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Soil aggregate formation in soils derived from loess reclamation

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Summary

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After the termination of open-cast mining, geological deposits lying above the coal layer are used in reclamation procedures as a new substrate for initializing soil formation. Soil formation after land restoration begins from the initial state of the parent material which is mostly characterized by destructed soil structure and its basic chemical and biological properties. Soil development in destructed soil is a complex biogeochemical process and successful reclamation is not only achieved by establishing sufficient state of soil fertility, but rather sustaining stable soil structure and soil functionality for a longer time period. The aim of this thesis is to characterize the soil structural development from the initial state of soil formation, when soil organic matter concentration is low and soil physicochemical properties are controlled by the parent material. We investigated young reclaimed loess soils by using a space-for-time chronosequence approach in agriculturally reclaimed sites in Garzweiler, Germany. We selected fields with six different soil formation stages (0, 1, 3, 6, 12, and 24 years after the first seeding of alfalfa) to examine soil formation factors along with ongoing biogeochemical processes. The objectives of the doctoral thesis were achieved by three independent studies.

The initial formation of soil structure, as well as the physicochemical and biological parameters in reclaimed loess was investigated in Study I. A combined laboratory and statistical analysis provided a comprehensive understanding of the initial soil formation factors in young calcareous loess. Observed fluctuations in soil physicochemical properties throughout the space-for-time chronosequence showed that loess parent material and reclamation management techniques were the most dominant factors influencing soil formation. Regardless of soil development over time, soft-rock like structures were predominant due to calcareous loess throughout the space-for-time chronosequence. Strong cementation led to domination of macroaggregates >6.3 mm, and no trend in dry aggregate size distribution was visible between reclamation sites, even when soil organic carbon (SOC) concentration increased significantly in bulk soil. During the first three years of the pioneering phase, alfalfa cultivation and litter decomposition were the greatest factors in the development of SOC content and microbial biomass. We observed higher SOC concentrations and increased contribution of recent carbon in the uppermost topsoil (1-5 cm). Despite alfalfa's deep rooting system, we could not observe any soil-plant

interaction in the subsoil even after 3 years of soil formation. Introducing tillage and organic fertilizer in year 4 significantly increased the quality of the soil's physical and chemical properties. Annual ploughing increased macroporosity and SOC content, and decreased bulk density in year 6. Application of organic fertilizer only had a short term effect on restoring SOC content. In years 12 and 24 we observed a substantial loss of SOC, which was related to the usage of inorganic fertilizers after year 7, showing that the loss of organic matter (OM) was not compensated by crop residues. These findings demonstrate that soil management, along with qualities of the parent material, play a leading role in defining the state and success of soil reclamation.

Heterogeneous arrangements of solids and voids define several soil functions at various spatial scales. In Study II, a novel correlative analysis of 2D imaging visible light near-infrared spectroscopy (imVNIR) and 3D X-ray computed microtomography (µCT) on intact soil cores was developed to gain a better understanding of the soil's inherent structure and its relation to SOM accumulation. Method combination enabled us to determine spatial changes in soil structure, as well as its chemical components, when soil agricultural management was introduced. When organic fertilizer was applied, we identified a higher spatial distribution of SOM and changes in aggregate shape. Besides displaying structural characteristics, correlative analysis of selected imaging methods expanded the accuracy of detecting SOM accumulation and the relation to the distance to pores and biopores. When normalizing SOM relative contribution by density shifts obtained by X-ray µCT, we could observe a direct contribution of SOM to the pore system. The novel combination of these methods showed that SOM accumulation was pre-dominant close to biopore walls in no-tilled topsoil and subsoil samples. When introducing tillage and organic fertilizer to the soil, it showed significant influence on soil structural heterogeneity and SOM spatial distribution. With wider distance between macropores, the relative abundance of SOM decreased in tilled topsoil. This developed method in Study II allows us to describe biogeochemical properties while taking into account the whole complexity of soil's inherent 3D structure.

The investigation on initial soil aggregate formation was conducted in Study III using the space-for-time chronosequence research sites. An aggregate size class fractionation provided an understanding of soil aggregate formation factors in calcareous loess. The study revealed that initial aggregate formation in calcareous soils had two mechanisms, and the aggregate formation in reclaimed soils did not follow aggregate hierarchy. The

formation of macroaggregates was influenced by the fresh OM input, whereas the silt- and clay-sized fraction (<63 µm) was consistently dominated by calcareous loess. After the application of organic fertilizer in year 4, we observed SOC and TN enrichment in large macroaggregates in year 6 suggesting that OM was crucial for building up a macroaggregate structure. These macroaggregates could not be sustained for a longer period due to reduced input of OM after year 7. In contrast to Study I, in which a substantial loss of SOC in years 12 and 24 was observed, the data from Study III revealed that the depletion of SOM in bulk soil was related to the loss of SOC in macroaggregates. The strong influence of $CaCO_3$ in microaggregates was presumably inherited from calcareous loess, which is used in the reclamation procedure as a parent material for soil formation. Despite ongoing soil development and the resulting enhancements in the soil's physical, chemical and biological properties, we did not observe any interactions between $CaCO_3$ and SOC and there was no SOC enrichment visible in microaggregates. This suggests that SOM through Ca-mediated organo-mineral interactions did not contribute to the formation of microaggregates. The structural stability analysis showed that cementation bonds diminished after submersion in water, which resulted in complete loss of soil aggregate structure. This ultimately reveals that the stability of soil structure is controlled by the inherent properties of calcareous loess.

The presented dissertation demonstrates that the soil formation is largely depending on the soil management strategies and inherent properties of the parent material. Both Studies I and III revealed how calcareous parent material defines the stability of soil and influences soil structural formation. Carbonates inherited from loess dominated the (in)stability of soil structure despite the development in SOC content and porosity along the space-for-time chronosequence. The stability of soil decreased directly when reclaimed soil was in contact with water. Soil management strategies influenced the dynamics of OC in soils, which was presumably connected to the formation of macroaggregates. It became evident that limited OM input (crop and root residues) could not sustain stable SOC content and macroaggregate formation. At the same time, we did not observe any correlation between $CaCO_3$ and SOC in any aggregate size class, which leads us to differentiate between biotic and abiotic factors on aggregate formation. In Study II we succeeded to develop a combinatory method, which provides a novel tool to describe biogeochemical interactions in the soils taking into account the whole complexity of soil structure, ultimately allowing us to work intact soil samples with greater volume. We observed SOM accumulation close

to biopore walls in no-tilled topsoil and subsoil and detected changes in soil structure and distribution of SOM when agricultural management was introduced together with organic fertilizer application. The results of this work provide evidence for processes and controlling factors (cementing effect, plowing, management practices) on defining the successful recovery of soil functions such as biological habitat, structural stability, nutrient cycling and SOC storage when calcareous soils are at the initial stage of soil formation.

Zusammenfassung

Bodenaggregatbildung in rekultivierten Lössböden

Nach der Beendigung des Tagebaus werden geologische Ablagerungen, die über der Kohleschicht liegen, in Rekultivierungsverfahren als neues Substrat zur Initialisierung der Bodenbildung genutzt. Nach der Bodensanierung beginnt die Bodenbildung beim Anfangszustand des Ausgangsmaterials, welcher meist durch eine zerstörte Bodenstruktur sowie grundlegende chemische und biologische Eigenschaften gekennzeichnet ist. Die Bodenentwicklung in gestörtem Boden ist ein komplexer biogeochemischer Prozess, und eine erfolgreiche Rekultivierung wird nicht nur durch das Erreichen eines zufriedenstellenden Zustands der Bodenfruchtbarkeit erreicht, sondern auch durch die Aufrechterhaltung einer stabilen Bodenstruktur und Bodenfunktionalität über einen längeren Zeitraum. Ziel der Dissertation ist es, die Bodenstrukturentwicklung aus dem Anfangszustand der Bodenbildung zu charakterisieren, wenn die Konzentration der organischen Bodensubstanz (SOM, „soil organic matter") gering ist und die physikalisch-chemischen Eigenschaften des Bodens durch das Ausgangsmaterial beeinflusst werden. Wir untersuchten junge rekultivierte Lössböden mit Hilfe eines räumlichen und zeitlichen Chronosequenz-Ansatzes in einem landwirtschaftlich rekultivierten Feld von Garzweiler (Deutschland). Wir wählten Felder mit sechs verschiedenen Bodenbildungsstadien (0, 1, 3, 6, 12 und 24 Jahre nach der ersten Aussaat) aus, um Bodenbildungsfaktoren mit den darunterliegenden biogeochemischen Prozessen zu untersuchen. Die Ziele der Doktorarbeit wurden durch drei unabhängige Studien erreicht.

Die initiale Ausbildung der Bodenstruktur sowie physikochemischer und biologischer Parameter in rekultiviertem Löß wurde in Studie I untersucht. Eine Kombination ausLabor- und statistische Analyse lieferte ein umfassendes Verständnis der initialen Bodenbildungsfaktoren in jungem kalkhaltigem Löß. Die beobachteten Fluktuationen der physikalisch-chemischen Eigenschaften des Bodens während der zeitlichen und räumlichen Chronosequenz zeigten, dass das Ausgangsmaterial des Lößes und die Techniken des Rekultivierungsmanagements die dominierenden Kontrollfaktoren für die Definition des Zustands der Bodenbildung waren. Unabhängig von der Entwicklung des Bodens im Laufe der Zeit war eine weichgesteinsähnliche Struktur durch kalkhaltigen Löß in der gesamten Raum-Zeit-Chronosequenz vorherrschend. Starke Zementierung führte zur Dominanz von trockengesiebten Aggregaten >6.3 mm, und es war kein Trend in der

trockengesiebten Aggregatgrößenverteilung zwischen den Rekultivierungsstandorten sichtbar, selbst wenn die SOC-Konzentration im lockeren Boden signifikant anstieg. Während der ersten drei Jahre der Pionierphase trugen Luzernekultivierung und Streuabbau am meisten zur Entwicklung von organischem Kohlenstoff im Boden (SOC, „soil organic carbon") und mikrobieller Biomasse bei. Wir beobachteten höhere SOC-Konzentrationen und einen erhöhten Beitrag von rezentem Kohlenstoff im obersten Oberboden (1-5 cm). Trotz des tiefen Wurzelsystems der Luzerne konnten wir selbst nach drei Jahren Bodenbildung keine Boden-Pflanzen-Interaktion im Unterboden beobachten. Die Einführung von Bodenbearbeitung und organischem Dünger im vierten Jahr.beschleunigte die Verbesserungen der physikalischen und chemischen Eigenschaften des Bodens erheblich. Jährliches Umpflügen erhöhte die Makroporosität, verringerte die Schüttdichte und wir beobachteten einen signifikanten Anstieg des SOC-Gehalts im sechsten Jahr. Die Anwendung von organischem Dünger hatte nur eine kurzfristige Auswirkung auf die Wiederherstellung des OC-Gehalts des Bodens. In den Jahren 12 und 24 beobachteten wir einen beträchtlichen Verlust an SOC, der mit dem Einsatz von anorganischem Dünger nach dem siebten Jahr zusammenhing, was zeigt, dass der Verlust an OM nicht durch Ernterückstände kompensiert wurde. Diese Ergebnisse zeigen, dass die Bodenbewirtschaftung zusammen mit dem Ausgangsmaterial eine prägende Rolle bei der Definition des Zustands und Erfolgs der Bodensanierung spielt.

Heterogene Anordnungen von Festkörpern und Hohlräumen definieren mehrere Bodenfunktionen auf verschiedenen räumlichen Skalen. In Studie II wurde eine neuartige korrelative Analyse von 2D-Bildgebung durch Spektroskopie mit sichtbarem Licht im nahen Infrarot (imVNIR) und 3D-Computer-Mikrotomographie (µCT) an intakten Bodenkernen entwickelt, um ein vollständiges Verständnis der inhärenten Architektur des Bodens und ihrer Beziehung zur SOM-Akkumulation zu erhalten. Die Methodenkombination ermöglichte es, die räumliche Verteilung von SOM als Funktion des Abstands zu Poren und Bioporen zu beobachten. Dies ermöglichte es, räumliche Veränderungen der Bodenstruktur und der chemischen Komponenten zu ermitteln, nachdem die landwirtschaftliche Bodenbewirtschaftung eingeführt wurde. Wir identifizierten einen höheren Gehalt an SOM und Veränderungen in der Aggregatform, wenn organischer Dünger ausgebracht wurde. Neben der Darstellung struktureller Merkmale erweitert die korrelative Analyse ausgewählter bildgebender Verfahren die Genauigkeit bei der Untersuchung der SOM-Akkumulation und der Beziehung zum

Abstand zu Poren und Bioporen. Bei der Normalisierung des relativen SOM-Beitrags durch Dichteverschiebungen, die mittels Röntgen-µCT erhalten wurden, können wir einen direkten SOM-Beitrag zum Porensystem beobachten. Die neuartige Kombination dieser Methoden zeigte, dass die SOM-Akkumulation in der Nähe von Bioporenwänden in nicht gepflügten Ober- und Unterbodenproben vorherrschend war. Nach der Anwendung von Bodenbearbeitung und organischem Dünger in den Böden zeigte sich ein signifikanter Einfluss auf die strukturelle Heterogenität des Bodens und die räumliche Verteilung der SOM. Mit größerem Abstand zu den Makroporen nahm die relative Häufigkeit von SOM im gepflügten Oberboden ab. Die in Studie II entwickelte Methode bietet die Möglichkeit, biogeochemische Wechselwirkungen unter Berücksichtigung der gesamten Komplexität der inhärenten 3D-Struktur des Bodens zu beschreiben.

