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Micro-structures generated by the mineral phase determine the fate of organic carbon and nitrogen in soil

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"Es giebt wohl kaum einen Gegenstand in der ganzen Natur, von welchem die Mehrheit der Menschen so unklare Begriffe hat und welcher bisher so völlig verkannt worden ist, als der Boden, auf welchem sie wandeln und welcher ihnen täglich vor Augen liegt."

Friedrich Albert Fallou, Pedologie oder allgemeine und besondere Bodenkunde, Dresden 1862

Summary

During soil formation, mineral particles, organic matter (OM) and microorganisms interact with each other and build up a complex soil structure with various biogeochemical interfaces. These biogeochemical interfaces are hotspots for soil processes and functions e.g. turnover of OM, fate of organic chemicals and storage of nutrients in soils. Under these aspects, the study of interactions between minerals, OM and microorganisms becomes essential for the overall understanding of soil functioning. In order to gain a mechanistic understanding of the interplay and interdependencies of physical, chemical and biological processes that occur at these highly reactive interfaces in soil, the German Research Foundation (DFG) started the Priority Program "Biogeochemical interfaces in soil" in 2007. This thesis is part of the second phase of the priority program, which started in 2011.

The main focus of this thesis was to advance the current knowledge on the formation and structure of biogeochemical interfaces by determining the effect of different soil components and their impact on the fate of carbon (C) and nitrogen (N) in soils. The overall aim was to evaluate the role of specific surfaces provided by different minerals and charcoal in the complex interplay between minerals, OM and microorganisms. Thereby, it was supposed that the formation of soil interfaces is controlled by the available particle surface. These points were addressed with two methodical approaches - artificial soils and isotopic tracing. On the one hand, artificial mixtures of minerals and OM incubated with a microbial inoculum from a natural soil were utilised to produce so called "artificial soils". These artificial soils were used to study the effect of different components on the formation of biogeochemical interfaces, aggregates and the establishment of microbial communities under well-defined conditions (Study II and III). In particular clay minerals, iron and aluminium oxides as well as charcoal with their specific surfaces were considered as important constituents affecting the development of biogeochemical interfaces. Thus, model materials of these groups were used to produce the artificial soils. On the other hand, isotopic labelled plant material (^{13}C and ^{15}N labelled litter) was applied to natural and artificial soils in order to trace the pathway of OM decomposition and sequestration as affected by the different soil compositions (Study I and III).

The aim of study I was to follow the formation of biogeochemical interfaces in a sieved (<2 mm) topsoil (Ap horizon from a Luvisol) amended with ^{13}C and ^{15}N labelled plant litter during a 42 days incubation experiment. This was done by analysing the complex interaction between soil mineral surfaces and OM as well as by imaging the spatial distribution of C and N in the clay-sized fraction. The association of OM with the mineral phase is known to be important for OM storage in soils. It is well accepted that especially the clay-sized particles have a strong effect on the sequestration of C and N in soils. Although a large number of studies investigated the properties of these organo-mineral associations, in most cases they remained a black box due to the common

approach to analyse soils and even soil fractions as a whole. The combination of nano-scale secondary ion mass spectrometry (NanoSIMS) with the application of isotopic tracers offered the possibility to study the spatial heterogeneity of organo-mineral associations down to the submicron-scale. A density and particle size fractionation was applied to separate the different fractions and get a process-oriented understanding of litter-derived C and N in organo-mineral associations and the development of biogeochemical interfaces. The biogeochemical interfaces formed in the clay-sized fraction were investigated by NanoSIMS. This technique enables the detection of up to seven secondary ion species simultaneously to generate a submicron-scale image of the elemental and isotopic composition down to a lateral resolution of around 150 nm. The results of this study showed that OM is mainly bound to mineral clusters with rough surfaces (Study I). The combination of stable isotope tracers with NanoSIMS gave the opportunity to visualise newly formed biogeochemical interfaces in soils and was used to distinguish between fresh litter-derived and pre-existing OM. Already existing OM clusters were found to be more important for the accumulation of new OM in the clay-sized fraction of a structured soil than a large area of mineral surfaces. Less than 19% of the visible mineral areas showed an OM attachment. Thus, the results from study I provide evidence that only a limited proportion of the clay-sized surfaces contribute to the interaction with OM. Overall, the results presented in this study are thought-provoking with respect to the current theory of C and N sequestration in soils and the widely used C saturation estimates, where the clay content or mineral surface area are used to estimate the sequestration potential of soils.

The establishment of the microbial communities and the formation of macro-aggregates as a function of the soil composition were studied with an approach using artificial soils (Study II). Five different artificial soil compositions with two types of clay minerals (illite, montmorillonite) and a varying presence of metal oxides (ferrihydrite, boehmite) or charcoal were used. The artificial soils, produced with sterile manure and a microbial community derived from a natural soil, were subjected to a 842 days incubation experiment. The defined compositions of the artificial soil systems allowed to directly relate the development of microbial communities and soil structure to the presence of specific components. After 562 days, sterile manure was applied a second time to the artificial soils to re-activate the system and analyse the response of the artificial soil systems. A changing microbial community composition and an effect on macro-aggregation after OM addition was expected, as the input of fresh substrate was supposed to re-activate the artificial soils. The additional OM input and longer incubation time (842 days) led to a re-formation of macro-aggregates which was significantly higher when montmorillonite was present. The second manure input resulted in higher CO_2 respiration rates. This indicated a stronger response of the microbial community in already established artificial soil systems. Furthermore, an increased bacterial population could be seen for all artificial soils after the second manure supply. The bacterial populations with strongly raised

abundance due to the second sterile manure application were affiliated to the genus of Pseudomonas, indicating that these had an advantage over other bacteria. The decrease in relative abundance of fast-responding Pseudomonas populations and the decline in CO_2 respiration over the incubation time suggest a succession in the active microbial community. The type of clay minerals was identified as the most important factor determining the composition of the bacterial communities. Thus, the properties of the two clay minerals, illite and montmorillonite, seemed to regulate the process of the bacterial community establishment over a long-term incubation experiment. In this study, it could be shown for the first time that clay minerals affect the microbial community composition even after a prolonged development of the system. This indicated that the effect of minerals on the microbial community is not solely dependent on the presence of fresh mineral surfaces, but points to a fundamental effect of minerals on the formation of microbial habitats in soil. The type of clay mineral present was therefore decisive for both microbial community composition as well as macro-aggregation. Even though different bacterial communities were established depending on the artificial soil composition, the amount and quality of the OM did not show significant differences, supporting the concept of functional redundancy. In contrast to the previous assumption, ferrihydrite, boehmite and charcoal had no additional effect on aggregation and the establishment of microbial communities.

In study III, the fate of OM in matured artificial soils (from study II developed over 842 days) and a natural soil (Ap, Luvisol) was analysed in an incubation experiment over 63 days. This was carried out by following the decomposition of ^{13}C and ^{15}N labelled plant litter and the investigating the distribution of organic C and N in fine-sized fractions with regard to different soil compositions. Furthermore, the response concerning the decomposition of freshly added litter material of the artificial soils regarding the processes occurring in a natural soil was determined to test, if the artificial soils can be used as a model system for natural soils. The microbial biomass, salt extractable organic C, the isotopic C and N composition in the bulk soil and the soil fractions (combined density and particle size fractionation) were determined. The decomposition of fresh litter in the matured artificial soils was affected by the type of clay minerals, as the results showed that the soil with montmorillonite exhibited a slower mineralisation compared to the artificial soil with illite, which was in line with a lower microbial biomass. Although a high specific surface area (SSA) is supposed to provide a high sequestration capacity for C and N, smaller amounts were sequestered in the soil containing montmorillonite with a higher SSA compared to the soil with illite. The effect of phyllosilicate minerals seemed to become evident only in developed soil systems. After several OM additions, clay minerals seem to be important for the differentiation of newly formed biogeochemical interfaces, whereas charcoal and iron oxides had no effect. It was assumed that a more intensive decomposition is associated with a higher microbial biomass leading to higher

amounts of microbial products sequestered in the clay-sized fraction. The results found in early artificial soil experiments compared to the differences in more mature artificial soils indicated that freshly added pure minerals react differently compared to minerals already incorporated in a soil-like structure. The comparison of the artificial soils with the natural soil showed that the produced soil-like systems have OM dynamics comparable to the natural soil.

The results of this dissertation contribute to the understanding of biogeochemical interfaces and structural arrangement of organic and mineral components in soils. This thesis provided evidence that only a limited proportion of the mineral surfaces interact with OM and that especially micro-structures serve as preferential binding sites for organic C and N. The studies presented in this thesis demonstrate that a detailed monitoring of the interactions between minerals, OM and microorganisms provide new insights and contribute to a better understanding of OM decomposition and sequestration of OM in soils.

Zusammenfassung

Während der Bodenbildung interagieren Mineralpartikel, organische Substanz und Mikroorganismen miteinander und bilden eine komplexe Bodenstruktur mit diversen biogeochemischen Grenzflächen. Diese Grenzflächen stellen hochreaktive Bereiche für Bodenprozesse und Bodenfunktionen dar, wie zum Beispiel für den Umsatz der organischen Substanz, den Verbleib organischer Chemikalien und die Speicherung von Nährstoffen in Böden. Für die Entwicklung eines ganzheitlichen Verständnisses dieser Prozesse und Funktionen, sind Untersuchungen über die Interaktionen zwischen Mineralen, organischer Substanz und Mikroorganismen unverzichtbar. Um die Mechanismen, Wechselwirkungen und gegenseitigen Abhängigkeiten physikalischer, chemischer und biologischer Prozesse an diesen reaktiven Grenzflächen besser zu verstehen, wurde 2007 das Schwerpunktprogramm „Biogeochemical interfaces in soil“ durch die Deutsche Forschungsgemeinschaft ins Leben gerufen. Die vorliegende Doktorarbeit ist Teil der zweiten Phase dieses Schwerpunktprogrammes, welche 2011 begonnen wurde.

Der Hauptschwerpunkt dieser Doktorarbeit besteht darin, das vorhandene Wissen über die Bildung und die Struktur biogeochemischer Grenzflächen zu erweitern, indem die Effekte verschiedener Bodenbestandteile und deren Einflüsse auf den Verbleib von Kohlenstoff und Stickstoff in Böden bestimmt wurden. Das übergeordnete Ziel der vorliegenden Arbeit war es zu ermitteln, welche Rolle verschiedene Oberflächen im komplexen Zusammenwirken von verschiedenen Mineralen, organischer Substanz und Mikroorganismen spielen. Dabei wurde angenommen, dass die Bildung reaktiver Grenzflächen von der verfügbaren Oberfläche der Partikel in Böden abhängt. Für die Untersuchung dieser Schwerpunkte wurden zwei methodische Ansätze verfolgt – künstliche Böden und Isotopenmarkierungstechnik. Einerseits wurden künstliche Gemische aus Mineralen, organischer Substanz und einem mikrobiellen Inokulum benutzt, um so genannte „künstliche Böden“ herzustellen. Diese künstlichen Böden dienten dazu, den Einfluss verschiedener Bodenbestandteile auf die Bildung biogeochemischer Grenzflächen sowie auf die Bildung von Aggregaten und die Besiedlung durch mikrobielle Gemeinschaften unter kontrollierten Bedingungen zu untersuchen (Studie II und III). Insbesondere Tonminerale, Eisen- und Aluminiumoxide und auch Holzkohle mit ihren spezifischen Oberflächen wurden als potentiell wichtige Bestandteile betrachtet, welche die Bildung biogeochemischer Grenzflächen beeinflussen. Vor diesem Hintergrund wurden Modelmaterialen der genannten Gruppen verwendet, um verschiedene Gemische von künstlichen Böden zu erzeugen. Andererseits wurden isotopenmarkierte Pflanzenmaterialien (^{13}C und ^{15}N markierte Streu) in Inkubationsversuchen mit künstlichen und natürlichen Böden verwendet, mit dem Ziel den Abbau sowie die Speicherung organischer Substanz unter dem Einfluss der sich unterscheidenden Bodenbestandteile zur verfolgen (Studie I und III).

Ziel von Studie I war es, die Bildung biogeochemischer Grenzflächen nachzuvollziehen. Dies wurde untersucht, indem ein gesiebter Oberboden (<2 mm, Ap Horizont eines Luvisols) mit ^{13}C und ^{15}N markiertem Pflanzenmaterial versetzt und einem 42 Tage dauerndem Inkubationsexperiment ausgesetzt wurde. Dazu wurden die komplexen Interaktionen zwischen Mineraloberflächen und organischer Substanz betrachtet sowie die räumliche Verteilung von Kohlenstoff und Stickstoff in der Tonfraktion mittels bildgebenden Verfahren untersucht. Es ist bekannt, dass die Assoziation von organischer Substanz mit der Mineralphase eine wichtige Rolle für die Speicherung von organischer Substanz in Böden spielt. Insbesondere ist anerkannt, dass vor allem die Tonfraktion einen starken Einfluss auf die Sequestrierung von Kohlenstoff und Stickstoff in Böden besitzt. Auch wenn sich bereits eine Vielzahl von Studien den Eigenschaften dieser organo-mineralischen Assoziationen widmeten, blieb die komplexe Struktur dieser Verbindung in den meisten Fällen unbekannt, da die üblichen Untersuchungsverfahren die Bodenproben und Bodenfraktionen im Ganzen betrachteten. Der Einsatz eines nanoskalischen Sekundärionenmassenspektrometers (NanoSIMS) in Kombination mit der Anwendung der Isotopentechnik, bot die Möglichkeit, die räumliche Heterogenität und Strukturen der gebildeten organo-mineralischen Assoziationen bis hin zum Submikrometerbereich abzubilden. Dabei wurde eine kombinierte Partikelgrößen- und Dichtefraktionierung zur Separierung verschiedener Bodenfraktionen verwendet, um ein prozessbezogenes Verständnis über den streubürtigen Kohlen- und Stickstoff innerhalb der organo-mineralischen Assoziationen und bei der Entwicklung biogeochemischer Grenzflächen zu erhalten. Die in der Tonfraktion ausgebildeten biogeochemischen Grenzflächen wurden mittels NanoSIMS untersucht. Diese Technik ermöglicht die Detektion von bis zu sieben Sekundärionen gleichzeitig und führt zu der Erstellung einer Abbildung der Element- und Isotopenzusammensetzung mit einer räumlichen Auflösung von etwa 150 nm. Die Ergebnisse dieser Studie zeigten, dass organische Substanz hauptsächlich an Mineralcluster gebunden wurde, welche eine raue Oberfläche besitzen. Ferner gibt die Kombination von NanoSIMS mit der Isotopentechnik die Möglichkeit, neu gebildete biogeochemische Grenzflächen abzubilden und wurde genutzt um zwischen streubürtiger und bereits vorhandener organischer Substanz zu unterscheiden. Dabei konnte festgestellt werden, dass solche Mineralcluster, die bereits gebildete organische Substanz binden, wichtiger für die Akkumulation frischer organischer Substanz sind, als die große Menge der restlichen Mineraloberflächen. Weniger als 19% der sichtbaren Mineraloberfläche zeigte überhaupt eine Bindung mit organischer Substanz. Die Ergebnisse von Studie I weisen demnach darauf hin, dass nur ein begrenzter Anteil der durch die Tonfraktion zur Verfügung gestellten Mineraloberflächen zur Interaktion mit der organischen Substanz beiträgt. Die in Studie I gewonnenen Resultate stellen bisherige Annahmen zur Speicherkapazität in Frage, da der Tongehalt und spezifische Mineraloberflächen üblicherweise zur Abschätzung des Kohlenstoffbindungspotentials von Böden genutzt werden.

Mit dem Ansatz künstlicher Böden in Studie II wurden die Entwicklung mikrobieller Gemeinschaften und die Bildung von Makroaggregaten in Abhängigkeit der Bodenzusammensetzung untersucht. In diesem Experiment wurden fünf verschiedene Zusammensetzungen künstlicher Böden mit den Tonmineralen Illit und Montmorillonit erzeugt. Daneben wurde die Anwesenheit von Metalloxiden (Ferrihydrit, Boehmit) bzw. von Holzkohle variiert. Neben diesen Komponenten wurden die künstlichen Böden aus sterilem Stallmist und einer aus einem natürlichen Boden stammenden mikrobiellen Gemeinschaft hergestellt. Diese künstlichen Böden wurden in Studie II einem Inkubationsexperiment über einen Zeitraum von 842 Tagen unterzogen. Die definierte Zusammensetzung der künstlichen Böden erlaubt den unmittelbaren Bezug der Entwicklung der mikrobiellen Gemeinschaften sowie der Bodenstruktur auf die Anwesenheit der spezifischen Komponenten. Nach 562 Tagen wurde ein zweites Mal steriler Stallmist appliziert, um das System zu reaktivieren und die Reaktion der künstlichen Böden auf diese zweite Zugabe hin zu untersuchen. Eine veränderte mikrobielle Gemeinschaft und ein Einfluss auf die Bildung von Makroaggregaten nach der Zugabe der organischen Substanz konnte erwartet werden, da der Eintrag frischer organischer Substanz vermutlich eine Reaktivierung der künstlichen Böden bewirkt. Der zusätzliche Eintrag von organischer Substanz und die längere Inkubationszeit (von 842 Tagen) führten zu einer Neubildung von Makroaggregaten mit signifikant höheren Mengen in den künstlichen Böden mit Montmorillonit. Die zweite Stallmistzugabe führte darüber hinaus zu höheren CO_2 Respirationsraten. Dies deutet auf eine verstärkte Reaktion der mikrobiellen Gemeinschaften in bereits etablierten künstlichen Bodensystemen hin. Weiterhin konnte in allen künstlichen Böden beobachtet werden, dass eine Bakterienpopulation durch die zweite Zugabe von Stallmist stimuliert wurde. Bakterienpopulationen, die in diesem Zusammenhang eine gesteigerte Häufigkeit zeigten, konnten der Gattung Pseudomonas zugewiesen werden. Es ist ein Hinweis darauf, dass diese im Vergleich zu anderen Bakterien deutlicher profitierten. Die Abnahme der relativen Häufigkeit dieser schnell reagierenden Pseudomonas Populationen, sowie ein festellbarer Abfall bezüglich der CO_2 Respiration über die Inkubationszeit hinweg, lässt eine Sukzession der aktiven mikrobellen Gemeinschaft vermuten. Das Vorhandensein von Tonmineral in den künstlichen Böden wurde allerdings als wichtigster Faktor für die Zusammensetzung der bakteriellen Gemeinschaft bestimmt. Demzufolge scheinen die Eigenschaften der beiden Tonminerale Illit und Montmorillonit, die Entwicklung der bakteriellen Gemeinschaften in diesem Langzeit Experiment zu regulieren. In dieser Studie konnte erstmals gezeigt werden, dass Tonminerale die Zusammensetzung der mikrobiellen Gemeinschaft auch nach einer längeren Entwicklungszeit beeinflussen. Dies lässt die Vermutung zu, dass der Einfluss von Tonmineralen nicht ausschließlich vom Vorhandensein frischer Mineraloberflächen abhängt, was auf eine eher grundlegende Wirkung der Minerale auf die Bildung spezifischer Mikrohabitate in Böden hinweist. Demzufolge waren die Tonminerale in dieser Studie

ausschlaggebend für beides, sowohl für die Zusammensetzung der mikrobiellen Gemeinschaft als auch für die Bildung von Makroaggregaten über eine längere Entwicklungszeit und nach einer zweiten Zugabe von Mist. Obwohl sich verschiedene bakterielle Gemeinschaften in Abhängigkeit der Zusammensetzung der künstlichen Böden entwickelt haben, zeigte die organische Substanz keine signifikanten Unterschiede. Dies stützt das Konzept der funktionalen Redundanz. Im Gegensatz zu den vorangestellten Annahmen wurde kein zusätzlicher Einfluss durch Ferrihydrit, Boehmit oder Holzkohle auf die Aggregation und die Entwicklung der mikrobiellen Gemeinschaften beobachtet.

