion

3.1. Carbon degradation

Carbon degradation in the Oi-beech solution (66%) was much larger than in the Oa-spruce solution (16%), despite a 13 days shorter incubation for the Oi-beech solution (Fig. IV.1). A large fraction of carbon was already degraded during the first three days of incubation (Fig. IV.1). The dynamics and the degree of C degradation are in accordance with results presented by Scheel *et al.* (2007). Kalbitz *et al.* (2003a) have demonstrated that during the first two weeks of incubation the largest fraction of DOM was mineralized and observed comparable values of C mineralization for the Oi-beech and Oa-spruce solution. The addition of Al to the DOM solutions resulted in significantly decreasing C degradation (Fig. IV.1). Larger Al/C ratios led to a larger reduction in C degradation when compared to the respective solutions without Al addition. However, Al/C ratios exceeding 0.1 resulted only in a small additional decrease in C degradation at the end of the experiment (Fig. IV.1). Decreasing C degradation with increasing Al/C ratios became more evident at the end of the experiment in comparison to 3 days of incubation. The observed reduction of C degradation by Al was larger than found by Schwesig *et al.* (2003). However, in their study the pH was not adjusted after Al addition so that precipitation

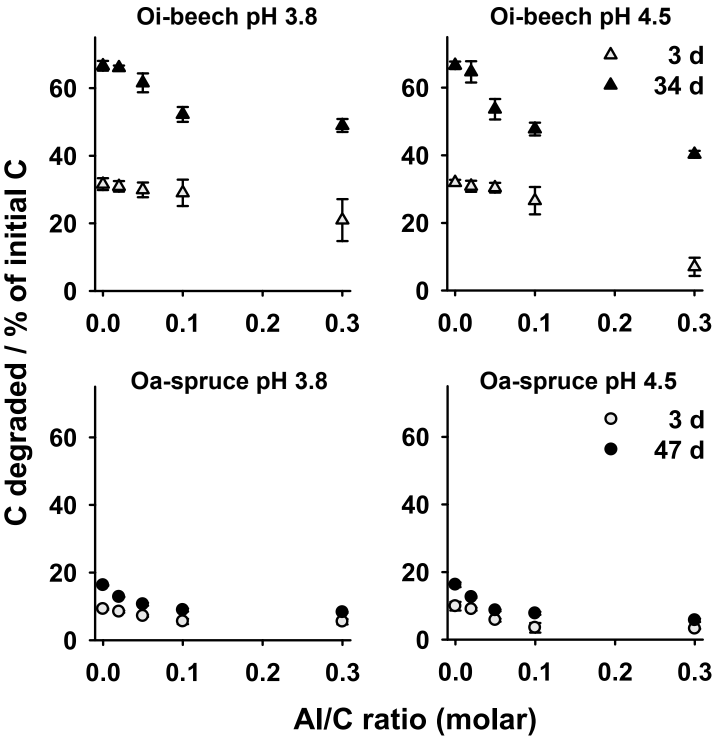

Fig. IV.1: Degradation of C at the beginning (after 3 days) and at the end of the experiment (Oi-beech: 34 days; Oa-spruce: 47 days) in dependence on the Al/C ratio used to precipitate organic matter. Two different organic matter solutions and 2 pH values were used. Mean values and standard error of 3 replicates are presented.

took place at different pH values. Large Al/C ratios will result in decreasing pH values with smaller amounts of C precipitated (Fig. IV.2) and consequently a smaller reduction in C degradation. Therefore, values of C degradation are not directly comparable between the two studies.

3.2. Carbon precipitation

The amount of carbon precipitated by Al increased strongly up to an initial Al/C ratio in solution of 0.1 (Fig. IV.2) whereas a further increase of the Al/C ratio to 0.3 did not strongly change the amount of precipitated C. Similar results were reported by Nierop *et al.* (2002) and Scheel *et al.* (2007). We observed a reduction in precipitation at Al/C ratios of 0.3 compared to 0.1 in the Oi-beech solutions at the beginning of our incubation, which was no longer observable at the end of the experiment. A larger fraction of OM was precipitated by Al at pH 4.5 than at pH 3.8, again in accordance with previous studies (Nierop *et al.*, 2002; Scheel *et al.*, 2007) and modelling results (Jansen *et al.*, 2003). As C was degraded during the incubation we expected also a slight reduction in the amount of precipitated C, but we observed always larger amounts of precipitated C at the end of the experiment than at the beginning. This increase in precipitation was slightly larger for precipitates formed at pH 3.8 (Fig. IV.2).

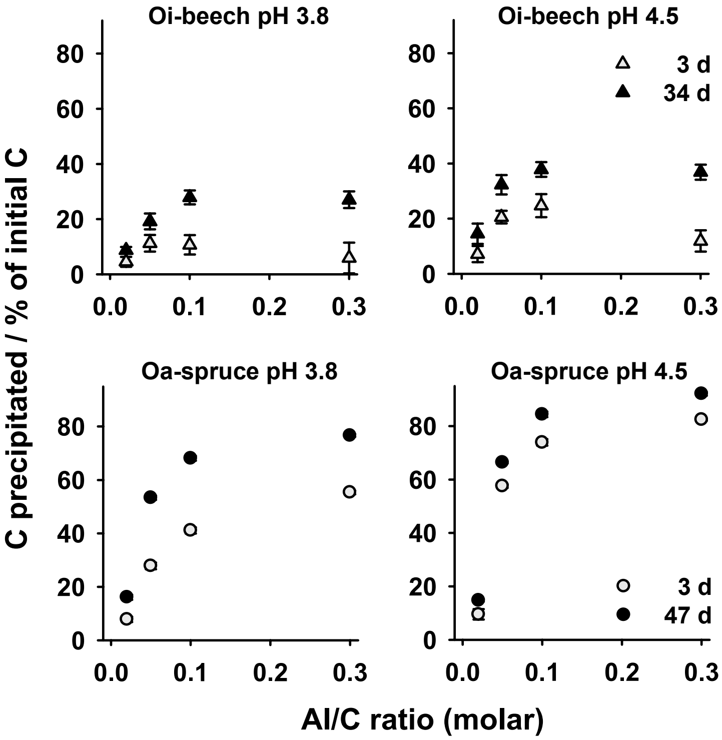

Fig. IV.2: Percentage of C precipitated at the beginning (after 3 days) and at the end of the experiment (Oi-beech: 34 days; Oa-spruce: 47 days) in dependence on the Al/C ratio used to precipitate organic matter. Two different organic matter solutions and 2 pH values were used. Mean values and standard error of 3 replicates are presented.

3.3. Distribution of Al in size classes

Generally, with increasing Al/C ratios the fraction of total Al not bound to OM (‘free’ Al) increased in both solutions and for both pH values (Fig. IV.3). The fraction of Al bound to OM was always larger at pH 4.5 when compared with pH 3.8 (Fig. IV.3) reflecting the larger fraction of Al bound to precipitated OM. In turn, the fraction of ‘free’ Al and the fraction of total Al bound in small soluble organo-Al complexes was larger at pH 3.8 compared to pH 4.5 (Fig. IV.3). For the large soluble organo-Al complexes no clear trend was observable.

The portion of Al bound in precipitates increased during incubation, whereas the fraction of ‘free’ Al declined. Furthermore, also the portion of Al bound in soluble complexes (small and large) decreased during incubation. In nearly all cases the fraction of small complexes decreased

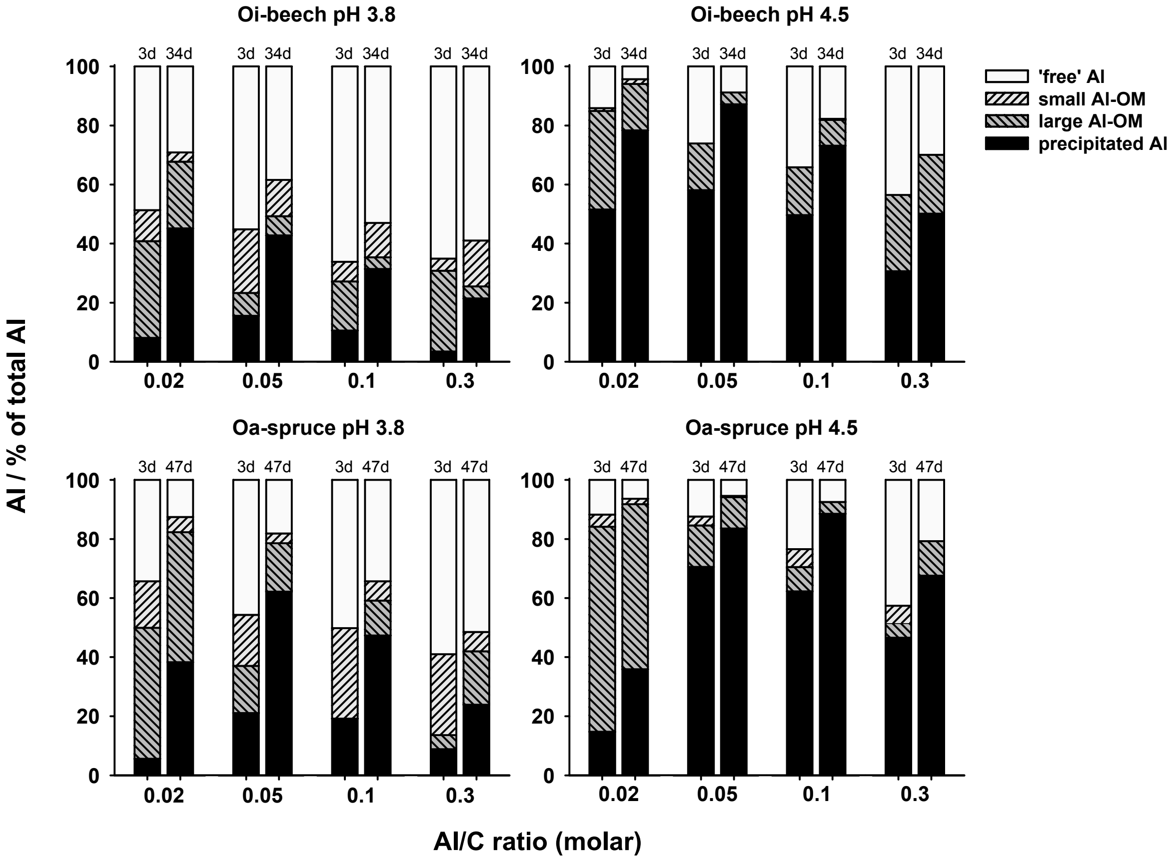

Fig. IV.3: Portions of aluminium not bound to organic matter (‘free’ Al), Al bound in small and large soluble organo Al complexes (small and large Al-OM) and Al bound to precipitated organic matter (> 0.4µm). Distribution of the Al fractions are shown at the start (after 3 days) and at the end of the experiment (Oi-beech: 34 days; Oa-spruce: 47 days). Al was added to two different organic matter solutions at 2 pH values and 4 Al/C ratios.

stronger than the fraction of large complexes. The decrease in soluble Al-OM complexes during incubation indicated that the OM of this fraction was either mineralized or the complexes grew in size and were precipitated. At the end of the incubation experiment there was still a considerable fraction of Al bound in soluble Al-OM complexes. Thus, the OM of that fraction must have been either hard to degrade or the OM to which the Al was bound was degraded and Al complexed other organic molecules.

At an Al/C ratio of 0.02 a much larger portion of Al was bound in soluble organic complexes compared with larger Al/C ratios, especially for the Oa-spruce solution. This confirms results of Nierop *et al.* 2002 who found a large increase in the fraction of C precipitating for Al/C ratios exceeding 0.03. Thus, at small Al/C ratios the Al is already bound to OM but the amount is too small to initiate precipitation of OM. Further effects of the Al/C ratio on the distribution of Al into the different size fractions could not be observed.

3.4. Toxic effects vs. decreased bioavailability

The decrease in C degradation of the OM solutions after Al addition could be caused by increased stability of precipitated OM against microbial decay (i.e. reduced bioavailability) or by toxic effects of ‘free’ Al on microorganisms. We consistently observed a larger fraction of ‘free’ Al at pH 3.8 than at pH 4.5 comparing similar Al/C ratios (Fig. IV.4). Assuming a toxic effect of Al, one would expect smaller C degradation at lower pH with larger amounts of ‘free’ Al. In contrast, we always found increased C degradation at lower pH and larger amounts of ‘free’ Al for the respective Al/C ratio (Fig. IV.4). However, the larger precipitation of OM by Al at pH 4.5 compared to pH 3.8 always led to a decrease of C degradation for each individual Al/C ratio (Fig. IV.4). The amount of Al bound in the precipitated OM had a similar effect on C degradation (Fig. IV.4).

We observed the largest reduction in C degradation for the three smallest Al addition steps (Al/C ratios: 0.02–0.10) with maximal ‘free’ Al concentrations of 4 mg Al l^{-1} (0.15 mM), being in the lower part of the range given for Al toxicity by Pina & Cervantes (1996). At the same time, the largest Al addition with maximal ‘free’ Al concentrations of 12 mg Al l^{-1} (0.45 mM) resulted only in a comparably small additional reduction of C degradation. This could be taken as further indication that the decrease in C degradation was not caused by toxic effects, but by the extent of C precipitation (Fig. IV.4).

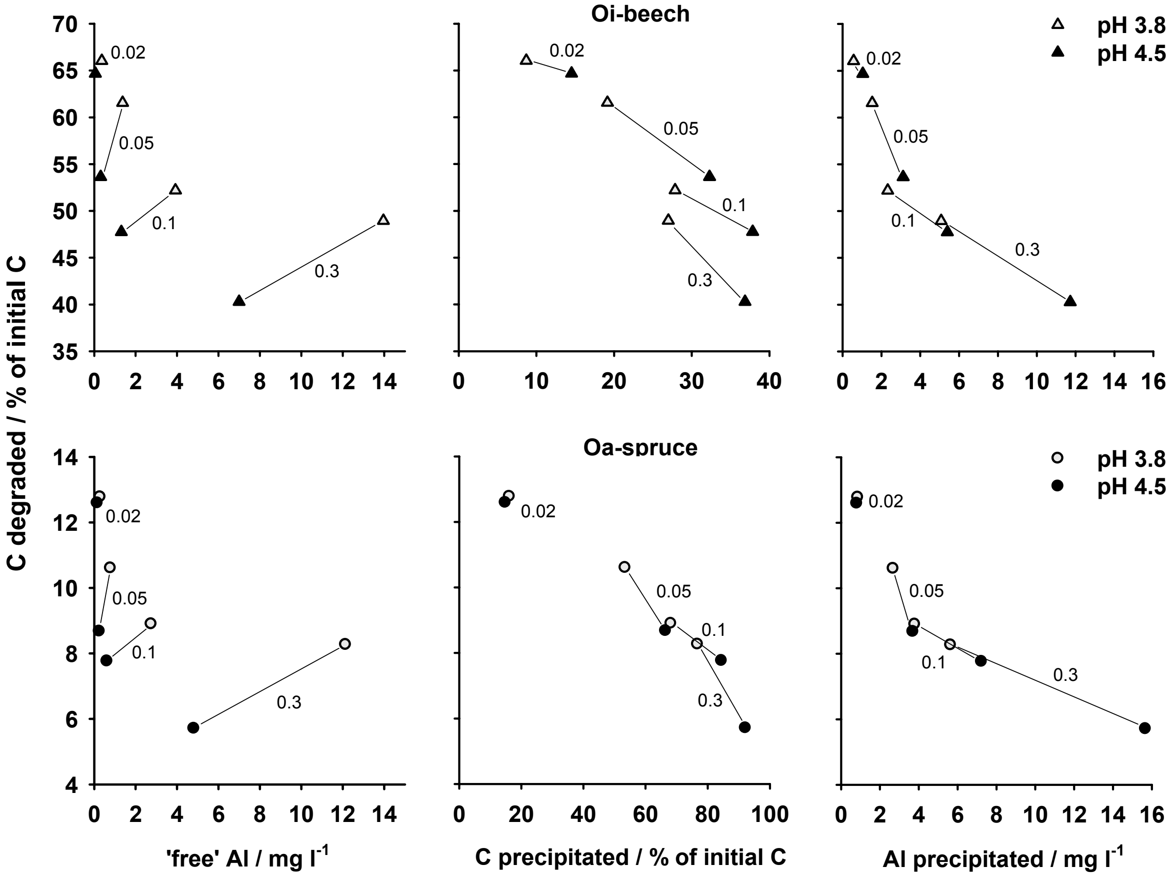

Fig. IV.4: Carbon degradation at the end of the experiment in dependence on the concentration of 'free' Al (left side), the percentage of C precipitated from solution (middle) and the amounts of precipitated Al (right side). Lines indicate the same Al/C ratios of 0.02, 0.05, 0.1 and 0.3 in solution. Precipitation of organic matter was induced in two solutions at two different pH values.

