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New Pathways in the Isolation, Investigation, and Evaluation of  
Functional Slow- and Fast-Cycling Soil Organic Matter Fractions

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# 1 Summary

Soil organic matter consists of constituents that differ in their specific stabilization/decomposition dynamics and turnovers. From a simplified viewpoint, two fractions in particular can be distinguished from one another. The first is particulate organic matter (POM), which is predominantly unbound in the soil matrix. Its turnover relies on its inherent structural chemical stability (recalcitrance) and the occlusion within aggregates. The second is mineral-associated organic matter (MAOM), which has a significantly smaller size and is adsorbed to mineral surfaces, thereby protected from microbial decomposition. It has longer mean residence times in soil than POM. Current environmental conditions, such as climate change, require that soil organic carbon (SOC) contents be increased to provide and maintain beneficial soil functions. Since SOC stocks are determined by C input/output balances, it is important to decrease carbon (C) output quantities by increasing the long-term stabilization of SOC within MAOM-C stocks. However, MAOM-C cannot be enriched indefinitely. It is limited by the amount of clay and fine silt particle surfaces it can adsorb to and to the general land-use management. Thus, MAOM-C can be increased until there is no further enrichment or uptake in this slow-cycling SOC fraction possible, and the soils are conceived as saturated. This thesis elaborates on new methods and tools in isolating and investigating the POM-C and MAOM-C fractions, i.e., fast- and slow-cycling organic matter pools concerning their functions in the environment and different land-use systems.

Thereby, a simple and fast particle-size fractionation method was compiled and tested to isolate POM-C and MAOM-C fractions ( $>20\ \mu\text{m}$  and  $<20\ \mu\text{m}$ ) using four mechanical and one chemical dispersion approach (water, water + glass beads, ultrasonication in two different intensities and sodium hexametaphosphate). Field experiments with a shift from C3 to C4 vegetation and  $\delta^{13}\text{C}$  natural abundance measurements were used to obtain data about the soil fraction turnovers and the respective quality of isolation. Moreover, data on cost and time efficiency, as well as the carbon recovery and reproducibility of the method, were evaluated. The newly compiled simple fractionation approach was then conducted on soils from 25 agricultural long-term field experiments (LTE) in Central Europe to evaluate differences in POM-C and MAOM-C fractions under different fertilization, crop rotation, and systemic management systems. Moreover, the MAOM-C

and POM-C contributions on the total SOC were predicted for arable soils using a variety of site inherent (soil texture, climate, elevation) and management parameters (fertilization, crop rotation, cropping system, below-ground OC inputs derived by belowground plant OC allocation) and linear (mixed-effects) models. SOC sequestration deficits were calculated and depicted and an indicator based on the ratio of POM-C/MAOM-C was developed.

In a further experiment, the molecular changes of POM during decomposition were evaluated using incubated artificially composed soil cores amended with POM, i.e., either green manure or straw amended to either mineral topsoil or subsoil of a Luvisol. The cores were analyzed using a combination of elemental analyzers and solid-state  $^{13}\text{C}$  CP-MAS nuclear magnetic resonance spectroscopy (NMR), as well as imaging visible-near-infrared spectroscopy (VNIR). The VNIR spectral data and measured elemental and chemical compositions were fitted into machine-learning algorithms to obtain spectral predictors to predict and map the alkyl/O-N alkyl ratio and the C/N ratio of the amended POM during decomposition.

The results from the fractionation development showed that the physical dispersion methods were able to isolate fractions with significantly different turnovers. However, the isolation quality of water dispersion with and without glass beads was reduced in soils with high clay content, indicating that they were missing the required disruptive power to reliably disperse all aggregates. The OC recovery was high overall (>95%) in the physical dispersion approaches. However, the chemical dispersion with sodium hexametaphosphate was time-consuming and caused strong SOC leaching, which is why it was discontinued. Since dispersion with ultrasound had the best results with good recovery and reproducibility, this methodology was used to fractionate the soils from the 25 long-term field experiments.

Investigating the LTE soil fractions indicated that only well-managed coarse-textured soils and fine-textured soils with extremely high organic fertilizer application reach estimated MAOM-C saturation levels. The MAOM-C was thereby significantly influenced by the soil texture, the organic fertilization, and the below-ground OC inputs from root exudates and the root biomass. The POM-C models had low predictive power, indicating high seasonal and spatial variability. Moreover, the developed POM-

C/MAOM-C ratio might be used to assess the sustainability of agricultural management in before/after management change comparisons. Accordingly, a sharply increasing ratio indicated that the change in management did not adequately affect the long-term MAOM-C storage of soil. Thereby, a dependence between the POM-C/MAOM-C ratio and the MAOM-C sequestration deficits in the soils was discovered, where arable soils with a POM-C/MAOM-C ratio indicator  $>0.35$  are close to MAOM-C saturation. The POM-C/MAOM-C ratio of 0.35 could thus be used as an agricultural management target to avoid organic over-fertilization and N loss, especially in coarse-textured soils. Thereby, the indicator might help optimize SOC management and sequestration on arable soils and support climate change mitigation strategies.

The combined VNIR imaging spectroscopy, NMR examination, and machine learning modeling approach successfully led to predictive models with high goodness-of-fit statistics at the sub-millimeter scale. It revealed the spatial heterogeneity of the decomposition of straw and green manure POM and allowed precise mapping of the alkyl/O-N alkyl and the C/N ratio. After 180 days, the alkyl/O-N alkyl ratio of the straw POM increased slightly indicating decomposition. Both organic amendments decomposed faster when embedded in topsoils compared to POM embedded in subsoil substrates highlighting the relevance of the mineral matrix for decomposition dynamics. Moreover, the C/N ratio decreased by approximately 40 units within 180 days. However, the green manure-derived POM decomposed heterogeneously and in layers, where, after the decomposition and drop off of a certain layer, e.g., the bark, a less decomposed plant tissue layer would emerge.

The newly developed tools and approaches can improve research on POM-C and MAOM-C fractions, the understanding of OC sequestration potentials under various management regimes, and the processes involved in the decomposition of organic amendments. For the study of short- and long-cycling OC pools, this means that, in the future, a simple method for isolating these pools will be available to assess them on regional and national scales. The compiled fractionation method could thereby be implemented in existing monitoring programs. In addition, the measured MAOM-C conditions can be compared with the MAOM-C maxima calculated in the models to identify MAOM-C sequestration deficits and to take into account measures for improvement. In this context, the POM-C/MAOM-C ratio indicator might be relevant to

determine the MAOM-C saturation behavior of arable sites in the context of annual monitoring and to adjust organic fertilization accordingly to minimize sequestration deficits without over-fertilizing the soils in the long-term. Although POM-C was not successfully modeled, its decomposing dynamics were investigated using VNIR imaging techniques. As the approach of using functional chemical groups combined with VNIR imaging techniques is completely new, much more research is needed before it might be implemented in standardized monitoring procedures. However, the results are promising for the spatial investigation of molecular changes within POM during decomposition and might help dismantle the roles of recalcitrance and accessibility within the decomposition cascade.

## 2 Zusammenfassung

Die organische Bodensubstanz (OBS) besteht aus Komponenten, die sich hinsichtlich ihrer Stabilisierungs- und Zersetzungsdynamiken unterscheiden und deshalb unterschiedliche Verweilzeiten in den Böden aufweisen. Vereinfacht betrachtet können zwei Fraktionen der organischen Bodensubstanz aufgrund dieser Charakteristika unterschieden werden.

Die partikuläre organische Bodensubstanz (POM oder auch Nährhumus) liegt ungebunden in der Bodenmatrix vor. Ihre Verweilzeit im Boden ist abhängig von ihrer spezifischen Rekalzitranz, d.h. ihrer strukturechemischen Stabilität, sowie der Okklusion, der räumlich eingeschränkten Zugänglichkeit für Zersetzer durch den Einschluss der POM in Bodenaggregaten. Die mineral-assoziierte organische Bodensubstanz (MAOM oder auch Dauerhumus) ist im Verhältnis zur POM deutlich kleiner. Sie ist an mineralische Oberflächen adsorbiert und dadurch vor Zersetzung und Mineralisierung durch Mikroorganismen geschützt. Die MAOM-Fraktion weist eine durchschnittlich höhere Verweilzeit im Boden auf als die POM-Fraktion.

Da die Vorräte an organischem Kohlenstoff (C) im Boden durch C-Input/Output-Bilanzen der OBS bestimmt werden, ist es wichtig, die C-Output-Mengen zu verringern, indem die OBS langfristig in der MAOM-Fraktion stabilisiert wird. Die MAOM Fraktion ist jedoch nicht unbegrenzt anreicherbar. Die Anreicherung entspricht einer Sättigungsfunktion, die durch die Menge der zur Adsorption geeigneten mineralischen Oberflächen in den Korngrößenbereichen Ton und Schluff sowie durch die generelle Landnutzung begrenzt ist. In dieser Dissertation werden neue Methoden und Instrumente zur Isolierung und zur Untersuchung der POM- und MAOM-Fractionen im Hinblick auf ihre Funktionen in der Umwelt und in verschiedenen Landnutzungssystemen entwickelt.

Dafür wurde in Laborexperimenten eine einfache und schnelle Partikelgrößenfraktionierungsmethode erarbeitet und getestet, um POM- und MAOM-Fractionen ( $>20\ \mu\text{m}$  und  $<20\ \mu\text{m}$ ) zu isolieren, wobei vier mechanische und ein chemischer Dispergierungsansatz (Wasser, Wasser + Glasperlen, Ultraschallbehandlung in zwei verschiedenen Intensitäten und Natriumhexametaphosphat) zum Aufbrechen der

Aggregate verwendet wurden. Anhand von Feldversuchen mit einem Wechsel von C3- zu C4-Vegetation und Messungen der natürlichen  $\delta^{13}\text{C}$ -Isotopenverhältnisse wurden Daten über die Umsätze und die Verweildauer der POM- und MAOM-Fraktionen und die Isolierungsqualität der untersuchten Methoden gewonnen. Darüber hinaus wurden die Kosten- und Zeiteffizienz, die Wiederauffindung des organischen Kohlenstoffs und die Reproduzierbarkeit der Methode ausgewertet.

Der Fraktionierungsansatz mit den besten Qualitätskriterien wurde dann an Böden von 25 mitteleuropäischen landwirtschaftlichen Langzeit-Feldexperimenten (LTE) durchgeführt, um die Unterschiede in den POM- und MAOM-Fraktionen unter verschiedenen Dünge-, Fruchtfolge- und Bewirtschaftungssystemen einzustufen und bewerten zu können. Des Weiteren wurden auf den Ackerstandorten die MAOM- und POM-Fraktionen mit Hilfe standörtlicher Parameter (Bodentextur, Klima, Höhe) und Bewirtschaftungsparameter (Düngung, Fruchtfolge, Anbausystem, unterirdische OC-Einträge abgeleitet aus der unterirdischen Kohlenstoffallokation der Pflanzen) in linearen (mixed-effects) Modellen modelliert. Es wurden standortspezifische MAOM-C-Sequestrierungsdefizite berechnet und ein Indikator für die nachhaltige organische Düngung auf der Grundlage des Verhältnisses von POM-C zu MAOM-C entwickelt.