In Studie III wurde mit der Durchführung eines Inkubationsexperiments über 63 Tage der Verbleib der organischen Substanz untersucht. Experimentelle Grundlage waren die über 842 Tage gealterten künstlichen Böden aus Studie II und ein natürlicher Boden (Ap, Luvisol). Analysiert wurde das Abbauverhalten der ^{13}C und ^{15}N markierten Streu und die Verteilung von organischem Kohlenstoff sowie Stickstoff in den feinen Bodenfraktionen in Abhängigkeit der Bodenzusammensetzung. Weiterhin wurde die Reaktion der gealterten künstlichen Böden mit Blick auf den Abbau der markierten Streu im Vergleich zu dem natürlichen Boden untersucht. Dieses zielte auf die Fragestellung ab, ob künstliche Böden als Modelsystem für natürliche Böden fungieren können. Inhalt dieser Studie war die Bestimmung der mikrobiellen Biomasse, des salzextrahierbaren Kohlenstoffs und der Isotopenmarkierung sowohl im Gesamtboden als auch in den Bodenfraktionen (gewonnen durch eine kombinierte Partikelgrößen- und Dichtefraktionierung). Der Abbau der frischen Streu in den gealterten künstlichen Böden war geprägt durch die Art des vorhandenen Tonminerals. Der künstliche Boden mit dem Tonmineral Montmorillonit zeigte eine langsamere Mineralisierung im Vergleich zum künstlichen Boden mit Illit. Dies erscheint mit Blick auf die geringen Mengen mikrobieller Biomasse im Boden mit Montmorillonit als plausibel. Obwohl vermutet wurde, dass höhere spezifische Oberflächen höhere Kohlenstoff- und Stickstoffspeicherkapazitäten nach sich ziehen, wurden in dem künstlichen Boden mit Montmorillonit trotz einer höheren spezifischen Oberfläche im Vergleich zum künstlichen Boden mit Illit kleinere Mengen organischer Substanz gespeichert. Der Einfluss der Tonminerale scheint daher erst in entwickelten Bodensystemen evident zu werden. Erst nach mehreren Zugaben von organischer Substanz scheinen die Tonminerale Bedeutung für die Differenzierung neu gebildeter biogeochemischer Grenzflächen erlangt zu haben. Dagegen zeigten Holzkohle und Eisenoxide keinen Effekt. Es wurde angenommen, dass eine intensivere Umsetzung der organischen Substanz verbunden mit einer höheren mikrobiellen Biomasse, zu höheren Mengen gespeicherter mikrobieller Produkte in der Tonfraktion führte. Die Ergebnisse der künstlichen Böden im Frühstadium im Vergleich zu denen im gealterten Zustand, weist darauf hin, dass frische reine Minerale eine andere Wirkungsweise haben, als Minerale, welche bereits in die Bodenstruktur eingebaut waren. Im Vergleich der künstlichen Böden mit dem natürlichen Boden konnte gezeigt werden, dass das entstandene bodenähnliche System hinsichtlich

der organischen Bodensubstanz vergleichbare Dynamiken aufwies, wie es auch am natürlichen Boden beobachtet werden konnte.

Zusammengefasst tragen die Ergebnisse dieser Doktorarbeit zum Verständnis von reaktiven Grenzflächen und der strukturellen Anordnung der organischen sowie mineralischen Bestandteile in Böden bei. Mit dieser Arbeit konnte nachgewiesen werden, dass nur ein begrenzter Anteil der Mineraloberfläche mit der organischen Substanz in Böden interagiert. Dabei fungieren insbesondere Bodenmikrostrukturen als bevorzugte Bindungsstellen für organischen Kohlenstoff und Stickstoff. Die Untersuchungen zeigten, dass detaillierte Beobachtungen der Interaktionen zwischen Mineralen, organischer Substanz und Mikroorganismen neue Erkenntnisse liefern und so zu einem besseren Verständnis des Umsatzes und der Speicherung organischer Substanz in Böden beitragen.

Content

List of Tables and Figures

Abbreviations

C	carbon
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
EPS	extracellular polymeric substance
ITS	internal transcribed spacer
IRMS	isotope ratio mass spectrometer
N	nitrogen
NanoSIMS	nano-scale secondary ion mass spectrometry
OC	organic carbon
OM	organic matter
POM	particulate organic matter
qPCR	real-time polymerase chain reaction
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SEM	scanning electron microscopy
SEOC	salt extractable organic carbon
SOM	soil organic matter
SSA	specific surface area
TC-DNA	Total community deoxyribonucleic acid x-Ray

List of publications and contributions

Research articles as first author

This scientific doctoral dissertation is based on the following research articles:

Publication I: **Cordula Vogel**, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter and Ingrid Kögel-Knabner (2014): Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils, Nature Communications 5, Article number: 2947, DOI: 10.1038/ncomms3947.

Publication II: **Cordula Vogel**, Doreen Babin, Geertje J. Pronk, Katja Heister, Kornelia Smalla and Ingrid Kögel-Knabner (2014): Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. Soil Biology and Biochemistry 79, 57-67, DOI: 10.1016/j.soilbio.2014.07.012.

Publication III: **Cordula Vogel**, Katja Heister, Franz Buegger, Irina Tanuwidjaja, Stephan Haug, Michael Schloter and Ingrid Kögel-Knabner (2015): Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions, Biology and Fertility of Soils, DOI: 10.1007/s00374-014-0987-7.

The published respectively accepted research papers are attached in the appendix.

Research article as co-author

Other related co-authored research articles from joint experiments in the Priority Program "Biogeochemical interfaces in soil":

Publication IV: Doreen Babin, **Cordula Vogel**, Sebastian Zühlke, Michael Schloter, Geertje J. Pronk, Katja Heister, Michael Spiteller, Ingrid Kögel-Knabner, Kornelia Smalla, 2014. Soil Mineral Composition Matters: Response of Microbial Communities to Phenanthrene and Plant Litter Addition in Long-Term Matured Artificial Soils. PLOS ONE 9, DOI: 10.1371/journal.pone.0106865.

My contributions to the publications were the following:

Publication I: I performed the experiments, collected and evaluated the data, developed analytical approaches to assess NanoSIMS output data and wrote the manuscript.

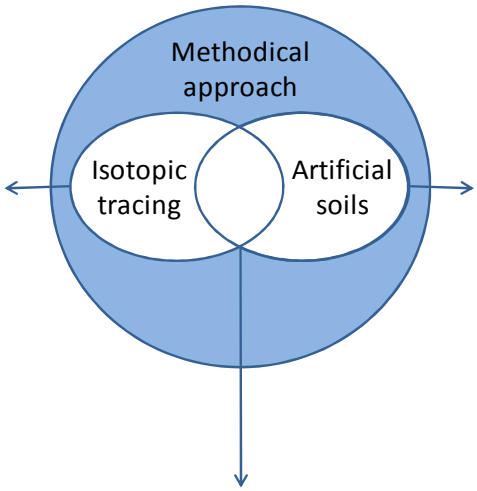
Publication II: I was involved in designing the experiment, conducted laboratory work, carried out laboratory analyses, evaluated the data and wrote the manuscript.

Publication III: I designed and performed the experiment, participated in conducting the laboratory experiments, collected and analyzed the data. I evaluated the data and wrote the manuscript.

Publication IV: I was involved in the development of the experimental design, participated in laboratory work, wrote parts of the methods section about the soils used in this study and commented on the whole manuscript.

Thesis at a glance

Micro-structures determine the fate of organic carbon and nitrogen in soil



Publication I:

Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils

Aim:

Study the formation of biogeochemical interfaces and the distribution of C and N by imaging the complex interaction between soil mineral surfaces and organic compounds.

Conclusion:

Present organo-mineral clusters with rough surfaces are crucial for the accumulation of new OM in the clay-sized fraction, rather than the large proportion of free mineral surfaces.

Publication II:

Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils

Aim:

Investigate the establishment of the microbial communities and the formation of macro-aggregates as a function of the mineral composition.

Conclusion:

The type of clay mineral was decisive for microbial community composition and macro-aggregation, but the amount and quality of the OM was similar.

Publication III:

Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions

Aim:

Analyse the fate of OM in matured artificial soils and a natural soil during litter decomposition regarding different soil compositions.

Conclusion:

A more intensive decomposition seems to be associated with a higher microbial biomass and thus leads to a higher amount of microbial products sequestered in the clay-sized fraction modified by the type of clay mineral present.

1 State of the art and objectives

1.1 The interaction between minerals, organic matter and microorganisms make soils to one of the most complex systems

1.1.1 Biogeochemical interfaces

The pedosphere is a diverse, biologically active and hierarchically structured interface between the biosphere, hydrosphere, atmosphere and lithosphere (Totsche et al., 2010) and represents therefore the integral part and central organiser of terrestrial ecosystems (Huang et al., 2005). Soils consist of a highly complex and heterogeneous soil structure and are characterised by a considerable heterogeneity in its physical, chemical and biological composition and properties. This heterogeneous structure is not fixed, but changes continuously with time (van Veen and Kuikman, 1990). Another point which makes soil to one of the most complex systems is that the emergent properties are not solely the result of the various soil components but also of interactions among them (Chorover et al., 2007). Jenny (1941) already recognised at his time the necessity of simplified methodological approaches to describe the complex soil system in order to make the multifaceted chains of cause and effect comprehensible.

Minerals, organic matter (OM) and microorganisms as major solid components in soils are in close contact and associated with each other. These associations form biogeochemical interfaces which serve as hotspots for soil processes (Chorover et al., 2007; Rennert et al., 2011). Biogeochemical interfaces are described as reactive zones mediated by microorganisms and developed in response to the interactions of soil OM (SOM), water, primary and secondary minerals and are defined by a complex three-dimensional association of various particles and aggregates (Chorover et al., 2007; Rennert et al., 2011; Totsche et al., 2010). The accurate characterisation of these reactive interfaces and the determination of the processes occurring at them pose unique challenges which cannot be achieved by analysing the bulk soil composition alone (Chorover et al., 2007). The investigation of biogeochemical interfaces at the nano- to micrometer-scale seems to be of fundamental importance, as interactions between mineral particles, OM and microorganisms at the small scale are thought to control the long term fate of e.g. SOM and nutrients (Chorover et al., 2007; Huang et al., 2005; Mueller et al., 2013; Totsche et al., 2010). Furthermore it is essential for the knowledge about restoring, enhancing and sustaining soils with its fundamental ecosystem services as for example food production (Huang et al., 2005; Totsche et al., 2010).

Enhancing the understanding about biogeochemical interface dynamics and architecture requires the assessment of the multifaceted interactions between microorganisms, OM and mineral surfaces and thus an integration of both biotic and abiotic factors (Huang et al., 2005; Totsche et al., 2010; Young and Crawford, 2004; Young et al., 2008). Although soil processes and properties are controlled by this

complex interplay, most studies look at soil processes either from a physical, chemical or from a microbiological point of view. Until today biogeochemical interfaces remained a black box due to the limited analytical tools and experimental designs.

1.1.2 Aggregate Formation

In the course of soil formation organo-mineral associations get produced, as mineral particles, OM and microorganisms are clustered and glued together and form aggregates of various sizes and complexity with highly reactive biogeochemical interfaces. Beside, these interactions polyvalent cations as well as microbial residues lead to the formation of micro-aggregates which serve as building blocks for macro-aggregates, enmeshed by fungal hyphae and roots (Tisdall and Oades, 1982). This aggregate hierarchy model was advanced by Oades (1984), who postulated that the OM within macro-aggregates is decomposed by microorganisms, becomes encapsulated with minerals and microbial residues and forms micro-aggregates within macro-aggregates. The major factors influencing aggregate formation and stabilisation are soil fauna, roots, microorganisms, environmental variables and inorganic binding agents (Six et al., 2004). The formation and stabilisation of aggregates is strongly influenced by the microbial-mediated substrate decomposition and the production of aggregate binding agents (Jastrow, 1996; Swaby, 1949). Aggregation is therefore a dynamic process, especially in regard to periodic input of OM (Abiven et al., 2009; Six et al., 2004). Microorganisms play a key role in this process of aggregate formation and stabilisation. Nowadays, microorganisms are seen as architects in shaping their immediate environment (Totsche et al., 2010; Young and Crawford, 2004). A more dominant role in soil macro-aggregation and stabilisation is assigned to fungi, as compared to bacteria, which are assumed to act at a smaller scale (Chenu and Cosentino, 2011; Chenu and Stotzky, 2002; Tisdall, 1994). Clay minerals as inorganic binding agents are known to be important for the formation and stabilisation of aggregates in soils (Edwards and Bremner, 1967; Six et al., 2004). However, only few studies have been carried out on the influence of a specific mineral composition on aggregation (Denef and Six, 2005; Denef et al., 2002; Fernández-Ugalde et al., 2013). Thus, our knowledge still lacks information regarding the role of different minerals like pedogenic oxides and clay minerals in soil aggregation (Abiven et al., 2009; Fernández-Ugalde et al., 2013).

1.2 OM turnover – Minerals and the microorganisms as driver

1.2.1 OM decomposition affected by minerals and microorganisms

The amount of OM stored in soils is mainly determined by the input from primary biomass production or fertilisation and its decomposition rate (Jenkinson, 1981; von Lützow et al., 2006). The decomposition of fresh OM entering the soil is usually described by a first phase which shows a fast turnover with a following second phase with slower decomposition rates (von Lützow et al., 2006).

The extent of OM decomposition in soils depends on the amount and quality of the substrate, abiotic factors (such as temperature, soil moisture, pH, oxygen, inorganic nutrients), the soil composition, the microbial activity and the accessibility of the substrate to the decomposer community (Jenkinson, 1981). Soil microorganisms act as major drivers for OM decomposition and the nutrient cycling in soils, as OM mineralisation and transformation in soils are microbially mediated. During decomposition of OM, the organic substrate gets incorporated into microbial biomass, used as energy source, converted to new products and partly released. Thus, microorganisms have been regarded as central transformation station (Van Veen et al., 1984). The microorganisms are the central players for the soil carbon balance by processing carbon from both fresh plant or fertiliser input and native SOM and is accordingly refereed to be the eye of the needle, where all SOM has to go through (Paterson et al., 2009). The microbial biomass itself, defined as the living microbial component of the soil (Sparling, 1985), is part of the OM in soils and accounts for approximately 0.3 to 7 % of the organic C (OC) content (Sparling, 1992; von Lützow et al., 2006). The diversity of the soil microbial community was supposed to be a key to the integrated functioning of nutrient cycling and OM decomposition in soil systems (Kennedy and Smith, 1995), although in certain cases no relationship has been found between microbial diversity and decomposition of OM (Nannipieri et al., 2003). A reduction in any group of species was found to have little effect on overall soil process and it was supposed that other microorganisms can carry out this function, indicating a functional microbial redundancy (Nannipieri et al., 2003). Nonetheless, until now it remained unclear how changes in microbial community composition alter microbial functions such as OM decomposition (Blagodatskaya et al., 2014).

