Department of Agronomy and Plant Breeding II

Chair of Organic Farming with focus on sustainable soil use

Justus Liebig University Giessen

Towards improved soil organic matter balance models in plant production systems:

a focus on aspects of parameter survey

DISSERTATION

For the doctoral degree (Dr. agr.)

at the Faculty Agricultural Sciences, Nutritional Sciences, and Environmental Management

Justus Liebig University Giessen

Submitted by

Lucas Ambrosius Dankward Knebl

Lindenfels, 2024

Authorised by the Faculty Agricultural Sciences,

Nutritional Sciences, and Environmental Management of the

Justus Liebig University Giessen

1st Assessor: Prof. Dr. Andreas Gattinger

2nd Assessor: Prof. Dr. Jan Siemens

Declaration

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an antiplagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen: "Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" in carrying out the investigations described in the dissertation.

Date Lucas A. D. Knebl

Annemarie, j'ai fini!

Table of content

List of tables

List of abbreviations

SOM:	soil organic matter
SOC:	soil organic carbon
STN:	soil total nitrogen
C:	carbon
N:	nitrogen
P:	phosphorus
S:	sulphur
GHG:	greenhouse gas
AFOLU:	agriculture, forestry and other land use
CCS:	carbon capture and storage
CO_2:	carbon dioxide
CO_2eq:	carbon dioxide equivalent
N_2O:	nitrous oxide
N_2:	dinitrogen
NO:	nitric oxide
NH_3:	ammonia
CH_4:	methane
H_2O_2:	hydrogen peroxide
POM:	particulate organic matter
DOM:	dissolved organic matter
LF:	light fraction
HF:	heavy fraction
NdfF:	nitrogen derived from fertilizer
FNU:	fertilizer nitrogen utilization
NUE:	nitrogen use efficiency
MDD:	minimum detectable difference
MRV:	measurement/monitoring, reporting and verification platforms

Summary

The increasing world population and advancing climate change are major challenges for current and future agriculture. Agricultural systems must be designed in such a way that they increase productivity without causing additional environmental pollution, and reduce expansion of agricultural area. Soils play an important role in this objective, in particular soil organic matter (SOM), which is ascribed a variety of functions (e.g. provisioning of nutrients for plant growth and CO_2 carbon sink). Farmers must therefore be able to organise their management in such a way that SOM stocks are safeguarded and, ideally, promoted.

To facilitate this aim, so-called "humus balances" have been developed as simple practice-applicable models to assess SOM supply in arable farming. These models relate to pools and fluxes of either organic carbon and/or nitrogen in the soil-plant system. Both soil organic carbon (SOC) and soil total nitrogen (STN) are important components and indicators of SOM that are closely related to each other. As the stoichiometry of carbon and nitrogen is an important factor in the development of SOM stocks, it is important to record both parameters when assessing SOM dynamics. However, quantification of SOC, STN as well as total SOM stocks is difficult, as their relatively small amounts are very heterogeneous in the soil and the possibilities of their measurement are limited (detectability of short-term/low quantity changes, lack of standards). SOM balance models can be useful planning tools and are also being discussed as political instruments against the background of carbon sequestration and carbon farming. The suitability of SOM balance models is often criticised, though. In particular, poor model validation is highlighted, but there is also room for improvement in the parameterization and calibration of the models. In addition, there are many possibilities to further develop the models, for example to better estimate the SOM dynamic in the subsoil or to evaluate new management practices (e.g. new crops, fertilizers, tillage). The aim is to develop models that are reliable and easy to use. Farmers and agricultural advisors are also interested in models that make it possible to balance the supply of nutrients to crops and provide information on carbon sequestration.

The aim of this thesis is to contribute to the improvement and development of SOM balance models, with a focus on the HU-MOD model that has been developed at the Chair of Organic Farming at the Justus Liebig University Giessen. One objective was to estimate the uptake of nitrogen from SOM mineralization by a crop that is fertilized with mineral nitrogen. This information is important for the calculation of SOM loss and the demand for organic matter supply. For this purpose, a greenhouse experiment with sorghum-sudangrass (*Sorghum bicolor X Sorghum sudanense*) and maize (*Zea mays*) was conducted. Plants were grown in bags and fertilized with ^{15}N-labelled ammonium nitrate. The aim was to contribute to the scarce data on sorghum-sudangrass as an energy crop with regards to nitrogen derived from fertilizer (NdfF) in the plant's biomass and fertilizer nitrogen utilization (FNU). It should also clarify whether it is advisable to use parameters of maize as proxies for sorghum-sudangrass if field data is missing for the latter. Results showed that FNU of sorghum-sudangrass (65 %) was significantly higher than that of maize (49 %), if grown in a greenhouse. Both crops accumulated more soil N than fertilizer N. The share of fertilizer N on total N uptake was also higher with sorghum sudangrass (NdfF = 38 %) compared to maize (NdfF = 34 %). This leads to the conclusion that parameters of maize should not be used as proxies for sorghum-sudangrass in SOM balance models. Parametrization of the crops in the model could not be validated though, as there was no sufficient data base (no long-term field experiment) for this purpose. With regard to model parametrization, it can be stated that the approach with ^{15}N fertilisation under clearly defined growth conditions and system boundaries is overall suitable for obtaining data. However, the conditions in the greenhouse differ significantly from those in the field and direct transfer of the results is difficult. Future studies could be carried out in a lysimeter experiment to provide a better comparison with real conditions. In addition, gas measurements and the detection of leachate could provide a more complete representation of the N pathways.

Another question was to what extent short-term field experiments can be used to assess SOM change under cropping systems as a basis for the validation of SOM balances and other models. To date, the validation of SOM balance models - if there is one - has been based on data from long-term field experiments. Although this is the most reliable approach, it is also time-consuming and expensive. The possibility of assessing parameters for new crops or fertilisers in short-term field experiments is therefore of great interest. To study this, a short-term field experiment (two series, each one year) was carried out at the experimental station Gladbacherhof of the Justus Liebig University Giessen. Target crops were winter wheat, potatoes, red clover for fodder (removed) and red clover for green manure (not removed). No additional fertilizer was applied. For SOC and STN assessment in the topsoil (0-30 cm), six subsamples per plot were analysed at the beginning and after harvest. Different outlier determination procedures were then applied to calculate plot means and subsequently measured data was compared to predictions by the HU-MOD balance model for each crop. The soil sampling design, paired with outlier determination, resulted in a decrease of minimum detectable differences (MDD) by a factor of 0.53 for SOC and 0.63 for STN masses. This allowed for detection of changes in the magnitude of 3.7 % and 2.6% of background SOC and STN levels, respectively. SOC and STN changes were significant with treatments that had the highest effects (potatoes and mulched red clover). The comparison of apparent and modelled changes with HU-MOD proved the suitability of this data collection for the purpose of parameter validation. In addition, the observation period needed for SOM change detection can be reduced noticeably. Field experiments that aim to develop SOM balance methods can profit from this sampling procedure and new management practices, fertilizers or crops can be validated in a shorter time period. Further improvements to the sampling design could include additional series, consideration of variable spatial impacts on the soil in crop stands and bulk density estimation parallel to each sampling procedure.

The third objective of this thesis was to clarify whether it is necessary to assess and consider SOM quantity changes in the subsoil in order to estimate and validate C and N balances in the soil. The rationale for this objective is that SOM balance models usually do not refer to a defined soil depth, but to organic matter inputs and outputs in general. For this study, mean SOC and STN changes were assessed in 30 cm depth increments down to 90 cm in the organic arable long-term field experiment Gladbacherhof over a 17-year observation period (three crop rotations). The experiment comprises three different farming types (mixed and stockless with either rotational ley or cash crops). Each farming type included four tillage treatments (full inversion, two-layer plough, reduced inversion and non-inversion). While differences in farming types could be recorded for topsoil SOC and STN, tillage treatments had effects on SOC and STN in 30-60 cm, where full inversion tillage resulted in an increase compared to reduced tillage treatments. This effect even led to a significant differentiation of these treatments with regards to the whole soil profile (0-90 cm). The results suggest that sampling depth should be extended to at least 60 cm in order to improve validation performance of SOM models for tillage effects on soil organic matter (SOM).

The three experiments of this thesis show that sampling procedure and experimental setup have a great potential to further develop SOM balance models in such a way that they become more reliable planning and monitoring tools and can react in a more flexible way to new developments in agricultural practice. The results of this thesis contribute to the standardised improvement and refinement of SOM balance models.

Zusammenfassung

Die wachsende Weltbevölkerung und der fortschreitende Klimawandel sind große Herausforderungen für die heutige und zukünftige Landwirtschaft. Landwirtschaftliche Systeme müssen so gestaltet werden, dass sie die Produktivität steigern, ohne zusätzliche Umweltbelastungen zu verursachen, und die Ausdehnung der landwirtschaftlichen Nutzfläche reduzieren. Dabei spielen die Böden eine wichtige Rolle, insbesondere die organische Bodensubstanz (soil organic matter, SOM[1]), der eine Vielzahl von Funktionen zugeschrieben wird (z. B. Bereitstellung von Nährstoffen für das Pflanzenwachstum und CO_2-Senke). Landwirt*innen müssen daher in der Lage sein, ihre Bewirtschaftung so zu gestalten, dass die SOM-Bestände gesichert und im Idealfall gefördert werden.

Um dieses Ziel zu erreichen, wurden so genannte „Humusbilanzen“ als einfache, in der Praxis anwendbare Modelle zur Bewertung der Versorgung der Böden mit organischer Substanz im Ackerbau entwickelt. Diese Modelle beziehen sich auf Pools und Flüsse von entweder organischem Kohlenstoff und/oder Stickstoff im System Boden-Pflanze. Sowohl der organische Kohlenstoff im Boden (SOC) als auch der Gesamtstickstoff im Boden (STN) sind wichtige Komponenten und Indikatoren von SOM, die eng miteinander verbunden sind. Da die Stöchiometrie von Kohlenstoff und Stickstoff ein wichtiger Faktor für die Entwicklung der SOM-Vorräte ist, ist es wichtig, beide Parameter bei der Bewertung der SOM-Dynamik zu erfassen. Die Quantifizierung von SOC, STN sowie der gesamten SOM-Menge ist jedoch schwierig, da ihre relativ geringen Mengen im Boden sehr heterogen und die Möglichkeiten ihrer Messung begrenzt sind (Nachweisbarkeit kurzfristiger/geringer Mengenänderungen, Mangel an Standards). SOM-Bilanzmodelle können nützliche Planungsinstrumente sein und werden auch als politische Instrumente vor dem Hintergrund von Kohlenstoffsequestrierung und Carbon Farming diskutiert. Die Eignung von SOM-Bilanzmodellen wird jedoch häufig kritisiert. Insbesondere wird die mangelhafte Modellvalidierung hervorgehoben, aber auch bei der Parametrisierung und Kalibrierung der Modelle gibt es Verbesserungs- und Entwicklungsmöglichkeiten. So ist es notwendig neue Bewirtschaftungsmethoden (z.B. neue Kulturen, Dünger, Formen der Bodenbearbeitung) zu parametrisieren, wobei die Möglichkeit einer Validierung in Kurzzeitexperimenten angestrebt werden sollte. Allgemein sollte bei der Validierung auch die Boden-Referenztiefe diskutiert werden und Effekte auf den Unterboden Berücksichtigung finden. Ziel ist es, Modelle zu entwickeln, die zuverlässig und einfach zu verwenden sind. Landwirt*innen und landwirtschaftliche Berater*innen sind auch an Modellen interessiert, die es ermöglichen, die Nährstoffzufuhr der Pflanzen zu bilanzieren und Informationen über die Kohlenstoffsequestrierung zu liefern.

[1] Im Folgenden werden die englischen Abkürzungen verwendet.

Ziel dieser Arbeit ist es, einen Beitrag zur Verbesserung und Weiterentwicklung von SOM-Bilanzmodellen zu leisten, wobei der Schwerpunkt auf dem HU-MOD-Modell liegt, das an der Professur für Organischen Landbau der Justus-Liebig-Universität Gießen entwickelt wurde. Ein Ziel war es, die Stickstoffmenge aus der SOM-Mineralisierung abzuschätzen, die von einer mit mineralischem Stickstoff gedüngten Pflanze aufgenommen wird. Zu diesem Zweck wurde ein Gewächshausversuch mit Sorghum-Sudangras (*Sorghum bicolor X Sorghum sudanense*) und Mais (*Zea mays*) durchgeführt. Die Pflanzen wurden in Säcken gezogen und mit ^{15}N-markiertem Ammoniumnitrat gedüngt. Ziel war es, einen Beitrag zu den spärlichen Daten über Sorghum-Sudangrass als Energiepflanze zu leisten, und zwar im Hinblick auf den Stickstoffanteil aus dem Dünger (nitrogen derived from fertilizer, NdfF) in der Biomasse der Pflanze und auf die Stickstoffverwertung des Düngers (fertilizer nitrogen utilization, FNU). Außerdem sollte geklärt werden, ob es ratsam ist, Parameter von Mais als Stellvertreter für Sorghum-Sudangras zu verwenden, wenn für letzteres keine Felddaten vorliegen. Die Ergebnisse zeigten, dass die FNU von Sorghum-Sudangras (65 %) unter Gewächshausbedingungen signifikant höher war als die von Mais (49 %). Beide Kulturen akkumulierten mehr Boden-N als Dünger-N. Der Anteil des Dünger-N an der Gesamt-N-Aufnahme war bei Sorghum-Sudangras (NdfF = 38 %) ebenfalls höher als bei Mais (NdfF = 34 %). Dies führt zu der Schlussfolgerung, dass Parameter von Mais nicht als Ersatz für Sorghum-Sudangras in SOM-Bilanzmodellen verwendet werden sollten. Die Parametrisierung der Kulturen im Modell konnte jedoch nicht validiert werden, da keine ausreichende Datenbasis (kein Langzeit-Feldexperiment) für diesen Zweck vorhanden war. Hinsichtlich der Modellparametrisierung lässt sich festhalten, dass der Ansatz mit einer ^{15}N-Düngung unter klar definierten Wachstumsbedingungen und Systemgrenzen prinzipiell zur Datengewinnung geeignet ist. Allerdings unterscheiden sich die Bedingungen im Gewächshaus deutlich von denen auf dem Feld und eine direkte Übertragung der Ergebnisse ist schwierig. Zukünftige Untersuchungen könnten in einem Lysimeter-Experiment durchgeführt werden, um einen besseren Vergleich mit realen Bedingungen zu ermöglichen. Darüber hinaus könnten Gasmessungen und der Nachweis von Sickerwasser eine vollständigere Darstellung der N-Pfade liefern.

Eine weitere Frage war, inwieweit Kurzzeit-Feldexperimente zur Bewertung von SOM-Veränderungen unter Anbausystemen als Grundlage für die Validierung von SOM-Bilanzen und anderen Modellen verwendet werden können. Bislang basiert die Validierung von SOM Bilanzmodellen - sofern es eine gibt – auf Daten aus Langzeit-Feldexperimenten. Diese, grundsätzlich verlässlichste Vorgehensweise ist jedoch zeit- und kostenaufwändig. Möglichkeiten Parameter für neue Kulturpflanzen oder Dünger in Kurzzeit-Feldexperimenten zu erheben sind daher von höchstem Interesse. Um dies zu untersuchen wurde auf der Versuchsstation Gladbacherhof der Justus-Liebig-Universität Gießen ein Kurzzeit-Feldversuch (zwei Serien, jeweils ein Jahr) durchgeführt. Zielkulturen waren Winterweizen, Kartoffeln, Rotklee als Futterpflanze (abgefahren) und Rotklee als Gründüngung (auf dem Feld belassen). Es wurde kein zusätzlicher Dünger ausgebracht. Zur Bestimmung von SOC und STN im Oberboden (0-30 cm) wurden zu Beginn und nach der Ernte sechs Unterproben pro Parzelle analysiert. Anschließend wurden verschiedene Verfahren zur Bestimmung von Ausreißern angewandt, um die Erfassung von verlässlichen Parzellenmittelwerte zu verbessern. Die gemessenen Daten wurden mit den Vorhersagen des HU-MOD-Bilanzmodells für jede Kultur verglichen. Das Design der Bodenprobenahme, gepaart mit der Bestimmung von Ausreißern, führte zu einer Verringerung der minimalen nachweisbaren Unterschiede (minimal detectable differences, MDD) um den Faktor 0,53 für SOC und 0,63 für STN-Mengen. Dadurch konnten Veränderungen in der Größenordnung von 3,7 % bzw. 2,6 % der Hintergrundwerte für SOC und STN festgestellt werden. Die Veränderungen von SOC und STN waren bei den Behandlungen mit den größten Effekten (Kartoffeln und gemulchter Rotklee) signifikant. Der Vergleich der offensichtlichen mit den modellierten Veränderungen beweist die Eignung dieser Datenerhebung für die Parametervalidierung. Darüber hinaus kann der Beobachtungszeitraum, der für die Erkennung von SOM-Veränderungen benötigt wird, deutlich reduziert werden. Feldversuche, die auf die Entwicklung von SOM-Bilanzierungsmethoden abzielen, können von diesem Probenahmeverfahren profitieren, und neue Bewirtschaftungsmethoden, Düngemittel oder Kulturen können in einem kürzeren Zeitraum validiert werden. Weitere Verbesserungen des Beprobungsdesigns könnten zusätzliche Serien, die Berücksichtigung variabler räumlicher Einflüsse auf den Boden in Pflanzenbeständen und die Schätzung der Trockenrohdichte des Bodens, parallel zu jedem Beprobungsverfahren umfassen.

Als letzter Aspekt sollte geklärt werden, ob es zur Abschätzung und Validierung von C- und N-Bilanzen im Boden notwendig ist, SOM-Mengenveränderungen im Unterboden zu erfassen bzw. zu berücksichtigen. Der Grund für diese Zielsetzung ist, dass sich SOM-Bilanzmodelle in der Regel nicht auf eine bestimmte Bodentiefe beziehen, sondern auf den Input und Output organischer Substanz im Allgemeinen. Für die Untersuchung wurden im ökologischen Ackerbau-Langzeitversuch Gladbacherhof, über einen 17-jährigen Beobachtungszeitraum (drei Fruchtfolgen), mittlere SOC- und STN-Veränderungen in 30 cm Tiefenschritten bis hinunter zu 90 cm erfasst. Der Versuch umfasst drei unterschiedliche Bewirtschaftungstypen (gemischt gegenüber viehlos mit entweder Rotationsbrache oder Marktfruchtanbau). Jeder Bewirtschaftungstyp umfasste vier Bodenbearbeitungsvarianten (Konventioneller Pflug, Zweischichtpflug, reduzierter Pflug und nicht wendende Bodenbearbeitung). Während für den Oberboden SOC- und STN-Unterschiede zwischen den Bewirtschaftungsformen festgestellt werden konnten, hatten die Bodenbearbeitungstypen Auswirkungen auf SOC und STN in 30-60 cm, wobei die Vollinversion im Vergleich zu den reduzierten Bodenbearbeitungsmethoden zu einem Anstieg führte. Dieser Effekt führte sogar zu einer signifikanten Differenzierung dieser Variante in Bezug auf das gesamte Bodenprofil (0-90 cm). Die Ergebnisse legen nahe, dass die Beprobungstiefe auf mindestens 60 cm ausgedehnt werden sollte, um die Validierungsleistung von SOM-Modellen für die Auswirkungen der Bodenbearbeitung auf die organische Substanz (SOM) zu verbessern.