3.5. Effects of phosphorous

Phosphorous was removed from soil solution by coprecipitation. With increased precipitation of OM the fraction of precipitated P also increased (Fig. IV.5A). The dissolved P concentrations decreased from about 1 mg P l^{-1} in the original solutions to about 50 µg P l^{-1} for the treatments with the largest C precipitation, a reduction of up to 95%. The removal of P from solution was significantly correlated to the precipitation of C (Fig. IV.5A) and the modelled fraction of P not complexed by Al (Fig. IV.5B). In the Oa-spruce and Oi-beech solution the fraction of P present as ortho-phosphate were 80% and 90%, respectively (Table IV.1). Guardado *et al.* (2007) report that Al has a large ability to complex PO_4 and bind it to OM. Precipitation of OM would then also lead to coprecipitation of P. But the formation of insoluble Al-PO_4

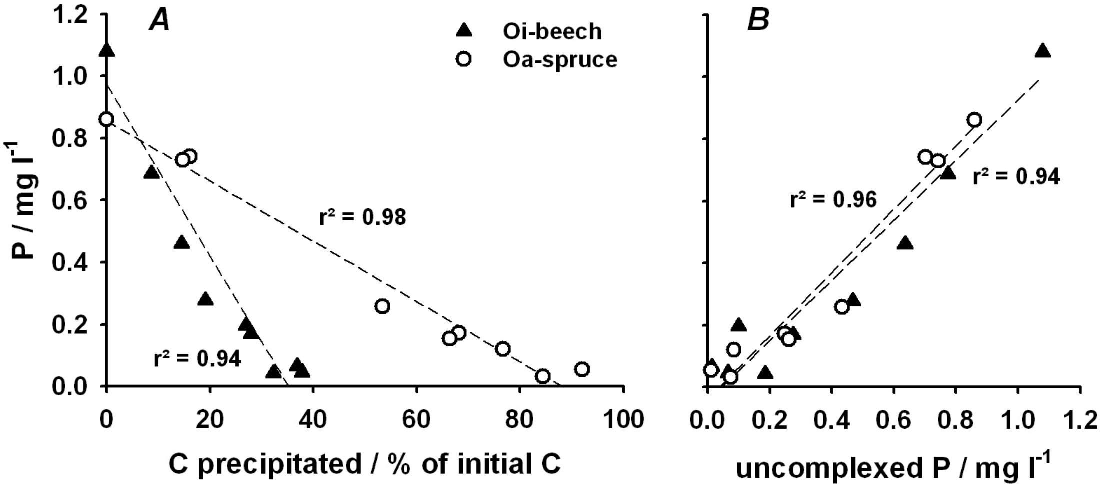

Fig. IV.5: Concentration of P in the filtered solutions in dependence on (A) precipitation of organic matter by Al and (B) the modelled fraction of P which was not complexed by Al. Dissolved organic matter was precipitated at two pH values from two different solutions.

complexes can also result in direct precipitation of P (Atkári et al., 1996). Therefore, we assume that either a large fraction of P must have been bound to DOM by Al before precipitation of OM (Fig. IV.5A) or the complexes formed by Al and PO_4 were precipitated from solution (Fig. IV.5B). We were not able to separate these processes, but microorganisms are able to use both organically bound P and Al-phosphate as P source (Illmer *et al.*, 1995; He & Zhu, 1998). Guardado *et al.* (2007) found that the binding of PO_4 to OM by Al was less stable when compared with PO_4 bound to OM by other metal cations, e.g. Fe or Cu. Nevertheless, we can not rule out the possibility that the reduction in C degradation was also driven by P deficiency.

3.6. Changes in organic matter composition

The humification indices deduced from synchronous fluorescence spectra (HIX_{syn}) of the filtered DOM solutions decreased with increasing Al/C ratios (Fig. IV.6). A decrease in HIX_{syn} indicates the removal of more aromatic and complex structures possibly deriving from lignin from the solutions (Kalbitz et al., 2004). At the end of the experiment these indices were smaller than at the beginning in nearly all cases, reflecting the larger portion of OM precipitated.

The dynamic of the humification indices deduced from fluorescence spectra (HIX_{syn}; Fig. IV.6) seems closely related to the amount of C precipitated (Fig. IV.2). Therefore, we related

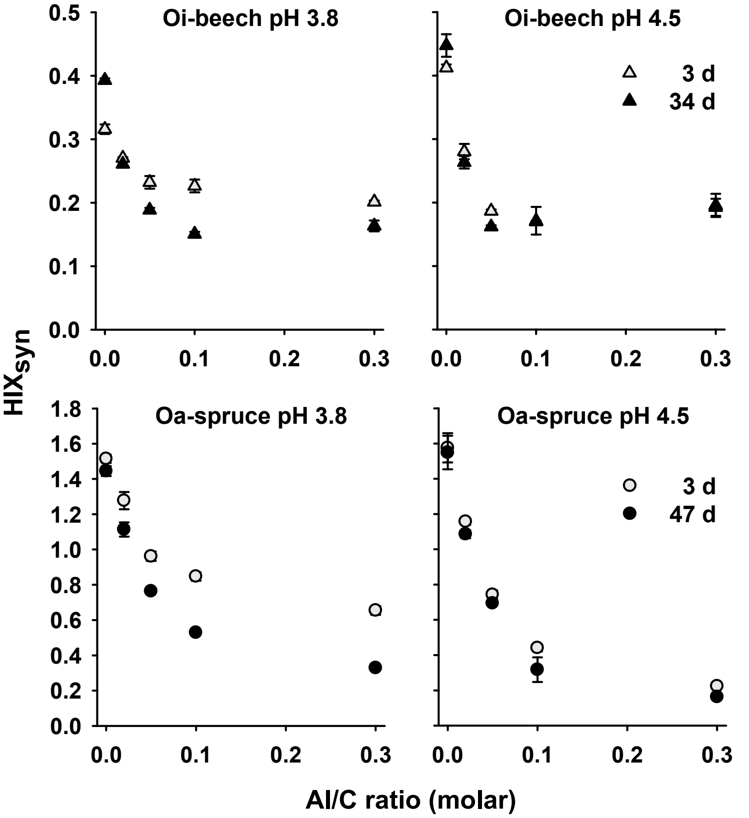

Fig. IV.6: Humification indices deduced from synchronous fluorescence spectra (HIX_{syn}) of the organic matter solutions after removal of precipitated organic matter in dependence on the Al/C ratio used for precipitation. Spectra were recorded at the beginning of the incubation (after 3 days) and at the end of the experiment (Oi-beech: 34 days; Oa-spruce: 47 days). Dissolved organic matter of two different solutions was precipitated at 2 pH values and 4 Al/C ratios. Mean values and standard error of 3 replicates.

the percentage of C precipitated to the reduction of HIX_{syn} (Fig. IV.7, top) which demonstrated that the precipitation process was clearly associated with the preferential removal of the more complex and aromatic structures from solution. Further it can be seen that the reduction in HIX_{syn} per mg C precipitated was larger for small amounts of precipitation (Fig. IV.7, bottom). After removal of about 30% of C from solutions the reduction in HIX_{syn} per mg C precipitated became almost constant, indicating that compounds with a particularly large humification index were precipitated first. Such compounds should be of utmost stability against microbial decay (Kalbitz *et al.*, 2003b).

3.7. Carbon stabilization

The amount of C stabilized by the Al addition was calculated from the loss of C during incubation in comparison to the C loss of the respective DOM solution. The DOC concentration in the Oa-spruce solution decreased from initial 40 mg C l^{-1} to 33.5 mg C l^{-1} after 47 days of incubation (Fig. IV.1). The smallest decrease in DOC concentration (37.8 mg C l^{-1}) was observed for the treatment with the largest Al addition at pH 4.5. This smaller decrease in C degradation of 4.3 mg C l^{-1} when compared with the untreated Oa solution is equal to a relative C stabilization

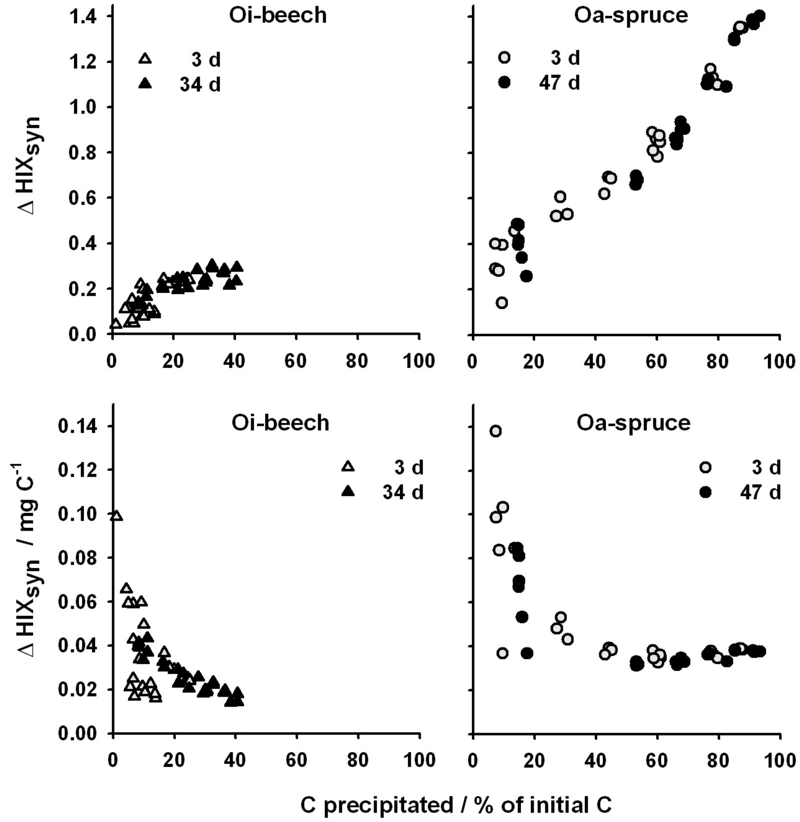

Fig. IV.7: Decrease in humification indices deduced from synchronous fluorescence spectra (HIX_{syn}) of all individual organic matter solutions induced by precipitation of organic matter by Al in relation to C precipitation. The figures on the top indicate relative decrease of the humification indices whereas the figures at the bottom illustrate the decrease in humification indices per mg C removed from solution.

by 65%. For the Oi-beech solution the C degradation was much larger with a decrease of DOC concentration from 40 mg C l^{-1} to 13.4 mg C l^{-1} after 34 days of incubation (Fig. IV.1). We observed also for the Oi solution that the treatment with the largest Al addition at pH 4.5 resulted in the smallest decrease in DOC concentrations during incubation (23.9 mg C l^{-1}). When compared with the C degradation of the untreated Oi solution this means that 10.5 mg C l^{-1} were less degraded; i.e. 40% of C were stabilized. The amount of stabilized C correlated significantly with the amount of precipitated C (Fig. IV.8). Larger Al additions resulting in increased precipitation led to an increased stabilization for both solutions and pH values (Fig. IV.8). The stabilization by Al was consistently larger at pH 4.5 than at pH 3.8, as a result of the larger amount of precipitates formed. As a consequence, the main factor for the observed stabilization was the amount of C and Al precipitated (Figs. IV.4 and IV.8). Therefore, it is likely that pH governs C degradation indirectly by determining the amount of C and Al precipitating. For equal amounts of C precipitated the stabilizing effect was larger in the Oi-beech solution, being a consequence of the better degradability of the solution itself. The extent of stabilization depends mainly on the amounts of precipitated OM than on preferential precipitation of certain compounds. The concentrations we used were in the range of acidic forest soils. Therefore, it can be assumed that the decrease in C degradation caused by Al might be representative for field conditions as well.

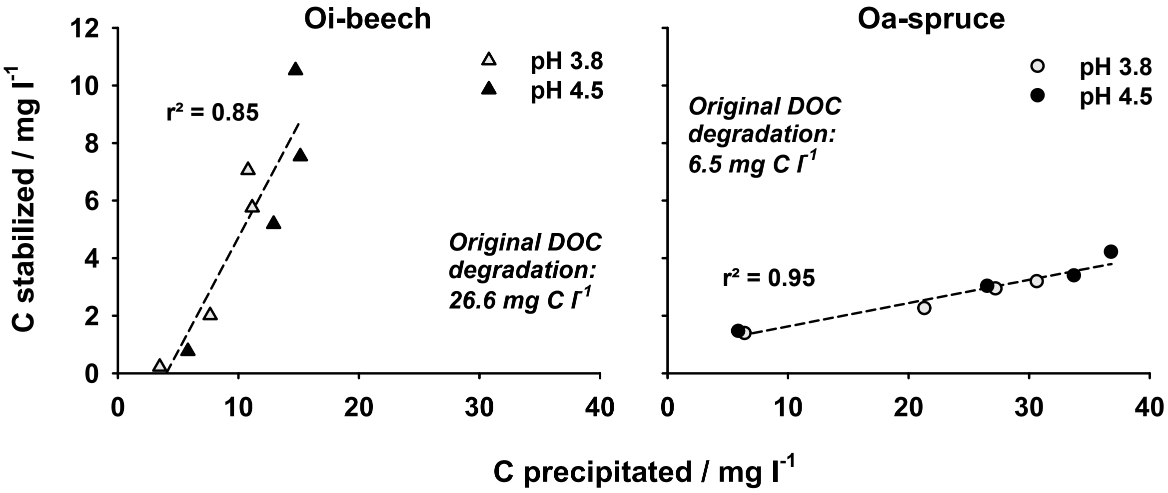

Fig. IV.8: Amounts of stabilized C (reduction in C degradation) in dependence on the amounts of C precipitated by Al for both solutions and pH values. Please note, the initial concentrations of dissolved organic C were 40 mg C l^{-1} and zero stabilization is equal to stabilization without addition of Al.

4. Conclusions

The DGT measurements revealed that a large fraction of Al is bound to OM, especially at low Al concentrations. We found no evidence of toxic effects of Al on microorganisms. The largest reduction in C degradation occurred while 'free' Al concentrations were still below 4 mg l^{-1}, a concentration commonly found in acidic forest soils (Schwesig *et al.*, 2003). Furthermore, degradation even increased with increasing concentrations of 'free' Al comparing identical Al/C ratios at the two pH values used in our study. Therefore, we can conclude that the extent of precipitation is of much larger importance for C stabilization than the concentration of 'free' Al. That means C stabilization is mainly caused by reduction of the bioavailability of C. Reduced availability of P might also contribute to C stabilization. Although we observed a preferential precipitation of compounds being more stable than the bulk OM, the fraction of stabilized C depended more on the amount of precipitated C than on its composition.

As the reduction in C degradation results from the formation of organo-Al precipitates the precipitated OM will be also stabilized after a potential decrease of Al concentrations in the soil solution. Thus, increasing pH values accompanied by decreasing Al concentrations do not lead to a quick rise in C mineralization in forest soils, as no re-dissolution of precipitated OM can be expected. Furthermore, precipitation of OM might further increase taking into account the expected increasing pH values after recovering from acidic deposition.

5. References

Atkári, K., Kiss, T., Bertani, R. & Martin, R.B. 1996. Interactions of aluminum(III) with phosphates. *Inorganic Chemistry*, **35**, 7089-7094.

Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, **47**, 151-163.