Die Untersuchung der anfänglichen Bildung von Bodenaggregaten wurde in Studie III unter Verwendung der Raum--Zeit-Chronosequnenz-Forschungsstandorte durchgeführt. Eine Fraktionierung der Aggregatgrößenklassen lieferte Erkenntnisse über die Faktoren der Bodenaggregatbildung in kalkhaltigem Löß. Die Studie zeigte, dass die anfängliche Aggregatbildung in kalkhaltigen Böden zwei Mechanismen aufweist und dass die Aggregatbildung in rekultivierten Böden keiner Aggregathierarchie folgt. Die Bildung von Makroaggregaten wurde durch den frischen OM-Eintrag beeinflusst, während die Schluff- und Tonfraktion (<63 µm) ständig von kalkhaltigem Löß dominiert wurde. Nach der Ausbringung von organischem Dünger in Jahr vier beobachteten wir in Jahr sechs eine SOC- und TN-Anreicherung in großen Makroaggregaten mit dem Hinweis, dass OM für den Aufbau einer Makroaggregatstruktur entscheidend war. Diese Makroaggregate konnten aufgrund der verminderten Zufuhr von OM nach dem siebten Jahr nicht länger über einen längeren Zeitraum bestehen. Als wir in Studie I einen beträchtlichen Verlust an OC im Boden in den Jahren 12 und 24 beobachteten, zeigten die Daten aus Studie III, dass die Abnahme von OM im lockeren Boden mit dem Verlust von SOC in Makroaggregaten zusammenhing. Der starke Einfluss von $CaCO_3$ in Mikroaggregaten wurde vermutlich von kalkhaltigem Löß geerbt, der im Rekultivierungsverfahren als Ausgangsmaterial für die Bodenbildung verwendet wird. Trotz der fortschreitenden Bodenentwicklung und Verbesserungen der physikalischen, chemischen und biologischen Eigenschaften des Bodens konnten wir keine Wechselwirkungen zwischen $CaCO_3$ und SOC beobachten, und es war keine SOC-Anreicherung in Mikroaggregaten sichtbar. Das bedeutet, dass SOM durch Ca-vermittelte organisch-mineralische Wechselwirkungen nicht zur Bildung von

Mikroaggregaten beitrug. Die Analyse der strukturellen Stabilität zeigte, dass die durch Karbonat zementierten Bindungen nach dem Eintauchen in Wasser abnahmen, was zum vollständigen Verlust der Bodenaggregatstruktur führte. Dies zeigt, dass die Stabilität der Bodenstruktur durch die inhärenten Eigenschaften des kalkhaltigen Lösses gesteuert wird.

Die vorgelegte Dissertation zeigte, dass die Bodenbildung weitgehend von den Bodenbearbeitungsstrategien und inhärenten Eigenschaften des Ausgangsmaterials abhängt. Sowohl Studie I als auch III zeigten, wie kalkhaltiges Ausgangsmaterial die Stabilität des Bodens definiert und die Bodenstrukturbildung beeinflusst. Vom Löss ererbte Karbonate dominierten trotz der Entwicklung des SOC-Gehalts und der Porosität entlang der Zeit-Raum-Chronosequenz über die (Un-)Stabilität der Bodenstruktur. Die Stabilität des Bodens nahm direkt ab, wenn der rekultivierte Boden mit Wasser in Kontakt kam. Bodenbearbeitungsstrategien beeinflussten die OC-Dynamik in Böden, was vermutlich mit der Bildung von Makroaggregaten zusammenhing. Es zeigte sich, dass, wenn keine ausreichende Menge frischer OM zugeführt wurde, nur Ernte- und Wurzelrückstände den stabilen SOC-Gehalt und die Bildung von Makroaggregaten nicht aufrechterhalten konnten. Gleichzeitig beobachteten wir in keiner Aggregatgrößenklasse einen Zusammenhang zwischen $CaCO_3$ und SOC, was uns dazu führt, biotische und abiotische Faktoren bei der Aggregatbildung zu unterscheiden. In Studie II ist es uns gelungen, eine kombinatorische Methode zu entwickeln, die ein neuartiges Werkzeug zur Beschreibung biogeochemischer Wechselwirkungen unter Berücksichtigung der gesamten Komplexität der Bodenstruktur bietet und es erlaubt, intakte Bodenproben mit grösserem Volumen zu bearbeiten. Wir beobachteten die Akkumulation von SOM in der Nähe von Bioporenwänden in nicht gepflügtem Ober- und Unterboden und stellten Veränderungen in der Bodenstruktur und der Verteilung von SOM fest, nachdem die landwirtschaftliche Bewirtschaftung zusammen mit der Ausbringung von organischem Dünger eingeführt wurde. Die Ergebnisse dieser Arbeit liefern Hinweise auf Prozesse und steuernde Faktoren (Zementierungseffekt, Pflügen, Düngungsstrategien) zur Definition der erfolgreichen Wiederherstellung von Bodenfunktionen wie biologischer Lebensraum, strukturelle Stabilität, Nährstoffkreislauf und SOC-Speicherung, wenn sich kalkhaltige Böden im Anfangszustand der Bodenbildung befinden.

Kokkuvõte

Mullaagregaatide teke rekultiveeritud lössis

Pealmaakaevandustes ehk karjäärides kaevandamise lõppemisel on tänapäeval reeglina nõutav kaevandamise käigus hävitatud maastiku taastamine või korrastamine. Peamiselt kasutatakse söekarjääride rekultiveerimisel substraadina söekihi peal lasunud geoloogilisi kihte, mille abil hakatakse mullageneesi ehk mulla arenemist taastama. Mullageneesi kulgemise tingimused sõltuvad lähtematerjali omadustest, mille esialgne struktuur, keemilised ja bioloogilised parameetrid on enamasti võrreldavad toitaine vaeste ja erodeeritud muldade omadustega. Mulla arenemine on kompleksne biogeokeemiline protsess, mille tulemuslikkust ei saa mõõta üksnes mullaviljakusele tuginedes, vaid võimekusest säilitada mullastruktuuri ja funktsionaalsust pikema ajaperioodi vältel. Käesoleva doktoritöö eesmärk on hinnata mullastruktuuri arenemist mullageneesi alghetkest, kui mulla orgaanilise aine sisaldus on madal ning füüsikalis-keemilised parameetrid peegeldavad eelkõige lähtematerjali omadusi. Mullageneesi hindamiseks analüüsitakse rekultiveeritud mulla lasuvustiheduse muutust, mulla orgaanilise süsiniku (C_{org}) dünaamikat, mullastruktuuri arenemist ja agregaatide teket aegreas. Käesolevas doktoritöös uuriti mulla arenemist Garzweileri (Saksamaa) rekultiveeritud karjääri aladel, kus ala korrastamise lähtematerjalina kasutati lössi ja alad taastati põllumajanduslikul eesmärgil kasutamiseks. Rekultiveeritud aladelt valiti aegreana kuus erinevat uurimisala (0, 1, 3, 6, 12 ja 24 aastat rekultiveerimisest), mis peegeldavad mullaarenemise erinevaid etappe. Käesoleva doktoritöö eesmärkide saavutatumiseks on läbi viidud kolmest iseseisvast uurimistööst koosnev uuringutsükkel.

Esimeses uuringus (*Study I*) analüüsiti mullastruktuuri, füüsikalis-keemiliste ja bioloogiliste parameetrite kujunemist reklutiveerimise vanuse aegreas. Kombineeritud laboratoorsed ja statistilised analüüsid tõid välja mullageneesi mõjutavad tegurid. Mulla füüsikalis-keemiliste omaduste muutlikkus ja toitainete kontsentratsioonid näitasid, et lähtematerjalina kasutatav löss ja rekultiveerimise tehnoloogia avaldasid tugevaimat mõju mulla seisundile. Lähtematerjalina kasutatav löss mõjutas mullastruktuuri kujunemist kõige enam karbonaatide sisalduse tõttu, mis soodustas pehme kivimitaolise struktuuri moodustumist ja seda sõltumata rekultiveeritud ala vanusest. Vaatamata mulla C_{org} sisalduse tõusule vastavalt rekultiveerimise läbiviimise vanusele, tekitas lähtematerjali tsementeerumine >6.3 mm suuruseid makroagregaate. Esimese kolme mullageneesi aasta

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jooksul aitas lutserni külvamine ja taimevarise lagunemine kaasa mulla orgaanilise aine ja mikroobse biomassi arengule. Selle tulemusel täheldasime mulla pindmises kihis C_{org} kontsentratsiooni ja värske süsinku osakaalu tõusu. Vaatamata lutserni sügavale juurestikule, ei suutnud me tuvastada juure ja mulla vastastikust koostoimet, mis avaldaks positiivset mõju mulla kvaliteedi tõstmiseks ka sügavamates kihtides. Põllumajandusliku tegevuse alustamine neljandal aastal ja orgaanilise väetise lisamine kiirendas märkimisväärselt mulla füüsikaliste ja keemiliste omaduste muutumist. Regulaarne külv ja künd mõjutas makropoorsuse tõusu ja mulla lasuvustiheduse vähenemist. Vaatamata kiirele mulla C_{org} sisalduse tõusule, avaldas orgaanilise väetise lisamine siiski kõigest lühiajalist mõju mulla orgaanilise aine varu taastumisele ja mullaviljakuse tõusule. 12 ja 24 aasta vanustel rekultiveeritud aladel täheldasime olulist mulla C_{org} sisalduse langust, mis oli otseselt seotud mineraalväetiste kasutusele võtmisega seitsmendal rekultiveerimisejärgsel aastal. Sellest võib järeldada, et põllule jäetud põhu lagunemine ei kompenseerinud mulla orgaanilise aine mineraliseerumist. Esimese uuringu tulemused osutavad, et rekultiveerimisel valitud lähtematerjalil ja majandamisviisil on oluline roll mullaarengu seisundi ja mulla funktsioonide taastamise edukuse määratlejana.

Mulla tahke faasi ja pooride asetuse heterogeensus mõjutavad mullafunktsioonide nagu bioproduktsiooni ja bioloogilise elurikkuse väljakujunemist. Selleks, et mõista mulla orgaanilise aine akumuleerumise ja mulla struktuuri omavahelist sõltuvust, töötasime teise uuringu (Study II) käigus välja kombinatoorse metoodika kasutades 2D nähtava valguse infrapunaspektroskoopia (*imaging visible light near-infrared spectroscopy* (imVNIR)) ja 3D kompuutertomograafia (*X-ray computed microtomography* (µCT)) tehnoloogiaid. Välja töötatud korrelatiivne analüüs pakub võimalusi analüüsida mulla orgaanilise aine ruumilise leviku sõltuvust poorde ja biopooride vahekaugusest. See võimaldas määrata põllumajandusliku tegevuse mõju rolli mulla struktuuri kujunemisele ja keemiliste ühendite ruumilise jaotuse muutustele. Proovidest, mis olid mõjutatud orgaanilise väetise lisamisest ja põllumajanduslikust tegevusest, tuvastasime mulla orgaanilise aine sisalduse suurenemise ja agregaatide kuju muutuse. Kui võtta lisaks ruumilisele jaotusele arvesse ka mulla tahke faasi elektrontihedust, mis on saavutatud µCT tehnoloogiaga, suurendas nende kahe meetodi kombineerimine analüüsi täpsust. Saadud tulemused näitasid, et biopoorid ja mulla struktuuri heterogeensus määravad mulla orgaanilise aine akumuleerimise intensiivsust. Leidsime, et põllumajanduslikust tegevusest mõjutamata proovides oli mulla orgaanilise aine talletamine suurem biopooride läheduses. Kui põllumajandusliku

tegevusega alustati, siis mullastruktuuri heterogeensus suurenes ja mulla orgaanilise aine akumuleerumine toimus makropooridest suuremal kaugusel. Uuringu raames välja töötatud metoodika pakub uudseid võimalusi biogeokeemiliste protsesside uurimiseks, võttes samal ajal arvesse ka mullale iseloomulikku heterogeense struktuuri keerukust.

Kolmandas uuringus (Study III) analüüsisime mulla agregatsiooni arengut rekultiveeritud aladel vastavalt vanusegradiendile. Agregaatide fraktsioneerimine võimaldas hinnata tegureid, mis mõjutasid agregaatide teket kõrge karbonaatsusega mullas. Uuringus selgus, et agregaatide moodustumine toimus biootilise ja abiootilise mehhanismi teel, mis ei järginud agregatsiooni hierarhilist mudelit. Kui makroagregaatide teke oli otseses sõltuvuses mulla orgaanilise aine sisalduse kasvust, siis tolmu- ja savi suuruse fraktsiooni (<63 μm) tekkel domineerisid lössi karbonaatsed omadused. Orgaanilise väetise lisamine neljandal aastal suurendas orgaanilise aine ja lämmastiku osakaalu, mis statistiliste seoste kaudu näitas, et mulla orgaaniline aine on oluline komponent makroagregaatide tekkel. Kiire orgaanilise aine lagunemine tõi kaasa makroagregaatide lühikese eluea, kui edasise põllumajandusliku tegevuse käigus kasutati värske orgaanilise aine allikana ainult põhku. Kui esimeses uuringus (*Study I*) tuvastasime 12 ja 24 aasta vanustel aladel olulise mulla C_{org} kontsentratsiooni languse, siis kolmanda uuringu tulemused näitasid, et C_{org} sisalduse langus toimus makroagregaatide arvelt. Karbonaatide tugev mõju mikroagregaatide tekkele pärines suure tõenäosusega lössist, mida kasutatakse rekultiveerimisel lähtematerjalina. Vaatamata pidevale mulla arengule ja mulla füüsikalis-keemiliste ja bioloogiliste omaduste paranemisele, ei täheldanud me karbonaatide ja mulla orgaanilise aine omavahelist seost ning ei tuvastanud vanusegradiendi järgi mikroagregaatides C_{org} kontsentratsiooni tõusu mullas. Sellest võib järeldada, et orgaanilis-mineraalne vastastikmõju ei mõjutanud mikroagregaatide teket. Mulla struktuuri analüüs näitas, et tsementeerumise tugevus vähenes märgatavalt vesikeskkonnas, mille tagajärjel mulla agregaadi struktuur hävines täielikult. See tõestas, et mulla struktuur ja stabiilsus on tugevalt seotud lössi karbonaatsete omadustega.

Käesolev doktoritöö näitab, et mullagenees on tugevalt mõjutatud mulla rekultiveerimise metoodikast ja kasutatava lähtematerjali omadustest. Uuringud I ja III kirjeldasid, kuidas karbonaatne lähtematerjal määras mulla struktuuri stabiilsust ja mõjutas pinnase struktuuri kujunemist. Karbonaadid domineerisid mullastruktuuri (eba)stabiilsuse üle vaatamata mulla C_{org} sisalduse kasvule ja struktuuri arenemisele. Kuivas keskkonnas avaldusid karbonaatse lössi tsementeerivad omadused, kuid vees vähenes mulla agregaadi stabiilsus

märgatavalt. Mulla majandamisviisid avaldasid olulist mõju mulla C_{org} dünaamikale, mis oli omakorda seotud makroagregaatide tekkega. Selgus, et kui värske orgaanilise aine lisamise osakaal vähenes, siis põllule jäetav põhk ei olnud piisav, et tagada püsivat mulla C_{org} sisaldust ja makroagregaatide teket. Samal ajal me ei tuvastanud karbonaatide ja orgaanilise süsiniku omavahelist koostoimet, mis lubas meil eristada abiootilised ja biootilised faktorid mulla agregaatsioonis. Teises uurimistöös õnnestus edukalt välja töötada korrelatiivne meetod, mis pakub uudset võimalust biogeokeemiliste protsesside kirjeldamiseks ning võimaldab samal ajal arvesse võtta mulla arhitektuuri keerukust suurema ruumalaga proovides. Me tuvastasime mitte kultiveeritud aladel suurema mulla orgaanilise aine akumuleerumise biopooride seinal, kuid põllumajandusliku tegevusega muutus mulla struktuur ja orgaanilise aine jaotus. Orgaanilise aine akumuleerumine leidis sel juhul aset makropooridest kaugemal. Doktoritöö tulemused toovad välja mulla arengu mehhanismid ja faktorid (tsementeerumine, kündmine, väetamise strateegia), mis mõjutavad rekultiveeritud mulla edukat taastumist ja tagavad mulla bioloogilise aktiivsuse, toitainete ringluse ja mulla C_{org} talletamise.