Die drei Versuche dieser Arbeit zeigen, dass Beprobungsdesign und Versuchsaufbau ein großes Potenzial haben, SOM-Bilanzmodelle so weiterzuentwickeln, dass sie zu einem zuverlässigen Planungs- und Überwachungsinstrument werden und flexibler auf neue Entwicklungen in der landwirtschaftlichen Praxis reagieren können. Die Ergebnisse dieser Arbeit tragen zur standardisierten Verbesserung und Verfeinerung von SOM-Bilanzmodellen bei.

Chapter 1: General introduction

1.1 Global challenges

In 2024, humankind is facing some serious challenges. While meeting the endeavour to feed an ever-growing world population, societies have to deal with ongoing and reoccurring social crises and the threat of pandemics such as COVID-19. At the same time, everything is taking place against the backdrop of environmental changes.

According to the United Nations Population Fund (UNFPA[2]), the global population will be 8.1 billion people in 2024 and is expected to reach 9.7 billion people by 2050. According to Van Dijk et al. (2021), this increase will be accompanied by an additional demand for food of 35% to 56%. In order to meet this demand, as well as necessary production of material for bio fuels and fibers, agricultural production has to increase by 70 % in 2050 compared to 2010 (FAO, 2009).
Already in 2022, around 690-783 million people are estimated to suffer from hunger (FAO, IFAD, UNICEF, WFP & WHO, 2023). The reasons for the unequal food situation are manifold and depend on natural conditions as well as social and political structures. More or less short-term events, such as wars or the COVID-19 pandemic, have worsened the situation, as does ongoing climate change (FAO, IFAD, UNICEF, WFP & WHO, 2023). These reasons will most likely continue to occur in the future. To ensure the resilience and stability of our planet's ecosystems, nine planetary boundaries have been defined, six of which may already have been surpassed, in particular: climate change, biosphere integrity and biogeochemical flows (disruption of the nitrogen and phosphorus cycle) (Richardson et al., 2023). Along with the growing population and increasing industrialization came an increase in greenhouse gas (GHG) emissions. A report by the IPCC (2023) lists the extent of GHG emissions and their impact. As this report was written by a large number of leading international experts in the field of climate research, some important key data is listed below.
The atmospheric CO_2 concentration in 2019 is higher than at any time in the last 2 million years and the methane concentration is higher than at any time in the last 800,000 years. A total production of 2,400 ± 240 Gt CO_2 is estimated for the period 1850 to 2019, of which 42 % falls within the last 30 years (1990-2019). According to the IPCC (2023), these amounts result from unsustainable energy use, land use and land-use change, lifestyles and patterns of consumption and production.

According to the same report, this results in a progressive climate change, entailing a warming of the atmosphere, oceans and land masses. In 2011-2020, the average temperature was 1.1 °C higher than in 1850-1900. In addition to the temperature change, sea levels continue to rise accompanied by

[2] www.unfpa.org/data; assessed 05.08.2024

periods of heat and drought as well as severe weather events in different parts of the world. The corresponding effects are largely irreversible and significant ecosystem losses (terrestrial, freshwater, cryospheric, coastal, ocean) are being recorded. Globally, an estimated 3.3-3.6 billion people live in contexts that are strongly influenced by climate change, mostly in low-income and developing countries. The supply of people with sufficient food and water is jeopardised in large parts of the world and heatwaves are increasingly leading to health problems. Human-induced global warming must be largely reduced. This requires net zero CO_2 emissions to limit warming to 1.5 °C, but no more than 2.0 °C. Adaptation strategies are currently being developed and implemented to combat negative effects of climate change. These are taking place in all sectors, more or less successfully (IPCC, 2023). As it becomes apparent by the given insights, environmental challenges reinforce each other (e.g. biodiversity loss through climate change) and are deeply entangled with food security now and in the future. At the same time, food production is not only affected by ecological change, but has a large impact on it, which leads to the next important topic.

1.2 Climate change and food security: The responsibility of agriculture

By 2050, global food systems must have undergone an eco-efficiency revolution (Keating et al. (2010). According to the authors, this means an increased utilization efficiency of the resources of land, water, nutrients and energy by 50 % to 100 %, while these resources are preserved and further GHG production is avoided. To meet this goal, strategies are needed that facilitate sustainable agricultural systems with regards to ecological as well as economic and social aspects.

In the last decades it has become clear that farms are not only feeling the effects of climate change, but that the agricultural sector itself has a significant impact on climate change (Praveen & Sharma, 2019).

Lamb et al. (2021) calculated the CO_2eq emissions (agricultural CH_4 and N_2O emissions, supplemented with CO_2 emissions from land-use) from various sectors for 2018. They summarise the contribution of agriculture, forestry and other land use in the "AFOLU" sector and attribute a contribution of 21 % of total CO_2eq emissions (11.6 Gt CO_2eq) to this sector, 47 % of which is due to land use change. This is in accordance with data reported by Tubiello et al. (2015) for the year 2010. Lamb et al. (2021) further attributed the contribution of global agriculture to 25 % from emissions from enteric fermentation, 11 % from managed soils and pasture, 9 % from rice cultivation and a further 9 % from manure management, biomass burning and synthetic fertilizer application. According to the authors, the emissions from agriculture are mainly CH_4 and N_2O emissions. Land use and land-use change activities, on the other hand, emit and remove mainly CO_2 gas (Tubiello et al., 2012). The study by Lamb et al (2021) showed that Africa, Asia and Latin America have the highest share of globally produced CO_2eq quantities in the AFOLU sector. The findings go along with the report of Praveen & Sharma (2019).

According to their study, low-income and so-called developing countries (Asia, Africa) not only depend heavily on agriculture to feed their population. These countries also lack in available modern technologies and use mineral fertilizers and agrochemicals to a high extent, which have negative effects both during production and through its application (runoff, leaching). However, these statements by Lamb et al. (2021) as well as Praveen & Sharma (2019) must be viewed in a differentiated way, as it does not take into account who is responsible for the goods produced in these countries. It is a fact that industrialised countries often outsource their production sites to developing countries and thus contribute to the cause (Vandergeten et al., 2016).

Reducing CO_2eq emissions is one of the most important tasks of modern agriculture due to its large contribution. In addition, land productivity has to increase in order to feed the growing world population (70 % increase by 2025). Whether and how agriculture can achieve this, is currently being discussed in a variety of ways and from different points of view (technical solutions vs. changes of entire systems).

There are several aspects to ensure a sustainable agriculture. On the one hand, agriculture must find adaptation strategies to be less affected by advancing climate change. On the other hand, agricultural practice itself should reduce its impact on climate change. Muhie (2022), among others, compares novel approaches and practices to sustainable agriculture. The author lists novel practices such as: *Rotating crops and embracing diversity, planting cover crops and perennials, reducing or eliminating tillage, integrating livestock and crops, applying integrated nutrient management,* and others. According to Muhie, novel approaches that both include socioeconomic and environmental factors are, e.g.: *Biodynamic & organic farming, regenerative farming and carbon farming,* and others.

Some of these practices and approaches are discussed controversially with regards to their actual suitability, as can be seen in the following chapters, e.g. reduced tillage or carbon farming. According to Seufert et al. (2012), a frequent criticism of organic farming is that it requires more land to achieve the same yields as conventional farming (due to lower average yields). Tuomisto et al. (2012) report that organic farming (without biogas production) requires 50 % more land area than conventional farming. Their results focussed on farming systems in England. With their meta-analysis, however, Seufert et al (2012) were able to show that the yield differences are strongly dependent on the cropping system and the site characteristics and yield gaps of organic vs conventional systems may only range from 5 % to 34 %. It must be noted that the lower land use efficiency of organic systems was compared for food systems that meet the current demand for food. If consumers adapt their diet and consume less meat, for example, the results may look different. A more recent meta-analysis on yield gaps between organic and conventional farming types was conducted by De La Cruz et al. (2023). The authors took four different climatic conditions (boreal, warm temperate, arid and equatorial), as well as study location, crop types and soil conditions into account. Their analysis showed that overall yields of organic farming systems were 18.4 % lower compared to conventional farming and 21.2 % lower in warm temperate climates. In the latter climate type, yield gaps were affected mainly by crop types but also regions and soil conditions. The importance of soils is also emphasised by Schreefel et al. (2020) who state that soils are the basis for low-input systems such as regenerative farming. Soil organic matter (SOM) is a driver for numerous ecosystem-services attributed to soil, and it plays a key role in sustainable agriculture (Bot & Benites, 2005). Therefore, it is important to understand the nature of SOM, its effects and how it is affected as well as have an understanding of the challenge in SOM assessment. The special role of SOM in securing food production and reducing climate change is described in the next sub-chapter.

1.3 Soil organic matter

1.3.1 General role of SOM

Before discussing the role of SOM, it must first be clarified that the terms SOM and humus are sometimes used synonymously in the literature (Lehmann & Kleber, 2016). When citing studies, this thesis attempts to avoid using the term humus when it is used synonymously with SOM. However, if the distinction is not clear, or if humus is specifically mentioned in a study, the original term from the study is retained.

The importance of humus in the soil with regard to plant production was already known to earlier societies (Waksman, 1936). According to Waksman (1936), humus began to be studied in more detail since the beginning of the last century, including its origin, composition and role for crop production. During the last decades, it became more common (especially in English literature) to use the term soil organic matter (SOM). Research has recognized the role of soil organic matter (SOM) for agricultural soils and terrestrial ecosystems (Wander, 2004). The role of SOM is attributed to its general function in providing plants and microorganisms with nutrients, thus controlling their growth and activity (which is associated with a phytosanitary effect); in improving soil structure and thereby its water retention, temperature and resilience towards erosion; in balancing chemical and biological processes and in reducing toxicity of metals (Fageria, 2012). Last but not least, SOM is crucial for global C and N cycles (Dick & Gregorich, 2004) as well as carbon sequestration (Poeplau & Don, 2015).

As a result, SOM has been extensively studied in the last decades and there are a number of review articles and books describing its nature, its effects on soil properties, crop production and carbon sequestration, as well as discussing methods for recording SOM stocks and explaining which factors in turn can influence SOM in terms of quality and quantity (e.g. Hoffland et al., 2020; Bashir et al., 2021; Krull et al., 2004; Wander, 2004 and Brock et al., 2023, among others). The following sub-chapters therefore do not provide an in-depth literature review. Instead, selected studies and corresponding review articles are presented, including studies on soil organic carbon (SOC) and soil total nitrogen (STN) as important compounds and indicators of SOM.

1.3.2 Nature of SOM

Definition

A look into the literature on SOM shows that there is still some disagreement about its definition. As can be deduced from the name, SOM are organic substances within the non-organic soil medium. While all studies on SOM agree on this part, some authors distinguish between living SOM (mainly bacteria, fungi, actinomycetes, protozoa, algea and nematodes) and non-living SOM, which includes decomposed and undecomposed products (Bashir et al. 2021). In contrast, other authors define SOM as dead organic matter in the soil that is in the process of decomposition (Trumbore, 1997 and Hoffland et al. 2020). Krull et al. (2004) consider SOM to be organic matter regardless of its degree of decomposition and Wander (2004) includes at least small amounts of living material in the definition. Ultimately, the underlying analysis method determines whether only dead or also living organic matter is assessed.

Overall, most SOM studies refer to the non-living part unless they refer to SOC. Studies on SOC mostly consider the particle size < 2mm, which includes both live and dead OM. However, numerous studies on SOM do not mention their underlying definition SOM at all. To facilitate definition of SOM, Lehmann & Kleber (2016) suggest considering SOM as a continuum that spans intact plant material to highly oxidized carbon in carboxylic acids.

SOM functions

Although it is generally recognised that an increase in SOM is desirable, the relation between soil functions and SOM contents are not linear (Krull et al., 2004). As the authors state in their review: “more SOM is not always better”. Some authors classify those multiple SOM functions into biological, physical and chemical functions (Bashir et al., 2021; Krull et al., 2004; Lal, 2020). The respective influences on soil properties are largely uniformly described in the aforementioned studies, although Bashir et al. (2021) provide a more detailed list. According to their study, the general functions are as follows:

<u>Physical functions</u>: *effects on soil structure, water retention, available water capacity, thermal conductivity, erodibility, infiltration, soil aggregate formation, soil colour, soil compaction, soil aeration, saturated and unsaturated hydraulic conductivity.*

<u>Chemical functions</u>: *effects on pH, buffering capacity, cation exchange capacity, base saturation, zeta potential, exchangeable cations, soil fertility, and nutrient release.*

Biological functions: *effects on soil microbial population, soil microbial biomass carbon, nitrogen transformation, mycorrhizal population, root length and root growth, dehydrogenase, phosphatase, and urease.*

Hoffland et al. (2020) further linked the most important ecosystem functions of SOM and processes supporting those functions. According to the authors the function of climate regulation is supported by carbon (C) sequestration; erosion protection is supported by water retention and aggregation. The later also supports the provision of a habitat for living organisms. The effect on primary production is supported by promoting plant health, nutrient mineralization, purification (retention of essential metal macro- and micronutrients, potentially toxic heavy metals, and organic pollutants), water retention and aeration. At last, the effect on water quality is supported by compound retention. The processes supported by labile SOM are typically relevant on a short (< 1 yr.) timescale, whereas the timescale of processes supported by stable SOM is highly context dependent (Hoffland et al., 2020).

SOM indicators and pools

C and N cycles in soil-plant systems play an important role for agricultural production and the environment (Gerke, 2022; Friedl et al., 2020). SOC and STN are often used as indicators for SOM.

According to Gerke (2022), SOC affects soil fertility, among other things by improving nutrient availability for plants and has influence on atmospheric CO_2-concentrations. Since C is a major element in living organisms (~50 % of dry mass) it became a basic indicator for energy and matter flows (Hessen et al., 2004). Janzen (2004) painted a quite generalized picture for C pathways into and out of terrestrial ecosystems. According to the author, atmospheric CO_2 enters the soil via photosynthesis of biomass and leaves the soil-plant system via CO_2 respiration from plants and microbes. Additional SOC losses can occur via leaching of dissolved organic carbon or gaseous CH_4 emission. In agricultural systems though, organic fertilizer application is an additional C entry point.

Entry points for N into the soil can be atmospheric deposition and N_2 fixation as well as fertilization while N losses occur due to leaching, gaseous losses (N_2, N_2O, NO, NH_3) or N uptake by plants as well as animals (e.g. through grazing) (Cameron, Di & Moir (2013). Since N is a central element for plant nutrition (Novoa & Loomis, 1981), gaseous N losses are of particular importance, as they can have a significant impact on nitrogen use efficiency (NUE) and total nitrogen uptake by plants (Scheer et al., 2020; Yankelzon et al., 2024). The climate relevance of gaseous N losses is equally important, especially in the case of N_2O, whose global warming potential is 298 times greater than CO_2 (Scheer et al., 2020). SOC and STN pathways are dependent on numerous factors, including quantity and quality of C and N compounds, as well as soil properties and climate factors.

In addition, the availability of C, N, phosphorus (P) and sulfur (S) affects SOM formation (Kirkby et al. (2011). The balance of these elements differs between ecosystems and can be analysed using "ecological stoichiometry" (Hessen et al., 2004). In order to receive a better understanding of C dynamics and how element ratios affect carbon sequestration, Kirkby et al. (2011) investigated the stoichiometry of SOM for a wide range of global soils. Their study resulted in rather constant ratios of C:N:organic P:S in the stable SOM pool. Van Groeningen et al (2017) stated that the average C:N ratio in SOM of agricultural soils is 12:1 and only changes, in the long term, if at all. Due to their importance, research is still going on to better understand SOC and STN pathways and turnover in soil-plant systems.

One approach in further studying the nature of SOM is its separation into different pools. These pools differ in their composition (measurable fractions), their proportional share in total SOM, their properties (e.g. turnover rates), as well as their effects on soil properties. In an overview of these pools, Wander (2004) criticises that the terms “fraction” and “pool” are not used uniformly in some of the studies, and the definitions of one or the other sometimes overlap, leading to confusion. The subdivision differs, for example, in whether the effects of the respective pools on the soil properties are analysed or the various turnover rates are taken as a basis. For example, Krull et al. (2004) and Bashir et al. (2021) subdivide non-living SOM into the pool of dissolved organic matter, particulate organic matter, humus and inert organic matter and describe their effects on various soil functions. Against the background of the respective half-lives, Trumbore (1997) and Wander (2004) state that SOM is generally divided into “labile or active SOM” with turnover-rates measured in years, “slow or intermediate SOM” with turnover-rates measured in decades and “recalcitrant, passive, stable and inert SOM” with turnover-rates of centuries or millennia.

In view of the unclear termination of the SOM pools, the specific functions of the individual pools are not described in detail at this point. However, Hoffland et al (2020) state that stable SOM plays an important role in soil aggregation and C sequestration, thereby serving primary production. Furthermore, according to the authors, most other soil functions are dependent on decomposition and are related to labile components.

It can be summarized that SOM pools do not form a static unit and are influenced by the factors of time, climate, soil properties and management. Looking at SOM (respectively SOC or STN) as a whole does not allow any statement to be made about the size or the development of different pools and the soil functions associated with these pools. This fact must be taken into account when interpreting results from studies on SOM. Hoffland et al. (2020) therefore call for future studies to link operationally defined SOM fractions to functions.

1.3.3 SOM, global food supply and climate change

Recently, Brock et al. (2023) published an overview of management practices, next to land use change, that are known to promote C sequestration in stable pools as well as dynamic C sequestration in labile pools and biomass. According to the authors, these practices are:

- *Increasing the diversity of cropping systems by integrating legumes, ley grassland, cover crops, under sown crops and perennial crops.*
- *Returning exported harvest residues to cropland via organic fertilizers.*
- *The application of biochar to cropland.*
- *Integrate trees on agricultural lands (agroforestry).*
- *The application and incorporation of mineral amendments (e.g. clay-rich materials, silicate rocks), which, however, refers to the inorganic C stock.*
- *Optimize grazing intensity, plan grazing holistically and promote plant diversity of grasslands.*

However, the frequently suggested avoidance of intensive tillage is controversially discussed by the authors, as they attribute the positive effects of reduced tillage on SOM quantities to the mere redistribution of SOM in the upper soil layers. According to the authors, the incorporation of OM into deeper soil layers can also lead to a reduced decomposition of these materials.

Nevertheless, all of these management practices on cropland soils (in many variations and combinations) are generally known to either increase C inputs, decrease C outputs and have further beneficial effects, such as promoting soil and plant health by improving soil structure, nutrient availability and water availability (Gebreyes, 2019). Schlesinger & Amundson (2018) state though, that most promising management practises (biochar application and enhanced silicate weathering) will be able to only balance ~5 % of annual CO_2 emissions from fossil fuel combustion.

In a global meta-analysis of the relationship between soil organic matter and crop yields, Oldfield, Bradford & Wood (2019) predicted how a SOC increase in the upper soil layer (0-15 cm) will affect global average yields of maize and wheat. Their quantitative model predicted yield increases of ~10 % for maize and ~23 % for wheat with an increase of SOC, levelling off at 2 % SOC. Lal (2020) proposed a critical SOC level of ~2 % in temperate zones and ~1 % in the tropics for securing crop yields. In an earlier study, Lal (2006) reports a possible increase of food-grain production in developing countries by 2 % to 5 % with additional 0.5-1 Mg C ha^{-1} yr^{-1} respectively (corresponding to a SOC increase of 0.02 % and 0.04 % per year). In addition, Lal expects far greater effects due to enhanced fertilizer and water use efficiencies. The importance of STN for crop productivity must be emphasised at this point. While the aforementioned studies analysed SOC as an indicator for SOM, another study by Oldfield et al. (2018) investigated the N uptake of the crops in correlation with SOM contents. According to the authors, higher SOM concentrations go along with an improved N availability for crops, which is accountable for the positive yield effect of SOM observed in their study (Oldfield et al., 2018).

