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Soil structure formation initiated by biochemical processing of organic matter

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I SUMMARY

The structure of a soil is formed by the three-dimensional arrangement of the particles and the surrounding voids. The architecture and stability of this arrangement defines a soil and is the basis for many soil processes and ecosystem services provided by soils. Soil structure formation requires a cohesion of the present soil particles. Within this process, the interaction of mineral particles and soil organic matter (SOM) is considered a major stabilizing factor.

The structural properties of a soil can be investigated through various approaches relying either on undisturbed or fractionated soil samples. A common tool in terms of fractionation is the isolation and analysis of soil aggregates, which are composite structures of various, agglomerated soil particles that are isolated as single entities from a soil. Although many studies have been conducted to investigate the properties of developed soil structure, there were only few attempts trying to elucidate the entire process of soil structure formation starting with individual, unconnected particles.

The main goal of this publication-based thesis was to decipher the processes of initial soil structure formation with regard to the biochemical processing of organic matter. The influence of soil texture, organic matter residues, and the developed microbial community were disentangled and systematically studied. In particular, the self-organized aspect of the structure formation was considered, which can happen without physical interference.

In order to achieve this, an experimental approach based on an incubation system with artificial soil microcosms was developed. The artificial soils were designed as mineral mixtures with the same mineral composition but differing texture (clay loam, loam, sandy loam), resulting in a textural range from clay-rich to sand-rich soils. Organic matter was added as individual residues (large and small plant litter (POM), dissolved organic matter (DOM), and bacterial necromass). The experimental design included an incubation of the microcosms at constant water tension to minimize mechanical and physical influences like stirring or changing water regime.

The incubation was suitable to support a stable water content and rapid development of microbial activity (CO_2-C release) in the artificial soils. The texture determined the bulk density, pore space, and water content in the soils. This shaped the abiotic infrastructure for microbial dwelling by determining the habitable pore space and water access. A higher sand content was accompanied by larger pores and limited water storage, which meant more challenging conditions for microbial life. The litter led to a higher water content in all textures, which was ascribed to a sponge-like behavior of the litter pieces. Dissolved organic matter and bacterial necromass induced a higher water content in the sand-rich texture, which was related to a higher microbial production of extracellular polymeric substances (EPS) for improved water storage.

Abiotic particle connections such as iron oxide containing coatings on quartz grains were established through initial particle arrangement during the preparation and incubation of the artificial soils. Although those mineral-mineral connections accounted for the majority of the mineral surface interactions, they did not lead to the formation of large water-stable aggregates. However, all tested organic residues were able to induce the formation of water-stable aggregates. It was shown that one month of incubation at constant water tension in presence of organic matter was sufficient to find approximately 90% of the particle mass bound within aggregates. This was remarkable, because both a longer timeframe and mechanical stress were previously considered a prerequisite for aggregate formation. Here, the presence of organic residues alone was sufficient to induce major soil structure formation, initiated by the biochemical processing of the added organic matter.

The various organic residues could induce microbial growth to a similar extent but led to a different microbial community composition. The litter supported both fungal and bacterial growth, while dissolved organic matter and bacterial necromass favored bacterial growth. Despite the differing community compositions, the microbial effect on soil structure formation was summarized to the formation of sticky organic patches that lead to water-resistant particle connections. Those patches covered less than one sixth of the mineral surfaces but were still sufficient to stabilize the majority of the particle mass within aggregates. This means that the OM-induced aggregates are loosely connected structures, bound together by some distinct patches and clusters of OM acting as gluing joints. This scaffold resists immersion in water, and thus may form large water-stable aggregates, but exhibits no stability towards mechanical pressure.

The bacterial growth led to small and large aggregates in the clay-rich soils and an enhanced macroaggregate formation in the sand-rich soils, because of the embedding of large sand grains within the formed aggregates. In the sand-rich soils, the limited water availability induced an increased pore filling by microbial products for water storage. The microbial products interact with mineral particles, which leads to the connection of the available sand grains as well. The litter induced a predominant formation of large macroaggregates irrespective of the soil texture and litter size. Here, the growth of fungal hyphae provided an additional, texture-independent stabilization of large aggregates by binding larger structural entities together.

The addition of dissolved organic matter was able to induce macroaggregates in all textures, showing that water-stable aggregate formation was possible without the presence of particulate organic matter nuclei. This gave insights into soil structure formation processes awaiting deeper soil regions.

The use of the developed incubation setup was applied to assess soil structural development in artificial soil mixtures for mine-site rehabilitation in Australia. The rehabilitation mixtures included both common and innovative amendments like paper mulch, fly ash, brown coal, and plant litter, which were mixed into the overburden substrate

in order to deliver organic carbon. The incubation led to a rapid development of microbial activity and water-stable aggregates, which was both enhanced in presence of litter. This showed that also innovative soil mixtures support a rapid soil structure formation if microbial growth was fostered. However, litter as sole amendment of the overburden indicated a rapid consumption of the present organic matter, implying an early organic matter depletion in the context of the studied (semi-)arid environment. It became apparent that a diverse mixture of both rapidly decomposable and more persistent substrates fosters both a sustainable microbial development and structure formation.

This thesis provides a conceptual understanding of initial soil structure formation mediated by biochemical processing of organic matter. It was shown that various organic residues can rapidly initiate the formation of soil structure, without requiring physical influences such as stirring or changing water regimes. This self-organized structure formation relies on small and distinct organic junctions that are water-stable but exhibit no mechanical stability. Those connections can hold the particles in place for further stabilization processes and their water-resistance promises soil structural resistance against water erosion. However, additional stabilization mechanisms are responsible and needed for further soil structural resistance against mechanical stressors (e.g., soil tillage).

II ZUSAMMENFASSUNG

Die Struktur eines Bodens entsteht durch die dreidimensionale Anordnung der Bodenpartikel und den sie umgebenden Hohlräumen. Die Architektur und Stabilität dieser Anordnung definiert den Boden und bildet die Grundlage für viele Bodenprozesse sowie Ökosystemdienstleistungen, welche die Böden liefern. Die Bildung der Bodenstruktur setzt eine Verknüpfung der vorhandenen Bodenpartikel voraus. Dabei ist das Zusammenspiel von mineralischen Bodenpartikeln und organischer Bodensubstanz ein wichtiger Faktor.

Um Bodenstruktureigenschaften zu analysieren, kann man verschiedene Methoden verwenden, welche entweder auf der Analyse von ungestörtem Boden oder aufgetrennten Bodenbestandteile basieren. Wenn der Boden aufgetrennt wird, werden oft Bodenaggregate isoliert und analysiert. Bodenaggregate sind zusammengesetzte Strukturen aus verschiedenen verknüpften Bodenpartikeln, welche als einzelne Einheiten aus dem Boden isoliert werden können.

Obwohl sich viele Studien mit den Eigenschaften von entwickelter Bodenstruktur befassen, gab es bisher nur wenige Versuche, den ganzen Prozess der Bodenstrukturbildung, ausgehend von einzelnen, unverbunden Partikeln, zu verstehen.

Der Fokus dieser publikationsbasierten Dissertation lag auf der Untersuchung von initialer Bodenbildung im Hinblick auf biochemische Umwandlungsprozesse von organischer Substanz. Es galt, die Rolle der Bodentextur, verschiedenen organischen Resten, sowie der sich entwickelnden Mikroben zu entschlüsseln und systematisch zu erforschen. Besonderes Augenmerk lag auf dem selbstorganisierten Aspekt der Bodenstrukturentwicklung, welcher ohne physikalische Einflussnahme vonstattengehen kann.

Im Rahmen dieser Arbeit wurde ein experimenteller Ansatz entwickelt, welcher auf einem Inkubationssystem mit künstlichen Böden in Mikrokosmen basierte. Die künstlichen Böden bestanden aus Mineralmischungen mit gleicher Mineralogie aber unterschiedlicher Textur (toniger Lehm, Lehm und sandiger Lehm), sodass tonreiche und sandreiche Böden untersucht werden konnten. Die organische Substanz (große und kleine Pflanzenstreu, gelöste organische Substanz und bakterielle Nekromasse) wurde den einzelnen Mikrokosmen in Form von separierten organischen Resten zugegeben. Der Versuchsaufbau beinhaltete eine Inkubation der Mikrokosmen unter konstanter Wasserspannung, wodurch mechanische und physikalische Einflussfaktoren wie Rühren oder veränderte Befeuchtungsregimes minimiert wurden.

Die Inkubation führte einer raschen Entwicklung eines stabilen Wassergehaltes sowie mikrobieller Aktivität in den Böden. Die Bodentextur bestimmte die Lagerungsdichte, den Porenraum und den Wassergehalt der Böden. Dies legte die abiotischen Rahmenbedingungen für das Mikrobenwachstum fest, indem der besiedelbare Porenraum

und die verfügbare Wassermenge definiert wurden. Ein höherer Sandgehalt führte zu größeren Poren und eingeschränkter Wasserverfügbarkeit, was schwierigere Bedingungen für die Mikroben bedeutete. Das Vorhandensein von Pflanzenstreu erhöhte den Wassergehalt in allen Bodentexturen, was einem schwammartigen Verhalten der organischen Substanz zugeschrieben wurde. Gelöste organische Substanz und bakterielle Nekromasse führte zu höheren Wassergehalten in sandreichen Böden, was mit einer erhöhten mikrobiellen Produktion von extrazellulären polymeren Substanzen (EPS) für eine bessere Wasserspeicherung in Verbindung gebracht wurde.

Die Herstellung und Inkubation der mineralischen Mischungen ohne organische Substanz führte zu abiotischen Partikelverbindungen wie zum Beispiel eisenoxidhaltigen Überzügen auf Sandkörnern. Obwohl diese Verbindungen für den Großteil der mineralischen Oberflächeninteraktionen verantwortlich waren, führten sie jedoch nicht zur Bildung von großen, wasserstabilen Aggregaten.

Wasserstabile Aggregate entstanden lediglich in Vorhandensein von organischer Substanz, wobei bis zu 90% der Partikelmasse in Form von Makroaggregaten gebunden sein konnte. Diese umfangreiche Aggregatentstehung war bemerkenswert, da dafür bisher sowohl längere Zeiträume, als auch mechanischer Druck für nötig erachtet wurden. Hier konnte gezeigt werden, dass das bloße Vorhandensein von organischer Substanz ausreicht, um eine durch biochemische Prozesse initiierte Bodenstrukturbildung hervorzurufen.

Verschiedene Arten von organischer Substanz konnten mikrobielles Wachstum gleichermaßen induzieren, was jedoch mit einer unterschiedlichen Zusammensetzung der mikrobiellen Gemeinschaft einherging. Bakterielles Material förderte Bakterienwachstum, während Pflanzenstreu sowohl Bakterien- als auch Pilzwachstum unterstützte. Trotz der unterschiedlichen Zusammensetzung der mikrobiellen Gemeinschaft konnte die Rolle der Mikroben bei der Bodenstrukturentwicklung diesbezüglich zusammengefasst werden, dass sie klebrige organische Flecken induzieren, wodurch wasserstabile Partikelverbindungen verursacht werden. Diese Klebeflecken bedeckten maximal ein Sechstel der mineralischen Oberfläche, reichten aber dennoch aus, um einen Großteil der Partikel in Form von großen Makroaggregaten zu verbinden. Das bedeutet, dass die durch organische Substanz induzierte Aggregate lose zusammengesetzte Strukturen sind, die durch einzelne, wie Klebepunkte wirkende, Flecken aus organischer Substanz zusammengehalten werden. Dieses Gerüst hält einem Eintauchen in Wasser stand, was große, wasserstabile Aggregate stabilisierte, zeigte aber keine Stabilität gegenüber mechanischem Druck.

Das Wachstum von Bakterien führte zu mehr Makroaggregaten in sandreichen Böden, was bedeutet, dass die Aggregate auch große Sandkörner enthielten. Die eingeschränkte Wasserverfügbarkeit induzierte mehr mikrobielle Produkte für die Wasserspeicherung, wodurch Poren verfüllt und Partikel verbunden wurden. Da in den sandigen Böden viele großen Sandkörnern vorhanden waren, wurden diese zu großen Aggregaten verknüpft.

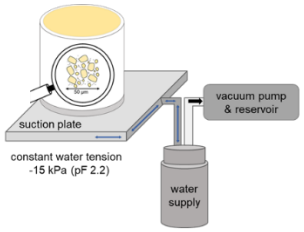
Die Pflanzenstreu führte, unabhängig von Bodentextur und Größe der organischen Partikel, zur überwiegenden Bildung von großen Makroaggregaten. Dies beruhte hautsächlich auf dem Effekt von Pilzhyphen, welche größere strukturelle Einheiten zusammenhalten können.

Die Zugabe von gelöster organischer Substanz konnte Makroaggregate in allen Texturen hervorrufen. Dies zeigte, dass die Bildung von wasserstabilen Aggregaten auch ohne das Vorhandensein von partikulären organischen Nuklei möglich ist und erweitert das bisherige Wissen über mögliche Bodenbildungsprozesse im Unterboden.

Der entwickelte Inkubationsaufbau wurde verwendet, um Bodenstrukturentwicklung in künstlichen Bodenmischungen für die Rekultivierung von Tagebauflächen zu studieren. Der experimentelle Testansatz beinhaltete verschiedene Mischungen aus herkömmlichen sowie innovativen Substraten wie Papiermulch, Flugasche, Braunkohle und Pflanzenstreu, welche in das vorhandene Abraummaterial eingemischt wurden, um unter anderem den organischen Kohlenstoffgehalt zu erhöhen. Die Inkubation führte zu einer raschen Entwicklung von mikrobieller Aktivität und wasserstabilen Aggregaten, wobei Pflanzenstreu bei beidem als verstärkender Faktor wirkte. Dies zeigte, dass auch innovative Bodenmischungen eine schnelle Bodenstrukturbildung unterstützen können, sofern Mikrobenwachstum gefördert wird. Allerdings führte die alleinige Beimischung von Pflanzenstreu in das Abraummaterial zu einem sehr schnellen Abbau der organischen Substanz, was eine baldige Erschöpfung des organischen Substrates im Kontext der betrachteten (semi-)ariden Umweltbedingungen bedeuten könnte. Damit wurde deutlich, dass eine diverse Substratmischung aus sowohl schnell abbaubaren, als auch persistenteren Substraten benötigt wird, um eine nachhaltige Entwicklung von Mikroben und Bodenstruktur zu gewährleisten.

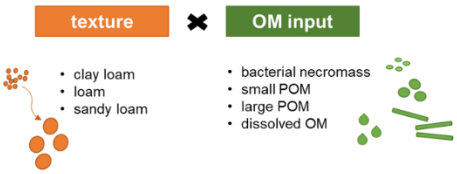
Diese Dissertation liefert ein konzeptionelles Verständnis initialer Bodenstrukturbildung, welche durch biochemische Prozesse an organischer Substanz hervorgerufen wird. Es konnte gezeigt werden, dass verschiedene Arten von organischer Substanz die Bildung von wasserstabiler Bodenstruktur hervorrufen können, welche ohne physikalische Einflüsse, wie zum Beispiel Umgraben oder wechselnde Befeuchtungsregimes ablaufen kann. Diese selbstorganisierte Bildung von Bodenstruktur wird durch einzelne, kleine organische Klebeverbindungen hervorgerufen. Die ausgebildeten Verknüpfungen können die Partikel für weitere Stabilisierungsprozesse fixieren. Die Wasserstabilität verspricht eine Stabilität der Böden gegenüber Wassererosion. Es bräuchte jedoch zusätzliche Mechanismen, um die Bodenstruktur gegenüber einer mechanischen Belastung zu stabilisieren, wie sie zum Beispiel beim Pflügen auftreten würde.