The formation of soil structure and the spatial distribution of SOM within this soil matrix has major implications for the accessibility to the decomposer community and thus for the bioavailability of the SOM (Ekschmitt et al., 2008). Frequently discussed mechanisms involved in the protection of OM against decomposition in soils are: selective preservation due to recalcitrance of OM, stabilisation by interaction with mineral surfaces and spatial inaccessibility of OM against decomposer organisms (Sollins et al., 1996; von Lützow et al., 2006). The stabilisation of OM against biological attack by the mineral phase is a function of the chemical nature of the soil mineral fraction and the presence of mineral surfaces capable of adsorbing organic materials on the one hand (Baldock and Skjemstad, 2000; Oades, 1984). Sorption and complexation reactions of OM with other organic substances or inorganic materials, such as clay surfaces or the surfaces of aluminum and iron oxides, are supposed to lower their potential to be turned over by microorganisms and their extracellular enzymes (Sollins et al., 1996). Sørensen (1981) assumed that clay particles increase the content of OM in soils by forming organo-mineral associations which partly protect OM against biodegradation. Saidy et al. (2012) analysed sand-clay mixtures supplemented with an OM solution

and revealed that the OC mineralisation was significantly affected by clay mineralogy, as they observed a higher OC mineralisation in the presence of kaolinite, with a smaller mineral surface area, than in the presence of illite or smectite with higher mineral surface areas. On the other hand depends the stabilisation of OM on the architecture of the soil matrix (Baldock and Skjemstad, 2000; Oades, 1984). The inaccessibility of OM and the subsequent protection against microorganisms is mainly attributed to the incorporation within aggregates (Sollins et al., 1996). Disturbance of soil structure has been found to result in a loss of SOM (Gregorich et al., 1989), which showed that the incorporation within aggregates is an important process against microbial decomposition. On the one hand, aggregates serve as microhabitats, where the physical soil structure determines the environmental conditions for soil microorganisms (Elliott et al., 1980; Hattori and Hattori, 1976). But on the other hand, microbial activity also affects soil structure by driving the formation and stabilisation of aggregates (Oades, 1993). Thus, there are complex interactions between soil microorganisms, minerals and soil structure affecting OM decomposition, which are essential for the understanding of OM turnover in soils.

1.2.2 Minerals and their relevance in OC and N sequestration

Sequestration of C in soils is widely discussed due to its potential role in reducing atmospheric CO_2 which contributes to global warming and thus to climate change. In this sense, sequestration implies transferring atmospheric CO_2 into long-lived pools and storing it securely, so it is not immediately reemitted (Lal, 2004). Beside the role of carbon sequestration in global warming, the accumulation of OC and N in soils and the storage over a longer time is of high importance for soil quality and subsequent for a sustained agricultural production. The capacity of soils to sequester OC and N is related to clay content and mineralogy, structural stability, landscape position, moisture and temperature regimes, and the ability to form and retain stable micro-aggregates (Lal, 2004).

It is widely accepted that during the OM decomposition OC and N gets partly accumulated in small-sized fractions (Christensen, 2001; Kögel-Knabner et al., 2008b; Lützow et al., 2006). Clay-sized fractions have been generally found to bind more OM than the sand fraction (Balabane and Plante, 2004) and it has often been observed that OC and N contents increases with decreasing particle size (Christensen, 2001). Soils sequester OC and N through the formation of strong bindings between OM and mineral surfaces in organo-mineral associations (Torn et al., 1997). Very high amounts of OM bound to minerals compared to the total C stocks demonstrate the essential sequestration role of organo-mineral interactions in agricultural soils (Kögel-Knabner et al., 2008a). The physico-chemical stabilisation is based on adsorption and chemical binding of soil OC and N onto mineral surfaces (Krull et al., 2003). Clay-sized particles such as layer silicates, Fe- and Al-oxides provide the most significant surface areas onto which OM can be bound (von Lützow et al., 2006). Early research on marine sediments showed that the availability of surface area controls the concentration of OM

(Mayer, 1994b). The author proposed a monolayer coverage of OM on clay mineral surfaces, as indicated by an indirect method due to surface area calculations derived from N_2 adsorption measurements (Mayer, 1994b). Direct analyses by transmission electron microscopy challenged the monolayer coverage concept and suggested that OM exists in patches associated with mineral surfaces (Chenu and Plante, 2006; Ransom et al., 1997). Nowadays, the sequestration potential of a soil is estimated from correlations between OC concentration and the mass proportions of fine soil particles or specific surface area (SSA) (Feng et al., 2013; Hassink, 1997). However, to understand and quantify the OM sequestration potential of soils, the dynamics of OC and N accumulation at the micro-scale must be studied. Therefore, advanced techniques operating at the appropriate spatial scale are required for a better understanding of the relationship between organic compounds and mineral grains (Kögel-Knabner et al., 2008b).

Most soils contain a mix of layer silicates, Fe- as well as Al-oxides, and there is good evidence that all of these materials play a role in binding and stabilising OM (Kögel-Knabner et al., 2008b). Although sequestration of OC and N by small particles is well established, the effect of different minerals on the formation of organo-mineral associations remains an open question. Soil mineralogy has been shown to be important for the determination of the amount of OC stored in soils (Barré et al., 2014; Torn et al., 1997). The interaction between small mineral particles and OM is likely affected by the soil mineralogy due to the different SSAs and the surface charge of the various minerals in soils (Feng et al., 2013; Six et al., 2002). Six et al. (2002) found out by regression analyses that soils dominated by 1:1 clay minerals (kaolinite) exhibited a lower sequestration of OC compared to soils dominated by 2:1 clay minerals (illite, montmorillonite, vermiculite). On the contrary, Wattel-Koekkoek et al. (2001) did not find differences in OC concentrations between soils containing 2:1 and 1:1 clay minerals. Our current knowledge of the effect of soil mineralogy on the storage of OM is based on limited and conflicting data. However, it is usually accepted that the SOM sequestration of minerals decreases in the following order: allophane > smectite > illite > kaolinite (Bruun et al., 2010; von Lützow et al., 2006). But direct studies on the effect of clay mineralogy on SOM dynamics are rare (Feng et al., 2013). Investigations especially considering differences between montmorillonite and illite are even neglected, although these clay minerals are abundant and provide different characteristics, e.g. cation exchange capacity and SSA.

1.3 Methods to disentangle the influence of minerals, OM and microorganisms on the formation and functionality of biogeochemical interfaces

1.3.1 Artificial soils as a tool in soil science

The study of interactions between mineral particles, OM and the soil microorganisms is needed to understand important soil functions, e.g. the OM turnover in soils. But the difficulty in analysing

these interactions in natural systems is the hampered comparability due to different environmental conditions under which different soils developed (Ding et al., 2013; Pronk et al., 2012). The investigation of direct relations between mineralogy and OM stabilisation in natural soils is for example impeded by the association of specific mineral species with different climates, which alters the SOM dynamics (Feng et al., 2013). The physical, chemical and biological complexity of soils further hampers the manipulation of soil characteristics independently (Guenet et al., 2011). Thus, this complexity makes it difficult to predict the role of single constituents in natural soil systems and complicates the identification of a direct link between mineralogy, OM and microorganisms. Moreover, the high microbial diversity in natural soils makes it problematic to identify the factors driving the establishment and maintenance of microbial communities and hampers the identification of microorganisms involved in particular aspects of soil functioning. Thus, experiments with simplified systems of known compositions and initial conditions appear to be useful for determining the role of different constituents in soils and the understanding of how various soil properties and functions develop. Simple model systems such as suspensions have been intensively used to evaluate the effect of clay minerals on the degradation of organic compounds (Barré et al., 2014) and were frequently applied for studying the interaction between clay minerals and microorganisms (Chenu and Stotzky, 2002). However, suspension experiments do not involve the interactions of microorganisms and OM with the micro-structure of the soil (Barré et al., 2014). Due to the fact that soils represent ecosystems dominated by solid particles, the interplay between minerals, OM and microorganisms building up a complex soil structure is essential for whole soil functioning and therefore has to be considered.

In an early study, Madhok (1937) produced so called ‘synthetic soils’ by the reasons that the availability of various nutrients to microorganisms depend on the physical and chemical composition and the arrangement of the solid part of the soil. The author tested the decomposition of cellulose in sand-bentonite mixtures and concluded that synthetic soils have distinct advantages over liquid media as well as over pure sand media for studying certain microbiological processes, as this system was more similar to a natural soil (Madhok, 1937). During the last years, artificial soils are more and more regarded as a good tool for studying the factors controlling soil functioning (Babin et al., 2013; Ding et al., 2013; Guenet et al., 2011; Pronk et al., 2012). Ellis (2004) used an artificial soil substrate composed of clay minerals, sand, humic acids and calcium carbonate to study microorganisms in a habitat similar to natural soils, but lacking in factors which complicate the experimental work. Guenet et al. (2011) enhanced the artificial soil protocol of Ellis (2004) by prior creation of aggregates of a defined size and the addition of mineral nutrients to take into account these points as important factors for microbial growth. Vos et al. (2013) mentioned in a review about the evolution and maintenance of bacterial diversity affected by the highly heterogeneous soil matrix, that artificial

systems can provide fundamental new insight into microbial interactions. So far, the most artificial soil approaches concentrated mainly on the microbiology of soils, although simplified model systems can also provide a good tool to advance the process understanding of e.g. aggregate formation in soil science. To study the development of biogeochemical interfaces in an early phase, Pronk et al. (2012) created an artificial soil incubation experiment from well-known compounds under defined conditions depending only on the soil mineral composition and the presence of charcoal (540 days). The authors showed that macro-aggregates and organo-mineral associations were formed on a very short time scale (90 days) from clean model materials (Pronk et al., 2012). The development of biogeochemical interfaces was accompanied by the colonisation of the initially clean particles by diverse microbial communities as a function of the mineral composition and charcoal (Babin et al., 2013; Ding et al., 2013). Microbial community studies of the artificial soils after 90 days of incubation demonstrated that charcoal and to a lesser extent clay minerals shaped the composition of bacterial communities (Ding et al., 2013). Analysis of bacterial and fungal communities after a longer incubation time (1 year) of these artificial soils showed an ongoing influence of clay minerals and charcoal (Babin et al., 2013); however, a remarkable increase of metal oxide effects on bacteria was observed. These studies demonstrated the formation of biogeochemical interfaces and the establishment of habitats for diverse microbial communities as a function of the artificial soil composition (Babin et al., 2013; Pronk et al., 2012). Besides, the recent studies showed a dynamic development of the microbial communities over the artificial soil incubation (Babin et al., 2013; Ding et al., 2013). Although the development of such artificial systems seems to be highly dynamic, such incubation experiments with model systems were carried out only for time periods up to 1.5 years (Guenet et al., 2011; Pronk et al., 2012). Therefore, it was not yet evident how model systems develop over a longer time period, and whether the observed soil composition-dependent differences persist in matured systems. During the artificial soil incubation, Pronk et al. (2013) observed no differences neither in the mineralisation nor in the OC quantity and quality between artificial soils with different mineralogical compositions after a single manure application and short maturation time of the artificial soils. Furthermore, the artificial soil systems were already declined with regard to microorganisms and macro-aggregation, which was attributed to the missing OM input (Pronk et al., 2012). This raised the question whether additional OM supply will reactivate the model system and how various model compounds affect the amount, composition and dynamics of SOM in pre-produced artificial soils with additional OM input under longer maturation times.

1.3.2 Isotope tracing to follow the fate of OC and N in soils

A difficulty in studying the complex OM dynamics affected by different factors in soils is that analysis of total OC and N does not provide detailed information on OM dynamics. The decomposition of OM present in the bulk soil or of plant residues entering the soil can mainly be accessed through the

determination of end-products, although these products are the result of several processes (van Veen and Kuikman, 1990). The use of isotopic labels gives the opportunity to comprehensively trace the pathways of OM decomposition and the dynamics of OM transformation in different forms and pools.

With the discovery and separation of isotopes in the 1930s, the use of isotopes as tracer in ecosystems began (Barrie and Prosser, 1996). Norman and Werkman (1943) published one of the first studies regarding the use of stable isotopes in a soil science context. The authors used ^{15}N labelled soybean plant residues mixed with a soil-sand composition to study the uptake of N by soybeans, mainly to illustrate that the use of isotopic N in decomposition studies provide a good aid to improve the scientific knowledge in this area. Broadbent and Norman (1947) applied ^{13}C, as stable isotope of C, to follow the decomposition of plant material in soil and found that the utilisation of SOM by microbial populations was accelerated by the addition of ^{13}C labelled Sudan grass. In the following years, ^{14}C as radioactive isotope of C was often used to trace the pathway of OM in soil research (Jenkinson, 1971) due to low costs and an easier measuring methodology (Barrie and Prosser, 1996). Since the study by Broadbent and Norman (1947), ^{13}C, ^{14}C and ^{15}N isotopic labelled compounds have been extensively used to study the process of OM decomposition and the influence of different biotic and abiotic factors on the OM decomposition in soils (Amato et al., 1984; Amato and Ladd, 1992; Chotte et al., 1998; Jenkinson, 1971; Ladd et al., 1981; Sørensen, 1974). For example, the discovery of priming action, nowadays called priming effect (Kuzyakov et al., 2000), stems from the application of isotopic techniques in soil science (Jenkinson, 1971). By using isotopic labelled compounds, Jenkinson (1966, 1968) carried out pioneering work in investigating the role of microorganisms in the SOM cycle. The author found that small amounts of the label during decomposition of ^{14}C labelled ryegrass were located in the biomass, establishing the soil microbial biomass as an important pool mediating the OC and N transformation in soils. Furthermore, Jenkinson (1977) and Sørensen (1981) found in decomposition studies with labelled substances a higher retention of labelled C in soils with higher clay contents, resulting in the assumption that small mineral particles play an important role in the sequestration of residues during decomposition. By combining tracer techniques with the chloroform fumigation method, Ladd et al. (1985; 1981) observed that soils of higher clay contents retained higher proportions of residual organic ^{14}C and ^{15}N in the microbial biomass, suggesting a stabilisation or protection of decomposer populations through the association with soil colloids. Amato and Ladd (1992) analysed soils of different clay contents amended with labelled glucose and demonstrated that the amounts of ^{14}C labelled residues and the microbial biomass ^{14}C were correlated with the clay content. Thus, the above mentioned studies emphasise the importance of the clay content in determining differences in the decomposer activities of soils as discovered by the application of isotopes. Especially the combination of labelled

substances with other methodical approaches strongly improved our understanding in soil science. The combination of density and particle size fractionation with isotopic labelling provide the possibility to obtain a process-oriented understanding about the fate of litter-derived OC and N through various soil fractions and the development of organo-mineral associations, as the label can be used to trace the incorporation of the released OC and N in the different soil fractions. Aita et al. (1997) for example studied the decomposition and the fate of ^{13}C and ^{15}N labelled wheat straw by separating the soil into different particle-size fractions and found that the straw-derived OC accumulated most rapidly and preferentially in fine-sized fractions. Accordingly, isotopic labelled plant material can be used for differentiating complex process of OC dynamics in soils between OM mineralisation and the concurrent stabilisation of OC and N in fine-mineral fractions.

The advances in the analytical precision and applications of isotope ratio mass spectrometry (IRMS) in soil science enhanced our understanding of ecosystem structure and function and facilitate the development of the knowledge about soils as ecosystems (Boutton and Yamasaki, 1996). Nonetheless, isotopic labelling techniques provide a very powerful tool for soil scientist, especially in the combination with new technologies. For example, elemental and isotopic imaging conducted via nano-scale secondary ion mass spectrometry (NanoSIMS) is a particularly promising technique for small-scale soil process research (Mueller et al., 2013). NanoSIMS enables the direct and spatial visualization of ^{13}C and ^{15}N down to a lateral resolution of 150 nm and can be used to study the localisation of isotopes in the soil matrix ranging from primary particles to intact soil cores. Mueller et al. (2013) noted that one of the most appealing aspects of NanoSIMS analysis for many soil scientists is the instrument's potential to quantitatively localise stable isotopes at a previously unresolved spatial scale. Thus, the combination of isotopic labelling as key technique of tracking biogeochemical cycles with NanoSIMS can advance the understanding of spatiotemporal soil process-level questions, as for example the dynamics of OC and N accumulation at the micro-scale can be studied. Overall, isotopic tracing under the aspect of the continuous development of new techniques and methodologies seems to be a very promising method for disentangling the influence of minerals, OM and microorganisms and in improving our understanding of soils as a complex three-dimensional system.

1.4 Objectives

This thesis was realised within the priority programme "Biogeochemical interfaces in soil" granted by the German Research Foundation (DFG) with the overall goal to gain a mechanistic understanding of the interplay and interdependencies of physical, chemical and biological processes at biogeochemical interfaces in soil and their effect on the fate and behaviour of organic chemicals. The interdisciplinary priority programme consisted of different research teams from different research fields within Germany and Austria. The individual project "Biogeochemical interface formation in soils controlled

by different components" in the second phase of the priority programme at the Chair of Soil Science in Freising-Weihenstephan aimed at gaining a better understanding of the spatial and temporal development of biogeochemical interfaces.

In this thesis, the main focus was to advance the current knowledge on the formation and structure of biogeochemical interfaces and their effect on the fate of OM as influenced by different soil components. In order to achieve this goal, two approaches were applied - firstly, artificial soils as a tool to study the effect of different soil constituents under well-defined laboratory conditions and secondly, ^{13}C and ^{15}N labelled litter as tracer to follow the fate of OM in soils. Artificial soils and two natural soils were used in the different incubation experiments (Figure 1, Figure 2, and Figure 3) to study the existing and newly formed interfaces of natural soils and the interfaces established and matured in the artificial soils. Thereby, it was supposed that the formation of soil interfaces is controlled by the available particle surface. The overall aim was to evaluate the role of surfaces provided by different minerals and charcoal in the complex interplay between OM-mineral-microorganism interactions and the subsequent effect on the fate of OM.

Based on the overall aim of the project, the following topics were studied and published in three articles:

Study I: Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils

The aim of study I was to follow the formation of biogeochemical interfaces over different time steps and the distribution of OC and N by imaging the complex interaction between mineral surfaces and decomposed organic compounds of a natural soil in an incubation experiment with ^{13}C and ^{15}N labelled plant litter. The main focus of this study was to analyse the composition and structure of the interfaces at a high spatial resolution by using NanoSIMS. The combination of NanoSIMS with isotopic tracing was used in this study, as this offers the direct visualisation of ^{13}C and ^{15}N down to a submicron-scale and can be used to identify reactive interfaces by the simultaneous detection of ion species derived from the organic and inorganic components of the associations.