With regards to the potential of global soils to sequester carbon, Lal (2018) suggested possible amounts of 1.45-3.44 Mg C yr^{-1}. According to the international "4 per 1000"[3] initiative, launched by France in 2015, CO_2 in the atmosphere could be significantly reduced if carbon in the upper 30-40 cm of soils would increase at a rate of 0.4 % yr^{-1}. The initiative also states that agricultural soils play a crucial role in climate change, as well as for food security (Minasny et al., 2017). However, the initiative is discussed controversially since the target of 0.4 % and its calculation is rather general and does not account for the progressing climate change or the limit of soils to sequester C (next to political and socio-economic issues) (Rumpel et al., 2020). On the basis of stoichiometry constraints, Van Groenigen et al. (2017) criticise the proposal of the "4 per 1000" initiative which would come down to a sequestration rate of 1200 Tg C yr^{-1} for all agricultural soils. Given average C:N ratios of 12:1 in these soils, the authors argue that an additional input of 100 Tg N yr^{-1} would be necessary to achieve this rate. Van Groenigen et al. (2017) question the origin of these N quantities, which would require an increase in N-fertiliser production or N_2-fixation. At the same time, according to the authors, these N quantities are not compatible with extensive agricultural systems (with usually less fertilization and less N uptake by plants), which makes a more differentiated approach to the "4 per 1000" initiative necessary. Against this background, it becomes apparent that an understanding of N dynamics is equally important as an understanding of C dynamics in the study of SOM dynamics. Wiesmeier et al (2020) studied the potential of agricultural soils in Bavaria to sequester this amount under different well-excepted management practices. They found that an annual increase of only 0.1 % seems possible until the SOC storage capacity of the soils is reached. The latter differs between soils, though and higher amounts might be sequestered in soils depleted of SOM. According to Lal (2018) the potential of a soil (and vegetation/wetlands) to function as a C sink depends on land use (historic and present), as well as depletion of C (actual C stock), soil properties (e.g. clay content and mineralogy, plant available water holding capacity, nutrient reserves, landscape position), climate, and management. Not to forget, the potential to sequester SOC is also limited by socio-economic and political barriers (Rumpel et al., 2020). The average global C sequestration potential of soils is estimated to range between 0.05- 1 Mg C ha^{-1} yr^{-1}, depending on SOC stocks that are protected against decomposition and have a long residence time (Lal, 2018).

It is not surprising that technologies and business models have developed around soil as a potential CO_2 sink. Carbon capture and storage (CCS) technique aims to mitigate climate change by capturing CO_2 from power plants and other industrial processes and store it in the subsurface (Bandilla, 2020). In contrast, the carbon credit trading approach compensates other sectors for offsetting the CO_2 emissions of one actor. This private control instrument is based on a certification system for carbon sequestration in agricultural soils. The certificates can be sold by farmers to companies and thus

[3] https://4p1000.org, assessed 11.08.2024

represent a new line of business for farmers – "carbon farming". An important aspect of this approach is that the amount of carbon sequestered must be on top of the amount that would be sequestered by usual practice anyway (additionality) and sequestration must be secured in the long term (Paul et al., 2023). Up to know, credible and reliable measurement/monitoring, reporting and verification (MRV) platforms have yet to be developed for national reporting of SOC stocks and for emissions trading (Smith et al., 2020). Therefore, its suitability to mitigate climate change has to be further discussed and studied. Paul et al. (2023), as well as Thamo & Paul (2016), questioned issues with carbon farming, such as: a.) securing and monitoring the long term C storage, b.) questions of liability if C storage is not permanent, c.) issues with guaranteeing additional C sequestration on top of usual C sequestration and d.) the possibility of misleading advertisement. Due to these issues, which they believe to be difficult to solve, the authors do not think that carbon farming is suitable for mitigating climate change. Paul et al. (2023) however, suggest to encourage carbon sequestration by public governance options and demand further research on private business models. According to Garsia et al. (2023), up to date protocols of voluntary carbon markets could profit from SOC prediction models, which results in a need for guidelines for model selection that are lacking to this day. Smith et al. (2020) propose benchmark sites as a central element of standardised MRV platforms to assess short-term and long-term effects on SOC stocks. The authors however, see a difficulty in mapping the diverse combinations of climate, land use, soil type and management practices and consider predictions from SOM models to be a valuable complement to measured SOC changes. Nevertheless, the respective models have to be tested across various parameters and combinations (see above) and could at best be further developed or parametrized on the basis of the same benchmark sites (Smith et al., 2020). The possibilities, limitations and development potential of SOM assessment and modelling are outlined in the next two sub-chapters.

1.3.4 Assessment of SOM

SOM analysis

The quantification of SOM is important with regards to climate change and agricultural production (Yang et al., 2024), not least due to its´ various functions already mentioned previously. SOM can be measured directly or calculated using indicators (e.g. SOC and STN). This can be done on a complete soil sample as well as for individual soil fractions. Some methods are suitable for assessing both the total SOM storage and its quantitative (and sometimes qualitative) changes. However, measurement of some indicators only allows for the assessment of SOM changes (quantitative and qualitative). Over time, a wide range of methods with even more variations have become established, including determination by mass loss, dry and wet combustion as well as Vis-NIR spectroscopy. These methods have been described in more detail by Tabatabai (1996), Shamrikova et al. (2022) and Yang et al. (2024), Bremner (1996) and Shi et al. (2013), for example. In addition, numerous fractionation methods were developed to divide SOM (or SOC) in fractions that belong to different functional groups. This separation can be done physically, by separating SOM into aggregate, particle size, and density fractions as well as by separation fractions according to their magnetic susceptibility. Elliott & Cambardella (1991), Christensen (1992; 2001) and Stevenson et al. (1989) have described the usual physical fractionation methods and treatment steps in detail. Another approach is the chemical fractionation, using various procedures that fractionate SOM according to solubility, hydrolysability, and resistance to oxidation or by destruction of the mineral phase. Next to this, a number of combinations exist (von Lützow et al., 2007).

However, SOM is spatially very heterogeneously distributed, and SOM changes are relatively small compared to a large background mass (Carter, 2002), usually smaller than their spatial variability (Brock et al., 2011). With today's methods of sampling and analysis, such small changes, often reflecting short-term-changes, are not easy to quantify. Smith (2004) states that short-term SOM changes can be detected by high sampling numbers, or if the changes are > 20 % (which usually takes at least five years). To reduce time effort and costs, some authors suggest to analyse SOM fractions that are more sensitive to short-term effects, such as recently added plant material (Conant et la., 2003). In their study, Leifeld & Kögel-Knabner (2005) analyse the suitability of SOM fractions as indicators of short-term changes. The fractions of non-complexed SOM were found to be particularly sensitive to management influences (Gregorich et al., 2006). Next to analytical approaches, Brock et al. (2011) hypothesize that an optimized sampling design can improve the quantification of short-term SOM changes while keeping sampling numbers relatively small. In addition, gaseous C and N losses can be used to predict short-term SOM losses. A common method is to assess CO_2, CH_4, N_2O and NH_3 emissions from soil by using closed chambers in which the gases are collected at the field site and collected and measured in regular intervals (Valujeva et al., 2017). Another method is the assessment of CO_2 emissions in incubation trails, indicating SOM decomposition. Weiglein et al. (2022) criticises that results of those studies are not included in most large-scale SOM studies, though. However, the measurement of in situ emissions is not easily done, since it is difficult to separate microbial respiration from plant respiration. As described earlier, N_2 emissions are of high importance (next to N_2O). However, the assessment of N_2 still comes with challenges due to high atmospheric background concentrations (Friedl et al., 2020).

All methods described above have their limitations, and studies with different analytical procedures are hard to compare at times. Roper et al. (2019), for example, compared four SOM measure methods (Walkley-Black method, mass loss on ignition, automated dry combustion, and humic matter colorimetry) on 84 soil samples and found significant differences between the results. The authors explain this by the varying components of SOM that are better assessed with one method or the other method and suggest either standardised methods in future research or at least great caution in the interpretation of studies using different methods. Next to differences resulting from analytical SOM methods, a lack of standardization affects the comparability of studies on SOM fractions. For example, size or density boundaries that link organic matter (OM) to a certain fraction or a pool are not yet generalized (Wander, 2004), and chemical fractionation is not suitable to definitely separate functional SOM pools from one another (von Lützow et al., 2007). Therefore, research is still ongoing to further improve quantitative recording of total SOM or individual fractions and the subdivision of SOM into pools.

It can be concluded that the analysis of individual SOM fractions is certainly important from a scientific point of view, but is not an option for practitioners to monitor the SOM stocks of their soils. In general, however, an increase in the SOM stock can be seen as positive, due to its importance for food production, carbon sequestration and a sustainable agriculture. This makes it necessary to assess the effects of agricultural management on SOM. According to Körschens (2006), the best way to monitor SOM in cropping systems is to set up long-term field experiments. However, these are rather costly and the time span (> 15 years) contrasts the often urgent need for data. In addition, research projects nowadays are usually funded for only two to three years and SOM changes are difficult or impossible to record during this time period, for the reasons already described (Myers, 1995). Against the background of management planning, there is a need for tools that are easy to use and that can provide at least a rough, yet reliable, indication of management effects on SOM. With food production and climate change in mind, these tools should ideally be suitable for assessing both effects on soil fertility and carbon sequestration. In addition to regular measurements, models for predicting SOM have proven to be useful planning aids for agricultural practise and are discussed in relation to facilitating carbon crediting.

SOM modelling

According to Campbell & Paustian (2015), SOM models have played a crucial role in research in recent decades, as they can be used to test hypotheses for soil processes by predicting SOM dynamics.

Some models are based on the assumption that SOM is present in different pools in the soil (the number of pools varies depending on the model) and that SOM is not a continuum of decomposition resistance (Myers, 1995). Those models view C and N flows between pools (e.g. the microbial biomass, labile OM and stabilized OM) and thus predict the C and N dynamics in soils (Gabrielle et al., 2002). Each SOM model depends on the quality and quantity of measured data to link this data to the conceptual understanding of the model (the mathematical presentation) (Campbell & Paustian, 2015).

A large number of models with different evaluation approaches have been developed worldwide. Campbell & Paustian (2015) conducted an in-depth literature study to evaluate the most cited SOM models. In total, they found 74 models to be effectively searchable (with increasing citations starting from the 1970s). Of these, the models CENTURY and RothC stood out due to direct citation numbers and because several studies were explicitly based on their underlying theory.

Regarding sustainable agricultural production, farmers should be able to assess the effects of their management on the SOM stocks of their soils, as these are crucial for the provision of plant nutrients, soil structure and pest management. Against this background, SOM balance models were developed as planning aids for agriculture (Brock et al., 2017). These SOM balance models (also referred to as “humus balance models”) focus only on one SOM pool against the background of primary production. These models compare SOM utilization by crops and SOM input via fertilizers (Körschens et al., 2005).

Brock et al. (2013) analysed and described most commonly used SOM balance models of central Europe in the 20th century. In the following, their study is summarised briefly, without a detailed description of the calculation approaches of the individual models. According to Brock et al. (2013), SOM balance models can be divided into those with an "ecological" and those with an "agronomical" approach. The usually simplified "ecological" approach is based on deterministic observations of long-term experiments and more complex modelling of SOC changes (as an indicator of SOM). Total SOM is assessed by offsetting site-specific conversion processes (ecological processes) and SOM reproduction (through crops, by products and organic amendments). In this approach, the SOM demand is equal to the total SOM loss due to management. This means that models with an "ecological" approach are best suited to estimate quantitative SOM changes and the associated carbon sequestration or GHG emissions. For this purpose, these models require information on site specific soil properties and climatic conditions, next to information on management.

SOM balance models with an "agronomical" approach evaluate SOM reproduction with different crop rotations and fertilization regimes, which goes hand in hand with crop productivity and environmental effects. In this context, however, no site-specific optimum contents are available. For this reason, SOM balance methods can be used as indirect indicators to estimate SOM build-up and depletion and to optimise management. Therefore, such models are less suitable for the assessment of carbon sequestration and GHG production, as they cannot quantify SOM changes. The calculations of these "agronomical" balance models are mostly based on empirical observations in long-term field experiments, the quality of organic fertilizers as well as other organic inputs, and on models that link nitrogen removal from plants to SOM turnover. Table 1 provides an overview of the European models and their approach as described in the study by Brock et al. (2013).

Table 1: Central European humus balance models divided into ecological and agronomical approach, according to Brock et al. (2013).

Balance models with ecological approach	Balance models with agronomical approach
CCB *(Franko et al. 2011)*	VDLUFA *(VDLUFA, 2004*)*
	Humus Unit *(Leithold et al. 1997)*
	Dynamic Humus Unit *(Hülsbergen, 2003)*
	SALCA *(Oberholzer et al. 2006)*
HU-MOD *(Brock et al. 2012)*	
STAND *(Kolbe, 2010)*	

*since further developed

Given the large number of SOM models and SOM balance models with different concepts, it is not easy to choose the right model for an underlying question. The selection of an unsuitable model can lead to considerable errors (Campbell & Paustian, 2015). Whether a model is suitable, next to the specific concept, depends on the data required to use the model and whether this data is readily available. The prediction accuracy is of course equally important. How accurate a model is, depends on the quality of the input parameters, the calibration and, last but not least, the validation performance of the model.

The large variety of models triggered a discussion regarding their basic assumptions, parameters, validation (e.g. Le Noë et al., 2023 and Garsia et al. 2023) and hence their suitability. Campbell & Paustian (2015) see a general opportunity in using SOM models as policy instruments to estimate the effects of land use change or on GHG budgets. According to Le Noë et al. (2023), about 60 % out of ~250 models analysed in their study are not suitable for predicting SOM dynamics, but rather provide information for a conceptional understanding of soil processes. Garsia et al (2023) reviewed 221 SOC models and found that the majority of the models (71 %) are not validated, or validation contexts are limited. In addition, they criticize that most models focus on the global north, do not provide clear reporting, and show major flaws in their performance evaluation. Therefore, Garsia et al. (2023) conclude that SOC models are not suitable to ensure reliable carbon crediting. Next to validation, the spatial dimension for which predictions are made have to be mentioned. Campbell & Paustian (2015) state that most SOM models predict SOM dynamics for the topsoil layer (0-30 cm) and propose to extend the modelling to deeper soil layers, as there is much evidence for their involvement in total SOM dynamics.

SOM balance models, on the other hand, do not refer to a fixed soil depth. However, data for the parametrization and validation of these models usually come from experiments that focus on SOM changes in the topsoil (Brock et al. 2017). This is a major issue when it comes to improving model performance. According to Brock et al. (2013), the major problem with SOM balance model validation is especially true for models with an "agronomical" approach. The authors also point out that the simplest methods, in contrast to more complex models, do not have a scientifically standardised parameterization. The latter come with higher data requirements and there is no reference validation data set for models with the same scope (Brock et al., 2013). In addition, SOM balance models need to integrate new research results and developments in cropping systems, such as new crops or fertilizers. As climate change progresses, new crops are being tested for agricultural use and new varieties of common crops are being bred in order to cultivate plants that are adapted to new climatic conditions. SOM balance models need to integrate new research results on these crops. Therefore, data requirements are high, yet long-term field experiments for these crops are scarce or not available. Because of this, proxies must be used for the required parameters or alternative experiments must be carried out to provide preliminary data. With regard to STN dynamics (and SOM dynamics, affected by it), it is important to investigate the extent to which a new crop utilizes an applied N fertilizer compared to STN. Campbell & Paustian (2015) list numerous possibilities to further develop SOM models, namely with regards to the microbial role in SOM stabilization, to SOM saturation, to temperature controls on SOM, as well as with regards to SOM in global models. Against the rising importance of SOM models as well as SOM balance models, there is an urgent need for reliable data with regards to their improvement and development.

To summarise, when developing SOM balance models, coefficients must be subjected to subsequent validation. Against this background and due to the high demand for data in connection with management effects on SOM, approaches must be examined that improve the assessment of SOM dynamics in short-term field experiments. In addition, the reference depth in the soil must be considered when validating coefficients. And since SOM contains C as well as N, and their relationship is important for SOM dynamics, results on plant-specific STN uptake are equally important, especially for crops that have been little studied to date. This is the basis of the objectives of this thesis.

1.4 Aim of the thesis

This thesis deals with the provision of STN for plant nutrition and the assessment of SOC in agricultural field experiments, aiming to contribute to the improvement and development of SOM balance models. It serves to achieve more reliable predictions of management effects on SOM. This is important against the background of maintaining and increasing primary food production and carbon sequestration. In this thesis, special focus was placed on the SOM balance model HU-MOD. However, the results are also relevant for other SOM balance models. The hypotheses were that an adaption in soil sampling procedure can improve data of both long-term and short-term field experiments for the validation of coefficients, and that pot or bag experiments are suitable for parametrization purposes.

The specific questions of the thesis were:

1. *What is the share of nitrogen from SOM-mineralisation taken up by a crop that is fertilised with mineral nitrogen?*
2. *Can short-term field experiments be used to assess SOM change under cropping systems as a basis for the validation of SOM balances and other models?*
3. *Is it necessary to assess SOM quantity changes in the subsoil in order to estimate and validate C and N balances in the soil?*

To answer these questions, three experiments were carried out, the results of which were published in scientific journals. The publications are presented in chapters 2, 3 and 4.

Chapter 2: First article

Article: Uptake of Fertilizer Nitrogen and Soil Nitrogen by Sorghum Sudangrass (*Sorghum bicolor X Sorghum sudanense*) in a Greenhouse Experiment with ^{15}N-Labelled Ammonium Nitrate

Published in *Soil Systems* (2023) 7, 71.

DOI: 10.3390/soilsystems7030071

soil systems

MDPI

Article

Uptake of Fertilizer Nitrogen and Soil Nitrogen by Sorghum Sudangrass (*Sorghum bicolor* × *Sorghum sudanense)* in a Greenhouse Experiment with ^{15}N-Labelled Ammonium Nitrate

Lucas Knebl [1,2,*], Andreas Gattinger [1], Wiebke Niether [1] and Christopher Brock [1,2]

1 Department of Agronomy and Plant Breeding II, Justus Liebig University Giessen, Karl-Glöckner Str. 21C, 35392 Giessen, Germany; andreas.gattinger@agrar.uni-giessen.de (A.G.); wiebke.niether@agrar.uni-giessen.de (W.N.); brock@forschungsring.de (C.B.)
2 Forschungsring e.V., Brandschneise 5, 64295 Darmstadt, Germany
* Correspondence: knebl@forschungsring.de

Abstract: A greenhouse experiment with sorghum sudangrass (*Sorghum bicolor* × *Sorghum sudanense*) and maize (*Zea mays*) was conducted to assess information on differences in their nitrogen and fertilizer utilization when used as energy crops. The aim was to contribute to the scarce data on sorghum sudangrass as an energy crop with regards to nitrogen derived from fertilizer (NdfF) in the plant's biomass and fertilizer nitrogen utilization (FNU). Sorghum sudangrass and maize were each grown in eight bags of 45 L volume and harvested at maturity after 154 days. Each crop treatment was further divided in a control treatment (four bags each) that did not receive N fertilization and a fertilization treatment (four bags each) that received 1.76 g N, applying a ^{15}N-labelled liquid ammonium nitrate fertilizer. Fertilization took place at the start of the experiment. After harvest, the whole plant was divided in the fractions "aboveground biomass" (ABM) and "stubble + rootstock" (S + R). Weight, N content and ^{15}N content were recorded for each fraction. In addition, N content and ^{15}N content were assessed in the soil before sowing and after harvest. The experiment showed that FNU of sorghum sudangrass (65%) was significantly higher than that of maize (49%). Both crops accumulated more soil N than fertilizer N. The share of fertilizer N on total N uptake was also higher with sorghum sudangrass (NdfF = 38%) compared to maize (NdfF = 34%). The observations made with our control plant (maize), showed that the results are plausible and comparable to other ^{15}N studies on maize regarding yields, NdfF, and FNU, leading to the assumption that results on sorghum sudangrass are plausible as well. We therefore conclude that the results of our study can be used for the preliminary parametrization of sorghum sudangrass in soil organic matter (SOM) balance at field level.