List of publications and contributions

The doctoral thesis is based on three first authored research studies.

Study I

<u>Title</u>	Initial soil formation in an agriculturally reclaimed open-cast mining area- the role of management and loess parent material
<u>Authors</u>	E. Pihlap, M. Vuko, M. Lucas, M. Steffens, M. Schloter, D. Vetterlein, M. Endenich, I. Kögel-Knabner
<u>Publication</u>	Soil & Tillage Research 191 (2019), 224-237, 10.1016/j.still.2019.03.023
<u>Summary</u>	Reclaimed soils from post open-cast mines are considered to have deficits in nutrient content, microbial activity and poor soil structure. Most reclamation techniques are focused on improving the nutrient transformation in soils, but soil formation is a complex process and it is important to understand the relation between soil's chemical, physical and biological processes. In the study we investigated the development of soil structure, physicochemical and biological parameters from the initial stage of soil formation. We used a chronosequence approach in the reclaimed open-cast lignite mining area in Garzweiler (Germany) with six time stages (0, 1, 3, 6, 12 and 24 years after the first seeding of alfalfa). Loess had a strong effect on aggregate formation by cementation effect, which did not change in older reclamation sites. In the pioneering phase, the alfalfa cultivation had high importance on developing microbial biomass and soil organic carbon (SOC) by litter decomposition in the uppermost topsoil. Soil physical parameters such as macroporosity and pore connectivity increased in year 6 after the transition from pioneering phase over to agricultural soil management. Along with organic fertilizer amendment the SOC increased directly in whole plough layer. However, in following years the crop and root residues, which were only source for organic matter (OM) after the year 7, were not sufficient for creating stable SOM pool for a longer period.
<u>Contribution</u>	I carried out the fieldwork, conducted laboratory analysis and data evaluation, wrote and revised the manuscript with input from co-authors.

Study II

<u>Title</u>	Combination of Imaging Infrared Spectroscopy and X-ray Computed Microtomography for the Investigation of Bio- and Physicochemical Processes in Structured Soils
<u>Authors</u>	M. Lucas*, <u>E. Pihlap</u>*, M. Steffens, D. Vetterlein, I. Kögel-Knabner
<u>Publication</u>	Frontiers in Environmental Science 8:42 (2020), 10.3389/fenvs.2020.00042

<u>Summary</u>

Soil is a complex mixture of solid (minerals, organic materials) and voids, which defines the structural heterogeneity and soil properties at various spatial scales. The objective of the study was to achieve complete image on soil architecture and characterize the interplay between soil structure and chemical composition. For that we developed a correlative image analysis technique using 2D imaging visible light near-infrared spectroscopy and 3D X-ray computed microtomography (µCT) on intact soil cores. Imaging spectroscopy gives spatial information on chemical composition from soil 2D surface and X-ray µCT provides observations on soil 3D structural characteristics. We selected three intact soil sample cores (Ø and height 3 cm) with different soil structures and development stages (3, 6 and 12 years after the reclamation) from the reclaimed open-cast mining area in Garzweiler (Germany). After 2D and 3D image registrations, and image analysis we evaluated SOM distribution as a function of distance to pores and biopores. The correlative image analysis gave new insights into the biopore network and the relation to SOM distribution. In no-tilled topsoil and tilled subsoil we found greater SOM contribution next to biopores, whereas in tilled topsoil SOM abundance was higher with greater distance from macropores. SOM accumulation and protection was related to the soil management practices, where alteration was induced by soil agricultural management.

<u>Contribution</u> I carried out the sample preparation, scanned samples with the hyperspectral camera, conducted imVNIR image analysis and data evaluation, wrote and revised the manuscript with input from co-authors.

*shared first authorship

Study III

<u>Title</u>	Initial soil aggregate formation and stabilisation in soils developed from calcareous loess
<u>Authors</u>	E. Pihlap, M. Steffens, I. Kögel-Knabner
<u>Publication</u>	Geoderma 385 (2021), 10.1016/j.geoderma.2020.114854

<u>Summary</u> Soil organic matter (SOM) is considered to be the major binding material in aggregation, but little is known about aggregate formation, when inorganic stabilising agents are present. The objective of the study was to investigate soil aggregate formation in calcareous loess from the initial state of soil development and understand cementation mechanisms on aggregate formation. Our study revealed that aggregation in calcareous loess had two mechanisms and aggregate structure did not follow aggregate hierarchy. Aggregate fractionation by wet sieving showed that the formation of macroaggregates was induced by fresh OM input. When organic fertilizer was applied in year 4, we observed in year 6 SOC enrichment in the large macroaggregates. These macroaggregates were also associated with the dynamics of bulk SOC. It became evident that the depletion of SOM in years 12 and 24 was associated with the loss of SOC in large macroaggregate size class. After changing the soil fertilization scheme in year 7, root and crop residues were the sole source of fresh OM input. This underlines strong relation between management practices and macroaggregate formation. Cementing effect by $CaCO_3$ dominated over the formation of silt- and clay-sized fraction ($<63\mu m$), which was most likely inherited from the loess used for reclamation. This was supported by the $CaCO_3$ enrichment in microaggregates, and strong relation between reclamation site and $CaCO_3$ content. The stability analysis revealed that carbonates dissolved easily in water, which resulted in significant degradation of soil aggregate structure. Despite continuing soil formation and improvements in SOC content, inherent properties of calcareous loess defined the structural stability of reclaimed soils.

<u>Contribution</u> I conducted laboratory analysis and data evaluation, wrote and revised the manuscript with input from co-authors.

1. Introduction

1.1. Soil formation in reclaimed soils

Open-cast mining destructs the landscape excessively, when huge amounts of material are removed during coal extraction. After the termination of open-cast mining processes, the main target is to recover the landscape and the functionality of the exploited area. In the reclamation procedure, leftover materials such as geological deposits that lie above the coal, are used frequently as a new substrate for soil recovery. When using nutrient poor deposits as a new parent material, reclaimed soils are often characterized by destructed soil structure and low biological activity (Shrestha and Lal, 2011). Deficits in soil functionality and fertility not only result in low agricultural productivity, but also in weak soil mechanical stability against soil erosion or poor protection of aquifers against hazardous substances.

The main focus in reclamation technique is to enhance the functionality of the soil by building up a stable nutrient pool. This means that careful selection of plant cultivation strategies and soil management play an important role in defining the reclamation success (Helingerová et al., 2010; Zhao et al., 2013). When open-cast mining fields are reclaimed with nutrient poor geological deposits, substrate quality reflects the initial state of soil formation. Besides the poor chemical properties of the substrate, the excavation process destructs the soil's initial physical structure and biological habitat. Changes in soil properties that appear after the soil reclamation of open-cast mining (Bradshaw, 1997; Harris et al., 1989; Liu et al., 2017; Zhou et al., 2017) demonstrate that reaching the original state of the natural soil would potentially take decades (Insam and Domsch, 1988; Liu et al., 2017).

Soil formation is a complex biogeochemical process, i.e. successful reclamation is not achieved only by focusing on nutrient transformation, but also by taking other physical and biological parameters into account. It is considered that organic matter (OM) is a crucial component in the reclamation process, due to its positive effect on soil structural properties, aggregate formation, and biolgical activity (Delschen, 1999; Pagliai et al., 2004; Qiang et al., 2007; Six et al., 2004). The establishment of a stable soil organic matter (SOM) pool in young, reclaimed soils is a complex process dictated by the input and subsequent degradation of organic material, ultimately making the return to a stable equilibrium state difficult. The major source for increasing OM content in reclaimed soils is vegetation.

Besides OM input by plant litter and root activities, plant growth accelerates biological activity and aggregate formation, ultimately supporting stable soil structural development (Kołodziej et al., 2016; Moreno-de las Heras, 2009; Six et al., 2004).

1.2. Soil structure as a key element in soil development

Soils are heterogeneous mixtures of constituents originating from the parent material, organic material and pore space. The inherent soil structure and soil functionality at various spatial scales emerges from the spatial arrangements of solids and voids (Portell et al., 2018; Ritz et al., 2004; Wanzek et al., 2018). Poor soil structure has a negative effect on soil formation and can counteract several soil functions (Kaufmann et al., 2009; Weisskopf et al., 2010). For example, aggregate break-up under unfavorable conditions can lead to soil compaction, i.e. poor soil structure, which in turn will affect negatively soil aeration, root penetration, and water permeability (Bronick and Lal, 2005; Czyz, 2004; Greenland, 1977; Rabot et al., 2018; Sheoran et al., 2010). This will have negative effect on soil microenvironmental processes, such as OC turnover by microbial decomposition. As SOM in soils is heterogeneously distributed (e.g. greater SOM content in biopores), soil's inherent architecture and the microenvironmental conditions play a significant role in controlling SOM mineralization intensity (Banfield et al., 2017; Dungait et al., 2012; Hoang et al., 2017; Hobley et al., 2018; Steffens et al., 2017). Since many soil functions are directly or indirectly related to soil structure, the structural formation can be considered as the main parameter for identifying reclamation success.

1.2.1. Characterization of soil structure by soil physical parameters

Several soil physical processes such as hydraulic conductivity, aeration, and plant root penetration are defined by soil structure. In studies on soil structure, it is difficult to determine one universal method characterizing structural formation together with its heterogeneity of solid phase arrangements (Diaz-Zorita et al., 2002; Rabot et al., 2018). When the focus is set on describing soil structure through the soil compaction, bulk density has been frequently used as the main measure. Despite this, bulk density does not take into account the complexity of soil structure that comes from different spatial arrangements of pores, soil texture, OM distribution, and soil habitat (Rabot et al., 2018). Amongst other soil physical parameters, macroporosity and pore connectivity are important measures for soil structure. Besides offering information on gas exchange and water infiltration, well connected pore systems enhance microbial activity and support plant growth (Lipiec and

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Hatano, 2003; Rabot et al., 2018). Management practices that increase soil macroporosity can also have a negative impact on soil structural formation. For instance, conventional tillage can damage fungi by destroying the hyphal network that entangles soil aggregates together (Boddington and Dodd, 2000; Jansa et al., 2002; Six et al., 2004). This demonstrates the importance of the interplay between solid and voids, and emphasizes the mechanisms that take place in the solid phase. i.e. processes that enhance aggregate formation and structural build-up.

1.2.2. Characterization of soil structure by aggregate formation

Soil aggregate size distribution and stability are often used to indicate the quality of soil structure (Baumert et al., 2018; Márquez et al., 2004; Six et al., 2000). Deficits in soil structure and soil aggregate stability are thought to contribute soil degradation (Boix-Fayos et al., 2001). It is proposed that aggregate formation follows an aggregate hierarchical model through complex interactions of biotic and abiotic binding mechanisms. Aggregates are formed when mineral building units are glued together by SOM, fungi, roots and also by inorganic cementing materials (Lehmann et al., 2020; Oades, 1993, 1984; Tisdall and Oades, 1982; Totsche et al., 2018). SOM is often recognized as a crucial component for the formation of aggregates (Six et al., 2004; Waters and Oades, 1991). According to the theory of aggregate hierarchy, macroaggregates (>250 μm) are formed and stabilized by temporary binding materials, such as fungi, bacteria, particulate OM and plant roots. The formation of microaggregates (<250 μm) is induced by organo-mineral interactions and due to their strong physicochemical bonding and presence of various cementing materials, microaggregates are persistent for a longer period (Bronick and Lal, 2005; Lehmann et al., 2007; Tisdall and Oades, 1982; Totsche et al., 2018). SOM-mediated aggregate formation and the relation to aggregate hierarchy is widely acknowledged, but little is known about the formation and stability of aggregates when stronger stabilizing agents are present in soil, which compete against the aggregate hierarchy induced by SOM (Bullinger-Weber et al., 2007; Fernández-Ugalde et al., 2011; Rowley et al., 2018; Waters and Oades, 1991). The aggregate stability can significantly increase when inorganic binding agents such as Al-, Fe-, and Mn-oxides and $CaCO_3$ are present in the soil (Bullinger-Weber et al., 2007; Rowley et al., 2018; Zhao et al., 2017).

1.3. The role of calcium carbonate on aggregation

Calcium carbonate ($CaCO_3$) is recognized as one of the most crucial inorganic binding materials that significantly influences aggregate stability and disrupts aggregate hierarchy (Fernández-Ugalde et al., 2011; Rowley et al., 2018). Unfortunately, aggregate formation and stabilization mechanisms promoted by carbonates remains unclear (Rowley et al., 2018; Virto et al., 2013, 2011). $CaCO_3$-mediated stabilization and aggregate formation can take place through several processes. In presence of Ca^{2+} ions, aggregate formation in calcareous soils can be induced by Ca^{2+} bridging mechanism between mineral surfaces and OM (Clough and Skjemstad, 2000; Fernández-Ugalde et al., 2011; Muneer and Oades, 1989). This promotes organo-mineral interactions and increases the stabilization of microaggregates. Ca-mediated aggregate formation and stabilization are widely acknowledged (Fernández-Ugalde et al., 2011; Rowley et al., 2018), but $CaCO_3$ induced aggregate formation and stability is not as well described. This aggregate stabilization mechanism is related to the cementation effect in which $CaCO_3$ precipitates on particle surfaces. Described coating effect is commonly found in calcareous parent materials such as loess, where the mechanical stability and plasticity of soil is predominantly defined by $CaCO_3$ (Meng and Li, 2019; Yates et al., 2017). In calcareous soils, carbonates can also dissolve and re-precipitate as a secondary carbonate on the particle surface. This mechanism can take place in calcareous soils, when CO_2 is released as a result of root and microbial respiration and acids are produced during the decomposition of OM and application of mineral fertilizers (Fernández-Ugalde et al., 2011; Zamanian et al., 2016). In the rooting zone, the solubility of finely distributed carbonates increases significantly, which is related to the greater CO_2 rate and production of weak organic acids that lowers soil pH (Zamanian et al., 2016). Re-precipitation of $CaCO_3$ provides a physical coating on aggregate surface, cementing them together and ultimately increasing the stability of aggregates.

1.4. Microscale studies on soil structural development

Microenvironmental conditions in soils are related to the heterogeneity of soil structure, which in turn defines the biological processes (e.g. microbial carbon turnover), root penetration, gas exchange, distribution of OM and physical protection of SOC (Dungait et al., 2012; Mueller et al., 2012; Rabot et al., 2018; Stockmann et al., 2013; Wiesmeier et al., 2019). Studies on soil structure focus either on applying soil physical methods with the goal

of gaining a better understanding of pore systems or extracting soil aggregates that are linked to the inherent arrangements in solid phase (Baveye et al., 2018; Young et al., 2008). Methods that focus only on one soil parameter, or that separate soil structure into fractions, destructs the linkage between the soil and its natural 3D structure. In order to understand the complex interplay between soil structure and biogeochemical processes, it requires analysis that investigates soils as a complete system.