In einem weiteren Laborversuch wurden die molekularen Veränderungen der POM-Fraktion während ihres mikrobiellen Abbaus in inkubierten künstlichen Bodenkernen untersucht. Dabei wurden Gründünger und Stroh als organische Dünger der POM-Fraktion in den mineralischen Ober- und Unterboden einer Parabraunerde eingebracht. Die Kerne wurden vor und nach Inkubation mit Elementaranalysatoren,  $^{13}\text{C}$ -CP-MAS-NMR-Festkörperspektroskopie sowie bildgebender Spektroskopie im sichtbaren- und nahen Infrarotspektrum (VNIR) auf ihre chemische Struktur und entsprechende Abbauprozesse analysiert. Die VNIR-Spektraldaten und die gemessene elementare und chemische Zusammensetzung wurden in Algorithmen des maschinellen Lernens eingepasst, um spektrale Prädiktoren zur Vorhersage und Abbildung des Alkyl/O-N Alkyl-Verhältnisses und des C/N-Verhältnisses der POM während ihres Abbaus zu erhalten.

Die Entwicklung einer einfachen Fraktionierungsmethode zeigte, dass mit allen physikalischen Dispergierungsmethoden Fraktionen mit deutlich unterschiedlichen Verweildauern isoliert werden können. Allerdings war die Isolierungsqualität nach Dispergierung mit Wasser (mit und ohne Glasperlen) in Böden mit feinerer Textur deutlich geringer, was darauf hindeutet, dass nicht alle Aggregate zuverlässig aufgebrochen werden. Die Wiederauffindung der organischen Bodensubstanz im Vergleich mit nicht fraktionierten Böden war bei den physikalischen Dispergierungsmethoden sehr hoch (>95 %). Die chemische Dispergierung mit Natriumhexametaphosphat führte jedoch zu hohen OBS-Verlusten und war darüber hinaus sehr zeitaufwendig. Deshalb wurde die chemische Dispergierung mit Natriumhexametaphosphat als Dispergenz im Rahmen des Experiments nicht weiterverfolgt. Die Dispergierung mit Ultraschall wies die besten Isolierungsqualitäten für die POM und MAOM-Fraktionen auf. Die OBS Wiederfindung und die generelle Reproduzierbarkeit der Methode waren zufriedenstellend. Deshalb wurde diese Methode zur Fraktionierung der Böden aus den 25 Langzeit-Feldversuchen verwendet.

Die Untersuchung der LTE ergab, dass gut bewirtschaftete grob-texturierte Böden und fein-texturierte Böden mit extrem hoher organischer Düngung die Schätzwerte für die MAOM-C-Sättigung erreichen. Der MAOM-C wurde dabei im Modell maßgeblich von der Bodentextur, der organischen Düngung und den unterirdischen OC-Einträgen aus Wurzelexsudaten und der Wurzelbiomasse beeinflusst. Die POM-C-Modelle hatten eine geringe Vorhersagekraft, was auf eine hohe saisonale und räumliche Variabilität der POM-Fraktion zurückzuführen ist. Darüber hinaus konnte der entwickelte POM-C/MAOM-C Indikator verwendet werden, um die Nachhaltigkeit der landwirtschaftlichen Bewirtschaftung, insbesondere der organischen Düngung, in vorher/nachher Vergleichen zu bewerten. Demnach deutet ein stark ansteigendes POM-C/MAOM-C Verhältnis darauf hin, dass die Bewirtschaftungsänderung die langfristige MAOM-C-Speicherung eines Bodens nicht maßgeblich erhöht, und die Permanenz der Kohlenstoffanreicherung im Boden deshalb nicht gegeben ist. Dabei wurde eine Abhängigkeit zwischen dem POM-C/MAOM-C-Verhältnis und den MAOM-C-Sequestrierungsdefiziten in Böden festgestellt, wobei Ackerböden mit einem POM-C/MAOM-C-Verhältnisindikator  $>0,35$  sich der MAOM-C-Sättigung annähern. Das POM-C/MAOM-C-Verhältnis von 0,35 könnte daher als Zielvorgabe für die

landwirtschaftliche Bewirtschaftung verwendet werden, um eine organische Überdüngung und N-Verluste insbesondere bei Böden mit grober Textur zu vermeiden. Auf diese Weise könnte der Indikator dazu beitragen, das OBS-Management und die MAOM-C-Sequestrierung auf Ackerböden zu optimieren und ackerbauliche Strategien zur Eindämmung des Klimawandels zu unterstützen.

Der kombinierte Ansatz aus bildgebender VNIR- und NMR-Spektroskopie und maschinellem Lernen war erfolgreich und führte zu Vorhersagemodellen mit hoher statistischer Güte. Dadurch konnte die räumliche Heterogenität des Abbaus von Stroh und Gründünger in den künstlichen Bodenkernen untersucht und eine pixelgetreue Kartierung der Alkyl/O-N-Alkyl- und der C/N-Verhältnisse im Submillimeter-Bereich durchgeführt werden. Der Abbau des Gründüngers und des Strohdüngers im Oberbodensubstrat erfolgte schneller als im Unterbodensubstrat. Nach 180 Tagen Inkubationszeit stieg das Alkyl/O-N Alkyl-Verhältnis der Stroh-POM leicht an, was auf fortschreitende Zersetzung/Mineralisierung hindeutet. Außerdem verringerte sich das C/N-Verhältnis innerhalb dieser Zeit um ca. 40 Einheiten. Die aus Gründüngung gewonnene POM zersetzte sich heterogen und schichtweise, wobei nach der Zersetzung und dem Zerfall einiger Gewebe, beispielsweise der Rinde, weniger zersetzte Pflanzengewebe zum Vorschein kam.

Die neu entwickelten Instrumente und methodischen Ansätze können die Forschung an POM- und MAOM-Fraktionen sowie das Verständnis der MAOM-Sequestrierungspotentiale unter verschiedener Bewirtschaftung und der Prozesse beim Abbau organischer Dünger verbessern. Für die Untersuchung von kurz- und langfristigen OBS-Pools bedeutet dies, dass in Zukunft eine einfache Fraktionierungsmethode zur Isolierung der POM- und MAOM-Fraktionen zur Verfügung steht, die in flächendeckenden großangelegten Untersuchungen zum Einsatz kommen kann. Sie könnte somit auch in bestehende Monitoringprogramme integriert werden. Darüber hinaus können die dabei erfassten MAOM-C-Verhältnisse mit den in den Modellen berechneten MAOM-C-Maxima abgeglichen werden, um MAOM-C-Sequestrierungsdefizite zu identifizieren und Maßnahmen zur Erhöhung des Dauerhumusgehalts zu ergreifen. In diesem Zusammenhang kann auch der POM-C/MAOM-C Indikator relevant sein, um im Rahmen von jährlichen

Monitoringprogrammen das MAOM-C-Sättigungsverhalten von Ackerstandorten zu bewerten. Auf dieser Basis kann das organische Düngemanagement entsprechend angepasst werden, um einerseits Sequestrierungsdefizite zu minimieren und andererseits die organische Überdüngung von Standorten zu vermeiden. Obwohl die POM-Fraktion mit Hilfe des Datensatzes der Langzeitversuche nicht erfolgreich modelliert wurde, konnte die Abbaudynamik mit VNIR-Bildgebungsverfahren untersucht werden. Da der Ansatz der Verwendung funktioneller chemischer Gruppen in Kombination mit VNIR-Bildgebungsverfahren völlig neu ist, ist jedoch weitere Forschung erforderlich, bevor diese Methodik in standardisierten Monitorings eingesetzt werden kann. Die Ergebnisse der räumlich aufgelösten und feinskaligen Untersuchung der POM auf molekularer Ebene sind jedoch vielversprechend und können dazu beitragen, die Einflüsse der konkurrierenden Stabilisierungsmechanismen, d.h. der eingeschränkten räumlichen Zugänglichkeit auf der einen Seite und der Rekalzitranz auf der anderen Seite, auf die mikrobielle Abbaudynamik zu entschlüsseln.

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## 4 List of Studies

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

### 4.1 Study I

Christopher Just, Christopher Poeplau, Axel Don, Bas van Wesemael, Ingrid Kögel-Knabner, Martin Wiesmeier

“A Simple Approach to Isolate Slow and Fast Cycling Organic Carbon Fractions in Central European Soils - Importance of Dispersion Method”

Frontiers in Soil Science, 08.10.2021: DOI: 10.3389/fsoil.2021.692583

#### Objectives:

1. Compiling a fractionation method that isolates fast and slow-cycling organic matter using delta <sup>13</sup>C natural abundance measurements
2. Investigating the influence of different aggregate dispersion pretreatments
3. Analyzing the isolation quality, recovery, reproducibility, and time/cost expenditures

#### Summary:

1. A fractionation method was compiled using </>20 µm particle-size fractionation and ultrasonication dispersion
2. Weak aggregate dispersion with only water or water and disruptive bodies showed poor isolation qualities in fine-textured soils
3. Chemical dispersion with sodium hexametaphosphate led to low OC recovery (high OC loss)
4. The fractionation method using a 20-µm threshold and ultrasonication dispersion had significant differences within the measured turnovers between the isolated fractions and were thus able to be used in further experiments.

#### Contribution:

I developed the experimental setup, conducted the experiment, performed the laboratory analyses of the POM-C and MAOM-C fractions, processed the data, wrote the manuscript, and handled the peer-review and publishing process.

## 4.2 Study II

Christopher Just, Martin Armbruster, Dietmar Barkusky, Michael Baumecker, Michael Diepolder, Thomas F. Döring, Lorenz Heigl, Bernd Honermeier, Melkamu Jate, Ines Merbach, Constanze Rusch, David Schubert, Franz Schulz, Kathlin Schweitzer, Sabine Seidel, Michael Sommer, Heide Spiegel, Ulrich Thumm, Peer Urbatzka, Jörg Zimmer, Ingrid Kögel-Knabner, Martin Wiesmeier

“Soil organic carbon sequestration in agricultural long-term field experiments as derived by particulate and mineral-associated organic matter”

Geoderma, 12.04.2023: <https://doi.org/10.1016/j.geoderma.2023.116472>

### Objectives:

1. Applying the newly compiled fractionation method from Study I to agricultural long-term field experiments with different management to depict and compare current POM-C and MAOM-C contents.
2. Evaluating MAOM-C saturation deficits.
3. Building predictive POM-C and MAOM-C models using inherent site parameters and management criteria.
4. Evaluating the POM-C/MAOM-C ratio as an indicator for sustainable OM enrichment.

### Summary:

1. POM-C and MAOM-C contents were depicted, compared, and related to estimated saturation potentials.
2. MAOM-C was modeled with high goodness-of-fit using soil texture, organic fertilization, and plant C-allocation factor-derived below-ground OC inputs.
3. The POM-C/MAOM-C ratio can be used as a long-term indicator for the sustainability of organic fertilization.

### Contribution:

I developed the experimental setup, led the sampling, conducted the experiment, performed the laboratory analyses, processed and modeled the data, wrote the manuscript, handled the peer-review and publishing process.