Keywords: mineral nitrogen utilization; ^{15}N-labelled fertilizer; N balance; sorghum sudangrass

check for updates

Citation: Knebl, L.; Gattinger, A.; Niether, W.; Brock, C. Uptake of Fertilizer Nitrogen and Soil Nitrogen by Sorghum Sudangrass (*Sorghum bicolor* × *Sorghum sudanense)* in a Greenhouse Experiment with ^{15}N-Labelled Ammonium Nitrate. *Soil Syst.* **2023**, *7*, 71. https://doi.org/10.3390/soilsystems7030071

Academic Editors: Douglas Guelfi, Heitor Cantarella and Flávio Henrique Silveira Rabêlo

Received: 21 May 2023
Revised: 20 July 2023
Accepted: 28 July 2023
Published: 3 August 2023

1. Introduction

The appropriate management of soil organic matter (SOM) is a major goal for sustainable agriculture [1]. Farmers must be able to estimate SOM contribution by demand of specific crops in order to adapt crop rotation and nutrient cycling to ensure optimal SOM reproduction [2]. Nitrogen is an important compound of SOM and a necessary nutrient for plants. However, even with standard mineral N fertilization that is adjusted to meet their demand, the total N uptake by crops consists of soil-derived N (NdfS) next to fertilizer-derived N (NdfF). Those soil-derived N amounts have to be replaced in order to maintain soil fertility. Fertilizer N that has not been taken up can either remain in the soil or be lost (e.g., leaching, volatilization). Fertilizer nitrogen utilization (FNU) provides an important indication of the potential amount of fertilizer N that remains in the soil (and may be available to the following crop) or is lost.

One way to estimate FNU of a crop is to use a ^{15}N-labelled fertilizer and thus record NdfF within the plant. Such experiments exist mainly for maize and cereal [3–13]. The studies on maize differ considerably in design and implementation, making comparability difficult. Differences exist in the fertilization (type, amount, fertilization timing, and application technique). In addition, the focus of the assessments often is on aboveground biomass (either in total or varying plant fractions). Fertilizer uptake by roots and remaining fertilizer in the soil is often neglected. Among available studies, only a few are comparable to our experimental setup based on fertilizer type (NH_4 and/or NO_3), crops, and sample size. A list of those studies can be found in Appendix A Table A1. Harris et al. [4] conducted a study on maize, examining grain, stem, and roots as well as soil after one growing season. In their experiment, 124 kg N*ha^{-1} was fertilized prior to seeding (granular, incorporated) in the form of ^{15}N-labelled $(^{15}NH_4)_2SO_4$. The authors found that corn had an overall FNU of 40%, while 23% of the applied fertilizer remained in the soil after harvest and about 38% of applied fertilizer could not be recovered in the samples taken and was assumed to possibly be lost. No data were provided on NdfF. Studies on maize that considered residual fertilizer N in the soil but not in the roots show comparable results to other studies on maize [3,4,6–8]. The NdfF reported in these studies ranged from 11% to 74% (median = 31%) and FNU of aboveground biomass ranged from 24% to 62% (median = 43%). Fertilizer N remaining in the soil in these studies was 14% to 46% of the original amount of N applied (median = 25%). Porter [6] only checked the amount of labelled N in the soil solution. Measured residual labelled NO_3 ranged from 1% to 10% of the applied fertilizer amount. The proportion of fertilizer N that was not recovered (see Appendix A Table A1) shows a high variance in the studies, ranging from 15% to 76% (median = 29%). In this regard, a positive relationship was observed between fertilizer quantity, and fertilizer N that could not be recovered [6].

However, some crops only recently received attention (<20 years), for example, plants cultivated for energy use. Against the background of climate change, plants for energy production are increasingly coming into focus. Plants that still achieve good yields under extreme conditions (e.g., drought, heat) and need low N input are advantageous in this regard. Sorghum species are characterized by comparatively good water use efficiency, tolerance to higher temperatures, lower fertilizer requirements, and advantages in erosion and weed control [14–16]. Under limited water availability and high temperature, sorghum species performance is higher than that of maize and other cereals [17]. Available studies in this regard focus on sorghum species for forage production, especially in arid and semiarid regions [16–21]. The species *Sorghum bicolor* × *Sorghum sudanense* (from here on called sorghum sudangrass) is particularly suitable and therefore commonly cultivated for bioenergy use. When growing this hybrids, farmers should be able to estimate N use efficiency in order to maximize yields without depleting their agricultural soils. However, for this crop, no long-term field experiments exist yet that can deliver a well-founded data base.

In cases without long-term observations, SOM balance models such as Roth-C, CCB, and HU-MOD [22,23] can provide first clues on how a specific crop might affect SOM. Usually, SOM balance models provide a decision tool in planning management strategies with regard to SOM maintenance or enhancement. Brock et al. [23] reviewed most commonly used SOM balance models (in the EU) in detail. Depending on the model, the specific effects of a cultivation system on the balance of soil organic carbon (SOC) and/or soil total nitrogen (STN) are calculated. The algorithms used are usually based on data derived from long-term field experiments on the corresponding cropping systems. The respective effects of individual crops are determined by the corresponding C and/or N inputs and withdrawals. If the data on one crop are scarce, SOM balance models can use data from an already studied physiologically similar plant. In the case of sorghum sudangrass, this could be *Zea mays* to make an initial estimate. However, the approach could as well result in a significant overestimation or underestimation of the true effects of sorghum sudangrass on SOM. Therefore, the aim of this study is to contribute to expanding the data situation on sorghum sudangrass as a bioenergy crop with regard to its fertilizer utilization (NdfF and FNU). In addition, we want to know whether a greenhouse experiment is sufficient to assess data that can be used to model SOM balance of crops in the field. The hypothesis of this study is that there are significant differences between sorghum sudangrass and maize regarding NdfF and FNU. This assumption is based on the reported differences between sorghum species and maize with regard to fertilizer use [14–17]. We further hypothesized that those differences can be assessed in a greenhouse experiment. For this purpose, sorghum sudangrass and maize were grown in a bag experiment in a greenhouse and fertilized with ammonium nitrate, labelled with ^{15}N to assess NdfF and FNU.

2. Materials and Methods

2.1. Experimental Set Up

The experiment was carried out in 2015 at the Experimental Station Rauischholzhausen of the Justus Liebig University Giessen, Germany (Mean annual temperature: 9 °C; geographic location: 50°75′79.7′′ N 8°88′75.1′′ E). Sorghum sudangrass (*S. bicolor* × *S. sudanense*) and maize (*Zea mays*) as control plant were grown in eight planting bags each (four fertilized, four not fertilized) (Table 1). Planting bags had a volume of 45 L, with a surface area of 0.126 m^2 and an approximate height of 35–36 cm. The trial started in mid-March with sowing and ended in mid-August with harvest. The planting bags were placed randomly in a greenhouse with daylight, which could be opened to the outside. Several holes at the bottom of the bags allowed for possible leaching. A standard soil substrate of the experimental station was used to fill the bags. The substrate consisted of an arable topsoil (stored air dry for a longer period prior to the experiment) that was sieved to <5 mm and mixed with quartz sand (2:1) when the experiment started. The mixture had a SOC and STN content of 0.95% and 0.10%, respectively, with a pH of 6.3. Plant available nitrogen (N_{min}) in the substrate corresponded to 200 kg N_{min}*ha^{-1}. Plant bags were filled with soil up to 36 cm height (compacted to 33 cm at harvest). The upper 15 cm were mixed with fertilizer homogeneously before filling the bag. Fertilized treatments (N1) received 1.76 g of nitrogen with liquid ^{15}N-labeled ammonium nitrate (50% ammonia, 50% nitrate, 1% enrichment). The fertilizer N amount corresponds to a fertilization of 140 kg N ha^{-1}. Control treatments (N0) did not receive any fertilization. Sorghum sudangrass (variety "Lussi") and maize (variety "Lorado") were sown immediately after filling the bags at a seed depth of 3 cm and 4 cm, respectively. For this purpose, five plants per bag were sown in four replicates each. For sorghum sudangrass, this seeding density and the N fertilization were in accordance with standard practice. Maize was seeded more densely than in practice, and the N fertilization was relatively low given the expected increased yields (due to the dense seeding). Seeding density for maize was five times higher than usually used in practice. This was applied to obtain a more homogeneous plant material (instead of only one plant). Seeds that did not germinate initially were replanted as soon as possible to reach five plants per bag at harvest time. Maize is a crop that has been sufficiently studied scientifically and the effects of seed density and N fertilization can be well estimated and evaluated. Plants were irrigated according to plant demand. The harvest of both crops took place after 154 days when plants had a dry matter content of 28–32%.

Table 1. Experimental setup of ^{15}N experiment with sorghum sudangrass and maize grown in bags (45 L vol.) in a greenhouse. DM = dry matter.

Plant	Treatment (ID)	Repetition (No.)	Fertilization (g N*Bag^{-1})	Seed Density (Seeds*Bag^{-1})	DM at Harvest (%)	Harvest (Day)
Sorghum sudangrass (*S. bicolor* × *S. sudanense*)	N0	4	0	5	30 ± 2	154
	N1	4	1.76	5	30 ± 2	154
Maize (*Zea mays*)	N0	4	0	5	30 ± 2	154
	N1	4	1.76	5	30 ± 2	154

2.2. Data Collection and Analysis

After sieving the soil substrate and mixing it with sand, three subsamples were taken before the substrate was fertilized and divided to be filled into the planting bags. After harvest, 3 soil samples per bag were taken with a soil auger (0–30 cm). The samples were oven-dried directly after collection at 40 °C for a period of 48 h, sieved to <2 mm, and grinded for analysis of STN, and ^{15}N content. At harvest, aboveground biomass (ABM) of the plants was cut at 10 cm height. To assess the main part of residues that stay on the field after harvest, stubble and rootstock of all plants within each bag were combined to the fraction stubble + rootstock (S + R). For total biomass (TBM), both plant fractions were summed up. Soil from rootstocks was removed by washing the rootstock with tap water over a sieve. Plant fractions were immediately oven-dried at 60 °C for a period of 48 h and grinded to be analyzed for carbon (C), nitrogen (N), and ^{15}N content. N content in both soil and plant biomass fractions, as well as organic carbon in soil, were assessed with a Vario EL (Elementar Analysensysteme GmbH, Langenselbold, Germany) with an analytical precision of 0.01 g kg^{-1} for C and N, respectively, according to DIN/ISO 13878:1998 and 10694:1996 [24,25].

Isotopic signature $\delta^{15}N$ was measured using EA-IRMS, i.e., an element analyzer coupled to an isotope ratio mass spectrometer (Flash-EA and DELTA V Advantage, Thermo Fisher Scientific GmbH, Dreieich, Germany). Measurements were calibrated through regression in three dimensions (signature × ion amount × time) against certified reference materials, which were treated and measured in the same way as the samples, and which covered the value ranges of the samples, i.e., $\delta^{15}N$: 1.18‰ to 19.6‰ air, sample weight: 0.3 mg to 1.0 mg (acetanilide #1, acetanilide #2, L-phenylalanine, Arndt Schimmelmann, Bloomington, IN, USA). Standard deviation of measured values against the certified value of the reference materials was less than 0.2‰ (n = 11) for $\delta^{15}N$. In accordance with Rocha et al. [8], the ^{15}N enrichment (atom % ^{15}N excess) in plant parts and soil were obtained by deducting the natural abundance. The latter was assessed in the not fertilized control treatments. The share of nitrogen derived from fertilizer (NdfF (%)) was calculated according to Equation (1) (in accordance with IAEA [26]).

$$\text{NdfF (\%)} = \text{atom \% } ^{15}\text{N excess in sample/atom \% } ^{15}\text{N excess in fertilizer} \times 100 \quad (1)$$

This results in the amount of fertilizer N in the corresponding plant fraction or in the soil according to:

$$\text{NdfF [g]} = \text{Ntot} \times \text{NdfF (\%)} \quad (2)$$

where Ntot describes the total amount of N (g) in the plant parts or the soil.

The amount of N derived from soil (NdfS) in the plant parts results from the subtraction of NdfF from Ntot:

$$\text{NdfS} = \text{Ntot - NdfF} \quad (3)$$

Calculation of FNU was then carried out according to IAEA [17]:

$$\text{FNU [\%]} = \text{NdfF in plant biomass/Nfert} \times 100 \quad (4)$$

where Nfert is the applied fertilizer N amount (g) and NdfF is the total N uptake (g) by plant biomass.

The fertilizer recovery rate in plant and soil (^{15}NRR) is calculated according to

$$^{15}\text{NRR [\%]} = 100/\text{Nfert} \times (\text{NdfFplant} + \text{NdfFsoil}) \quad (5)$$

where NdfFplant and NdfFsoil is nitrogen derived from fertilizer in plant biomass and soil, respectively.

2.3. Statistical Analysis

For the independent variables (weights, N and ^{15}N amounts of plant fractions), mean values were tested for significant differences using two-way ANOVA (crop * fertilization), followed by Tukey HSD. For data that did not meet the requirements for ANOVA, the Kruskal–Wallis test followed by the Wilcoxon rank sum exact test was carried out. Dependent variables (changes in soil total N and soil ^{15}N) were analyzed using *t*-test in the case of existing normal distribution. If the variables were not normally distributed, Kruskal–Wallis test (Dunn's post-hoc test with Bonferroni adjustment) was applied. Analysis was carried out with Rstudio statistical software, version 1.3.1056-1 [27].

3. Results

3.1. Biomass and Soil

At the start of the experiment, there were some inhibitions in plant germination, followed be a delayed plant development. This resulted in a slightly longer growing season compared to the field (154 days). N fertilization resulted in higher dry matter production of ABM and TBM of sorghum sudangrass (Table 2). For maize, a positive fertilization effect was only observed with regards to TBM yield. In contrast, fertilization did not affect growth of S + R of both crops. The ratio of ABM: S + R of sorghum sudangrass increased with N fertilization from 6.7 to 8.2. An increase in the ratio ABM:S + R of maize was not observed, and the ratio accounted for 7.3 with both fertilization treatments. Sorghum sudangrass achieved higher overall TBM yields compared to maize in both fertilization treatments, but ABM of sorghum sudangrass was higher than maize only in fertilization treatment N1. Extrapolated to one ha, the TBM yields obtained correspond to about 39 and 28 Mg*ha^{-1} each for fertilized sorghum sudangrass and maize, respectively.

Table 2. Dry matter yields (g DM*bag^{-1}) and N content (%) of aboveground biomass (ABM), stubble + rootstock (S + R) and total biomass (TBM) for sorghum sudangrass and maize with fertilization treatment N0 (0 g N*bag^{-1}), and N1 (1.76 g N*bag^{-1}). Letters denote significant differences for each plant part between crops and fertilization treatments.

	Crops	Sorghum Sudangrass				Maize			
	Fertilization Treatment	N0		N1		N0		N1	
ABM	(g DM*bag^{-1})	328.6	a	431.6	b	305.3	c	373.9	ac
	N (%)	0.61	a	0.64	a	0.58	a	0.58	a
S + R	(g DM*bag^{-1})	55.7	abc	59.8	abc	48.4	b	64.9	c
	N (%)	0.24	a	0.31	b	0.19	a	0.23	b
TBM	(g DM*bag^{-1})	384.3	a	491.4	b	353.7	a	438.8	b
	N (%)	0.55	a	0.59	b	0.53	a	0.53	b

For both sorghum sudangrass and maize, N fertilization resulted in higher N content in all plant fractions (Table 2). These were on average higher in N1 compared to N0. Differences between crops were not observed. The N content (%) of ABM on average was about two to three times higher than of S + R. Sorghum sudangrass absorbed more NdfF than maize in all plant fractions (Figure 1). Furthermore, no differences were observed between the crops in ABM, nor was there any fertilizer effect in ABM of the respective crops. In the S + R fraction, N fertilization led to increased Ntot compared to the unfertilized treatment N0. Without N fertilization, sorghum sudangrass accumulated more Ntot in S + R than maize in that fraction, while there were no differences in Ntot uptake of TBM of unfertilized plants. The only difference was observed for fertilized sorghum sudangrass, that took up more Ntot than unfertilized maize (Figure 1). Remaining amounts of NdfF in soil after harvest ranged between 12% after maize and 15% after sorghum sudangrass of the initially applied amount (1.76 g N*bag^{-1}) and was not different for the two crops.

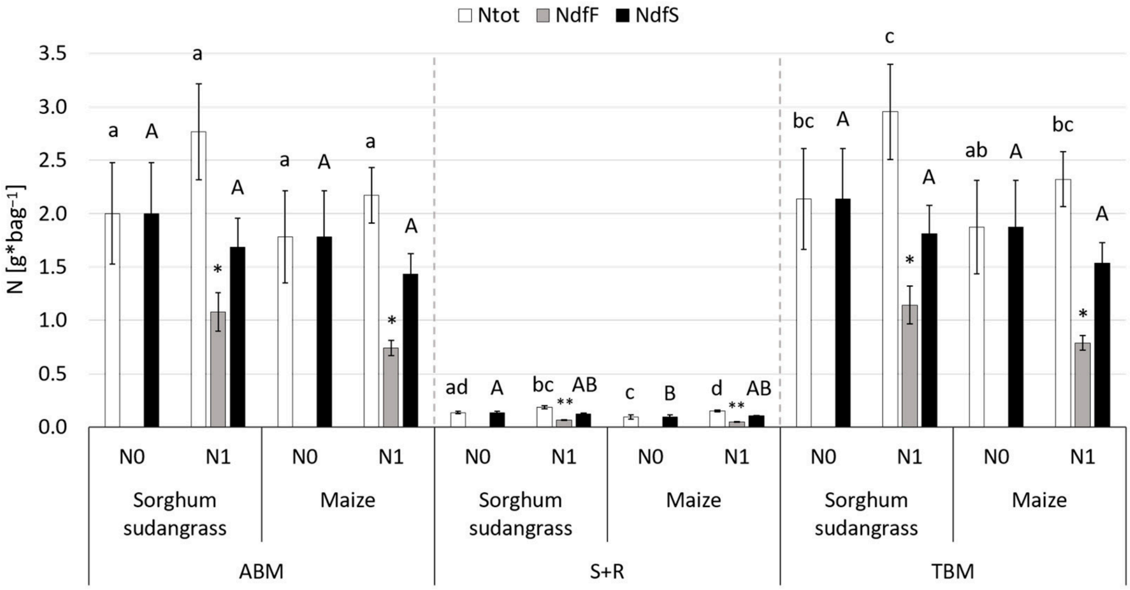

Figure 1. Total N uptake (Ntot), fertilizer N (NdfF) and soil N (NdfS) for plant parts of sorghum sudangrass and maize with the respective fertilization treatment (N0 and N1). Error bars show standard deviation. Lower case letters denote differences of Ntot between sorghum sudangrass and maize as well as fertilization treatments (N0 and N1) within respective plant parts. Upper case letters denote differences of NdfS between sorghum sudangrass and maize as well as fertilization treatments (N0 and N1) within respective plant parts. * and ** denote significant differences (at alpha = 0.5 and 0.1, respectively) of NdfF between sorghum sudangrass and maize within respective plant fractions.