In soil science, combinatory applications of various imaging methods have been progressively used (Hapca et al., 2011; Juyal et al., 2019; Kravchenko et al., 2019). Combining methods that provide different but corroborating information on soil structure aide in explaining the relationship between soil microenvironment, biogeochemical and physical processes (Schlüter et al., 2019). There are several known methods that investigate soil structure without disturbing its initial architecture. For example, visible light near-infrared spectroscopy (Vis-NIR) is a method, that allows for a simultaneous analyzation of several soil properties, based on their spectral characteristics (Soriano-Disla et al., 2014; Stenberg et al., 2010). Soil science methodology has been enhanced to include promising, emerging technology, including an imaging Vis-NIR (imVNIR) spectroscopy, which provides a rapid evaluation on 2D spatial distribution of chemical components. This technique has the potential to enhance observations of soil chemical composition from microscale to whole pedon (Buddenbaum and Steffens, 2011; Hobley et al., 2018; Steffens et al., 2014; Steffens and Buddenbaum, 2013). Soil's inherent physical 3D architecture (pore size distribution and pore connectivity) is commonly analyzed in undisturbed soils by X-ray computed microtomography (X-ray μCT) (Rabot et al., 2018). As those two techniques corroborate each other by expanding observations on the same soil, the combinatory application of 2D imVNIR and 3D X-ray μCT may have the potential to provide comprehensive information on biogeochemical processes and the relation to soil inherent structure in intact soil samples.

1.5. Objectives and approach

The aim of the dissertation is to understand the formation of soil structure from the stage at which soil organic carbon content is low and the main soil physicochemical properties are controlled by the parent material. We used a space-for-time chronosequence approach in agriculturally reclaimed sites in Garzweiler post open-cast lignite mining. We investigated young reclaimed soils to examine the complex relationship between soil parent material and the soil structural formation, and analyzed this together with ongoing soil biogeochemical processes. This close linkage between soil physicochemical and structural properties determines the recovery rate of important soil functions in nutrient poor environments (e.g. nutrient supply, microbial abundance, aeration and root penetration).

The dissertation has three main objectives and approaches:

<u>Objective 1.</u> *Initial soil formation in an agriculturally reclaimed open-cast mining area - the role of management and loess parent material (Study I).* The objective of Study I aimed to determine factors influencial to soil development and understand the interaction between soil's chemical, physical and biological processes, which ultimately contribute to the formation of a stable soil structure and restoration of soil functions. We investigated reclaimed soils with six different time stages during the reclamation (0, 1, 3, 6, 12 and 24 years after reclamation). Post-mining fields in Garzweiler provided three main criterions for studying soil formation: a) the soils originated from the similar parent material; b) soils were in the initial state of soil development, and c) soils went through the same soil formation phases. Additionally, we selected a mature soil located next to the mining area as a reference field to describe typical soil properties within the region. The approach combined soil physicochemical and biological analysis from three different depth increments (1-5 cm, 16-20 cm and 41-45 cm).

The study approach included:

a) characterizing soil chemical, physical and biological properties along the space-for-time chronosequence;
b) identifying soil formation stages throughout the space-for-time chronosequence and evaluating the reclamation success by the state of mature soil;
c) determining controlling factors on soil formation after the reclamation of open-cast pits;

d) observing the correlation between soil physical, chemical and biological parameters and their role on contributing to soil development;

e) evaluating loess inherent properties and their impact on soil structure;

f) assessing the role of parent material in defining the state of soil development.

Objective 2. *Combination of Imaging Infrared Spectroscopy and X-ray Computed Microtomography for the Investigation of Bio- and Physicochemical Processes in Structured Soils (Study II).* Study I showed that soil management had a great influence on building up soil physical, chemical and biological properties and significantly affected soil OC dynamics in young developing soils. Soils are complex mixtures and the spatial arrangements of mineral particles and organic material influence several soil functions. The objective of Study II was to develop a technique that enable a simultaneous investigation of soil biogeochemical properties and its inherent architecture. To build up the method and visualize the soil architecture role on SOM accumulation, three different soil cores under different management methods (tilled and no-tilled) were chosen from the reclaimed sites in Garzweiler.

The approach included the following steps:

a) visualizing soil inherent structure and the 2D spatial distribution of chemical components by imVNIR image processing techniques;

b) developing a correlative analysis of 2D imaging visible light near-infrared spectroscopy and 3D X-ray computed microtomography (μCT);

c) characterizing the distribution and protection of SOM in structured soils;

d) determining how soil management influences SOM spatial variability and how the pore network contributes to the SOC decomposition.

Objective 3. *Initial soil aggregate formation and stabilisation in soils developed from calcareous loess (Study III).* In Study I we determined a strong cementing effect caused by loess parent material, which dominated the macroaggregate formation despite the increase in soil OC content in year 6. The aggregate hierarchy model proposes that soil aggregate formation is induced by OM, but the importance of OM in aggregation was not visible in Study I. Although it is known that inorganic binding agents can significantly influence aggregate formation, the role of cementation induced by $CaCO_3$ on aggregate stabilization has received less attention. The objective of Study III was to enhance understanding on aggregate formation and stabilization mechanisms in calcareous soils. Here, we focused on

the whole space-for-time chronosequence in order to determine aggregate formation stages during the soil development.

The approach in the study included:

a) fractionating aggregate size classes using a gentle wet sieving method;

b) characterizing trends in aggregate formation and the dependence on the soil management phases;

c) determining aggregate size class contribution to the total soil OC, TN and $CaCO_3$ concentration throughout the space-for-time chronosequence;

d) determining parameters that influence the aggregate formation in reclaimed soils and investigating the interplay between SOC and $CaCO_3$ on aggregation;

e) observing soil structural stability by applying ultrasonication energy to macroaggregates obtained in Study I;

f) evaluating aggregate hierarchy and SOC dynamics in calcareous loess.

2. Material and methods

2.1. Study area (Studies I-III)

The study area is located in Garzweiler post-mining fields (51°5´N, 6°28´E), which is part of the lignite mining area in the German state of North-Rhine Westphalia. Garzweiler is situated 5 km west of Grevenbroich and located between bigger cities such as Cologne, Mönchengladbach, and Aachen. In the region of North-Rhine Westphalia, the mean annual rainfall is 720 mm and mean annual temperature is 9.5 °C. Haplic Luvisol (Aric, Hypereutric, Ochric, Siltic) is the common soil type in the area (IUSS Working Group WRB, 2015) and it is developed from loess. Loess is an Aeolian sediment and originates from the Weichselian glaciation period (deposited 12,000–100,000 years ago) and can be characterized by its light yellow color and high proportion of silt content, high $CaCO_3$ concentration (10-200 mg g^{-1}) and cementing properties (Kels and Schirmer, 2010; Lehmkuhl et al., 2016; Pecsi, 1990; Schirmer, 2016). Loess in the region is the natural parent material and belongs to Lower Rhine Embayment.

2.2. Reclamation method in Garzweiler

In Garzweiler the open-cast mining technique is characterized by simultaneous mining and reclamation processes on exploited sites. Today, about 33,000 ha of land have been excavated and about 23,000 ha have been reclaimed either as agricultural land (12,000 ha) or forest (9,000 ha). The reclamation technique currently utilized in Garzweiler was developed in the 1990's (Dumbeck, 1996, 1992). During the surface mining process, approximately 20 m thick layer of bedrock (loess) is removed together with developed topsoil (Luvisol). The excavated material has a Luvisol/loess ratio of about 1:10 and is used in the reclamation technology as a new source material for soil formation. The mixture of loess is deposited on top of exploited sites to a thickness of two meters and left there in stockpiles for settling. After three months of settling, the loess mixture is levelled with caterpillars and the reclamation procedure begins (Fig. 1). The reclamation consists of two phases: the pioneering phase and the agricultural management phase. The pioneering phase begins with the cultivation of alfalfa (*Medicago sativa*), which stabilizes the soil with a deep rooting system and stimulates biological activity through nitrogen and carbon enrichment. The alfalfa growth period in the pioneering phase lasts for three years without any additional soil disturbances by management. The agricultural management phase

consistently begins in year 4, when alfalfa residues and organic fertilizer are mixed to the topsoil, and crop rotation begins. Until year 6, crop rotation includes *Triticum aestivum L.* (wheat) and *Hordeum vulgare L.* (barley) and in the following years a variation of *Triticum aestivum L.* (winter wheat and summer wheat), *Hordeum vulgare L.* (winter barley), *Brassica napus L.* (rapeseed) and *Zea mays L.* (maize) are planted. During the agricultural soil management period, organic fertilizer (compost or manure) is added in years 4 and 7, and in the following years mineral fertilizers are used by farmers. Reclaimed sites are managed by the mining company (RWE power AG) for the first 7 years before being returned to local farmers.
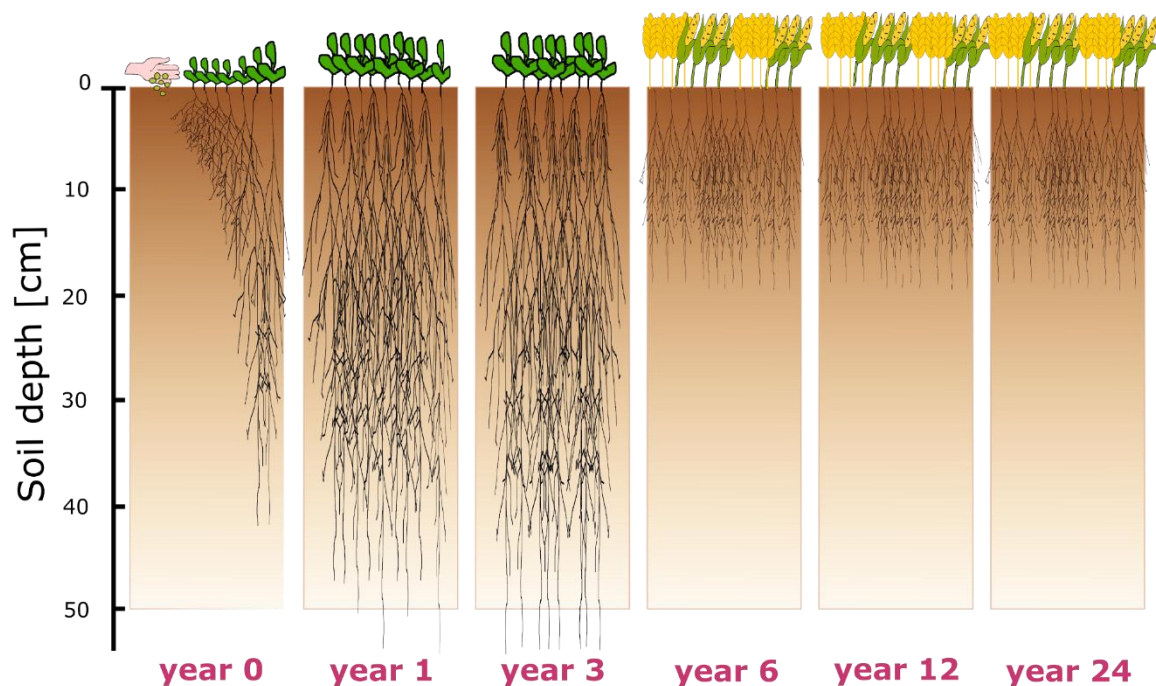

Figure 1. Reclamation scheme across the chronosequence, where years 0-3 represent the pioneering phase and years 6-24 agricultural soil management phase.

2.3. Space-for-time chronosequence (Studies I-III)

In the study, we used a space-for-time chronosequence approach (Fig. 1) with six different time stages. To study the reclamation process, we selected fields of 0, 1, 3, 6, 12 and 24 years after the first seeding of alfalfa. The location of study sites in Garweiler are presented in Figure 2. Our space-for-time chronosequence consists of reclamation fields that originated from the similar loess parent material, and that went through the same soil reclamation steps as described in the chapter 2.2. The youngest reclamation sites (years 0, 1 and 3) in the space-for-time chronosequence represent the pioneering phase with the

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cultivation of alfalfa, whereas the older reclamation stages (years 6, 12 and 24) are fields with agricultural soil management with crop rotation. Additionally, we selected a mature soil as a reference field, which describes the typical soil properties in the region before the mining activities started. The mature soil site was situated next to the open-cast mining pit, but was not yet affected by mining activities. We selected three replicate plots for each study site (areas between 2 and 35 ha). Each replicate plot was approximately 200 m^2. In total, we ended up with 21 plots, in which two different sampling strategies were applied.
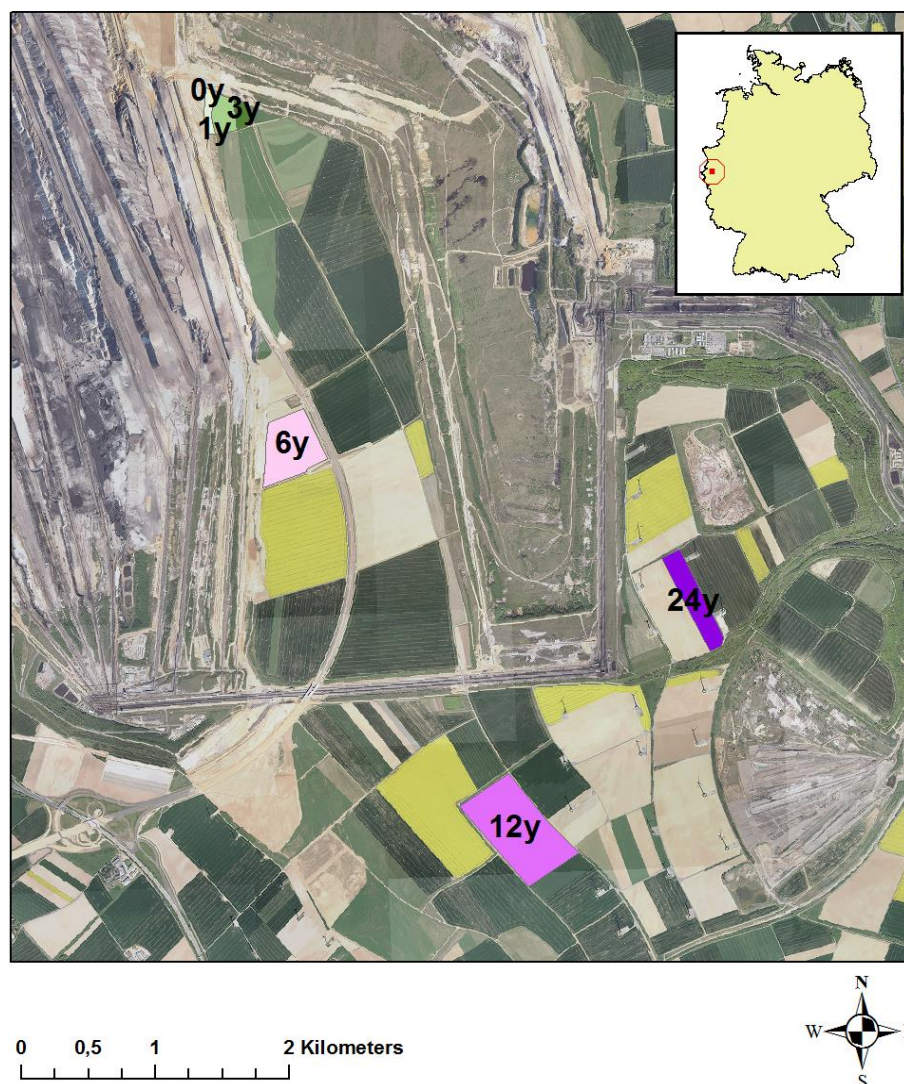

Figure 2. *Location of mining area in Germany (the map above) and study fields in Garzweiler reclamation area. Numbers on the field represent the time (years) from the first seeding of alfalfa.*

Sampling from reclamation sites were done in October 2016 and from mature soil in November 2017. In each of three replicate plots, we took undisturbed samples from four independent sampling points. Undisturbed samples were taken with 100 cm^3 steel cylinders in triplicate (total volume 300 cm^3) at three sampling depths. In each sampling point we sampled topsoil at two different sampling depths of 1-5 cm and 16-20 cm, in which subsoil was sampled at the depth of 41-45 cm only once per field replicate plot. As the substrate was similar throughout the space-for-time chronosequence, we did not expect high heterogeneity in subsoil. In order to standardize the weight and moisture content, each sample was oven-dried at 40 °C.