The main objectives were:

i) ... to get a process-oriented understanding about litter-derived OC and N turnover in the various soil compartments and the development of biogeochemical interfaces.

ii) ... to study the spatial heterogeneity of organo-mineral associations down to the submicron-scale.

iii) ... to identify and quantitatively assess the OM-reactive surfaces in organo-mineral associations that control the OC and N sequestration potential of soils.

Study II: Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils

The aim of this interdisciplinary approach using artificial soils was to understand the impact of different soil constituents on the development of biogeochemical interfaces, the establishment of microbial communities and the formation of macro-aggregates with the resulting effect on the processes of OM turnover during maturation of the artificial soils. To take the proposed dynamic processes into account, artificial soils were subjected to a prolonged incubation period of more than two years (842 days). Encouraged by the results obtained from the artificial soil studies in the first phase of the priority programme, we implemented a second sterile manure input in order to avoid a declining of the system (Figure 1). Thus, a further focus was to analyse the reaction of the established artificial soil systems to an additional manure input with regard to microbial community response and macro-aggregation.

The objectives of the second study were:

i) ... to study the microbial community composition depending on the artificial soil composition in a long-term incubation experiment.

ii) ... to evaluate the response of an additional sterile manure input on the soil microorganisms and macro-aggregate formation in the artificial soils with various compositions.

iii) ... to evaluate if the SOM properties depend on the microbial community established according on the artificial soil composition.

Study III: Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions

The aim of the third study was to elucidate the fate of OM in matured artificial soils and a natural soil during fresh litter decomposition in a short-term incubation by following the decomposition of the labelled litter and the distribution of OC and N in fine-sized fractions with regard to the different soil compositions. By using pre-produced artificial soils in an incubation experiment with ^{13}C and ^{15}N labelled plant litter in combination with a physical fractionation approach, we were able to differentiate between OM mineralisation and the concurrent stabilisation of OC and N affected by the soil composition over time.

The objectives of this study were:

i) ... to study the decomposition of added labelled litter in the light of different the mineral compositions in matured artificial soils.

ii) ... to investigate the sequestration of OC and N dependent on the composition of the artificial soils.

iii) ... to assess the response of the artificial soils during the decomposition of freshly added litter material compared to the processes occurring in a natural soil.

2 Materials and methods

2.1 Materials

2.1.1 Natural soils

In this thesis, two natural soil materials from the uppermost 5 cm of Ap horizons (<2 mm, Luvisol, Scheyern, Bavaria, Germany) with slightly different characteristics were used. The natural soil material applied in study I, Luvisol I had an OC content of 13.5±0.9 mg g^{-1}, a N content of 1.4±0.0 mg g^{-1}, a C/N-ratio of 9.6±0.4 and a pH value ($CaCl_2$) of 5.5±0.1. The other natural soil, Luvisol II was used to extract the microbial inoculum for the artificial soils and as natural soil material in study III. This soil material was characterised by OC contents of 16.0±0.8 mg g^{-1}, N contents of 1.8±0.1 mg g^{-1}, a C/N-ratio of 9.0±0.4 and a pH value ($CaCl_2$) of 6.6±0.1. The clay-sized fraction of both natural soil materials was determined by X-ray diffraction and showed a comparable mineral composition dominated by illite, chlorite, quartz and mixed-layer minerals.

2.1.2 Model materials of the artificial soils

The artificial soils were created of the model materials montmorillonite, illite, ferrihydrite, boehmite and charcoal. All mixtures contained sand-sized (Quartz Sand H33, Haltern, Germany) and silt-sized (Millisil W11 H) quartz (Quarzwerke GmbH, Frechen, Germany) to provide a similar texture and were composed of 40-42 % sand (>63 µm), 52-54 % coarse and medium silt (6.3-63 µm) and 6 % fine silt and clay (<6.3 µm). The montmorillonite (Ceratosil WG, Süd-Chemie AG, Moosburg, Germany) and illite (Inter-ILI Mérnöki Iroda, Hungary) were used as model clay minerals. A 6-line ferrihydrite, synthesised in the laboratory at TUM according to the method of Schwertmann and Cornell (1991), and boehmite (Capatal A Alumina, Sasol North America, Westlake, Louisiana) were used as model metal oxides. The charcoal was produced from commercial barbecue charcoal by grinding and dry sieving to a grain size of 63-200 µm. The model materials (illite, montmorillonite, ferrihydrite, boehmite and charcoal) were characterised before the experiment was set-up (Table 1). Five different compositions indicated by montmorillonite (MT), illite (IL), montmorillonite + charcoal (MT+CH), illite + ferrihydrite (IL+FH) and illite + boehmite (IL+B) were used in study II (Figure 1). The mixture with illite and boehmite was omitted in study III.

2.1.3 Organic matter amendments

Dry and sterilised manure (4.5 wt-%) sieved to <2 mm was used as OM source during the artificial soil incubation. Sterilisation was done by repeated autoclaving (4x) at 121 °C over a period of four weeks. The manure of the first addition, supplied at the beginning of the experiment (Figure 1), is characterised by an OC content of 338.5±6.9 mg g^{-1}, a N content of 30.7±1.6 mg g^{-1} and a C/N ratio of 11.0±0.4 (Study II). A second addition of sterilised manure (4.5 wt-%) was performed after 562 days to simulate the effect of fresh OM input in study II (Figure 1). The manure used for the second addition exhibited an OC content of 165.4±2.7 mg g^{-1}, a N content of 11.5±0.2 mg g^{-1} and a C/N ratio of 14.4±0.2.

Table 1: Properties of the model materials as determined by Pronk and modified from Pronk et al. (2012). For the materials for which particle size distribution between two categories is not known, total % content for both is indicated, e.g. – 100 –.

Model component	Texture			CEC	C	N	SSA BET-N_2
	sand %	silt %	clay %	$cmol_c\ kg^{-1}$	mg g^{-1}	mg g^{-1}	$m^2\ g^{-1}$
quartz sand	100				0.05	0.01	0.1
silt-sized quartz	6	94			0.05	0.01	1
montmorillonite	8	25	67	85.97	2.38	0.01	71
illite		50	50	16.65	0.18	0.16	40
ferrihydrite		– 100 –			3.5	3.4	247
boehmite	– 100 –				4.6	0.1	298
charcoal	100				750	3.8	45

For the short-term incubations with stable isotopes, a plant litter derived from a 1:1 mixture of maize (*Zea mays*) and potato (*Solanum tuberosum*) both enriched in ^{13}C and ^{15}N was used (Study I and III). The plant litter material was labelled homogeneously. Therefore, plants were grown in a green house setting using tents made out of airtight transparent plastic (Esperschütz et al., 2011). Air recirculation was achieved using six fans, which were located in the tent corners and in the middle of the longitudinal sides of the tent. The CO_2 in the tent's atmosphere was subsequently replaced with enriched $^{13}CO_2$ ($\delta^{13}C$ +170‰ V-PDB, Air Liquide, Düsseldorf, Germany) and the CO_2 concentration within the tent was maintained at 350 and 400 µmol mol^{-1} (monitored by a photo-acoustic CO_2-controller, calibration at 300 to 600 µmol mol^{-1} ±2%). Using this experimental setup, an enriched ^{13}C-atmosphere of +140‰ V-PDB was established during the whole plant growth period. The ^{15}N label was introduced into the plant biomass by adding ^{15}N ammonium-nitrate to the soil as fertiliser which corresponded to a fertiliser amount of 100 kg ha^{-1}, before plant growth started. The labelled plants

were harvested before flowering after a total growth time of six to eight weeks. The ^{15}N and ^{13}C concentrations of the mixed litter material were 6.1 and 4.9 atom-%, respectively. The OC content of the litter was 404.2±1.8 mg g^{-1}, and the N content was 23.3±0.0 mg g^{-1}, resulting in a C/N ratio of 17.3±0.1.

2.2 Incubation experiments

2.2.1 Artificial soil incubation

Five different artificial soil compositions (see 2.1.2.) were analysed to study their effect on the establishment of microbial communities, the OM turnover and aggregate formation (Figure 1). The artificial soils have been produced with a microbial inoculum derived from a Luvisol under agricultural use (Luvisol II, Scheyern, Germany; 30 ml were used to inoculate 500 g of artificial soil) and sterile manure under well-defined conditions. Additionally, a second manure supply was done after 562 days (Figure 1). The initial wetting was realised with a 0.01 M $CaCl_2$ solution to provide a soil solution with an ionic strength typical of soils.

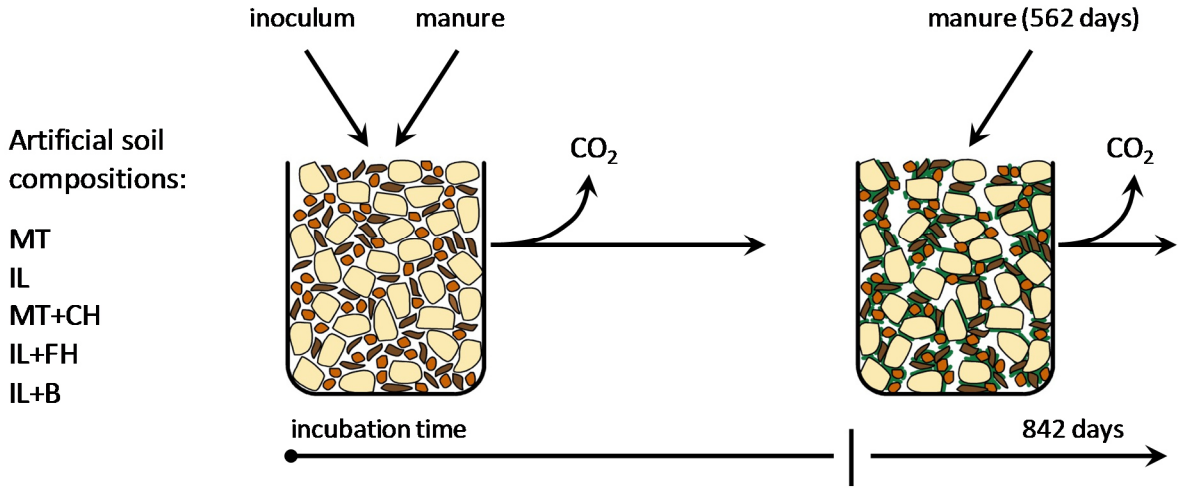


Figure 1: Study II - Long-term artificial soil incubation experiment with a second manure application after 562 days. Montmorillonite (MT), illite (IL), montmorillonite + charcoal (MT+CH), illite + ferrihydrite (IL+FH) and illite + boehmite (IL+B) indicate the artificial soil mixtures used in study II.

The artificial soils were weekly moistened with deionised water to maintain constant water content and gently mixed after water addition to ensure a homogenous distribution of water. The artificial soils were incubated in the dark at 20 °C and at a constant water content of 60 % of maximum water holding capacity for 842 days. Mixing was done carefully in order to avoid disruption of the aggregates present in the mixtures. The artificial mixtures MT, IL, MT+CH and IL+FH matured over 842 days were further used for a short-term incubation with stable isotopic labelled plant litter in study III (Figure 3).

2.2.2 Short-term incubation experiments with stable isotopes

For the incubation experiment of study I, 50 g of sieved soil (<2 mm) of the uppermost 5 cm of the Ap horizon (Luvisol I) was thoroughly mixed with 0.5 g of the labelled plant litter material (Figure 2). Three treatments were incubated: (1) labelled litter and soil (three independent replicates), (2) unlabelled litter and soil (three independent replicates), and (3) an additional treatment per point in time without litter, as a control (Figure 2). After litter addition, the soils were incubated in the dark for 42 days under defined conditions at 14 °C and at 60 % of maximum water holding capacity. The soil columns were moistened very other day with deionised water to maintain the water content.

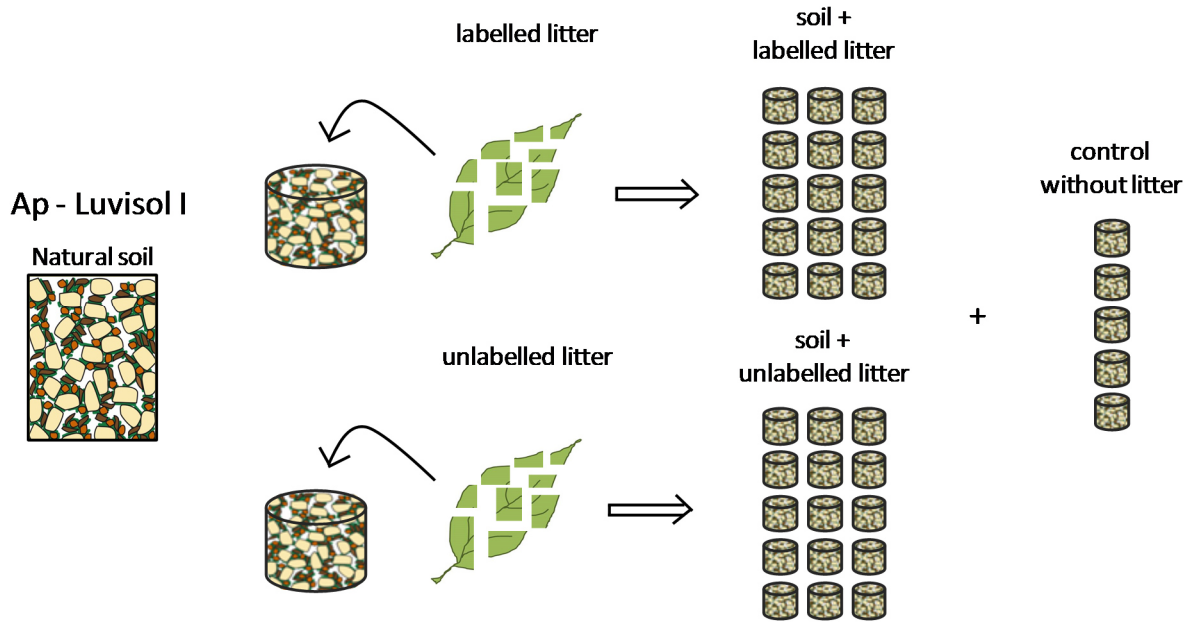


Figure 2: Study I - Incubation experiment of an Ap material (Luvisol I) with ^{13}C and ^{15}N labelled plant litter over 42 days.

In the study III, four artificial soil compositions and a natural soil (Luvisol II) were used for the short-term incubation experiment over 63 days. For this incubation, artificial soils matured over 842 days with compositions varying in the presence of illite, montmorillonite, ferrihydrite and charcoal and a natural soil were used (Figure 3). After a maturation time of 842 days, the artificial soils were sieved to <2 mm under moderately moist conditions. For the experiment, 35 g of the sieved soil (<2 mm) was homogenised with 0.350 g litter (<200 µm). Three treatments were incubated (Study III): Soil with labelled litter, control treatments without litter addition and additionally, a treatment with unlabelled litter to follow the natural abundance of ^{13}C and ^{15}N. The control treatments were set up in the same way without litter supply. After litter addition, the soils were incubated for 63 days at 14 °C in the dark and 60 % maximum water holding capacity. The soil columns were moistened in a two days interval using deionised water, to maintain the requested water content.

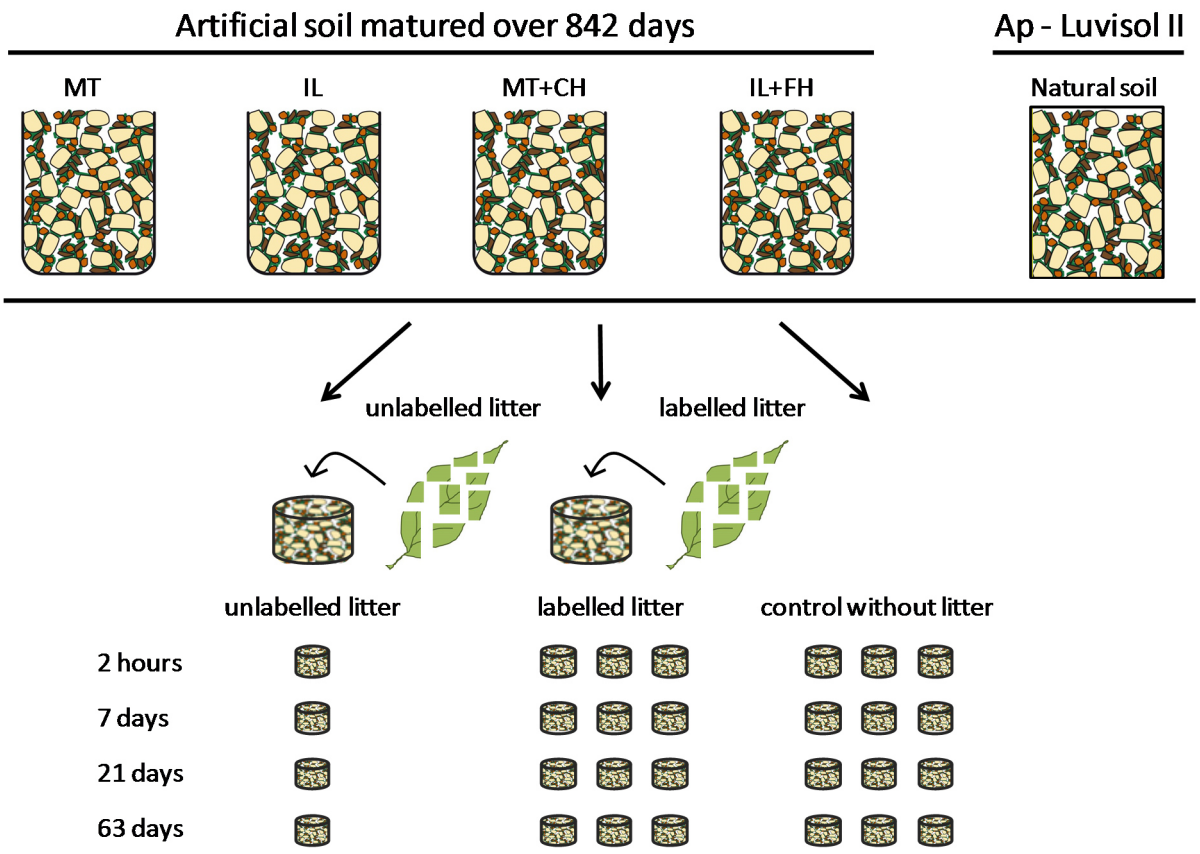


Figure 3: Study III - Incubation experiment of matured artificial soils and a natural soil (Luvisol II) with ^{13}C and ^{15}N labelled plant litter over 63 days.