3.2. Nitrogen Uptake Characteristics

The recovery rate (^{15}NRR) of Nfert (Table 3) was higher with sorghum sudangrass (81.1%) compared to maize (56.9%). TBM of sorghum sudangrass had a higher NdfF compared to maize (38.7% and 34.0%, respectively). Both plants accumulated more N than was applied through fertilization. The FNU is higher with sorghum sudangrass (65.0%) compared to maize (44.8%).

Table 3. Recovery rate (^{15}NRR) of fertilizer, not recovered fertilizer N amount (Nfert), share of fertilizer N on total N uptake by plants (NdfF), and plant utilization of fertilizer (FNU). Mean values displayed for fertilization treatment N1 (1.76 g N*bag^{-1}) of sorghum sudangrass and maize. TBM = total plant biomass. Letters denote significant differences between sorghum sudangrass and maize, respectively.

Crops	Sorghum Sudangrass		Maize	
Fertilizer recovery rate (^{15}NRR (%))	81.1	a	56.9	b
Nfert not recovered (%)	19.9	a	43.1	b
Nitrogen in TBM derived from fertilizer (NdfF (%))	38.7	a	34.0	b
Fertilizer nitrogen utilization of TBM (FNU (%))	65.0	a	44.8	b

4. Discussion

4.1. Observed Biomass Production

In the experiment, sorghum sudangrass produced yields of ABM corresponding to 34.2 Mg*ha^{-1}. This yield is somewhat high, yet still plausible compared to yield ranges in practice of 18.0 to 33.1 Mg*ha^{-1} [28]. For maize, yields have been reported in the range of 15.8 to 22.7 Mg*ha^{-1} [29]. Against high seed density of maize in the bag experiment, the rather high production of ABM (corresponding to 29.6 Mg*ha^{-1}) is plausible as well. The aim of the discussion is—in addition to the interpretation of differences between sorghum sudangrass and maize—also the examination of a possible transferability of the results of the bag experiment to the field level.

The S + R fraction was chosen in our study as the bulk of the crop and root residues and is not reported in this form by other studies. The fraction contains both a portion of the aboveground biomass (stubble) and a significant portion of the root biomass, the rootstock. The total root biomass for maize averages 31 g*plant^{-1} [30]; thus, for our experiment, it should account for approximately 155 g*bag^{-1} (with five plants per bag). Since the measured masses of the S + R fraction were min. 48 and max. 69 g*bag^{-1}, it must be assumed that a considerable part of the roots was not recorded. On the other hand, it is extremely difficult to draw conclusions about root development, especially root mass growth of a plant under field conditions, from observations made in pot experiments or bag experiments. This is due to the severely compromised soil conditions in the pots or bags. After all, roots could have been underdeveloped compared to the field, since fertilization was more direct and there was no need (and no space) to expand. For this reason, it is not so much the root masses as the N uptakes of fraction S + R that are discussed here. Other studies report N contents in the organic biomass of maize ranging from 0.65% to 0.98% [31,32]. On this basis, the observed N contents of ABM from maize in our experiment are plausible (0.58%). In the Lynch et al. study [32], the biomass is plant residues, and no further definition is provided as to which residues are involved. The authors also reported mean N contents of 1.09% in sorghum sudangrass residues. These contents are again a little higher than observed in our experiment for sorghum sudangrass (0.61% to 0.64%). The sometimes two- to threefold lower N contents in the fraction S + R of both sorghum sudangrass and maize might indicate N translocation from vegetative plant parts (e.g., stubble mass and rootstock) during grain filling.

4.2. NdfF and Implications for FNU

As already described in the introduction, studies on TBM of maize report NdfF ranging from 11% to 74% with a median of 31% [3–8]. In our experiment, NdfF of sorghum sudangrass and maize lies within this reported range (with 39% and 34%, respectively) and is comparable to the calculated median. Although NdfF was significantly higher for sorghum sudangrass than for maize, the magnitude was rather low.

FNU of maize is reported to range from 24% to 62% with a median of 43% (see Appendix A Table A1), which is close to observed FNU of cereal that ranges from 27% to 66% with a median of 46% (see Appendix A Table A1). In our study, FNU of maize (45%) is in line with that range and the median is similar.

Sorghum sudangrass, on the other hand, had a much higher FNU of 65%. In search of an explanation, differences in root growth are an obvious possibility, since roots play a key role in the specific use efficiency [33]. Clark [34] highlights the special growth performance and the good root system of sorghum sudangrass, as well as its adaptability to stressful conditions. However, in our experiment, there were no differences found in total root mass of sorghum sudangrass and maize. A difference in root architecture and/or speed of growth is highly possible. A higher growing rate of roots might lead to a higher utilization of fertilizer N before it is transported to deeper soil layers and/or possibly is lost. Therefore, Olson et al. and Olson and Swallow [11,12] underlined the importance of the time of fertilization for NdfF in crops. The authors stated that FNU will be higher if the fertilization takes place contemporary with the plant's demand for N. The authors, in addition, speculated about a "nitrogen sink" in the soil that has to be filled up before plants can utilize the fertilizer N [12]. This assumption comes from the observation of higher FNU with higher N fertilization. Therefore, higher FNU with higher fertilization rates might be expected for sorghum sudangrass as well. When extrapolating those findings to the field level, one has to keep in mind the higher seed density of maize in our bag experiment. At field level and with typical seed density, the comparison of root growth might have resulted in different observations and should not be overestimated.

After harvest of sorghum sudangrass and maize, about 13.5% of the applied fertilizer N was recovered in the soil. This share is on the lower end of shares reported in other studies on maize, which range from 14% to 46% (median = 25). However, it can be assumed that fertilizer N that was not found in the soil material might have been transformed by microorganisms with an unknown magnitude (e.g., NO_3 to N_2) and subsequently lost to volatilization. At the end of our experiment, about 20% and 43% of fertilizer N was not accounted for with sorghum sudangrass and maize treatments, respectively. This is in line with findings of comparable studies that include the assessment of fertilizer N in TBM as well as in soil or that at least assessed the effect of possible sources of N loss (see Appendix A Table A1). Those studies report a fertilizer N loss of 29% (median) in experiments with maize, ranging from 15% to 76%. There are different reasons and explanations for a loss of fertilizer N in such experiments (see Appendix A Table A1). However, the quantification of those losses is reported to be specifically difficult [35,36]. Most studies assign the observed losses to, e.g., denitrification, leaching, or NH_3 volatilization, even though this was not quantified in the study. Our data show a strong relationship between fertilizer N loss and FNU with sorghum sudangrass and maize (R^2 = 0.87), which could be expected. However, the correlation does not provide full information on the causal relationship. From the data, it cannot be concluded whether higher fertilizer N loss reduced FNU of the plants or whether an inhibited FNU consequently resulted in higher fertilizer N losses. The magnitude of losses observed in our experiment are reasonable with regard to possible pathways. After irrigation, some leachate could be observed under the bags, which were placed directly on the ground of the greenhouse. However, irrigation was carried out very carefully to avoid leachate. It is still likely that NdfF was lost within leachate, especially after the early fertilizer application (no plant uptake in the initial period). Next to that, nitrification and denitrification are reported to be an important source for N_2O losses from soils [33]. In contrast, other studies have stated that nitrification is probably less important for N losses of arable soils [37]. Kastori [38] observed up to 80 kg N^*ha^{-1} lost through volatilization during one growing and that NH_3 released from aboveground biomass can hold 52% to 73% of observed fertilizer N losses as well as 15% to 20% of FNU [3]. In our study, the initial procedure of mixing the soil substrate with the liquid fertilizer was rather intense compared to arable soils. Thus, we assume the initial microbial activity in the substrate increased significantly, resulting in higher ^{15}N losses due to nitrification and volatilization.

It must also be taken into account that the present study did not record and analyze the entire root mass. Root stock (including coarse roots) and stubble mass represent a great part of the crop residue that remains in the soil when whole crops are harvested for energy use. Because the assessment of fine roots is quite expensive and not always viewed as totally objective [39], we decided to limit our observations to the combined S + R fraction. However, it can be expected that the ^{15}N recovery and the observed fertilizer use efficiency would be higher if the entire root mass had been included.

In addition to in situ losses of nitrogen, sampling procedures and further treatment of samples contribute to nutrient loss. Possible errors can occur due to, e.g., dry matter loss during washing of roots, root respiration after sampling, C and N losses during drying, and storage of samples [40–43].

4.3. Data Suitability for SOM Balance at Field Level

One of our experimental questions was to what extent the data collected in our experiment are suitable to be used in SOM balance models. At this point, the disadvantages and advantages of a bag experiment in a greenhouse have to be discussed against the background of this specific question.

A clear shortcoming of bag experiments lies in the transferability of data to the field level. Differences in soil and climatic conditions can influence overall biomass production and root development as well as nutrient uptake of the plants. Therefore, the interpretation of results must be made with care. The different space availability, but also the different water and temperature regimes in bags most likely alter root development of a plant in comparison to the field. Next to effects on plant growth, this can lead to a misinterpretation of the rhizodeposition when transferring from the bag experiment to field level. At the same time, complete root coverage carries some risks for error [37]. Due to the high costs of complete root collection, its necessity should be discussed before the experiment. In addition, temperature regimes in a greenhouse and controlled water supply to the bags distinguishes such experiments from normal field conditions. Against this background, it is better to study plants under actual field conditions if the subject matter addresses the assessment of absolute values (e.g., C sequestration). However, bag experiments offer a great advantage if the subject matter addresses the assessment of relative values, such as, in our case, NdfF and FNU of one plant compared to another. Reasons for can be found in homogeneous and overall known experimental parameters (including soil). This leads to better comparability of results.

As already introduced, maize is a well-studied crop and parameters needed for SOM balancing are available to a great extent. In contrast to maize, considering data on sorghum sudangrass, especially the hybrid *sorghum bicolor* × *Sorghum sudanense,* the data situation looks much thinner. Since maize shows some physiological similarities to sorghum species, parameters of maize may be used as substitutes for sorghum in SOM balance models. Due to reported differences between maize and sorghum [14–17], we question the admissibility of this procedure. In our experiment, maize and sorghum sudangrass significantly differed with regard to NdfF and FNU (parameters needed for SOM balance). At the same time, observed data for maize are plausible and comparable with other studies. For this reason, we assume that the data collected for sorghum sudangrass on NdfF and FNU are equally plausible. However, we have to stress the fact that our experiment was carried out on only one soil substrate, with one fertilizer type and only two fertilizer levels. In addition, we did not record the amounts of N loss and N in fine roots. These are methodologically as difficult to capture in field experiments as in bag experiments. In future studies, N leachate, volatile N losses, and fine roots may receive a higher focus to better estimate the N pathways of one plant versus another. In addition, further experiments with different soil types, fertilizer types, fertilizer rates, different varieties and seed densities would further complete the dataset on sorghum sudangrass.

Nevertheless, for the first evaluation of sorghum sudangrass with SOM balance models, we recommend using the data presented here on NdfF and FNU for sorghum sudangrass and not relying on data from maize in this case. We base this recommendation on the significant differences compared to maize observed in our experiment and the physiological differences of the two plants mentioned in the literature. Those estimates, however, should be verified in long-term studies on sorghum sudangrass at field level. Against this background, long-term experiments have to be designed that deliver suitable data.

5. Conclusions

From the findings of our study, we conclude that sorghum sudangrass differs significantly from maize with regards to nitrogen utilization and FNU. In conclusion, parameters of maize should not be used as a substitute value in the SOM balance of sorghum sudangrass at field level. Since the observations made for maize are comparable to similar studies, we assume that the results for sorghum sudangrass are plausible as well. We therefore prefer to use the collected results of our experiment on sorghum sudangrass for the preliminary parametrization of this crop in SOM balance. However, a greenhouse experiment differs in conditions like soil and water conditions, climate, etc., compared to the field. With regards to the increasing importance of bioenergy plants, we hence highly suggest further studying crops like sorghum sudangrass, especially with regard to nitrogen utilization, N gas emission, and N leachate, both in the greenhouse and in long-term field studies. Our study is a first valuable contribution to this collection of data.

Author Contributions: Conceptualization, L.K. and C.B.; methodology, L.K.; software, L.K.; validation, L.K., C.B. and A.G.; formal analysis, L.K.; investigation, L.K.; resources, C.B.; data curation, L.K.; writing—original draft preparation, L.K.; writing—review and editing, C.B., A.G. and W.N.; visualization, L.K.; supervision, C.B. and A.G.; project administration, C.B.; funding acquisition, C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FACHAGENTUR NACHWACHSENDE ROHSTOFFE e.V. (FNR), grant number 22402112 and the APC was funded by Justus Liebig University Giessen.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data from this study will be shared with justified request to the corresponding author.

Acknowledgments: We would like to thank the FACHAGENTUR NACHWACHSENDE ROHSTOFFE for funding the project as well as our project partners at the Humboldt University of Berlin (Christof Engels). Special thanks goes to Klemens Eckschmitt from JLU Gießen for coordinating the ^{15}N analysis. We are also grateful to our staff in the greenhouse and laboratory.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Overview on key data from studies used for discussion (crops, parameters and specifications, fertilization, NdfF (%), FNU (%), and ^{15}N, either remaining in soil or not recovered; Exp. = experiment).

Crop	Parameters and Specifications	Fertilization	NdfF	FNU	^{15}N Remaining in Soil	^{15}N Lost or Not Recovered	References
Maize (irrigated in two experiments)	Leaves, stalks, and grain in both experiments; 240 cm soil depth in Exp. 1	Exp. 1: 50, 100, and 150 kg N*ha^{-1} as NH_4NO_3 at V3 stage; Exp. 2: 75 to 300 kg N*ha^{-1} as $(NH_4)_2SO_4$ at V3 stage.	Exp. 1: 12 to 31% (at maturity); Exp. 2: 21 to 55% (at maturity)	Exp. 1: 48 to 53%; Exp. 2: 24 to 36%	Exp. 1: 24% (with 50 kg N rate) and 20% (with 100 and 150 kg N rate)	Exp. 1: 29% (mean) of which 52 to 73% lost by NH_3 volatilization of plants between blister and maturity (15 to 20% of FNU) [1]; Exp. 2: 64 to 76%	Francis, Schepers and Vigil (1993) [3]
Maize	Grain, stover, roots, and soil (after one growing season)	124 kg N*ha^{-1} before sowing (granular, incorporated) as $(NH_4)_2SO_4$	Not assessed	40%	23%	38% (due to leaching and denitrification) [2]	Harris et al. (1994) [4]
Maize (irrigated, 2 yr observation)	Grains, cobs, and stover	50 kg N*ha^{-1} and 150 kg N*ha^{-1} before seeding as $(NH_4)_2SO_4$	11% (with 50 kg N rate); 30% (with 150 kg N rate)	42% and 49% at 50 kg N rate and 150 kg N	After 1 year 30% with 50 kg N rate and 27% with 150 kg N rate	17 to 18% (due to leaching and denitrification) [2]	Olson (1980) [5]
Maize	Aboveground biomass (at dent), (3 yr observation period and 3 different water regimes)	0 kg, 125 kg, 251 kg, and 376 kg N*ha^{-1} (surface-applied and raked in) as $(NH_4)_2SO_4$	43% to 74%	43% to 62%	Not assessed	Only presented in diagrams, but rather high (due to detectability of soil ^{15}N amounts) [2]	Porter (1995) [6]
Maize	Grains, stover, and litter (after first growing season)	140 kg N*ha^{-1} (30 kg at planting and 110 kg top-dressed at V5) as $(NH_4)_2SO_4$ (granular)	21% in grain, 65% in stover, 33% in shoots	35% with shoots and 4% with litter	46%	15% (2% due to NH_3 volatilization [1], rest rather due to leaching than N_2O) [2]	Rocha et al. (2019) [7]

Table A1. *Cont.*

Crop	Parameters and Specifications	Fertilization	NdfF	FNU	^{15}N Remaining in Soil	^{15}N Lost or Not Recovered	References
Maize	Grain, stover (1 plant per microplot), and soil (15 cm soil depth)	200 kg N*ha^{-1} as $(NH_4)_2SO_4$, 133 kg N*ha^{-1}, 10 days prior to planting (incorporated), second application at V6 (raked in)	Not assessed	32% (with application before seeding) and 48% (with application at V6)	15% (with application before seeding) and 14% (with application at V6)	53% (with application before seeding) and 38% (with application at V6) (due to leaching) [2]	Seo, Meisinger, and Lee (2006) [8]
Wheat	grain and straw at ripe stage; soil (90 cm soil depth)	50 kg N*ha^{-1} before sowing as $(NH_4)_2SO_4$ and KNO_3	25% in average, (in wheat tops)	47% in average, (in wheat tops)	29% on average, mostly in organic form (>8%)	19% in average	Ladd and Amato (1986) [9]
Winter wheat	Plant tops, large roots, soil (180 cm soil depth); 1 to 5 yr obs. period	50 kg and 100 kg N*ha^{-1} as $(NH_4)_2SO_4$ (incorporated in fall, surface-applied in spring)	22% and 26% (with 50 kg N rate in fall or spring); 38% and 40% (with 100 kg N rate in fall or spring)	After 1 year 44% and 46% (with 50 kg N rate in fall or spring); 48% and 57% (with 100 kg N rate in fall or spring)	After 1 year 36% and 34% (with 50 kg N rate in fall or spring); 29% and 23% (with 100 kg N rate in fall or spring)	After 1 year 20% loss on average (due to leaching, but mainly denitrification) [2]	Olson et al. (1979) [10]; Olson and Swallow (1984) [11]
Barley	Grain, straw, stubble, and soil (70 cm soil depth)	About 140 kg N*ha^{-1} as NH_4NO_3 (in a solution), 6 weeks after sowing	36 to 48%	34 to 47% Including weed	34 to 37%	18.1 to 27.6%	Glendining et al. (1997) [12]
Spring barley	Straw and grain at two sites (subsequent years)	30 to 150 kg N*ha^{-1} as NH_4NO_3 with ^{15}N enrichment either at sowing or booting stage	6% in average (being increased at booting stage for one site)	27 to 41% (with application at sowing), 45 to 66% (with application at booting)	Not assessed	59 to 73% (calculated by difference)	Tran and Tremblay (2000) [13]

NdfF (%) = fertilizer N share on total N in plant biomass; FNU (%) = percentage of fertilizer N utilization by plant. Percentages are either reported in the reference study or have been calculated based on values shown (percentages are rounded up or down, respectively). [1] Measured/observed in reference study or previous study by same authors. [2] Assumed, based on literature discussion and experimental conditions.