Boltz, D.F. & Mellon, M.G. 1948. Spectrophotometric determination of Phosphorus as molydiphosphoric acid. *Analytical Chemistry*, **20**, 749-751.

Borggaard, O.K., Raben-Lange, B., Gimsing, A.L. & Strobel, B.W. 2005. Influence of humic substances on phosphate adsorption by aluminium and iron oxides. *Geoderma*, **127**, 270-279.

Burford, J.R. & Bremner, J.M. 1975. Relation between denitrification capacities of soils and total water soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry*, **7**, 389-394.

Giller, K.E., Witter, E. & McGrath, S.P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biology and Biochemistry*, **30**, 1389-1414.

Gimpel, J., Zhang, H., Hutchinson, W. & Davison, W. 2001. Effect of solution composition, flow and deployment time on the measurement of trace metals by the diffusive gradients in thin films technique. *Analytica Chimica Acta*, **44**8, 93-103.

Guardado, I., Urrutia, O. & Garcia-Mina, J.M. 2007. Size distribution, complexing capacity, and stability of phophate-metal-humic complexes. *Journal of Agricultrual and Food Chemistry*, **55**, 408-413.

He, Z. & Zhu, J. 1998. Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biology and Biochemistry*, **30**, 917-923.

Illmer, P. & Mutschlechner, W. 2004. Effect of temperature and pH on the toxicity of aluminium towards two new, soil born species of *Arthrobacter* sp. *Journal of Basic Microbiology*, **44**, 98-105.

Illmer, P., Barbato, A. & Schinner, F. 1995. Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganisms. *Soil Biology and Biochemistry*, **27**, 265-270.

Jansen, B., Nierop, K.G.J. & Verstraten, J.M. 2002. Influence of pH and metal/carbon ratios on soluble organic complexation of Fe(II), Fe(III) and Al(III) in soil solutions determined by diffusive gradients in thin films. *Analytica Chimica Acta*, **454**, 259-270.

Jansen, B., Mulder, J. & Verstraten, J.M. 2003. Organic complexation of Al and Fe in acidic soil solutions. Comparison of diffusive gradients in thin films analyses with Models V and VI predictions. *Analytica Chimica Acta*, **498**, 105-117.

Jones, D.L. 1998. Organic acids in the rhizosphere – a critical review. *Plant and Soil*, **205**, 25-44.

Kalbitz, K. & Kaiser, K. 2008. Contribution of dissolved organic matter to carbon storage in forest mineral soils. *Journal of Plant Nutrition and Soil Science*, in press.

Kalbitz, K., Geyer, W. & Geyer, S. 1999. Spectroscopic properties of dissolved humic substances – a reflection of land use history in a fen area. *Biochemistry*, **47**, 219-238.

Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E. 2003a. Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma*, **113**, 273–291.

Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumaier, L., Glaser, B., Ellerbrock, R. & Leinweber, P. 2003b. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. *Soil Biology and Biochemistry*, **35**, 1129-1142.

Kalbitz, K., Glaser, B. & Bol, R. 2004. Clear-cutting of a Norway spruce stand: Implications for controls on the dynamics of dissolved organic matter in the forest floor. *European Journal of Soil Science*, **55**, 401–413.

Kawai, F., Zhang, D. & Sugimoto, M. 2000. Isolation and characterization of acid- and Al-tolerant microorganisms. *FEMS Microbiology Letters*, **189**, 143-147.

Khare, N., Hesterberg, D. & Martin, J.D. 2005. XANES investigation of phosphate sorption in single and binary systems of Iron and Aluminum oxide minerals. *Environment Science & Technology*, **39**, 2152–2160.

Marschner, B. & Kalbitz, K. 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma*, **113**, 211-235.

Michalzik, B. & Matzner, E. 1999. Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. *European Journal of Soil Science*, **50**, 579–590.

Nierop, K.G.J., Jansen, B. & Verstraten, J.M. 2002. Dissolved organic matter, aluminium and iron interactions: Precipitation induced by metal/carbon ratio, pH and competition. *Science of the Total Environment*, **300**, 201–211.

Pesavento, M. & Biesuz, R. 1997. Sorption of divalent metal ions on an iminodiacetic resin from artificial seawater. *Analytica Chimica Acta*, **346**, 381-391.

Pina, R.G. & Cervantes, C. 1996. Microbial interactions with aluminium. *BioMetals*, **9**, 311-316.

Scally, S., Davison, W. & Zhang, H. 2006. Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films. *Analytica Chimica Acta*, **558**, 222-229.

Scheel, T., Dörfler, C. & Kalbitz, K. 2007. Precipitation of dissolved organic matter by Al stabilizes C in acidic forest soils. *Soil Science Society of America Journal*, **71**, 64-74.

Schwesig, D., Kalbitz, K. & Matzner, E. 2003. Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. *European Journal of Soil Science*, **54**, 311-322.

Solinger, S., Kalbitz, K. & Matzner, E. 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. *Biogeochemistry*, **55**, 327–349.

Warnken, K.W., Davison, W., Zhang, H., Galceran, J. & Puy, J. 2007. In situ measurements of metal complex exchange kinetics in freshwater. *Environment Science & Technology*, **41**, 3179–3185.

Zhang, D., Duine, J.A. & Kawai, F. 2002. The extremely high Al resistance of *Penicillium janthineleum* F-13 is not caused by internal or external sequestration of Al. *BioMetals*, **15**, 167-174.

Zhang, H. & Davison, W. 2000. Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using Diffusive Gradients in Thin Films. *Analytical Chemistry*, **72**, 4447-4457.

Chapter V

Properties of organic matter precipitated from acidic forest soil solutions

Thorsten Scheel[a]

Ludwig Haumaier[a], Ruth H. Ellerbrock[b], Jörg Rühlmann[c] and Karsten Kalbitz[a]

[a]Department of Soil Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany.

[b]Leibniz Centre for Agricultural Landscape Research (ZALF), Institute for Soil Landscape Research, Eberswalder Straße 84, 15374 Müncheberg, Germany

[c]Institute of Vegetable and Ornamental Crops Großbeeren (IGZ), Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany

Organic Geochemistry 39 (2008), 1439-1453

0. Abstract

From soil solutions of acidic forest soils, up to 90% of organic matter (OM) can be precipitated by Al or Fe. Precipitation of OM contributes to its stabilization against microbial decay. Thus, this process should be of major relevance for C cycling and sequestration, but little is known about the properties and the composition of precipitated OM. Therefore, we investigated the sizes of precipitated OM flocs and determined the chemical components which were preferentially precipitated. We used four highly different organic matter solutions and precipitated OM at different Al/C ratios and pH values. The results of ^{13}C NMR, ^{1}H NMR and UV/Vis analysis clearly showed a preferential precipitation of aromatic components with a high content of carboxylic groups at all pH and Al/C ratios. This selective precipitation was supported by FTIR measurements which additionally revealed that binding mechanisms were independent of pH and Al/C ratios. However, we observed larger Al/C ratios in OM precipitated at pH 4.5 than at 3.8, which could be related to the amount of $Al(OH)^{2+}$ cations present in solution or the formation of small Al-hydroxide primary particles. The diameters of precipitated OM flocs ranged from 3 to 110 µm with smaller sizes at pH 3.8 (average: 16.6 µm) than at pH 4.5 (average: 27.6 µm). We estimated that a floc (10 µm diameter) consists of approximately 80 million organic molecules linked by Al, forming on average 2 to 3 bonds. As a consequence, the spatial accessibility of the OM should be considerably reduced in these flocs leading to additional stabilization, besides the intrinsic stability of the precipitated organic compounds and the bonds to Al.

1. Introduction

Dissolved organic matter (DOM) can be precipitated from acidic soil solutions by polyvalent cations, e.g. Al, Fe. The fraction of DOM which is prone to precipitation by metal cations (Fe, Al) and their hydroxides can range up to 90% of DOM derived from organic horizons of acidic forest soils (Nierop et al., 2002; Scheel et al., 2007). Furthermore, it has been shown that precipitation of DOM by Al results in a distinct reduction of C mineralization (Scheel et al., 2007) when compared with bulk DOM. The resulting carbon mineralization of precipitated OM ranged from 0.5 to 7.7% of initial C after 7 weeks of incubation, whereas the respective DOM solutions had with 5 to 49% a much larger C mineralization (Scheel et al., 2007). Thus, precipitated OM comprises a stable carbon source.

Precipitation of OM could be of large importance for C cycling in soils, but little is known about the properties of precipitated OM and its composition. The decline of UV absorbance in samples, where precipitated OM was removed gave an indication that probably aromatic components were preferentially removed from solution (Scheel et al., 2007). Kaiser et al. (1997) observed a preferential removal of aromatic and carboxylic C from the soil solution by sorption. Thus, it can be assumed that carboxylic groups play an essential role for the binding of OM to metal cations. But also phenolic groups are of importance for bond formation of organic matter with aluminium hydroxide surfaces (Guan et al., 2006a, b).

The amount of OM precipitated by Al depends on the prevailing pH of the solution (Nierop et al., 2002; Scheel et al., 2007). Also the stability of the precipitates against microbial decay was influenced by pH (Scheel et al., 2007). Possibly, this is related to Al speciation which is highly pH dependent, with increasing hydroxide species at larger pH. Also the protonation of functional groups, e.g. carboxylic groups, depends on pH. Thus, these factors may influence precipitated OM composition, but also change the binding mechanisms between the metal cations and OM. This would directly affect the stability of the OM, as the binding mechanism can strongly influence its biodegradability (Mikutta et al., 2007).

Besides the composition also the size of the precipitated OM flocs should be important e.g., for transport processes in soil profiles. Smaller flocs will be transported through smaller pores into greater depth with less microbial activity probably resulting in larger stabilization against microbial decay. The size of the flocs is also of interest in aquatic systems, as it determines the settling velocity and thus transportation distances. We are not aware of any study which determined floc sizes of natural OM precipitated by Al in terrestrial systems. Flocs formed during sewage water treatment are well investigated, but often applied metal cation concentrations are hardly comparable to concentrations observed in forest soils. Jarvis et al. (2006) observed flocs of up to 1000 µm, but floc sizes were strongly influenced by inorganic precipitation of iron hydroxides. Rapid stirring of solutions led to a considerable decrease in floc diameters to about 300 µm (Jarvis et al., 2005). In Scheel et al. (2007) separation of precipitated OM was done by filtration through 0.4 µm, meaning that the size of precipitated flocs must be at least larger than this. As the average organic molecule is at least a factor of 100 smaller, the metal cations must arrange a large number of organic molecules. This may be achieved by ligand exchange of water molecules from the hydrate sphere of Al by carboxylic groups of the organic molecules (Vreysen and Maes, 2006). To cross-link the organic molecules, they must possess two or more functional groups, each forming monodentate bonds with an Al cation. If these groups are adjacent to each other then one metal cation possibly form bi- or tridentate bonds, which is disadvantageous for cross-linking. Therefore, low molecular weight organic acids are not the most likely candidates to be precipitated, despite a large content of functional groups. High molecular weight structures, with large contents of functional groups, e.g. deriving from lignin or tannin, seem to be likely candidates for precipitation.

In our experiment we used four different DOM solution and precipitates formed at pH values of 3.8 and 4.5 at Al/C ratios of 0.05, 0.1 and 0.3. These are similar to the samples used by Scheel et al. (2007) and Scheel et al. (2008a) to quantify the stabilization of precipitated OM against microbial decay. We analyzed the original DOM, the precipitated OM and the remaining filtrates with a large variety of methods, like elemental analysis, ^{13}C and ^{1}H NMR spectra, FTIR and UV/Vis spectra and laser scanning microscopy (LSM). The questions we wanted to answer were (i) which DOM components are preferentially precipitated, (ii) what are the differences in composition and the type of bonds between OM precipitated at pH 3.8 and 4.5 and (iii) what are average diameters of precipitated OM flocs. The gathered information should enable us to draw further conclusions about the mechanisms leading to the stabilization of precipitated OM.

2. Materials and Methods

2.1. Samples

Samples of Oi and Oa horizons from a Norway spruce (Waldstein-Fichtelgebirge, Germany; Michalzik and Matzner, 1999) and a European beech (Steinkreuz-Steigerwald, Germany; Solinger et al., 2001) forest site were homogenized and stored frozen. Water extracts were prepared at 5°C with a soil to (ultrapure) water ratio of 1:10 (Scheel et al., 2007). After one day, DOM solutions were filtered through a preconditioned ceramic filter plate and filtered at 5°C through a 0.2-µm membrane filter (OE 66, Schleicher and Schuell) to exclude microorganisms. The DOM solutions were diluted with ultrapure water to 40 mg C l^{-1} to ensure comparability. The chemical composition of the solutions was given by Scheel et al. (2007).

2.2. Precipitation of dissolved organic matter

To precipitate the DOM at conditions similar to those in acidic forest soils, Al was added as $AlCl_3 * 6H_2O$ solution (Al/C ratios: 0.05, 0.1 and 0.3) at pH values of 3.8 and 4.5 to these 4 different DOM solutions (Scheel et al., 2007). All bottles were gently shaken for 24 h at 5°C. Then precipitates were separated by passing the solution through a prewashed polycarbonate membrane filter (0.4 µm, HTTP Millipore). Dissolved organic matter, precipitated OM and the filtrates were freeze-dried, ground, homogenized and stored at 2 °C prior to analysis. We produced large amounts of precipitates because of the sample requirements of the different methods. Therefore, our composite samples are representative for the different treatments (kind of soil solution, pH, Al/C ratio) although precipitation could not be done with replicates.

2.3. Elemental analysis

The carbon, nitrogen and sulphur contents of the freeze-dried and ground samples of dissolved and precipitated OM were determined by a CNS elemental analyzer (Vario EL, Elementar). The content of aluminium and phosphorus in dissolved and precipitated OM were determined by complete dissolution of defined amounts of sample material in 10 ml 0.01 M NaOH and subsequent analysis (ICP-OES, GCP Electronics). In DOM and the solutions from which precipitated OM was removed we further measured NO_3 (ion chromatography, Dionex DX

100), NH_4 (flow injection, photometric mLE– FIA LAB) and Ca, Fe, K and Mg (ICP–OES, GCP Electronics).

2.4. ^{13}C and ^{1}H NMR

NMR spectra of freeze-dried samples dissolved in 3 ml 0.5 M NaOD solution were obtained on a Bruker Avance DRX 500 NMR spectrometer (11.7 T) (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) at a temperature of 290 K under the following conditions:

^{13}C NMR: 10-mm sample tubes; spectrometer frequency, 125 MHz; inverse-gated decoupling; acquisition time, 0.16 s; delay time, 1.84 s; line-broadening factor, 100 Hz.

^{1}H NMR: 5-mm sample tubes; spectrometer frequency, 500 MHz; homonuclear presaturation for solvent suppression; acquisition time, 1.16 s; delay time, 1 s; line-broadening factor, 2 Hz. The ^{13}C NMR spectra were not recorded for all samples, because of the large amount of sample material needed (150 mg).

In the ^{13}C NMR spectra the peak area from 0-60 ppm was assigned to alkyl C (C_{alkyl}), 60-105 ppm to O-alkyl C ($C_{O\text{-}alkyl}$), 105-160 ppm to aromatic C ($C_{aromatic}$) and 160-200 ppm to carboxylic C ($C_{carboxyl}$). For the ^{1}H NMR spectra the peak areas were assigned to alkyl H (H_{alkyl}) from 0-1.8 ppm, carbohydrate H ($H_{c.hydrate}$) from 3.0-4.5 ppm and aromatic H ($H_{aromatic}$) from 5.5-10 ppm.