III GRAPHICAL SUMMARY



Development of an incubation set-up to investigate initial soil structure formation
(Study I)

Water-stable aggregation is possible within one month maturation without physical disturbance and particulate organic matter nuclei

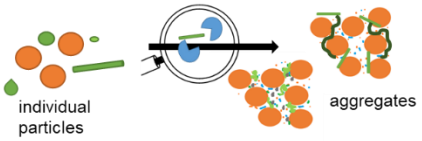


Investigating the influence of soil texture and distinct organic matter (OM) residues
(Study II and III)

Aggregate formation can be induced by various organic residues and can take place in various soil textures, because is mediated by microbial degradation of the organic matter

Soil texture defines the characteristics of the microbial habitat

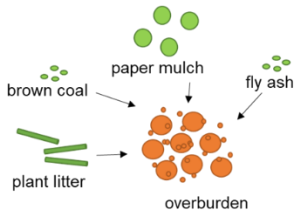
Various organic residues induce a different microbial community composition



Tracing the process of aggregate formation
(Study II and III)

OM-induced aggregates are a loosely connected scaffold of small sticky organic patches causing water-stable particle connections

Solely OM-induced aggregates have no mechanical stability



Expanding the experimental set-up to investigate soil structure development of artificial soils for mine rehabilitation
(Study IV)

Rapid OM-induced aggregate formation can also be observed in artificial mine rehabilitation mixtures, sustainable microbial development is important for long-term perspective

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IV LIST OF STUDIES

This publication-based doctoral dissertation is based on the following first-authored scientific research articles:

Study I

Franziska B. Bucka, Angelika Kölbl, Daniel Uteau, Stephan Peth, Ingrid Kögel-Knabner
"Organic matter input determines structure development and aggregate formation in artificial soils"
Geoderma 354 (2019) 113881, DOI: 10.1016/j.geoderma.2019.113881

Objectives

- Developing an experimental set-up based on artificial soil microcosms to study initial aggregate formation under controlled laboratory conditions
- Investigating the influence of organic matter (OM) derived from particulate organic matter (POM) and dissolved organic matter (DOM) on aggregate formation
- Analyzing the formed aggregates, microbial activity, organic carbon (OC) allocation, and pore system in the microcosms

Summary

- It could be demonstrated that aggregate formation in a loamy textured artificial soil is possible within 30 days and can take place in the absence of physical interference like stirring or repeated wet-dry cycles
- The OM was rapidly accessed and degraded by microbes
- The OM presence led to the predominant formation of water-stable macroaggregates. The OC allocation between the aggregates was closely linked to their size distribution, indicating a functional link with respect to the aggregate forming process
- The microcosms with POM had less large and more fine macropores and a highly connected pore system after incubation, indicating a reorganization of the pore space towards a pore system supporting sufficient gas, water and nutrient exchange. The DOM induced an overall

smaller pore connectivity than the POM, suggesting that changes in pore structure depend on OM type

- The two OM types induce a specific aggregate formation and development of the associated pore system, initiated by the microbial decomposition of the OM and the interaction of OM-compounds with mineral surfaces

Contribution I developed the experimental set-up, conducted the experiment, performed the laboratory analyses of the aggregates and OC, processed the laboratory data of the aggregates and OC, wrote the manuscript, and handled the peer-review and publishing process

Study II

Franziska B. Bucka, Vincent J.M.N.L. Felde, Stephan Peth, Ingrid Kögel-Knabner
"Disentangling the effects of OM quality and soil texture on microbially mediated structure formation in artificial model soils"
Geoderma 403 (2021) 115213, DOI: 10.1016/j.geoderma.2021.115213

Objectives

- Disentangling the effects of soil texture (clay loam, loam, sandy loam) and distinct organic matter (OM) residues (large and small particulate organic matter (POM), bacterial necromass) within the process of soil structure formation
- Tracing the aggregate forming process in order to develop a general understanding of initial soil structure formation
- Elucidating the role of the developed microbial community
- Investigating the properties and mechanical stability of the formed aggregates

Summary

- The study showed that all the formed aggregates were a loosely connected scaffold, bound together by some distinct spots of processed OM as gluing joints. Those aggregates are water-stable, but have a very low mechanical stability
- The mixing and incubation of the mineral mixtures led to abiotic particle-particle interactions and fine mineral and iron oxide coatings of the sand grains as shown by a reduction of the specific surface area
- The OM residues were quickly accessed and degraded by microbes, leading to OM patches occupying < 17% of the mineral surfaces after the incubation
- The POM of both sizes induced predominantly the formation of large macroaggregates regardless of the mixtures' texture. The bacterial necromass induced a texture-dependent formation of macro- and microaggregates (63-200 µm), with larger aggregates in sand-rich texture
- The different aggregate sizes could be related to differences in the developed microbial community. The bacterial necromass induced a

microbial community dominated by bacteria, whereas the POM fostered a high relative abundance of fungi, whose hyphae could enmesh and stabilize large aggregates in all textures

Contribution I conducted the experiment, performed the laboratory analyses, processed the data, wrote the manuscript, and handled the peer-review and publishing process

Study III

Franziska B. Bucka, Vincent J.M.N.L. Felde, Stephan Peth, Ingrid Kögel-Knabner
"Percolating dissolved OM initiates water-stable soil structure in various soil textures"
In preparation for publication

Objectives
- Investigating the effects of varying soil texture (clay loam, loam, sandy loam) on soil structure formation induced by percolating dissolved organic matter (DOM)
- Identifying the influence of abiotic and biotic processes within the initial structure formation
- Characterizing the developed microbial community composition
- Investigating the properties and mechanical stability of the formed aggregates

Summary
- The soil texture defines the pore system and flow characteristics, causing a coarser pore system and more rapid soil solution exchange at higher sand contents
- The organic carbon (OC) retention depends on illite, montmorillonite and goethite surfaces
- The microbial activity and total microbial biomass is defined by the available OC and not by the texture
- The microbial community is dominated by bacteria and a higher sand content leads to a higher proportion of gram positive bacteria
- The DOM induces aggregate formation by biochemical processing in all textures without the presence of particulate organic matter (OM)
- The water-stability of the aggregates can be established by very low OC contents
- The aggregates have a very low mechanical stability and the breakdown pattern indicates a homogenous aggregate architecture in the clay-rich texture and stepwise breaking off of sand grains in the sand-rich texture

Contribution I conducted the experiment, performed the laboratory analyses, processed the laboratory data, and wrote the manuscript draft

Study IV

Franziska B. Bucka, Evelin Pihlap, Jara Kaiser, Thomas Baumgartl, Ingrid Kögel-Knabner "A small-scale test for rapid assessment of the soil development potential in post-mining soils"

Soil & Tillage Research 211 (2021) 105016, DOI: 10.1016/j.still.2021.105016

Objectives

- Using the developed laboratory approach to investigate initial steps of soil development in artificial soil mixtures for mine rehabilitation in arid and semi-arid climatic conditions
- Testing the performance of six different mixtures composed of increasing complexity including paper mulch, fly ash, brown coal, and plant litter as amendments for the overburden material
- Assessing the mixtures' properties and the potential for initial development of soil structure, in order to identify suitable soil mixtures for subsequent field trials.

Summary

- Soil structure formation, as measured by isolating water-stable aggregates, was induced in all mixtures and intensified in presence of litter
- The addition of fly ash to the overburden led to a higher moisture content
- Fly ash together with paper mulch and brown coal improved nutrient supply and organic carbon (OC) content but led to a very wide C/N ratio. The molecular composition of the paper mulch and brown coal OC showed the potential for long-term OC storage because of slow microbial degradation
- The microbial activity was high in all mixtures with litter addition, but only the additional presence of fly ash, brown coal and paper mulch led to a higher microbial carbon use efficiency.
- The complex rehabilitation mixture showed the potential for soil structure development within a short timeframe also in field scale, because the tested substrates stored moisture, delivered nutrients and OC for sustainable microbial growth.

Contribution	I developed the experimental setup, conducted the incubation, performed the analyses, processed the data, wrote the manuscript, and handled the peer-review and publishing process

1 INTRODUCTION

1.1 Soil aggregates as a tool for investigating soil structure

The term soil structure describes the three-dimensional arrangement of particles and voids within a soil (Letey, 1991). The nature and stability of this arrangement defines a soil and is the basis for many soil processes and ecosystem services provided by soils (Amezketa, 1999; Baer and Birgé, 2018; Dexter, 1988). The structure of a soil has significant effects on plant development, water balance or workability (Dexter, 1988). Plant roots and soil organisms need nutrients, water and oxygen, which have to be supplied by the surrounding soil, especially through the soil pores (Dexter, 1988). A stable soil structure can provide slope stability during heavy rainfall events or chemical buffer capacity to prevent leaching of potentially harmful substances (Barthes and Roose, 2002; Dayton et al., 2010; Doran et al., 1996).

The architecture of the soil particle arrangement defines the surrounding voids and thus shapes the pore network of a soil. The large variety of particles and their possible arrangement causes a huge diversity of potential pore sizes and properties. This pore network defines the various microsites, which can be biologically and chemically reactive and provide the space for gas, water, and nutrient exchange (Blaud et al., 2012; Horn and Smucker, 2005; Jarvis, 2007; Nunan et al., 2003; Schaaf et al., 2011; Steffens et al., 2017).

Despite its prominence and frequent use, the term soil structure describes rather a concept than one distinct feature and can be approached from various angles using different techniques. Soil structural investigations rely on tools for the quantification of related features and properties (Letey, 1991; Young et al., 2001), which can rely either on undisturbed or fractionated soil samples. A common tool in terms of fractionation is the isolation and analysis of soil aggregates. Soil aggregates are composite structures of various, agglomerated soil particles that can be isolated as a single entities from a soil (Amezketa, 1999; Kemper and Rosenau, 1986). Common aggregate fractionation procedures rely on either their resistance to mechanical force, submersion in water or a combination of both. The aggregates are isolated by sieving the soil with sieves of different size, allowing to sort the obtained fractions into distinct size classes, which usually range from several centimeters to tens of micrometers (Asano and Wagai, 2014; Chenu and Plante, 2006; Edwards and Bremner, 1967; Totsche et al., 2018).

The isolation of water-stable aggregates that resist submersion in water is a commonly used approach that is easy to obtain. Water-stable aggregates are linked to soil stability (Amezketa, 1999; Dexter, 1988; Tisdall and Oades, 1982) and organic carbon (OC) sequestration (Baldock and Skjemstad, 2000; Lehmann et al., 2007; McCarthy et al., 2008). The size distribution of isolated water-stable aggregates in a soil has proven to respond quickly to soil management adjustments and therefore is frequently used as soil quality indicator (Barthes and Roose, 2002; Elliott, 1986; Sparke et al., 2011).
Because the investigation of soil aggregates is a useful tool to address soil structure, there are many studies focusing on the properties of aggregates and changes in their size distribution. In contrast, there have been only few attempts trying to elucidate the entire process of soil structure formation starting with individual, unconnected particles.

1.2 The complex process of soil structure formation

The formation of soil aggregates requires the grouping and cohesion of the present soil particles. Within this process, the interaction of mineral particles and soil organic matter (SOM) is considered to be a major factor for the formation and stabilization. To understand the complex process of soil structure formation, it is necessary to disentangle the texture-dependent interplay of organic matter (OM) and mineral particles and to elucidate the role of the microorganisms.

1.2.1 Soil mineral particles: The inherited infrastructure

The mineral particles in a soil include weathering products inherited of the parent rock material and newly formed minerals during pedogenesis. During pedogenesis, the parent rock is weathered which leads to the formation of smaller particles. Dependent of their mineral constituents, some particles can be more prone to weathering than others. This means that the mineral particles occur in different sizes, shapes, structures, and chemical compositions, which affects their surface and capacity to interact with each other and organic matter (OM) (Dultz et al., 2019; Kleber et al., 2015).
Since the most abundant elements in the earth's crust are oxygen and silicon, the most abundant minerals are silicates that are built up by SiO_4-tetrahedrons (Mackenzie, 1975). The silicates can be classified according to the arrangement of the basic SiO_4-tetrahedrons

(Brown, 1955; Mackenzie, 1975). Pedogenic silicate minerals < 2 µm are commonly referred to as clay minerals and define the plasticity and ion exchange capacity in soils (Mackenzie and Mitchell, 1966). The most abundant soil mineral is quartz (technically classified as silica oxide), which consists solely of SiO_4-tetrahedrons (Drees et al., 1989). Quartz is quite resistant to weathering and its density of 2.65 g cm^{-3} is the dominating particle density in a soil (Drees et al., 1989). Phyllosilicates are one group within the silicate minerals that are classified according to their 2-dimentional cross-linking of the basic tetrahedron building blocks, which leads to a sheet-like shape (Mackenzie and Mitchell, 1966). Illites are three-layer minerals, consisting of two silica tetrahedron layers and one alumina octahedron layer (Mackenzie, 1965). The space between the silicate layers is occupied by potassium, which leads to a very narrow inter-layer space, where water molecules cannot enter (Mackenzie, 1975). Montmorillonite is also a three-layer clay mineral, but its lower layer charge leads to a possible water storage in between the silicate layers and thus the ability for expanding, i.e., swelling (Brown, 1955; Mackenzie and Mitchell, 1966). Clay mineral surfaces are permanently negative charged due to isomorphic substitution, whereas the edges contain variable pH-dependent charges, which are negative at common soil pH values (Chorom et al., 1994).

Soils also contain iron, which is weathered from the parent material and mostly bound within iron oxides and hydroxides (Schwertmann, 1985; Schwertmann and Taylor, 1989). One of the most abundant iron(III) oxide-hydroxides in the soil is goethite (α-FeOOH) (Schroeder, 1988; Schwertmann and Taylor, 1989). It is typically needle-shaped, has a density of 4.3 g cm^{-3} and its point of zero charge is at pH 8.5-9.5 (Schwertmann and Taylor, 1989).

The mineral particles in a soil include high range of particle sizes, which is described by the soil texture. According to Atterberg (1908) and the German soil classification (Atterberg, 1908; Atterberg, 1917; Eckelmann et al., 2006), the particles (< 2 mm) are grouped into three size categories: clay (< 0.002 mm), silt (0.002-0.063 mm), and sand (0.063-2 mm). In soils, the size distribution of the reacting particles (i.e., soil texture) is an essential characteristic and defines many physical and chemical properties. Fine particles are known to have a much larger specific surface area than coarse particles and thus provide relatively more contact points with other particles. Clay particles have been reported to be crucial for aggregation, due to their size and charge (Edwards and Bremner, 1967; Krause et al., 2018), whereas sand particles are considered not to contribute significantly to aggregate formation (Bronick and Lal, 2005). However, recent studies highlighted the incorporation of sand-sized mineral particles within aggregates (Felde et al., 2020; Paradis et al., 2017). To

characterize the soil texture effect, a systematic study of the influence of distinct particle size distributions on initial soil structure formation is required.

1.2.2 Soil organic matter: The entangled network

The term soil organic matter (SOM) describes all dead plant and animal residues and their transformation products in a soil (Waksman, 1926). This implies that SOM is simultaneously present in numerous forms, states, and origins, ranging from fresh and undecomposed material to highly degraded, transformed, or stabilized compounds (Kleber et al., 2015; Kögel-Knabner, 2002; Schmidt et al., 2011). Organic matter (OM) enters the soil mainly as particulate organic matter (POM) by plant debris of aboveground or belowground biomass (Kögel-Knabner, 2002; Scholes et al., 1997). Within the soil, macro- and microorganisms process and degrade the OM, causing a size reduction and a change in the chemical composition (Baldock et al., 1992; Golchin et al., 1994b). Growing microbes excrete extracellular polymeric substances (EPS), during their growth and metabolism, which lead to biofilm patches (Costa et al., 2018; Flemming and Wingender, 2010). Those biofilm patches are sticky and may adhere to surrounding minerals leading to soil structure formation by cross-linking of particles (Chenu and Jaunet, 1992; Gaillard et al., 1999; Rabbi et al., 2020; Young and Crawford, 2004). Within this process, the POM is believed to serve as a physical nucleus for particle accumulation in the course of aggregate formation (Golchin et al., 1994b; Jastrow, 1996). Until now, there is not much information, how the size of the POM can affect this process and shape the properties of the formed aggregates. During OM degradation, and also originating from OM itself, dissolved organic matter (DOM) compounds are formed and distributed in the soil (Kalbitz et al., 2000). DOM is a collective term for OM smaller than 0.45 µm (Kalbitz et al., 2000) and may contain a varying amount of organic carbon (OC) within complex molecules like organic acids, sugars, amino acids, depending of its origin (Kaiser and Kalbitz, 2012). Especially in deeper parts of the soil, where no plant roots are present, the percolating DOM originating form upper soil horizons is considered to be the main OM source in temperate soils (Angst et al., 2018; Kaiser and Guggenberger, 2000). In subsoils, this DOM meets mineral surfaces that are mostly free of OM and prone to OC sorption (Kaiser and Guggenberger, 2003). The interaction of DOM with minerals has been addressed by many studies (cf. Jardine et al., 1989; Kaiser and Guggenberger, 2000; Shen, 1999; Sokol et al., 2019), but there is only limited knowledge about the role of DOM within soil structure formation.

When focusing on OM induced soil structure formation, the microbial degradation of the OM is usually implied, but the role of the microbial biomass itself is rarely taken into

account. When microbes degrade the OM, they use the OC to build their biomass, which eventually becomes transformed to microbial necromass (Miltner et al., 2012). This microbial necromass can form a substantial part of the SOM, which may encounter further degradation by the living microbes in a soil (Miltner et al., 2012). The microbial cells itself can adhere to mineral particles and thus induce particle agglomeration (Chenu et al., 2002; Foster, 1988). Since most research is focused on the effect of plant residues, there is the need for a systematic investigation of the necromass effect within this process.

In soils, the SOM components face microbial degradation, which is influenced by the OM properties and may affect the structure inducing effect. For example, macromolecules like lignin, which are found in plant residues only, decompose slowly, whereas polysaccharides, which are found in bacterial cell walls, decompose easily (Kögel-Knabner, 2002). The C:N ratio of an OM substrate can function as a proxy for the degradability as it accounts for the nitrogen availability, which is often a limiting factor for microbial decay (Cotrufo et al., 1995; Eiland et al., 2001). It is necessary to disentangle the effects of distinct OM residues to identify and understand selected process complexes within soil structure formation.

1.2.3 Living microorganisms: The hidden residents

Soils harbor a huge number of microorganisms, with estimates assuming that up to 10 billion microorganisms live in one gram of soil (Rosselló-Mora and Amann, 2001; Torsvik and Øvreås, 2002). The microbes are linked to key processes in soils like organic matter (OM) degradation and soil structure formation (Chenu et al., 2002; Schimel and Schaeffer, 2012).