### 4.3 Study III

Christopher Just, Julien Guigue\*, Siwei Luo, Marta Fogt, Michael Schloter, Ingrid Kögel-Knabner, Eleanor Hobley

\*equal first-authorship contribution

“Spatial molecular heterogeneity of POM during decomposition at different soil depths resolved by VNIR hyperspectral imaging”

European Journal of Soil Sciences, 15.12.2021: DOI: 10.1111/ejss.13207

#### Objectives:

1. Combining VNIR spectroscopy, NMR spectroscopy, elemental analysis, and machine learning modeling to model the molecular composition and decomposition dynamics of different POM amendments (organic fertilizers) within a chronosequence.
2. Using state-of-the-art imaging techniques to resolve the spatial distribution of the C/N ratio and alkyl/O-N alkyl ratio for individual OM particles at a sub-millimeter scale.
3. Depicting the influence of topsoil and subsoil substrates on the decomposition of POM in artificial soil columns.

#### Summary:

1. Combining VNIR spectroscopy, NMR spectroscopy, elemental analysis, and machine learning modeling was successful and led to models with very high goodness-of-fit statistics.
2. The spatial distribution of the alkyl/O-N alkyl ratio and the C/N ratio was resolved and depicted in graphs. The alkyl/O-N alkyl ratio and the C/N ratio changed during decomposition. The molecular composition and decomposition dynamics were disentangled showing linear decomposition in the straw amendment and heterogeneous layered decomposition in the green manure amendment.
3. POM amendments decomposed faster in topsoil substrates compared to subsoil substrates.

#### Contribution:

I participated in developing the experimental setup, supervised and instructed the laboratory work, and engaged in data processing, modeling, and writing the manuscript.

## **5 Introduction**

### **5.1 A general definition of soil organic matter and related soil functions**

Soil organic matter (SOM) includes all dead plant and animal matter in and on soils as well as their organic transformation products. It is derived from different origins and present in a variety of states covering a gradient from undecomposed to highly altered and processed (Kögel-Knabner, 2002; Kleber et al., 2015). SOM is an important component in soils and provides a variety of ecosystem functions, some of which have a positive impact on human use of the soil. It fosters soil aggregation, thus enhancing soil capacities for water storage (Lal, 2016). Moreover, it provides nutrients to the soil biome and the vegetation promoting soil health and fertility (Bossuyt et al., 2005; Kravchenko et al., 2019). Soil organic matter withdraws carbon (C) from the atmosphere and contains about two to three times more C than the atmosphere (Ciais et al., 2013), thus contributing to climate change mitigation (Bruce et al., 1999; Lal et al., 2015; Minasny et al., 2017). Soil management influences SOC stocks, and adequate management has the potential to enhance C sequestration and nutrient availability (Jarecki and Lal, 2003; Vogel et al., 2019). Therefore, soil organic matter is often considered a keystone for their sustainable use (Chenu et al., 2019; Wiesmeier et al., 2019). The major immission pathway of OM into soils is by the dead plant above- and below-ground biomass. Once there, a composting cascade begins, in which the OM is distributed within the soil and decomposed/mineralized by the soil macrofauna and soil microorganisms (Grandy and Neff, 2008; Bridgham and Ye, 2015)

### **5.2 Soil organic matter fractionation – a historical review**

The exploration of soil organic matter has a long history. The first reported studies dealing with alkaline extraction of soil organic matter fractions date back to the 18th century (Achard, 1786). In the following century, SOM fractions received attention from numerous chemists trying to extract different fractions from the soil by using a variety of chemical agents (Berzelius, 1813, 1839; Hermann, 1837, 1841; Hoppe-Seyler, 1889; as cited in Waksman, 1936). As more and more extracted fractions were described, scientists categorized them related to their color and/or specific extraction method. Amongst

countless different extractions and fraction descriptions, fulvic acids, humic acids and humins with a variety of sub-classes or overlapping classifications prevailed as the most frequently mentioned fractions (Oden, 1919a, 1919b; as cited in Waksman, 1936). They were extracted in so-called acid/base extractions, mostly using sodium hydroxide and hydrochloric acid or sulfuric acid in combination with a variety of solvents such as pyridine or potassium permanganate (Vincent et al.; Piettre, 1923; as cited in Waksman, 1936). The prevailing view was that “stable” soil organic matter with a long residence time was formed due to chemical breakdowns and the consequent formation of new stable macromolecules. The related process was called humification and deemed to be similar to the Maillard reaction, thus being indicated by dark colors (Maillard, 1913; Oden, 1919a; as cited in Waksman, 1936). In the 1930s, studies hypothesized that acid-base soil extracts were heterogeneous and artificial operational constructs that were unsuited for studies on soil-related functions and the persistence of OM. It was stated that “a system [...] merely founded upon the isolation of a few substances by arbitrary chemical procedures fails to give a picture of the true nature of humus, its origin, and its dynamic condition in the soil” (Waksman, 1936). However, the process of humification as derived by chemically extracted humins remained in the spotlight for the following decades.

Eventually, new physicochemical methods for the further analytical breakdown and fractionation of soils and SOM were developed and introduced to soil science. Sodium-containing salts were found highly effective for the flocculation of clay minerals (Tyner, 1940) by the exchange of sorbed mostly polyvalent binding cations (having small hydrated ion radius) with monovalent sodium cations (having large hydrated ion radius) (Degrève et al., 1996). Furthermore, soil dispersion with sonochemistry was introduced in the 1960s (Edwards and Bremner, 1964). Sonochemistry utilizes ultrasound, which does not directly interact with the soil or molecules within the soil solution. Instead, ultrasonication in liquids leads to the growth of cavities, i.e., implosive bubbles that collapse and emit high pressure and heat waves of up to 500 atm and 5000°C (Suslick, 1990). Thereby, adjacent aggregates are broken down by the omitted energy without any change to the chemical properties of the dispersed medium. Furthermore, fractionation techniques were improved by the introduction of halogenated hydrocarbons ( $C_2H_2Br_4$ ,  $CHBr_3$ ,  $CCl_4$ ) that allowed the separation of fractions with varying densities (Ford et al., 1969; Richter et al., 1975). Due to their toxicity, these halogenated hydrocarbons were

eventually replaced with heavy inorganic salt solutions such as sodium polytungstate (Golchin et al., 1994). In the 1970-1990s, new methods such as VNIR, short-wave infrared (SWIR), NMR, and isotope-ratio mass spectrometry (IRMS) were implemented in soil science (Condit, 1970; Baldock et al., 1992; Ben-Dor and Banin, 1995; Kögel-Knabner, 1997). Since there was neither evidence of functional uniform extract groups (humic acids, fulvic acids) nor correlations between those fractions and their general persistence and residence time within soils, the impact of humification for the stabilization of SOM and the associated methodology of acid and base extraction was eventually contested (Tiessen and Stewart, 1983; Tiessen et al., 1984; Lehmann and Kleber, 2015; Baveye and Wander, 2019).

Divergent theories emerged that decoupled the OM turnover times from the recalcitrance of the acid/base extracted fractions (Cambardella and Elliot, 1992; Christensen, 1992; von Lützow et al., 2006; von Lützow et al., 2008). OM stabilization mechanisms due to occlusion in soil aggregates, as well as protection from decomposition by association with mineral surfaces, were explored as possible C storage mechanisms using radiocarbon dating (Paul et al., 1964; Jenkinson, 1968; Jenkinson and Rayner, 1977). Particle sizes of OM were found to directly relate to C/N ratios in soils (Turchenek and Oades, 1979), with smaller particles having lower C/N ratios. Within a fixed aggregate hierarchy, different forces and processes for OM storage come into play according to the size order of the respective stabilized aggregates (Oades and Waters, 1991). By using the natural  $^{13}\text{C}$  discrimination of C3 (Calvin cycle metabolism) and C4 plants (Hatch-Slack cycle metabolism), SOM turnovers of different fractions were determined with the temporal precision of one vegetation period (Balesdent, 1987). This allowed physical and chemical fractionation methods to be investigated in terms of their precision to isolate OM fractions with specific turnovers (Cambardella and Elliot, 1992; Poeplau et al., 2018). Moreover, studies revealed a correlation between the abundance of clay (<2  $\mu\text{m}$ ) and silt (2 - 63  $\mu\text{m}$ ) mineral particles, i.e., their mineral surfaces, and the association of OM with these surfaces (Hassink, 1997; Six et al., 2002). As the abundance of clay and silt surfaces is limited within the soil, it was deduced that saturation with mineral-associated organic matter must occur as a function of the availability of mineral surfaces within the given fractions (Stewart et al., 2007).

### **5.3 Soil organic matter fractionation – a state-of-the-art review**

Today, physical and chemical fractionation methods are mostly used to separate functional SOC fractions with either different turnover times or implications for the aggregation and the soil biome. Fractionation schemes often combine physical and chemical methods (Moni et al., 2012; Poeplau et al., 2018).

Physical fractionations are classified into aggregate and particle size fractionation schemes (Oades, 1988; Moni et al., 2012; Chenu et al., 2015). Particle fractionations rely on the assumption that bio-accessibility is the main driver of SOM decomposition and, therefore, the associations between soil particles are important (von Lützow et al., 2007). Thereby they are mostly utilized to isolate organic matter fractions with different turnovers (Cambardella and Elliot, 1992; Christensen, 1992; Chenu et al., 2015). However, aggregate fractionations are used to investigate the impact of soil management on the pore space and microbial habitats of soil aggregates and the spacing between these aggregates. Thereby, density fractionations are used to isolate light SOM fractions (LF) that are not associated with soil minerals and consist of plant debris particles that are either occluded within the aggregates or are completely unbound within the soil matrix (Christensen, 1992; von Lützow et al., 2007). Particle size and particle density fractionation schemes can be combined, for example, to further investigate LF in the course of a turnover assessment (Six et al., 1998; Zimmermann et al., 2007; Steffens et al., 2009). While particle size fractionations are faster and cheaper to accomplish, density fractionations enable a more detailed view of heavy and light fractions in particular (Balesdent, 1987, 1996; Cambardella and Elliot, 1992; Hassink, 1997; Amelung et al., 1999; Rumpel et al., 2000; von Lützow et al., 2007; Moni et al., 2012; Chenu et al., 2015; Poeplau et al., 2018).

In contrast with aggregate fractionation protocols, all particle size fractionation protocols require soil aggregate dispersion. However, the effects of different dispersion methods on the isolation qualities of fractionation methods have merely been investigated. The most common dispersion methods are treatments with water, in which sometimes also disruptive bodies like glass beads are added (Balesdent, 1987; Chenu and Plante, 2006; Plante et al., 2006; Rovira et al., 2010), ultrasonication (Tiessen and Stewart, 1983; Kölbl

et al., 2005; Kaiser et al., 2012), and chemical peptization (Tyner, 1940; Six et al., 2000; Sanderman et al., 2013).

While the fields of application of the various fractionation methods vary according to research hypotheses, they are also often specialized to the characteristics of particular soil types, soil textures, and clay mineralogy (Wagai et al., 2015; Inagaki et al., 2019). Thereby, many different physical fractionation approaches may contribute to understanding the prevailing views of SOC stabilization (Lützow et al., 2006; Poeplau et al., 2018).