2.5. FTIR analysis

FTIR spectra of freeze-dried dissolved and precipitated OM were recorded in the range of wavenumbers between 3900 and 400 cm^{-1} with a BioRad® FTS 135 (BIO-RAD, Cambridge, Massachusetts, USA). The sample material (0.5 mg) was mixed with 80 mg KBr and finely ground using an agate mortar. The resulting mixture was dried for 12 h over silica gel in a desiccator to standardize the water content. All spectra were recorded under the same conditions with a resolution of 1 cm^{-1} and 16 scans (Ellerbrock et al., 1999). The peak maximum in the region 1640 to 1600 cm^{-1} was in all spectra close to 1625 cm^{-1} and can be assigned to the C=O stretching of deprotonated carboxylic groups (Ellerbrock and Kaiser, 2005). The peak for protonated carboxylic groups at 1710 cm^{-1} was negligible, because of the possible interference of the KBr (Niemeyer et al., 1992). The peak at 1400 cm^{-1} was attributed to complexed carboxylic groups, either by forming chelates or binding of metals. The nitrate peak at 1384 cm^{-1}, when present, was not included in the interpretation. At wavenumbers close to 1080 cm^{-1} the peak maximum represents C-O-C groups, as found in polysaccharides.

2.6. UV-Vis analysis

Ultraviolet (UV) absorbance at 280 nm was recorded (UVIKON 930, Bio-Tek Instruments, Bad Friedrichshall, Germany) from DOM solutions and the filtrates after removal of precipitated OM as an estimate of the aromaticity of DOM (Traina et al., 1990). The C concentrations were adjusted to 10 mg C l^{-1} to ensure comparability.

2.7. Laser Scanning Microscopy (LSM) analysis

To determine the size of precipitated OM, the flocs were not removed from the solutions by filtration (see section 2.2). The suspensions were shaken manually to ensure homogeneity before measurement. Then, the suspension containing flocs of precipitated OM was transferred between two glass slides with a fixed distance of 0.15 mm to avoid squeezing of the flocs. At least three micrographs were taken from each solution with a Laser Scanning Microscope (LSM 510 META, Zeiss) and the homonymous software (Version 3.2 SP2). Precipitated OM was easily identifiable as cloud-like white structures. Afterwards, the recorded micrographs (size: 687x867 µm) were analyzed with the software DAIME (Daims et al., 2006), which calculated the following parameters: number of flocs per micrograph, the area of each floc, its perimeter and the length of the major axis. From this information we calculated the corresponding circular diameter of the flocs in the plane. We did not calculate the spherical diameter of the flocs, as we had no information about diameters in the third dimension. Our visual observation was that flocs had settled on the glass plane in the physically most stable position or simply said, the flocs were lying flat. Therefore, the spherical diameters should be smaller than the circular diameters of the flocs, especially for large ones. Assuming that the diameter of the flocs in the third dimension should have been only one fourth of the circular diameter, the reduction of the spherical diameter would have been still less than 40%. This experiment was performed in triplicate with the Oa-spruce and Oi-beech solution at an Al/C ratio of 0.1 and both pH values of 3.8 and 4.5.

For a particularly large floc (Oa-spruce solution: pH 4.5; Al/C 0.1) we recorded a xyz-stack (98 slices) with a HeNe laser (λ = 633 nm, 5 mW); the thickness of the optical slices was 0.5 µm.

The minimal resolution in the picture was 0.45x0.45 µm. The slices were afterwards reconstructed to a 3D image using the 3D module of the LSM 510 META software.

2.8. Statistics & Modelling

We calculated linear regressions between the percentage of Al in precipitated OM and the summed percentage of C, N, S and P with SPSS (significance $p<0.01$). Further linear regressions were calculated between parameters derived from the FTIR spectra (significance $p<0.01$). The modelling of the Al speciation in the used OM solutions was done with VisualMinteq v2.51 which can also calculate organic complexation. The solution compositions were taken from Scheel et al. (2007) including DOM and only the Al concentration and pH values of the respective treatment were varied.

3. Results

3.1. Elemental analysis

Precipitated OM had mostly lower C contents than the freeze dried DOM from which it was precipitated (Fig. V.1, Table V.1). The amount of organic N in the precipitates decreased along with the reduction in C (Fig. V.1, top). The C to organic N ratio in precipitated OM was in nearly all cases larger than in the respective DOM (Table V.1). We did not observe a clear response of the C to organic N ratio to differences in pH and the Al/C ratio. However, phosphorus was enriched in precipitated OM when compared with DOM, indicating precipitation of organic and inorganic P species (Fig 1, bottom). We were not able to observe any precipitation of K, Ca, Mg, NO_3 or NH_4 at the pH values investigated (data not shown). In contrast, a large fraction of Fe, Al, P and organic N was precipitated from solution. Also S was precipitated, but to a lesser extent. The OM precipitated at pH 3.8 showed only a slight increase in its Al/C ratio with increasing Al/C ratios (0.05-0.3) in solution (Table V.1). In contrast, the Al/C ratio of OM precipitated at pH 4.5 did strongly increase with larger Al/C ratios in solution. The Al content of OM precipitated at pH 4.5 was significantly larger than at pH 3.8 (Table V.1). The relative decrease in

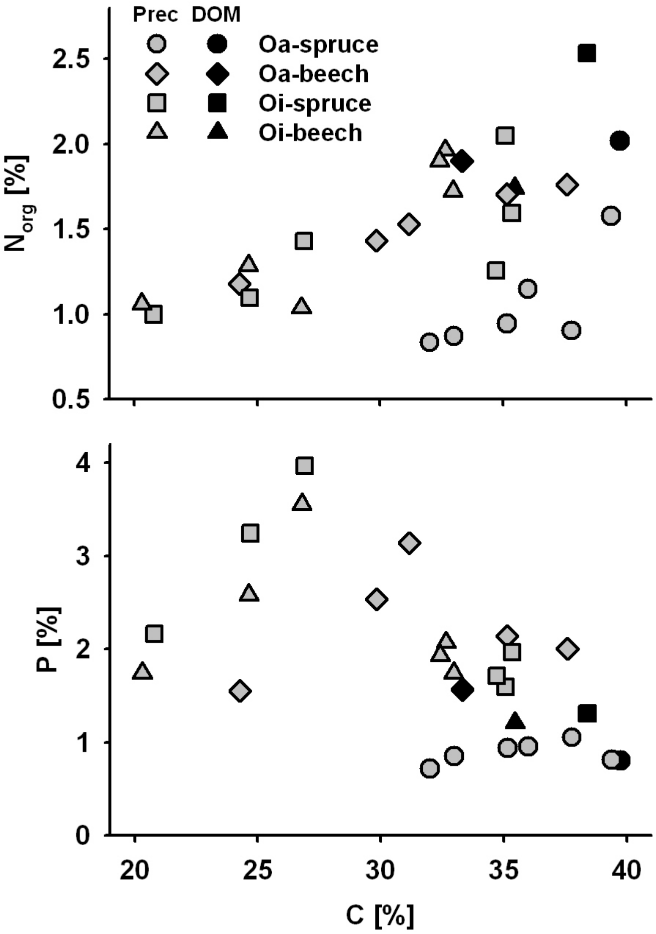

Fig. V.1: Relationship between the organic N (top) and P content (bottom) and the C content of freeze-dried dissolved organic matter (DOM) (black symbols) from four different solutions and corresponding precipitated organic matter (Prec) formed at two pH values and three Al/C ratios (grey symbols).

the weight fraction of C, N, S and P taken together was correlated linear to the increasing Al content of precipitated OM (Fig. V.2).

Table V.1: Elemental composition of freeze-dried dissolved organic matter derived from four sources and precipitated organic matter formed at two different pH values and three Al/C ratios.

	Solution	pH	Al/C ratio	C %	N %	Al %	P %	S %	C/N_{org}	Al/C prec
Dissolved organic matter	Oa-spruce			39.8	2.2	0.6	0.8	0.6	37.6	
	Oa-beech			33.3	5.2	0.3	1.6	0.6	20.5	
	Oi-spruce			38.4	3.7	0.1	1.3	0.5	17.7	
	Oi-beech			35.5	3.1	0.1	1.2	0.3	14.5	
Precipitated organic matter	Oa-spruce	3.8	0.05	39.4	1.6	3.1	0.8	0.2	29.2	0.03
			0.1	36.0	1.1	3.7	1.0	0.2	36.6	0.05
			0.3	35.2	0.9	5.6	0.9	0.3	43.5	0.07
		4.5	0.05	37.8	0.9	5.1	1.1	0.2	48.8	0.06
			0.1	33.0	0.9	6.7	0.8	0.2	44.2	0.09
			0.3	32.0	0.8	10.5	0.7	0.4	44.8	0.15
	Oa-beech	3.8	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0.1	37.6	1.8	4.7	2.0	0.3	24.9	0.06
			0.3	35.1	1.7	5.9	2.1	0.3	24.1	0.07
		4.5	0.05	31.2	1.5	6.9	3.1	0.2	23.8	0.10
			0.1	29.8	1.4	7.9	2.5	0.2	24.3	0.12
			0.3	24.3	1.2	12.5	1.6	0.5	24.0	0.23
	Oi-spruce	3.8	0.05	35.1	2.0	4.0	1.6	0.2	20.0	0.05
			0.1	35.4	1.6	4.0	2.0	0.2	25.9	0.05
			0.3	34.7	1.3	4.3	1.7	0.2	32.2	0.06
		4.5	0.05	26.9	1.4	8.2	4.0	0.2	22.0	0.14
			0.1	24.7	1.1	10.7	3.2	0.2	26.3	0.19
			0.3	20.8	1.0	13.6	2.2	0.4	24.3	0.29
	Oi-beech	3.8	0.05	33.0	1.7	4.1	1.7	0.2	22.3	0.05
			0.1	32.7	2.0	4.3	2.1	0.2	19.4	0.06
			0.3	32.4	1.9	4.5	1.9	0.2	19.9	0.06
		4.5	0.05	26.8	1.0	7.4	3.6	0.2	30.2	0.12
			0.1	24.6	1.3	8.3	2.6	0.2	22.3	0.15
			0.3	20.3	1.1	12.1	1.7	0.3	22.3	0.26

3.2. ^{13}C and ^{1}H NMR analysis

We observed for precipitated OM an enrichment of aromatic and carboxylic C, accompanied by a depletion of O-alkyl and alkyl C when compared with the respective DOM solution (Fig. V.3; Table V.2). The ^{1}H NMR spectra showed a similar trend with an enrichment of aromatic H in precipitated OM and a depletion of carbohydrate H (Fig. V.4; Table V.2). Differences in the ^{13}C NMR spectra between dissolved and precipitated OM were more pronounced for the Oi-beech than for the Oa-spruce solution. This was explicable by the fraction of DOM which was precipitated, being in the Oa-spruce solution about 40 and 75%, whereas from the Oi-beech solution about 15 and 30% of OM

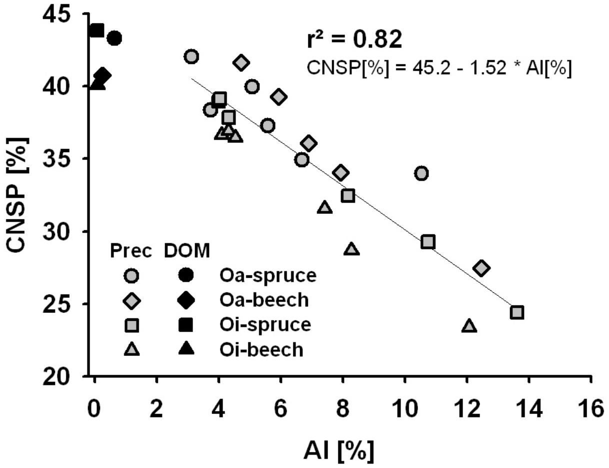

Fig. V.2: Relationship between the Al content in precipitated organic matter (Prec) and its cumulated content of C, N, S and P (CNSP). The values of Al and CNSP in dissolved organic matter (DOM) are given for comparison.

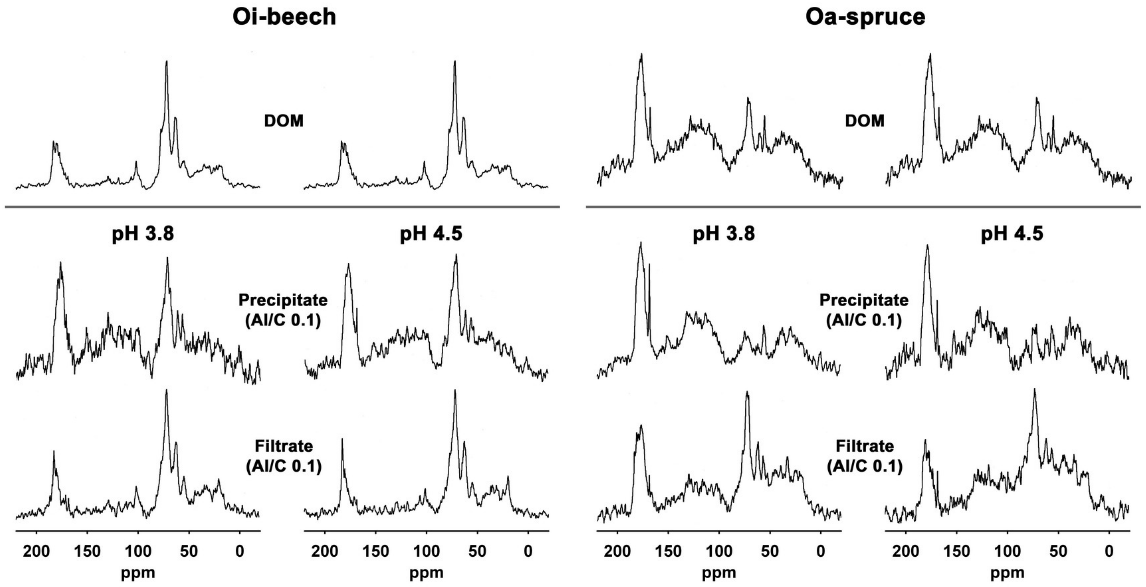

Fig. V.3: ^{13}C NMR spectra of dissolved organic matter (DOM) from the Oi beech and Oa spruce solutions are shown (top). The spectra of the DOM solutions are displayed twice to enable direct comparison to OM precipitated (middle) from these solutions at pH 3.8 (left) and pH 4.5 (right) at an Al/C ratio of 0.1. Further, spectra of the remaining filtrate (bottom) are shown for both solutions and pH-values.

Table V.2: Carbon and H moieties (as a percentage of the total) of dissolved organic matter (DOM) and selected precipitates and the remaining filtrates, as determined by ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy.

	pH	Al/C ratio	C_{alkyl} %	$C_{O\text{-}alkyl}$ %	$C_{aromatic}$ %	$C_{carboxyl}$ %	H_{alkyl} %	$H_{c.hydrate}$ %	$H_{aromatic}$ %
Oa-spruce									
DOM			22.8	22.5	30.1	24.6	25.2	32.9	14.8
Filtrate	3.8	0.05	30.7	30.1	19.7	19.5	25.8	34.0	12.1
		0.1					25.3	36.4	9.6
		0.3					40.5	29.6	5.4
	4.5	0.05	29.6	38.8	19.2	12.4	25.3	42.6	7.3
		0.1					26.2	42.5	6.4
		0.3					33.3	37.2	3.5
Precipitate	3.8	0.1	22.8	16.6	33.4	27.1	25.8	32.1	19.3
	4.5	0.1	24.0	17.4	32.2	26.4	23.0	28.6	23.0
Oa-beech									
DOM			30.2	26.7	20.4	22.7	35.4	29.9	8.3
Filtrate	3.8	0.05	34.3	30.9	15.9	18.9	40.5	21.5	9.1
		0.1					38.2	29.4	7.8
		0.3					34.8	35.6	8.0
	4.5	0.05	37.5	28.0	16.2	18.3	45.6	22.5	6.8
		0.1					44.0	25.0	7.7
		0.3					35.6	35.7	4.6
Oi-spruce									
DOM			26.1	45.5	13.9	14.6	30.9	42.8	6.2
Filtrate	3.8	0.05	29.9	45.7	10.0	14.4	30.0	46.8	3.8
		0.1					25.2	51.7	4.7
		0.3					23.5	57.2	3.7
	4.5	0.05	30.3	44.6	11.0	14.1	25.5	53.8	3.6
		0.1					25.9	53.0	3.3
		0.3					28.7	50.5	3.6
Oi-beech									
DOM			27.6	46.3	8.5	17.7	33.4	44.6	2.9
Filtrate	3.8	0.05	30.1	42.9	10.8	16.2	31.8	43.1	3.9
		0.1					31.6	45.0	3.2
		0.3					25.4	55.7	4.7
	4.5	0.05	27.5	45.5	11.4	15.6	35.1	40.3	3.4
		0.1					27.3	52.0	3.2
		0.3					24.9	55.7	2.6
Precipitate	3.8	0.1	22.3	29.2	26.8	21.7	26.9	39.9	10.4
	4.5	0.1	23.6	30.2	24.7	21.4	22.6	41.8	10.8

were removed at a pH of 3.8 and 4.5 (Scheel et al., 2007), respectively. Therefore, it was not surprising that for the Oi-beech solution the spectra of the DOM and filtrates were almost similar, whereas the spectra of the precipitates were distinctly different from DOM (Fig. V.3). The differences between the ^{13}C and ^{1}H NMR spectra of OM precipitated at pH 4.5 and pH 3.8 were very small and not significant (Fig. V.3, Fig. V.4). The same applied to the classified groups derived from the ^{13}C and ^{1}H NMR spectra (Table V.2).