Microbial life requires a suitable habitat, a sufficient supply of water and nutrients, and a functional gas exchange (Esperschütz et al., 2013). In soils, the spatial arrangement of solid particles and pores filled with water or air define the habitat of the microorganisms and can provide protected spaces and substrate access (Chenu et al., 2002; Voltolini et al., 2017). Despite the high number of microbes in a soil, their growth is usually limited to several, distinct growth patches (Foster, 1988) that occupy much less than 1 % of the available surface area (Young and Crawford, 2004). This makes it difficult to find and quantify the microbial population.

Microbial life covers many different niches, which implies functional differentiation by various phenotypes and metabolisms (Schimel et al., 2007; Torsvik and Øvreås, 2002). For example, different aggregate sizes have been linked to different microbial communities,

suggesting that the properties of the isolated aggregates may relate to their microbial colonization (Harvey et al., 2020; Oades and Waters, 1991).

The introduction of OM into soils delivers substrates for microbial decay. Growing microorganisms shape their environment by excreting enzymes and other extracellular polymeric substances (EPS) that facilitate chemical reactions, nutrient entrapment, and provide protection against environmental stressors (Costa et al., 2018). Those microbial products may be sticky and adhere to solid particles (Chenu and Jaunet, 1992; Costa et al., 2018). This can lead to aggregate formation when OM becomes tightly associated by mineral particles, which prevents further degradation (Costa et al., 2018; Oades, 1984). Microbial products have also shown to fill up soil pores, leading to further encapsulation of the OM by shutting out microbes and enzymes (McCarthy et al., 2008).

The incorporation of fresh particulate OM has proven to increase soil microbial biomass by offering new sites for microorganisms, while soluble OM showed to stimulate microbes located in the soil matrix (Chotte et al., 1998). In an isolated soil aggregate, several microdomains with different spatial and functional properties were identified and linked to different OM qualities (Steffens et al., 2017). These observations suggest that various OM substrates may create specific microenvironments for microbial degradation with implications for the developed microbial community composition and soil structure development. There is the need to investigate the effects of distinct OM substrates in combination with varying soil textures on the microbial community development and subsequent soil structure formation.

1.3 Fostering initial soil structure development at former mine-sites

Brown coal is mined in open cut mines that imply a large-scale soil destruction because the mine pits can occupy footprints of several square kilometers, while being more than one hundred meters deep. After ceasing the mining activities, the landscape needs to be restored and functional soils need to be re-established to ensure plant growth and stability against erosion (DeJong et al., 2011).

In some areas, when there is not enough topsoil material available for rehabilitation, the only alternative would be the spreading of the previously excavated overburden material. The overburden is the material that was overlying the coal seam and has been removed prior to the coal extraction. It is usually poorly structured and does not contain organic

carbon (OC), which means that soil structural development would start at point zero. A simple overburden application as a reclamation strategy would need substantial time and subsequent management efforts like fertilizer application to ensure soil structure formation and rehabilitation (Pihlap et al., 2019; Shrestha and Lal, 2011). In some remote mine sites, such long-term intensive management is not possible, because the transport of the immense amount of fertilizer is not feasible. Especially in drylands, rehabilitation approaches are challenging, because there is the need for amendments that improve the water-holding capacity of the poorly structured overburden substrate (Hueso-González et al., 2018). Therefore, alternative approaches are required to accelerate soil development and rehabilitation. One newly developed approach at mine sites is the use of locally available OC-rich substrates that are added to the overburden in order to create a suitable topsoil replacement (Belyaeva and Haynes, 2009; Dayton et al., 2010; Taylor et al., 2014). Testing the soil structural development of such rehabilitation mixtures in field trials is expensive, time and resource consuming, and highly dependent on weather conditions. This means that there is a need for reasonable and straightforward test systems for investigating the early soil development processes within a controlled lab approach. The gained knowledge gives insight into the relevant processes of soil structure development within those mixtures. Furthermore, a first assessment of the mixtures within a short timeframe would facilitate the selection of the most promising mixtures to make subsequent field trials more predictable.

1.4 Objectives and approach of the thesis

The main goal of this publication-based thesis was to decipher the processes of initial soil structure formation with respect to the biochemical processing of organic matter. The influence of soil texture, organic matter residues, and the developed microbial community were disentangled within this process. In particular, the self-organized aspect of the structure formation was elucidated, which can happen without mechanical pressure.

In order to address the main goal, seven objectives were formulated. Together with their corresponding approach, they are listed in the following:

1. *Establishing initial self-organized soil structure formation under controlled conditions (Study I)*

   An experimental approach based on an incubation system with artificial soil microcosms was developed. The artificial model soils were designed to mimic a soil development starting with single, unconnected particles. The experimental design included an incubation of the microcosms at constant water tension to minimize mechanical and physical influences like stirring or changing water regime. This allowed the detailed investigation of initial, self-organized soil structure formation in a highly controlled environment.

2. *Elucidating the aggregate forming capacity of various, distinct organic matter residues (Study I, II, III)*

   Various individual organic matter (OM) residues, which are contributing to the natural soil organic matter (SOM) pool, were selected and used for incubation. The organic matter was added as individual residues (large and small plant litter (POM), dissolved organic matter (DOM), and bacterial necromass) to the respective microcosms. The effects of these individual OM residues on aggregate formation were elucidated by monitoring microbial parameters and structure-related properties of the bulk soils and isolated aggregates after the incubation.

3. *Investigating the effects of soil texture on aggregate formation (Study II, III)*

   Diverse artificial soils were designed as mineral mixtures with the same mineral composition (Quartz, Illite, Montmorillonite, Goethite) but differing texture (clay loam, loam, sandy loam), resulting in a textural range from clay-rich to sand-rich

soils. This allowed studying the effect of particle size separated from mineral composition on soil structure formation by monitoring microbial parameters and structure-related properties of the bulk soils and isolated aggregates.

4. *Understanding the process of organic matter induced soil structure formation (Study II, III, IV)*

   The transformation of single particles to compound, interconnected structures was traced by investigating soil structural and chemical properties of the artificial soils before and after the incubation. In order to separate the abiotic processes from OM-induced structure formation, the structural development of artificial soils with the same texture but without OM was investigated and compared to soils with OM. By individually studying the soil structural development of artificial soils with three different textures in combination with one distinct OM residue (or no OM input), selected process complexes during OM-induced soil structure formation could be identified.

5. *Elucidating the properties and stability of the organic matter induced soil structure (Study I, II, III, IV)*

   The distinct properties of OM-induced soil structure were identified by comparing the stability, architecture and chemical properties of the artificial soils with OM to the soils without OM. Bulk soil analyses were used to study bulk density, water storage, and pore characteristics. Isolated aggregates were used to study their size, texture, stability against water and mechanical force, breakdown pattern, and organic carbon content. The measured properties of bulk soil and aggregates were related to the distinct soil textures and organic residues, and the formed soil structural and microbial properties during the incubation.

6. *Understanding the role of the microbial community (Study I, II, III, IV)*

   The microbial effect within the initial soil structure formation processes was elucidated by monitoring the microbial activity and measuring the developed microbial biomass, and community composition during incubation. The developed microbial features were related to the distinct soil textures and organic residues and the soil structural and chemical properties and aggregates after the incubation.

7. *Investigating initial soil structure development in artificial soil mixtures for mine-site rehabilitation (Study IV)*

   The developed incubation approach was applied to study early soil development processes in artificial substrate mixtures used for mine-site remediation in Australia. Various mixtures of both known and innovative OC-rich amendments (paper mulch, fly ash, brown coal, and plant litter) of the overburden substrate were prepared and incubated. The mixtures' properties, microbial development, and soil structural formation were investigated in order to identify the main drivers and processes for OM-induced soil structural development within the studied (semi-)arid environment.

2 MATERIAL AND METHODS

2.1 Experimental setup and preparation of the artificial soils

2.1.1 Microcosm design and incubation (Study I, II, III, IV)

The experimental setup consisted of individual microcosms that were incubated at constant water-tension on a suction plate. Figure 1 gives a schematic overview of the experimental design.

The microcosms were designed as cylindrical columns (unplasticized PVC, height: 9.1 cm, diameter: 8.1 cm, resulting in a total volume of 470 cm^3). The bottom consisted of a glass microfiber filter (pore size 1.1 µm, size 90 mm, VWR, Leuven, Belgium) and a layer of fine meshed polyester cloth (37 µm mesh size) for protecting and fixing the filter disk. Each microcosm was filled with 300 g of artificial soil of various composition, which occupied a volume between 200 and 350 cm^3 depending on the bulk density.

The microcosms were placed on a suction plate (plastic suction plate with polyamide membrane, pore size 0.45 µm, EcoTech Umwelt-Messsysteme, Bonn, Germany). The suction plate was integrated into a closed hydraulic system, and a suction pressure of -15 kPa (corresponding to a pF value of 2.2) was applied to the suction plate throughout the whole incubation period. Water flow into and out of the microcosms was controlled by a vacuum pump, resulting in a constant water tension (fluctuation of ±1 kPa; electronic vacuum controller combined with vacuum reservoir and surge tank, EcoTech Umwelt-Messsysteme, Bonn, Germany), reached after a less than 5-days wetting period. The microcosms were incubated in the dark at 20°C.

During the initial wetting period, the microcosms were irrigated daily to reach a stable water content. During the incubation, the irrigation solution was carefully dropped onto the surface twice per week for compensating the water-loss due to evaporation. The water content in the microcosms was measured during the incubation by weighing the microcosms 48 hrs. past each irrigation event (24 hrs. during the initial wetting phase).

CO_2-release from the samples was monitored twice a week by placing the microcosms for several hours in an airtight container, while trapping the released CO_2 with 0.1 M NaOH (Bimueller et al., 2014; Luxhøi et al., 2006). The amount of consumed NaOH was determined by means of titration (Mettler-Toledo, Giessen, German) with 0.1 M HCl to pH

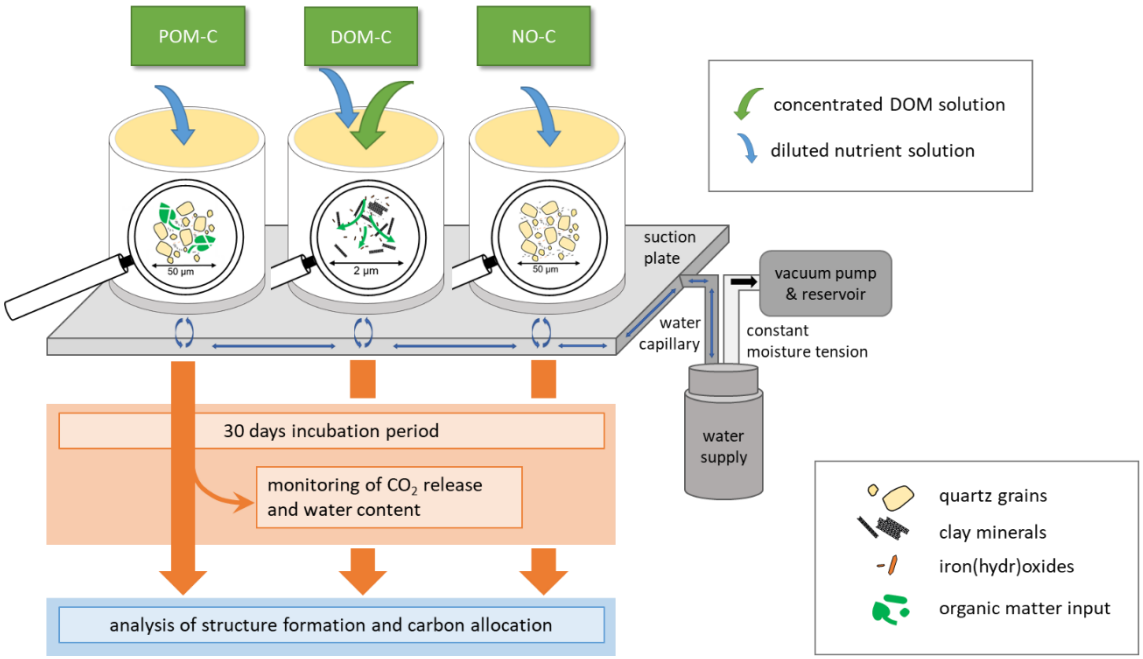

Figure 1: Experimental setup of the incubation of individual microcosms at constant water-tension. (Figure taken from Study I).

8.3, and the amount of released CO_2 was calculated using the HCl amount that was required for titration. The periodically measured CO_2 release was extrapolated and summed up to the total CO_2 release.

After incubation end, the material in the microcosms was sampled and divided into various aliquots that were stored by air-drying, freeze-drying or moist short-term storage at 4°C for further analyses.

2.1.2 Mineral mixture (Study I, II, III)

For the basic mineral mixture, quartz grains of different size were mixed in order to create a defined bulk texture (Euroquarz, Laußnitz, Germany and Quarzwerke, Frechen, Germany). For creating reactive surfaces, 3% of montmorillonite (Franz Mandt, Wunsiedel, Germany), 7% of illite (Aspanger, Aspang, Austria) and 1% of goethite (Sigma-Aldrich, Steinheim, Germany) in fine silt and clay size were added to the quartz mixtures. The

materials were mixed throughout in a dry state. Overall, three different textures (classified according to FAO) with increasing sand content were created for the studies (Figure 2) and used for the incubation: (i) clay loam (31% clay, 48% silt, 21% sand); (ii) loam (16% clay, 39% silt, 45% sand); (iii) sandy loam (12% clay, 15% silt, 73% sand).

2.1.3 Organic matter residues (Study I, II, III)

Four different types of organic matter were isolated and used for the incubation: (i) large POM (lPOM), (ii) small POM (sPOM), (ii) bacterial necromass (BAC), and (iv) dissolved OM (DOM) (Figure 2). The POM was added as milled hay litter (organically produced grass-clover hay; *Trifolium* spec., *Lolium perenne* L., and *Lolium multiflorum* L.), dry sieved to two different size classes: 0.63-2 mm (lPOM) and < 63 µm (sPOM). The bacterial necromass was added as *Bacillus subtilis* (freeze-dried material by Lactopia, Saarbrücken, Germany, sterilized with 50 kGy γ-radiation). The DOM was produced as concentrated solution of 4 mg OC ml^{-1} by water extraction (35 °C, 48 h) of ground hay (hay:water, 1:20, w:w) and filtration to <0.45 µm by pressure filtration.

The lPOM, sPOM and bacterial necromass were added in a concentration of 13 mg OC g^{-1} soil and were mixed in a dry state before the incubation. The DOM solution was added regularly with the irrigation solution during the incubation, leading to a total input of 2.6 mg OC g^{-1}. Each microcosm type was prepared with 3 replicates and there were 3 control microcosms prepared, which received no organic matter addition.

2.1.4 Microbial inoculum and nutrient supply (Study I, II, III)

The mineral mixtures were inoculated with a microbial inoculum isolated from an arable Cambisol in southern Germany (Krause et al., 2018). The inoculum was prepared by water-extraction (Lehmann et al., 2018; Pronk et al., 2012), using 1.2 g topsoil material per microcosm. An 1:5 (w:w) soil:water suspension was shaken for 2 hrs. with gravel and the microbial pellet was isolated subsequently by two centrifugation steps. The inoculum was suspended in 15 mL distilled water and used for inoculation.

To prevent nutrient shortage, an 1:10 diluted Hoagland's solution (pH 5.5, Hoagland's No. 2 basal salt mixture, Sigma-Aldrich, Steinheim, Germany) was used for the irrigation during the incubation.

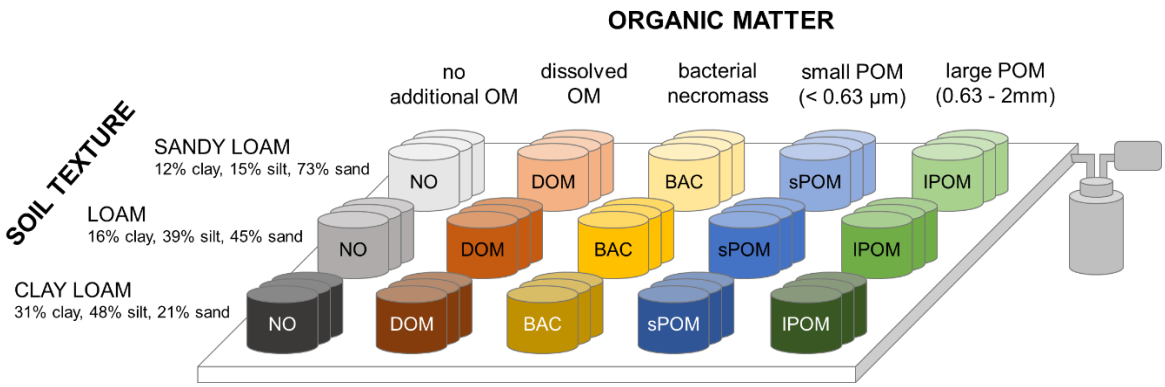

Figure 2: Overview of the artificial model soils with three different textures (prepared with 3 replicates) and a distinct type of organic matter input that were prepared for incubation. (Figure taken and modified from Study II).