#### **5.4 POM-C and MAOM-C fractionation – a simplified concept**

Many studies have developed new fractionation approaches tailored to their specific needs, and in many cases, have eventually increased the number of fractions separated and thus the cost and labor involved. Although it was already known that simple particle size fractionations allowed clear conclusions to be drawn about the stabilization mechanisms and the turnover times of SOM, they did not find widespread use during the last few decades (Tiessen and Stewart, 1983; Cambardella and Elliot, 1992). Therefore, there were calls from both the scientific and applied agricultural communities to develop simple fractionation schemes that could be applied on a large scale and provide comprehensive information on fast- and slow-cycling OM pools or particulate and mineral-associated OC fractions (Lavallee et al., 2019).

Depending on the underlying soil classification system, the POM-C fraction is assigned to particle size classes of either >20, >53, or >62  $\mu\text{m}$ , and the MAOM-C fraction is assigned to particle sizes below these thresholds (Christensen, 1996; Cotrufo et al., 2019; Lavallee et al., 2019). The simplified concept relies on the observation that certain soil textures are associated with the abundance of different chemical classes and microbial pathways. The clay and silt-sized fractions contain more lipids, aliphatic carbohydrates, proteins, and amino acids and the sand-sized fraction contains more lignin and lignin subunits (Grandy and Neff, 2008). Thus, the POM-C fraction is decomposed related to either the inherent chemical recalcitrance of its biopolymers, the limited spatial accessibility (compartmentalization) due to occlusion within aggregates, or other

environmental conditions that affect the biotic and abiotic interactions and decomposition dynamics (Six et al., 2002; Marschner et al., 2008; Kleber, 2010).

Mineral-associated organic matter (MAOM) is significantly smaller and is protected from decomposition by its adsorption to mineral surfaces (Tiessen et al., 1984; Baldock and Skjemstad, 2000; Kleber et al., 2015). Furthermore, the MAOM-C fraction is deemed to be saturated according to the saturation concept (Stewart et al., 2007) and evolves around equilibrium dynamics over longer periods, so-called steady-state assumptions (Stewart et al., 2007; Castellano et al., 2015). In a large-scale approach, Poeplau et al. (2018) evaluated the isolation quality and the time and cost requirements for different fractionation methods. They showed that the isolation of silt-, clay- and sand-sized fractions is adequately successful as more sophisticated methods while being faster and more reliable. However, there is a general demand to characterize and evaluate the qualities and quantities of all work modules within a particle-size fractionation protocol, i.e., the pre-treatment of the soil, the dispersion procedure, the isolation step, and the post-treatment.

### **5.5 VNIR spectroscopy in the scientific investigation of SOM (fractions)**

VNIR spectroscopy determines the absorption of light when it meets a solid, liquid, or gas. The light is absorbed in different wavelengths dependent on the composition of the absorbent, allowing the analysis of qualitative and quantitative characteristics. The use of VNIR spectral information was introduced to soil science in the 1970s (Condit, 1970). Over the following decades, spectral analysis was established as a reliable instrument to model different chemical and physical properties of soil samples, e.g., soil organic carbon/nitrogen content, soil moisture, lignin-derived carbon, exogenous organic matter, or SOM fractions (Stoner and Baumgardner, 1981; Dalal and Henry, 1986; Ben-Dor and Banin, 1995; Rumpel et al., 2001; Peltre et al., 2011; Baldock et al., 2013). With the development of machine learning algorithms, large data sets as derived from spatially resolved hyperspectral imaging were used to model and visualize SOM along soil profiles using partial-least squares regression (PLSR), Support Vector Machines (SVM), decision tree-based algorithms, and backpropagation neural nets (Mouazen et al., 2010; Rossel and Behrens, 2010; Steffens and Buddenbaum, 2013; Hobley et al., 2015, 2018; Hobley and

Prater, 2019). Using these advanced algorithms, POM-C and MAOM-C fractions were also detected and classified (Vos et al., 2018). The logical consequence would not only be to quantitatively determine OM, POM, or MAOM using imaging hyperspectral analyses but to create models that also gather information on the functional quality, i.e., the chemical composition and the spatial variability of these organic pools.

## **5.6 Objectives and approach of the thesis**

The main goal of this publication-based thesis was to elaborate new ways and tools in the investigation of fast- and slow-cycling organic matter pools (POM-C and MAOM-C) concerning their specific functions in the environment and land-use systems. To address the primary goal, six objectives were formulated as achievable milestones in the course of the studies:

a) **Compiling a simplified SOM fractionation scheme (Study I)**

Experimental development of a quick and inexpensive SOM fractionation method that allows the quantification of fast- and slow-cycling SOC pools for different soils in consideration of different soil dispersion scenarios and time requirements. The qualities to isolate slow- and fast-cycling SOC pools were analyzed using soils from C3/C4 vegetation change field experiments and  $\delta^{13}\text{C}$ -natural abundance measurements.

b) **Quantifying fast- and slow-cycling POM-C and MAOM-C fractions on soils of long-term field experiments with known management history (Study II)**

Based on the newly compiled fractionation scheme, soils of 25 long-term field experiments with different fertilization, crop rotation, and management scenarios were sampled and checked for OC, POM-C, and MAOM-C contents. MAOM-C levels were compared under consideration of current MAOM-C saturation models and differences were depicted between arable soils and grassland soils.

c) **Understanding influential factors for the establishment of different POM-C and MAOM-C levels in arable soils (Study II)**

Factors that influence inherent site characteristics (soil texture, climate) and management regimes including fertilization and crop rotation were modeled for their significance to POM-C and MAOM-C levels. Mixed-effects modeling and leave-one-group-out cross-validation were used to establish validated predictive POM-C and MAOM-C models for agricultural soils.

d) Investigating a new POM-C/MAOM-C ratio indicator (Study II)

Testing and depicting a POM-C/MAOM-C ratio indicator for the sustainable enrichment of arable soils with slow-cycling MAOM-C and as a cross-check for local MAOM-C saturation deficits

e) Developing new tools to visualize changes in the composition and the state of decomposition of POM in soils (Study III)

VNIR hyperspectral imaging, state-of-the-art machine learning modeling, and  $^{13}\text{C}$ -NMR-Spectroscopy were combined in a newly developed approach for high-resolution mapping of the composition and decomposition of POM in incubated soil cores amended with organic matter.

f) Elucidating the composition and decomposition of POM using the newly developed synthesis of remote sensing, machine learning, and  $^{13}\text{C}$ -NMR-spectroscopy (Study III)

Evaluating the influence of soil depth and the quality of organic amendments on decomposition dynamics of POM using the newly developed combination of VNIR hyperspectral imaging, state-of-the-art machine learning algorithms, and the chemical composition of POM.

## 6 Material and Methods

### 6.1 Experimental sites and soil collection (Study I, II, III)

The scientific approaches of this thesis made it necessary to sample soils from different field experiments for all three studies. To develop a simplified fractionation method (Study I), five different soils from field experiments in central Europe: Braunschweig, Hohenheim, Jyndeavat, Rotthalmünster, and Tännikon were used. To verify differences in turnover of soil fractions, the experimental setup required soils from long-term field experiments that had undergone a change from C3 vegetation (various crops) to C4 vegetation (*Miscanthus x giganteus*, *Zea mays*, *Sorghum spec.*) featuring adjacent reference plots cultivated exclusively with C3 vegetation. The long-term experiments were selected to cover a wide soil texture gradient, covering the natural conditions of the arable soils in Central Europe. The clay content varied between 4% in Braunschweig and 29% in Tännikon, and the silt content between 6% in Jyndeavat and 75% in Rotthalmünster. The average climatic conditions in these areas are typical for temperate regions in Central Europe and range from 7.9 °C in Jyndeavat to 8.8 °C in Braunschweig, and 620 mm and 1,185 mm of precipitation in Braunschweig and Tännikon, respectively. The soil samples were collected after 17-36 years of C4/C3 plant cultivation as composite samples on ~ 300 m<sup>2</sup> grids within a depth of 10 cm. Eventually, the samples were oven dried at 60 °C and sieved to 2 mm.

Study II required soils from long-term field experiments with a well-known history of diverse management practices. Therefore, soils from 25 different agricultural LTEs from 18 experimental stations in Central Europe were sampled: Bernburg-Strenzfeld, Berlin-Dahlem, Wesseling, Dülmen, Gießen, Groß Kreutz, Hennef, Bad Lauchstädt, Kempton, Linz (AT), Müncheberg, Puch, Rauischholzhausen, Renningen, Speyer, Thyrow, Viehhausen, and Villmar. The experimental stations were chosen based on a study on LTE data (Grosse et al., 2020) concerning their fertilization and crop rotation management. Two out of twenty-five experimental sites were grasslands (Renningen, Kempton). All other LTEs comprise cultivated arable soils and are plowed to a depth of 25-30 cm. One LTE was included as a reference site for extremely high organic fertilization regimes (Bad Lauchstädt, V494).

The majority of the LTEs were planned in block and split-plot experimental designs with at least three real replications. However, some experiments do not have randomization and/or true replication due to their advanced age (Wesseling (Dikopshof), Bad Lauchstädt (Stat. Düng. Vers.), Berlin-Dahlem (BDa\_D3), Thyrow (Thy\_D6), and Puch (V22, V24, V25)). Due to their spatial and scientific proximity, the LTEs V22, V24, and V25 (Puch) were evaluated together. In study II, as in study I, care was taken to cover as wide a variety of soil textures and climatic conditions as possible with the LTEs. The soil clay contents were between 3% and 36%, the silt contents between 12% and 75%, and the sand contents between 3% and 80%. Thereby, the soil textures of the sampled LTEs represent about 90% of the soil texture classes of LTEs recorded by Grosse et al., (2020) according to the German soil classification system (Ad-hoc-Arbeitsgruppe-Boden, 2005). Moreover, they represent 76% of the major soil texture classes recorded in the German soil condition survey for arable soils (Jacobs et al., 2018). Mean annual temperatures range from 8.2 °C to 11.3 °C. Mean annual precipitation ranges from 501 mm in Bad Lauchstädt to 1223 mm in Kempten. The soils were sampled over a period of 6 weeks from February to April 2019. The soil was collected from the plowed topsoil within a depth of 0-20 cm using 100 cm<sup>3</sup> sampling cylinders and spades. The samples were oven-dried at 60 °C and sieved to 2 mm. The soil bulk density was calculated after drying at 105° C.

Study III required the use of soils that also take into account the subsoils as a study variable in the experimental layout. Soils from a field trial at the University of Bonn in Klein-Altendorf were therefore used for this study. The soil is a Haplic Luvisol derived from quaternary loess. The clay content ranged from 25% to 43%, the silt content from 50% to 68%, and the sand content from 4% to 6%. The clay content increased with increasing depth while sand and silt contents decreased. Soil samples were taken from the plowed topsoil within a depth of 0-30 cm and the upper part of the unplowed subsoils within a depth of 30-60 cm. Soil cores were prepared in 90-cm-high PVC pipes with a diameter of 7.5 cm by means of stacking (bottom to top) 20 cm of quartz sand, 30 cm of subsoil, 5 cm of mixed topsoil and subsoil, and 25 cm of topsoil. Either the top or subsoils were amended with POM per soil core. The POM amendment was either wheat straw from harvest residues or composted green manure from blended green wastes of a composting station. The organic amendments were mixed into the mineral soil in a 1:4 volumetric ratio. The rate of application was related to ongoing deep-plowing scenarios

within the field experiment. The core halves without organic amendments were packed at densities of  $1.35 \text{ g cm}^{-3}$  and  $1.5 \text{ g cm}^{-3}$  with the top and subsoil material. The organically amended core halves were packed at  $1.20 \text{ g cm}^{-3}$  and  $1.35 \text{ g cm}^{-3}$  with the amended top and subsoil material. A total of 16 soil cores were prepared to cover the following variables. Two organic amendments (straw/green manure), admixed into one of the two soil materials (top-/subsoil) within the cores, incubated for either zero or 180 days, with a total of 2 replicates (2 amendments, 2 depths, 2 incubation times, 2 replications). The soil cores were kept at  $20 \text{ }^{\circ}\text{C}$  and 60% of field capacity.