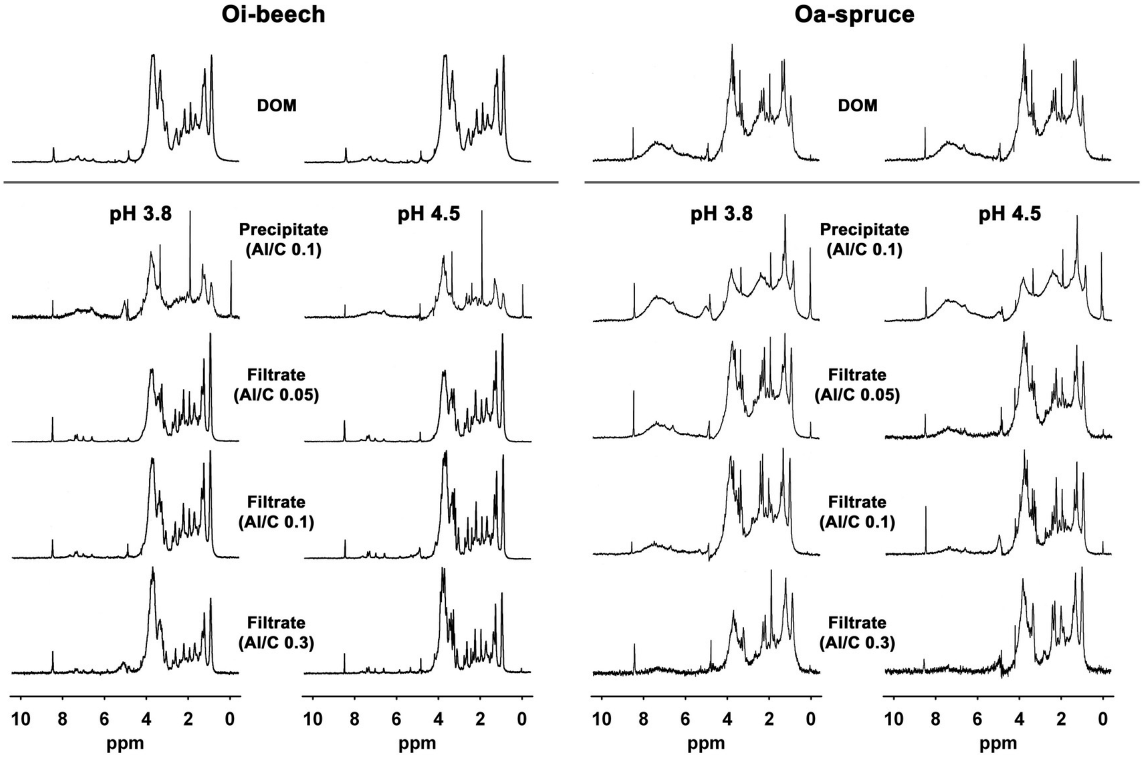

Fig. V.4: ^{1}H NMR spectra of dissolved organic matter (DOM) from the Oi beech and Oa spruce solutions are shown (top). The spectra of the DOM solutions are displayed twice to enable direct comparison to OM precipitated (middle) from these solutions at pH 3.8 (left) and pH 4.5 (right) at an Al/C ratio of 0.1. Further, spectra of the remaining filtrates (bottom) are shown for both solutions and pH-values and Al/C ratios of 0.05, 0.1 and 0.3.

The ratios of O-alkyl C and alkyl C to carboxylic C were lower in precipitated OM than in DOM (Fig. V.5, left). Also the ratios of O-alkyl C and alkyl C to aromatic C were considerably lower in precipitated OM than in the DOM solutions (Fig 5, right). The differences were largest for the Oi-beech solution with little precipitation. In the filtrates these ratios increased in almost all cases, when compared with the respective DOM solutions (Fig. V.5). Only the filtrates of the Oi-beech solutions did not reflect this trend as the differences in the ^{13}C NMR spectra between DOM and filtrate were fairly small because of little precipitation. The filtrates of the Oa solutions showed the largest increase in the ratios due to the large amount of OM removed from solution.

The same patterns were also found in the ratios of the ^{1}H NMR spectra, when comparing the ratios of carbohydrate H and alkyl H to aromatic H (Fig. V.6; Table V.2). Also here the precipitated OM had smaller ratios than the DOM, whereas the ratios of the filtrates were mostly larger than in the respective DOM solutions.

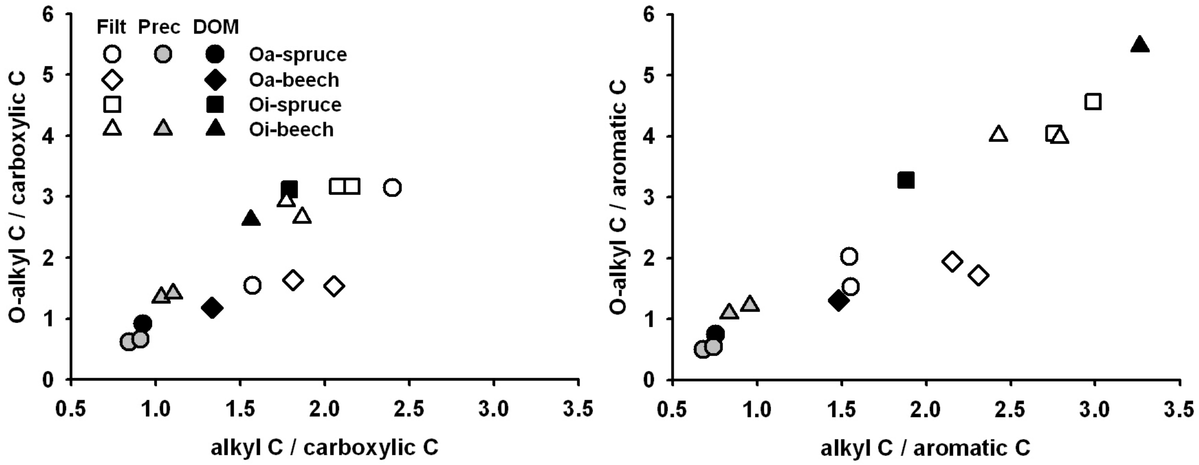

Fig. V.5: The fractions of alkyl C and O-alkyl C were related to carboxylic C (left) and aromatic C (right) determined from ^{13}C NMR spectra of the four dissolved organic matter (DOM) solutions, selected precipitated organic matter (Prec) and the remaining filtrates (Filt).

3.3. UV/Vis analysis

The UV absorbance decreased considerably after precipitation of OM when compared to the original values of the respective solutions before precipitation (Fig. V.7). We found linear correlations between the UV absorbance at 280 nm and the fraction of aromatic H ($r^2 = 0.74$) and aromatic C ($r^2 = 0.85$) for DOM and filtrates (Fig. V.7). The fraction of carboxylic C did correlate with the UV absorbance to a lesser extent ($r^2 = 0.44$).

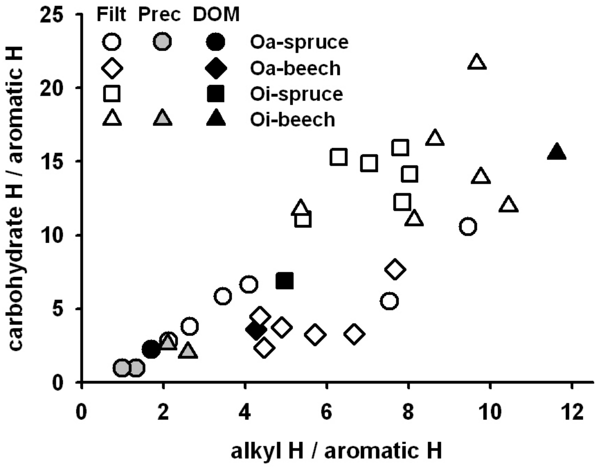

Fig. V.6: The fraction of alkyl H and carbohydrate H was related to aromatic H determined from ^{1}H NMR spectra of the four dissolved organic matter (DOM) solutions, selected precipitated organic matter (Prec) and the remaining filtrates (Filt).

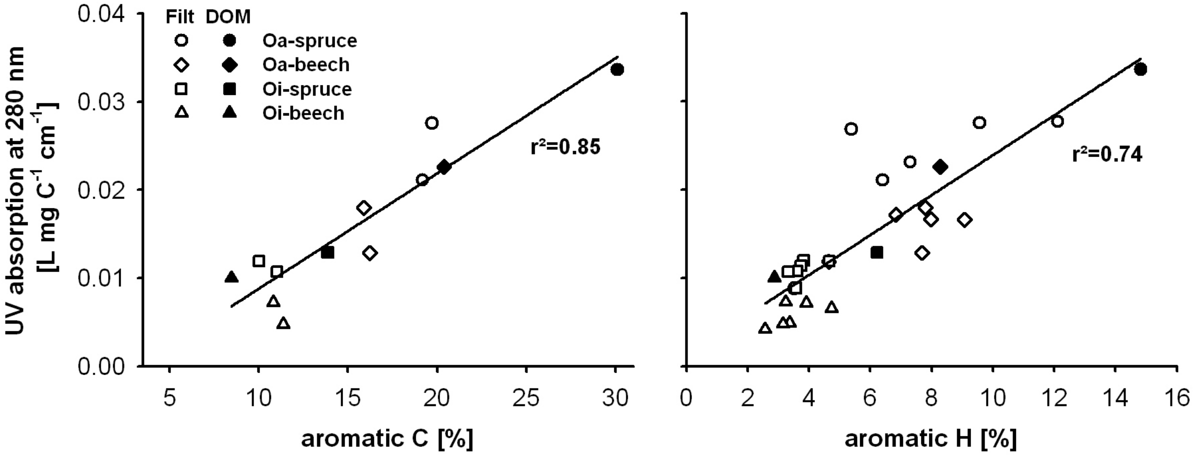

Fig. V.7: Influence of the aromatic C content determined from ^{13}C NMR spectra (left) and the aromatic H content determined from ^{1}H NMR spectra (right) on the UV absorption at 280 nm. Four dissolved organic matter (DOM) solutions and the remaining filtrates (Filt) after precipitation of organic matter were analyzed.

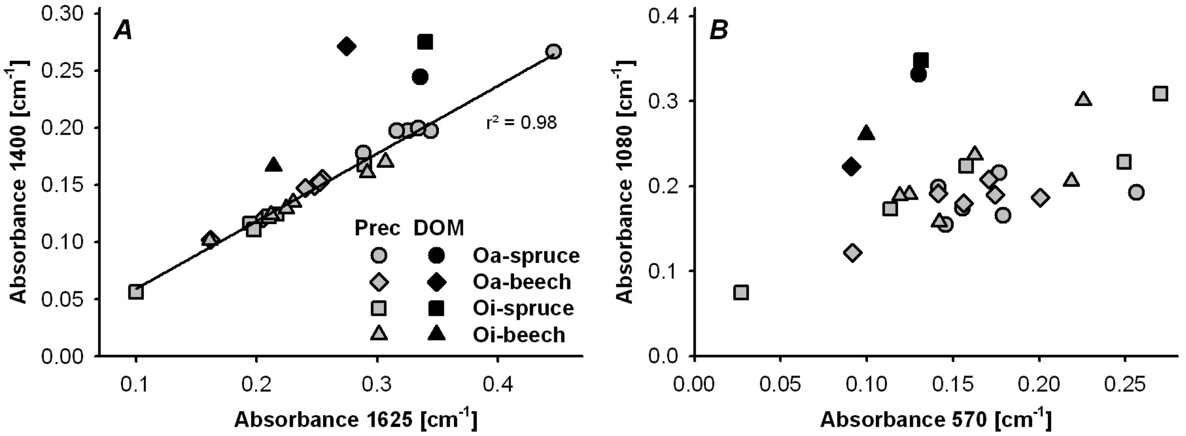

Fig. V.8: Relative intensities of absorption bands A) at 1400 cm^{-1} vs that at 1625 cm^{-1} and B) Intensities of absorption bands at 1080 cm^{-1} vs that at 570 cm^{-1} determined from Fourier transformed infrared spectra recorded for four DOM solutions and the precipitated organic matter (Prec) formed at pH values of 3.8 and 4.5 (Al/C ratios: 0.05, 0.1, 0.3).

3.4. FTIR analysis

We observed a clear increase in the ratio of the carboxylate to the complex band (wavenumber 1625 vs 1400 cm^{-1}) in precipitated OM compared to the respective DOM solution (Fig. V.8A). The complex peak was significantly correlated with the carboxylate peak for precipitated OM (constant ratio of: ~0.60), independent of the OM source, the pH of

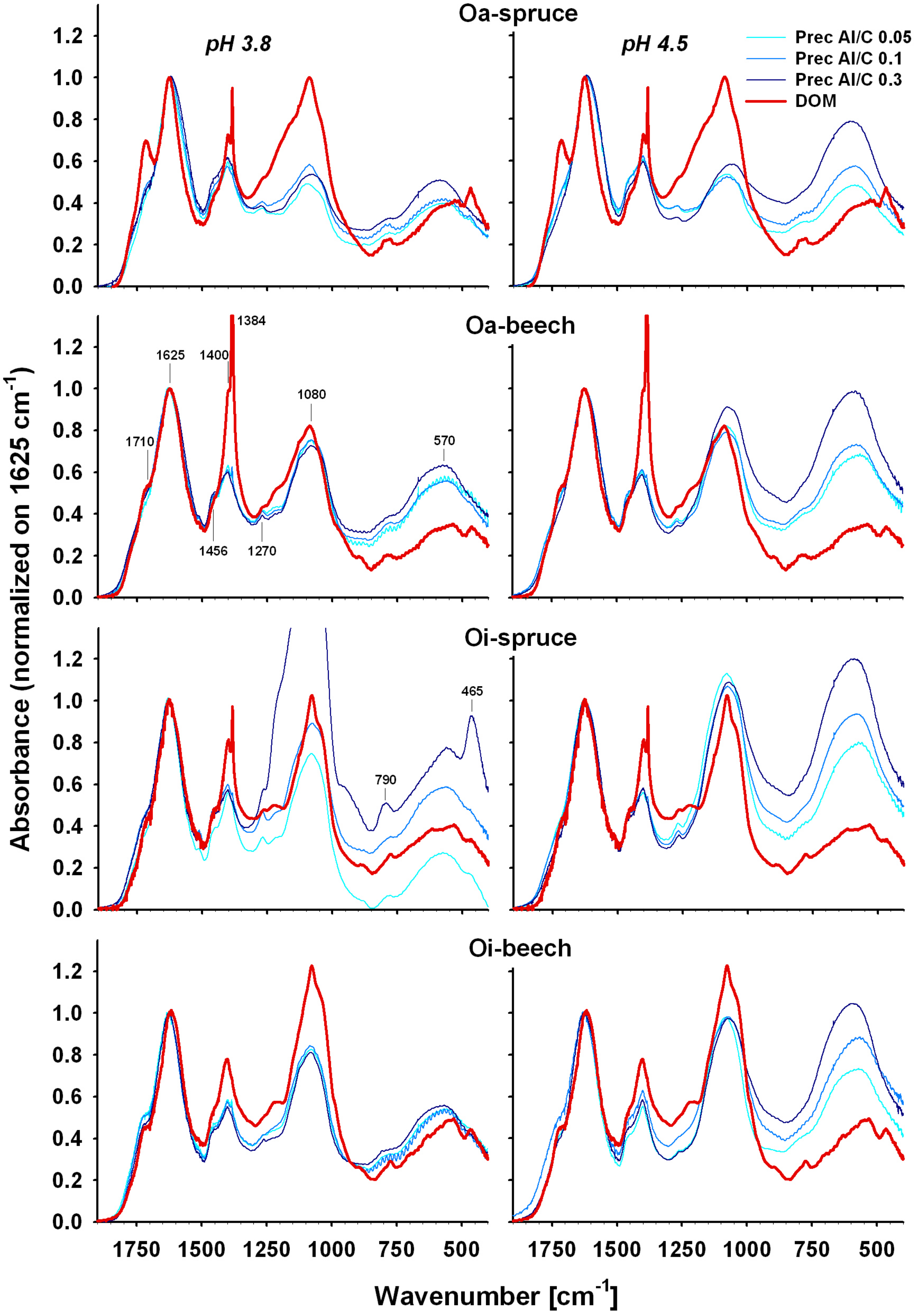

Fig. V.9: Fourier transformed infrared spectra of the four dissolved organic matter (DOM) solutions and the precipitated organic matter (Prec) formed at pH values of 3.8 and 4.5 (Al/C ratios: 0.05, 0.1, 0.3). The spectra were normalized to the peak height at 1625 cm^{-1}.