2.4. Intact soil cores for correlative imaging analysis (Study II)

Undisturbed cylindrical cores (diameter 10 cm and height 20 cm) were taken by custom-made drill (UGT GmbH, Germany) on the basis of the study of Lucas et al., (2019). Samples were taken after the first sowing from each reclamation site (Fig. 1) during four sampling campaigns (October 2017-March 2018). From each replicate plot one undisturbed cylindrical core was taken from topsoil (0-20 cm) and subsoil (40-60 cm). For the correlative analysis we selected samples from different soil formation (years 3, 6 and 12) and management stages (no tillage and tillage), which provided method application and evaluation in a diversely structured soil. Year 3 represents an immature soil that had not yet been affected by soil tillage and had a low SOC content. Tilled topsoil sample in year 6 characterizes changes in soil structure and SOM content due to the transition of soil reclamation management from the pioneering phase to agricultural management and organic fertilizer amendment. The subsoil sample from year 12 represents a soil structure with high content of macropores and biopores, which have developed under the topsoil. To increase the resolution for μCT analysis, from each cylindrical core three subsamples were taken with the diameter and height of 3 cm.

2.5. Analytical methods

Samples of the Study I and III were oven-dried at 40 °C and samples for the study II were stored at 4°C to reduce biological activity until further analysis. The overview of applied methodologies are concluded in Table 1.

Table 1. *Overview of the methods and measured parameters in Studies I-III*

Method	Obtained fractions and description of methods	Study
Dry crushing followed by dry sieving	Dry crushed macroaggregates with sizes of 6.3-20 mm, 2-6.3 mm and <2 mm	I
Aggregate fractionation with wet sieving	Aggregate fractions with sizes of >630 μm, 200-630 μm, 63-200 μm and <63 μm	III
Macroaggregate stability with ultra-sonication	Aggregate size distribution with the size range of >630 μm, 200-630 μm, 63-200 μm and <63 μm	III
Imaging Infrared Spectroscopy (imVNIR)	OM distribution as a function to pores and biopores	II

Parameter	Method	Study	Sample type
Bulk density	Drying sample at 105° and dividing the oven dry soil mass by sample volume	I	Bulk soil
pH	0.01 M CaCl$_2$ solution (soil/CaCl$_2$ solution ratio 1:5)	I	Bulk soil
Texture	H$_2$O$_2$ oxidation, CaCO$_3$ removal by HCl, wet sieving, X-ray sedimentation (Sedigraph III Plus, Micrometrics, Aachen, Germany)	I	Bulk soil
^{14}C	Accelerator mass spectrometry (AMS) carried out at the CologneAMS Centre	I	Bulk soil
TC and TN concentrations	Dry combustion at 1,000°C, EuroEA Elementar analyser (Hekatech GmbH, Wegberg, Germany)	I	Bulk soil
		I	Dry crushed macro-aggregates
		III	Wet-sieved aggregate fractions
CaCO$_3$ concentration	Scheibler Calcimeter (Eijkelkamp, Giesbeek, The Netherlands)	I	Bulk soil
		I	Dry crushed macro-aggregates
		III	Wet sieved aggregate fractions

Parameter	Method	Study	Sample type
OM composition	Solid state ^{13}C NMR CPMAS spectroscopy (Brucker DSX 200 NMR spectrometer, Karlsruhe, Germany)	III	Wet sieved aggregate fractions

2.5.1. Bulk density and soil texture analysis

Bulk density was measured from each sampling point along the space-for-time chronosequence. Bulk density was obtained with dividing oven-dry (105°C) sample mass by the total sample volume. The particle size distribution of reclamation soil was analyzed only from subsoil samples, because we did not expect significant texture gradient changes along the profile. A whole profile from the mature soil was taken for the particle size distribution analysis. After sample homogenization (<2 mm) the organic material was oxidized in the mature soil with submerging samples with H_2O_2 (30%) and carbonates were removed from reclamation field samples with 1M HCl solution. Organic matter removal was not applied to samples originating from reclamation sites, because subsoil samples contained very low SOC (2.7 ± 0.8 mgOC g^{-1}). Each sample was suspended in 0.025 M $Na_4P_2O_7$ solution and dispersed by ultrasonication at 450 J ml^{-1}. Dispersed samples were wet sieved into three sand fractions (63-200 µm, 200-630 µm, >630 µm) and dried in an oven at 105°C (Emerson, 1971; Schmidt et al., 1999). Silt- and clay-sized fractions (<63 µm) were freeze dried and their distribution was subsequently measured by X-ray sedimentation (Sedigraph III Plus, Micrometrics GmbH, Germany).

2.5.2. SOC determination, SOC stocks and the origin of SOC

All bulk soil samples in Study I and aggregate size class fractions in Studies I and III were analyzed for TC and $CaCO_3$. TC and TN were analyzed simultaneously by dry combustion with an EuroEA Elementar analyzer (HEKAtech GmbH, Wegberg, Germany) and the $CaCO_3$ concentration by a Scheibler Calcimeter (Eijkelkamp, Giesbeek, The Netherlands). SIC was calculated with dividing $CaCO_3$ concentration by the constant of 8.33 and soil organic carbon (SOC) concentration was obtained by subtracting SIC from the TC. In mature soil all samples were free of $CaCO_3$, therefore, the TC concentration equals to SOC concentration.

Soil OC stocks were calculated in Study I using the equivalent mass approach according to Ellert and Bettany, (1995). Different reclamation management stages influenced significantly bulk densities along the space-for-time chronosequence, which can lead to false SOC stock assessment (Wendt and Hauser, 2013). Therefore, for the SOC stock calculation we normalized soil masses according to the soil management stages (pioneering phase, transition from pioneering phase over to agricultural management phase, continuous soil tillage and reference soil). Total SOC stock was calculated for three depth increments (0-10 cm, 10-30 cm and 30-50 cm) with following equation (Ellert and Bettany, 1995):

$$SOC_{stock} = EM \times SOC \times 10^{-3} \tag{1}$$

where SOC$_{stock}$ is the total soil organic carbon stock (kg SOC m^{-2}), EM is the equivalent mass (kg m^{-2}) and SOC is measured soil organic carbon concentration (mgOC g^{-1}).

The ^{14}C content of selected bulk soil samples was measured by accelerator mass spectrometry (AMS) at the CologneAMS Centre (Rethemeyer et al., 2013). Carbonates were removed by submerging samples with 1% HCl solution for 72h with 1 h of heating samples at 60°. From the radiocarbon ^{14}C activity, the proportion of "dead" carbon was calculated by following the equation (Rumpel et al., 2000):

$$\text{"Dead" carbon [\%]} = [1 - (^{14}C_{activity} / 115)] \times 100 \tag{2}$$

where ^{14}C$_{activity}$ is measured radiocarbon activity.

2.5.3. Aggregate size class fractionation

Two different aggregate fractionation schemes were applied. An overview of the aggregate fractionation schemes is given in Figure 3. In Study I we determined dry macroaggregate size distribution by breaking samples into aggregates by jaw crusher (set to 20 mm) and a dry sieving them into three macroaggregate size classes (6.3-20 mm, 2-6.3 mm, <2 mm).

Additionally, aggregate fractionation of selected bulk samples was conducted by gentle wet sieving method, obtaining different water-stable aggregate size classes (Baumert et al., 2018; Bucka et al., 2019; Puget et al., 1998). Bulk samples (8 g) were gently rewetted in distilled water for 30 min and subsequently wet sieved into four aggregate size classes, which separates soil into large macroaggregates (>630 µm), small macroaggregates (200-630 µm), large microaggregates (63-200 µm) and silt- and clay-sized fractions (<63 µm). Silt- and clay-sized fraction will further be denominated as S + C-fraction. Submerged samples were transferred into a sieve tower, which was moved vertically about 2 cm in the

distilled water for 5 minutes (30 cycles per minute). Obtained aggregate size classes of >630 μm, 200-630 μm and 63-200 μm were dried in an oven at 60°C and S + C-fraction (<63 μm) was freeze dried. Each sample was fractionated with a duplicate in order to produce a sufficient amount of fractions for further elemental analysis.

SOC, TN and $CaCO_3$ concentrations were measured from selected dry sieved macroaggregate size class fractions and from each wet sieved aggregate size class fractions. After obtaining the aggregate mass proportions and corresponding elemental concentrations, each aggregate size class contribution to total SOC, TN and $CaCO_3$ was calculated. Additionally, the SOC, TN and $CaCO_3$ enrichment factors were calculated from wet sieved aggregate fractions according to the equation (Guggenberger et al., 1994):

$$E_{SOC/TN/CaCO3} = \frac{SOC/TN/CaCO_3 \; mg \; g^{-1} \; (aggregate)}{SOC/TN/CaCO_3 mg \; g^{-1} \; (total \; soil)} \qquad (3)$$

where "aggregate" is the elemental concentration of the aggregate fraction, and "total soil" is elemental concentration in the bulk soil.
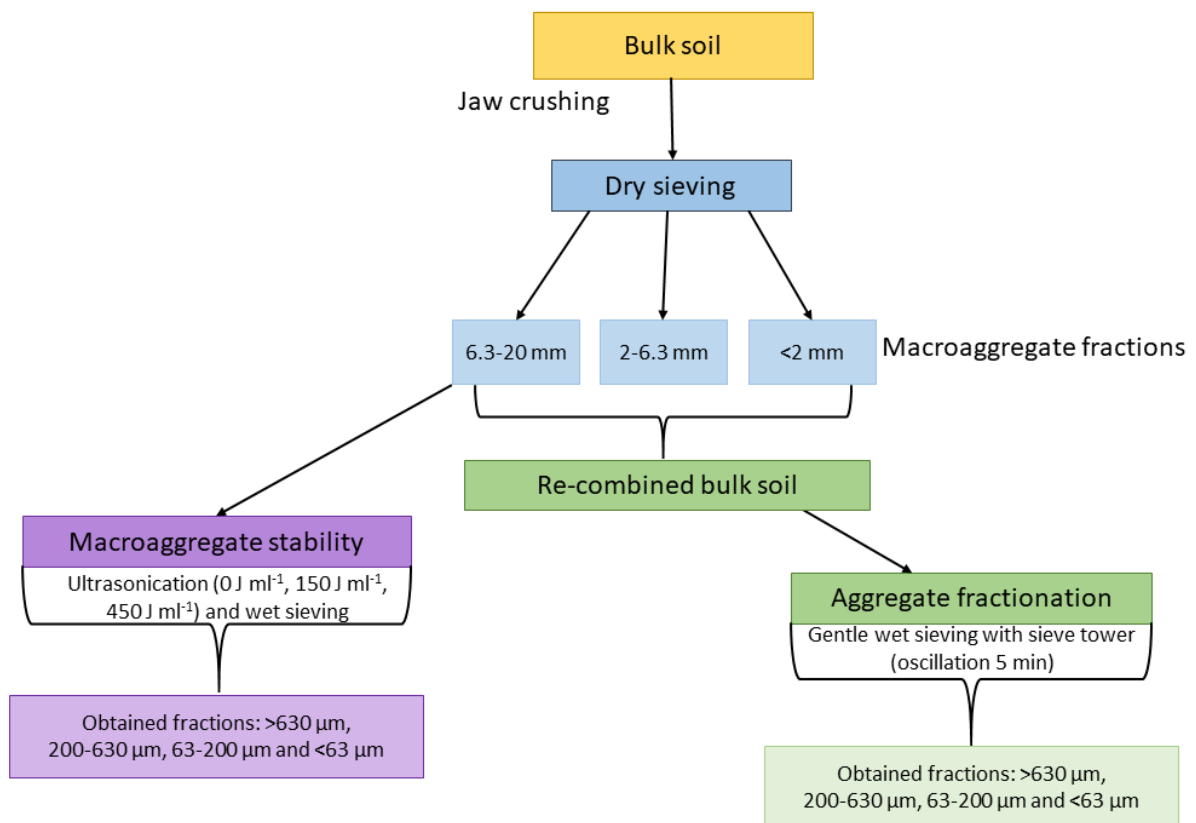
Figure 3. *Scheme of the aggregate fractionation and aggregate stability methods*

The chemical composition of >630 µm, 200-630 µm and 63-200 µm fractions were characterized by solid-state ^{13}C NMR spectroscopy (Bruker Biospin DSX 200 NMR spectrometer, Rheinstetten, Germany). Before the application of ^{13}C NMR spectroscopy, all aggregate fractions (2 g) were treated with 10% (w/w) HF five times to enrich SOC concentration by removing minerals that interfere with the analysis. Signal intensities of the chemical shifts were integrated according to four chemical shift regions: 0–45 ppm (alkyl-C), 45–110 ppm (O/N-alkyl-C). 110–160 ppm (aryl-C), and 160–220 ppm (carboxyl-C) (Knicker and Lüdemann, 1995). The degree of OM decomposition rate was assessed with the O/N-alkyl-C: alkyl-C ratio (Baldock et al., 1997).