References

1. Brock, C.; Hoyer, U.; Leithold, G.; Hülsbergen, K.-J. The humus balance model (HU-MOD): A simple tool for the assessment of management change impact on soil organic matter levels in arable soils. *Nutr. Cycl. Agroecosyst.* **2012**, *92*, 239–254. [CrossRef]
2. Leithold, G.; Brock, C.; Hoyer, U.; Hülsbergen, K.J. Anpassung der Humusbilanzierung an die Bedingungen des ökologischen Landbaus. In *KTBL: Bewertung ökologischer Betriebssysteme–Bodenfruchtbarkeit, Stoffkreisläufe, Biodiversität, KTBL-Schrift 458*; KTBL: Darmstadt, Germany, 2007; pp. 24–50.
3. Francis, D.D.; Schepers, J.S.; Vigil, M.F. Post-Anthesis Nitrogen Loss from Corn. *Agron. J.* **1993**, *85*, 659–663. [CrossRef]
4. Harris, G.H.; Hestermann, O.B.; Paul, E.A.; Peters, S.E.; Janke, R.R. Fate of Legume and Fertilizer Nitrogen-15 in a Long-Term Cropping Systems Experiment. *Agron. J.* **1994**, *86*, 910–915. [CrossRef]
5. Olson, R.V. Fate of Tagged Nitrogen Fertilizer Applied to Irrigated Corn. *Soil Sci. Soc. Am. J.* **1980**, *44*, 514–517. [CrossRef]
6. Porter, L.K. Depleted 15N carryover, leaching and uptake for three years of irrigated corn. *J. Contam. Hydrol.* **1995**, *20*, 209–226. [CrossRef]
7. Rocha, K.F.; Mariano, E.; Grassmann, C.S.; Trivelin, P.C.O. Fate of 15N fertilizer applied to maize in rotation with tropical forage grasses. *Field Crop. Res.* **2019**, *238*, 35–44. [CrossRef]
8. Seo, J.-H.; Meisinger, J.J.; Lee, H.-J. Recovery of Nitrogen-15–Labeled Hairy Vetch and Fertilizer Applied to Corn. *Agron. J.* **2006**, *98*, 245–254. [CrossRef]
9. Ladd, J.N.; Amato, M. The fate of nitrogen from legume and fertilizer sources in soils successively cropped with wheat under field conditions. *Soil Biol. Biochem.* **1986**, *18*, 417–425. [CrossRef]
10. Olson, R.V.; Murphy, L.S.; Moser, H.C.; Swallow, C.W. Fate of Tagged Fertilizer Nitrogen Applied to Winter Wheat. *Soil Sci. Soc. Am. J.* **1979**, *43*, 973–975. [CrossRef]
11. Olson, R.V.; Swallow, C.W. Fate of Labeled Nitrogen Fertilizer Applied to Winter Wheat for Five Years. *Soil Sci. Soc. Am. J.* **1984**, *48*, 583–586. [CrossRef]
12. Glendining, M.J.; Poulton, P.R.; Powlson, D.S.; Jenkinson, D.S. Fate of 15N-labelled fertilizer applied to spring barley grown on soils of contrasting nutrient status. *Plant Soil* **1997**, *195*, 83–98. [CrossRef]
13. Tran, T.S.; Tremblay, G. Recovery of ^{15}N-labeled fertilizer by spring bread wheat at different N rates and application times. *Can. J. Soil Sci.* **2000**, *80*, 533–539. [CrossRef]
14. Bartzialis, D.; Giannoulis, K.D.; Gintsioudis, I.; Danalatos, N.G. Assessing the Efficiency of Different Nitrogen Fertilization Levels on Sorghum Yield and Quality Characteristics. *Agriculture* **2023**, *13*, 1253. [CrossRef]
15. Gönülal, E. Performance of sorghum sudangrass hybrid (*Sorghum bicolor* L. *Sorhgum sudanense*) cultivars under water stress conditions of arid and semi-arid regions. *J. Glob. Innov. Agric. Soc. Sci.* **2020**, *8*, 78–82.
16. Kır, H.; Dursun Şahan, B. Yield and Quality Feature of Some Silage Sorghum and Sorghum-Sudangrass Hybrid Cultivars in Ecological Conditions of Kırşehir Province. *Türk Tarım Doğa Bilim. Derg.* **2019**, *6*, 388–395. [CrossRef]
17. Khalaf, A.A.; Issazadeh, L.; Abdullah, Z.A.; Hassanpour, J. Growth and Yield Assessment of Two Types of Sorghum-Sudangrass Hybrids as Affected by Deficit Irrigation. *Int. J. Agric. Biol. Eng.* **2019**, *3*, 214–218.
18. Çelik, B.; Türk, M. The Determination of Forage Yield and Quality of Some Silage Sorghum Cultivars in Ecological Conditions of Uşak Province. *Turk. J. Range Forage Sci.* **2021**, *2*, 1–7. [CrossRef]
19. Prajapati, B.; Shrivastava, A.; Sarvade, S.; Agrawal, S.; Solanki, R. Nutrient Management for Optimizing Fodder Production of Sorghum—A Review. *Int. J. Bioresour. Stress Manag.* **2023**, *14*, 83–93.
20. Ghalkhani, A.; Golzardi, F.; Khazaei, A.; Mahrokh, A.; Illés, Á.; Bojtor, C.; Mousavi, S.M.N.; Széles, A. Irrigation Management Strategies to Enhance Forage Yield, Feed Value, and Water-Use Efficiency of Sorghum Cultivars. *Plants* **2023**, *12*, 2154. [CrossRef]
21. Ozkan, U.; Yildiz, N.; Peker, C. Influence of N fertilizing on Single-cut Sorgum × Sudangrass Hybrids' Forage Yield and Nutritive Profile. *Turk. J. Agric. Eng. Res.* **2023**, *4*, 61–72. [CrossRef]
22. Shibu, M.E.; Leffelaar, P.A.; Van Keulen, H.; Aggarwal, P.K. Quantitative description of soil organic matter dynamics—A review of approaches with reference to rice-based cropping systems. *Geoderma* **2006**, *137*, 1–18. [CrossRef]
23. Brock, C.; Franko, U.; Oberholzer, H.-R.; Kuka, K.; Leithold, G.; Kolbe, H.; Reinhold, J. Humus balancing in Central Europe—Concepts, state of the art, and further challenges. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 3–11. [CrossRef]
24. *ISO 13878:1998*; International Standards Organization (ISO). SI Units and Recommendations for the Use of their Multiples and of Certain Other Units. Soil Quality–Determination of Total Nitrogen Content by Dry Combustion (Elementary Analysis). Beuth-Verlag GmbH: Berlin, Germany, 1998.
25. *ISO 10694:1996–08*; International Standards Organization (ISO). SI Units and Recommendations for the Use of their Multiples and of Certain Other Units. Soil Quality–Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). Beuth-Verlag GmbH: Berlin, Germany, 1996.
26. IAEA, International Atomic Energy Agency. *Guide on the Use of Nitrogen-15 and Radioisotopes in Studies of Plant Nutrition: Calculations and Interpretation of Data*; IAEA: Vienna, Austria, 1983.
27. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2020. Available online: https://www.r-project.org/ (accessed on 31 July 2023).
28. Venuto, B.; Kindiger, B. Forage and biomass feedstock production from hybrid forage sorghum and sorghum-sudangrass hybrids. *Grassl. Sci.* **2008**, *54*, 189–196. [CrossRef]

29. Millner, J.P.; Aver, R.V.; Hardcare, A.K. The yield and nutritive value of maize hybrids grown for silage. *N. Z. J. Agric. Res.* **2005**, *48*, 101–108. [CrossRef]
30. Amos, B.; Walters, D.T. Maize Root Biomass and Net Rhizodeposited Carbon—An Analysis of the Literature. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1489–1503. [CrossRef]
31. Barbosa, J.Z.; Ferreira, C.F.; Santos, N.Z.D.; Motta, A.C.V.; Prior, S.; Gabardo, J. Production, carbon and nitrogen in stover fractions of corn (*Zea mays* L.) in response to cultivar development. *Ciência E Agrotecnologia* **2016**, *40*, 665–675. [CrossRef]
32. Lynch, M.; Mulvaney, M.; Hodges, S.; Thompson, T.L.; Thomason, W. Decomposition, nitrogen and carbon mineralization from food and cover crop residues in the central plateau of Haiti. *Springerplus* **2016**, *5*, 973. [CrossRef]
33. Gastal, F.; Lemaire, G. N uptake and distribution in crops: An agronomical and ecophysiological perspective. *J. Exp. Bot.* **2002**, *53*, 789–799. [CrossRef] [PubMed]
34. Clark, A. *Managing Cover Crops Profitably*, 3rd ed.; Sustainable Agriculture Network: Beltsville, MD, USA, 2007.
35. Gardner, J.B.; Drinkwater, L.E. The fate of nitrogen in grain cropping systems: A meta-analysis of^{15}N field experiments. *Ecol. Appl.* **2009**, *19*, 2167–2184. [CrossRef] [PubMed]
36. van Groenigen, J.W.; Huygens, D.; Boeckx, P.; Kuyper, T.W.; Lubbers, I.M.; Rütting, T.; Groffman, P.M. The soil N cycle: New insights and key challenges. *Soil* **2015**, *1*, 235–256. [CrossRef]
37. Cameron, K.C.; Di, H.J.; Moir, J.L. Nitrogen losses from the soil/plant system: A review. *Ann. Appl. Biol.* **2013**, *162*, 145–173. [CrossRef]
38. Kastori, R. Nitrogen volatilization form plants. *Zb. Matice Srp. Prir. Nauke.* **2004**, *107*, 111–118. [CrossRef]
39. Hupe, A.; Schulz, H.; Bruns, C.; Haase, T.; Heß, J.; Dyckmans, J.; Joergensen, R.G.; Wichern, F. Get on your boots: Estimating root biomass and rhizodeposition of peas under field conditions reveals the necessity of field experiments. *Plant Soil* **2019**, *443*, 449–462. [CrossRef]
40. Lak, Z.A.; Sandén, H.; Mayer, M.; Rewald, B. Specific root respiration of three plant species as influenced by storage time and conditions. *Plant Soil* **2020**, *453*, 615–626. [CrossRef]
41. Nina, D.O.; Sigunda, D.O. Effects of drying method, storage period and carbon: Nitrogen ratio on inorganic nitrogen contents of Vertisols. *AJEST* **2012**, *6*, 476–482.
42. Swinnen, J.; Van Veen, J.A.; Merckx, R. Losses of14C from roots of pulse-labelled wheat and barley during washing from soil. *Plant Soil* **1994**, *166*, 93–99. [CrossRef]
43. van Noorwijk, M.; Floris, J. Loss of dry weight during washing and storage of root samples. *Plant Soil* **1979**, *53*, 239–243. [CrossRef]

Chapter 3: Second article

Article: Improving minimum detectable differences in the assessment of soil organic matter change in short-term field experiments

Published in *Journal of Plant Nutrition and Soil Science* (2015) 178(1): 35-42

DOI: 10.1002/jpln.201400409

J. Plant Nutr. Soil Sci. 2015, 178, 35–42 DOI: 10.1002/jpln.201400409 35

Improving minimum detectable differences in the assessment of soil organic matter change in short-term field experiments[#]

Lucas Knebl[1]*, Günter Leithold[1], and **Christopher Brock[1]**

[1] Justus Liebig University Gießen, Organic Farming, Karl-Glöckner-Straße 21c, 35394 Gießen, Germany

Abstract

The demand for information on cropping system impact on soil organic matter (SOM) calls for efforts to improve the utilization of short-term field experiments (*e.g.*, to evaluate the parameterization of cropping systems in models). Those approaches have coped with the problem of determining small SOM changes within a large background mass. Thus, objectives of this survey are (1) the improvement of the minimum detectable difference (MDD) in SOM in the hudycrop short-term field experiment by methods of sampling design and data treatment, (2) the verification to what extend the *hudycrop* short-term field experiment allows for the determination of management induced effects on SOM, and (3) the investigation to what extent the obtained results may be suitable to evaluate the parameterization of a SOM balance model. The design of the *hudycrop* is suitable for excluding outliers plotwise. The estimation of plot means can be improved by the sampling design. Instead of determining a single plot mean in a mixed sampling procedure, the design provides multiple values for each plot, allowing for the identification of extreme values before calculating plot means. In consequence, minimum detectable differences decrease by a factor of 0.53 for soil organic C (SOC) and 0.63 for soil total N (STN) masses, allowing for detection of changes in the magnitude of 3.7 and 2.6% of background SOC and STN levels, respectively. Differences between treatments, however, are significant with corrected values (after outlier exclusion) for the crop production systems with the highest impact (potatoes and mulched red clover). Determining outliers based on Student's t-test gives the lowest MDD and is therefore considered to be the most suitable method in this case. Correlations between apparent changes and SOM balances according to the HU-MOD–2 model, used in this survey, indicate that the experimental design, in principal, is suitable for the evaluation of the parameterization of crop production systems in models. Still, an improved precision in SOM change detection is necessary. Reasonable options for that purpose are discussed in the paper.

Key words: soil organic matter / short-term field experiments / minimum detectable differences / outlier determination

Accepted November 30, 2014

1 Introduction

Maintaining the productivity of soils is an important goal in sustainable agriculture. Studies about the dynamics of soil organic matter (SOM) support this goal by improving the development of appropriate management systems (*Christensen*, 2001). SOM is linked to several soil chemical, biological, and physical properties. For that reason SOM is a key factor in arable crop production systems (*Leifeld* and *Kögel-Knabner*, 2005). Therefore, knowledge of its structure, functions and dynamics helps to increase the maintenance of soil quality (*Guggenberger* et al., 1994). However, the assessment of SOM changes in cropping systems is challenging. Under conditions of farming practice, simple models (like SOM balances) may be applied for *ex ante* decision support in OM management (*Brock* et al., 2013). Yet, as *Körschens* (2006) observed, most often only long-term experiments provide the opportunity to assess the management impact on soil properties in farming systems. Accordingly, the best option to develop and evaluate SOM balance models may be provided by long-term observations of the effects of crop production on SOM. However, such experiments are time-consuming and cannot react to short-term demand for information. Further, model parameters for a single crop production system could only be validated in factorial field experiments. So it is hardly surprising that corresponding experiments have rarely been conducted. Therefore, there is a need for methodical solutions utilizing short-term field experiments for the evaluation

* Correspondence: L. Knebl;
e-mail: lucas.a.knebl@agrar.uni-giessen.de

[#]Focus Issue "Soil organic matter balance methods". Selected papers presented on the international workshop "Evaluation of soil organic matter balance methods as practice-applicable tools for environmental impact assessment and farm management support (SOMpatic)" held in Rauischholzhausen (Germany), November 20–22, 2013 (Conveners: Christopher Brock, Uwe Franko, and Hans-Rudolf Oberholzer).

and improvement of SOM balances and other models for environmental impact assessment and management support. Approaches to measure SOM change under production systems of single arable crops have to cope with the problem of determining small changes within a large background mass (*Carter*, 2002; *Rodeghiero* et al., 2009). Further, many soil properties show a considerable variability over space (*Körschens*, 2006). Against this background, the assessment of SOM mass change in short time periods may be facilitated by enlarging sample size (*Smith*, 2004), application of sensitive SOM indicators (*Conant* et al., 2003), and/or adaptations of the spatial sampling design (*Brock* et al., 2011). Despite the current efforts, the assessment of the effects of cropping systems on SOM in short-time periods remains difficult. In an attempt to adapt the sampling design, *Brock* et al. (2011) succeeded with an assessment of soil organic C (SOC) and soil organic N (STN) change under carrot cropping with repeated sampling in a tight grid. Based on their findings, the *hudycrop* short-term field experiment was conducted to assess effects of single cropping systems on SOM. The purpose of the *hudycrop* short-term field experiment is to allow for an improved estimation of treatment means with a sampling design that reduces the input of within-plot variability of soil properties. Aims of this survey are (1) the improvement of the minimum detectable difference (MDD) in SOM by sampling design and data treatment, (2) the verification to what extend the *hudycrop* short-term field experiment allows for the determination of management induced effects on SOM, and (3) the assessment to what extend the obtained results may be suitable to evaluate the parameterization of a SOM balance model.

2 Material and methods

The basic idea of the *hudycrop* experiment was to reduce the impact of within-plot spatial heterogeneity on the estimation of plot values by disaggregating the single subsamples in the commonly applied bulk sampling. Therefore, the design comprises six subsamples per plot that were each analyzed separately. Subsequently, different outlier determination procedures were applied to calculate plot means based on the most representative data for the plot. In a second step, measured changes were related to calculated SOM balances, in order to analyze the suitability of the experimental design for the evaluation of SOM balances and other models on the impact of crop production systems on SOM.

2.1 Experimental setup

The short-term field experiment "humus dynamics in crop production" (*hudycrop*) was carried out at the experimental station Gladbacherhof of Gießen University. The station is located at Villmar in the Taunus hill landscape in Hesse, Germany. The location has a mean altitude of 170 m asl., a mean annual temperature of 9.5°C, and a mean annual precipitation of 649 mm. The soil is an Orthic Luvisol with a high clay content (2.3 / 66.0 / 31.7 g kg^{-1} sand / silt / clay). Initial background levels of SOC and STN at the experiment site accounted for 47.3 Mg SOC ha^{-1} and 5.4 Mg STN ha^{-1} in the 0–30 cm soil layer. The aim of the *hudycrop* experiment was to reduce the impact of within-plot variability of soil properties on the estimation of treatment means. For that purpose, a grid of six miniplots (1 m^2 each) within each plot was used for data collection. The *hudycrop* experiment included winterwheat (WW- ···), potatoes (POT), red clover for fodder (CFD), and red clover mulched (CGM). Red clover treatments were cut 3 times. Only treatments without additional fertilizer application were considered in this study.

All crop production treatments were established in late summer after summer barley as pre-crop and were followed by unfertilized winter wheat to allow for the assessment of specific pre-crop treatment effects on the succeeding wheat. The experiment was set up in a single factor complete block design (Fig.1) with 4 replications in two series, resulting in

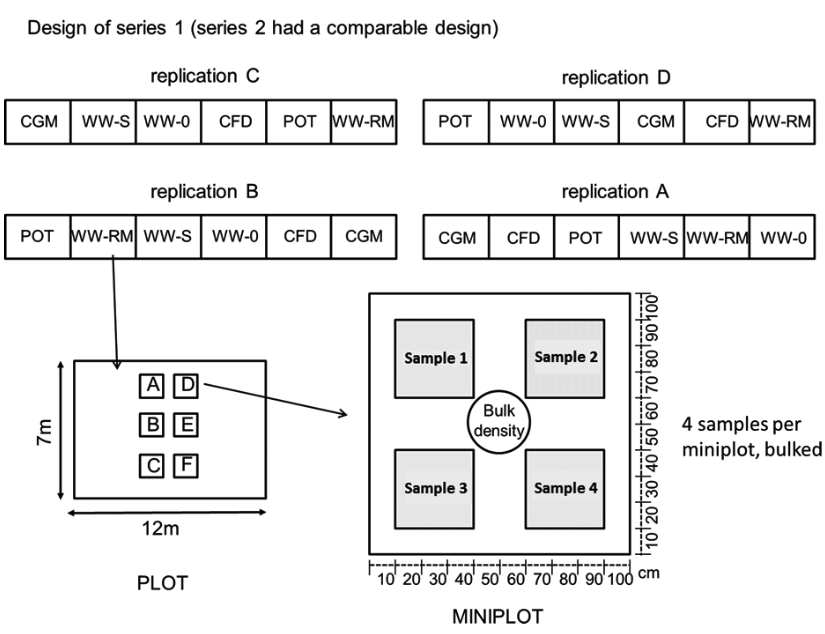

Figure 1: Experimental setup of the *hudycrop* experiment. WW-0 = winter wheat; WW-RM = winter wheat, rotten cattle manure application; WW-S = winter wheat, cattle slurry application; POT = potatoes; CFD = clover/fodder; CGM = clover/green manure. WW-0, POT, CFD, CGM unfertilized (NB: CGM = mulched).

6 treatments × 4 replications = 24 plots per series, or 48 plots altogether. Series 1 was conducted during the period 2010–2012, and series 2 was run during 2011–2013. The series were located on adjacent fields. In order to avoid errors caused by soil movement due to tillage operations, initial soil samples were taken shortly after tillage and flattening with harrow. Post treatment sampling took place after harvest before any tillage operation. Within each plot, 6 miniplots (1 m^2 each) were established in a regular grid covering the center area (6 m ×5 m) of each plot (12 m × 7 m). All data, except for samples to determine N fixation by legumes, were collected in the single miniplots, yielding 24 plots × 6 miniplots = 144 data points per sampling per series. N fixation was measured based on plot instead of miniplot values (n = 24).