precipitation and Al/C ratio (Fig. V.8A). As we observed this strong relation to the carboxylate peak we normalized all spectra to the peak at 1625 cm^{-1}. For all four solutions we observed clearly different spectra between DOM and precipitated OM (Fig. V.9). Surprisingly, all precipitated OM displayed a highly similar spectra shape between 1900 and 1300 cm^{-1} (Fig. V.9) independent of the original solution. Like NMR analysis the FTIR analysis indicate that precipitation seems to equalize OM regarding to its composition. The Oi-spruce sample precipitated at pH 3.8 and Al/C 0.3 showed an unusual large peak height at 1080 cm^{-1} accompanied by additional peaks at 790 and 465 cm^{-1} (Fig. V.9). Since, these are characteristic peaks for silica compounds (Bosch Reig et al., 2002) we assume that this sample was contaminated by, e.g. laboratory glass. Therefore this sample was excluded from the further discussion of the FTIR spectral data. The absorbance of the Oi-spruce sample (pH 3.8, Al/C 0.05) was comparably low at small wavenumbers, but we do not have an explanation for this.

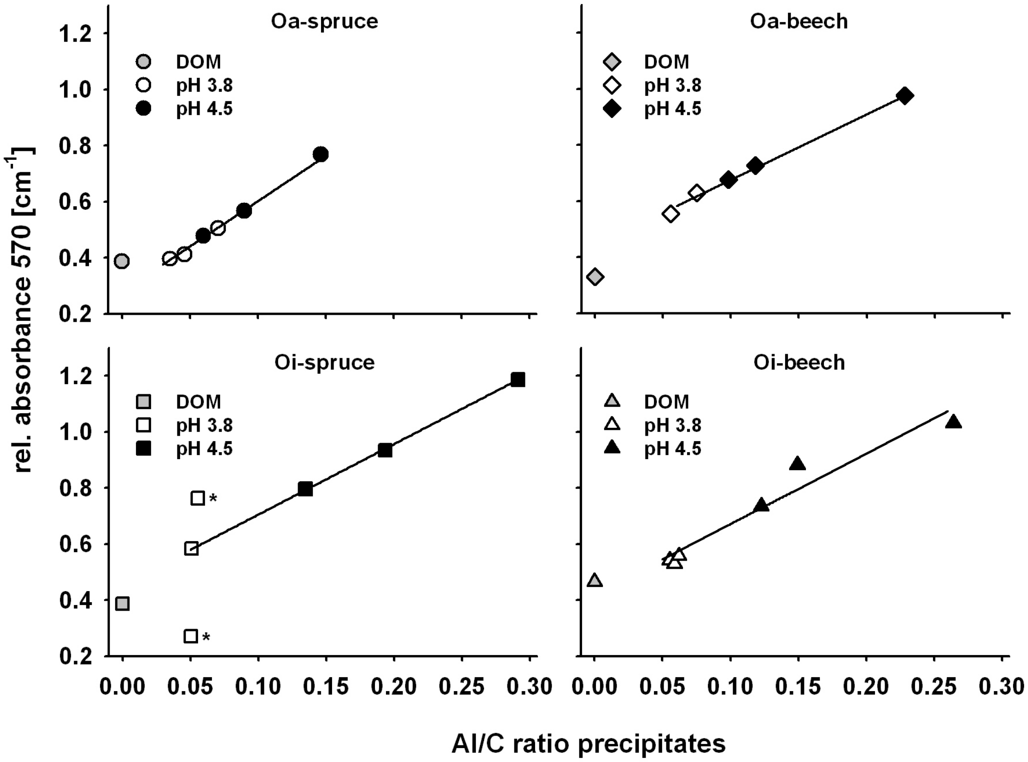

Fig. V.10: Relationship between the Al/C ratio of organic matter precipitated at pH 3.8 (white symbols) and pH 4.5 (black symbols) from four dissolved organic matter (DOM, grey symbols) solutions and the normalized (1625 cm^{-1}) absorbance at 570 cm^{-1} of the FTIR spectra. The two samples of the Oi-spruce solution marked with "*" were not included in the regression.

The small peak at 1270 cm^{-1} can be attributed to the C-O stretching of phenolic groups (Kaiser et al., 1997) and is indicative of a lignin backbone (Artz et al., 2008). Also Guan et al. (2006b) assigned the wavenumbers between 1278 and 1212 cm^{-1} to complexed phenolic C-O-Al groups. Further we observed a shoulder at 1456 cm^{-1} of the complex band. According to Cai et al. (2006) the band at 1456 cm^{-1} can be attributed to the carboxylate stretching vibration overlapped with the C-C asymmetrical deformation. The spectra of precipitated OM were clearly dominated by carboxylic groups, but there was also clear indication for phenolic groups being precipitated. The long wide peak in the fingerprint region below 1000 cm^{-1} with its maximum at 570 cm^{-1} is typically observed for amorphous Al-hydroxide (Huang et al., 1977; Karthikeyan et al., 1999, Pigna et al., 2006). We found for all four DOM solutions a linear relationship between the Al/C ratios of precipitated OM and the normalized peak height at 570 cm^{-1} (Fig. V.10). The Al content in the samples also influenced the polysaccharide peak at 1080 cm^{-1} (Fig. V.8B) which was consequently difficult to interpret in terms of polysaccharide alone. Nevertheless, we observed a clearly larger polysaccharide peak in the DOM samples related to the peak at 570 cm^{-1} when compared to the precipitated OM (Fig. V.8B), indicating that polysaccharides were not preferentially precipitated.

3.5. Laser Scanning Microscopy analysis

The diameters of precipitated OM flocs ranged from 3 to 110 µm (Fig. V.11, Table V.3). The minimum was defined by the optical system, so that we cannot exclude the possible formation of flocs smaller than 3 µm. The shape of the flocs differed largely and had a highly

Table V.3: The number of flocs per µl solution, their average, minimal and maximal circular diameter and their size distribution over the range of <5 to >50 µm as determined with a Laser Scanning Microscope. Precipitated organic matter flocs were produced from Oa-spruce and Oi-beech solutions at an Al/C ratio of 0.1 and two pH values. Mean values and standard error of three replicates in brackets.

Solution	pH	Flocs / µl	diameter [µm]			Flocs in size range [%]					
			Average	Min.	Max.	<5 µm	5-10 µm	10-20 µm	20-30 µm	30-50 µm	>50 µm
Oa-spruce	3.8	1224 (177)	16.8 (1.2)	3.2 (0.6)	87.6 (7.4)	9.4 (2.9)	33.3 (2.8)	33.9 (2.6)	10.8 (1.0)	7.8 (1.1)	4.7 (1.0)
	4.5	759 (76)	27.6 (2.0)	4.0 (0.9)	111.6 (8.4)	1.5 (0.5)	6.2 (2.0)	44.2 (2.8)	20.8 (2.0)	14.8 (1.7)	12.5 (2.3)
Oi-beech	3.8	724 (61)	16.4 (1.3)	3.2 (0.5)	70.9 (6.8)	10.3 (3.6)	27.4 (2.8)	41.9 (5.0)	9.0 (1.6)	7.7 (1.5)	3.8 (1.0)
	4.5	596 (64)	27.5 (1.9)	6.7 (1.1)	100.2 (2.8)	0.9 (0.7)	10.7 (2.2)	40.4 (2.4)	18.2 (1.6)	18.4 (2.3)	11.3 (1.8)

irregular cloud-like structure (Fig. V.11).

We consistently observed a larger amount of flocs when OM was precipitated at pH 3.8 than at pH 4.5 (Table V.3). However, the average diameters of the flocs were larger at pH 4.5 (~27.6 µm) than at pH 3.8 (~16.6 µm). At pH 4.5 we did not find a significant number of flocs with diameters below 5 µm, but a considerable fraction was still larger than 50 µm (Table V.3). In contrast, at pH 3.8 there was a much larger fraction of flocs smaller than 5 µm, and clearly less flocs larger than 50 µm when compared with pH 4.5. Further, we observed a larger number of flocs in the Oa-spruce solutions when compared with the Oi-beech solutions, reflecting the larger amount of OM precipitated from solution (Scheel et al., 2007). Surprisingly, the average sizes of (i) flocs and (ii) their size class distribution were almost similar for both DOM solutions at both pH values (Table V.3).

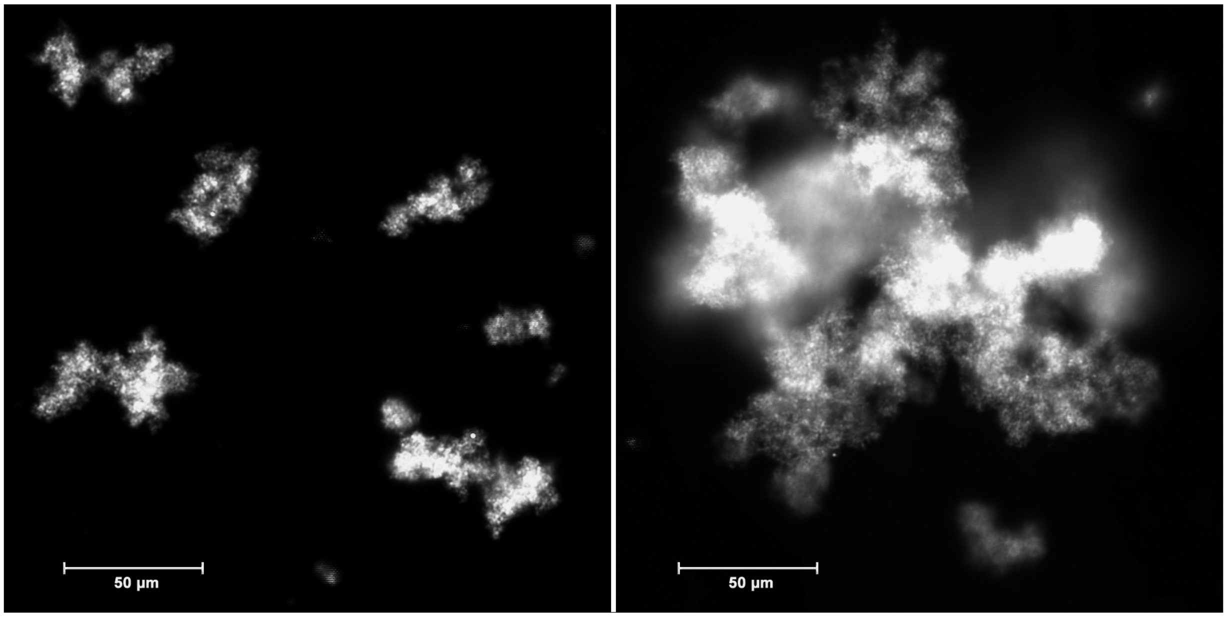

Fig. V.11: Micrographs of precipitated organic matter flocs (magnification: 400 fold). Micrographs represent flocs formed from Oa-spruce solution at pH 4.5 and an Al/C ratio of 0.1. The average circular diameter of flocs in this solution was 27.6 µm (left), but also much larger flocs could be observed (right).

A 3D micrograph taken from a large floc (Oa-spruce, pH 4.5, Al/C 0.1) showed the very porous structure of the flocs (Fig. V.12) indicating its large surface area. The maximal diameter of this floc was 130 µm and the minimal diameter 45 µm. We calculated a volume of 111228 µm^3 (0.00011 mm^3) and a surface of 114891 µm^2 (0.115 mm^2) for this floc. A sphere calculated from this volume would have a diameter of 59.6 µm and a respective surface of 11184 µm². This means that the surface of the floc is 10 times larger than the comparable spherical surface.

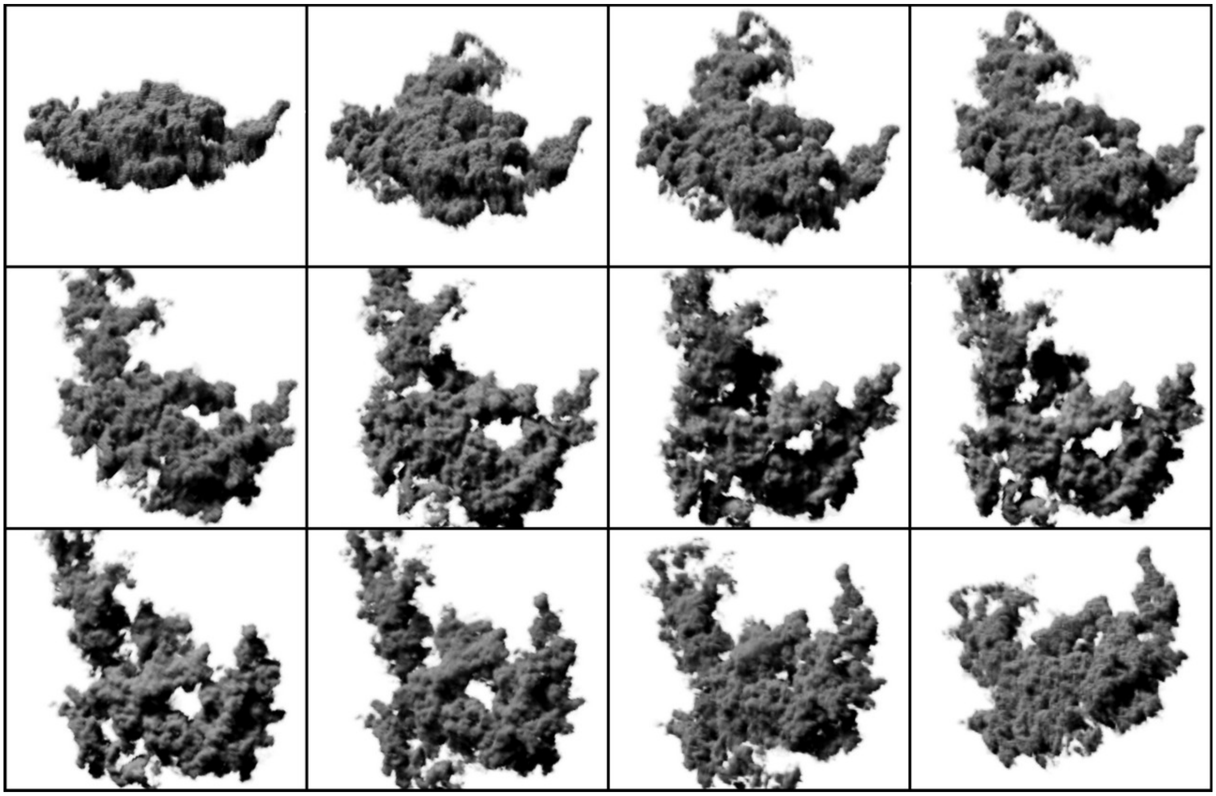

Fig. V.12: 3D reconstruction (different perspectives) of a precipitated organic matter floc (Oa-spruce, pH 4.5, Al/C 0.1) from a xyz-stack recorded with a Laser Scanning Microscope. The maximum diameter of this floc was 130 µm and the minimum 45 µm.