2.1.5 Artificial rehabilitation mixtures (Study IV)

The rehabilitation mixtures were based on overburden material and four different substrates that were mixed in: (i) fly ash, (ii) brown coal, (iii) paper mulch, and (iv) plant litter (Figure 3). The overburden and the brown coal were taken from the Loy Yang brown coal mine, an open cut mine in the Latrobe Valley, Victoria, Australia. The overburden material consisted of Tertiary and Quaternary deposits, predominantly silt and clay-rich alluvial sediments (Cochrane et al., 1991; Durie, 2013) with quartz being the predominant mineral, followed by kaolinite, muscovite, illite, and montmorillonite (Brockway et al., 1991; Durie, 2013).

The fly ash was sourced from the Loy Yang Power Station, where the feedstock was supplied by the Loy Yang mine. The paper mulch was provided as a by-product from the Maryvale Paper Mill (Maryvale, Australia). The overburden, brown coal, fly ash, and paper mulch were air-dried, gently crushed, and dry sieved to < 2 mm before mixing to ensure a homogenous mixture in the lab scale set-up. The litter was produced by milling (ultra-centrifugal mill with 0.5 mm sieve, Retsch, Haan, Germany) air-dried native grassland vegetation from the Loy Yang mine area. The following six mixtures were used in the incubation experiment: (i) overburden only, (ii) overburden mixed with litter, (iii) overburden mixed with fly ash, (iv) overburden mixed with fly ash and litter, (v) overburden mixed with fly ash, paper mulch and coal (rehabilitation mixture), and (vi) overburden mixed with fly ash, paper mulch, coal, and litter (rehabilitation mixture plus litter). Figure 3 gives a detailed

overview of the prepared mixtures. The mixing ratios of overburden, fly ash, paper mulch and coal were equivalent to the mixing ratio used for creating an artificial soil in a field trial at the Loy Yang mine area. The litter was added to the mixtures in a concentration of 13 mg OC g^{-1} soil mixture.

The microcosms were filled with 300 g of the mixture material and each mixture was replicated three times.

						OC content [% ± *SD*]	**CN ratio**
Overburden material	Overburden					0.4 ± *0.0*	15
Overburden plus litter	Overburden	*Plant litter* (*13 mg OC g^{-1} soil*)				1.7 ± *0.1*	33
Overburden plus ash	Overburden	Fly ash				3.5 ± *0.3*	95
Overburden plus ash plus litter	Overburden	Fly ash	*Plant litter* (*13 mg OC g^{-1} soil*)			4.5 ± *0.2*	75
Rehabilitation mixture	Overburden	Fly ash	Paper mulch	Brown coal		7.3 ± *3.2*	150
Rehabilitation mixture plus litter	Overburden	Fly ash	Paper mulch	Brown coal	*Plant litter* (*13 mg OC g^{-1} soil*)	8.2 ± *0.9*	110
Mixing ratio (w:w)	1	0.6	0.2	0.1			
OC content (% ± *SD*)	0.35 ± *0.03*	6.97 ± *0.24*	30.73 ± *4.76*	35.54 ± *0.48*	41.15 ± *0.11*		
CN ratio	15 ± *0.03*	162 ± *0.2*	247 ± *5*	112 ± *0.5*	66 ± *0.2*		

Figure 3: Composition of the artificial rehabilitation mixtures. (Figure taken form Study IV).

2.2 Soil analyses

2.2.1 Porosity and water-filled pore space (Study I, II, III, IV)

The porosity (*n*) of the microcosms was calculated according to equation (1), using the bulk density (d_B) and literature values for the particle density (d_P).

$$n = 1 - \frac{d_B}{d_P} \quad (1)$$

The maximum mean diameter of 20 µm for the water-filled pores at the applied water tension of -15 kPa was calculated by using the Young-Laplace-Equation for capillary rise (Tuller and Or, 2004).

The water-filled pore space (*WFPS*; %) was calculated at the given water tension according to equation (2), using the pore volume (V_V; cm^3) and water volume (V_W; cm^3) in the microcosms.

$$WFPS\ [\%] = \frac{100}{V_V} * V_W \quad (2)$$

A higher proportion of water-filled pore space implies a higher proportion of pores with a mean diameter < 20 µm.

2.2.2 Soil solution (Study IV)

The pH and electric conductivity (EC) of the soil solution were determined for the substrates as well as the bulk mixtures before and after incubation in a soil and water solution of 1:2.5 (w:w).

The total elemental concentration of 21 beneficial and potentially toxic elements in the soil solution of the substrates and the bulk mixtures were measured by means of inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian, Darmstadt, Germany) in a soil and water solution of 1:2.5 (w:w).

2.2.3 Aggregate fractionation (Study I, II, III, IV)

The mass contribution of the aggregate size fractions was determined by submerged sieving before and after the incubation. About 20 g of fresh soil was loaded onto a sieve stack (630 µm, 200 µm, 63 µm sieve), submerged in deionized water and sieved for 2 min, by moving the sieves 1 cm up and down at 30 rpm. The sieve fractions were oven-dried at 60 °C and weighed to calculate the mass contribution of the aggregate size fractions. The maximum size of the aggregates > 630 µm was determined with a caliper (Feret diameter) on the largest aggregate of each sieving process. Four different aggregate size fractions were obtained: large macroaggregates (> 630 µm), small macroaggregates (200 – 630 µm), large microaggregates (63 – 200 µm), and small microaggregates (< 63 µm).
A dry separation of single aggregates 2 - 4 mm was done by dry sieving of air-dried bulk soil. The aggregates that passed a 4 mm sieve and were collected on a 2 mm sieve were picked with tweezers.

2.2.4 Density fractionation (Study I)

The moist soil was submerged with sodium polytungstate solution (density of 1.7 g cm^{-3}, determined by pre-experiments; TC Tungsten Compounds, Grub am Forst, Germany) and floating POM was removed by vacuum suction. The sediment was submerged again, and the occluded POM was extracted step-by-step using increasing levels of ultrasonic energy (100, 200 and 300 J ml^{-1}, 13-mm probe tip, Bandelin, Berlin, Germany) for aggregate disruption. The floating POM was separated by centrifugation (3500 rpm, 30 min, 20 °C) and collected by vacuum suction. The isolated POM was washed salt-free by pressure filtration (pore size 0.2 µm), until the electric conductivity of the washing fluid was < 2 µS cm^{-1}, freeze-dried and weighed.

2.2.5 Texture analysis (Study I, II, IV)

The texture of the bulk soil and aggregate size fractions was determined by wet sieving (630 µm, 200 µm, 63 µm sieve) after complete ultrasonic disruption (450 J ml^{-1}). The overburden material was pre-treated before sieving by suspending 30 g of dry material (<

2 mm) in 0.025 M sodium pyrophosphate ($Na_4P_2O_7$) and adding a 30% H_2O_2 solution until all of the OC was removed. The sieve fractions were oven-dried and weighted.
The sample material < 63 µm was re-submerged with 0.025 M sodium pyrophosphate and analyzed with X-ray sedimentation analysis with a sedigraph (Micromeritics, Aachen, Germany). If there was not enough sample material for the X-ray sedimentation analysis, the material was wet-sieved to < 20 µm, oven-dried and weighted to estimate the combined proportion of medium, fine silt and clay.

2.2.6 Specific surface area (Study II, III)

The specific surface area (SSA) of the mineral components and the mixtures before and after the incubation was determined by N_2 adsorption at 77 K according to the Brunauer-Emmett-Teller (BET) method (multipoint N_2-BET SSA) (Brunauer et al., 1938). The measurement was done as described in Heister, 2016 using 0.5 - 2 g of dry material after outgassing under vacuum using He at 40 °C for > 16 hrs. The total available surface area was calculated for each mixture by summing up the measured SSA of the mineral components. Mineral surfaces covered with OM were assumed to be negligible in the BET-N_2 SSA measurement (Heister, 2014), allowing to calculate the OM covered surface area by subtracting the SSA of the OM mixtures from the SSA of the mixtures without OM.

2.2.7 Mechanical stability of the aggregates (Study II, III)

The mechanical stability of the formed aggregates was analyzed by dry crushing with a mechanical loading frame for material testing (Zwick Roell, Ulm, Germany) equipped with a high-resolution load cell (Xforce HP 10 N) for force recording as described in (Felde et al., 2020; Skidmore and Powers, 1982). Air-dry aggregates (2 - 4 mm) were randomly chosen with 10 replicates for each OM type and texture. The aggregates were crushed one by one by reducing the distance between piston and table (at a speed of 500 µm min^{-1}) from 4.1 mm to 1.5 mm. Pre-tests showed a maximum diameter of 1.5 mm for the sand grains in the mixtures, so the test was stopped at this distance to prevent the crushing of sand-sized mineral particles.
To obtain a measure of the mechanical stability of the aggregates, the applied work for a plastic deformation in the magnitude of 20% (10%, 5%) of the aggregate size was

calculated (area of the force-displacement curves). The number of force drops (maxima of the force-displacement curves) of several magnitudes (> 5%, > 10%, > 25%, > 40%, > 50%, > 75% of the force) was counted to record breaks of different magnitudes during the crushing process. Figure 4 shows an example of a force-displacement curve and its characteristics as obtained during the crushing.

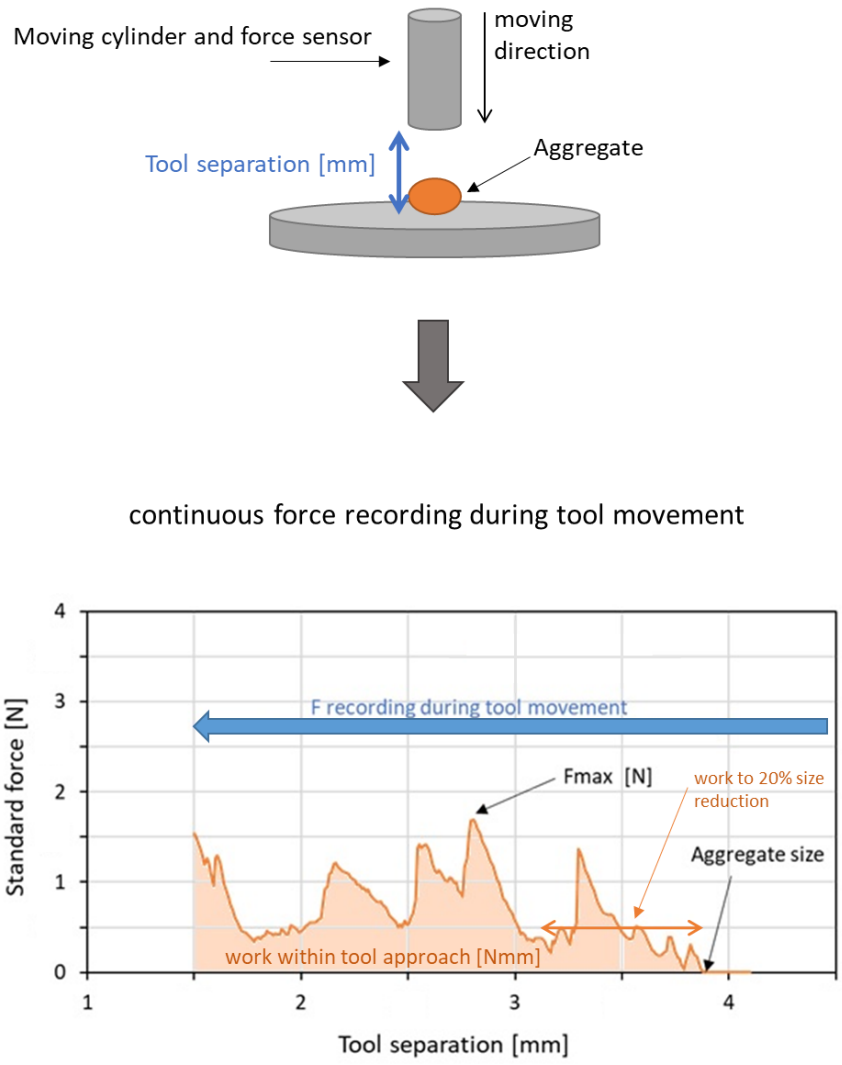

Figure 4: Example of the data acquisition and analysis during the dry crushing. During the crushing process, the force sensor moves towards the crushing table, which continuously reduces the tool separation (mm). During the movement, the force (N) is recorded, which leads to a force-displacement curve (read from right to left). The integral of the force-displacement curve describes the applied work (Nmm) during the crushing process, sudden force drops (maxima of the curve) relate to breaks. The tool movement was started at 4 mm tool separation, the first increase in the recorded force denotes the encounter between tool and aggregate surface (aggregate size). (Figure taken and modified from Study II).

2.3 Organic carbon analyses

2.3.1 Organic carbon content, leaching and allocation (Study I, II, III, IV)

The OC and N content of the residues, dry bulk mixtures before and after the incubation, and the dried aggregate fractions was determined by dry combustion of milled sample material using a CN analyzer (HEKAtech, Wegberg, Germany). It was checked before the measurement that the measured sample material did not contain inorganic C, so the OC content corresponded to the total C content.

By subtracting the sum of total CO_2-C release and bulk OC content after the incubation from the starting bulk OC, leached OC during the incubation was calculated.

The OC contribution of the aggregate size classes to the total OC was calculated by using the mass contribution and OC concentration of each aggregate size class.

OC enrichment factors of the aggregate size classes were calculated according to Guggenberger et al., 1994, by dividing the OC concentration of the aggregate size classes by the bulk OC concentration. Values > 1 indicated a relative carbon enrichment compared to the bulk soil, while values < 1 indicated a carbon depletion.

2.3.2 Dissolved organic carbon (Study I, III, and IV)

The dissolved OC content of the hay extract (DOM treatment) and the soil solution of the substrates and the bulk mixtures for mine-site rehabilitation before and after the incubation was determined by catalytic oxidation with a TOC analyzer (Shimadzu, Duisburg, Germany) in a soil and water solution of 1:2.5 (w:w) filtered to < 0.45 µm.

2.3.3 Organic carbon molecular characterization (Study III, IV)

The molecular composition of the OC-containing substrates and the rehabilitation mixture were analyzed by means of solid-state ^{13}C-NMR spectroscopy (Bruker, Rheinstetten, Germany) (Baldock et al., 1992). The dried and milled samples were measured by using cross-polarization magic angle spinning (CPMAS) at 6.8 kHz with 1 ms contact time and 1 s of pulse delay (Schaefer and Stejskal, 1976). Four major chemical shift regions were

integrated within the spectra to calculate the relative abundancies of alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), aryl C (110-160 ppm), and carboxyl C (160-220 ppm), including spinning side bands (Knicker et al., 2005; Wilson, 1987). The alkyl C/O-alkyl C ratio was calculated as a proxy for decomposition (Baldock et al., 1997). The molecular mixing model was applied according to (Nelson and Baldock, 2005) in order to calculate the proportions of the following six abundant biomolecules from the spectral data: carbohydrate, protein, lignin, lipid, carbonyl, and char.

2.4 Microbial community analyses

2.4.1 Microbial biomass and carbon use efficiency (Study IV)

The OC related to microbial biomass was determined by chloroform fumigation-extraction at the beginning and end of the incubation period (Vance et al., 1987). 5 g of fresh sample material was fumigated with $CHCl_3$ for 20 hrs. in a desiccator. 20 mL of 0.05 M K_2SO_4 was used for extraction, and the OC in the extracts was determined with a TOC analyzer (Shimadzu, Duisburg, Germany). The microbial biomass OC was calculated by subtracting the extracted OC of a non-fumigated control from the extracted OC of the fumigated soil. The result was divided by a correction factor of 0.45 to account for the non-extractable microbial biomass C (Joergensen, 1996). The OC related to microbial biomass growth was calculated by subtracting the microbial biomass OC at the beginning from the biomass OC at the end of the incubation period. The microbial carbon use efficiency (CUE) was calculated by dividing OC related to microbial growth by microbial uptake (the sum of OC microbial growth and OC respired as CO_2-C) (Manzoni et al., 2012; Spohn et al., 2016).

2.4.2 Microbial community composition (Study II, III)

The microbial community composition as addressed by analyzing the phospholipid fatty acid (PLFA) pattern of the soils before and after the incubation. The extraction was done according to (Baumert et al., 2018; Frostegård et al., 1991), using a Bligh and Dyer solution for lipid extraction, followed by a solid phase extraction on silica columns (Chromabond, Machery-Nagel, Düren, Germany). The lipids were transformed into fatty acid methyl esters

(FAME) by a mild alkaline methanolysis and measured by gas chromatography with flame ionization detection (GC-FID; Thermo Fisher Scientific, Waltham, USA with silica capillary column, Phenomenex Ltd., Aschaffenburg, Germany). Nine microbial PLFAs were assigned to be of bacterial origin (15:0, 17:0, a15:0, i15:0, i16:0, i17:0, 273 16:1ω7, cy17:0, cy19:0), one PLFA was assigned to be of fungal origin (18:2ω6) (Frostegård et al., 1993; Frostegård et al., 2011). Five PLFAs (14:0, 16:0, 18:0, 20:0, 18:1ω9t) were not assigned to one specific microbial group, but were still considered to be of microbial origin, leading to a total of 15 PLFAs used for the calculation of the total microbial PLFAs (Baumert et al., 2018). The fungal:bacterial$_{PLFA}$ ratio was used as an indicator for the microbial community composition. According to Kaneda, 1977, Zarnowski et al., 2004, and pre-test results, the a15:0 and i15:0 PLFAs were used as a qualitative indicator of the relative abundance of *Bacillus subtilis* necromass within the extracted PLFAs and were not taken into account for calculating bacterial growth in those mixtures.