## **6.2 Soil fractionation (Study I, II)**

In Study I, the fractionation approach was compiled, which was then applied to fully describe the POM and MAOM fractions within Study II. The fractionation methodology from Study I is therefore presented below. The experiments were set up using approx. 10 g of dried soil per sample. Within the compilation of a well-functioning fractionation scheme, four physical soil dispersion methods were tested, featuring a gradient in dispersion intensity:

- a) Dispersion in 100 ml of deionized water on a flatbed horizontal shaker for 25 minutes at 250 rpm (adapted and modified from van Wesemael et al., 2019)
- b) Dispersion using 10 glass beads ( $\text{\O} 4 \text{ mm}$ ) within 100 ml of deionized water on a flatbed horizontal shaker for 25 minutes at 250 rpm (adapted and modified from Rovira et al., 2010)
- c) Dispersion using ultrasonication (Sonopuls HD2200, Bandelin electronic GmbH, Germany) in 150 ml of deionized water with a low energy input of  $100 \text{ J ml}^{-1}$  (calorimetric calibration) (according to Hassink, 1997) with a 13-mm-diameter sonotrode, an oscillation frequency of 20 kHz, and an immersion depth 15 mm (following Graf-Rosenfellner et al., 2018)
- d) Dispersion using ultrasonication (Sonopuls HD2200, Bandelin electronic GmbH, Germany) in 150 ml of deionized water with a high energy input of  $450 \text{ J ml}^{-1}$  (calorimetric calibration) (according to Schmidt et al., 1999) with a 13-mm-diameter sonotrode, an oscillation frequency of 20 kHz, and an immersion depth 15 mm (following Graf-Rosenfellner et al., 2018))

The experiment also featured a chemical dispersion method using sodium hexametaphosphate as a dispersion agent. The application of this method was canceled for a variety of issues evolving with the use of chemical dispersion agents.

- e) Chemical dispersion in 40 ml of 0.5% sodium hexametaphosphate ( $\text{NaPO}_3$ )<sub>6</sub> shaken on a flatbed horizontal shaker (following Cambardella and Elliot, 1992; Six et al., 2000; Sanderman et al., 2013)

Due to the data's incompleteness, sodium hexametaphosphate was isolatedly evaluated from the other dispersion methods. In Study II, as a result of Study I, only ultrasonication with  $450 \text{ J ml}^{-1}$  was used to disperse the soils. After the dispersion, the samples were wet sieved using  $20 \text{ }\mu\text{m}$  stainless-steel sieves mounted on a horizontal shaking sieving machine (Analysette 03.502, Fritsch GmbH, Germany) with a frequency of  $\sim 50 \text{ Hz}$  and an amplitude of 2-3 mm. The resulting fractions  $<20 \text{ }\mu\text{m}$  and  $>20 \text{ }\mu\text{m}$  contained the MAOM-C and the POM-C, respectively.

### **6.3 Determining C and N (Study I, II, III)**

The total C and N concentration were measured in all samples (bulk soils, fractions and ROI samples) and experiments using a CN analyzer (HEKAtech EuraEa 3000) and dry combustion. The samples were measured against standard references of 2,5-bisthiophen ( $\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_2\text{S}$ ) and sulfanilamide ( $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ ). The inorganic carbon content was measured using a calcimetric reaction in 4M HCl (Scheibler calcimeter). Organic C concentrations were calculated as the difference between total C and inorganic C concentrations.

### **6.4 Determining $\delta^{13}\text{C}$ , the coefficient of C4-C (*fC4*), and the isolation quality indicator (*Range fC4*) (Study I)**

To acquire information on the abundance of C3 and C4 plant material to evaluate the isolation quality of the fractionation approaches, the  $\delta^{13}\text{C}$  natural abundance signatures were measured using an isotope mass spectrometer (Delta V Advantage coupled to EA Isolink CN, Thermo Fisher Scientific, Waltham, USA). The *fC4* addresses the

incorporation of new C4-derived C into SOM fractions (Equation 1)

$$f_{C4\text{fraction}_i} = \frac{\delta^{13}C_{\text{fraction}_i (C4\text{ soil})} - \delta^{13}C_{\text{fraction}_i (C3\text{ soil})}}{\delta^{13}C_{\text{fraction}_i (C4\text{ plant})} - \delta^{13}C_{\text{fraction}_i (C3\text{ soil})}} \quad (1)$$

where  $f_{C4}$  is the factor of C4-derived C in the soil fraction<sub>i</sub> in the C4-vegetated soil and the  $\delta^{13}C$  values of the fraction<sub>i</sub> (C4 soil / C3 soil) and C4 plant refers to the  $\delta^{13}C$  values of the SOC fraction of interest in the C4 vegetated soil, the same SOC fraction in the C3 reference soil, and the  $\delta^{13}C$  value of the grown C4 plant. The  $\delta^{13}C$  reference value was defined as -12‰ (Menichetti et al., 2013). An  $f_{C4}$  of 1 indicated a sample or C fraction having all C derived from new C4-plant input. An  $f_{C4}$  of 0 indicated a sample or C fraction without any fresh C4-plant material. The difference between the  $f_{C4}$  of the POM-C fraction and the MAOM-C fraction is used as an indicator for the isolation quality of the used fractionation method (Equation 2).

$$\text{Range } f_{C4} = f_{C4\text{POM}} - f_{C4\text{MAOM}} \quad (2)$$

Thereby, a high *Range*  $f_{C4}$  indicates a high isolation quality of fractions with different turnover.

## 6.5 Fractionation and C-detection quality indicators (I, II)

In study I and II, the OC recovery was calculated as the difference between the summarized OC content of the fractions and the bulk soil. Since in Study I the fractionation scheme was not only applied but compiled, further quality indicators were obligatory. Therefore, the mass recovery was calculated. Moreover, the mass and OC reproducibility were calculated based on differences in mass (M) and OC losses between analytical replicates (Equation 3).

$$\text{Range } \frac{M}{OC_{\text{fraction}_i}} \quad (\text{in } mg\ g^{-1}\ \text{soil}) = \left| \frac{\frac{M}{OC_{\text{fraction}_i\text{replicate } 1}} \times 1000}{\frac{M}{OC_{\text{total}_i\text{replicate } 1}}} - \frac{\frac{M}{OC_{\text{fraction}_i\text{replicate } 2}} \times 1000}{\frac{M}{OC_{\text{total}_i\text{replicate } 2}}} \right| \quad (3)$$

Due to high OC losses in the chemically dispersed soils with sodium hexametaphosphate, the OC recovery was compared against an untreated control group. Additionally, the  $\delta^{13}\text{C}$  recovery was calculated to verify the validity and consistency of the measured  $\delta^{13}\text{C}$  values (Equation 4).

$$\delta^{13}\text{C}_{recovery} = 100 - \left( \frac{100}{\delta^{13}\text{C}_{bulk}} \right) \times \left( (M\%_{fraction1} \times \delta^{13}\text{C}_{fraction1}) + (M\%_{fraction2} \times \delta^{13}\text{C}_{fraction2}) - \delta^{13}\text{C}_{bulk} \right) \quad (4)$$

where  $\delta^{13}\text{C}_{bulk/fraction}$  is the  $\delta^{13}\text{C}$  value of a certain bulk soil or fraction and  $M\%_{fraction}$  is the mass percentage of a certain fraction on their referring bulk soil without consideration of the mass loss.

The  $\delta^{13}\text{C}$ -recovery comprises all the errors of mass and OC loss and is therefore a very conservative quality indicator with high expressive power.

Moreover, Study I assessed the costs and workloads of the tested dispersion approaches, manual and automated sieving, and different drying techniques using drying cabinets, freeze dryers, and rotary evaporators.

## **6.6 Variable selection and definition for POM-C and MAOM-C modeling (Study II)**

As target variables for the predictive modeling of POM-C and MAOM-C of the LTE soils, POM-C and MAOM-C contributions were calculated as the measured SOC concentration of the fractions  $<20 \mu\text{m}$  and  $>20 \mu\text{m}$ , multiplied by the mass coefficient of the respective fractions on the entire soil sample (Study II, Equations 1+2). Inherent site variables (MAT, MAP, elevation, and texture), management variables (mineral and organic fertilization, belowground OC inputs as derived by crop rotation and C allocation factors), and logical interactions were used for predictive POM-C contribution and MAOM-C contribution (both in  $\text{mg g}^{-1}$ ) models (Study II, Table 2). Specific elemental fertilization inputs (OC, N, P, K) were either directly set and measured by the LTE administrators or calculated with fertilization rate tables (Paulsen et al., 2013; DLG e.V. Fachzentrum Landwirtschaft, 2017; Landesanstalt für Landwirtschaft Bayern, 2021). C

allocation coefficients were used to estimate below-ground OC inputs (Bolinder et al., 2007, 2015). Thereby, the net primary productivity (NPP) of different crops is calculated proportionally for the harvested product, the crop residues, the stubbles, the roots, and OC-containing root exudates (Equation 5):

$$NPP_{cultivar} = NPP_{yield} + NPP_{residues} + NPP_{stubble} + NPP_{root} + NPP_{exudates} \quad (5)$$

where  $NPP_{yield}$  are the crop yields,  $NPP_{residues}$  are the crop residues,  $NPP_{stubble}$  are the stubble residues after harvest,  $NPP_{root}$  is the root biomass and  $NPP_{exudates}$  are the root exudates (all quantified in  $t\ ha^{-1}\ a^{-1}$ ).

The input of plant-derived OC into an agricultural system can then be calculated using the crop-specific C allocation coefficients based on recently determined values valid for Central Europe (Jacobs et al., 2018) and the yield biomass ( $NPP_{yield}$ ) (Equations 6,7,8,9):

$$NPP_{residues} = \frac{AC_{residues}}{AC_{yield}} \times NPP_{yield} \quad (6)$$

$$NPP_{stubble} = \frac{AC_{stubble}}{AC_{yield}} \times NPP_{yield} \quad (7)$$

$$NPP_{root} = \frac{AC_{root}}{AC_{yield}} \times NPP_{yield} \quad (8)$$

$$NPP_{exudates} = \frac{AC_{exudates}}{AC_{yield}} \times NPP_{yield} \quad (9)$$

where  $AC_{yield}$ ,  $AC_{residues}$ ,  $AC_{stubble}$ ,  $AC_{root}$ , and  $AC_{exudates}$  are the allocation coefficients of the yield, the crop residues, the stubble, the root biomass, and the root exudates as derived from Jacobs et al. (2018).

The below-ground OC inputs were calculated for each crop as the sum of  $NPP_{root}$  and  $NPP_{exudates}$  and averaged over 10 years (2009-2018).