3.6. Modelling

The fraction of Al present as Al^{3+} cation was clearly larger at pH 3.8 than at pH 4.5 and increases for both pH values with increasing Al/C ratios in all solutions (Table V.4). Also the $Al(OH)^{2+}$ species was more present at pH 4.5, but the increase with the Al/C ratio was not observed at pH 3.8 (Table V.4). The contribution of dimeric Al species was negligible (<0.3%) and values for trimeric species were even smaller. The model calculated also an organically complexed amount of Al (Al_{org}) which was larger at pH 4.5 than at pH 3.8 (Table V.4). This fraction decreased with increasing Al/C ratios. The $AlHPO_4^+$ species behaved similar, whereas the $Al_2PO_4^{3+}$ fraction mostly increased with increasing Al/C ratios (Table V.4). The large affinity of phosphate for Al was also demonstrated by the substantial reduction of the phosphate concentrations to below 2% of the initial values (Table V.4).

Table V.4: Modelled Al species distribution and dissolved phosphate (% of initial value) of the four solutions with the respective Al concentrations and pH values used in our experiment. The solution composition was taken from Scheel et al. (2007) and the modelling performed with VisualMinteq. Aluminium species with a contribution of less than 0.1 % are not shown.

Solution	pH	Al/C ratio	Al^{3+}	$Al(OH)^{2+}$	$Al_2(OH)_2^{4+}$	$AlSO_4^{+}$	$AlHPO_4^{+}$	$Al_2PO_4^{3+}$	Al_{org}	PO_4
			% of Al							%
Oa-spruce	**3.8**	**0.05**	53.0	0.7	0.0	1.6	4.7	0.5	39.5	59.1
		0.1	68.9	0.9	0.0	1.4	3.4	0.9	24.5	36.8
		0.3	85.9	0.9	0.0	0.8	1.2	1.1	10.0	13.7
	4.5	**0.05**	24.4	1.7	0.0	0.8	6.9	1.6	64.6	36.4
		0.1	50.2	3.2	0.0	1.2	3.7	3.4	38.2	10.7
		0.3	77.1	4.2	0.2	0.8	0.6	2.7	14.2	1.6
Oa-beech	**3.8**	**0.05**	51.9	0.7	0.0	1.8	13.4	1.3	30.8	59.7
		0.1	66.5	0.8	0.0	1.6	9.7	2.4	19.0	37.8
		0.3	83.8	0.9	0.0	0.9	3.4	3.1	7.8	14.1
	4.5	**0.05**	23.4	1.6	0.0	0.9	19.7	4.4	49.9	37.4
		0.1	46.2	3.0	0.0	1.3	10.8	9.3	29.4	11.7
		0.3	74.3	4.0	0.2	0.9	1.9	7.6	10.9	1.7
Oi-spruce	**3.8**	**0.05**	65.7	0.9	0.0	1.2	8.3	1.0	22.8	54.0
		0.1	77.2	1.0	0.0	1.0	5.5	1.6	13.7	34.2
		0.3	89.3	1.0	0.0	0.5	1.9	1.8	5.5	13.2
	4.5	**0.05**	41.6	2.8	0.0	0.8	12.2	4.8	37.6	24.0
		0.1	61.9	3.9	0.1	0.9	5.6	6.4	21.1	8.3
		0.3	81.7	4.4	0.3	0.5	1.0	4.3	7.7	1.5
Oi-beech	**3.8**	**0.05**	69.8	0.9	0.0	0.8	6.9	0.9	20.6	52.5
		0.1	80.2	1.0	0.0	0.7	4.5	1.3	12.2	33.4
		0.3	90.8	1.0	0.0	0.4	1.5	1.5	4.9	13.0
	4.5	**0.05**	47.7	3.2	0.0	0.6	9.9	4.5	34.0	21.4
		0.1	66.3	4.2	0.1	0.6	4.4	5.4	18.8	7.7
		0.3	83.6	4.5	0.3	0.3	0.8	3.5	6.8	1.4

4. Discussion

4.1. Change in composition between dissolved and precipitated organic matter

The changes in composition of OM caused by precipitation can be considered as an important pathway of its stabilization against microbial decay. The smaller C to organic N ratios in precipitated OM are unfavourable for C mineralization by microorganisms (Scheel et al., 2007). Phosphorus is an essential nutrient for microorganisms, but as most of the P is probably complexed by Al (Guardado et al., 2007) it is questionable if the increased P content of the precipitates would be beneficial for C mineralization.

The results of the ^{13}C and ^{1}H NMR data indicate a selective precipitation of a certain group of compounds, having large aromatic contents together with associated carboxylic groups (Table V.2). The results of the UV absorption measurements also indicated a removal of aromatic compounds (Fig. V.7) which should be the most stable components of DOM (Kalbitz et al., 2003), whereas the correlation to the carboxylic C was less strong. This seems to be contradictory as the Al cations are supposed to bind to the carboxylic groups and not to aromatic C atoms. Probably only carboxylic groups attached to aromatic rings were precipitated after binding, whereas carboxylic groups in low molecular weight organic acids remained as colloidal organo-metal complexes in solution (Scheel et al., 2008b). Therefore, the correlations to the aromatic C contents were stronger.

The large similarity of precipitated OM in the FTIR spectra (Fig. V.9) clearly indicated an equalization of OM deriving from various sources by precipitation. The relative increase of the carboxylate band in precipitated OM when compared with DOM hints at ligand exchange as an important mechanism of precipitate formation. In turn, the relative decrease of the complex band indicates that chelation might be of minor importance. This further indicates that carboxylic groups must be involved in the precipitation process. Furthermore, the relative decrease in absorption intensity at wavenumber 1080 cm^{-1} (Fig. V.8B) within the FTIR spectra illustrates that polysaccharides were not preferentially precipitated, being in contrast to the observations made by Schwertmann et al. (2005). Thus, the small rates of C mineralization observed for precipitated OM (Scheel et al., 2007) are surely related to the enrichment of aromatic compounds and the complexation by Al.

4.2. Influence of pH on precipitated organic matter and the formed bonds

NMR data, FTIR spectra and C/N ratios did not indicate differences in the composition of OM precipitated at the two different pH values. However, the molar Al/C ratio in the precipitated OM was by a factor of 2-3 larger at pH 4.5 than at pH 3.8 (Table V.1). Also the peak at 570 cm^{-1} of the FTIR spectra, being closely related to the Al/C ratio of precipitated OM (Fig. V.10), showed a clear increase for precipitates formed at pH 4.5 in comparison to pH 3.8 (Fig. V.9). This pattern was also found for the modelled $Al(OH)^{2+}$ species fraction (Table V.4). Thus, we assume that the $Al(OH)^{2+}$ species may be of particular importance because precipitation of OM was also larger at pH 4.5 than at 3.8. Possibly, also larger Al/C ratios in precipitated OM can result from the concentration of $Al(OH)^{2+}$ in solution. Increasing Al concentrations will lead to increased competition between the cations for interaction with the OM functional groups. Consequently, this leads to less functional groups bound by one Al cation und thus to increasing Al/C ratios of precipitated OM. However, the strong increase in the Al/C ratios of OM precipitated at pH 4.5 could also be related to increasing precipitation of amorphous Al-hydroxide primary particles, as indicated by saturation indices for Al-hydroxide found by Scheel et al. (2007).

Currently, no information is available if Al-OM complexes will show a FTIR pattern similar to those of amorphous Al-hydroxide at the region of 570 cm^{-1}. In our experiments the most favourable conditions for precipitation of amorphous Al-hydroxide should have been at pH 4.5 and large Al/C ratios. Surprisingly, we found a linear relationship between the Al/C ratio of precipitated OM at pH 3.8 and 4.5 (Fig. V.10) and the peak at 570 cm^{-1}, related to the carboxylate peak. This indicates that the peak at 570 cm^{-1} reflects not only Al-hydroxide, but Al-O bonds in general.

The strong correlation between the carboxylate and complex bands of the FTIR spectra (Fig. V.8A) implied that the type of bonds between carboxylic groups and Al cations formed in all precipitated OM were similar. This is remarkable since the formation of Al-C=O bonds seem to be independent on (i) composition of OM, (ii) differences in pH, and (iii) Al/C ratios during precipitation. Further, the constant ratio between the carboxylate and complex peak implies that the functional groups of the precipitated OM were complexed to a similar degree, no matter if they were formed in solutions with small or large Al contents. The most likely explanation was that all carboxylic groups of the precipitated OM were involved in binding to Al.

4.3. Implications of floc structure and composition

The observed pH dependence of the floc sizes could be important for transport processes in soil profiles. For podzols an increase in pH with depth can be observed which would also lead to an increase in floc size. This could decrease the mobility of the precipitated OM or even completely disable the movement into smaller pores. This might be a central part of the podzolization theory and an explanation for the observed morphological structure of OM in podzols (Buurman and Jongmans, 2005). Furthermore, the smaller flocs formed at lower pH could enter smaller pores. Strong et al. (2004) reported that OM in pores from 15-60 µm was readily decomposed, whereas OM in smaller pores was protected against biodegradation.

The embedding of OM in the inner part of the floc may have a comparable protective effect. Therefore, we wanted to estimate the number of molecules aggregated in one floc. For this, we needed information about the size of the joined molecules, the size of the floc and its porosity. High molecular weight organic molecules in soil solution are in the literature often referred to as fulvic and humic acids. The average radius of such molecules was determined by de Wit et al. (1993) to be 0.6-4.4 nm for humic acids (Median 0.85 nm), being in accordance with radii reported by Buffle et al. (1998) and Pranzas et al. (2003). Also latest research shows similar results with molecular weights of 4.2-4.9 kDa for fulvic compounds (Fong and Mohamed, 2007). To make a conservative estimation we assumed an average diameter of 5 nm for the organic molecules being precipitated. As we had no information about the porosity of the flocs we assumed that the volume fraction of the flocs taken up by organic molecules would be only 1% (porosity of the floc: 99%) making also here a very conservative estimation. For the average spherical diameter of the floc we used 10 µm (Table V.3). Under these assumptions about 80 million organic molecules need to be linked by cations to form an average OM floc of this size. Smaller organic molecules, a less porous structure or larger precipitated OM flocs would result in an even larger number.

This linking of millions of molecules requires the formation of a large number of bonds. Each Al cation would need to perform at least two bonds to achieve this kind of network between the organic molecules. Therefore we tried to estimate the number of bonds per Al from the

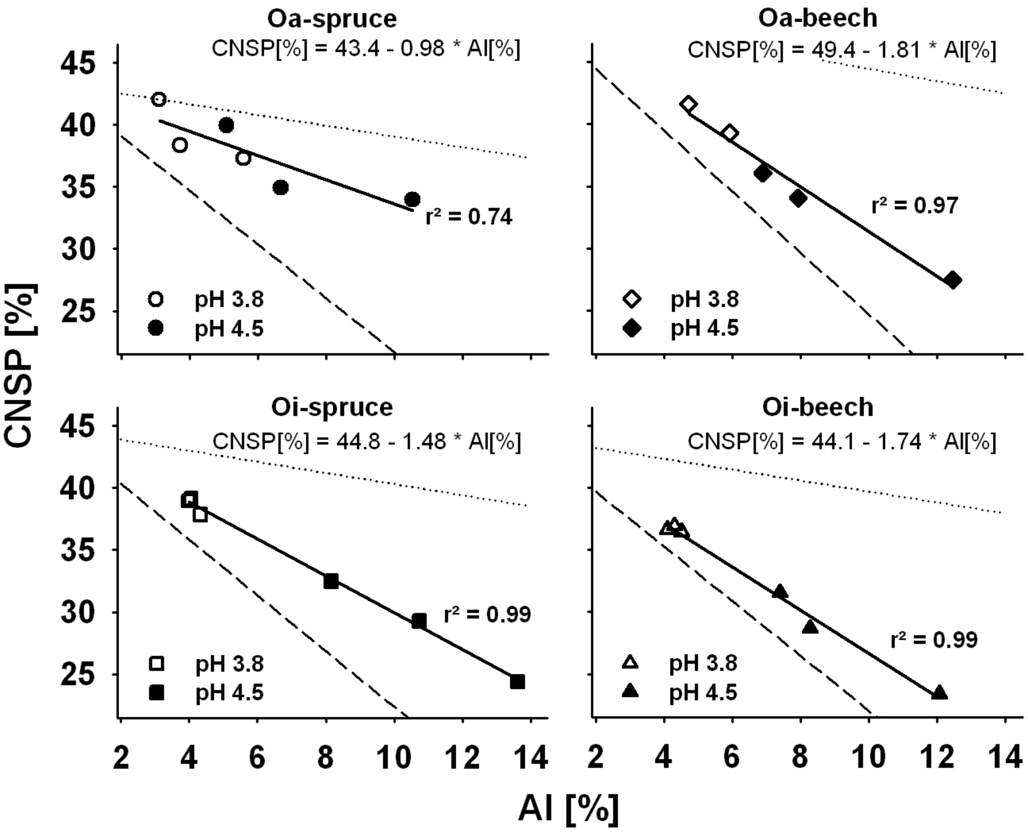

Fig. V.13: Influence of the Al content of precipitated organic matter on the cumulated amount of C, N, S and P of precipitated organic matter from four dissolved organic matter solutions. From the slope of the linear regressions we calculated the amount of water still present in the hydrate sphere after binding to the organic matter. The lower dashed line indicates the relative reduction in CNSP in case Al would completely keep its hydrate sphere after binding and the upper dotted line supposes a complete loss of the hydrate sphere.

regressions in Fig. V.13. Assuming that Al cations would be bound to the OM without any substitution of H_2O in their hydrate sphere, the percentage of C, N, S and P in precipitated OM (assuming constant O/C and H/C ratios of OM) should decrease not only proportional with the increasing Al contents, but proportional to the Al content including six water molecules in its hydrate sphere. This hypothetical decrease in the fraction of C, N, S and P is indicated by the dashed line in Fig. V.13, whereas the dotted line represents the decrease if the hydrate sphere would have been completely substituted by organic ligands. The decrease in C, N, S and P in the precipitated OM indicated that the Al cations contained still a part of their hydrate sphere (Fig. V.13). From the slope of the regressions we estimated the number of the remaining hydroxide or water molecules in the hydrate sphere of the precipitated Al to 1.9 (Oa-spruce), 4.0 (Oa-beech), 3.5 (Oi-spruce) and 4.4 (Oi-beech). This implies that per Al cation on average 1.6 to 4.1 bonds were formed, with an overall average number of 2.5. We assume that the two axial positions of the Al cation are more favourable for bond formation, with only a minor fraction of the bonds formed in the equatorial sites. The bonds between Al and the functional groups could be formed by ligand exchange reactions of carboxylic and phenolic groups with the hydrate sphere of the Al cation. The formation of Al-O-Al bonds should not be ruled out, but the fraction of Al species present as dimers or trimers in solution should have been fairly low (Table V.4).

Further, substantial precipitation of amorphous Al-hydroxide should be visible in the Al/C ratios of the precipitated OM, which range between 0.03 and 0.29 (Table V.1). We used the same 4 DOM solutions for a sorption experiment with amorphous Al-hydroxide which led to Al/C ratios of 1.0-2.0 for the samples with the maximal OM load (unpublished data). Similar findings were reported by Kaiser and Guggenberger (2007) who reported for OM sorbed to amorphous Al-hydroxide Al/C ratios of 0.6-1.1 for samples with large OM loadings. The DOM solution used in their experiment is most comparable to the Oa-spruce solution used in our experiment. There the Al/C ratios of precipitated OM were clearly smaller (0.03 to 0.15; Table V.1) indicating that the fraction of precipitated amorphous Al-hydroxide was probably little when compared with the precipitation of organically bound Al.