2.5 Imaging techniques

2.5.1 Resin embedding (Study I, III)

To investigate the soil structure and pore space, randomly selected, undisturbed subsample cores and single, dry separated aggregates were embedded in resin. The embedding was done according to Herrmann et al., 2007 and Mueller et al., 2012. The cores were slowly dehydrated in a graded series of acetone:water mixtures (30%, 50%, 70%, 90%, 100% acetone) in a desiccator. Araldite 502 (Electron Microscopy Sciences, Hatfield, USA) was used for embedding. The resin infiltration was done by slow capillary saturation of a graded series of araldite:acetone mixtures (1:3, 1:1, pure araldite, v:v) and the resin blocks were cured at 60 °C for > 48 hrs.

2.5.2 X-ray microtomography (Study I, III)

The resin embedded subsample cores were scanned with an X-ray microtomograph (Zeiss Xradia Versa 520). The scanning, 3D image reconstruction, and data processing was done by Daniel Uteau at the University Kassel. The scanning was performed with a voltage of 80 kV and power of 9 W and total of 1600 projections were taken at an exposure time of 1

s. For 3D reconstruction, a beam hardening correction of 0.05 was applied, leading to a voxel resolution of 15 µm by which pores with a diameter > 60 µm (4 voxels), i.e., macropores, could be resolved. The image analysis was done with the software MAVI (Modular Algorithms for Volume Images, Fraunhofer ITWM, Kaiserslautern, Germany), implemented the ToolIP framework. A median filter was applied to the grey-scale images (window size 33 voxels) to reduce noise. Thresholds for binarization were selected by applying Otsu's algorithm (Otsu, 1979), which segmented pore space from unresoluted soil matrix. All isolated pores were labelled to calculate the ratio between the largest connected pore cluster (LP) and the total resolved porosity (TP) as an indicator for pore connectivity ($0 < LP/TP < 1$). A LP/TP value close to 1 indicates a highly connected pore space. The Euler number of each sample and of the largest pore was calculated as intensity parameter for pore complexity as it accounts for the number of self-connections (Vogel and Roth, 2001). The hydraulic diameter of the pores was measured by the maximal ball method, which locally fits the biggest inscribed sphere into the pore space. A Young- Laplace model was applied to the resulting pore size distribution to derivate a water retention curve for each sample. The van Genuchten function parameters (α, n and m) were fitted by the “nlxb”-funtion of the R-Package “nlmrt”.

2.5.3 Light microscopy (Study I, II)

The architecture of the embedded soil cores and aggregates was investigated with light microscopy. The resin embedded samples were cut into 1.5 mm slices with a diamond saw and the surface was polished with a sequence of polishing paper (coarse to fine grit) to remove scratches from the sawing.
The slices were glued onto a glass slide and examined with a reflected light microscope with a 5x to 100x objective.

2.6 Statistical analyses (Study I, II, III, IV)

Tests for detecting statistically significant differences between the microcosms and aggregates properties were performed on the measured data from the soil analyses, organic carbon analyses, and microbial community analyses that could be obtained with three replicates. In those cases, it was tested for normal distribution and homogeneity of variances using the Shapiro-Wilk test, a graphical inspection of the QQ-plots, followed by

the Bartlett test and Levene's test. A statistical significance of the differences was detected by using a one-way ANOVA in combination of a post-hoc Tukey HSD test. A significance level of $\alpha=0.05$ was chosen for all tests. The test results were calculated with RStudio using the R version 3.4.3 and the packages “stats” and “agricolae” (RCoreTeam, 2017).

The data of the imaging techniques and organic carbon molecular characterization were obtained by measuring selected samples only and were therefore presented without standard deviation.

3. RESULTS AND DISCUSSION

3.1 An incubation system for rapid and precise investigation of initial soil structure formation

The newly developed artificial soil microcosms were incubated for one month in presence of organic matter (OM) in order to initiate aggregate formation (Study I). The experimental setup proved to be suitable to maintain a stable water content in the soils throughout the incubation after a 3-day moistening phase (Study I, Supporting information 1). This showed that despite of the regular irrigation events, the physical disturbance, e.g., by changing moisture regimes, could be kept to a minimum due to the application of a constant water-tension.

The incubation in presence of OM as solid, particulate OM as well as dissolved OM solution induced a significant formation of water-stable aggregates in the artificial soils within the 30-day incubation (Study I, Figure 3).

This aggregate formation after only one month of incubation was a novel observation, as previous investigations have always been focused on longer timeframes (Pronk et al., 2012; Pronk et al., 2017; Vogel et al., 2014). This was later confirmed by a study by Rabbi et al., 2020, who also found a rapid aggregate formation after a short-term incubation of 42 days in an artificial soil experiment with glucose and cellulose addition.

Furthermore, it could be shown that the presence of OM alone can induce aggregate formation without requiring the additional impact of physical interference like stirring of the mixtures or wetting and drying cycles. This is in contrast to previous concepts, where mechanical stress was considered a prerequisite for aggregate formation in soils (Dexter, 1988; Six et al., 2004). Here, the incubation results suggest that the OM degradation by microbes in a moist environment, as revealed by the CO_2 release (Study I, Table 1), initiated the formation of water-stable aggregates originating from primary particles (Study I). This indicates a self-organization of the soil structure formation according to Young and Crawford, 2004, who suggested that microbes actively shape the soil structure towards further favorable sites for growth and exploitation, by influencing water distribution, solute flow and diffusion rate with their growth patches.

There was a predominant formation of macroaggregates, as shown by an increase in the mass proportion of this fraction from 6% to up to 88% after the incubation (Study I, Figure 3). This is in line with the theoretical aggregate hierarchy concept by Oades and Waters, 1991, which proposes the early formation of OM-stabilized large aggregates, followed by

the formation of smaller aggregates after the degradation of the initially stabilizing OM components. The initial formation of large macroaggregates could also be observed in the incubation of artificial substrate mixtures for mine site rehabilitation during Study IV and was similarly observed in natural soils at former mine sites (Pihlap et al., 2019).

In summary, the results of the 30-day short-term incubation experiment conducted for Study I showed that the experimental setup and the chosen timeframe were sufficient to induce reproducible aggregate formation in the artificial soils. This allows investigating selected process complexes within initial soil structure formation. Furthermore, it proved that the presence of organic matter alone could induce water-stable aggregate formation within one month of incubation without requiring physical interference like stirring or repeated wet-dry cycles.

3.2 The soil texture and initial particle arrangement determine the abiotic infrastructure

The three different textures (clay loam, loam, sandy loam) of the mineral mixtures that were investigated for Study II and III, shaped the underlying physical conditions of the artificial soils. Those were characterized by a higher bulk density and lower porosity at higher sand contents (Study II, Table 1). The texture also defined the pore size-related water content: the relatively larger pores in the sand-rich mixtures were associated with a lower water content and lower proportion of water-filled pore space (WFPS) (Study II, Table 1) at the given water tension of - 15 kPa during the incubation period. This corresponded to a shorter retention time, suggesting a faster exchange of the soil solution in the sand-rich microcosms (Study III).

Although the OM presence increased the water content and WFPS in almost all mixtures (Study II, Table 1 and Supplement 2), it had a weaker effect than the soil texture.

X-ray microtomography scans revealed some distinct large macropores in the clay loam microcosms without OM after the incubation (Study I, Figure 2), which appeared as isolated round holes in the scanning images. These circular holes in the matrix are probably so-called vesicular pores (Dietze et al., 2012; Peth et al., 2010) established by the initial irrigation when the advancing wetting front induced menisci forces and elevated gas pressure within the soil matrix (Study I).

The agglomeration of particles reduces the available specific surface area (SSA) that can be quantified by N_2-BET adsorption. The sum of the SSA of all used mineral components

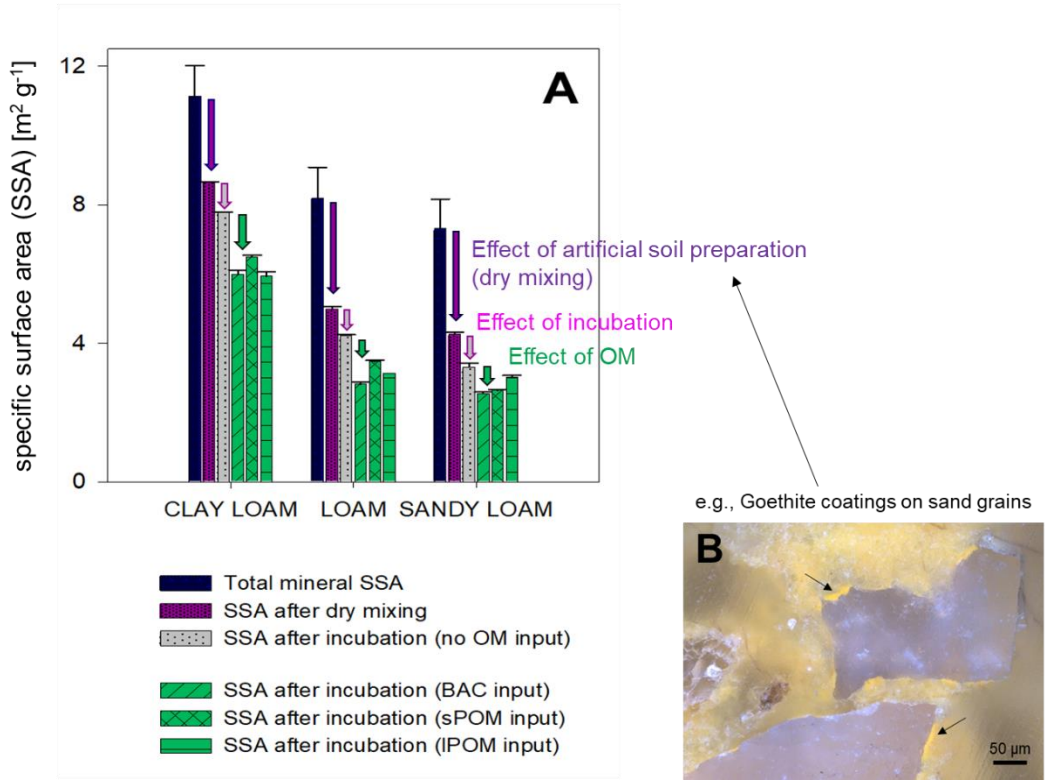

Figure 5: (A) Specific surface area (SSA) [m^2 g^{-1}] of the bulk mixtures with three different textures (clay loam, loam, sandy loam) and OM residues (BAC, sPOM, lPOM) before and after the incubation, compared to the calculated total mineral SSA in the mixtures. The colored arrows depict the effects of dry mixing and incubation in presence with and without OM on the SSA reduction (i.e., particle interactions and OM coatings). (B) Goethite containing coatings on sand grains as obtained by microscopic images. (Figure taken and modified from Study II)

was used to calculate the maximum SSA for each mineral mixture (Figure 5). The dry mixing of the mineral components during preparation of the artificial soils led to a mean SSA reduction of 34 ± 6% (Study II, Figure 2), which was the result of more particle-particle interfaces, caused by the physical contact during the mixing. The slightly higher SSA reduction in the sand-rich mixtures indicates a coating of the sand grains with finer particles (Study II). Microscopic images of the artificial mixtures (after incubation) showed a yellowish rim around the quartz grains (Study II, Supplement 6), indicating the presence of goethite in the coatings, which could later be confirmed by elemental mapping (NanoSIMS) as well. Goethite can form stable coatings on quartz grains (Scheidegger et al., 1993) with a thickness of up to tens of micrometers (Penn et al., 2001) that can act as bridge for the adhesion of illite (Guhra et al., 2019) and montmorillonite (Krause et al., 2019). However, the submerged sieving after the incubation showed no significant changes in the aggregate

size distribution in comparison to the original mixtures' textures (Study II, Figure 3). This shows that the formed abiotic particle-particle interfaces are not able to form large water-stable aggregates in the absence of OM (Study II).

In summary, the texture defined the bulk density, pore space, and water content in the soils. The dry mixing and incubation of the mineral mixtures led to particle arrangement and coatings that could be traced by SSA measurements but did not lead to the formation of large water-stable aggregates.

3.3 Biochemical processing rapidly forms water-stable aggregates

The series of incubation experiments conducted for the studies I, II, III and IV showed that the presence of various OM residues can induce a significant formation of large, water-stable aggregates in varying soil textures.

Study II showed that there could be up to 93 wt-% of the mixture material be bound in large macroaggregates (> 630 µm) after incubation (Figure 6), whereas there was never more than 17% of the available mineral SSA covered by OM (Study II, Figure 2). That surprisingly small OM covers on the mineral surface suggests the formation of some small and distinct OM spots, which are nevertheless sufficient for stabilizing a large number of water-stable aggregates. Those connections probably function like a scaffold that connects the particles and holds them in place during the submerged sieving process (Study II).

The present OM was quickly accessed and degraded by microbes in all mixtures, as shown by the CO_2-C release (Study I, Supplement 2 and Study II, Supplement 3). The microbial processing produces and accumulates OM of microbial origin (Kallenbach et al., 2016), which is able to stick to minerals and thus form aggregates (Chenu, 1989; Costa et al., 2018).

Dry crushing of macroaggregates (2-4 mm) revealed a very low mechanical stability of the particle connections, as forces < 4 N were already sufficient for a breakdown of all tested aggregates (cf. Study II, Figure 5). In contrast, aggregates isolated from mature soils with comparable texture exhibited a more than ten times higher mechanical stability (Felde et al., 2020). The OM presence increased the aggregates' tensile strength only slightly, which stronger effects in the clay-rich mixtures (Study II).

This suggests that microbial processing of OM can rapidly produce water-stable soil aggregates. Those initially formed aggregates are loosely connected structures, bound together by some distinct OM clusters acting as gluing joints that connect less than 17% of

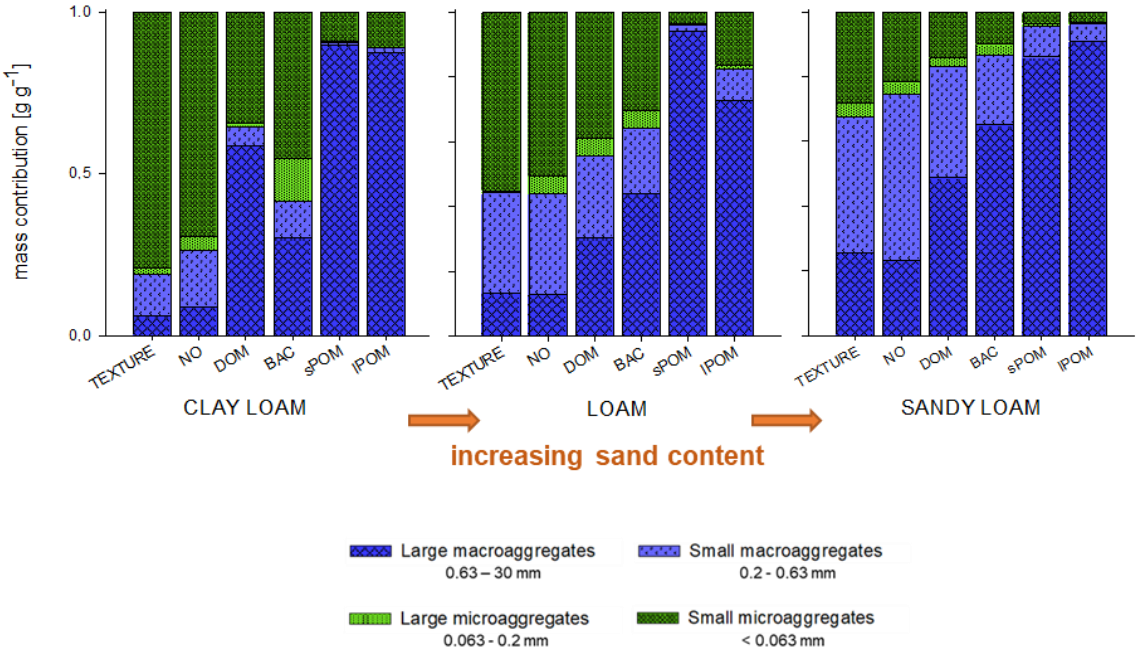

Figure 6: Formation of water-stable aggreagtes during the incubation. Mass contribution of the size fractions gained by submerged sieving after the incubation period (the first bar of each figure represents the mass contribution of mineral particles in a dispersed sample according to the mixtures texture). (Figure taken and modified from Study II)

the mineral surfaces (Study II). This solely OM-induced scaffold resists immersion in water, and thus may form large water-stable aggregates, but exhibits no stability towards mechanical forces.

3.4 Disentangling the interactions between organic matter, soil texture, and microbial community

All tested organic residues were able to induce water-stable soil structure formation. However, the used OM residue type influenced the aggregates' properties, which could be related to the effects of soil texture and differences in the developed microbial community. Figure 7 shows a general overview of the developed concept of aggregate formation taking into account the microbial community and soil texture.