2.5.4. Macroaggregate stability

Selected samples of macroaggregate size class (6.3-20 mm) were analyzed for aggregate stability (Fig. 3) using ultrasonication at three different energies (0 J ml^{-1}, 150 J ml^{-1} and 450 J ml^{-1}). The dry macroaggregate size class (6.3-20 mm) fraction was selected due to its highest mass contribution to the total soil throughout space-for-time chronosequence. 15 g of 6.3-20 mm macroaggregate fractions from selected samples were immersed in distilled water to remove light organic material. To destroy aggregates, samples were dispersed ultrasonically in distilled water at selected energy and subsequently wet sieved by immerging fractions on the sieve into four size classes (>630 µm, 200-630 µm, 63-200 µm and <63 µm). All fractions of >630 µm, 200-630 µm, 63-200 µm were dried at 60° and <63 µm fraction was freeze dried.

2.6. Statistical analyses

To compute significant differences (p<0.05) within a sampling depth and throughout the space-for-time chronosequence in Study I, the Kurskal-Wallis test followed by the Dunn's multiple comparison test were conducted using RStudio software (R version 3.4.2) (Dunn, 1964; Kruskal and Wallis, 1952). A two-step cluster analysis followed by discriminant analysis was used in SPSS Statistics software (version 25.0, SPSS Inc., Chicago, USA) to cluster the phases of soil formation and to identify parameters contributing to the soil development. When residuals did not follow normal distribution, a log transformation was applied. In order to understand the correlation between measured parameters, a Pearson's correlation test was conducted.

In Study III, significant differences between aggregate size classes were tested in SPSS Statistics 25.0 by Kurskal-Wallis test and Dunn's post hoc test. To analyze factors influential to aggregate formation, a path analysis was computed in RStudio (version 3.6.1) with the lavaan package (Rosseel, 2020). For the path analysis, a min-max normalization was applied in order to normalize the dataset into the same scale.

2.7. Correlative analysis

Study II focuses on building up a methodology for the correlative analysis of 2D imaging visible light near-infrared spectroscopy (imVNIR) and 3D X-ray computed microtomography (μCT), which can evaluate the interaction between the 3D soil architecture and the biogeochemical processes in intact soil samples.

2.7.1. Sample preparation and imVNIR scanning

After the X-ray computed microtomography (μCT) registration, the intact soil cylinders (height and diameter of 3 cm) were subsequently impregnated with a resin in order to avoid creation of artifacts when slicing the soil sample. Each sample was dehydrated with acetone (graded series from 30-100% (v/v)) and impregnated with polyester resin (PALATAL P 6-01, BÜFA, Germany). After polyester resin application, samples were left for 5 weeks for polymerization and then placed to an oven at 40°C for 48 hours. Cured samples were cut into two slices (thickness of 1 cm) and subsequently polished. Each sample was scanned with a hyperspectral camera (VNIR-1800, Norsk Elektro Optikk Ås, Norway). The distance of the camera lens from the sample surface was set approximately to 30 cm. To achieve balanced illumination on the sample surface, two light sources in front and behind the camera were used with an angle about 45°. After the registration, an image with 53 μm resolution (1800 pixels per line) and spectral range with 196 bands (400-900 nm) was obtained. Each image was normalized by a reflectance standard (reflectance 50%) using following equation (Peddle et al., 2001; Steffens and Buddenbaum, 2013):

$$\rho_{obj} = \frac{L_{obj}}{L_{ref}} \times \rho_{ref}$$

(4)

where L_{obj} is the radiance of the recorded sample, L_{ref} is the radiance of the certified reflectance standard and ρ_{ref} is the reflectance of the certified reflectance standard.

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2.7.2. Hyperspectral image processing

Hyperspectral images were processed in ENVI Classic (Version 5.2, Exelis Visual Information Solutions, Boulder, Colorado, United States) software. The overview of the image processing steps is concluded in Figure 4. In the first image processing step, a principal component analysis was computed in order to display the spatial variability of components on the sample and to remove correlation between neighboring bands (Steffens et al., 2014). Subsequently, principal component images were used to select pure spectral classes as endmembers for linear spectral unmixing (LSU). LSU calculates relative abundance of selected classes in each pixel and generates abundance maps (Fig. 4) (Ravel et al., 2018). For computing the LSU, we selected manually in total six different endmembers: a) pores filled with polyester resin; b) pure polyester resin; c) soil matrix; d) OM dominated regions; e) fresh OM dominated region; f) Fe-oxide dominated regions, and g) iron-manganese concretions. A Jeffries-Matusita distance test was applied to test statistical significance of selected endmembers (Dabboor et al., 2014). Abundance maps from LSU were subsequently used for supervised classification and for the correlative analysis of imVNIR and X-ray μCT. We used a maximum likelihood supervised classification in order to display the area coverage of selected components (Borra et al., 2019). For the supervised classification new class regions were selected and their significant difference were tested as well by Jeffries-Matusita distance test. The accuracy of the maximum likelihood classification was computed in ENVI Classic software with a confusion matrix. For the confusion matrix a random pixel selection was conducted and compared with the results of maximum likelihood classification. To estimate the agreement of the supervised classification, the kappa coefficient was calculated in the software by following equation (L3 Harris Geospatial Solutions, 2020):

$$k = \frac{N \sum_{i=1}^{n} m_{i,i} - \sum_{i=1}^{n}(G_i C_i)}{N^2 - \sum_{i=1}^{n}(G_i C_i)} \tag{5}$$

where i represents the number of classes, N is the total number of classified values compared to the truth values, $m_{i,i}$ represents the amount of values that belong to the truth class i and have also been classified as the class i, C_i represents the total number of predicted values that belong to the class i, and G_i is the total number of truth values, that belong to class i.
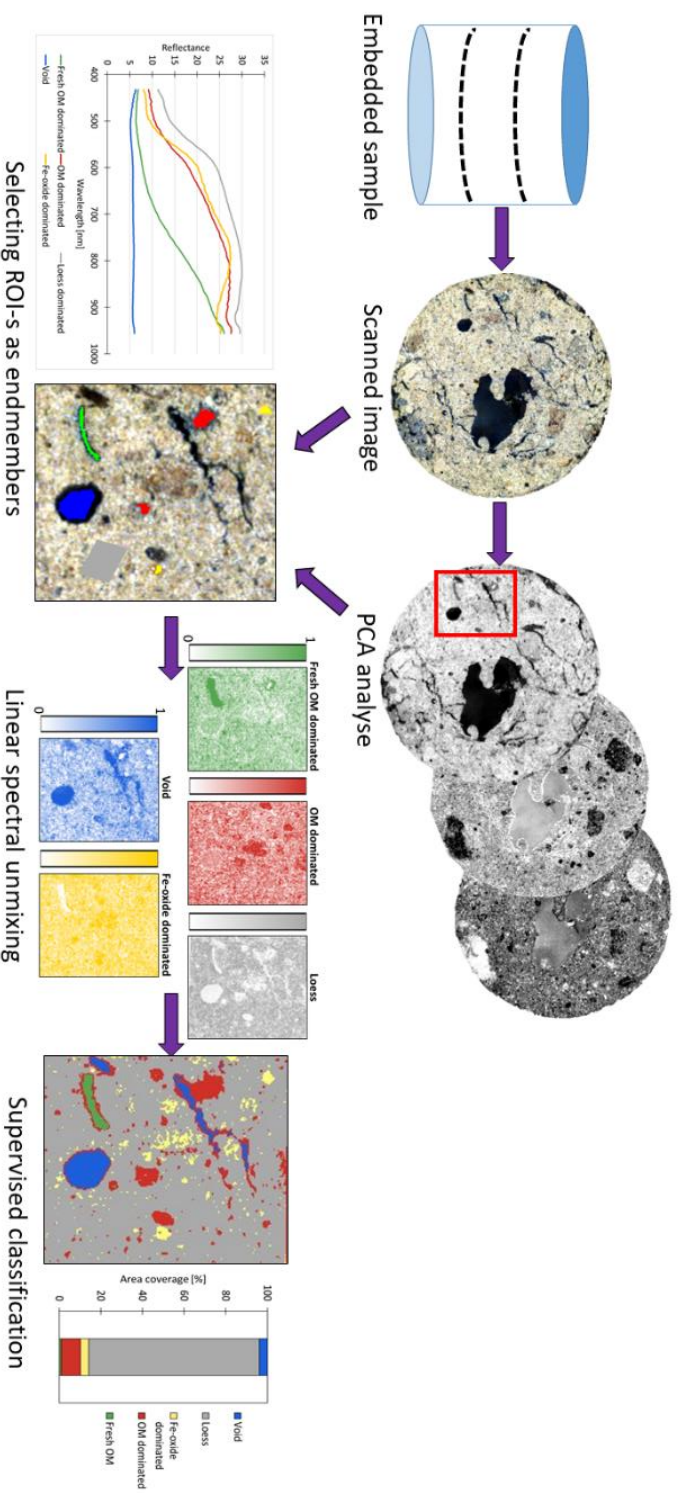
Figure 4. *imVNIR image processing steps. Intact samples (height and diameter of 3 cm) were impregnated with polyester resin and subsequently cut in two slices (thickness of 1 cm). Sample slices were scanned with a hyperspectral camera, where images with 53 µm² pixel resolution were obtained. Following image processing steps, PCA analysis was conducted to concentrate spatial variability of each component on the sample 2D surface. This allowed us to separate pure spectral endmembers (ROI-s) for linear spectral unmixing (LSU). In this current example we selected five classes as endmembers. With LSU, the relative abundance of selected endmember was calculated in each pixel and abundance maps were created. In the abundance maps, the strongest colour intensity shows the highest contribution of selected endmember to the pixel. In the final image processing step, abundance maps were used for maximum likelihood supervised classification, which allowed to calculate the area coverage of each component on 2D sample surface.*

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2.7.3. Correlative image analysis

For the correlative image analysis of 2D imaging visible light near-infrared spectroscopy (imVNIR) and 3D X-ray computed microtomography (µCT) the image registration was applied using the elastix software. This allowed to set the orientation and match images from two methods with different resolution (Klein et al., 2010; Schlüter et al., 2019; Shamonin et al., 2014). For the correlative analysis, from imVNIR we used the relative abundance maps of OM computed with LSU and from X-ray µCT registration we used both the binary images and the Euclidean distance images of pores and biopores. Multichannel images were created in Fiji, which combined the information obtained from the abundance maps and Euclidean distances to pores and biopores. Subsequently, multichannel images allowed us to calculate the relative contribution of OM-rich pixels with distance to pores and biopores. Additionally, we took into account the density shifts around the pores and biopores and normalized the relative contribution of OM-rich pixels according to the density shifts from X-ray µCT.
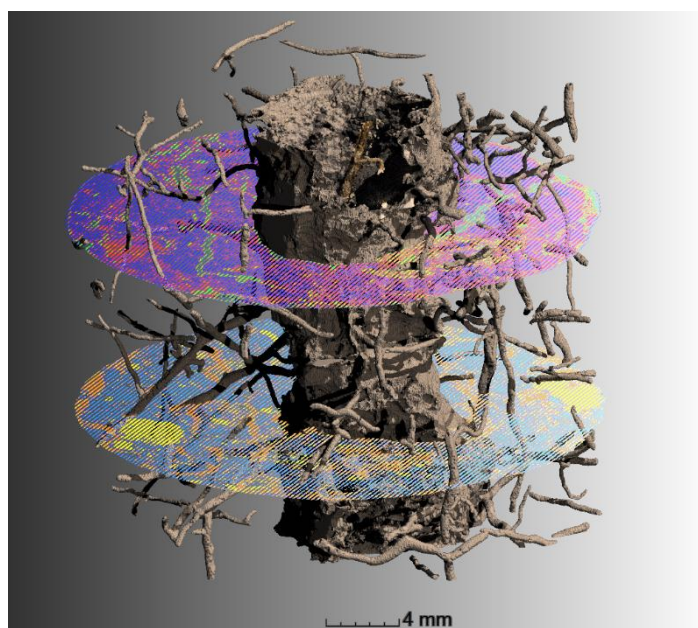
Figure 5. *Correlation of 2D imVNIR and biopores from 3D X-ray µCT.*

3. Results and discussion

3.1. Soil formation factors in reclaimed loess

The similarity in soil texture (Study I), high $CaCO_3$ concentration (Study I, Table 1) and a predominant cementation effect (Study I, Fig. 4) seen in all reclamation sites indicate that the study sites originate from the same parent material. The soil texture in each reclamation site throughout the space-for-time chronosequence was silty clay loam with a slightly alkaline pH (pH_{CaCl2}=7.5) and observed high $CaCO_3$ concentrations (33.1-124.5 mg $CaCO_3 \, g^{-1}$) (Study 1). This confirms that the investigated soil characteristics are adequate to show the soil development stages over the course of the the space-for-time chronosequence.

According to the two-step cluster analysis in Study I, we can differentiate between two soil formation phases on reclamation sites. The analysis separated the pioneering phase (years 0-3) and agricultural management phase (years 6-24) from each other, where the most contributing parameters were macroporosity, SOC and TN content in soil (Study I, Fig. 8). This is in line with applied reclamation management scheme (Fig. 1) and suggests that that the soil formation is driven predominantly by the soil management strategies.

Soil physical parameters in Study I revealed that the pioneering phase (years 0-3) was most typically characterized by a post-mined soil structure with a low macroporosity and high bulk density (Study I, Fig. 1) (King, 1988; Krümmelbein and Raab, 2012; Mukhopadhyay et al., 2016; Shrestha and Lal, 2011; Zhao et al., 2013). Although soil compaction can negatively influence root growth (Nosalewicz and Lipiec, 2014), alfalfa's deep rooting system was not affected by high bulk densities. Observed rooting depth in years 1 and 3 was more than 1 m (Study I) and in the subsoil (40-60 cm) the dense biopore system created by alfalfa growth existed even after 24 years of continued soil management (Lucas et al., 2019). In the pioneering phase, we observed low SOC and TN concentrations (Study I, Table 1), and also low microbial abundance (Study I, Fig. 6). The high proportion of "dead" carbon in the pioneering phase (Study I, Table 1) reflected that an ancient carbon was an important source for microbes at the beginning of soil formation (Rumpel and Kögel-Knabner, 2004). Despite the available carbon (Study I, Table 1) in the early stages of soil formation, the microbial community could not benefit from it (Study I, Fig. 6, Table 1) under low SOC and TN content (Study I, Table 1). During the first three years of soil

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management, the SOC stocks (Study I, Fig. 3) and contribution of recent carbon (Study I, Table 1) increased in the uppermost topsoil. Simultaneous developments in bacterial and fungal gene abundances (Study I, Fig. 6) in the first sampling depth (1-5 cm) reveals that the alfalfa litter decomposition was extensively incorporated to the ecosystem development (Bardgett et al., 2005). The incorporation of plant residues is also evident by the concentration peaks of microbial nitrogen and TN in year 3 (Study I, Table 1). In the nutrient poor subsoil the formation of microbial biomass depends predominantly on fresh OM input by plant roots (Grayston et al., 2004; Paterson et al., 2007). In the pioneering phase, the development in soil chemical and biological parameters decreased rapidly with increasing depth (Study I, Table 1, Fig. 5, Fig. 6), indicating that rhizodeposition and dead roots of alfalfa were either not sufficient enough to contribute to the formation of SOM and microbial community, or that changes in soil parameters that provide evidence of soil-plant interactions were not yet detectable after 3 years of soil management.