2.3 Analytical methods

2.3.1 Soil characterisation

2.3.1.1 Bulk soil characterisation

The mass contribution of macro-aggregates >2 mm in the artificial soil experiment (Study II) was determined by sieving under moderately moist conditions. The pH of the samples was measured potentiometrically in 0.01 M $CaCl_2$ solution at a soil to solution ratio of 1:2.5. The OC and N contents of bulk soils and particle size fractions were determined after grinding in duplicate by an elemental analyser (Hekatech, Euro EA 3000, Wegberg, Germany). Acid hydrolysis adapted from Stevenson (1996) was used to determine the protein N, non-hydrolysable N and unidentified hydrolysable N content of the bulk samples and in the fractions <20 µm (Study II). For the determination of the salt extractable organic carbon (SEOC), fresh soil was shaken with 0.01 M $CaCl_2$ (Study I and III). Afterwards, the supernatant was filtered. SEOC was determined on a Total Carbon Analyzer (Shimadzu TOC 5050, Tokyo, Japan) by catalytic high-temperature oxidation.

2.3.1.2 Fractionation procedures

In this thesis, two different fractionation methods were used. In study II, artificial soils and the applied manure were separated into particle size fractions >200 µm, 63-200 µm, 20-63 µm and <20 µm by wet sieving. Ultrasonication was used to disrupt aggregates before this fractionation with an energy of 60 J ml^{-1} (Bandelin, Sonopuls HD 2200, Berlin, Germany; VS 70 T Sonotrode Ø 13 mm with a liquid coverage of 1.5 cm) (Schmidt et al., 1999). In the other studies, a combined density and particle size fractionation with a sodium polytungstate solution was applied in order to remove all free labelled litter and to isolate the organo-mineral associations (Table 2, 4). The floating particulate organic matter (POM) fraction was extracted using a water jet pump. The remaining slurry was ultrasonically treated to disrupt soil aggregates. The residues were wet-sieved at 63 µm and 20 µm to separate the sand-sized (> 63 µm) and coarse silt-sized fraction (20-63 µm). The soil material <20 µm was separated into medium and fine silt (2-20 µm) as well as in clay (<2 µm) sized fractions via sedimentation. Further details can be found in Publication I and II.

Table 2: Overview of the determined soil parameters and the applied methods in study I.

Study I **Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils** Incubation experiment of an Ap horizon (Luvisol I) with ^{13}C and ^{15}N labelled plant litter over 42 days
Soil parameters
• SEOC by extraction with 0.01M $CaCl_2$ • OC ,N,^{13}C and ^{15}N by isotope ratio mass spectrometer • POM, 20-63 µm , 2-20 µm and <2 µm by combined density and particle size fractionation (sodium polytungstate solution with a density of 1.8 g cm^{-3}, ultrasonically dispersion with 450 J ml^{-1})
Microbial parameters
• Microbial biomass by fumigation-extraction method
Soil fraction parameters
• OC ,N,^{13}C and ^{15}N by isotope ratio mass spectrometer • Specific surface area (clay-sized fractions) by physisorption of N_2 gas before and after OM removal by H_2O_2 • Imaging of the cay-sized fraction by SEM • Elemental mapping of the clay-sized fraction (5 clay-sized fractions): unlabelled, by NanoSIMS
Data presentation and statistical analyses
• Data presentation by SigmaPlot and ImageJ • Statistical analyses by SPSS

Table 3: Overview of the determined soil parameters and the applied methods in study II.

Study II **Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils** Artificial soils incubation experiment over 842 days with a second manure addition after 562 days
Soil parameters
• macro-aggregates >2 mm by sieving • pH in 0.01 M $CaCl_2$ solution • OC and N by an elemental analyser • protein N, non-hydrolysable N and unidentified hydrolysable N by acid hydrolysis • >200 µm, 63-200 µm, 20-63 µm and <20 µm by particle size fractionation (ultrasonically dispersion with 60 J ml^{-1})
Microbial parameters
• CO_2 respiration by titration • microbial community composition by TC-DNA extraction, DGGE fingerprint and quantitative real-time (qPCR) of 16S rRNA gene and ITS fragments
Soil fraction parameters
• OC and N by an elemental analyser • OC composition (<20 µm fraction) by solid-state ^{13}C NMR spectroscopy • protein N, non-hydrolysable N and unidentified hydrolysable N (<20 µm fraction) by acid hydrolysis
Data presentation and statistical analyses
• Data presentation by SigmaPlot • Statistical analyses by SPSS

2.3.1.3 Isotope ratio mass spectrometer (IRMS) measurements

Aliquots of the bulk soil and soil fractions from the labelling experiments (Study I and III) were ground and homogenized before determination of OC, ^{13}C, N and ^{15}N in duplicate, using an IRMS (Delta V Advantage, Thermo Fisher Scientific, Dreieich, Germany) coupled with an elemental analyser (Euro EA, Eurovector, Milan, Italy). Values of the ^{15}N and ^{13}C atom% excess were calculated by subtracting the ^{13}C and ^{15}N enrichment of the respective unlabelled soil (treatments with unlabelled litter) from the enrichments obtained from the labelled soil.

2.3.1.4 Specific surface area measurements

In study I, the specific surface areas of the clay-sized fractions were determined by N_2 adsorption (Autosorb-1 analyser, Quantachrome, Syosset, NY, USA) from the Brunauer-Emmett-Teller equation before and after OM removal with 10 % H_2O_2 solution. These values were used to calculate the specific surfaces area covered by OM.

2.3.1.5 Solid-state ^{13}C NMR spectroscopy

The chemical composition of the OC present in the OM amendments and the small-sized fractions (<20 µm - study II; the clay-sized fraction – study III) were determined by solid-state ^{13}C NMR spectroscopy (Bruker DSX 200 NMR spectrometer, Rheinstetten, Germany) using the cross-polarisation magic angle spinning (CPMAS) technique. A line broadening of 50 Hz was applied. The ^{13}C chemical shifts were calibrated against tetramethylsilane (0 ppm). The relative contributions of the various C groups were determined by integration of the signal intensity in their respective chemical shift regions and were divided into four major chemical shift regions (0 to 45 ppm: alkyl-C, 45 to 110 ppm: O-alkyl-C, 110 to 160 ppm: aromatic C, 160 to 220 ppm: carboxyl-C).

2.3.1.6 Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) and scanning electron microscopy (SEM)

In study I, the clay-sized fractions at the time points of 2 h and 7, 21 and 42 days, as well as a clay-sized fraction with unlabelled litter as natural abundance (5 clay-sized fractions), were analysed using NanoSIMS (Cameca NanoSIMS 50L, France). A sample of 1 mg dried clay-sized fraction was dispersed in 20 ml of deionised water, and 100 µl of the dispersion was dropped on a silica wafer, which was dried overnight in a desiccator. Reflected-light microscopic images of the whole sample were taken for documentation and later orientation on the sample using the CCD camera of the NanoSIMS. SEM images (Jeol JSM-5900LV, Eching, Germany) were taken to select regions of interest (ROIs) to support interpretation of the NanoSIMS results. Prior to SEM measurements, the samples were coated with gold (~5 nm; SCD 005 sputter coater, Bal-tec GmbH, Germany) to avoid charging. For NanoSIMS analysis, the Cs^+ primary ion probe was used with primary ion impact energy of 16 keV. Prior to analysis, contaminants and an additional gold coating layer (~30 nm) were sputtered away by a high primary beam current (pre-sputtering). During the pre-sputtering, the reactive Cs^+ ions were implanted into the sample to enhance the secondary ion yields. The primary beam (~1.2 pA) focused at a lateral resolution of 100-200 nm was scanned over the sample (40 × 40 µm), and $^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, $^{16}O^-$, $^{28}Si^-$ and $^{27}Al^{16}O^-$ secondary ions were collected on electron multipliers with an electronic dead time fixed at 44 ns. The measurements were performed at high mass resolving power to circumvent the occurrence of mass interferences (e.g., $^{12}C^1H^-$ vs. $^{13}C^-$, $^{12}C^{14}N^-$ vs. $^{12}C_2{}^1H_2{}^-$ and $^{12}C^{15}N^-$ vs. $^{13}C^{14}N^-$). Charging of the non-conductive mineral particles was compensated by using the

electron flood gun of the NanoSIMS instrument. All measurements were done in imaging mode. The ion images were acquired using a dwell time of 1-3 ms/pixel, 512 × 512 pixels and 1 cycle. For every sample, 10-12 spots were analysed to obtain a reliable data basis for the calculation of the fate of ^{13}C and ^{15}N. NanoSIMS images were evaluated using the Open MIMS Image plugin for the ImageJ software. ROIs were chosen according to the distribution of the $^{12}C^-$ and $^{12}C^{14}N^-$ secondary ions as an OM indicator. Areas and sums of secondary ion counts for each ROI were extracted from all images. All ROIs with an area greater than 10 pixels were used for further calculations. Minerals were selected as ROIs on the basis of $^{16}O^-$ and $^{27}Al^{16}O^-$ images. For structural analyses, the visible mineral surface areas were divided into clustered particles and individual particles on the basis of the corresponding SEM images.

2.3.2 Microbiological characterisation

2.3.2.1 Microbial biomass and soil respiration

Microbial biomass (C_{mic}) was determined according to the fumigation-extraction method (Study I and III) of Vance et al. (1987) by the Research Unit Environmental Genomics at the Helmholtz Zentrum München. C_{mic} was calculated as the difference between C_{total} in fumigated (fum) and non-fumigated (n-fum) samples using a k_{EC} value of 0.45 (Joergensen, 1995). Atom-% ^{13}C in the extracts was measured via online liquid chromatography coupled with stable isotope ratio mass spectrometry (MAT 253, Thermo Fisher Scientific, Dreieich, Germany) according to Krummen et al. (2004). The atom-% ^{13}C in microbial biomass (atom-% $^{13}C_{mic}$) was calculated as described by Marx et al. (2007). The CO_2 respiration rate was recorded at several time steps during the artificial soil experiment (Study II) according to the method of Isermeyer (1952). For the cumulative CO_2 respiration, the mean values between time steps and the standard deviations were accumulated over the incubation time.

2.3.2.2 Total community DNA (TC-DNA) extraction

Microbial communities in the artificial soils were analysed at the Institute for Epidemiology and Pathogen Diagnostics, Ferderal Research Centre for Cultivated Plants (JKI) in Braunschweig one day before the second manure addition (after 561 days of incubation), after another 7 days (569 days) and after 839 days (Study II). Per artificial soil mixture, five replicates were subjected to TC-DNA extraction using FastPrep FP24 bead-beating system (twice, 30 s, 5.5 m s^{-1}; MP Biomedicals, Santa Ana, CA) and FastDNA spin kit for soil (MP Biomedicals). Purification of extracted TC-DNA was performed using GeneClean spin kit (Qbiogene, Inc., Carlsbad, CA).

2.3.2.3 Denaturing gradient gel electrophoresis (DGGE) fingerprint and quantitative qPCR of 16S rRNA gene and ITS fragments

Bacterial structural diversity was determined based on 16S rRNA gene fragments amplified in a PCR reaction described by Gomes et al. (2001) and modified by Babin et al. (2013) in study II. The fungal community was studied based on the ITS fragment amplified, as described by Weinert et al. (2009). Fingerprints were generated by DGGE using the Ingeny PhorU system (Ingeny, Goes, The Netherlands) and the protocol by Weinert et al. (2009). The identification of time-dependent upcoming bacterial populations was performed by excision and cloning of the respective bands from DGGE gels as described by Smalla et al. (2001) with modifications from Babin et al. (2013). Bacterial 16S rRNA genes were quantified by a qPCR 5'-nuclease assay with primer and TaqMan-probe described by Suzuki et al. (2000). Quantification of the fungal ITS fragment was carried out according to the protocol established by Gschwendtner et al. (2010). Both amplifications for 16S rRNA gene and ITS fragments were carried out in CFX96 Real-Time System (Biorad, Munich, Germany). These analyses were also performed at the Institute for Epidemiology and Pathogen Diagnostics, Ferderal Research Centre for Cultivated Plants (JKI) in Braunschweig**.**

Table 4: Overview of the determined soil parameters and the applied methods in study III.

Study III **Enhanced organic matter decomposition leads to higher organic carbon and nitrogen sequestration in fine-sized soil fractions modified by clay mineralogy** Short-term incubation experiment with matured artificial soils and a natural soil with ^{13}C and ^{15}N labelled litter
Soil parameters
• SEOC by extraction with 0.01M $CaCl_2$ • OC ,N,^{13}C and ^{15}N by isotope ratio mass spectrometer • POM, 2-20 µm and <2 µm by combined density and particle size fractionation (sodium polytungstate solution with a density 1.6 g cm^{-3}, ultrasonically dispersion with 200 J ml^{-1})
Microbial parameters
• Microbial biomass by fumigation-extraction method
Soil fraction parameters
• OC ,N,^{13}C and ^{15}N by isotope ratio mass spectrometer coupled to an elemental analyser • Composition of OC by solid-state ^{13}C NMR spectroscopy
Data presentation and statistical analyses
• Data presentation by SigmaPlot • Statistical analyses by R 3.0.2

3 Discussion

3.1 Distribution of litter-derived OM in organo-mineral associations as indicator for the development of biogeochemical interfaces

The development of biogeochemical interfaces in established soil systems was investigated in study I and III, in which labelled plant litter provided the possibility to follow their formation in addition to already present organo-mineral associations. Density and particle size fractionation in combination with isotopic labelling was used to extract the organo-mineral associations and to study the distribution of litter-derived OC and N through various soil fractions which was used as indicator for the formation of new biogeochemical interfaces. The data obtained by the isotope tracing experiments demonstrated a fast formation of new organo-mineral associations due to the rapid decomposition of the added litter material (Study I and III). The litter-derived ^{13}C and ^{15}N tracer were found in all fine mineral fractions right from the beginning which pointed to an immediate attachment of the labelled OC and N on the mineral particles of the fine-sized fractions. The silt-sized fractions appeared as a transient pool for the litter-derived OC and N, as the label got attached immediately, but got lost thereafter over the incubation time. The ^{13}C concentrations in the clay-sized fractions firstly got enriched, followed by a small decrease over the incubation times, while the ^{15}N concentrations increased. This was also reflected by a slight decrease in the C/N ratios of the clay-sized fractions, indicating an enrichment of N over time. In study III, the accumulation of ^{15}N in the clay-sized fraction followed a growth curve over the incubation time. The results from study I and III showed a high contribution of fresh, litter-derived N-rich material to the clay-sized fractions, suggesting that the chemical composition of the OM became dominated to a greater extent by microbial residues and metabolites during the incubation due to the microbial processing of the fresh litter material.

To summarise, the development of new biogeochemical interfaces in the isotopic labelling experiments can be divided in two phases. The first phase reflected a fast development, indicated by an immediate enrichment of ^{13}C and ^{15}N in the fine organo-mineral fractions, which resulted possibly from leaching of soluble litter-derived OM and sorption to the fine mineral fractions. The second phase was driven by the transformation and maturation of added OM by microbial activity, as demonstrated by the specific progression of ^{13}C and ^{15}N in the various fine-sized fractions. Thus, the formation of new biogeochemical interfaces was caused by sorption and microbial mediated transformation processes. These observations are supported by a study of Aita et al. (1997), even though the authors considered a longer incubation time in the field. They observed that straw-derived C accumulated rapidly in the <50 µm faction and assumed that the label in the <50 µm fraction initially came from the water soluble fraction of the residues, and that over the longer term microbial biomass and its associated metabolites contribute to a larger proportion (Aita et al., 1997).

Overall, the clay-sized fractions showed generally the highest amounts of OC and N as well as the highest amounts of litter-derived ^{13}C and ^{15}N and exhibit therefore the most newly formed biogeochemical interfaces in addition to the currently present interfaces. Thus, the clay-sized fraction represents an important soil compartment for the sequestration of OC and N (Study I and III). This observation matched the widely accepted assumption that minerals in clay-sized fraction stimulate the storage of OC and N, providing the most significant surface areas on which OM can be bound (Kögel-Knabner et al., 2008b; von Lützow et al., 2006).