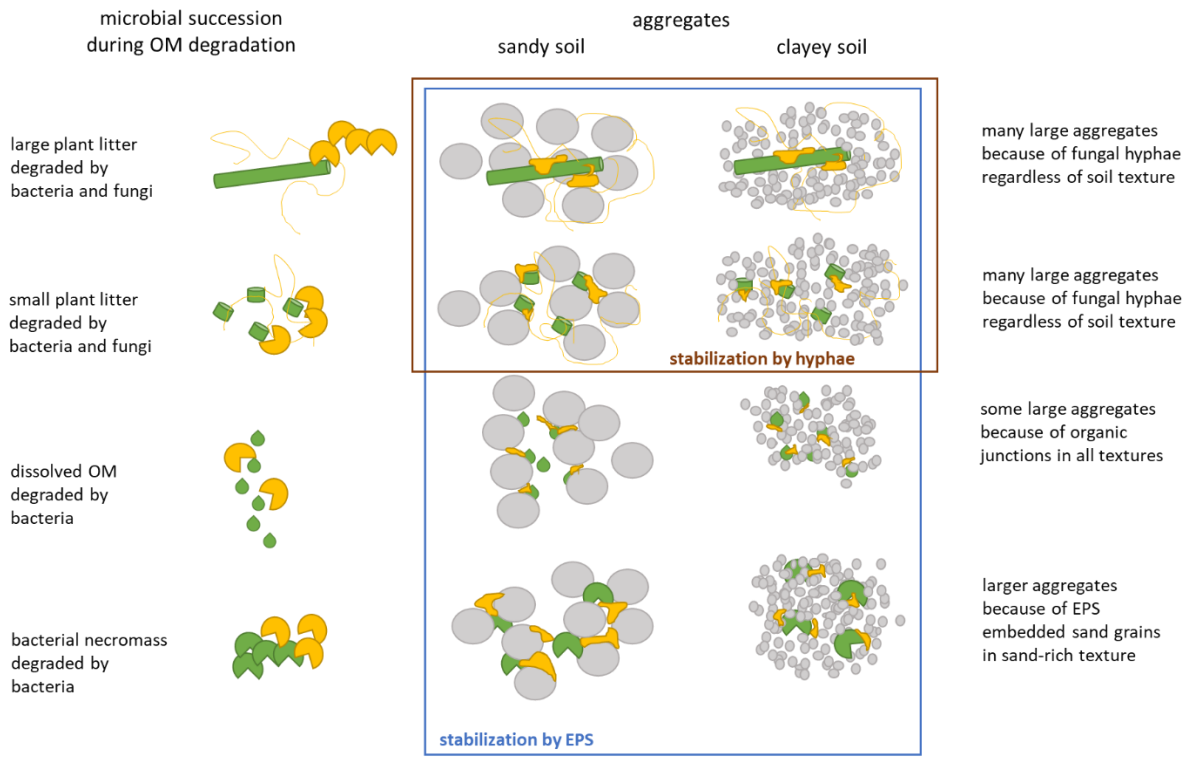

Figure 7: General concept of aggregate formation and stabilization taking into account microbial succession during organic matter degradation and soil texture.

3.4.1 Bacterial necromass: Challenging conditions for bacteria foster larger aggregates

The *Bacillus subtilis* necromass consisted of bacterial cells and colonies that were spread across the mineral matrix forming small, OC-rich substrate spots (< 20 µm) that were easily degradable by the living microbes (Study II). The relative decline of the *Bacillus subtilis*-marker PLFAs (Kaneda, 1977; Zarnowski et al., 2004) after the incubation (Study II, Supplement 4) indicated the degradation of the added necromass accompanied by the growth of other microbial taxa, but an unchanged microbial community composition dominated by bacteria (fungal:bacterial$_{PLFA}$ ratio <0.03, Study II, Table 3). The newly formed microbial biomass can directly adhere to clay particles and induce a changed microenvironment by particle attachment around the cells (Dorioz et al., 1993; Foster, 1988; Foster, 1993).

In the clay loam, fine particles dominate the mineral matrix with some interspersed sand grains, which have a greater distance from each other than in the sand-rich mixtures (3:1 volume ratio of sand grains to OM in the clay loam, compared to 10:1 in the sandy loam, Study II). Thus, there is a higher probability that the uniformly distributed OM-microbe patches are surrounded by fine particles only, leading to the formation of small structures, i.e., microaggregates (< 200 µm), as observed in the clay loam mixtures (Study II, Figure 3). However, the formation of large macroaggregates (0.63-10 mm) was more pronounced in this texture and surprisingly even higher with increasing sand contents (Study II, Figure 3). An increasing sand content caused an increasing bulk density, fewer, but larger pores and therefore a lower water content and less water-filled pore space in the mixtures (Study II, Table 1). Because bacteria are known to be located in or close to water-filled pores (Juyal et al., 2018; Ruamps et al., 2011), those initial conditions are less favorable and the microbes might invest in an extended production of extracellular polymeric substances (EPS) (Roberson and Firestone, 1992). Those EPS help microorganisms to ameliorate environmental constraints like drought stress, e.g., by acting like a sponge to store water (Benard et al., 2019; Couradeau et al., 2018; Roberson and Firestone, 1992). In the sand-rich mixtures, the necromass presence induced a higher water-filled pore space in the mixtures (Study II, Table 1), suggesting a pore filling by microbial products. The microbial products interact with mineral particles (Flemming and Wingender, 2010). If there are many sand grains available, they become connected as well (Costa et al., 2018) leading to a larger size of the formed aggregates. When the aggregates are crushed down, the breaking pattern indicates that the sand grains are prone to break off one after the other (Study II, Figure 5), because their connection is established by some small glue dots.

There can be concluded that the easily degradable bacterial necromass substrate fosters microbial growth dominated by bacterial taxa (cf. Figure 7). The newly formed OM interacts with surrounding particles, inducing small aggregates in the clay-rich and larger aggregates (> 630 µm) in the sand-rich textures.

3.4.2 Particulate OM: Fugal hyphae overrule the texture effect

The incubation in presence of POM induced the predominant formation of large macroaggregates (Study I, II). Regardless of the soil texture or the POM size, there was 72-91% of the particle mass bound in water-stable large macroaggregates (0.63-30 mm) after incubation (Study II, Figure 3).

From a microbial point of view, the large POM pieces appear as solid bridges, larger than many sand grains. A short-term incubation experiment with maize litter showed that the initial OC mineralization takes place directly on the plant residues, whereas only a minor OC amount was transported to a small, adjacent detritusphere (Védère et al., 2020). This implies that sticky microbial growth patches might establish on several, distinct locations on the POM surface. By this, the POM particles might act as a bridge that connects mineral particles over their entire length of up to 2 mm.

The existence of microbial products was revealed by very narrow C:N ratio of < 9 in the small microaggregate size fractions (< 63 µm) (Study II, Figure 4). Because the large POM pieces are too large to be found in the < 63 µm size fraction, and the leached DOC has a higher C:N ratio, the OC in those fractions suggests OM of microbial origin. A decrease in the C:N ratio from coarser to finer size fractions is well described for various developed soils (Guggenberger et al., 1994) and also for artificial soils (Kallenbach et al., 2016), but in the present study it could be shown that 30 days of incubation are sufficient for the formation of detectable microbial products (Study II).

The small POM particles had a rounded shape, which was more alike the *Bacillus subtilis* necromass than to the pole-shaped large POM pieces (Study II). However, the small POM induced -like the large POM- a predominant formation of large macroaggregates with a mass contribution > 86%, regardless of the texture (Study II, Figure 3). Thus, the aggregate formation was more influenced by the residue origin (plant- or bacteria-derived) than the residue size.

The POM of both sizes induced a microbial community composition highly influenced by fungi (fungal:bacterial$_{PLFA}$ ratio close to 1 for the small POM and > 1 for the large POM, Study II, Table 3). Fungal growth includes hyphae, which are filamentous structures that can act as stabilizing factors binding aggregates together (Baumert et al., 2018; Foster, 1988; Ivanov et al., 2020; Went and Stark, 1968). Those hyphae could be observed in microscopic images (Study II, Supplement 6). The action of those fungal hyphae has proven to be strong enough to stabilize water-stable aggregates in sand dunes (Forster, 1979; Koske and Polson, 1984), which can explain the formation of almost only large macroaggregates even in the sand-rich mixtures. However, the dry crushing revealed that this stabilization mechanism still does not lead to a high tensile strength. Even though single sand grains were less prone from breaking apart than in the aggregates induced by the bacterial necromass, there were still extremely low forces sufficient for a breakdown of the aggregates, (Study II, Figure 5).

There can be concluded that the POM of both sizes induces the predominant formation of large macroaggregates irrespective of the texture (cf. Figure 7). Those aggregates are

formed by microbial growth, forming sticky growth patches on the POM surface. In addition, the POM supports fungal growth, whose hyphae enmesh and stabilize the aggregates against slaking when immersed in water. However, both stabilization mechanisms provide no stability against mechanical pressure.

3.4.3 Dissolved OM: Aggregate formation without particulate organic nuclei

The dissolved organic matter (DOM) solution that was regularly added throughout the incubation (Study I, III) caused a significant aggregate formation in all microcosms. There were predominantly large macroaggregates formed (Study III, Figure 2). Most aggregate formation occurred in the clay-rich mixtures, but even the sand-rich mixture showed a significant aggregate formation (Study III, Figure 2). This shows that the biochemical processing of the available OM leads to the water-stable cohesion of mineral particles into large aggregates, without requiring the presence of a particulate OM nucleus. This extends the conventional understanding of aggregate formation, which is still based on nuclei of particulate OM becoming occluded and stabilized in an organo-mineral conglomerate (Golchin et al., 1994a; Oades and Waters, 1991).

Initial wetting of dry unstructured soils will create regions of water flow and no-flow soil matrix (Morales et al., 2010). This implies that at initial stage, the DOM solution is present predominately inside those flow regions, leading to spatial inhomogeneity of OC concentration (Study I and Study III). Those areas are favorable sites for microbial colonization and the establishment of biofilms that include polysaccharides, nucleic acids and proteins (Flemming and Wingender, 2010) can glue bigger aggregates together (Costerton et al., 1995; Rabbi et al., 2016; Ruamps et al., 2011).

With 0.5-1 m^2 g^{-1}, approximately 10% of the mineral surfaces were occupied by OM after the incubation (Study III, Table 1). When assuming that the OC is only available in preferential flow paths, only the pore surfaces of those pores can be taken into account for the potential sorption, creating OC-enriched layers on the pore surfaces in contrast to the OC-free surrounding matrix. In the clay-rich texture, the high abundance of clay-sized quartz particles dilutes the adsorption surface (goethite, illite, montmorillonite), possibly leading to patchy OC adsorption patterns at the pore surface. In the sand-rich texture, the combined surface area of illite, montmorillonite, and goethite occupies 84% of the total available specific surface area, the large sand grains have clay mineral and goethite coatings, and almost no clay-sized quartz grains are available to dilute the adsorption surface. This suggests that the OC sorption can occur almost on the entire pore surface (Study III).

Approximately 15% of the OC input was respired as CO_2 in all mixtures, and the same total amount of extracted PLFA in all mixtures indicated the same overall microbial biomass in all textures (Study III, Table 2). Because the microbes were introduced by a liquid inoculum, they were initially placed into the same pores of preferential flow than the DOM solution. The increased proportion of water-filled pore space in the sand-rich mixtures with DOM compared to the control indicates the presence of microbial products, causing water storage in previously air-filled large pores, which is linked to aggregate formation (Costa et al., 2018; Roberson and Firestone, 1992).

The breakdown pattern during the crushing process as recorded by counting force drops of various magnitude of gave information about the architecture of the aggregates (Study III, Figure 4). This suggests a relatively homogenous aggregate structure in the clay-rich mixtures, stabilized by many small, interconnected particles with small and separated pores in between, causing a quite force-resistant, but brittle structure. The aggregates of the sand-rich mixtures broke down with many larger force drops that were ascribed to the breaking off of the large sand grains. The sand grains are probably stabilized with small glue dots within a relatively porous structure, which makes them prone to break off gradually at very low mechanical forces.

There can be concluded that the biochemical processing of the percolating DOM was able to stabilize large macroaggregates without requiring the presence of physical OM-nuclei (cf. Figure 7). Hereby, the sorption and microbial processing of locally accumulated OC were considered as the main processes.

3.5 Rapid soil structure formation in artificial rehabilitation mixtures

For Study IV, the developed incubation setup was applied to investigate early soil structure development within a more applied setting by investigating artificial soil mixtures for mine-site rehabilitation in Australia.

The testing set-up included several mixtures of common and innovative organic carbon (OC)-containing rehabilitation substrates like paper mulch, fly ash, brown coal, and plant litter, which were mixed into the mineral overburden substrate. The incubation produced functioning microcosms that showed a rapid development of microbial activity and structure formation (Study IV).

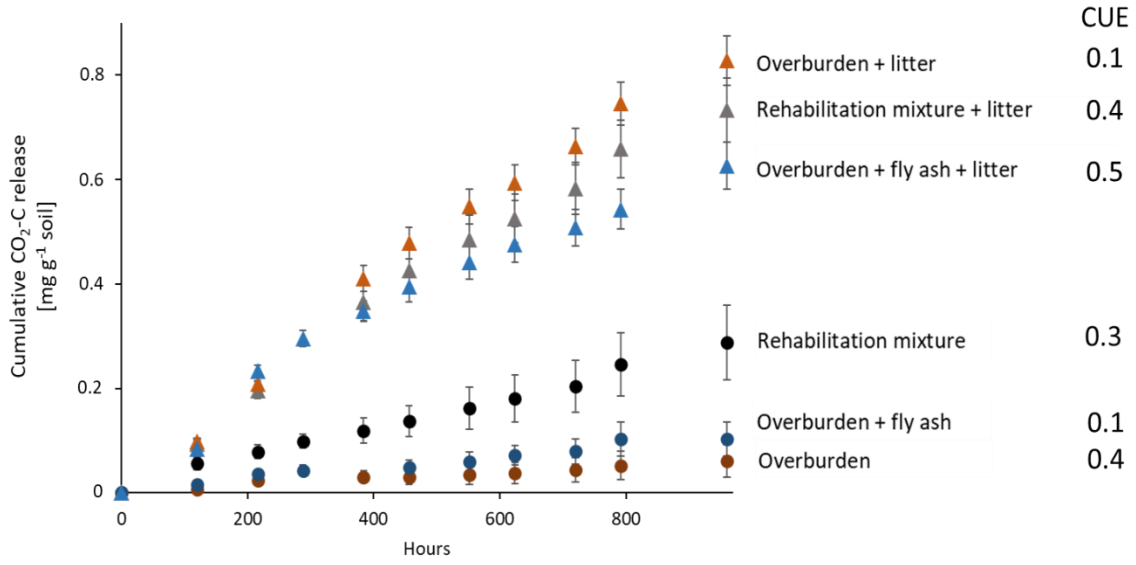

Figure 8: Cumulative CO_2-C release from the artificial rehabilitation mixtures during incubation. The CUE indicates the microbial carbon use efficiency (microbial biomass growth divided by microbial OC uptake).

The main characteristics were a constant water content after a short wetting phase, the development of microbial biomass, and the formation of water-stable aggregates (Study IV). After incubation, there were significantly more large macroaggregates (> 630 µm) present in all mixtures (Study IV, Figure 3). Most macroaggregate formation occurred in the mixtures with litter addition, where approximately 85% of the particle mass was bound in large macroaggregates after incubation, regardless of the other substrates in the mixture. Therefore, litter was the strongest driver for the formation of water-stable aggregates in these soils. The litter pieces become colonized by microbes (Aneja et al., 2006), which produce microbial products and biofilms that glue soil aggregates together (Chenu and Jaunet, 1992; Chenu et al., 2002; Costerton et al., 1995; Nunan, 2017; Rabbi et al., 2016; Redmile-Gordon et al., 2014; Spohn and Giani, 2010). In the mixtures with high macroaggregate formation, > 85% of the bulk carbon was stored in this size fraction (Study IV, Figure 3), which underlines the importance of OC for aggregate stabilization.

The complex rehabilitation mixture (containing all components except of the litter) promoted the formation of large macroaggregates to a large extent as well (Study IV, Figure 2), indicating that the added substrates provide the capacity to form soil structure, which makes the mixture promising for rehabilitation attempts under beneficial environmental conditions, e.g., without water limitation. The microbial carbon use efficiency (CUE), i.e.,

the ratio of microbial growth to OC uptake, was higher in the more complex mixture, indicating a higher OC sequestration by conversion of OC into microbial growth and products instead of CO_2 release (Figure 8). This suggests that the substrates in the complex rehabilitation mixture provide OC components linked to long-term OC storage, while still supporting microbial growth necessary for a functional early soil development. In contrast, the litter as sole amendment of the overburden induced a low microbial CUE, indicating a high OC consumption, but low microbial growth. These results show that a diverse mixture of both rapidly decomposable and more persistent substrates promises to foster both sustainable microbial development and structure formation.

CONCLUSIONS AND OUTLOOK

Within the frame of this thesis, an experimental approach was developed to elucidate the initial steps and processes of soil structure formation. The influence of various, distinct organic matter (OM) residues and soil textures was investigated. The approach was then applied to investigate those processes in artificial soil mixtures for rehabilitation attempts at former mine-sites.

The formation of water-stable soil structure was observed in the artificial soils within one month of incubation in presence of OM without requiring the influence of mechanical pressure. The biochemical processing of the added organic input is the mediating process, which leads to the predominant formation of large macroaggregates. The addition of dissolved OM was able to initiate the formation of large macroaggregates as well. This shows that macroaggregate formation is possible without the presence of particulate OM nuclei.