Changing the reclamation management from the pioneering phase over to the agricultural management phase resulted in a sharp stratification between topsoil and subsoil. The transition in reclamation management strategies also illustrated the control of soil management over to soil's physicochemical and biological properties. Introducing agricultural management and annual ploughing significantly decreased bulk density and increased macroporosity in the whole ploughing layer (Study I, Fig.1). The developed biopore system from the pioneering phase was directly interrupted when tillage was introduced, which resulted in high macropororisty and low pore connectivity (Lucas et al., 2019). After the organic fertilizer amendment in year 4, SOC stocks and SOC concentration increased in year 6 at both sampling depths (1-5 cm and 16-20 cm) (Study I, Table 1, Fig. 3). In year 6 the SOC stocks (4.0 kg OC m^{-2}) in ploughing layer (0-30 cm) were comparable to the mature soil (4.6 kg OC m^{-2}) in the region (Study I, Fig. 3). Although, changes in reclamation management supported improvements in soil chemical and physical parameters (Study I, Table 1, Fig. 1, Fig. 3), it affected microbial abundance negatively (Study I, Fig. 6). In year 6 at the first sampling depth (1-5 cm), we observed a shift in the composition of microbial community with having lower fungal and bacterial abundances compared to year 3 (Study I). Changes in microbial community reflect that plowing together with introducing new source of SOM largely defines the composition of microbial community (Roy et al., 2017; Vuko et al., 2020). Although organic fertilizer significantly improved soil OC content in year 6 in the topsoil, the fertilization only showed a short term

effect. Continuous plowing can increase mineralization rates over time, which in turn can result in substantial OC losses in soil (Böhme et al., 2005; Delschen, 1999; Rochette and Gregorich, 1998). Higher macroporosity observed in the agricultural management phase (Lucas et al., 2019) and the significant decrease in SOC stocks in years 12 and 24 can be related to the higher mineralization rates, but C_{mic} values (Study I, Table 1) did not follow the assumption. Observed dynamics of SOC and microbial abundances in Study I suggest that reclaimed soil contained large amounts of labile OM which was easily used up by microorganisms. It is also supported by the radiocarbon ^{14}C activity (Study I, Table 1), where after the organic fertilizer amendment the proportion of "dead" carbon increased again in year 12. This demonstrates that crop and plant root residues as the sole source for fresh OM in following years were not able to build up stable SOC pool, and thus compensate for OM mineralization. This in turn suggests that the soil formation was predominantly mediated by OM amendments. This is supported by the discriminant analysis and general linear model (Study I, Fig. 7, Fig. 8, Fig. 9), where the most contributing parameters on soil formation phases were SOC, TN, macroporosity, bulk density. The general linear model revealed that these physicochemical properties were significantly related to the reclamation age and soil management (Study I, Fig. 8). Defined soil formation factors demonstrate that soil management practices play a dominant role in defining the success of soil development in reclaimed soils and repeated C input over a longer time will have a beneficial effect on building up stable soil OC dynamics and microbial composition.

3.2. Soil structural stability is driven by calcareous loess

$CaCO_3$ concentration in the space-for-time chronosequence varied between 30-120 mg $CaCO_3$ g^{-1} (Study I, Table 1), which was consistent with the concentrations observed from loess deposits (Laszlo et al., 1995; Smalley et al., 2011). Due to the presence of $CaCO_3$, loess has a tendency to form soft rock-like structures under dry environmental conditions (Li, 2016; Yates et al., 2017), which were visible in each sample from our study sites regardless of the sampling depth, stage of soil formation or applied soil management strategy. This is evident by high $CaCO_3$ concentrations that were observed throughout the space-for-time chronosequence (Study I, Table 1) and by the macroaggregate size distribution (Study I, Fig. 4), in which no correlation between the mass proportion of aggregates and reclamation age was observed. The cementation predominantly formed and stabilized macroaggregates with an aggregate size of 6.3-20 mm, demonstrating that the

dominance of cementation greatly affected the dry macroaggregate size class distribution (Study I, Fig. 4), despite the developments in soil chemical and physical properties.

Soil stability analysis in Study III revealed that the stability of aggregates and soil architecture was driven by the inherent properties of loess. After submerging dry macroaggregates (6.3-20 mm) in water, the aggregate structure was completely lost and the size distribution (Study III, Fig. S2) was similar to the primary particle size distribution of reclaimed soils (Study III, Table 1). Even when no ultrasonication energy was applied, the <63 μm had the highest contribution to the total soil mass. Although the cementing effect increases the stability of loess in dry conditions, it also defines the plasticity and wettability of the soil (Meng and Li, 2019). Loess tends to lose these soft-rock like structures when the moisture content increases (Yates et al., 2017), which was also evident in our Study III. The loss of cementation bonds between particles resulted in a substantial degradation of the aggregate structure (Study III, Fig. S2). This shows severe water instability and low plasticity of soil, which indicates that soils in our study sites are prone to soil erosion (Quijano et al., 2019). The aggregate (in)stability and dominance of cementation in Study III demonstrates that even when soils have developed a soil structure (Lucas et al., 2019), there is no development in aggregate hierarchy. In non-calcareous silty or clayey soils the aggregate hierarchy is commonly observed, but there the aggregation is predominately mediated by different fractions of OM (John et al., 2005; Puget et al., 2000; Šimansky, 2012). The stability analyses in Study III underscore that the soil structural stability was directly associated with the inherent properties of loess and $CaCO_3$ as a cementing agent, despite the development in SOM content and ongoing soil formation along the space-for-time chronosequence.

3.3. Aggregate formation mechanisms on calcareous loess

Study I showed that the cementation by $CaCO_3$ dominated over soil structural formation under dry conditions despite the soil development. The aggregate size class fractionation in Study III revealed and differentiated biotic and abiotic mechanisms of aggregate formation on calcareous loess. In the Study III, we found that the input of fresh OM influenced the formation of large macroaggregates. Higher SOC enrichment (Study III, Table 3) was visible already in year 3 in the large macroaggregates at the first sampling depth (1-5 cm), when alfalfa litter decomposition contributed to the increase in SOC content (Study I). In year 4, when the organic fertilizer was applied, we determined higher SOC

and TN concentrations (Study III, Fig. 4a and 4b), the highest enrichment (Study III, Table 3) and higher relative contribution to the macroaggregates in year 6 compared to the pioneering phase (Study III, Fig. 5a and 5b). Findings in Study III confirm that the input of fresh OM promotes the aggregate formation in young unstructured soils (Bucka et al., 2019). This underlines the importance of OM for being a critical material for building up a soil macroaggregate structure.

When SOM accumulation was predominant in macroaggregates, the highest $CaCO_3$ enrichment (Study III, Table 3) and contribution to total $CaCO_3$ (Study III, Fig. 5c) were observed in the S + C-sized fraction (<63 µm). The predominance of $CaCO_3$ in microaggregates (Study III, Table 3) is most likely inherited from the calcareous loess. Loess is the major component in the reclamation mixture used as a new parent material in the reclamation process, which can be characterized by high silt and clay content (Study III, Table 1) (Dumbeck, 1996, 1992; Lehmkuhl et al., 2016; Pecsi, 1990). Along the space-for-time chronosequence we detected high variability in $CaCO_3$ content (Study III, Table 2, Fig. 4c), which is most likely related to the different strata of loess geological deposits in the region (Schirmer, 2016). When we excluded differences in the $CaCO_3$ concentration, it showed that the contribution of carbonates to aggregate formation did not change despite the differences in geological deposits (Study III, Table 3). This is also evident by the strong relationship between $CaCO_3$ content and reclamation age in the path analyses (Study III, Fig. 6). We observed carbonates in each aggregate size class (Study III, Fig. 4c), but the S + C-sized fraction differentiated from other aggregate size classes by being predominantly controlled by $CaCO_3$. It is supported by the high contribution of S + C-sized fraction to total $CaCO_3$ content (Study III, Fig. 5c) and also by similar $CaCO_3$ enrichment in the finest fraction in each reclamation site (Study III, Table 3). When soil has a low SOC concentration, the domination of calcareous loess becomes more evident (Boix-Fayos et al., 2001). In the S + C-sized fraction, we did not observe any SOC enrichment (Study III, Table 3) or any correlation between $CaCO_3$ and SOC (Study III, Fig. 6). This indicates that microaggregate formation in calcareous loess is not mediated by organo-mineral interactions between SOM and Ca^{2+}, as we would have expected in calcium rich soils (Fernández-Ugalde et al., 2014; Rowley et al., 2018). The data in Study III concludes that initial aggregate formation in calcareous loess has two mechanisms, where we can differentiate OM-induced macroaggregate formation, and $CaCO_3$-mediated microaggregate formation.

3.4. Does the cementing effect stabilize SOM?

The analysis of soil aggregate size class fractionation together with their contribution to total soil OC content revealed SOM dynamics present in calcareous loess. In Study I, we observed that macroaggregates of mature soil contributed equally to total soil SOC content (Study I, Fig. 5), whereas in reclaimed soils finer aggregates contributed significantly less to total SOC content even after 24 years of soil formation processes (Study I, Fig. 5). This indicates that there was not enough input of OM in the reclaimed soil, which could build up SOC content in finer macroaggregate size classes. The aggregate fractionation analysis in Study III provided clearer understanding on SOC dynamics. The solid state ^{13}C NMR spectra of aggregates showed that SOM chemical quality was similar despite the increasing reclamation age and sampling depth (Study III, Fig. S1, Table S2). The OM amendment significantly increased SOC concentration (Study I, Table 1, Study III, Fig. 4a) and greater enrichment in macroaggregates (Study III, Table 3) in year 6. This is in line with the spectra of large macroaggregate (>630 µm) size class in year 6 (Study III, Fig. S1), where we observed higher proportion of labile and less decomposed SOM (A/OA=0.35). Our data shows that these macroaggregates had a short lifetime due to reduced input of fresh OM in years 12 and 24 (Buyanovsky et al., 1994; Pronk et al., 2012; Puget et al., 2000; Tisdall and Oades, 1982). The last organic fertilizer amendment was conducted in year 7, and in following years root and plant litter were the sole sources for fresh OM. It becomes evident that the loss of SOC in bulk soil observed in Study I (Study I, Table 1, Fig. 3) was associated with the loss of SOC in macroaggregates in older reclamation sites (years 12 and 24) (Study III, Fig. 4a, Fig. 5a, Table 3). Our finding underscores that the cementing effect by $CaCO_3$ contributed to microaggregation, but it did not provide physical protection of SOM in aggregates. This is also evident from the path analysis, in which no correlation between carbonates and SOC was observed (Study III, Fig. 6). In calcareous soils, the SOM mineralization can be promoted by continuous cultivation. Higher CO_2 rates in calcareous soil from microbial respiration and OM decomposition can increase the dissolution of finely distributed carbonates, resulting in the loss of physical protection, making OM more vulnerable to microbial decomposition (Ye et al., 2020; Zamanian et al., 2016). Study III underscores that the interaction between $CaCO_3$, SOM dynamics and aggregate formation is a complex process, and careful soil management has a decisive role in preserving SOC in calcareous loess soils.

3.5. Correlative image analysis reveals SOM distribution in structured soils

The correlative analysis of imVNIR and X-ray µCT provided new opportunities to investigate the heterogeneous distribution of SOM in structured soils. An applied sample preparation that impregnated undisturbed soil samples with polyester resin allowed to sustain the initial macropore system, perfecting conditions for segmenting structural and compositional features produced by imVNIR image processing (Study II, Fig. 3) (Gutiérrez Castorena et al., 2016; Jangorzo et al., 2014; Mueller et al., 2017; Rasa et al., 2012; Schlüter et al., 2019). Polyester resin prevented the creation of several artifacts when slicing the samples, e.g. smearing the macropore system and creating shadows by surface topography.

Polyester impregnation maintained perfect conditions for segmenting voids from the loess, which allowed us to distinguish soil structural characteristics induced by soil management. In year 3, the soil can be described by dense soil matrix and spherical aggregates, i.e. "rolled aggregates" formed on the conveyor belt (Study II, Fig. 3). The spherical aggregates are visible in the areas 5 E-F, 1-2 L, 3M in no-tilled topsoil (Study II, Fig. 3). Those aggregates were most probably derived from the old developed topsoil, because the reclamation mixture contains small amounts of Luvisol (Luvisol/ loess ratio 1:10) (Study I). Soil's inherent structure in year 3 is in line with high bulk density and low marcoporosity from Study I (Study I, Fig. 1). Tillage influences SOM distribution and homogenization (Kay and Vandenbygaart, 2002; Yang and Wander, 1999), which was visible in tilled topsoil sample. Tillage and compost application in year 6 resulted in an increase of SOM spatial distribution per area and changes in the aggregate shapes. In tilled topsoil the OM induced aggregates are visible in areas 11 B-C, 12 B, 11-12 K, 10 L-M and 11 L-M (Study II, Fig. 3). Although the total area coverage of SOM in year 6 was rather similar to the year 3, SOC concentration in those soils were significantly different (Study I, Table 1). Observed changes in the spatial arrangements of solid (loess and SOM) and voids, and fluctuations of soil chemical properties (Study I, Table 1) illustrates that the soil management significantly affects the soil structural composition (Study II, Fig. 3).

Combination of imVNIR and X-ray µCT is a novel tool that greatly increases understanding of soil structure and chemical composition in larger sample volume, i.e. OM distribution to the distance to pores and biopores (Study II, Fig. 5). There are several imaging combinatory methods available, however, mostly high-resolution analysis is limited to microscale and

thus focuses only on understanding certain microenvironmental condition (Baveye et al., 2018; Schlüter et al., 2019). Study II provided a novel tool to describe biogeochemical interactions in larger sample volume with taking into account whole complexity in soil structure. When investigating the relationship between SOM and macroporosity, we found that OM showed greater accumulation with increasing distance to macropores (Study II, Fig. 6). In greater distance to pores, SOM was most probably physically protected against mineralization by microorganisms (Dungait et al., 2012). Besides the general characterization of the spatial arrangements in soil, those two imaging methods gave corroborating information on the same material, which allowed to expand knowledge of the relationship between SOM accumulation and biopore system. When taking into account the density shifts from X-ray μCT, it allowed to normalize the relative contribution of SOM to the pore system. Besides increasing the accuracy on observing the contribution of OM in soil, the application also expanded information level on structural features. The correlative analysis revealed that after the normalization by the density shifts, we found in no-tilled topsoil and subsoil samples greater OM contribution next to biopores (Study II, Fig. 6), reflecting that the quantity of SOM per gram soil was greater close to the pore walls. Correlative imaging of imVNIR and X-ray μCT allowed us to work with larger sample volumes and to characterize the chemical and structural features that are related to the different stages of soil formation. Working with samples with greater sample volume can expand our understanding of microscale processes and the association of SOM mineralization rates with soil's inherent architecture.