3.2 The importance of micro-structures for the fate of OC and N

The composition of reactive interfaces analysed at high resolution by NanoSIMS showed that almost all of the OM existing in the clay-sized fraction was bound in organo-mineral clusters (Study I). The small mineral particles in organo-mineral clusters with rough surfaces were thereby identified as essential reactive interfaces for OM sequestration. The results of study I clearly indicate that mineral particles with a smooth surface are not suited for substantial OM sequestration, presumably because they do not have etch pits, micropores or cracks. Surface roughness of mineral particles due to edges and micropores was supposed as sites of increased reactivity providing preferential binding sites for OM (Kaiser and Guggenberger, 2003; Kögel-Knabner et al., 2008b). Kaiser and Guggenberger (2003) suggested that micropores may contribute to the preferential sorption of OM during the early stages of OM accumulation. Zimmerman et al. (2004) tested the hypothesis of mesopores as sorption sites in batch experiments with aqueous suspensions by examining the adsorption of amino acids onto mesoporous compared to nonporous materials. The authors oberserved greater adsorption to rough surfaces with mesopores compared with their nonporous analogues (Zimmerman et al., 2004). Chenu annd Plante (2006) suggested that micro-aggregates are favoured sites of OM storage both by entrapment and by adsorption to minerals. The combination of etch pits, micropores or cracks at the mineral particles as well as the roughness formed by clustering shaped a micro-structure which seemed to determine the sequestration of OC and N. However, the 2-D visually generated data have to be validated by surface roughness measurements for example by Atomic Force Microscopy, although the results of the SSA determination indicated that especially small mineral particles with a high surface roughness got covered by OM, which confirmed the findings by NanoSIMS. The OC loading of 1.1 ± 0.1 mg m^{-2} determined for the clay-sized fractions in study I fell into the range proposed for the monolayer coverage (Keil and Mayer, 2014; Keil et al., 1994; Mayer, 1994a). But the clustered particles contained most of the OM, which means that a small proportion of the mineral surface exhibit a several times higher amount of OM as needed to create a single-layer. Thus, the organo-mineral clusters with rough surfaces indicate a much higher capacity for OC storage. Preferential OM sequestration on mineral clusters with rough surfaces also might be due to better

hydration and nutrient conditions at rough surfaces, conditions that offer an advantageous microhabitat for microorganisms (Wang and Or, 2013).

The identification and quantification of the OM-reactive surfaces in organo-mineral associations of the clay-sized fraction showed that most of the mineral surfaces did not show any OM coverage at all (Study I). Less than 19 % of the total visible mineral area was covered by OM and a large proportion of the total area in the clay-sized fractions did not contribute to the binding of OM. This finding was unexpected because the clay-sized mineral particles in this soil (illite, mixed layer clay minerals and pedogenic iron oxides, such as goethite) are all considered to serve as reactive surfaces for OM binding. The clay content and the SSA of mineral particles are often used as a proxy for the estimation of the soil OC sequestration potential (Feng et al., 2013). The findings presented in study I may explain why the clay content is sometimes poorly related to OC concentration (Percival et al., 2000) and provide evidence that only a limited proportion of the clay-sized surfaces contribute to OM sequestration. The data of study I point to the necessity of a careful identification and quantification of the reactive mineral complexes that are responsible for OM sequestration controlling the OM saturation capacity of soils and sediments.

The visualisation of the biogeochemical interfaces by NanoSIMS confirmed the heterogeneous distribution of the OM in small patches on the mineral surfaces. Several studies in soils and sediments gave evidence that mineral bound OM is attached to minerals in form of discrete accumulations rather than as continuous layer coating to the mineral surfaces (Bock and Mayer, 2000; Chenu and Plante, 2006; Lehmann et al., 2007; Ransom et al., 1997). The OM spots enriched in both isotopes as shown in study I represent OM bound to mineral surfaces derived either directly from the litter material due to sorption in the early phase of the incubation and/or from the microbial products originating from litter decomposition. The areas enriched solely in ^{15}N increased as well as the heterogeneity in the enrichment of the ^{15}N spots over the incubation time pointing to a more intensively microbial turned material. Keiluweit et al. (2012) observed by NanoSIMS in an incubation experiment with tracer substances that soil micro-structures revealed preferential associations of ^{15}N with iron-rich particles. The authors found by means of synchrotron-based scanning transmission X-ray spectromicroscopy that these micro-structures were enriched in aliphatic C and amide N matching those of microbial biopolymers like proteins and lipids, which implied that intensive microbial processing occurred prior to association with mineral surfaces (Keiluweit et al., 2012). Synchrotron-based near-edge X-ray spectromicroscopy of mineral assemblages illustrated that OM is present in extremely heterogeneous forms, such as plant or microbial biopolymers, at distinct locations (Lehmann et al., 2008). The heterogeneity of organic patches bound to mineral surfaces is generally not detected by bulk soil analyses and point to the necessity of small scale techniques to advance the current knowledge about organo-mineral

associations. NanoSIMS in this case can provide detailed insights into the spatial variability of OM accumulation during such an incubation experiment, especially in combination with other advanced small-scale techniques. Nevertheless, small-scale technologies like NanoSIMS have to be used in conjunction with bulk analytical methods as the combined information of different analytical scales will lead to advanced understanding of the complex soil system.

The study of the spatial distribution of the litter-derived OM on the mineral surfaces showed that the ^{13}C and ^{15}N tracer were extensively incorporated over about half of the organo-mineral cluster areas. The high proportion of the areas covered with new labelled OM indicated a preferential attachment of new OM to organo-mineral clusters with rough surfaces containing pre-existing OM. This finding was demonstrated by an increase of area enriched in ^{13}C and ^{15}N, while the total area covered with OM remained constant (Study I). Our data are in agreement with a conceptual model of preferential sorption of OM on surfaces already containing OM (Kleber et al., 2007; Sollins et al., 2006). This multizone sorption model proposed a structure for organo-mineral associations referring to the concept of OM sorption in different layers onto mineral surfaces. The onion layering model is based on the preferential accumulation of carboxyl and amino compounds directly to mineral surfaces by ligand exchange and electrostatic binding (Sollins et al., 2006). Other organics could sorb then more readily on this inner organic layer than onto the unconditioned mineral surfaces forming a secondary membrane-like bilayer which may exchange more easily with the surrounding solution (Kleber et al., 2007). Consequently, NanoSIMS enabled a combined determination of newly formed and pre-existing biogeochemical interfaces by the combination of stable isotope tracing and elemental mapping and therefore gave the chance to peer into the black box of micro-scale interfaces. Overall, with the results obtained in study I, it could be shown that micro-structures generated by the mineral particles provide preferential spots for OC and N sequestration in soils. These organo-mineral clusters with rough surfaces are furthermore crucial for the accumulation of new OM in the clay-sized fraction, rather than the large proportion of free mineral surfaces.

3.3 Establishment and functionality of microbial communities in the artificial soils

The artificial soil approach provided the opportunity to focus on the establishment of microbial communities during the development of the soil-like system affected by the mineral composition. In an interdisciplinary approach using artificial soils, the composition of the microbial communities depending on the artificial soil composition were studied in a long-term incubation experiment (Figure 4). Over the long-term (842 days), the clay minerals illite and montmorillonite were identified as main drivers of the bacterial community structure compared to the other constituents, charcoal and metal oxides (Study II). This observation was in contrast to the assumption based on previous results from the artificial soils, expecting a further differentiation of bacterial community

composition depending on the various artificial soils with longer incubation time (Babin et al., 2013; Ding et al., 2013). The influence of charcoal on the establishment of the bacterial community declined during the artificial maturation. Ding et al. (2013) found that charcoal was an important driver for the initial bacterial communities establishment in the artificial soils. Thus, charcoal seems to be an important constituent in the early stage of the bacterial community establishment, whereas charcoal plays a minor role over the long-term incubation for the further development of diverse bacterial communities. The influence of metal oxides on the bacterial community composition was also rather small compared to the clay minerals over the more than two years of incubation. Carson et al. (2009) showed that the structure of bacterial communities is influenced by the mineral substrate and concluded that minerals play a greater role in bacterial ecology than simply providing an inert matrix for bacterial growth. It was supposed by previous studies that small soil particles affect microorganisms either by direct effects through surface interaction, e.g. adhesion of microorganisms, influence of their metabolic activity or by indirect effects by influencing the characteristics of the microbial habitat with respect to e.g. nutrient supply and buffering the pH (Chenu and Stotzky, 2002; Stotzky, 1986). Recently, Heckman et al. (2013) found that microbial community composition was significantly affected by the presence of goethite and gibbsite perhaps due to their effects on N and P availability. A tentative explanation for strong influence of the different clay minerals on the composition of the bacterial communities could be an advantageous or disadvantageous effect on specific microorganisms generated by the different reactive surfaces provided in the various artificial soil constituents. For example, Stotzky (1986) reported an inhibiting effect of higher montmorillonite concentrations related to limitations in O_2 diffusion. However, experimental observations of the influence by different clay minerals on microorganisms are often contradictory (Chenu and Stotzky, 2002). Direct effects of the mineral surfaces might also be more important in the early phase of microbial establishment. The mineral and charcoal surfaces already interacted with the microorganisms and the OM during the artificial soil development of the initial phase, as Pronk et al. (2012) showed that organo-mineral associations formed on a short time scale from previous clean model materials. Thus, the establishment of different microhabitats became probably more relevant than direct effects of the surfaces present. In study II, it was supposed that the compositions of the artificial soils, which varied in the fine-sized fractions, built specific micro-structures as microbial habitats over the long-term due to different physicochemical properties depending on the two clay minerals present. The clay minerals illite and montmorillonite had also a strong effect on the amount of macro-aggregates, thus it was assumed that the mineral-driven bacterial communities were further influenced by a structural distinction on the larger scale in the artificial soils (Study II). Structural developments depending on the two different clay minerals seem to become more important for the microbial community composition over the long-term.

The evaluation of the OM properties in the artificial soils in relation to the microbial community depending on the artificial soil composition showed no significant differences (Study II). During the incubation time, a part of the original manure added in study II was broken up, partly lost as CO_2 and N as well as OC got distributed to the fine fractions (<20 µm). However, the respiration rates, OM degradation and quality did not differ significantly between the artificial soils in study II. Accumulation of OC and especially N in the fractions <20 µm were similar for the artificial soils. Further, no differences in the chemical composition as analysed by solid state ^{13}C NMR spectroscopy of these fractions (<20 µm) could be seen between artificial soil compositions. Hence, the selection and establishment of different bacterial populations during incubation depending on the clay mineral composition of the artificial soils had no effect on the OM degradation, accumulation and quality. Heckman et al. (2013) obtained similar results. The authors found a significantly different microbial community composition, but the respiration rates did not differ significantly (Heckman et al., 2013). This is in accordance with a functional microbial redundancy in soil as hypothesized in earlier studies (Bell et al., 2005; Nannipieri et al., 2003). Overall, it seems that different bacterial populations were able to undertake identical functions in the decomposition of OM as well as in the accumulation of OM in the fine-sized fractions of the artificial soils. Therefore, it is certainly possible that the overall balance of soil processes could alter as a function of the relative activities of the component microbial populations, rather than simply the presence or absence of individual species (Paterson et al., 2009). However, further research is needed to determine whether differences among soil compositions can be seen on a functional level.

3.4 Effect of additional manure input on the microorganisms and macro-aggregate formation

The response of the microorganisms in the artificial soils on an additional sterile manure input showed slight changes in the bacterial community compositions independent of the artificial soil mixtures detected by comparing bacterial DGGE-fingerprints before and after the second manure addition (Study II). Previous studies pointed to the strong influence of nutrient additions on the microbial community composition (Blaud et al., 2012; Griffiths et al., 1998). In study II, bacterial populations with strongly increased abundance in response to the addition of sterile manure displayed a high similarity to *Pseudomonas resinovorans, Pseudomonas stutzeri* and *Pseudomonas protegens* (Study II). *Pseudomonas* represents typical soil *r*-strategists. Therefore, it is likely that they had an advantage over other bacteria and benefitted from the available substrate (Fierer et al., 2007; Smit et al., 2001). The decrease in relative abundance of fast-responding *Pseudomonas* populations with incubation time and the decline in CO_2 respiration over the incubation time suggest a succession in the active microbial community (Blagodatskaya and Kuzyakov, 2008; Ekschmitt et al., 2005; Fontaine et al., 2003).

The response of the additional sterile manure input to the artificial soils revealed furthermore a re-activation of the artificial soil system (Figure 4). After the fresh substrate was brought into the system, increased microbial gene copy numbers and longer-lasting higher respiration rates were observed in all artificial soils. This might be on the one hand caused by an increased microbial activity due to increased availability of easily decomposable OM. On the other hand, this could be due to a larger microbial biomass than introduced with the inoculum at the beginning of the experiment indicated by the increased microbial gene copy numbers (Study II). The additional substrate supply affected the microorganisms also over a longer time than after the start of the incubation with the first manure input, as a higher and longer response was observed due to the additional manure addition. Thus, the adaption of the established microbial communities to prevailing physicochemical conditions and the microbial habitats established might have led to the stronger and longer response of the microbial communities. Overall, the response of the artificial soil systems to OM addition regarding respiration seems to be more likely a result of the established microhabitats than an effect of the different soil compositions.

The additional sterile manure input led to a re-formation of macro-aggregates, as a significant proportion of the artificial soils (47-66 %) was present in macro-aggregates >2 mm at the end of the incubation (842 days). Pronk et al. (2012) discovered in the artificial soil experiment after 180 days a decrease in the amount of macro-aggregates, explained by a decline of the available OM and the decreasing biological activity in the system. The stability of aggregates underlies a dynamic process after OM addition depending on the degradation of the added OM (Chenu and Cosentino, 2011). The availability of fresh OM substrate was essential for the sustainability of macro-aggregation, as stable macro-aggregates decline with the OM content due to decomposition (Tisdall and Oades, 1982). The additional OM and longer incubation time led to a re-formation of macro-aggregates which was significantly higher when montmorillonite was present compared to illite (Figure 4). The differentiation in the amount of macro-aggregates depending on the clay mineral present was in contrast to Pronk et al. (2012). The specific mechanisms causing this effect are not yet clear. The amount of OM, operating as binding agent for soil aggregates, does not really serve as explanation for the higher amounts of macro-aggregates in the artificial soils with montmorillonite, as only trends to higher OC concentration of the bulk soil in these artificial mixtures were found. A tentative explanation would be that the macro-aggregates produced by different minerals exhibit different structural stability and therefore a different aggregate evolution after the addition of OM similar to a conceptual model developed for different OM sources (Chenu and Cosentino, 2011). It can be speculated that the presence of the different microbial communities established according to the two clay minerals in the artificial soils serve as possible explanation for the different amounts of macro-aggregates dependent on the clay minerals present. Previous studies indicated an influence of

soil microorganisms on aggregation by the production of different binding agents (Dorioz et al., 1993; Geoghegan and Brian, 1948; Six et al., 2004). Even though fungi are known to influence the macro-aggregation (Oades and Waters, 1991; Tisdall, 1994), no difference in fungal abundance and community composition in the artificial soils was found. The clay minerals were decisive for the composition of the bacterial community and the macro-aggregation, although the influence of bacteria on the soil structure was so far confined to the formation of micro-aggregates (Chenu and Cosentino, 2011; Tisdall, 1994). However, a heterogeneous mixture of EPS released by diverse communities, probably differing in amount or quality, might glue particles together to different extents and could be responsible for the differentiation in macro-aggregation. The clay mineral environment alters the functionality of bacterial species resulting in different EPS production (Richaume et al., 1989; Weinberg and Stotzky, 1972), but this would not be seen by analysis of the bacterial community composition. Regarding the macro-aggregate formation in the artificial soils it needs to be considered that the amounts of macro-aggregates were only measured at one point in time over the aggregate evolution in study II.

3.5 The clay mineralogy matters for the fate of OM in artificial soils

In study III, the artificial soils with illite and montmorillonite showed a differentiation in OM decomposition and in the sequestration of OC and N in the clay-sized fractions. Due to the use of isotope tracing, a significant difference in amount as well as in the curve progression of the ^{13}C label remaining in the artificial soil containing montmorillonite compared to illite was found (Study III). The artificial soil with montmorillonite exhibited a slower decomposition of the fresh added litter material, which was in line with significantly higher ^{13}C and ^{15}N amounts in the POM fraction, whereas the artificial soil with illite showed a faster decomposition. Differences in the sorption capacity of the diverse clay minerals may have an effect on the decomposition of OM, by influencing the substrate availability to the decomposer community. The observed lower SEOC values directly after the incubation start in the artificial soil with montmorillonite could indicate that the easily available OC was more and/or stronger bound and therefore protected from microorganisms. Saggar et al. (1996) assumed that stabilisation of OM by adsorption on soil surfaces is the principal mechanism controlling the OM decomposition, as they found that the ^{14}C decomposition was lower in smectitic soils. Sanderman et al. (2014) found in experiments with clay-sized fractions from natural soils differing in mineralogy that the long-term stability of the sorbed OM was a function of the mineralogy involved in the binding process. Recently, Wei et al. (2014) found out that higher clay contents promote the OM decomposition by sustaining a greater microbial biomass. The results of study III demonstrated that the lower microbial biomass was in agreement with the slower mineralisation rates in the artificial soil with montmorillonite and hence seems to be the reason for the slower decomposition of the fresh added litter material. Clay minerals were often assumed to

influence microorganisms, and thus modify their activity and consequently the degradation of OM (Sollins et al., 1996; Stotzky, 1967, 1986). However, in the literature, contradictory effects of montmorillonite on the degradation of OM are reported (Filip, 1973; Haider et al., 1970). For example, Stotzky (1986) reported about an inhibiting effect of montmorillonite due to limitations in O_2 diffusion. Also, Filip (1973) stated in a review that clay minerals of the smectite group were usually found to reduce the rate of decomposition. On the other hand, it was also indicated that montmorillonite stimulates microbial activity and therefore the decomposition of OM, mainly attributed to the buffering capacity of montmorillonite (Haider et al., 1970; Kunc and Stotzky, 1974; Stotzky and Rem, 1966). As the previous artificial soil studies revealed no effect of mineralogy on the OM decomposition (Pronk et al., 2013), it is unlikely that effects closely related to the clay mineral surfaces, either directly on the activity of the microorganisms or indirectly by sorption on the mineral surfaces, are responsible for the difference in decomposition. It has to be kept in mind that the differences in decomposition between the two clay minerals were small and that only the first phase during OM turnover was analysed in this short-term study. As the aggregation of soils is supposed to affect OM dynamics by influencing the microbial activity (Sollins et al., 1996), the clay-mineral dependent aggregate formation of the artificial soils could serve as possible explanation for the differences in decomposition. In study II, it was shown that artificial soils with montmorillonite exhibited more macro-aggregates than the artificial soils with illite. As the litter was mixed with the soils at the beginning of the experiment, the reduced growth of the microorganisms in the artificial soil with montmorillonite might be explained by higher aggregation resulting in an inaccessibility of substrate to the microorganisms.