Mineral particles of all sizes were incorporated in the formed macroaggregates. This suggests that the OM-induced particle connections can connect mineral particles of all sizes, which even includes large sand grains.

Although abiotic particle connections were established as well and even though they accounted for the majority of the mineral surface interactions, they did not lead to the formation of large, water-stable aggregates. The water-stability of the OM-induced aggregates was established by sticky organic patches that covered less than one sixth of the available mineral surfaces. This means the aggregates' architecture resembles a loosely connected scaffold, which may hold particles in place for further stabilization processes. The aggregates exhibited no resistance to mechanical pressure. This means, there are different stabilization mechanisms responsible for water-stability and mechanical stability of the aggregates, which may also occur within a different timescale.

The soil texture defined the abiotic infrastructure of the soils. A higher sand content was accompanied by larger pores and lower water storage. All tested organic residues fostered the microbial activity to a similar extend but the residue type influenced the developed microbial community composition. Plant litter supported both fungal and bacterial growth, while dissolved OM and bacterial necromass favored bacterial growth. Despite the differences in the community composition, the microbial effect on soil structure formation can be summarized to the formation of sticky organic patches leading to water-resistant particle connections. Fungal hyphae deliver an add-on effect by binding larger structural entities together.

The interplay between the biochemical processing of the present organic residues and soil texture defined the aggregates' size. Bacterial necromass induced small and large aggregates in clay-rich soils and large aggregates in sand-rich soils. Here, the texture-dependent pore filling by microbial products causes a larger aggregate size in sand-rich soils. The dissolved OM-induced formation of large aggregates in all textures was caused by sorption and microbial processing of locally accumulated organic carbon (OC) within water-filled pores. The plant litter induced a predominant formation of large macroaggregates regardless of the soil texture and litter size. Here, the additional growth of fungal hyphae was able to bind large aggregates together.

The investigation of initial soil development in artificial soil mixtures for mine-site rehabilitation showed that the aggregate formation followed the same principles. There was a rapid formation of large macroaggregates observed, which was intensified in presence of plant litter. This means that the innovative rehabilitation mixtures support a rapid and self-organized development of soil structure in presence of OM as well as the previously tested artificial soils. This suggests that the developed concept of the OM-induced aggregation can be applied to other soils.

When the obtained observations are transferred to natural soils, it becomes evident that the separately studied OM-induced biochemical process complexes are intertwined. All selected OM residues can be present in a natural soil. However, they represented natural OM within several states of degradation from larger plant litter to smaller litter pieces, very small OM compounds < 0.45 µm, and finally microbial biomass. This implies that their occurrence might be spatially and temporally decoupled (and so are the induced processes). When OM enters a soil as plant litter, fungal and bacterial growth will be fostered, leading to the rapid formation of large aggregates stabilized by bacterial products like EPS and fungal hyphae irrespective of the soil texture. At later stages of the degradation, when the OM has been transformed into smallest components and microbial biomass, mostly bacterial growth is supported (succession of the degrading community), which implies a change into a texture-dependent aggregate stabilization mainly by extracellular polymeric substances (EPS) excreted by bacteria.

Because the microbes play an important role within the OM-induced structure formation, the process depends on beneficial conditions for microbial growth, such as a sufficient water, nutrient, air, and temperature supply. Furthermore, there is a sufficient supply of degradable OM required. When a continuous supply cannot be provided (e.g., by climatic constraints) the addition of OM substrates can be beneficial, as an OC substrate depletion might slow down or even cease microbial action, which might impair the structure formation.

For example, the results of Study IV underlined that a mixture of both rapidly decomposable and more persistent OC-containing amendments of the overburden promised a rapid soil structure development in combination with a sustainable microbial growth in the studied Australian context.
The results suggest that the biochemically induced structure formation can be expected to happen very rapidly also in natural soils. The established water-stable connections might provide resistance against water-induced erosion and can hold particles in place for further stabilization processes. However, additional stabilization mechanisms are responsible and necessary to establish the resistance against mechanical stressors, e.g., soil tillage within agricultural soil management.

The rapid establishment of initial soil structure observed in artificial substrates is a promising result encouraging the use of short-term incubation experiments in order to assess the suitability of innovative substrates or management strategies for soil development. The controlled environment of lab experiments allows the investigation of specifically manipulated variables while other influences are kept on a minimum level. There are no limits to creativity and the experiments can be designed to answer highly specific research questions or to disentangle complex interactions. Even though the experimental approach is limited to the laboratory conditions and cannot fully predict the actual soil development under natural conditions, it can help to assess the pedogenetic potential of substrates and management approaches. If key processes and main influencing factors are identified and understood, it sheds light onto the potential development awaiting the natural soils.

REFERENCES

Amezketa, E., 1999. Soil aggregate stability: A review. Journal of Sustainable Agriculture 14(2-3), 83-151.

Aneja, M.K., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J.C., Schloter, M., 2006. Microbial Colonization of Beech and Spruce Litter—Influence of Decomposition Site and Plant Litter Species on the Diversity of Microbial Community. Microbial Ecology 52(1), 127-135.

Asano, M., Wagai, R., 2014. Evidence of aggregate hierarchy at micro- to submicron scales in an allophanic Andisol. Geoderma 216, 62-74.

Atterberg, A., 1908. Om lerornas natur. Geologiska Föreningen i Stockholm Förhandlingar 30(1), 31-44.

Atterberg, A., 1917. Die Konsistenzlehre — Eine neue physikalische Lehre. Kolloid-Zeitschrift 20(1), 1-7.

Baer, S., Birgé, H., 2018. Soil ecosystem services: An overview. Managing soil health for sustainable agriculture 1, 1-22.

Baldock, J., Oades, J., Waters, A., Peng, X., Vassallo, A., Wilson, M., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state 13 C NMR spectroscopy. Biogeochemistry 16(1), 1-42.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997. Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. Soil Research 35(5), 1061-1084.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31(7-8), 697-710.

Barthes, B., Roose, E., 2002. Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels. Catena 47(2), 133-149.

Baumert, V.L., Vasilyeva, N.A., Vladimirov, A.A., Meier, I.C., Kögel-Knabner, I., Mueller, C.W., 2018. Root exudates induce soil macroaggregation facilitated by fungi in subsoil. Frontiers in Environmental Science 6, 140.

Belyaeva, O.N., Haynes, R.J., 2009. Chemical, microbial and physical properties of manufactured soils produced by co-composting municipal green waste with coal fly ash. Bioresource Technology 100(21), 5203-5209.

Benard, P., Zarebanadkouki, M., Brax, M., Kaltenbach, R., Jerjen, I., Marone, F., Couradeau, E., Felde, V.J., Kaestner, A., Carminati, A., 2019. Microhydrological niches in soils: How mucilage and EPS alter the biophysical properties of the rhizosphere and other biological hotspots. Vadose Zone Journal 18(1), 1-10.

Bimueller, C., Mueller, C.W., von Luetzow, M., Kreyling, O., Koelbl, A., Haug, S., Schloter, M., Koegel-Knabner, I., 2014. Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. Soil Biol Biochem 78, 263-273.

Blaud, A., Lerch, T., Chevallier, T., Nunan, N., Chenu, C., Brauman, A., 2012. Dynamics of bacterial communities in relation to soil aggregate formation during the decomposition of 13C-labelled rice straw. Applied soil ecology 53, 1-9.

Brockway, D., Ottrey, A., Higgins, R., 1991. The science of victorian brown coal. Durie, RA, Ed.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124(1-2), 3-22.

Brown, G., 1955. Report of the clay minerals group sub-committee on nomenclature of clay minerals. Clay Minerals Bulletin 2(13), 294-302.

Brunauer, S., Emmett, P.H., Teller, E., 1938. Adsorption of gases in multimolecular layers. Journal of the American chemical society 60(2), 309-319.

Chenu, C., 1989. Influence of a Fungal Polysaccharide, Scleroglucan, on Clay Microstructures. Soil Biol Biochem 21(2), 299-305.

Chenu, C., Jaunet, A., 1992. Cryoscanning electron microscopy of microbial extracellular polysaccharides and their association with minerals. Scanning 14(6), 360-364.
Chenu, C., Plante, A., 2006. Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the 'primary organo-mineral complex'. European Journal of Soil Science 57(4), 596-607.
Chenu, C., Stotzky, G., Huang, P., Bollag, J., 2002. Interactions between microorganisms and soil particles: an overview. Interactions between soil particles and microorganisms: Impact on the terrestrial ecosystem 1, 1-40.
Chorom, M., Rengasamy, P., Murray, R., 1994. Clay dispersion as influenced by pH and net particle charge of sodic soils. Soil Research 32(6), 1243-1252.
Chotte, J.L., Ladd, J.N., Amato, M., 1998. Sites of microbial assimilation, and turnover of soluble and particulate 14C-labelled substrates decomposing in a clay soil. Soil Biology and Biochemistry 30(2), 205-218.
Cochrane, G., Quick, G., Spencer-Jones, D., 1991. Introducing victorian geology. Melbourne, Vic., Geological Society of Australia (Victorian Division).
Costa, O.Y., Raaijmakers, J.M., Kuramae, E.E., 2018. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Frontiers in microbiology 9, 1636.
Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M., 1995. Microbial biofilms. Annual Reviews in Microbiology 49(1), 711-745.
Cotrufo, M.F., Ineson, P., Derek Roberts, J., 1995. Decomposition of birch leaf litters with varying C-to-N ratios. Soil Biology and Biochemistry 27(9), 1219-1221.
Couradeau, E., Felde, V.J., Parkinson, D., Uteau, D., Rochet, A., Cuellar, C., Winegar, G., Peth, S., Northen, T.R., Garcia-Pichel, F., 2018. In situ X-ray tomography imaging of soil water and cyanobacteria from biological soil crusts undergoing desiccation. Frontiers in Environmental Science 6, 65.
Dayton, E.A., Whitacre, S.D., Dungan, R.S., Basta, N.T., 2010. Characterization of physical and chemical properties of spent foundry sands pertinent to beneficial use in manufactured soils. Plant and Soil 329(1), 27-33.
DeJong, J.T., Soga, K., Banwart, S.A., Whalley, W.R., Ginn, T.R., Nelson, D.C., Mortensen, B.M., Martinez, B.C., Barkouki, T., 2011. Soil engineering in vivo: harnessing natural biogeochemical systems for sustainable, multi-functional engineering solutions. Journal of the Royal Society Interface 8(54), 1-15.
Dexter, A.R., 1988. Advances in Characterization of Soil Structure. Soil Till Res 11(3-4), 199-238.
Dietze, M., Bartel, S., Lindner, M., Kleber, A., 2012. Formation mechanisms and control factors of vesicular soil structure. Catena 99, 83-96.
Doran, J.W., Jones, A.J., Doran, J.W., Parkin, T.B., 1996. Quantitative Indicators of Soil Quality: A Minimum Data Set, Methods for Assessing Soil Quality. SSSA Special Publication, pp. 25-37.
Dorioz, J.M., Robert, M., Chenu, C., 1993. The role of roots, fungi and bacteria on clay particle organization. An experimental approach. Geoderma 56(1), 179-194.
Drees, L.R., Wilding, L.P., Smeck, N.E., Senkayi, A.L., 1989. Silica in Soils: Quartz and Disordered Silica Polymorphs, Minerals in Soil Environments, pp. 913-974.
Dultz, S., Woche, S.K., Mikutta, R., Schrapel, M., Guggenberger, G., 2019. Size and charge constraints in microaggregation: Model experiments with mineral particle size fractions. Applied Clay Science 170, 29-40.
Durie, R., 2013. The science of Victorian brown coal: structure, properties and consequences for utilization. Butterworth-Heinemann.
Eckelmann, W., Sponagel, H., Grottenthaler, W., Hartmann, K.-J., Hartwich, R., Janetzko, P., Joisten, H., Kühn, D., Sabel, K.-J., Traidl, R., 2006. Bodenkundliche Kartieranleitung. KA5.
Edwards, A.P., Bremner, J.M., 1967. Microaggregates in Soils. Journal of Soil Science 18(1), 64-&.

Eiland, F., Klamer, M., Lind, A.M., Leth, M., Bååth, E., 2001. Influence of Initial C/N Ratio on Chemical and Microbial Composition during Long Term Composting of Straw. Microbial Ecology 41(3), 272-280.
Elliott, E., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil science society of America journal 50(3), 627-633.
Esperschütz, J., Zimmermann, C., Dümig, A., Welzl, G., Buegger, F., Elmer, M., Munch, J.C., Schloter, M., 2013. Dynamics of microbial communities during decomposition of litter from pioneering plants in initial soil ecosystems. Biogeosciences 10(7), 5115-5124.
Felde, V.J., Schweizer, S.A., Biesgen, D., Ulbrich, A., Uteau, D., Knief, C., Graf-Rosenfellner, M., Kögel-Knabner, I., Peth, S., 2020. Wet sieving versus dry crushing: Soil microaggregates reveal different physical structure, bacterial diversity and organic matter composition in a clay gradient. European Journal of Soil Science.
Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. Nature Reviews Microbiology 8(9), 623-633.
Forster, S.M., 1979. Microbial aggregation of sand in an embryo dune system. Soil Biology and Biochemistry 11(5), 537-543.
Foster, R., 1988. Microenvironments of soil microorganisms. Biology and fertility of soils 6(3), 189-203.
Foster, R.C., 1993. The ultramicromorphology of soil biota in situ in natural soils: a review. In: A.J. Ringrose-Voase, G.S. Humphreys (Eds.), Developments in Soil Science. Elsevier, pp. 381-393.
Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14(3), 151-163.
Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59(11), 3605-3617.
Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43(8), 1621-1625.
Gaillard, V., Chenu, C., Recous, S., Richard, G., 1999. Carbon, nitrogen and microbial gradients induced by plant residues decomposing in soil. European Journal of Soil Science 50(4), 567-578.
Golchin, A., Oades, J., Skjemstad, J., Clarke, P., 1994a. Study of free and occluded particulate organic matter in soils by solid state ¹³C Cp/MAS NMR spectroscopy and scanning electron microscopy. Soil Research 32(2), 285-309.
Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994b. Soil-Structure and Carbon Cycling. Aust J Soil Res 32(5), 1043-1068.
Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. European journal of soil Science 45(4), 449-458.
Guhra, T., Ritschel, T., Totsche, K.U., 2019. Formation of mineral–mineral and organo–mineral composite building units from microaggregate-forming materials including microbially produced extracellular polymeric substances. European Journal of Soil Science 70(3), 604-615.
Harvey, H.J., Wildman, R.D., Mooney, S.J., Avery, S.V., 2020. Soil aggregates by design: Manufactured aggregates with defined microbial composition for interrogating microbial activities in soil microhabitats. Soil Biology and Biochemistry 148.
Heister, K., 2014. The measurement of the specific surface area of soils by gas and polar liquid adsorption methods—Limitations and potentials. Geoderma 216, 75-87.
Heister, K., 2016. How accessible is the specific surface area of minerals? A comparative study with Al-containing minerals as model substances. Geoderma 263, 8-15.

Herrmann, A.M., Clode, P.L., Fletcher, I.R., Nunan, N., Stockdale, E.A., O'Donnell, A.G., Murphy, D.V., 2007. A novel method for the study of the biophysical interface in soils using nano-scale secondary ion mass spectrometry. Rapid Commun Mass Spectrom 21(1), 29-34.

Horn, R., Smucker, A., 2005. Structure formation and its consequences for gas and water transport in unsaturated arable and forest soils. Soil and Tillage Research 82(1), 5-14.

Hueso-González, P., Muñoz-Rojas, M., Martínez-Murillo, J., 2018. The role of organic amendments in drylands restoration. Current Opinion in Environmental Science & Health 5, 1-6.

Ivanov, P., Manucharova, N., Nikolaeva, S., Safonov, A., Krupskaya, V., Chernov, M., Eusterhues, K., Totsche, K.U., 2020. Glucose-stimulation of natural microbial activity changes composition, structure and engineering properties of sandy and loamy soils. Engineering Geology 265, 105381.

Jardine, P.M., Weber, N.L., Mccarthy, J.F., 1989. Mechanisms of Dissolved Organic-Carbon Adsorption on Soil. Soil Science Society of America Journal 53(5), 1378-1385.

Jarvis, N.J., 2007. A review of non-equilibrium water flow and solute transport in soil macropores: principles, controlling factors and consequences for water quality. European Journal of Soil Science 58(3), 523-546.

Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biol Biochem 28(4-5), 665-676.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. Soil Biology and Biochemistry 28(1), 25-31.

Juyal, A., Eickhorst, T., Falconer, R., Baveye, P.C., Spiers, A., Otten, W., 2018. Control of Pore Geometry in Soil Microcosms and Its Effect on the Growth and Spread of Pseudomonas and Bacillus sp. Frontiers in Environmental Science 6(73).

Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry 31(7-8), 711-725.

Kaiser, K., Guggenberger, G., 2003. Mineral surfaces and soil organic matter. European Journal of Soil Science 54(2), 219-236.

Kaiser, K., Kalbitz, K., 2012. Cycling downwards–dissolved organic matter in soils. Soil Biology and Biochemistry 52, 29-32.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science 165(4), 277-304.