4. Conclusions and outlook

The dissertation focused on the initial stage of soil structural formation, in which soil organic carbon content is low and the main soil physicochemical properties are controlled by calcareous loess. In the post open-cast mining area of Garzweiler (Germany), we investigated young reclaimed loess soils by using a space-for-time chronosequence approach in agriculturally reclaimed sites and selected fields that displayed different stages of soil formation (years 0, 1, 3, 6, 12 and 24 after the first seeding of alfalfa). We investigated young reclaimed soils to examine the relationship between soil parent material and the soil structure formation, pairing this with analyses of soil physical and chemical properties.

In Study I we characterized soil formation along the space-for-time chronosequence. We found that loess parent material and the soil management strategies were the most dominant factors that controlled the state of soil formation. Loess material can be characterized by its ability to form soft-rock like structures, which was visible in each reclamation site despite the improvements in soil OC content and structure. Strong cementation by $CaCO_3$ led to the domination of dry macroaggregates (size class 6.3-20 mm) and no trend in macroaggregate size distribution could be detected. During the first three years of the pioneering phase, the alfalfa cultivation and litter decomposition were the greatest factors that contributed to SOC development and microbial biomass in the uppermost topsoil. When agricultural management was applied with organic fertilizer amendment, the physical and chemical properties of the soil improved significantly in year 6. Despite this, organic fertilizer only showed a short term effect on improving soil OC content. In years 12 and 24, we detected substantial loss of SOC, which was related to the usage of mineral fertilizers after year 7 and small OM input by crop residues. This indicates that soil management predominantly defines the state of soil formation and calcareous loess controls the structural stability.

Soils are complex and heterogeneous mixtures of solids and voids. In order to achieve complete understanding of soil's inherent architecture and related biogeochemical processes, a novel correlative analysis of 2D imaging visible light near-infrared spectroscopy and 3D X-ray computed microtomography (μCT) on intact soil cores was developed in Study II. Method combination allowed for the observation of SOM spatial distribution as a function of distance to pores and biopores. We observed higher content of

SOM, as well as changes in aggregate shape when reclamation management changed from pioneering phase over to agricultural management phase. A correlative analysis of selected imaging methods expanded knowledge of the relationship between SOM accumulation and the pore system. After normalizing SOM relative contribution by density shifts, it was revealed that SOM accumulation was predominantly close to biopore walls in the no-tilled topsoil and subsoil samples. In year 6, when tillage and organic fertilizer was introduced to the soil, we found a significant influence on soil structural heterogeneity and spatial distribution of SOM. In particular, the relative abundance of SOM increased with a higher distance to macropores. The developed method in Study II confirmed that correlative analysis of 2D imVNIR and 3D μCT provides new opportunities to describe biogeochemical interactions together with the complexity of soil's inherent structure.

An aggregate size class fractionation in Study III provided more detailed knowledge on soil aggregate formation in calcareous soil. The study revealed that aggregate formation in calcareous loess had two mechanisms, in which macroaggregate formation was induced by the input of OM. When organic fertilizer was applied in year 4, we observed in year 6 SOC enrichment in the large macroaggregate (>630 μm) size class. It became evident that these macroaggregates were also associated with the dynamics of bulk SOC, while the loss of OM in bulk soil in following years (Study I) were connected with the loss of SOC in large macroaggregates (Study III). Microaggregate formation was dominated by the $CaCO_3$ inherited from the loess parent material. Despite ongoing soil formation, the small microaggregate size class had the highest $CaCO_3$ enrichment and contribution to total $CaCO_3$ content in reclaimed sites. At the same time, we did not observe any correlation between $CaCO_3$ and SOC, suggesting that SOM did not contribute to microaggregate formation through Ca-mediated organo-mineral interactions. The stability analysis revealed that finely distributed carbonates dissolved easily in water, which resulted in significant degradation of soil aggregate structure. This showed that the structural stability of reclaimed soil was defined by the inherent properties of calcareous loess even after 24 years of soil formation.

The dissertation identified that the soil management and calcareous parent material affected the formation of soil physicochemical properties in reclaimed sites. All conducted studies underlined that the state of soil formation depended on soil management strategies, however, the properties of calcareous loess plays a dominant role in the soil's structural stability. With Study II we successfully developed a combinatory method, which allowed

us to analyze chemical properties without losing the soil's inherent structure, as well as work with intact samples with greater volume.

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Evelin Pihlap

Freising, 23 May 2020

7. Appendix

Curriculum Vitae

Personal Information:

Name	Evelin Pihlap
Born	07.10.1990 in Tartu, Estonia
Nationality	Estonian

Education:

2016-2020	Doctoral candidate in Soil Science, Technical University of Munich, Germany
2016-2020	Doctoral candidate in Environmental Technology, University of Tartu (joint degree agreement), Estonia
2013-2016	MSc. in Environmental Technology, University of Tartu, Estonia
2014-2015	Environmental Management, Christian-Albrechts University of Kiel, Germany
2010- 2013	BSc. in Environmental Technology, University of Tartu, Estonia

Awards:

2019	Scholarship holder of the Estonian Students Fund in USA
2017	Soil Pitch award at the conference of German Soil Science Society
2015	Scholarship holder of the Estonian Students Fund in USA
2014-2015	Kiel City scholarship holder
2014	Mare Taagepera Scholarship holder

Participation at international conferences:

2019	International Symposium on Soil Organic Matter, Adelaide, Australia
2018 & 2019	European Geosciences Union (EGU) General Assemblies, Vienna, Austria
2018	International Symposium: Soil organic matter management in agriculture- Assessing the potential of 4per1000 initiative, Braunschweig, Germany
2017 & 2019	Annual conference of the German Soil Science Society (DBG)

International experiences and courses:

2019	Visiting researcher at GHERG, Federation University Australia, Victoria, Australia
2019	Statistical modelling and regressions using R for soil scientists, University Kassel, Germany
2018	Argumentation in Scientific Writing, TU München, Germany
2018	International field workshop WRB soil classification in Romania
2017	International field workshop WRB soil classification in Latvia and Estonia
2017	Argumentation & Negotiation Skills, TU München

List of Scientific Contributions

Peer-reviewed publications (first-authored):

Pihlap, E., Steffens, M., Kögel-Knabner, I. (2021) Initial soil aggregate formation and stabilisation in soils developed from calcareous loess. *Geoderma* 385, 114854. 10.1016/j.geoderma.2020.114854

Lucas, M.*, **Pihlap, E.*,** Steffens, M., Vetterlein, D., Kögel-Knabner, I. (2020). Combination of Imaging Infrared Spectroscopy and X-ray Computed Microtomography for the Investigation of Bio- and Physicochemical Processes in Structured Soils, *Frontiers in Environmental Science*, 8, 1–12. 10.3389/fenvs.2020.00042

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Pihlap, E., Vuko, M., Lucas, M., Steffens, M., Schloter, M., Vetterlein, D., Endenich, M. Kögel-Knabner, I. (2019). Initial soil formation in an agriculturally reclaimed open-cast mining area - the role of management and loess parent material. *Soil and Tillage Research*, *191*, 224–237. 10.1016/j.still.2019.03.023

Oral presentations:

Pihlap, E., Lucas, M., Steffens, M., Vetterlein, D., Kögel-Knabner, I. (2019) 2D imaging spectroscopy and 3D X-ray CT high spatial resolution analysis ─ method combination for investigating potential interplay of SOM and soil structure development in intact soil samples. In 7th Internation Symposium on Soil Organic Matter 06.-11.10.2019, Adelaide, South Australia.

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Pihlap, E., Steffens, M. and Kögel-Knabner, I. (2017) Initial development of soil structure and soil organic matter in an agriculturally managed chronosequence on recultivated loess. In Jahrestagung der Deutschen Bodenkundlichen Gesellschaft (DBG) 02.-07.09.2017, Göttingen, Germany.

Poster presentations:

Pihlap, E., Steffens, M. and Kögel-Knabner, I. (2018) Development of SOM and aggregation in an agriculturally managed re-cultivated loess. In European Geosciences Union (EGU) General Assembly 2018, 08.-13.04.2018, Vienna, Austria.

Study I

Pihlap, E., Vuko, M., Lucas, M., Steffens, M., Schloter, M., Vetterlein, D., Endenich, M. Kögel-Knabner, I. (2019). Initial soil formation in an agriculturally reclaimed open-cast mining area - the role of management and loess parent material. *Soil and Tillage Research*, *191*, 224–237. 10.1016/j.still.2019.03.023
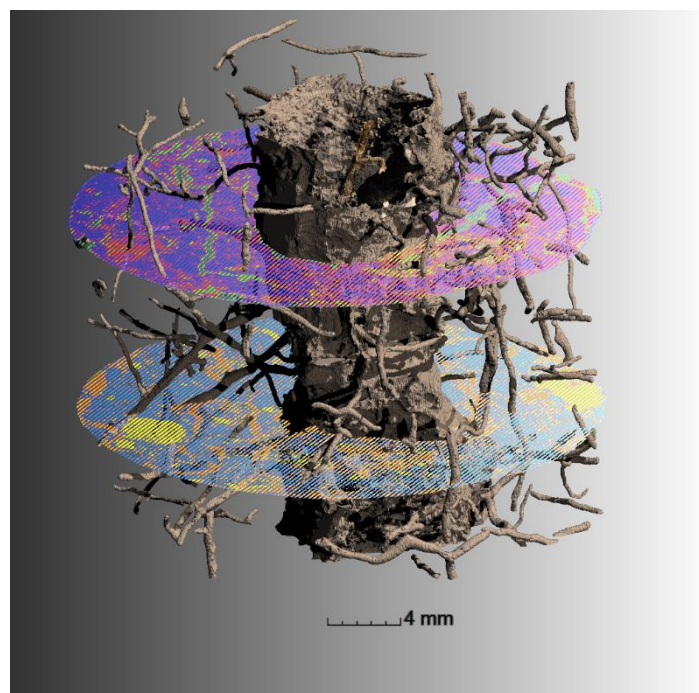
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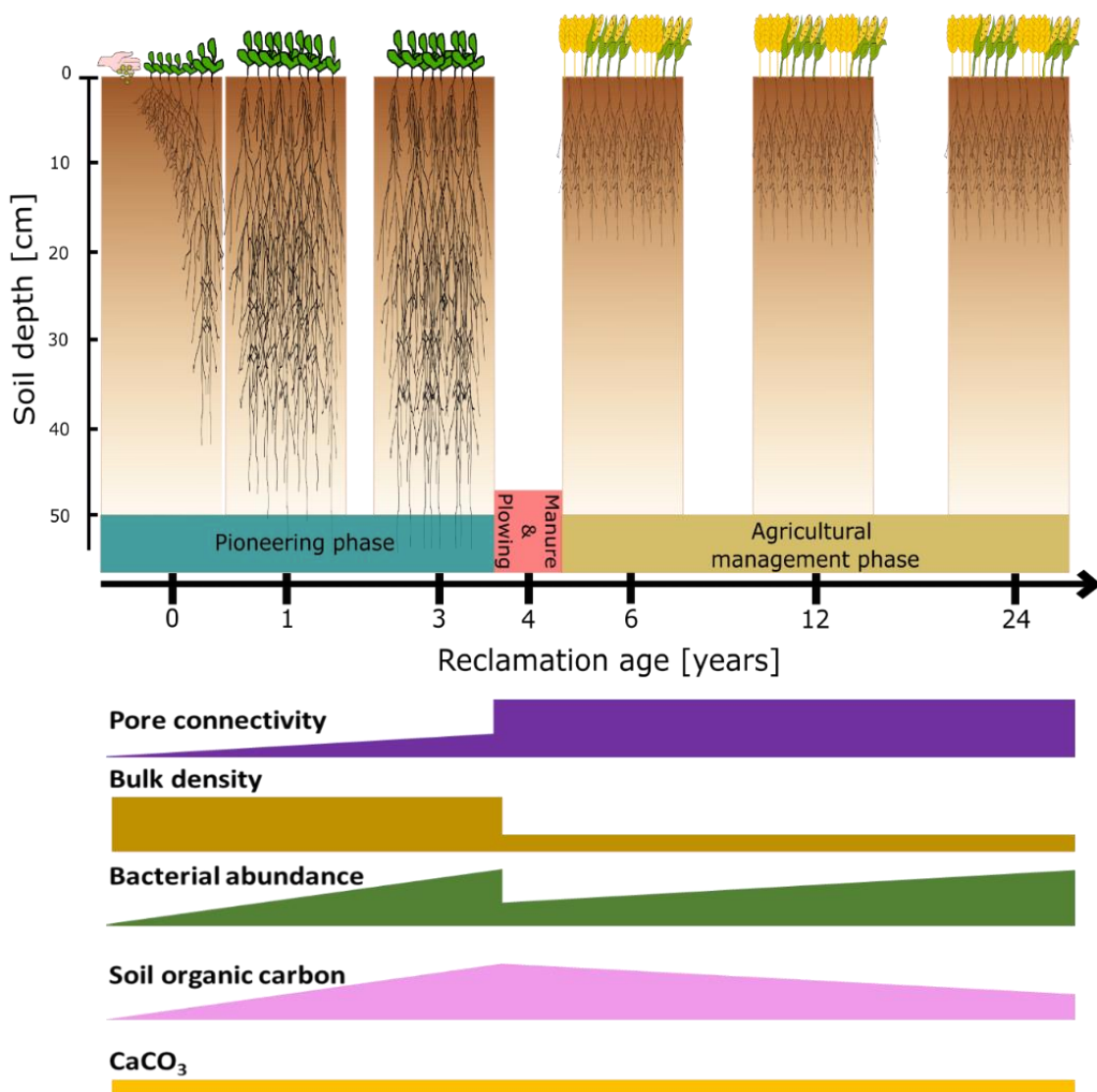
Study II

Lucas, M.*, **Pihlap, E.*,** Steffens, M., Vetterlein, D., Kögel-Knabner, I. (2020). Combination of Imaging Infrared Spectroscopy and X-ray Computed Microtomography for the Investigation of Bio- and Physicochemical Processes in Structured Soils, *Frontiers in Environmental Science*, 8, 1–12. 10.3389/fenvs.2020.00042

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Study III

Pihlap, E., Steffens, M., Kögel-Knabner, I. (2021) Initial soil aggregate formation and stabilisation in soils developed from calcareous loess. *Geoderma* 385, 114854. 10.1016/j.geoderma.2020.114854