Despite lower decomposition rates of artificial soils containing montmorillonite, the artificial soil with illite sequestered more OC and N in the clay-sized fraction than the artificial soil with montmorillonite. This observation was unexpected as it is supposed that clays with a higher SSA like montmorillonite favour more and/or stronger OC-clay interactions than illite with a smaller SSA (von Lützow et al., 2006). It might be that freshly added pure minerals react differently compared to minerals already incorporated in a soil-like structure.

While differences in the amount of sequestered OC and N in the clay-sized fraction depended on the mineralogy, the quality of the OC bound to the mineral surfaces did not differ. This was also in contrast to the assumption that the composition of clay-associated OM is influenced by the type of clay minerals present, as it was found that smectite-associated OM is higher in aromatic compounds (Wattel-Koekkoek et al., 2001).

Overall, it was proposed that the faster decomposition in the artificial soils with illite resulted in higher amounts of microbial metabolites accumulated in the clay-sized fraction and generated

higher amounts of mineral-associated OM (Study III), although a faster mineralisation is commonly associated with a lower sequestration of OC (Six et al., 2002).

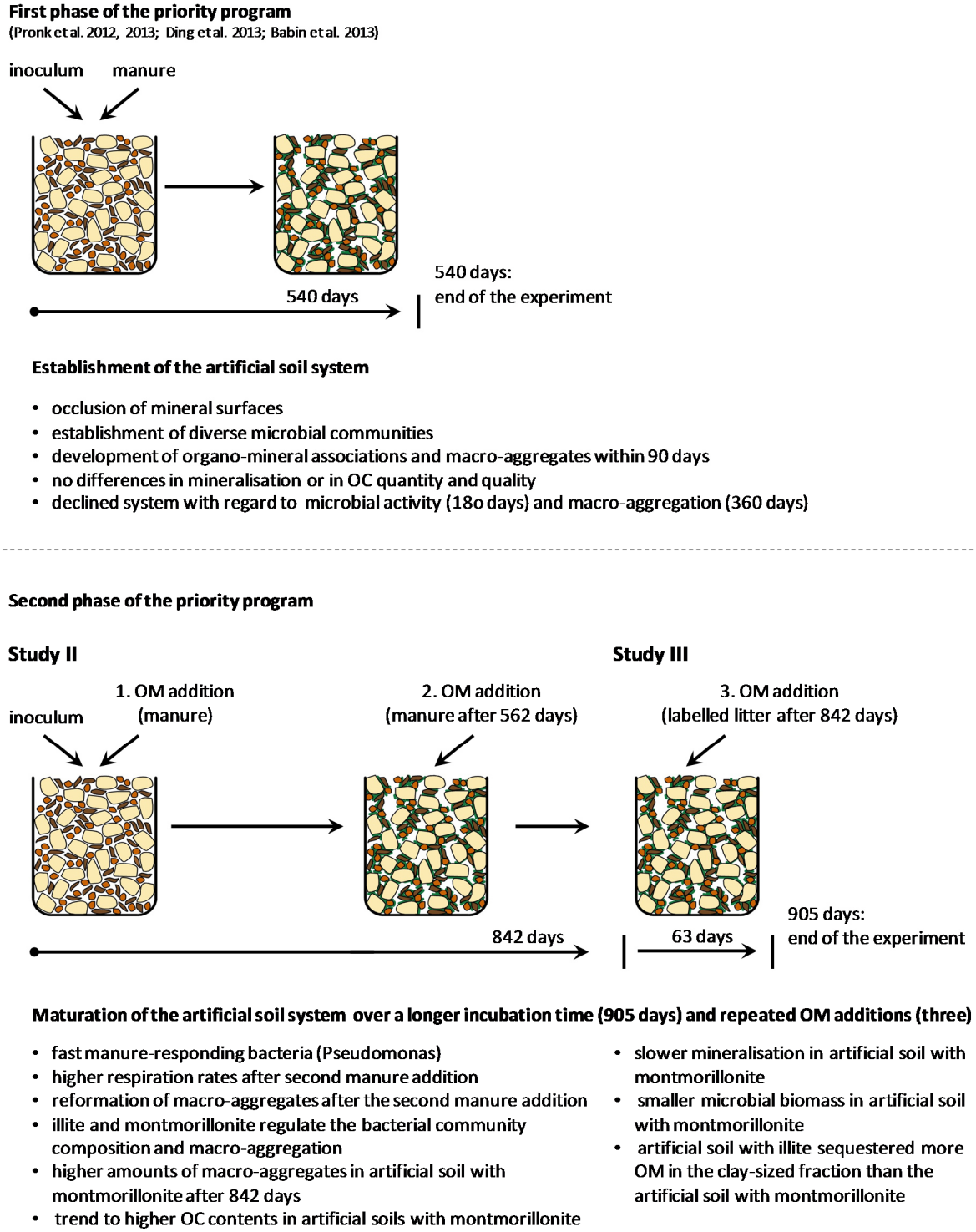


Figure 4: Summary of the artificial soil experiments from the establishment in the early phase and maturation after repeated OM additions and a longer incubation time.

It is conceivable that over the longer maturation time (905 days) and repeated OM additions (three OM additions), a soil structure developed in the artificial soils, which controls the OM decomposition and sequestration on the long-term (Figure 4). During the first 540 days of artificial soil development, Pronk et al. (2013) observed no differences neither in the mineralisation nor in the OC quantity and quality between artificial soils with different mineralogical compositions after a single manure application. In study II, evidence for higher OC contents in artificial soils containing montmorillonite compared to artificial soils formed from illite were found after an additional manure application and longer maturation time, although no significant differences were found. Nevertheless, a differentiation in the amounts of macro-aggregates depending on the two clay minerals was found in study II. In the following study with a further addition of fresh OM material a differentiation in decomposition and sequestration of OM was observed (Study III). Thus, the structural development in the artificial soils after several OM additions may provide a potential reason for the observed development.

3.6 The role of the specific surface area – a potential size of the total interface?

To evaluate the role of surfaces provided by different minerals and charcoal in the complex interplay between OM-mineral-microorganism interactions and the subsequent effect on the fate of OM was a main objective of all studies. In particular clay minerals (illite and montmorillonite), iron (ferrihydrite) and aluminium oxides (boehmite) as well as charcoal, each with a large SSA, were considered as important constituents affecting the development of biogeochemical interfaces and aggregation in the artificial soil approach. It was supposed that the formation of biogeochemical interfaces as indicated by organo-mineral association and aggregates will be controlled by the particle surface area and type of particle surface, as the availability of SSA in a soil or sediment was assumed to control its concentration of OM (Mayer, 1994b). Pronk et al. (2012) showed in the artificial soils that the mineral and charcoal surfaces already interacted with the microorganisms and OM during the initial phase, as organo-mineral associations formed on a short time scale from previous clean model materials in the artificial soils. But although charcoal and the metal oxides provide large SSAs, which would make them important potential sorption sites in the artificial soils, the partly substitution of the clay minerals by these materials had no significant effect on the organo-mineral association and the formation of macro-aggregates (Study II and III). In accordance, Pronk et al. (2012) did not observe that metal oxides and charcoal supported organo-mineral associations and macro-aggregate formation. It was unexpected that the metal oxides had no effect on aggregation (Study II), as ferrihydrite was assumed to be important for the formation of organo-mineral associates and aggregates in natural soils (Duiker et al., 2003; Goldberg, 1989). From results of study II, one may conclude that all constituents, despite the fact that they provided different SSAs, exhibited the same options for interactions to form macro-aggregates. Furthermore, no effect of charcoal and metal

oxides surfaces was found (charcoal, ferrihydrite - Study II and III, boehmite - Study II) on decomposition and sequestration of the added OM. This was in contrast to assumptions that sorption of organic substrates to mineral particles like ferrihydrite provides an important mechanism by which the bioavailability and capability for biodegradation through soil microorganisms is reduced (Jones and Edwards, 1998). Sorptive protection by minerals like ferrihydrite was assumed to be a major mechanism of the OC sequestration (Kalbitz et al., 2005). However, the relatively high crystallinity of the ferrihydrite and the neutral pH of the artificial soils made the ferrihydrite probably less reactive than respective minerals occurring in natural soils. Eusterhues et al. (2005) assumed that the reactive surface sites with singly coordinated hydroxyl groups provided by Fe oxides are the preferred sites for OM sorption in acid soils. The neutral pH of the artificial soils is close to the point of zero charge of the ferrihydrite, which could have decreased the affinity of OM to ferrihydrite.

Even though the observation of study I and III matched the widely accepted assumption that minerals in clay-sized fraction stimulate the storage of OC and N by providing the most significant surface areas on which OM can be bound (Kögel-Knabner et al., 2008b; von Lützow et al., 2006), the SSA itself seems to be not decisive for the sequestration of OC and N. It was considered by several authors that mineral surface area reflected the sorptive capacity of a system, and that SSA is predictive of carbon storage capacity in soils and sediments (Keil and Mayer, 2014; Keil et al., 1994; Mayer, 1994a). However, in study III, higher amounts of OC and N were found in the fine-sized fractions of the artificial soils with illite compared to montmorillonite, although the montmorillonite had a higher SSA. This observation is in contrast to the assumption that clays favour more and/or stronger OM-mineral interactions due to their larger SSA (von Lützow et al., 2006; Wattel-Koekkoek et al., 2003). Thus, it was concluded that the size of SSA and the sorption of OM on those are not decisive for decomposition and sequestration, as we further did not find a reduced mineralisation in the artificial soils with ferrihydrite with high SSA (Study II and III). However, it has to be kept in mind that over the long maturation time of the artificial soils of more than two years, organo-mineral associations have already developed, which may react differently compared to clean mineral surfaces, although there might be enough free mineral surfaces available as found in study I. The fact that the mineral surface areas were of minor importance for the decomposition and sequestration of OC and N (Study I, II, III) could also indicate that sorption processes were less important in the analysed soil materials. The association of minerals with OM in soils occurs mainly via a combination of sorption and aggregation processes (Chenu and Plante, 2006; Keil and Mayer, 2014; Krull et al., 2003). However, sorption is presumable more important in the early phase of organo-mineral interactions, whereas aggregation processes gain more importance in soil material with higher OM contents and already established organo-mineral associations. Overall, the data obtained in this thesis showed that the shape and roughness of mineral clusters as well as the soil structure are more

important determinants for the formation of interfaces and the sequestration of OC and N than the size of the SSA.

3.7 Can artificial soils serve as model system for natural soils?

The complexity of natural soils and the different environmental conditions, under which they develop, complicate finding direct links between minerals, OM and microorganisms. Thus, experiments with simplified systems of known compositions and initial conditions can be useful for understanding the complex interplay between minerals, OM and microorganisms. Therefore, artificial soils were more and more regarded as a good tool for studying the factors controlling soil functioning (Babin et al., 2013; Ding et al., 2013; Guenet et al., 2011; Pronk et al., 2012). In the early development of the artificial soils, the potential to produce a soil-like system from clean minerals after a single OM addition and the application of a microbial inoculum was demonstrated, as organo-mineral associations and aggregates were formed after several months (Pronk et al., 2012). Nevertheless, it has to be kept in mind that the processes in the artificial soils were restricted compared to those occurring in nature, for example regarding the aggregation processes. Several important factors for soil aggregation like wetting/drying and freezing/thawing cycles, the effect of plants and larger soil animals on aggregation (earthworms) were excluded from this experiment. Moreover, the weekly stirring of the artificial soil used for homogenisation of the batches, represented a mechanical action which does not occur in natural systems. This action could have had an additional influence on the aggregate formation in the artificial soils, although stirring was done very carefully to avoid compressing the artificial soils and aggregate disruption. However, as all artificial soils were treated in the same way, this action does not present a restriction in the comparability. The differences in natural soils and the artificial soil approach used in this thesis are summarised in Table 4.

Furthermore, the clay minerals and oxides used in the artificial approach are generally very different from the mineral phases available in natural soil systems. For example, phyllosilicates in natural soils do not occur as individual types of minerals, but as mixture of several phyllosilicates or different types of interstratified minerals (Barré et al., 2014). The ferrihydrite used in the artificial soils was also different from ferrihydrite occurring in nature. The 6-line ferrihydrite that was synthesised represented a relatively well-crystallised form of ferrihydrite (Pronk et al., 2012) showing distinct particles (Heister et al., 2012), whereas ferrihydrite in natural soil systems occurs as nano-particles and coatings on other minerals (Hochella et al., 2008; Wang et al., 1993). This could be a possible explanation for the apparent lack of effects on OM decomposition and sequestration as well as on the formation of organo-mineral associations and aggregation by ferrihydrite, besides the neutral pH.

Table 5: Summary of the differences between natural and artificial soil systems.

natural soil	versus	artificial soil
complex system		simplified system
unknown initial conditions		known initial conditions
various OM sources		restricted OM sources
changing environmental conditions		controlled environmental conditions
naturally developed mineral composition		model mineral materials
diverse microbial community		known initial microbial community
continuous OM input		controlled application of OM
variety of processes		restricted soil processes
memory effect		no memory effect
open system		closed system

Overall, it remained an interesting open question, if results and relationships found in artificial soils can be transferred to processes taking place in natural soils. The question if an artificially produced soil-like system can be used to study processes occurring in natural soils was tried to answer in study III. In this study, the response concerning the decomposition of freshly added litter material of the artificial soils in comparison to the processes occurring in a natural soil was analysed. Therefore, the artificial soils were compared with a natural Ap horizon (Luvisol). Although the natural soil exhibited the highest amount of microbial biomass compared to the artificial soils in the control treatment, the mineralisation rate of the added labelled litter showed no difference to the artificial soils. In the natural soil, 55% of the initial ^{13}C tracer was mineralised in the natural soil, which was almost the same percentage found for the artificial soils (47-60%). Also Chotte et al. (1998) observed a comparable amount of OC mineralised after 66 days in natural soils amended with ^{14}C labelled glucose, starch, legume and wheat. Thus, the mineralisation of OC in the artificial soils was in the same range as found for natural soils. A multidimensional scaling plot, used to illustrate dissimilarities between the artificial soils and the natural soil, showed that the dissimilarities between the sampling time points were higher than between the different soils (Study III). Furthermore, it was observed that the microbial biomass in the natural soil was in the same range of values found for the artificial soil after addition of the labelled litter. Consequently, the artificial soils were assumed to be appropriate as model systems for studying processes occurring in natural soils, as they react similarly in laboratory incubation experiments. This was not the case in a study by Babin et al. (2014), using the same natural soil and artificial soils analysed in study III. The authors observed differences in the response of the two systems regarding spiking with litter material and phenanthrene (Babin et al., 2014). Furthermore, different and more complex patterns of bacterial community were revealed in the natural soil than in the artificial soils (Babin et al., 2014). Babin et al. (2014) observed that the microbial communities in all artificial soils were more strongly affected by

spiking than in the natural soil, which might indicate the importance of higher microbial diversity to compensate perturbations. It is conceivable that the natural soil showed a memory effect to this perturbation due to repeated encounter with such interferences.

Overall, there is no doubt that the natural soil and the artificial soils used in study III differ from each other with respect to their properties and functions. It should also be noted that the artificial soil systems still remain a simplified system and do not reflect the natural soil system. Nevertheless, the artificial soil approach offers a great opportunity to study various soil processes in simplified and well-defined systems from known initial conditions, which can be used to disentangle the complex interplay between minerals, OM and microorganisms.

3.8 The soil structure: Rip it apart or keep it intact?

Soils are seen as complex highly structured heterogeneous environments with large zones of little activity and hotspots full of activity (Kooistra and Noordwijk, 1996; Totsche et al., 2010). The complex soil structure can be investigated by different procedures. However, usually the principle question is, if the soil structure should be kept intact or separated into smaller units. Two commonly used ways exist. On the one hand, soil fractionation including complete disruption of the soil structure is widely used, with subsequent physical and chemical characterisation of the isolated units. On the other hand, soil fixation by for example embedding with resins is used for microscopic and/or spectroscopic analysis, keeping the soil structure intact. Fractionation methods are usually utilised to separate the complex soil structure into various compartments, based on the concept that the association of soil particles and their spatial arrangement play a key role in SOM dynamics (von Lützow et al., 2007). Thus, the provided fractions can be related to functional or structural components in soil (Cambardella and Elliott, 1993). Much of what is known for example about the relationships between soil structure, microorganisms and microbial-mediated processes is based on studies where soil has been broken up (Frey and Hillel, 2005). Next to the extensively used physical fractionation due to particle size, density fractionations are usually applied in soil science to separate light or particulate OM from more mineral-associated OM (von Lützow et al., 2007). In the present thesis, both fractionation methods were employed to study organo-mineral associations as indicator for biogeochemical interfaces. In study II, a particle size fractionation was used, whereas in study I and III, a combined density and particle size fractionation was applied to remove particulate OM and to isolate as well as to quantify the organo-mineral associations. Although physical and density fractionations are frequently applied in soil science, several uncertainties exist. For example, chemical or ultrasonic dispersion used to disrupt soil aggregates during the fractionation are known to alter the distribution of SOM recovered in the density or size fractions (Elliott and Cambardella, 1991; Mueller et al., 2012). As ultrasound was applied during soil fractionation, a redistribution of ground labelled material to the fine-sized fractions cannot be excluded and may partly explain the

isotope enrichment of the fine-sized fractions in the early phase of the studies I and III. However, as the particulate OM fraction was removed by floating with sodium polytungstate, the redistribution of the labelled material was minimised. Other critical issues concerning soil fractionation procedures are for example related to losses of soluble OM and the separation of aggregates up to primary particles (Cerli et al., 2012; Elliott and Cambardella, 1991; Moni et al., 2012).