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature communications 7(1), 1-10.

Kaneda, T., 1977. Fatty acids of the genus Bacillus: an example of branched-chain preference. Bacteriol Rev 41(2), 391-418.

Kemper, W., Rosenau, R., 1986. Aggregate stability and size distribution. Methods of soil analysis: Part 1 Physical and mineralogical methods 5, 425-442.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral–organic associations: formation, properties, and relevance in soil environments, Advances in agronomy. Elsevier, pp. 1-140.

Knicker, H., González-Vila, F.J., Polvillo, O., González, J.A., Almendros, G., 2005. Fire-induced transformation of C- and N- forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (Pinus pinaster). Soil Biology and Biochemistry 37(4), 701-718.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34(2), 139-162.

Koske, R., Polson, W., 1984. Are VA mycorrhizae required for sand dune stabilization? Bioscience, 420-424.
Krause, L., Biesgen, D., Treder, A., Schweizer, S.A., Klumpp, E., Knief, C., Siebers, N., 2019. Initial microaggregate formation: Association of microorganisms to montmorillonite-goethite aggregates under wetting and drying cycles. Geoderma 351, 250-260.
Krause, L., Rodionov, A., Schweizer, S.A., Siebers, N., Lehndorff, E., Klumpp, E., Amelung, W., 2018. Microaggregate stability and storage of organic carbon is affected by clay content in arable Luvisols. Soil and Tillage Research 182, 123-129.
Lehmann, J., Kinyangi, J., Solomon, D., 2007. Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry 85(1), 45-57.
Lehmann, K., Schaefer, S., Babin, D., Köhne, J.M., Schlüter, S., Smalla, K., Vogel, H.-J., Totsche, K.U., 2018. Selective transport and retention of organic matter and bacteria shapes initial pedogenesis in artificial soil - A two-layer column study. Geoderma 325, 37-48.
Letey, J., 1991. The study of soil structure-science or art. Soil Research 29(6), 699-707.
Luxhøi, J., Bruun, S., Stenberg, B., Breland, T.A., Jensen, L.S., 2006. Prediction of gross and net nitrogen mineralization-immobilization-turnover from respiration. Soil Science Society of America Journal 70(4), 1121-1128.
Mackenzie, R.C., 1965. Nomenclature Sub-Committee of C.I.P.E.A. Clay Minerals 6(2), 123-126.
Mackenzie, R.C., 1975. The Classification of Soil Silicates and Oxides. In: J.E. Gieseking (Ed.), Soil Components: Vol. 2: Inorganic Components. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1-25.
Mackenzie, R.C., Mitchell, B.D., 1966. Clay mineralogy. Earth-Science Reviews 2, 47-91.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196(1), 79-91.
McCarthy, J.F., Ilavsky, J., Jastrow, J.D., Mayer, L.M., Perfect, E., Zhuang, J., 2008. Protection of organic carbon in soil microaggregates via restructuring of aggregate porosity and filling of pores with accumulating organic matter. Geochimica Et Cosmochimica Acta 72(19), 4725-4744.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111(1-3), 41-55.
Morales, V.L., Parlange, J.Y., Steenhuis, T.S., 2010. Are preferential flow paths perpetuated by microbial activity in the soil matrix? A review. Journal of Hydrology 393(1), 29-36.
Mueller, C.W., Kölbl, A., Hoeschen, C., Hillion, F., Heister, K., Herrmann, A.M., Kögel-Knabner, I., 2012. Submicron scale imaging of soil organic matter dynamics using NanoSIMS–from single particles to intact aggregates. Organic geochemistry 42(12), 1476-1488.
Nelson, P.N., Baldock, J.A., 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid-state 13C NMR and elemental analyses. Biogeochemistry 72(1), 1-34.
Nunan, N., 2017. The microbial habitat in soil: Scale, heterogeneity and functional consequences. Journal of Plant Nutrition and Soil Science 180(4), 425-429.
Nunan, N., Wu, K.J., Young, I.M., Crawford, J.W., Ritz, K., 2003. Spatial distribution of bacterial communities and their relationships with the micro-architecture of soil. Fems Microbiology Ecology 44(2), 203-215.
Oades, J., Waters, A., 1991. Aggregate hierarchy in soils. Soil Research 29(6), 815-828.
Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant and soil 76(1), 319-337.

Otsu, N., 1979. A threshold selection method from gray-level histograms. IEEE transactions on systems, man, and cybernetics 9(1), 62-66.
Paradis, A., Brueck, C., Meisenheimer, D., Wanzek, T., Dragila, M.I., 2017. Sandy Soil Microaggregates: Rethinking Our Understanding of Hydraulic Function. Vadose Zone Journal 16(9).
Penn, R., Zhu, C., Xu, H., Veblen, D., 2001. Iron oxide coatings on sand grains from the Atlantic coastal plain: High-resolution transmission electron microscopy characterization. Geology 29(9), 843-846.
Peth, S., Nellesen, J., Fischer, G., Horn, R., 2010. Non-invasive 3D analysis of local soil deformation under mechanical and hydraulic stresses by μCT and digital image correlation. Soil and Tillage Research 111(1), 3-18.
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.
Pronk, G.J., Heister, K., Ding, G.C., Smalla, K., Kögel-Knabner, I., 2012. Development of biogeochemical interfaces in an artificial soil incubation experiment; aggregation and formation of organo-mineral associations. Geoderma 189, 585-594.
Pronk, G.J., Heister, K., Vogel, C., Babin, D., Bachmann, J., Ding, G.C., Ditterich, F., Gerzabek, M.H., Giebler, J., Hemkemeyer, M., Kandeler, E., Mouvenchery, Y.K., Miltner, A., Poll, C., Schaumann, G.E., Smalla, K., Steinbach, A., Tanuwidjaja, I., Tebbe, C.C., Wick, L.Y., Woche, S.K., Totsche, K.U., Schloter, M., Kogel-Knabner, I., 2017. Interaction of minerals, organic matter, and microorganisms during biogeochemical interface formation as shown by a series of artificial soil experiments. Biology and Fertility of Soils 53(1), 9-22.
Rabbi, S.M., Daniel, H., Lockwood, P.V., Macdonald, C., Pereg, L., Tighe, M., Wilson, B.R., Young, I.M., 2016. Physical soil architectural traits are functionally linked to carbon decomposition and bacterial diversity. Sci Rep 6, 33012.
Rabbi, S.M.F., Minasny, B., McBratney, A.B., Young, I.M., 2020. Microbial processing of organic matter drives stability and pore geometry of soil aggregates. Geoderma 360, 114033.
RCoreTeam, 2017. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.
Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: Extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biology and Biochemistry 72, 163-171.
Roberson, E.B., Firestone, M.K., 1992. Relationship between Desiccation and Exopolysaccharide Production in a Soil Pseudomonas sp. Applied and Environmental Microbiology 58(4), 1284-1291.
Rosselló-Mora, R., Amann, R., 2001. The species concept for prokaryotes. FEMS Microbiology Reviews 25(1), 39-67.
Ruamps, L.S., Nunan, N., Chenu, C., 2011. Microbial biogeography at the soil pore scale. Soil Biol Biochem 43(2), 280-286.
Schaaf, W., Bens, O., Fischer, A., Gerke, H.H., Gerwin, W., Grünewald, U., Holländer, H.M., Kögel-Knabner, I., Mutz, M., Schloter, M., Schulin, R., Veste, M., Winter, S., Hüttl, R.F., 2011. Patterns and processes of initial terrestrial-ecosystem development. Journal of Plant Nutrition and Soil Science 174(2), 229-239.
Schaefer, J., Stejskal, E., 1976. Carbon-13 nuclear magnetic resonance of polymers spinning at the magic angle. Journal of the American Chemical Society 98(4), 1031-1032.
Scheidegger, A., Borkovec, M., Sticher, H., 1993. Coating of silica sand with goethite: preparation and analytical identification. Geoderma 58(1), 43-65.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88(6), 1386-1394.

Schimel, J., Schaeffer, S., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3(348).
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478(7367), 49-56.
Scholes, M.C., Powlson, D., Tian, G., 1997. Input control of organic matter dynamics. Geoderma 79(1), 25-47.
Schroeder, D., 1988. Zur Geschichte des Mineralnamens “Goethit”. Zeitschrift für Pflanzenernährung und Bodenkunde 151(2), 137-139.
Schwertmann, U., 1985. The Effect of Pedogenic Environments on Iron Oxide Minerals. In: B.A. Stewart (Ed.), Advances in Soil Science. Springer New York, New York, NY, pp. 171-200.
Schwertmann, U., Taylor, R.M., 1989. Iron Oxides, Minerals in Soil Environments, pp. 379-438.
Shen, Y.H., 1999. Sorption of natural dissolved organic matter on soil. Chemosphere 38(7), 1505-1515.
Shrestha, R.K., Lal, R., 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161(3), 168-176.
Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till Res 79(1), 7-31.
Skidmore, E., Powers, D., 1982. Dry soil-aggregate stability: Energy-based index. Soil Science Society of America Journal 46(6), 1274-1279.
Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology 25(1), 12-24.
Sparke, S., Putwain, P., Jones, J., 2011. The development of soil physical properties and vegetation establishment on brownfield sites using manufactured soils. Ecological engineering 37(11), 1700-1708.
Spohn, M., Giani, L., 2010. Water-stable aggregates, glomalin-related soil protein, and carbohydrates in a chronosequence of sandy hydromorphic soils. Soil Biology and Biochemistry 42(9), 1505-1511.
Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biology and Biochemistry 97, 168-175.
Steffens, M., Rogge, D.M., Mueller, C.W., Höschen, C., Lugmeier, J., Kölbl, A., Kögel-Knabner, I., 2017. Identification of Distinct Functional Microstructural Domains Controlling C Storage in Soil. Environ Sci Technol 51(21), 12182-12189.
Taylor, M., Yellishetty, M., Panther, B.C., 2014. Geotechnical and Hydrogeological Evaluation of Artificial Soils to Remediate Acid Mine Drainage and Improve Mine Rehabilitation–An Australian Case Study, Mine Planning and Equipment Selection. Springer, pp. 855-865.
Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. European Journal of Soil Science 33(2), 141-163.
Torsvik, V., Øvreås, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5(3), 240-245.
Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kogel-Knabner, I., 2018. Microaggregates in soils. Journal of Plant Nutrition and Soil Science 181(1), 104-136.
Tuller, M., Or, D., 2004. Retention of water in soil and the soil water characteristic curve. Encyclopedia of Soils in the Environment 4, 278-289.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19(6), 703-707.
Védère, C., Vieublé Gonod, L., Pouteau, V., Girardin, C., Chenu, C., 2020. Spatial and temporal evolution of detritusphere hotspots at different soil moistures. Soil Biology and Biochemistry 150, 107975.
Vogel, C., Babin, D., Pronk, G.J., Heister, K., Smalla, K., Kögel-Knabner, I., 2014. Establishment of macro-aggregates and organic matter turnover by microbial communities in long-term incubated artificial soils. Soil Biol Biochem 79, 57-67.
Vogel, H.J., Roth, K., 2001. Quantitative morphology and network representation of soil pore structure. Advances in Water Resources 24(3), 233-242.
Voltolini, M., Taş, N., Wang, S., Brodie, E.L., Ajo-Franklin, J.B., 2017. Quantitative characterization of soil micro-aggregates: New opportunities from sub-micron resolution synchrotron X-ray microtomography. Geoderma 305, 382-393.
Waksman, S.A., 1926. The origin and nature of the soil organic matter or soil “humus”: I. Introductory and historical. Soil Science 22(2), 123.
Went, F., Stark, N., 1968. The biological and mechanical role of soil fungi. Proceedings of the National Academy of Sciences of the United States of America 60(2), 497.
Wilson, M., 1987. NMR Techniques and Applications in Geochemistry and Soil Chemistry. vii Geological Magazine, 125. 05. Cambridge University Press.
Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. Science 304(5677), 1634-1637.
Young, I.M., Crawford, J.W., Rappoldt, C., 2001. New methods and models for characterising structural heterogeneity of soil. Soil and Tillage Research 61(1-2), 33-45.
Zarnowski, R., Ellis, R.J., Lewicka, T., Pietr, S.J., 2004. Effect of age on the fatty acid composition of the Bacillus subtilis PO270 isolated from wheat rhizosphere. Polish journal of microbiology 53(4), 267-272.

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APPENDIX

A Studies

A1. Study I

Franziska B. Bucka, Angelika Kölbl, Daniel Uteau, Stephan Peth, Ingrid Kögel-Knabner

Organic matter input determines structure development and aggregate formation in artificial soils

Geoderma 354 (2019) 113881

https://doi.org/10.1016/j.geoderma.2019.113881

A2. Study II

Franziska B. Bucka, Vincent J.M.N.L. Felde, Stephan Peth, Ingrid Kögel-Knabner

Disentangling the effects of OM quality and soil texture on microbially mediated structure formation in artificial model soils

Geoderma 403 (2021) 115213

https://doi.org/10.1016/j.geoderma.2021.115213

A3. Study III

Franziska B. Bucka, Vincent J.M.N.L. Felde, Stephan Peth, Ingrid Kögel-Knabner

“Percolating dissolved OM initiates water-stable soil structure
in various soil textures”

In preparation for publication

A4. Study IV

Franziska B. Bucka, Evelin Pihlap, Jara Kaiser, Thomas Baumgartl, Ingrid Kögel-Knabner

A small-scale test for rapid assessment of the soil development potential in post-mining soils

Soil & Tillage Research 211 (2021) 105016

https://doi.org/10.1016/j.still.2021.105016

B Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung TUM School of Life Sciences (Weihenstephan) der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel „Soil structure formation initiated by biochemical processing of organic matter“ am Lehrstuhl für Bodenkunde unter der Anleitung und Betreuung durch Frau Prof. Dr. Dr. h.c. Ingrid Kögel-Knabner ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Ab. 6 und 7 Satz 2 angebotenen Hilfsmittel benutzt habe.

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

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

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

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

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

C Scientific contributions

Peer-reviewed publications

Bucka, F.B., Felde, V.J.M.N.L., Peth, S., Kögel-Knabner, I., 2021. Disentangling the effects of OM quality and soil texture on microbially mediated structure formation in artificial model soils. Geoderma 403, 115213.

Bucka, F.B., Pihlap, E., Kaiser, J., Baumgartl, T., Kögel-Knabner, I., 2021. A small-scale test for rapid assessment of the soil development potential in post-mining soils. Soil and Tillage Research 211, 105016.

Bucka, F.B., Kölbl, A., Uteau, D., Peth, S., Kögel-Knabner, I., 2019. Organic matter input determines structure development and aggregate formation in artificial soils. Geoderma 354, 113881.

Schweizer, S.A., **Bucka, F.B.**, Graf-Rosenfellner, M., Kögel-Knabner, I., 2019. Soil microaggregate size composition and organic matter distribution as affected by clay content. Geoderma 355, 113901.

Kölbl, A., **Bucka, F.**, Marschner, P., Mosley, L., Fitzpatrick, R., Schulz, S., Lueders, T., Kögel-Knabner, I., 2019. Consumption and alteration of different organic matter sources during remediation of a sandy sulfuric soil. Geoderma 347, 220-232.

Conference contributions (first-authored)

2021

Bucka, F. B., Felde, V. J. M. N. L., Peth, S., Kögel-Knabner, I., 2021. Initial aggregate formation: Disentangling the effects of soil texture, OM properties and microbial community using artificial model soils. EGU General Assembly 2021, online. vPICO presentation

2020

Bucka, F., 2020. Processing of organic matter input influences aggregate formation in artificial soils with different texture. EGU General Assembly, Sharing Geoscience Online. Display presentation

Bucka, F., Pihlap, E., Kaiser, J., Baumgartl, T., Kögel-Knabner, I., 2020. Testing the rehabilitation potential of post-mining soils: soil organic matter, microbial biomass and aggregate formation. EGU General Assembly, Sharing Geoscience Online. Display presentation

2019

Bucka, F., Kölbl, A., Uteau, D., Peth, S., Kögel-Knabner, I., 2019. Organic matter input determines structure development and aggregate formation in artificial soils. 7th International Symposium on Soil Organic Matter, Adelaide, Australia. Oral presentation

Bucka, F.B., Kölbl, A., Uteau, D., Peth, S., Kögel-Knabner, I., 2019. Processing of organic matter input leads to aggregate formation in artificial soils. DBG annual meeting, Bern, Switzerland. Oral presentation

Bucka, F., Kölbl, A., Uteau, D., Peth, S., Kögel-Knabner, I., 2019. Organic matter treatment determines structure development and aggregate formation in artificial soils. EGU General Assembly, Vienna, Austria. Poster presentation

2018

Bucka, F., Kölbl, A., Marschner, P., Fitzpatrick, R., Mosley, L., Kögel-Knabner, I., 2018. Remediation of acid sulphate soils by addition of organic matter. EGU General Assembly, Vienna, Austria. Oral presentation

2017

Bucka, F., Schweizer, S., Graf-Rosenfellner, M., Kögel-Knabner, I., 2017. Die Lösung von organischem Kohlenstoff führt zu einer verminderten Wasserstabilität bei Mikroaggregaten in einem Ackerboden mit Texturgradienten. DBG annual meeting, Göttingen, Germany. Oral presentation

D Curriculum vitae