## 6.7 Experimental plot classification (Study II)

To make the different treatments of the LTE in Study II comparable, they were classified into different categories. The groups “organic fertilization”, “mineral fertilization” and “mixed fertilization” contain treatments that were fertilized in the named manner without any general changes in management or crop rotation compared to their corresponding null variants. The group “system” includes treatments that have variable fertilization and crop rotations or general changes in management, e.g., conversion to organic farming compared to their corresponding null variants. In the “control” group the corresponding unfertilized null variants were combined, if available. Due to their particularly high OC content, grasslands were presented as a separate group. However, the groups were only descriptively evaluated using graphs, as statistical comparability is not expedient due to the different LTE designs.

## 6.8 VNIR hyperspectral imaging (Study III)

Before hyperspectral imaging of the artificial soil columns was carried out, the columns were divided into two longitudinal halves and dried at room temperature. A Hypsplex-VNIR-1800 camera (Norsk Elektro Optikk, Norway) was used for imaging the inside surfaces of the illuminated core halves. The lens featured a field of view (FOV) of 9 cm. With 1,800 detectors, the spatial resolution was approx. 53 x 53  $\mu\text{m}^2$ . For each pixel, the intensity of the light reflection was measured in 186 spectral bands. The spectral bandwidth was 400-990 nm. This results in a spectral resolution of 3.17 nm per band. The spectral intensity (I) of the raw data was normalized for each wavelength ( $\lambda$ ) and pixel (x) to a calibration target with a calibrated reflectance (R) of 50% (Equation 10).

$$R_{Sample,\lambda,x} = \frac{I_{Sample,\lambda,x}}{I_{Target,\lambda,x}} \cdot R_{Target,\lambda,x,defined} \quad (10)$$

Areas featuring light overexposure, shadow, background, and the plastic pipes were masked using the software ENVI version 5.2 (L3Harris Geospatial Solutions, Broomfield, USA).

### **6.9 VNIR POM classification and sampling in soil cores (Study III)**

After preprocessing and masking, the remaining pixels were classified into two groups containing either mineral soil or POM using a spectral angle mapper classification algorithm within the ENVI software. Then, a principal component transformation was applied to the images. Thereby, regions of contrasting variance were identified within the first three principal components, accounting for more than 95% of the spectral variability. In the soil cores amended with straw, two regions of interest (n=16) were sampled. Due to the greater spectral heterogeneity of the green manure amendments, the number of samples was increased to n=31 within these soil cores. A micro spatula was used for POM sampling. The average ROI includes  $9,267 \pm 1,478$  pixels, which corresponds to an area of  $26.0 \pm 4.2$  mm<sup>2</sup>. The sampled POM was milled using mortar and pestle for further analysis.

### **6.10 Solid-state <sup>13</sup>C CP-MAS-NMR spectroscopy (Study III)**

Solid-state <sup>13</sup>C-CPMAS-NMR spectra of the ROI samples were obtained at a frequency of 50.3 MHz (Bruker DSX 200). The powdered samples were measured in zirconium dioxide rotors and spun at a magic angle under a magnetic field of 6,800 Hz with an acquisition time of 0.01024 s. To prevent Hartmann-Hahn mismatches, a ramped <sup>1</sup>H pulse was applied during a 1 ms contact time. An average of 5,500 scans were accumulated in 1 s intervals dependent on the mass and carbon content of the sample. As a reference for the chemical shifts, Tetramethylsilane was equalized at 0 ppm. A Fourier transformation was applied to the spectra for phase and baseline correction. The spectra were integrated into chemical shift regions (Beudert et al., 1989) and modified to the specifications of the device (Mueller and Koegel-Knabner, 2008). The chemical shift regions were defined as -10 to 45 ppm (alkyl C), 45 to 110 ppm (O/N alkyl C), 110 to 160 ppm (aromatic C), and 160 to 220 ppm (carboxyl C). Spinning sidebands were included. As a determinant of the decomposition state of the POM, the ratio of alkyl C to O/N alkyl C was calculated (Baldock and Preston, 1995; Baldock et al., 1997).

### **6.11 Statistical analysis (Study I)**

All calculations were made using the software R (versions 3.2 to 4.2). To test for the

differences in the isolation qualities of the fractionation procedures, Kruskal-Wallis tests and analysis of variance models were used. Multiple comparisons were carried out using Tukey's Honestly Significance Difference tests from the package "multcomp" (Hothorn et al., 2008). Fisher's t-test was applied to test for mean value differences within the proportion of C4-derived carbon of the fractions <20 and >20  $\mu\text{m}$ . Chemical leaching due to the use of chemical dispersing agents was evaluated using Welsh's t-test for data with unequal variance. The data were checked for Gaussian distribution and homoscedasticity using Shapiro-Wilk tests on either the variables or the residues. The significance level was defined as  $p < 0.05$  for all tests.

## **6.12 Statistical analysis and modeling (Study II)**

To model typical MAOM-C and POM-C contributions on commonly managed arable sites, the grassland sites and Chernozems were excluded after residual inspection. Eventually, linear models used for the POM-C models and mixed effect models (Bates et al., 2015) with a random intercept for site effects were used to obtain significant influential predictors for the MAOM-C models. To avoid multicollinearity, variance inflation factors (VIF) were calculated and variables with  $VIF > 3$  were excluded from the models. The models were simplified by eliminating non-significant interactions (Study II, Table 2) followed by non-significant main predictors (Crawley, 2012). Only significant fixed effects were considered in the final models. For the POM-C model, coefficients of determination were calculated. For the MAOM-C mixed-effects models, marginal ( $R^2_m$ ) and conditional pseudo-coefficients ( $R^2_c$ ) were calculated to explain the variance of fixed effects ( $R^2_m$ ) and the combination of fixed effects and random effects ( $R^2_c$ ) (Nakagawa and Schielzeth, 2013).

The independent variables were checked for normality using a Lilliefors test and a graphical inspection of QQ plots. A variable transformation was applied when necessary. Minimal models were checked for homoscedasticity. Due to the absence of crop yield data to calculate below-ground OC inputs, some LTE and observations were excluded from the models. The final MAOM-C model contained 66 observations; each averaged out of 2 to 3 replicates from 11 sites.

Moreover, the correlation of MAOM-C and the C/N ratio of the fraction <20  $\mu\text{m}$  was checked to evaluate the influence of micro-POM <20  $\mu\text{m}$  as an error source given by the

particle-size fractionation protocol. To visualize the effects of soil texture (particles <20  $\mu\text{m}$ ) and management effects on the MAOM-C contributions, linear models and quantile regression models (Koenker, 2005) were applied. Thereby, modeled sites and the grassland and Chernozem site excluded from the modeling data set were depicted against estimated MAOM-C saturation levels derived from relevant literature (Hassink, 1997; Six et al., 2002).

According to the named literature, the MAOM-C saturation potential was defined as the difference between current MAOM-C content and the highest MAOM-C saturation levels (cultivated soils, mean + 95% confidence interval boundary in Six et al., (2002)) given as (Equation 11):

$$MAOM - C_{potential} (mg g^{-1}) = 4.15 + 0.5 \times fraction < 20 \mu m (\%) - MAOM - C_{actual} (mg g^{-1}) \quad (11)$$

To identify the relevance of a POM-C/MAOM-C ratio indicator, the POM-C/MAOM-C ratio was plotted against the MAOM-C saturation potential.

### 6.13 Statistical analysis and modeling (Study III)

The C/N and alkyl ratios of the VNIR-mapped soil columns were modeled using the obtained VNIR reflectance spectra after z-transformation (standard normal variate) as predictors in artificial neural networks and random forest algorithms. Before modeling, the ground material spectra were compared to the reflectance spectra before grinding as a consistency check using Euclidean distance. Eventually, five samples with a Euclidean distance >1.5 and four samples with spectra corresponding to light overexposure or shadows were removed, and 4 samples from a core replicate with spectral scattering were removed from the modeling data set. The final model training data contained 34 ROI samples. The mean spectra were calculated out of each ROI sample, extracted, and fitted as predictors for the C/N and alkyl ratio.

The modeling was conducted using two algorithms; an unconstrained random forest (RF) algorithm from the “party” package (Hothorn et al., 2006), which was then optimized for the number of decision trees, and a random ensemble of fully connected artificial neural

networks (ensemble ANN) and optimized for the number of models within the ensemble. The ANN modeling was conducted using the “neuralnet” package (Fritsch et al., 2019). Both model results were evaluated using weighted and unweighted estimate averaging, where the weighting applied proportional to the goodness-of-fit of the respective model estimates.

The random ensemble ANN was compiled using randomized initial starting weights for each participating ANN model and an architecture of two hidden layers with a randomized number of nodes. The randomization for the first layer obtained 3 to 185 nodes and 2 to  $x-1$  in the second layer, where  $x$  is the number of nodes given in the first layer. The ensemble ANN model is preferential to singular ANN models due to the high sensitivity and instability caused by the initialization parameters (Kolen and Pollack, 1990). The randomized structure was selected to prevent the overfitting of the acquired models. The random ensemble was compiled using  $n$ -out-of- $n$  bootstrapping and was independently optimized for the number of models for each of the variables using the out-of-bag samples in each bootstrap. Goodness-of-fit was calculated using the mean squared error of prediction (MSE), the coefficient of determination. The final number of models being used in the ensemble models was determined according to the stability of the variance with an increasing number of models. The target variables within each ANN model were scaled to a range of 0-1 before the fitting and the estimates were transformed back into the original scale after prediction. The final model evaluation was carried out using  $n$ -out-of- $n$  bootstrap estimation for the RF and the ensemble ANN out-of-bag estimates using the coefficient of determination, the root-mean-squared error (RMSE), and the mean average error (MAE). Moreover, for the ANN, not only the mean out-of-bag estimates were evaluated, but also the median out-of-bag estimates.

The predictive models were applied to the pixels classified as POM material, and the alkyl ratio, as well as the C/N ratio, were predicted. The plausibility of the data was checked against the calibration range and against data published in diverse literature.

The changes in the chemical characteristics were checked for the different treatments (incubation time, type of amendment, top-, or subsoil using 3-way ANOVA and Kolmogorov-Smirnov non-parametric tests).

## 7 Results and Discussion

### 7.1 A simple method to isolate fast and slow-cycling organic matter – Compilation of a fractionation method (Study I)

The isolated fractions  $<20\ \mu\text{m}$  had significantly different C<sub>4</sub>-derived organic carbon content, indicating that the isolated fractions had been matching the POM-C and MAOM-C pool with different turnovers (Study I, Figure 2) to a satisfying degree. However, differences in the isolation quality parameter were observed for both – the different dispersion methods and the soil texture gradient – with a high *Range fC<sub>4</sub>* indicating a high quality of isolation. The fractionation of coarse-textured soils had, in general, lower isolation quality, while isolation was significantly more accurate in fine-grained soils (Study I, Figure 3). Clay particles organize themselves in very stable micro-aggregates (Tisdall and Oades, 1982; Wagner et al., 2007) that can bond to other micro-aggregates, also increasing the macro-aggregation of the soil (Krause et al., 2018; Schweizer et al., 2019). Moreover, the weaker dispersion methods using only water with or without glass beads had lower isolation quality, indicating that they lacked the disruptive power to fully break up the soil aggregates (Study I, Figure 3). Likely, weaker dispersion methods using water and glass beads were not capable of breaking up the soil macro-aggregates. This assumption is backed up by the data, showing that the shift in the isolation quality (*Range fC<sub>4</sub>*) is mostly based on changes in the C<sub>4</sub>-derived OC within the fraction  $>20\ \mu\text{m}$ .