As in the study I organo-mineral clusters were found to react as preferential spots for OC and N sequestration, it would be interesting to localise these hot spots within the intact soil structure. As soil fractionation was used in this study to analyse the OC and N distribution exclusively in the clay-sized fraction, it is not clear where and how these micro-structures are arranged in the soil matrix. It could probably be that the rough surfaces are excellently suited as microbial habitats and that the microorganisms living there promote the OM binding at such spots. The question, if this spot develops due to the fact that not all mineral surfaces come in contact with OM and microorganisms, could probably be answered in a next step by keeping the soil structure intact. Embedding with resin was recently supposed to be currently the best option for examining intact macro-aggregates or whole soil cores (Mueller et al., 2013). Even though resin-embedding is typically applied for the fixation of intact soil structures with subsequent cutting and polishing (Mueller et al., 2013), this procedure has also several uncertainties, as the resin for example introduce an artificial C source. One of the most critical parts during the fixation procedure is the drying process of the sample material prior to the impregnation (Murphy, 1982). A common method used for drying is the replacement by an organic solvent such as acetone (Dexter, 1988; Murphy, 1982), which could change the natural composition and structure of the organic components as well as redistribute OM within the soil structure. Several limitations in the sample preparation over different size ranges, from individual soil particles to intact soil structures, as well as possible alternatives regarding NanoSIMS measurements were discussed by Mueller et al. (2013).

Although the uncertainties of both methodical approaches have to be kept in mind, soil fractionation and fixation of the soil structure due to embedding can increase the knowledge about how OM becomes arranged within the soil structure, where soil microorganisms are living and which role minerals play in this sense. Especially the combination of fractionation methods with approaches analysing intact aggregates or soil cores in one and the same experiment could have great potential to extend our knowledge in soil science.

4. Conclusion and Outlook

The key objective of this thesis was to evaluate the role of specific surfaces provided by different minerals and charcoal as reactive interfaces in the complex interplay between OM-mineral-microorganism interactions and the subsequent effect on the fate of OM. Thereby it was supposed

that the formation of soil interfaces is controlled by the type of particle surface present and the assemblage of OM with mineral particles. Stable isotopic labelling and an artificial soil have been used as approaches to study the interactions between minerals, OM and microorganisms and to trace the influence on OM decomposition and the sequestration of OC and N in fine-sized fractions. The stable isotope tracing offered the possibility to observe small differences between the analysed soil materials which remain hidden, if only total OC is analysed.

The clay-sized fraction was shown to provide the highest amounts of OC and N as well as the highest amounts of litter-derived ^{13}C and ^{15}N tracer and exhibit therefore the most newly formed biogeochemical interfaces in addition to the currently present interfaces (Study I and III). Thus, the clay-sized fraction represents an important soil compartment for the sequestration of OC and N (Study I and III). Due to the results obtained in study I, it could be demonstrated that micro-structures generated by the mineral particles provide preferential spots for OC and N sequestration in soils. These organo-mineral clusters with rough surfaces are furthermore crucial for the accumulation of new OM in the clay-sized fraction, rather than the large proportion of free mineral surfaces (Study I).

With regard to the microbial community composition, it was observed that the surface properties of illite versus montmorillonite seemed to regulate the process of bacterial community establishment over a long-term incubation time (Study II). This indicated a fundamental effect of clay minerals on the formation of microbial habitats in soils. The second manure addition resulted in a re-activation of the artificial soil system and induced a re-formation of macro-aggregates. This in combination with a longer maturation time led to a differentiation in the amount of macro-aggregates depending on the type of clay mineral present, whereby more macro-aggregates were found in the artificial soils with montmorillonite compared to illite (Study II). The microbial communities were able to process the added organic substrate much more efficiently in the developed, i.e. aggregated soil system after the second manure supply than after initial addition pointing to the adaption of the microorganisms. With a further addition of fresh OM to the artificial soil approach, the soil with montmorillonite exhibited a slower mineralisation compared to the artificial soil with illite, which was in line with a smaller microbial biomass in this soil (Study III). The artificial soil with illite sequestered more OC and N in the clay-sized fraction than the artificial soil with montmorillonite, although the montmorillonite had a higher SSA. Consequently, a more intensive decomposition seems to be associated with a higher microbial biomass and thus leads to a higher amount of microbial products sequestered in the clay-sized fraction modified by the type of clay mineral present. As the discrepancies in the decomposition of the labelled litter between the different clay minerals are small, we suppose that several OM inputs and longer maturation times

are needed to reveal a differentiation in the amount of stored OC and N depending on the different clay minerals due to the structural development of the artificial soils.

It was supposed in the beginning that the formation of soil interfaces is controlled by the type of particle surface present and a high SSA are usually considered to provide a high storage capacity for OC and N, however the mineral surface area seemed to be of minor importance for the storage capacity and the formation of interfaces in the studies presented here. On the whole, the data obtained in this thesis showed the higher importance of the shape and roughness of mineral clusters as well as the soil structure for the sequestration of OM than the SSA of soil constituents.

Although, incubation experiments are usually carried out with a single OM input, there seems to be a need for incubation experiments with repeated OM additions not only because natural system usually receive regular OM inputs. The type of clay minerals for example seemed to be important for the fate of OM only after several OM additions and microorganisms showed a stronger response to a second manure input than to the initial addition. Thus experiments with repeated OM additions can advance our knowledge about the fate of OM in soils. A further interesting issue for the future would be to test if macro-aggregates produced by different minerals exhibit different structural stabilities and therefore possibly different aggregate evolutions, as the stability of aggregates underlie dynamic processes after OM addition. In this context, the location of microorganisms within the soil matrix would be important, especially regarding the formation of microhabitats and their role for the fate of OM. Direct after the second OM addition, manure responding bacteria were found similar for all artificial soil, although the clay minerals were the driver of microbial community composition on the long-term (Study II). This led to the assumption that microorganisms colonise specific microbial habitats. As r-strategists (Pseudomonas) responded to the manure supply similar for all artificial soils, it could be supposed that these bacteria are more associated with particulate OM which was the same for the artificial soils. The clay minerals were found to be decisive for the bacterial community composition over the long-term, thus it might be possible that the k-strategists are more influenced by the different mineral phase which forms their microhabitat. It also remained an open question if the clay minerals affect the microorganisms directly or indirectly, e.g. by aggregation.

Next steps regarding the observations about micro-structures could be: (1) the further investigation of the 2-D visually identified spatial structures in particular regarding their surfaces properties, for example by surface roughness measurements and (2) to study the arrangement of the examined micro-structures providing hot spots for OC and N sequestration within the soil structure. The question if these spots develop due to the fact that not all mineral surfaces come in contact with OM and microorganisms, or the rough surfaces serve as excellently microbial habitats where

microorganisms promote the OM binding to such spots, could probably be answered by keeping the soil structure intact.

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Appendix

Publication I

Publication II

Publication III

Eidesstattliche Erklärung

Curriculum vitae

List of Publications

Publication I

The results of study I are summarised and published in the article:

Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils

by Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter and Ingrid Kögel-Knabner

published 2014 in Nature Communications 5, Article number: 2947.

DOI: 10.1038/ncomms3947.

Publication II

The results of study II are summarised and published in the article:

Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils

by Cordula Vogel, Doreen Babin, Geertje J. Pronk, Katja Heister, Kornelia Smalla and Ingrid Kögel-Knabner

published 2014 in Soil Biology and Biochemistry 79, 57-67.

DOI: 10.1016/j.soilbio.2014.07.012.

Publication III

The results of study III are summarised and published in the article:

Clay mineral composition modifies decomposition and sequestration of organic carbon and nitrogen in fine soil fractions

by Cordula Vogel, Katja Heister, Franz Buegger, Irina Tanuwidjaja, Stephan Haug, Michael Schloter and Ingrid Kögel-Knabner

published 2015 in Biology and Fertility of Soils.

DOI: 10.1007/s00374-014-0987-7.

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung bzw. Fakultät Wissenschaftszentrum Weihenstephan der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:

Micro-structures generated by the mineral phase determine the fate of organic carbon and nitrogen in soil

am Lehrstuhl für Bodenkunde unter der Anleitung und Betreuung durch Frau Prof. Dr. Ingrid Kögel-Knabner ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Abs. 6 und 7 Satz 2 angegebenen Hilfsmittel benutzt habe.

Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

Ich habe den angestrebten Doktorgrad **noch nicht** erworben und bin **nicht** in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

Die öffentlich zugängliche Promotionsordnung der TUM ist mir bekannt, insbesondere habe ich die Bedeutung von § 28 (Nichtigkeit der Promotion) und § 29 (Entzug des Doktorgrades) zur Kenntnis genommen. Ich bin mir der Konsequenzen einer falschen Eidesstattlichen Erklärung bewusst.

Mit der Aufnahme meiner personenbezogenen Daten in die Alumni-Datei bei der TUM bin ich einverstanden.

München, den 29.01.2015

Unterschrift

Curriculum vitae

Cordula Vogel

Personal details

date of birth	23 March 1986
place of birth	Grimma, Germany
marital status	single

Current position

Since 04.2011	Ph.D. student Lehrstuhl für Bodenkunde Technische Universität München, Germany Project: Biogeochemical interfaces in soil - SPP1315 Member of TUM Graduate School and TUM IGSSE (International Graduate School of Science and Engineering)

Education

02.2011	Graduation as Diplom Agraringenieur Diploma thesis: "Initiale Bodenbildung im Vorfeld des Arolla-Gletschers." Grade (1.1)
10.2005-03.2011	University: Study of agriculture Martin-Luther University Halle-Wittenberg Specialisation: soil protection and landscaping Grade (1.1)
08.2002-07.2005	Secondary School: Berufliches Schulzentrum Markkleeberg Specialisation: agronomy
08.1996-07.2002	Primary School: Mittelschule Zschaitz-Ottewig

Work experience

02.2008-06.2010	Student research assistant at the Martin-Luther University Halle-Wittenberg, Soil Science and Soil Protection
11.2006-09.2007	Student research assistant at the Georg-August-University Göttingen, Plant Nutrition

International experience

08.2014-11.2014	Research and Training stay at the CSIRO Land and Water in the group of Jeffrey Baldock Adelaide/Australia
03.2010-04.2010	Higrade excursion to Philippines “Land-use conflicts and conservation of natural resources in Philippine Rice Terraces”
11.2009	Student research assistant, field work trip to Cixi/China, maintenance servicing of redox electrodes and suction cups in the field, research project Biogeochemistry of paddy soil evolution

Awards

Dr. Heinrich-Baur-Förderpreis 2014 of the Dr. Heinrich-Baur-Stiftung des Wissenschaftszentrums für Ernährung, Landnutzung und Umwelt Weihenstephan, TU München

Best poster award, DBG Kommissionssitzung „Soil processes- is the whole system regulated at hotspots? From micro-scales to the pedon”, 04.-06.05.2014

Best poster award, Jahrestagung der Deutsche Bodenkundliche Gesellschaft, 03.-09.9.2011

Publications

Peer-reviewed publications:

2015

Cordula Vogel, Katja Heister, Franz Buegger, Irina Tanuwidjaja,, Stephan Haug, Michael Schloter and Ingrid Kögel-Knabner (2015): Enhanced organic matter decomposition leads to higher organic carbon and nitrogen sequestration in fine-sized soil fractions modified by clay mineralogy, Biology and Fertility of Soils, Biology and Fertility of Soils, doi: 10.1007/s00374-014-0987-7.

2014

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter and Ingrid Kögel-Knabner (2014): Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils, Nature Communications 5, Article number: 2947, doi:10.1038/ncomms3947.

Cordula Vogel, Doreen Babin, Geertje Pronk, Katja Heister, Kornelia Smalla and Ingrid Kögel-Knabner (2014): Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. Soil Biology and Biochemistry 79, 57-67, doi:10.1016/j.soilbio.2014.07.012.

Doreen Babin, Cordula Vogel, Sebastian Zühlke, Michael Schloter, Geertje J. Pronk, Katja Heister, Michael Spiteller, Ingrid Kögel-Knabner, Kornelia Smalla, (2014): Soil Mineral Composition Matters: Response of Microbial Communities to Phenanthrene and Plant Litter Addition in Long-Term Matured Artificial Soils. PLoS ONE 9, doi:10.1371/journal.pone.0106865.

Oral presentations:

2014

Cordula Vogel (2014): Sequestration of organic matter in organo-mineral association - NanoSIMS and artificial soils as tools in soil science, University of South Australia, Adelaide, Australia, 24.10.2014 (invited)

Cordula Vogel (2014): Sequestration of organic matter - NanoSIMS and Artificial soils as tools in soil science, CSIRO, Adelaide, Australia, 29.10.2014

Cordula Vogel (2014): Sequestration of organic matter by organo-mineral associations, La Trobe University, Melbourne, Australia, 31.10.2014 (invited)

2013

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Katja Heister, Michael Schloter, Franz Buegger and Ingrid Kögel-Knabner (2013): Die räumliche Verteilung organischer Substanz auf mineralischen Oberflächen-mittels nanoskalischem Sekundärionen-massenspektrometer (NanoSIMS), Jahrestagung der Deutsche Bodenkundliche Gesellschaft 2013, Rostock, Germany, 07.-12.09.2013

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter and Ingrid Kögel-Knabner (2013): Sequestration of organic matter (OM) by mineral particles, Conference of Soils in Space and Time, Ulm, Germany, 30.9.-5.10.2013

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter and Ingrid Kögel-Knabner (2013): Mineral particles clustered in submicron structures provide preferential organic matter sequestration spots, DMG-GV-Sediment 2013 Meeting, Tübingen, Germany, 16-19.9.2013

2012
Cordula Vogel, Katja Heister, Franz Buegger, Andrea Bannert and Ingrid Kögel-Knabner (2012): Investigation of biogeochemical interface development by a short-term incubation experiment using ^{15}N and ^{13}C labeled litter, General Assembly 2012 of the European Geosciences Union, Vienna, Austria, 22-27.4.2012

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Katja Heister, Andrea Bannert, Franz Buegger and Ingrid Kögel-Knabner (2012): Micro-scale distribution of litter-derived organic matter in mineral fractions as revealed by nano-scale secondary ion mass spectrometry (NanoSIMS), 5th International Workshop on Soil and Sedimentary Organic Matter Stabilization and Destabilization, Ascona, Switzerland, 7-11.10.2012

Poster presentations:

2014
Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter, Ingrid Kögel-Knabner (2014): Submicron mineral structures control the sequestration of litter-derived organic matter in soils – A NanoSIMS study, General Assembly 2014 of the European Geosciences Union, Vienna, Austria, 27.4-2.5.2014

Cordula Vogel, Carsten W. Müller, Carmen Höschen, Franz Buegger, Katja Heister, Stefanie Schulz, Michael Schloter, Ingrid Kögel-Knabner (2014): Submicron mineral structures control the Sequestration of litter-derived organic matter in soils – A NanoSIMS study, DBG Kommissionssitzung, Freising, Germany, 04-06.5.2014

2013
Cordula Vogel, Stefanie Schulz, Katja Heister, Franz Buegger, Michael Schloter and Ingrid Kögel-Knabner (2013): Mineral composition control the formation of organo-mineral associations -An artificial soil experiment using ^{15}N and ^{13}C labeled litter, DMG-GV-Sediment 2013 Meeting, Tübingen, Germany, 16-19.9.2013

Cordula Vogel, Doreen Babin, Geertje Pronk, Katja Heister, Kornelia Smalla and Ingrid Kögel-Knabner (2013): Clay mineralogy shapes microbial habitats and macroaggregates during a long-term artificial soil incubation experiment, Annual SPP 1315 Meeting, Jena, Germany, 9-11.10.2013

Cordula Vogel, Stefanie Schulz, Katja Heister, Franz Buegger, Michael Schloter and Ingrid Kögel-Knabner (2013): Investigation of organo-mineral association in an artificial soil experiment using ^{15}N and ^{13}C labelled litter, Annual SPP 1315 Meeting, Jena, Germany, 9-11.10.2013

2012
Cordula Vogel, Katja Heister, Angelika Kölbl and Ingrid Kögel-Knabner (2012): How does oxidative degradation of organic matter affect surface area and microporosity? – A comparison of three common removing agents, 4th International Congress of the European Confederation of Soil Science Societies (ECSSS), Bari, Italy, 2-6.7.2012

Cordula Vogel, Carsten W. Mueller, Carmen Höschen, Katja Heister, Andrea Bannert, Franz Buegger and Ingrid Kögel-Knabner (2012): Micro-scale distribution of litter-derived organic matter in mineral fractions as revealed by nano-scale secondary ion mass spectrometry (NanoSIMS), Annual SPP 1315 Meeting, Jena, Germany, 10-12.10.2012

Cordula Vogel, Katja Heister and Ingrid Kögel-Knabner (2012): Selective removal of organic matter as a method to investigate biogeochemical interfaces – A comparison of three common removing agents, Annual SPP 1315 Meeting, Jena, Germany, 10-12.10.2012

2011

Cordula Vogel, Katja Heister and Ingrid Kögel-Knabner (2011): Biogeochemical interface formation in soils as controlled by different components -SECOND PHASE- Microporosity and architecture of biogeochemical interfaces at the nanoscale, Annual SPP 1315 Meeting, Jena, Germany, 6-7.10.2011

Cordula Vogel, Thomas Kühn, Klaus Kaiser and Reinhold Jahn (2011): Initiale Bodenentwicklung im Vorfeld des Arolla Gletschers, Wallis, Schweiz, Jahrestagung der Deutsche Bodenkundliche Gesellschaft 2011, Berlin, Germany, 03.-09.9.2011