2.2 SOM change assessment

2.2.1 Sampling and analyses

SOC and STN changes (ΔSOC, ΔSTN) under the treatments were determined with the help of repeated measurements in the miniplots at the beginning (after harvest of the barley precrop, before establishment of clover treatments CFD and CGM as the earliest crops) and the end (before sowing of the winter wheat succeeding crop) of the central survey period. Soil samples considered in this survey were taken at 0–30 cm below ground. For each miniplot four subsamples were bulked for the sample (Fig. 1). Samples were dried at 40°C for 72 h. SOC and STN were analyzed by dry combustion according to *ISO 10694* (1996; STC) and *ISO 13878* (1998; STN). The device used was a Vario EL (Elementar Analysensysteme GmbH, Germany) with an analytical precision of 0.01 g kg^{-1} for C and N, respectively. SOC and STN contents were transferred to masses based on bulk density (BD) within the respective sample horizon of each miniplot (Fig. 1). Bulk density (BD) was determined with sample rings at the start of each survey period.

2.2.2 Estimation of treatment means

Treatment means were calculated after applying three different procedures of outlier control. Outliers were identified on miniplot level to improve plot means $\overline{X}$. Procedures of outlier control are based on (1) the standard deviation *s* (cor. SD), (2) the exclusion of the most extreme values (cor. MinMax), and (3) the Student's t-test (cor. t-test). In mathematical terms:

cor. SD: If $\overline{X} - s > x_i > \overline{X} + s$ *then* x_i = outlier, (1)

cor. MinMax: Min,Max = outlier, (2)

cor. t-test: *If* $\overline{X} - t_{\alpha(2),v} s_{\overline{X}} > x_i > \overline{X} + t_{\alpha(2),v} s_{\overline{X}}$ *then* x_i = outlier, (3)

Miniplot values on SOC and STN change, which were removed after outlier control, were also deleted in the SOM balances.

2.2.3 Evaluations

Minimum detectable differences (MDD) of SOM changes were calculated according to *Zar* (2010):

$$MDD = \sqrt{\frac{s^2}{n}(t_\alpha,\ v + t_\beta, v)}, \quad (4)$$

with s^2 = variance, n = sample size, t_α, v = critical values of t-distribution at significance level α with V degrees of freedom, t_β, v = critical value of t-distribution at statistical power of 1-α with V degrees of freedom.

Treatment differences were identified with one-way ANOVA separately for series 1, series 2, and series 1+2 together. ANOVA and preceding tests were conducted with Statistica 10.0 (Statsoft Inc.). All other evaluations were conducted with Microsoft Excel 2010 after implementation of the algorithms above and the connected tables (t-values).

2.3 SOM balance calculation

SOM balances were calculated with the HU-MOD–2 SOM balance model (*Brock* et al., 2012). It has to be noted that the model version applied here differs from the original model description (*Brock* et al., 2012) in the calculation of SOM supply. The original version calculated SOM supply based on C inputs and substrate-specific 'humification' rate coefficients. In order to allow for an easier adaptation of the model to different site conditions and with regard to the inclusion of new crops and cropping systems, this concept was abandoned. Instead, the model now uses the coupled C and N balance approach denoted in Eqs. 2–4. The applicability of this approach was successfully tested with the dataset used in the validation of the original model version (*Brock* and *Leithold*, 2014). HU-MOD–2 is a decision support tool for application in farming practice and, thus, has a low demand for input data, especially with regard to soil data. Briefly, the model calculates SOM loss under a crop production system based on N withdrawal with harvest products (considering N supply from other sources), and estimates SOM supply based on C and N inputs to the soil and their dynamics. In mathematical terms the calculations are:

SBA = SOMSUP – (SOMLOSS * SITECN), (5)

SOMSUP = MINIMUM (CSUP; NSUP * SITECN), (6)

CSUP = CCR + COF, (7)

NSUP = NCR + NOF + NREM, (8)

SOMLOSS = NPB – NDEP - NFIX – NFTLZ + NYRN, (9)

with SBA = SOM balance (kg SOM C ha^{-1});
SOMSUP = SOM supply (kg SOM C ha^{-1});
SOMLOSS = SOM loss (kg SOM N ha^{-1});
SITECN = topsoil C:N ratio at the site under assessment;
CSUP, NSUP = effective total C, N supply (kg SOM [C, N] ha^{-1});
CCR, NCR = effective C, N input by harvest residues, roots, rhizodeposition (kg SOM [C, N] ha^{-1});
COF, NOF = effective C, N input by fertilizers (kg SOM [C, N] ha^{-1});
NREM = remaining inorganic N in the soil profile that has not been taken up by the plants (kg N ha^{-1});
NPB = N in plant (crop) biomass (kg N ha^{-1});

NDEP = effective N supply to plant N uptake by atmospheric deposition (kg N ha^{-1});
NFIX = symbiotic N fixation by legumes (kg N ha^{-1});
NFTLZ = effective N supply to plant N uptake by fertilizers (kg N ha^{-1});
NYRN = not yield-related N losses caused by high mechanical impact intensity in the crop production system *etc*. (kg N ha^{-1}).

The model output can refer either to SOC (SBAC = SOC-related SOM balance) or STN (SBAN = STN-related SOM balance). The conversion factor is determined by the soil C/N ratio at the site under assessment. In the *hudycrop*, the initial C/N ratio in the miniplots was applied as conversion factor, respectively.

Measurement data were available for aboveground biomass yields and dry matter. In the case of legumes (treatments CGM and CFD), N contents of the aboveground biomass and NFIX have been analyzed on the plot level as additional parameters. N contents were analyzed according to *ISO 13878* (1998), and NFIX was measured with the $^{14/15}$N natural abundance method according to *Shearer* and *Kohl* (1986). Balances were related to measured SOC and STN changes applying linear regression, with the balances as independent (x) and measured changes as dependent (y) variable.

3 Results

After correction for outliers on the miniplot level (Eqs. 1, 2 and 3), MDD were calculated (Eq. 4) for each treatment on the basis of obtained results. Afterwards, data were aggregated to the overall treatment level. Table 1 shows the results for each series separately as well as for both series together.

The original data, as expected, result in higher MDD (*i.e.*, reduced detectability of differences) compared to MDD after outlier correction. With all corrections the detectability of changes is considerably better in series 2 than in series 1. Comparing the different outlier elimination procedures, *cor. t-test* yields the best MDD values, slightly lower than those with *cor. SD*. The *cor. t-test* allows for the detection of overall mean changes at a magnitude of 1771.8 kg SOC ha^{-1} and 142.6 kg STN ha^{-1} under cropping systems (Table1). In comparison to the original data, the detectability of SOM changes was improved by factor 0.53 (SOC) and factor 0.62 (STN) in this case.

Table 2 displays mean SOC and STN changes on treatment level for series 1, 2 and in overall perspective. Differences between treatments are denoted by different letters.

Without correction of outliers (original data), the *hudycrop* sampling design is not able to identify differences between treatments regardless of the series. However, in series 2 treatment differences can be identified after all control procedures. Differences obtained vary between the three procedures. The aggregation of means of both series to overall treatment means is shown at the bottom of the table. At this level, treatment differences can be identified mainly between POT and CGM, except after correction with *cor. t-test*. The latter procedure results in differences between WW-0 and CGM for STN.

The SOM balances indicate SOC changes in the magnitude between roughly –900 and +2300 kg SOM-C ha^{-1} under the treatments, and between –105 and +270 kg SOM-N ha^{-1} (Fig. 2). The balances are not significantly altered when miniplot values are removed that have been identified as outliers in SOC change calculations. As for apparent changes, SOM balances indicate changes below MDD in the experiment except for CGM (even after applying the outlier determination procedures). However, the differentiation of treatments with regard to SOC and STN changes correlated with the differentiation of SOM balances (Fig. 2).

4 Discussion

4.1 Detectable differences

As shown in the results, neither the spatial sampling design of the *hudycrop* nor the additional control for outliers is able to quantify significant SOM changes under the treatments WW-0, POT, and CFD (see Tables 1 and 2). Only apparent SOC and STN changes under CGM, in average of both series, are higher than the within-plot variation of this parameter and therefore are significant. A definite success, however, is the improvement of detectable changes in SOM due to the applied data treatment (outlier control). Comparable studies obtained detectable differences after a 5 year period of 2 Mg

Table 1: Minimum detectable differences (MDD) for SOC and STN in the *hudycrop* experiment. Significance level α = 0.05.

Treatment	Series	Parameter	Mean MDD / kg ha^{-1}			
			original	cor. SD	cor. MinMax	cor. t-test
ALL	1	ΔSOC	3907.9	2170.9	2442.6	2131.8
ALL	2	ΔSOC	3560.2	1425.8	1653.9	1411.9
ALL	1+2	ΔSOC	3734.1	1798.4	2048.2	1771.8
ALL	1	ΔSTN	358.7	192.7	215.1	162.4
ALL	2	ΔSTN	394.3	137.8	199.2	121.5
ALL	1+2	ΔSTN	376.5	165.2	207.2	142.6

Table 2: Comparison of measured change in SOC and STN under the treatments. The evaluation period covers the cropping year of the treatments only (1 y). Differences between treatments at α = 0.05 within an assessment unit (series 1, series 2, series 1+2) are denoted with different letters. WW-0 = winter wheat; POT = potatoes; CFD = clover/fodder; CGM = clover/green manure. All treatments unfertilized (except CGM = mulched).

Treatment	Series	Object	Mean change / kg ha^{-1}							
			original		cor. SD		cor. MinMax		cor. t-test	
WW-0	1	ΔSOC	1001.2	a	688.7	a	911.7	a	1251.7	a
POT	1	ΔSOC	–456.5	a	379.8	a	–220.9	a	–113.8	a
CFD	1	ΔSOC	497.5	a	55.4	a	55.7	a	–49.5	a
CGM	1	ΔSOC	1771.0	a	2382.1	a	2065.3	a	2393.1	a
WW-0	2	ΔSOC	–2168.4	A	–1458.8	AB	–1110.3	AB	–1210.2	AB
POT	2	ΔSOC	–2108.8	A	–1587.6	A	–1960.9	A	–1894.1	A
CFD	2	ΔSOC	–341.7	A	–401.6	AB	–96.5	B	–272.6	B
CGM	2	ΔSOC	–405.2	A	39.8	B	318.0	B	190.0	B
WW-0	1	ΔSTN	140.7	a	106.1	a	110.7	a	109.5	a
POT	1	ΔSTN	39.1	a	62.0	a	42.9	a	66.8	a
CFD	1	ΔSTN	93.5	a	58.2	a	56.6	a	41.2	a
CGM	1	ΔSTN	225.1	a	292.2	a	272.0	a	326.6	a
WW-0	2	ΔSTN	–274.3	A	–103.8	AB	–117.6	AB	–116.9	AB
POT	2	ΔSTN	–227.2	A	–190.8	A	–223.1	A	–179.5	A
CFD	2	ΔSTN	–28.0	A	9.4	BC	12.4	AB	9.4	AB
CGM	2	ΔSTN	–34.0	A	87.6	C	46.6	B	77.0	B
WW-0	1+2	ΔSOC	–514.7	a	–448.2	ab	–99.3	ab	–191.5	ab
POT	1+2	ΔSOC	–1282.6	a	–603.9	a	–1090.9	a	–1102.8	a
CFD	1+2	ΔSOC	77.9	a	–173.1	ab	–20.4	ab	–161.1	ab
CGM	1+2	ΔSOC	682.9	a	1211.0	b	1191.7	b	1250.7	b
WW-0	1+2	ΔSTN	–57.8	a	–5.0	ab	–3.4	ab	–16.3	a
POT	1+2	ΔSTN	–94.1	a	–56.5	a	–90.1	a	–40.3	ab
CFD	1+2	ΔSTN	32.8	a	34.5	ab	34.5	ab	24.8	ab
CGM	1+2	ΔSTN	95.5	a	186.8	b	159.3	b	193.5	b

SOC ha^{-1} with a sampling size of n = 5 (*Conant* et al., 2003) and 5 Mg SOC ha^{-1} with a sample size of n = 16 (*Garten* and *Wullschleger*, 1999). The spatially accurate sampling in the *hudycrop* already allows for the quantification of changes in a magnitude of 3.70 Mg SOC ha^{-1} and 0.37 Mg STN ha^{-1} after only a 1-y time period. The spatial sampling grid is designed to allow for a detailed control for outliers, which further decreases MDD from 3.73 to 1.77 Mg SOC ha^{-1} and 0.37 to 0.14 Mg ha^{-1} respectively, at the overall series level. The elimination of extreme values, in order to minimize the MDD of a plot, requires an adjusted methodical approach. Common outlier detection procedures (*Grubbs*, 1950; *Dean* and *Dixon*, 1951) are designed not to exclude extreme values too easily. In this survey though, procedures are chosen to identify outliers in plot data rather rigorously. This decision is based on the assumption that the assessed parameters (SOC and STN as indicators for SOM), are affected by management in interaction with site specific soil properties (*e.g.*, texture, water holding capacity, bulk density, *etc.*). The heterogeneity of those soil properties is a substantial reason for the difficulties in assessing management impact on SOM over short time periods (*Papritz* and *Webster*, 1995). Additionally, the collection of soil samples is rather punctual and, therefore, reinforces the picture of a heterogeneous distribution of the according parameter. The data treatment applied in this survey is an attempt to isolate the group of subsamples that apparently is most representative for the respective plot. In this case, deviating values identified as 'outliers' do not have to be incorrect, but are considered as being less representative for the plot. Therefore, the threshold limits for the acceptance of values are set more narrow than with the common procedures mentioned above. In cases where data within a miniplot is distrib-

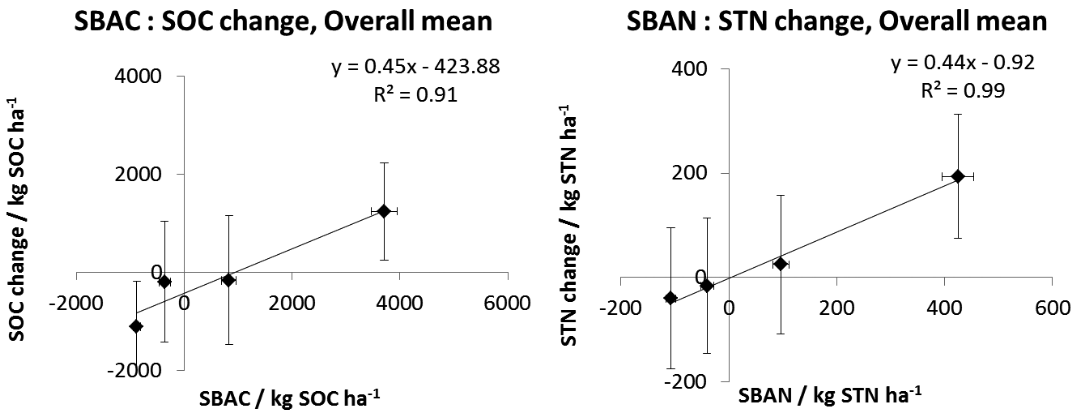

Figure 2: Relation between SOM balances and measured SOM changes in the *hudycrop* experiment at the treatment level. The evaluation period covers the cropping year of the treatments only (1 y). No subdivision in miniplots, plots and series. Error bars denote standard errors of the estimate (SEE).

uted normally, the outlier determination results in neglecting up to 4 out of 6 values. This rigorous isolation, nevertheless, has little influence on the miniplot mean, due to its distribution. Fewer values (mostly 2 out of 6 values) are determined as outliers in cases where data are distributed not normally. In this survey, one aim is to improve the minimum detectable difference of SOC and STN changes. In this context the outlier determination with student's t-test (cor. t-test) performs best.

4.2 Methodical considerations concerning the experimental design

According to background masses within 0–30 cm soil depths on the experimental site, the detectable differences account for about 3.7 and 2.6% of total SOC and STN, respectively. The variability of miniplot values is higher than the overall change in SOM. Next to spatial variability and analytical limits, explanations for this finding could also be derived from the sampling procedure. Variability among several soil samples of one plot is attributable to within plot location (*Ellert* et al., 2002). Other studies show that the sampling location, for example, has a mentionable effect on the nutrient content of the soil sample (*Thomsen* and *Sørensen*, 2006; *Clay* et al., 1995). The authors of both studies observed higher mineralization rates within plant rows compared to rates between the rows. Based on these findings, a balanced ratio of soil samples taken within and in between plant rows appears appropriate. In the *hudycrop* sampling design this fact however remained unconsidered and may have increased already existing differences between miniplots. Further, some authors have argued that the use of the fixed depth equation for quantifying changes in SOM results in incorrect assessments of changes when bulk densities differ substantially between treatments or between sampling dates (*Ellert* and *Bettany*, 1995; *Wendt* and *Hauser*, 2013; *Lee* et al., 2009). Probable differences in bulk density can be corrected by using the calculation of an equivalent soil mass (mass-depth), resulting in a more accurate assessment of changes of soil constituents (*Wuest*, 2009).

In this study, the SOM content within a specified soil depth (0–30cm) as well as the corresponding bulk density (BD) were used to calculate the SOM mass on an area basis (kg SOM ha^{-1}). Determination of BD in the respective soil layers was carried out at the beginning of the survey period. A possible change in BD due to the cropping systems therefore remains unconsidered. Soil BD, however, is reported to be affected by farming systems (*i.e.*, tillage, residue management, *etc.*; *Allen* et al., 2010). In addition, BD was determined by using the complete soil core within the used sampling ring. After drying the soil, there was no further subdivision into a fraction bigger or smaller than 2 mm. For analytical reasons, though, SOC and STN content were evaluated in soil material < 2 mm. The calculation on an area basis therefore might include slight errors. Soil material > 2 mm, however, accounts for a very small amount in the analyzed soil. *Rodeghiero* et al. (2009) even consider it necessary to include stone and root content in the calculation of SOC density. It is assumed that improving the sampling design according to these considerations will result in even smaller detectable differences. Further parameters that may function as indicators for small SOM changes offer an additional perspective in the assessment of short-term cropping system impact. Parameters of interest are particulate organic matter, the so called light fraction (*Gregorich* et al., 2006; *Wander*, 2004), biological indicators related to soil functions (*Bell* and *Raczkowski*, 2008), and CO_2 fluxes as a net indicator of ecosystem C exchange (*Kutsch* et al., 2010). Not least, the consideration of additional series might overcome uncertainty of apparent changes as there are obvious differences between series 1 and series 2 (see Tables 1 and 2). Expanding the experimental setup of short-term field experiments on the series level would increase the effort. On the other hand it would provide the possibility to enlarge the information range (obtain different soil types, crop rotations, management types) and, therefore, might be an advantage towards long-term field experiments.

4.3 Applicability of short-term field experiments for the evaluation of SOM balance models

The aim of the *hudycrop* experiment was to contribute to methodical approaches for the assessment of agricultural management impact on SOM in short-term field experiments, *e.g.*, as a basis for the evaluation of the parameterization of agricultural management measures in models. For this application the prediction accuracy (indicated by minimal detectable differences) still needs further improvement. SOM changes under crop production systems, indicated by SOM balances, will in many cases be < 1,000 kg SOM-C ha^{-1} y^{-1}. However, if apparent SOM changes under the treatments are considered as a derived variable based on the repeated measurement data, it is possible to evaluate the ability of SOM balance models to differentiate between treatments, regardless of whether the change itself is significant or not. In fact, in the *hudycrop* experiment a positive correlation between calculated SOM balances and apparent SOM changes is observed on the treatment level. As the differentiation between treatments in apparent SOM change is supported by ANOVA according to Table 2, the observed correlation is taken as a hint towards the applicability of short term field experiments for SOM balance model evaluations in principal.