In contrast, the fraction  $<20\ \mu\text{m}$  barely changed in the use of the different dispersion approaches. That means that the most relevant criterion for high-quality isolation of POM and MAOM fractions is the degree of purity of the POM fraction ( $>20\ \mu\text{m}$ ). In the comparison of the low and high energy input ultrasonication methods, higher energy input implies increasing proportions of new C<sub>4</sub> plant material within the POM fraction ( $>20\ \mu\text{m}$ ) and the MAOM fraction ( $<20\ \mu\text{m}$ ). While the higher energy input disrupts more micro aggregates and shifts clay and silt particles with adsorbed old C<sub>3</sub> plant carbon to the fraction  $<20\ \mu\text{m}$ , this also indicates that new C<sub>4</sub> plant POM particles partly get broken down to a size of  $<20\ \mu\text{m}$  (micro-POM). One solution for this issue could be to compare the C/N ratios of the resulting POM-C and MAOM-C fractions. As MAOM-C is attached to mineral soil with low C/N ratios; one would expect the MAOM-C fractions C/N ratio to lie between 8-12, while the fibrous POM fractions have higher C/N ratios dependent on their origin and state of decomposition.

Comparing the isolation quality indicator to the results of a study using a variety of different chemical and physical fractionation approaches on similar soils showed that having more particle-size fractions isolated does not necessarily lead to better results (Rovira et al., 2010; Poeplau et al., 2018), while eventually increasing the amount of work and the procedural costs involved. Methods that included chemical agents for density fractionation (sodium polytungstate), SOM oxidation ( $H_2O_2$ ,  $NaOCl_2$ ), or alkaline extraction (sodium diphosphate) within the fractionation protocols (Balesdent, 1987; Leifeld and Kögel-Knabner, 2001; Zimmermann et al., 2007; Sollins et al., 2009; Kaiser et al., 2016, as tested in Poeplau et al., 2018) were likely to achieve better isolation results in general. However, this is associated with the risk that the chemical agents could change the chemical composition of POM, while the use of water appears to be harmless (Chen et al., 2019; Ohno et al., 2019).

Moreover, the experiment (Study I) provided evidence that chemical agents can lead to strong SOC leaching. Samples treated with the dispersant sodium hexametaphosphate lost approximately 25% of their initial OC contents. Accordingly, there is the possibility that higher isolation qualities related to chemical agents are caused by the leaching and loss of an artificially dissolved organic matter (DOM) fraction.

After approving the validity of the compiled particle-sized fractionation approach, it was decided that the fractionation scheme to be applied within the following experiment should use ultrasonication dispersion in demineralized water at  $450 \text{ J ml}^{-1}$  for its reliability and high quality of isolation.

## **7.2 Soil organic carbon sequestration in agricultural long-term field experiments as derived by POM and MAOM fractions (Study II)**

A first evaluation of the different management classes within the 25 long-term field experiments (control, organic fertilization, mineral fertilization, mixed fertilization, multiple systemic changes, and grassland management) showed that the managed OM, POM, and MAOM contents within the arable fields were almost identical (Study 2, Figure 4). The control group had slightly lower OC, POM-C, and MAOM-C contents. In contrast, the grasslands had genuinely higher OC MAOM-C and POM-C levels, pointing

to the general OC depletion of arable farmland soils in contrast to grassland soils (Cambardella and Elliot, 1992; Angers et al., 2011). Approx. 80% of OC was within the MAOM-C fractions of the soils, while the POM-C fraction contained only 20% of the OC. Furthermore, it was found that the variance of the MAOM-C contents between nested site-specific parameters (soil texture) was greater than the effects of the treatments (management effects). This finding confirmed the validity of the hierarchical mixed-effects models to be used for the prediction of MAOM-C.

The mixed effects modeling disentangled the significant predictors for the MAOM-C contents of arable soils. Besides the soil texture, the organic fertilization management and the crop rotation were found to significantly influence the abundance of MAOM-C. If crops with a large below-ground C allocation (high root biomass formation and root exudation) are used within the crop rotation, this will consequently increase the slow-cycling OC pool of arable soils (Pausch and Kuzyakov, 2018; Ball et al., 2020; van der Pol et al., 2022). Furthermore, this emphasizes the use of easily accessible yield biomass as an indicator for below-ground OC enrichment (Kätterer et al., 2011; Dechow et al., 2019), even if yield-independent methodologies for the estimation of below-ground OC inputs might be favorable (Hirte et al., 2018).

Recent studies highlighted that exchangeable metals and organo-metal complexes can also be used to predict SOC contents (Rasmussen et al., 2018; Fukumasu et al., 2021). However, the exchangeability of metals, in this case, iron- and aluminum-oxyhydroxides, is related to low pH values. Since arable soils in Central Europe are treated with  $\text{CaCO}_3$  to maintain neutral pH values (liming), this makes them unsuitable for the use of such SOC predictors and points out the necessity to understand arable soils not as natural but as artificial soil systems.

To evaluate the impact of the agricultural management effects, the MAOM-C contents of each soil sample were plotted against the soil texture. Thereby, all differences between the control plots and the managed plots can be attributed to the remaining significant predictors, i.e., the organic fertilization and the crop rotation management (Study II, Figure 6). Furthermore, the figure depicts the estimated MAOM-C saturation area (Hassink, 1997; Six et al., 2002; Wiesmeier et al., 2019). The results show that commonly

managed coarse-textured arable soils occasionally reach MAOM-C saturation levels. However, fine-textured arable soils have significant MAOM-C deficits and only reach the estimated saturation area when they receive extremely high amounts of organic fertilizers. As MAOM-C saturation deficits are estimated based on natural grassland soils, this highlights the particular importance of fine-textured arable soils for extensification or land-use change to grassland (Vuichard et al., 2008; Chang et al., 2017). Moreover, the results imply that MAOM-C saturation is not a realistic goal for fine-textured arable soils. As such, MAOM-C saturation can only be reached when exceeding the allowed amounts of farmyard manure and/or slurry, which are set at  $170 \text{ kg ha}^{-1} \text{ a}^{-1}$  N equivalents according to the European Union Nitrates Directive.

A quantile regression approach was used to estimate the management effects on MAOM-C contents. Thereby, soils from control sites were compared to well-managed soils in relation to the soil texture using the 95<sup>th</sup> quantile of MAOM-C content to resemble well-managed soils. The well-managed soils are estimated to have  $4.1 \text{ mg g}^{-1}$  more MAOM-C than the control sites. This corresponds to higher MAOM-C stocks of  $1.38 - 1.84 \text{ t ha}^{-1}$  in the topsoils of well-managed soils, or  $5.06 - 10.1 \text{ t ha}^{-1}$  sequestered  $\text{CO}_2$ -equivalents respectively. However, this steady-state calculation neither accounts for expected SOC losses due to climatic conditions nor for the possibilities of recent climate-smart agricultural practices that were not investigated within the study (Bellamy et al., 2005; Capriel, 2013; Hartley et al., 2021). The effects of intercropping, agroforestry, and no-till management to increase SOC stocks are still highly debated and might provide further possibilities to increase SOC in the long run (Sapkota et al., 2012; Haddaway et al., 2017; Chenu et al., 2019; Mayer et al., 2022).

The POM-C/MAOM-C ratio was influenced by soil texture and was therefore not suitable for comparing soils with different textures unless a proper correction for the textural bias is applied. However, it can be a tool for before/after comparisons in soil carbon sequestration studies under consistent agricultural management. A stable POM-C/MAOM-C ratio can indicate the long-term development of MAOM-C and a sustainable supply with POM-C in agricultural soils. A significantly increasing ratio under increasing organic carbon concentrations signals that organic fertilization does, not effectively contribute to long-term carbon sequestration (Study II, Figure 7). This might either be the

case either when the soil is saturated in MAOM-C saturation or when decomposition dynamics in relation to POM input are unbalanced. Conversely, a decreasing ratio indicates a lack of a stable POM-C pool for soil biome nutrition, especially for the fungal community (Emilia Hannula and Morrien, 2022). It is assumable that a specific POM-C/MAOM-C ratio reflects a healthy soil state that supports the entire soil ecology including a functional food web. A POM-C/MAOM-C ratio above 0.35 indicates approximation to the soil's MAOM-C saturation potential (Study II, Figure 8), although soils with a low ratio can still be saturated in MAOM-C under certain conditions. In the discussions about slow- and fast-cycling OM fractions and MAOM-C saturation, the POM-C/MAOM-C ratio can be utilized as an additional tool for tracking relative changes in dependence to management and environmental factors.

### **7.3 Visualizing the molecular heterogeneity of POM during decomposition (Study III)**

To get further insights into non-modelable and highly variable POM-C fractions, imaging techniques were used by linking VNIR spectra, C/N measurements, and  $^{13}\text{C}$ -CPMAS-NMR spectroscopic data in random forest and ensemble artificial neural nets algorithms. Thereby, it was possible to successfully model the alkyl/O-N alkyl ratio and the C/N ratio of POM during decomposition. It was shown that VNIR imaging is a valid tool to differentiate POM within the soil matrix (Study III, Figures 3 & 4), to classify it according to its molecular composition, and to highlight its heterogeneity at a sub-millimeter scale (Study III, Table 2 & Figure 2). Since the approach of the experiment is completely new, there are currently no studies with which the modeling results could be compared. For the sake of the novelty of the approach, there are also limitations in the methodology, which demand for improvement in subsequent studies. Other studies using machine-learning algorithms already point out that the choice of modeling algorithms strongly affects the results (Hobley et al., 2018; Xu et al., 2020; Granlund et al., 2021). Random forest algorithms do not extrapolate any predictions, i.e., the end-members of the training data set are automatically close to the predictive end-members. Predictions out of the calibration range might appear disadvantageous in the first place; this, however, leads to a compression of the number of predictions at both extremes of the calibration range. In contrast, the ensemble artificial neural net might extrapolate its predictions, resulting in very high goodness-of-fit parameters. However, the extrapolated values are not bound to logical constraints, leading to a high proportion of either impossible or nonsensical

predictions. Thus extrapolations have to be interpreted with great care (Study III, Figure 2).

Furthermore, the calibration of the alkyl/O-N alkyl and the C/N ratio is related to regions of interest that have to fulfill certain criteria. Sampling the regions of interest has to be proceeded as shallow as possible to avoid obtaining soil or POM material with different spectral signatures, all the while a minimum amount of approx. 100 mg is still required for the following measurements. These requirements could be circumvented, if the molecular information was assessed on a similar scale to hyperspectral imaging, for example, by combining scanning electron microscopes and energy-dispersive X-ray spectroscopy (Hapca et al., 2015).