Precipitated OM contains substantial amounts of aromatic C with carboxylic and phenolic groups being cross-linked by Al cations. However, the large number of linked organic molecules should not only lead to a stabilization of OM because of strong chemical bonds by the organic-metal complexes or preferential precipitation of aromatic compounds. This cross-linking can also lead to spatial inaccessibility of the molecules in the flocs. Enzymes like laccase, β-glucosidase or phosphatase are important for the degradation of organic matter. The molecular weight of laccase is about 50 kDa (Jaouani et al., 2005). For β-glucosidase molecular weights of 66 kDa (Xie et al., 2004) and 120-360 kDa (Yan et al., 1998) were measured. Turowski et al. (1997) determined the molecular weight of phosphatase subunits to 31.5-105.5 kDa, equalling Stokes radii of 2.51-4.24 nm. So an average diameter for enzymes of about 5-10 nm can be assumed. Thus, the inner part of precipitated OM is probably not easily accessible for enzymes, which explains the finding of Scheel et al. (2008a), that only a certain fraction of precipitated OM is prone to degradation by enzymes. Henriques et al. (2005) showed that the availability of substances in larger flocs clearly decreased. Therefore, precipitated OM should be also additionally stabilized by spatial inaccessibility of OM for microorganisms and their enzymes.

5. Conclusions

The combination of a large variety of methods gave us a coherent view of the composition, structure and size of precipitated OM and considerably improved our understanding of mechanisms of OM stabilization. We observed that precipitated OM was clearly enriched in P when compared with the respective DOM solutions. Further, we found a preferential precipitation of aromatic compounds and an enrichment of carboxylic C in precipitated OM. This indicated that bonds between Al and OM were preferably formed with functional groups attached to molecules having high contents of aromatic C. The binding mechanism between OM and the Al cation was independent of pH, Al/C ratio and the composition of the solution. On average each Al cation bound in precipitated OM had lost 2.5 water molecules of its hydrate sphere, indicating a ligand exchange reaction. This should result in particularly stable OM, as for OM bound by ligand exchange a low biodegradability was observed (Mikutta et al., 2007). The Al cations link different molecules of organic matter up to floc sizes of 110 µm in diameter, with larger diameters at higher pH. So we assume that precipitated OM is not only stabilized by bonding of Al cations, but also through spatial inaccessibility of OM in the flocs.

The precipitation of OM was highly sensitive to changes in pH and DOM composition and also floc sizes did depend on pH. Therefore, the recovery from acidification and climate related changes in biomass production, accompanied by changes in DOM fluxes and composition, could lead to large changes in the amount of OM precipitated and the size of the formed flocs. This is of large relevance for transport and stabilization of OM in soil profiles. Furthermore, precipitated OM flocs can be destroyed by drought and dry up in soil pores. This may be beneficial for aggregate formation, but can also lead to strongly hydrophobic surfaces due to the large aromatic C content in precipitated OM. These hydrophobic surfaces are no ideal habitat for microorganisms as rewetting of these surfaces is considerably hampered. The dried precipitated OM flocs would surely not be longer recognizable as such in a soil profile, but contribute additionally to OM stabilization in soils, besides the large chemical stability and spatial inaccessibility of precipitated OM.

6. References

Artz, R.R.E., Chapman, S.J., Robertson, A.H.J., Potts, J.M., Laggoun-Défarge, F., Gogo, S., Comont, L., Disnar, J.-R., Francez, A.-J., 2008. FTIR spectroscopy can be used as a screening tool for organic matter quality in regenerating cutover peatlands. Soil Biology & Biochemistry 40, 515-527.

Bosch Reig, F., Gimeno Adelantado, J.V., Moya Moreno, M.C.M., 2002. FTIR quantitative analysis of calcium carbonate (calcite) and silica (quartz) mixtures using the constant ratio method. Application to geological samples. Talanta 58, 811-821.

Buffle, J., Wilkinson, K.J., Stoll, S., Filella, M., Zhang, J., 1998. A generalized description of aquatic colloidal interactions: The three-colloidal component approach. Environmental Science & Technology 32, 2887-2899.

Buurman, P., Jongmans, A.G., 2005. Podzolisation and soil organic matter dynamics. Geoderma 125, 71-83.

Cai, K., Ode, M., Murakami, H., 2006. Influence of polyelectrolyte dispersants on the surface chemical properties of aluminium in aqueous suspension. Colloids and Surfaces A: Physicochemical and Engineering Aspects 284-285, 458-463.

Daims, H., Lücker, S., Wagner, M., 2006. daime, a novel image analysis program for microbial ecology and biofilm research. Environmental Microbiology 8, 200-213.

de Wit, J.C.M., van Riemsdijk, W.H., Koopal, L.K., 1993. Proton binding to humic substances. 1. Electrostatic effects. Environmental Science & Technology 27, 2005-2014.

Ellerbrock, R.H., Kaiser, M., 2005. Stability and composition of different soluble soil organic matter fractions-evidence from δ^{13}C and FTIR signatures. Geoderma 128, 28-37.

Ellerbrock, R.H., Höhn, A., Rogasik, J., 1999. Functional analysis of soil organic matter as affected by long-term manurial treatment. European Journal of Soil Science 50, 65-71.

Fong, S.S., Mohamed, M., 2007. Chemical characterization of humic substances occurring in the peats of Sarawak, Malaysia. Organic Geochemistry 38, 967-976.

Guan, X.H., Chen, C.-H., Shang, C., 2006a. Combining kinetic investigation with surface spectroscopic examination to study the role of aromatic carboxyl groups in NOM adsorption by aluminum hydroxide. Journal of Colloid and Interface Science 301, 419-427.

Guan, X.H., Shang, C., Chen, C.-H., 2006b. ATR-FTIR investigation of the role of phenolic groups in the interaction of some NOM model compounds with aluminium hydroxide. Chemosphere 65, 2074-2081.

Guardado, I., Urrutia, O., Garcia-Mina, J.M., 2007. Size distribution, complexing capacity, and stability of phophate-metal-humic complexes. Journal of Agricultural and Food Chemistry 55, 408-413.

Henriques, I.D.S., Holbrook, R.D., Kelly II, R.T., Love, N.G., 2005. The impact of floc size on respiration inhibition by soluble toxicants – a comparative investigation. Water Research 39, 2559-2568.

Huang, P.M., Wang, T.S.C., Wang, M.K., Wu, M.H., Hsu, N.W., 1977. Retention of phenolic acids by noncrystalline hydroxy-aluminium and –iron compounds and clay minerals of soils. Soil Science 123, 213-219.

Jaouani, A., Guillén, F., Penninckx, M.J., Martínez, A.T., Martínez, M.J., 2005. Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzyme and Microbial Technology 36, 478-486.

Jarvis, P., Jefferson, B., Parsons, S.A., 2005. Breakage, regrowth, and fractal nature of natural organic matter flocs. Environmental Science & Technology 39, 2307-2314.

Jarvis, P., Jefferson, B., Parsons, S.A., 2006. Floc structural characteristics using conventional coagulation for a high doc, low alkalinity surface water source. Water Research 40, 2727-2737.

Kaiser, K., Guggenberger, G., Haumaier, L., Zech, W., 1997. Dissolved organic matter sorption on subsoils and minerals studied by ^{13}C-NMR and DRIFT spectroscopy. European Journal of Soil Science 48, 301-310.

Kaiser, K., Guggenberger, G., 2007. Distribution of hydrous aluminium and iron over density fractions depends on organic matter load and ultrasonic dispersion. Geoderma 140, 140-146.

Kalbitz, K., Schwesig, D., Schmerwitz, J., Kaiser, K., Haumaier, L., Glaser, B., Ellerbrock, R., Leinweber, P., 2003. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biology & Biochemistry 35, 1129-1142.

Karthikeyan, K.G., Chorover, J., Bortiatynski, J.M., Hatcher, P.G., 1999. Interaction of 1-Naphthol and its oxidation products with aluminium hydroxide. Environmental Science & Technology 33, 4009-4015.

Michalzik, B., Matzner, E., 1999. Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. European Journal of Soil Science 50, 579–590.

Mikutta, R., Mikutta, C., Kalbitz, K., Scheel, T., Kaiser, K., Jahn, R., 2007. Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica et Cosmochimica Acta 71, 2569-2590.

Niemeyer, J., Chen, Y., Bollag, J-M., 1992. Characterization of humic acids, composts, and peat by diffuse reflectance fourier-transform infrared spectroscopy. Soil Science Society of America Journal 56, 135-140.

Nierop, K.G.J., Jansen, B., Verstraten, J.M, 2002. Dissolved organic matter, aluminium and iron interactions: Precipitation induced by metal/carbon ratio, pH and competition. The Science of the Total Environment 300, 201–211.

Pigna, M., Krishnamurti, G.S.R., Violante, A., 2006. Kinetics of arsenate sorption-desorption from metal oxides: Effects of residence time. Soil Science Society of America Journal 70, 2017-2027.

Pranzas, K.P., Willumeit, R., Gehrke, R., Thieme, J., Knöchel, A., 2003. Characterisation of structure and aggregation processes of aquatic humic substances using small-angle scattering and X-ray microscopy. Analytical and Bioanalytical Chemistry 376, 618-625.

Scheel, T., Dörfler, C., Kalbitz, K., 2007. Precipitation of dissolved organic matter by Al stabilizes C in acidic forest soils. Soil Science Society of America Journal 71, 64-74.

Scheel, T., Pritsch, K., Schloter, M., Kalbitz, K., 2008a. Precipitation of enzymes and organic matter by aluminium – impacts on carbon mineralization. Journal of Plant Nutrition and Soil Science, in press.

Scheel, T., Jansen. B., van Wijk, A.J., Verstraten, J.M., Kalbitz, K. 2008b. Stabilization of dissolved organic matter by Aluminium – A toxic effect or stabilization through precipitation? European Journal of Soil Science, submitted.

Schwertmann, U., Wagner, F., Knicker, H, 2005. Ferrihydrite-humic associations: Magnetic hyperfine interactions. Soil Science Society of America Journal 69, 1009-1015.

Solinger, S., Kalbitz, K., Matzner, E., 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55, 327–349.

Strong, D.T., de Wever, H., Merckx, R., Recous, S., 2004. Spatial location of carbon decomposition in the soil pore system. European Journal of Soil Science 55, 739–750.

Traina, S., Novak, J., Smeck, N.E., 1990. An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. Journal of Environmental Quality 19, 151–153.

Turowski, P., Favre, B., Campbell, K.S., Lamb, N.J.C., Hemmings, B.A., 1997. Modulation of the enzymatic properties of protein phosphatase 2A catalytic subunit by the recombinant 65-kDa regulatory subunit PR65α. European Journal of Biochemistry 248, 200-208.

Vreysen, S., Maes, A., 2006. Adsorption mechanism of fulvic acid onto freeze dried poly(hydroxo aluminum) intercalated bentonites. Applied Clay Science 32, 190-196.

Xie, Y., Gao, Y., Chen, Z., 2004. Purification and characterization of an extracellular β-glucosidase with high transglucosylation activity and stability from *Aspergillus niger* No. 5.1. Applied Biochemistry and Biotechnology 119, 229-240.

Yan, T.-R., Lin, Y.-H., Lin, C.-L., 1998. Purification and characterization of an extracellular β-glucosidase II with high hydrolysis and transglucosylation activities from *Aspergillus niger*. Journal of Agricultural and Food Chemistry 46, 431-437.

Appendix

Own contribution of the candidate

Chapter II - Precipitation of dissolved organic matter by Al stabilizes C in acidic forest soils

Experimental design: In cooperation with K. Kalbitz
Measurements: C precipitation, C mineralization, UV absorption, determined mainly by C. Dörfler, NMR analysis L. Haumaier – all other measurements own contribution
Data analysis: Own contribution
Graphs & Tables: Own contribution
Manuscript preparation: Own contribution (75%), K. Kalbitz (25%)

Chapter III - Precipitation of enzymes and organic matter by aluminium – impacts on carbon mineralization

Experimental design: In cooperation with K. Kalbitz
Measurements: Own contribution with help by K. Pritsch
Data analysis: Own contribution with help by K. Pritsch
Graphs & Tables: Own contribution
Manuscript preparation: Own contribution (80%), K. Kalbitz (10%), further co-authors (10%)

Chapter IV - Stabilization of dissolved organic matter by Aluminium – A toxic effect or stabilization through precipitation?

Experimental design: In cooperation with B. Jansen
Measurements: Elemental analysis by T. van Wijk and P. Wartenbergh – DOC, fluorescence and DGT measurements, own contribution
Data analysis: Own contribution with help by B. Jansen
Graphs & Tables: Own contribution
Manuscript preparation: Own contribution (80%), K. Kalbitz (10%), further co-authors (10%)

Chapter V - Properties of organic matter precipitated from acidic forest soil solutions

Experimental design: In cooperation with K. Kalbitz
Measurements: NMR analysis by L. Haumaier, FTIR spectra by R. Ellerbrock – all other analysis, own contribution
Data analysis: Own contribution with help by R. Ellerbrock
Graphs & Tables: Own contribution
Manuscript preparation: Own contribution (85%), K. Kalbitz (10%), further co-authors (5%)

Publications

Mikutta, R., Mikutta, C., Kalbitz, K., **Scheel, T.**, Kaiser, K., Jahn, R., 2007. Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica et Cosmochimica Acta 71, 2569-2590.

Scheel, T., Dörfler, C., Kalbitz, K., 2007. Precipitation of dissolved organic matter by Al stabilizes C in acidic forest soils. Soil Science Society of America Journal 71, 64-74.

Scheel, T., Pritsch, K., Schloter, M., Kalbitz, K., 2008a. Precipitation of enzymes and organic matter by aluminium – impacts on carbon mineralization. Journal of Plant Nutrition and Soil Science 171, 900-907.

Scheel, T., Jansen. B., van Wijk, A.J., Verstraten, J.M., Kalbitz, K. 2008b. Stabilization of dissolved organic matter by Aluminium – A toxic effect or stabilization through precipitation? European Journal of Soil Science 59, 1122-1132.

Scheel, T., Haumaier, L., Ellerbrock, R.H., Rühlmann, J., Kalbitz, K. 2008c. Properties of organic matter precipitated from acidic forest soil solutions. Organic geochemistry 39, 1439-1453.

Acknowledgements

I need to specially thank Karsten Kalbitz for giving me the opportunity to work on this fascinating topic. Further, I greatly acknowledge the assistance and encouragement he gave me and all his helpful suggestions and discussions.

I kindly thank Egbert Matzner for supporting my work and also Ludwig Haumaier, Uwe Hell and Ingeborg Vogler as well as all other people from the Soil Ecology workgroup for their help. Further, I want to thank Catharina Dörfler and Maximilian Schneider, which both did a great master thesis on the topic.

My special thanks also go to Boris Jansen, Klaas Nierop and Koos Verstraten and the whole IBED workgroup for great collaboration and a wonderful time in Amsterdam.

A big thank to Karin Pritsch and Michael Schloter and all other people from GSF in Munich for the nice working atmosphere and the possibility for excellent work in the laboratory.

I gratefully acknowledge Ruth Ellerbrock for measuring the FTIR spectra and patiently answering all my questions.

Furthermore, I wish to thank Jörg Rühlmann and his staff for enabling the LSM measurements and the warm welcome at their institute.

A big thank also to Klaus Kaiser for fruitful discussions and opposing views.

I also kindly thank all members of the central analytical department for their help and flexibility with their analyses.

I gratefully acknowledge the funding by the Deutsche Forschungsgemeinschaft (DFG) within the priority programme SPP 1090 'Soils as source and sink of CO2'.

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig verfaßt und keine anderen als die angegeben Quellen und Hilfsmittel benutzt habe. Ich versichere weiterhin, dass ich nicht diese oder eine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden habe.

____

Thorsten Scheel