Despite the limitations, the molecular characteristics of straw and green manure POM during decomposition were disentangled. The alkyl/O-N alkyl ratio of the straw-derived POM increased, indicating a change of substituted aliphatic alcohols and ethers to unsubstituted C in paraffinic structures (Wilson et al., 1983; Kögel-Knabner, 2002). The C/N ratio decreased due to microbial respiration (Geissen and Brümmer, 1999). The heterogeneity of both parameters increased during incubation, which highlights that decomposition does not proceed uniformly but is spatially heterogeneous. This can be attributed to either preferential microbial decomposition dynamics (Golchin et al., 1998) or the spatial arrangement of microbial habitats driven by the pore space-induced moisture and the microbial accessibility of the POM (Dungait et al., 2012).

The decomposition dynamics of green manure POM were less uniform than in straw POM. Opposing the expected trend, the modeled C/N ratios of the surface VNIR spectra increased while the alkyl/O-N alkyl ratio decreased (Golchin et al., 1994; Baldock et al., 1997; Kögel-Knabner, 2002). After careful investigation, it was found that the exposed surfaces had changed during incubation. While the initially pre-fermented green manure consisted mainly of leaves, woody parts, and bark materials, the samples contained significantly fewer bark structures after incubation than before incubation. It was observed that the bark was decomposed and peeled off, revealing new woody plant tissues that had undergone less decomposition, thus having higher C/N ratios and lower alkyl/O-N alkyl ratios. This points out the need for further research on the molecular characterization of the used organic amendments when using surface imaging techniques.

Furthermore, it was found that straw amendments decomposed faster when added to topsoil than when added to subsoil material. Topsoils have more SOC, higher microbial activity, and also more diverse microbial communities than subsoils (Taylor et al., 2002). High SOC content and the abundance of microorganisms foster the release of dissolved organic matter (DOM) (Guigue et al., 2015), leading to the enrichment of DOM within topsoils (Kalbitz et al., 2000; Kaiser and Kalbitz, 2012). Microbes use DOM for their production of extracellular hydrolytic enzymes, which accelerates the decomposition of SOM in general (Berg and McClaugherty, 2014).

## 8 Conclusions and Outlook

This thesis contributed to the elaboration of new ways and tools to investigate fast- and slow-cycling organic matter pools concerning their specific functions in the soils, their environments, and land-use systems. Study I showed the plausibility of simple physical particle-size fractionation protocols using a threshold of 20  $\mu\text{m}$  for the assessment of information on SOM pools with different soil residence times. It enables a fast and cheap investigation of SOM fractions. The differentiation between slow- and fast-cycling OM fractions is, however, dependent on the soil texture and the used dispersion method.

It was shown that fine-textured soils required higher dispersion intensities, while the choice of the dispersion method was neglectable in coarse-textured soils. As a result, ultrasonication using 450  $\text{J ml}^{-1}$  energy input had the best mean isolation quality of all tested soil dispersion methods. The method might therefore be used to estimate management influences on the main soil functions of the fast- and slow-cycling OM fractions, i.e., the nutrient turnover and the C sequestration. Thereby, it has the potential to track the state and fate of these two meaningful fractions when added to ongoing soil monitoring programs. Furthermore, it might also be used to fractionate archived soil samples to improve the understanding of SOM development that led to the recent soils.

The compiled fractionation method was applied to soils from diverse agricultural long-term experiments to evaluate POM-C and MAOM-C contents under common management practices (Study II). Cross-validated predictive MAOM-C models revealed the soil texture, the organic fertilization, and the below-ground OC input as the major drivers of MAOM-C in the investigated soils. The model enables the estimation of MAOM-C contents for commonly managed farmlands on a broad level. The differences between the control plots and well-managed sites were estimated using a linear (quantile) regression. Thereby, the long-term adaptation of improved organic fertilization and crop management accounted for an increase of about 4.1  $\text{mg g}^{-1}$  MAOM-C compared to not organically fertilized sites with the least diverse crop rotations (Study II). Thereby, the contribution of improved organic fertilization and crop rotation to climate change mitigation was estimated to increase topsoil MAOM-C stocks of 1.38 - 1.84  $\text{t ha}^{-1}$ . Thus, the identification of ill-managed arable soils is essential to improve agricultural

management where needed, and appropriately reduce MAOM-C sequestration deficits. With this regard, a POM-C/MAOM-C ratio indicator was developed and tested as a benchmark test for sustainable organic fertilization measures. The ratio should be used within before/after comparisons of fast- and slow-cycling OM contents or long-term monitoring of the management, where POM-C/MAOM-C ratios  $>0.35$  indicate slow-cycling OM contents close to saturation. The MAOM-C model and the POM-C/MAOM-C ratio indicator might be implemented into existing soil monitoring programs after further validation. As the POM-C contents could not be successfully modeled, VNIR spectroscopy imaging combined with machine learning modeling was used to obtain a more detailed view.

A novel approach was designed that combined techniques to build models fitted with measured data from NMR-spectroscopy and C/N elemental analyzers to map the molecular composition of POM during decomposition within the top and subsoil. The models were based on averaged ensemble artificial neural nets and random forest algorithms (Study III). Thereby, the changes within the spatial heterogeneity of the C/N and the alkyl/O-N alkyl ratio were revealed for straw and green manure amendments. The straw decomposed almost uniformly over the entire surface with an increasing alkyl/O-N alkyl ratio and a decreasing C/N ratio. The green manure amendment had opposite trends caused by the preferential decomposition of N-rich OM tissues, leaving less decomposable C-rich OM behind. The decomposition was slower within the subsoil than in the topsoil, as indicated by less pronounced shifts within the alkyl and C/N ratios. The visualization shows great potential for the exploration of the spatial molecular heterogeneity of OM and might provide insight into concurrent OM stabilization mechanisms, i.e., OM inherent recalcitrance and the accessibility of the OM particles during decomposition.

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## **11 Appendix**

### **11.1 Study I**

Christopher Just, Christopher Poeplau, Axel Don, Bas van Wesemael,  
Ingrid Kögel-Knabner, Martin Wiesmeier

“A Simple Approach to Isolate Slow and Fast Cycling Organic Carbon  
Fractions in Central European Soils - Importance of Dispersion Method”

Frontiers in Soil Science, 08.10.2021:

<https://doi.org/10.3389/fsoil.2021.692583>

## 11.2 Study II

Christopher Just, Martin Armbruster, Dietmar Barkusky, Michael Baumecker, Michael Diepolder, Thomas F. Döring, Lorenz Heigl, Bernd Honermeier, Melkamu Jate, Ines Merbach, Constanze Rusch, David Schubert, Franz Schulz, Kathlin Schweitzer, Sabine Seidel, Michael Sommer, Heide Spiegel, Ulrich Thumm, Peer Urbatzka, Jörg Zimmer, Ingrid Kögel-Knabner, Martin Wiesmeier

“Soil organic carbon sequestration in agricultural long-term field experiments as derived by particulate and mineral-associated organic matter”

Geoderma, 12.04.2023:

<https://doi.org/10.1016/j.geoderma.2023.116472>

### 11.3 Study III

Christopher Just, Julien Guigue\*, Siwei Luo, Marta Fogt, Michael Schloter,  
Ingrid Kögel-Knabner, Eleanor Hobbey  
\*equal first-authorship contribution

“Spatial molecular heterogeneity of POM during decomposition at different  
soil depths resolved by VNIR hyperspectral imaging”

European Journal of Soil Sciences, 15.12.2021:

<https://doi.org/10.1111/ejss.13207>

## 11.5 Scientific Contributions

### Peer-reviewed publications

**Just C.**, Poeplau C., Don A., van Wesemael B., Kögel-Knabner I. and Wiesmeier M. (2021): A Simple Approach to Isolate Slow and Fast Cycling Organic Carbon Fractions in Central European Soils—Importance of Dispersion Method. *Front. Soil Sci.* 1:692583. doi: 10.3389/fsoil.2021.692583

**Just C.**, Armbruster M., Barkusky D., Baumecker M., Diepolder M., Döring T. F., Heigl L., Honermeier B., Jate M., Merbach I., Rusch C., Schubert D., Schulz F., Schweitzer K., Seidel S., Sommer M., Spiegel H., Thumm U., Urbatzka P., Zimmer J., Kögel-Knabner I. and Wiesmeier M. (2023): Soil organic carbon sequestration in agricultural long-term field experiments as derived from particulate and mineral-associated organic matter. *Geoderma* (434), doi: 10.1016/j.geoderma.2023.116472

**Just C.**, Guigue J., Luo S., Fogt M., Schloter M., Kögel-Knabner I., and Hobley E. (2022). Spatial molecular heterogeneity of POM during decomposition at different soil depths resolved by VNIR hyperspectral imaging. *European Journal of Soil Science*, 73( 1), e13207. <https://doi.org/10.1111/ejss.13207>

Dorau K., Pohl L., **Just C.**, Höschen C., Ufer K., Mansfeldt T. and Mueller C.W. (2019): Soil Organic Matter and Phosphate Sorption on Natural and Synthetic Fe Oxides under in Situ Conditions. *Environ Sci Technol.* 53(22):13081-13087. doi: 10.1021/acs.est.9b03260

## **Conference contributions (first-authored)**

### **2019**

**Just C.,** Poeplau C., Don A., van Wesemael B., Wiesmeier M. and Kögel-Knabner I.:  
A simple method to quantify labile and stable carbon in temperate agricultural soils, EGU  
General Assembly 2019, Vienna, Poster presentation

### **2020**

**Just C.,** Poeplau C., Don A., van Wesemael B., Kögel-Knabner I. and Wiesmeier M.:  
Assessing slow and fast cycling SOM pools by particle-size fractionation – A perspective of  
modelling SOC sequestration potentials, BonaRes Status Seminar 2020, Leipzig, Poster  
presentation

### **2021**

**Just C.,** Poeplau C., Don A., van Wesemael B., Kögel-Knabner I. and Wiesmeier M.:  
A simple approach to isolate slow and fast cycling organic carbon fractions in Central European  
soils – importance of dispersion method, Eurosoil 2021, Geneva, Poster presentation

**Just C.,** Poeplau C., Don A., van Wesemael B., Kögel-Knabner I. and Wiesmeier M.:  
A simple approach to isolate slow and fast cycling organic carbon fractions & Development of  
an indicator to estimate SOC sequestration potentials, EUF-Tagung (digital), Oral presentation

### **2022**

**Just C.,** Kögel-Knabner I. and Wiesmeier, M.: Effects of agricultural management practices on  
POM-C and MAOM-C as derived from 25 long-term field experiments in Central Europe,  
World Congress of Soil Science 2022, Glasgow, Poster presentation

**Just C.,** Kögel-Knabner I. and Wiesmeier, M.: Partikuläre und mineral-assoziierte organische  
Substanz als Indikatoren für die landwirtschaftliche Bewirtschaftung, DBG-Jahrestagung 2022,  
Trier, Oral presentation

### **2023**

**Just C.,** Kögel-Knabner I. and Wiesmeier, M.: The POM-C / MAOM-C ratio – A compliance  
indicator for sustainable soil organic carbon management of arable soils in Central Europe?  
EGU General Assembly 2023, Vienna, Oral presentation

**Just C., Paul, C. and Wiesmeier M.:** Soils as a key to climate change mitigation: private and public governance instruments to unlock the potential, International BonaRes Conference 2023, Berlin, Convener

**Just C., Kögel-Knabner I., Wiesmeier, M.:** The POM-C / MAOM-C ratio as an indicator for sustainable soil organic carbon management of arable soils, International BonaRes Conference 2023, Berlin, Oral presentation