It should also be considered that the HU-MOD–2 model proves applicability for SOM change assessment in long-term field experiments. A systematic error of the model cannot be observed in these evaluations (*Brock* and *Leithold*, 2014). The apparent systematic error of the model in the *hudycrop* experiment therefore should not be overestimated. This is even more relevant as the model does not refer to a fixed soil depth—whereas the measurements do—but to the rooted soil layer in total. Still, the model tends to overestimate SOM supply in a crop rotation with mulched rotational alfalfa grass ley (*Brock* and *Leithold*, 2014). As the long-term field experiment is a complex multi-factor farming system experiment (*Schulz* et al., 2014), it is difficult to identify flawed parameters for single crop production systems. The factorial design of the *hudycrop* experiment could contribute to an improved and differentiated evaluation of the model.

5 Conclusions

The sampling design and the opportunities for data treatment provided by the design improved minimum detectable differences in the assessment of SOM changes. Due to the use of rigorous outlier determination methods, detectable differences range between 3.7 and 2.6% of background SOC and STN levels after only 1 y. Significant differences between treatments with the strongest positive and negative impact on SOM can be observed. It therefore is assumed that short-term field experiments can be suitable for the assessment of management impact on SOM in crop production systems. Further improvements of the experimental design could include additional series, consideration of variable spatial impacts on the soil in crop stands and bulk density estimation parallel to each sampling procedure. The consideration of subsoil SOM levels (in layers below 30 cm soil depth) is assumed to be another valuable improvement of the data collection. The latter would further improve the suitability of the obtained results to evaluate the parameterization of SOM balance models if they relate to mass fluxes in the soil and not to a fixed soil layer.

Acknowlegment

Special thanks to *Dr. Franz Schulz* and team as well as *Maria Nägele* and team for their excellent work, which was a decisive factor in the conduction of the experiment.

References

Allen, D. E., *Pringle, M. J.*, *Page, K. L.*, *Dalal, R. C.* (2010): A review of sampling designs for the measurement of soil organic carbon in Australian grazing lands. *Rangeland J.* 32, 227–246.

Bell, M. C., *Raczkowski, C. W.* (2008): Soil property indices for assessing short-term changes in soil quality. *Renew. Agr. Food Syst.* 23, 70–79.

Brock, C., *Knies-Deventer, H.*, *Leithold, G.* (2011): Assessment of cropping-system impact on soil organic matter levels in short-term field experiments. *J. Plant Nutr. Soil Sci.* 174, 867–870.

Brock, C., *Hoyer, U.*, *Leithold, G.*, *Hülsbergen, K. J.* (2012): The humus balance model (HU-MOD): a simple tool for the assessment of management change impact on soil organic matter levels in arable soils. *Nutr. Cycl. Agroecosys.* 92, 239–254.

Brock, C., *Franko, U.*, *Oberholzer, H. R.*, *Kuka, K.*, *Leithold, G.*, *Kolbe, H.*, *Reinhold, J.* (2013): Humus balancing in Central Europe—concepts, state of the art, and further challenges. *J. Plant Nutr. Soil Sci.* 176, 3–11.

Brock, C., *Leithold, G.* (2014): A Practice-Applicable Model for the Assessment of Management Impact on Organic Matter in Arable Soils, in Rahmann, G., Aksoy, U. (eds.): Building Organic Bridges, Johann Heinrich von Thünen-Institut, Braunschweig, Germany, Thuenen Report, no. 20, pp. 457–458.

Carter, M. R. (2002): Soil quality for sustainable land management. Organic matter and aggregation interactions that maintain soil functions. *Agron. J.* 94, 38–47.

Christensen, B. T. (2001): Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur. J. Soil Sci.* 52, 345–353.

Clay, D. E., *Schumacher, T. E.*, *Brix-Davis, K. A.* (1995): Carbon and nitrogen mineralization in row and interrow areas of chisel and ridge tillage systems. *Soil Till. Res.* 35, 167–174.

Conant, R. T., *Smith, G. R.*, *Paustian, K.* (2003): Spatial variability of soil carbon in forested and cultivated sites: implications for change detection. *J. Environ. Qual.* 32, 278–286.

Dean, R. B., *Dixon, W. J.* (1951): Simplified statistics for small numbers of observations. *Anal. Chem.* 23, 636–638.

Ellert, B. H., *Bettany, J. R.* (1995): Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75, 529–538.

Ellert, B. H., *Janzen, H. H.*, *Entz, T.* (2002): Assessment of a method to measure temporal change in soil carbon storage. *Soil Sci. Soc. Am. J.* 66, 1687–1695.

Garten, C. T., *Wullschleger, S. D.* (1999): Soil carbon inventories under a bioenergy crop (Switchgrass): Measurement limitations. *J. Environ. Qual.* 28, 1359–1364.

Gregorich, E. G., *Beare, M. H.*, *McKim, U. F.*, *Skjemstad, J. O.* (2006): Chemical and biological characteristics of physically uncomplexed organic matter. *Soil Sci. Soc. Am. J.* 70, 975–985.

Grubbs, F. (1950): Sample criteria for testing outlying observations. *Ann. Math. Stat.* 21, 27–58.

Guggenberger, G., *Christensen, B. T.*, *Zech, W.* (1994): Land-use effects on the composition of organic matter in particle-size separates of soil. I. Lignin and carbohydrate signature. *Eur. J. Soil Sci.* 45, 449–458.

ISO 10694 (1996): Soil Quality—Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). Beuth Verlag, Berlin, Germany.

ISO 13878 (1998): Soil Quality—Determination of Organic and Total Nitrogen Content by Dry Combustion (Elementary Analysis). Beuth Verlag, Berlin, Germany.

Körschens, M. (2006): The importance of long-term field experiments for soil science and environmental research—a review. *Plant Soil Environ.* 52, 1–8.

Kutsch, W. L., *Aubinet, M.*, *Buchmann, N.*, *Smith, P.*, *Osborne, B.*, *Eugster, W.*, *Wattenbach, M.*, *Schrumpf, M.*, *Schulze, E. D.*, *Tomelleri, E.*, *Ceschia, E.*, *Bernhofer, C.*, *Béziat, P.*, *Carrara, A.*, *Di Tommasi, P.*, *Grünwald, T.*, *Jones, M.*, *Magliulo, V.*, *Marloie, O.*, *Moureaux, C.*, *Olioso, A.*, *Sanz, M. J.*, *Saunders, M.*, *Søgaard, H.*, *Ziegler, W.* (2010): The net biome production of full crop rotations in Europe. *Agr. Ecosyst. Environ.* 139, 336–345.

Lee, J., *Hopmans, J. W.*, *Rolston, D. E.*, *Baer, S. G.*, *Six, J.* (2009): Determining soil carbon stock changes: simple bulk density corrections fail. *Agr. Ecosyst. Environ.* 134, 251–256.

Leifeld, J., *Kögel-Knabner, I.* (2005): Soil organic matter fractions as early indicators for carbon stock change under different land-use? *Geoderma* 124, 143–155.

Papritz, A., *Webster, R.* (1995): Estimating temporal change in soil monitoring: II. Sampling from simulated fields. *Eur. J. Soil Sci.* 46, 13–27.

Rodeghiero, M., *Heinemeyer, A.*, *Schrumpf, M.*, *Bellamy, P.* (2009): Determination of Soil Organic Carbon Stocks and Changes, in Kutsch, W. L., Bahn, M., Heinemeyer, A. (eds.): Soil Carbon Dynamics. An Integrated Methology. Cambridge University Press, Cambridge, UK, pp. 49–75.

Shearer, G., *Kohl, D. H.* (1986): N_2-fixation in field settings: estimations based on natural ^{15}N abundance. *Funct. Plant Biol.* 13, 699–756.

Smith, P. (2004): How long before a change in soil organic carbon can be detected? *Global Change Biol.* 10, 1878–1883.

Schulz, F., *Brock, C.*, *Schmidt, H.*, *Franz, K.-P.*, *Leithold, G.* (2014): Development of soil organic matter stocks under different farm types and tillage systems in the Organic Arable Farming Experiment Gladbacherhof. *Arch. Agron. Soil. Sci.* 60, 313–326.

Thomsen, I. K., *Sørensen, P.* (2006): Tillage-induced N mineralization and N uptake in winter wheat on a coarse sandy loam. *Soil Till. Res.* 89, 58–69.

Wander, M. (2004): Soil Organic Matter Fraction and Their Relevance to Soil Function, in Magdoff, F., Weil, R. (eds.): Advances in Agroecology. CRC Press, Boca Raton, FL, USA, pp. 67–102.

Wendt, J. W., *Hauser, S.* (2013): An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64, 58–65.

Wuest, S. B. (2009): Correction of bulk density and sampling method biases using soil mass per unit area. *Soil Sci. Soc. Am. J.* 73, 312–316.

Zar, J. H. (2010): Biostatistical Analysis. 5^{th} ed., Pearson Education, London, UK.

Chapter 4: Third article

Article: The role of soil depth in the evaluation of management-induced effects on soil organic matter: Soil depth and soil organic matter

Published in *European Journal of Soil Science* (2017) 68(6) : 979-987

DOI: 10.1111/ejss.12492

European Journal of Soil Science

European Journal of Soil Science, November 2017, **68**, 979–987 doi: 10.1111/ejss.12492

Special issue article

The role of soil depth in the evaluation of management-induced effects on soil organic matter

L. Knebl, G. Leithold, F. Schulz & C. Brock
Department of Agronomy and Plant Breeding II, Organic Farming, Justus Liebig University, Karl–Gloeckner–Street 21c, Giessen 35394, Germany

Summary

The aim of the current survey was to determine the relevance of the subsoil in the assessment of management effects on soil organic matter (SOM) in arable farming. Data in this survey were provided by the organic arable long-term field experiment Gladbacherhof (OAFEG). Three different ‘farming types’ were compared: mixed (MF), stockless with rotational ley (SFL) and stockless with cash crops (SFC). Each type had four different tillage treatments: full inversion (FIT), two-layer plough (TLP), reduced inversion (RIT) and non-inversion (NIT). The different mean masses of soil organic carbon (SOC) and soil total nitrogen (STN) at 0–30-, 30–60-, 60–90- and 0–90-cm soil depths after three crop rotation cycles (17-year observation period) were evaluated and considered as an indicator of SOM. ‘Farming types’ differ in their effect on SOC and STN mass of the topsoil layer (0–30 cm) in the order MF > SFL > SFC ($P < 0.01$). For the 0–90-cm soil depth there were no differences between the treatments. Treatments with different tillage intensity did not show any effect on the contents of SOC or STN within the 0–30-cm soil depth. However, within the 30–60-cm depth full-inversion tillage (FIT) was associated with larger amounts of SOC and STN than the reduced tillage intensity treatments TLP, RIT and NIT ($P < 0.01$). This situation even resulted in significantly larger SOC and STN masses under FIT ($P < 0.05$) over the whole soil profile under study (0–90 cm). Our results suggest that sampling depth should be extended to include the effects on the upper subsoil in assessment of the effects of arable management on soil organic matter.

Highlights

- Role of subsoil in the assessment of management effects on soil organic matter in arable farming is rarely accounted for.
- We investigated SOC and STN contents below 0–30-cm depth in a long-term field experiment.
- Consideration of the subsoil had a marked effect on the differentiation of the treatments.
- Sampling depth should be extended to include effects of management on the upper subsoil in the assessment of soil organic matter.

Introduction

Sustainable agriculture requires well-developed management practices that are appropriate for the specific site where crop production takes place. Soil organic matter (SOM) dynamics are often used as an indicator of management effects on soil fertility. An important factor in sampling design is the reference soil depth. Studies refer most frequently to either the first 0–30-cm soil depth or the soil layer in which cultivation takes place. Consequently, the sampling depth is rarely deeper than 30 cm. Evidence suggests, however, that the subsoil contributes considerably to plant nutrition, yet little is known about the dynamics of nutrients in the deeper soil layers (Kautz *et al.*, 2013). This is mainly because of the costs and the extent of analyses required to provide the necessary information. Therefore, the potential effects of various management practices on

Correspondence: L. Knebl. E-mail: lucas.a.knebl@agrar.uni-giessen.de
Received 28 September 2017; revised version accepted 28 September 2017

subsoil nutrient concentrations have not yet been quantified (Kautz *et al*., 2013). The analysis by West & Post (2002) of global data on rates of sequestration of soil organic carbon by tillage and crop rotation showed that there is a shortage of data on tillage effects on SOM in soil layers deeper than 30 cm. Van den Bygaart *et al*. (2003) presented a compendium of studies on management effects on SOC sequestration, and even though Canadian studies only were reviewed, the current lack of research on SOM dynamics of the subsoil (>30 cm) was emphasized.

Baker *et al*. (2007) referred to the two latter studies and were critical of standardized soil sampling procedures in most of the long-term experiments on management effects on SOM. The authors pointed to several studies that showed root growth and distribution, rhizodeposition and microbial activity were directly or indirectly affected by tillage practices. In fact, several studies did not restrict their investigations to the upper soil layer (e.g. Sanchez *et al*., 2002; Wilts *et al*., 2004; Olson & Al-Kaisi, 2015). Based on the observations in these studies, Baker *et al*. (2007) expressed concern about the disregard of subsoil layers. Dolan *et al*. (2006) suggested that certain observed tillage effects might occur in the upper soil layers only, but these effects might not necessarily be consistent with observations in the whole soil profile. Their suggestions conform to the study by Machado *et al*. (2003), who emphasized the effect of different tillage treatments on the vertical distribution of SOM.

This study therefore addresses the relevance of subsoil layers for the assessment of management effects on soil organic matter based on the Organic Arable Long-term Field Experiment Gladbacherhof (OAFEG). The experiment was established in 1998 and compares the effects of three different types of farming in combination with four different tillage treatments on agroeconomic and agroecological properties (Schmidt *et al*., 2006). Results of changes in SOM (indicated by soil organic carbon and soil total nitrogen) after two crop rotational cycles over 11 years at 0–30-cm soil depth revealed no significant differences in tillage treatments, but an increase in soil organic carbon (SOC) and soil total nitrogen (STN) masses at 30–60-cm soil depth with full inversion tillage (Schulz *et al*., 2014). The present study assesses in detail the effect of treatment in relation to soil depth with an extended database that covers the first three rotational cycles in the long-term experiment (1998–2015).

Materials and methods

Field experiment

The OAFEG experiment has been operational since 1998 at the research station Gladbacherhof in Villmar in the Taunus hill landscape in Hesse, Germany (Table 1). Soil properties examined are listed in Table 2. The site has been under organic management since 1983. Manure supply before the start of the experiment was equivalent to 0.7 livestock units (LU) cattle per ha arable land. The experiment includes three different crop rotations and fertilizer applications as the major factor (Table 3) and four tillage treatments as the minor factor (Table 4) in a two-factorial split plot design with four replications, as shown in Figure 1.

Table 1 Site conditions and basic set-up of the Organic Arable Farming Experiment. Gladbacherhof (OAFEG) according to Schulz *et al*. (2014).

Location	North-western Taunus hills, Germany (50.39706°N, 8.250238°E)
Elevation	174 m a.s.l.
Relief	Hill ridge, slight inclination (< 4%)
Soil type (according to WRB)	Haplic Luvisol
Texture	285, 680, 35 g kg^{-1} clay, silt, sand, respectively
pH ($CaCl_2$)	6.5–7.5
Mean annual precipitation	655 mm
Mean annual temperature	9.3°C
Two-factorial split-plot experiment, four replicates	Major factor: ‘farming type’ (crop rotation and/or fertilization)
	Minor factor: tillage intensity
Overall size	86 m × 144 m
Plot size	14 m × 36 m (main plot), 14 m × 9 m (subplot)

WRB, World Reference Base for Soil Resources (FAO, 2014).

Sampling and analysis

Soil samples for SOC and STN analyses were taken regularly in the early spring to a depth of 90 cm (at 0–30-, 30–60- and 60–90-cm depths) in all plots with a hand auger. This study is based on data from the complete analysis of topsoil (0–30-cm) and subsoil at depths of 30–60 and 60–90 cm at the start of the experiment in 1998 and after the second and third crop rotation cycles in 2009 and 2015, respectively. To calculate SOC and STN masses, soil samples were taken in the middle of each sampled soil layer with a 100-cm^3 sampling ring for each plot. Bulk density was analysed by weighing the volumetric samples after desiccation at 105°C. The determination of bulk density for each soil depth was carried out with a sample ring kit (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) with a closed ring holder. The SOC and STN contents in samples were measured according to ISO norms 10694 (ISO, 1996) and 13878 (ISO, 1998) with a Vario–EL apparatus (Elementar Analysesysteme, Langenselbold, Germany) with a measurement precision of 0.01 g kg^{-1} for total carbon and nitrogen analysis, and a SCHEIBLER apparatus (Eijkelkamp Agrisearch Equipment) to quantify carbonate. According to ISO 10694, SOC equals soil total carbon minus carbonate carbon.

Soil mass (Mg ha^{-1}) was calculated according to the following formula:

$$\text{Soil mass} = BD_{2015} \times \text{soil depth} \times 100, \quad (1)$$

where BD_{2015} (g cm^{-3}) is soil bulk density recorded in 2015 and soil depth (cm) is the reference soil depth interval.

In 1998, BD was assessed for the topsoil layer only (0–30 cm). In 2009 and 2015, BD was assessed in stages to a depth of 90 cm in all plots. Between 2009 and 2015 there were no significant differences in BD. Therefore, BD measured in 2015 was used to calculate SOC and STN masses for 1998, 2009 and 2015. In our assessment,

Table 2 Soil properties at the experimental site at the 0–30-cm soil depth. Clay, silt and sand content (%) (equivalent diameter in μm), soil class and pH for each block, according to Schulz (2012)

Block	Clay / % < 2.0 μm	Silt / % 2–63 μm	Sand / % 63–2000 μm	Soil class	pH
I	31.9	64.8	3.2	Silty clay loam	6.5
II	29.3	67.0	3.7	Silty clay loam	6.8
III	23.8	71.6	4.6	Silt loam	7.5
IV	29.0	68.5	2.6	Silty clay loam	7.2

Table 3 Experimental set-up of the Organic Arable Farming Experiment Gladbacherhof (OAFEG), major factor 'farming type', according to Schulz *et al.* (2014)

Major factor: 'farming type'				
Crop rotation field	Year	MF, 'mixed farm' with 1 LU ha^{-1} cattle	SFL, 'stockless farm' with mulched ley	SFC, 'stockless farm' with cash crops in all years
1	1998, 2004, 2010	Lucerne clovergrass mixture (*Medicago sativa*, *Trifolium pratense*, *3 Poaceae*)	Oats[a] (*Avena sativa*)	Oats
			Undersown crop[b]	Stubble crop[c]
2	1999, 2005, 2011	Lucerne clovergrass mixture	Green fallow/cropped ley (Lucerne clovergrass mixture)	Field beans (*Vicia faba*)
				Undersown crop[d]
3	2000, 2006, 2012	Winter wheat I (*Triticum aestivum*)	Winter wheat	Winter wheat
		Stubble crop[c]	Stubble crop[c]	Stubble crop[c]
4	2001, 2007, 2013	Potatoes (*Solanum tuberosum*)	Potatoes	Potatoes
5	2002, 2008, 2014	Winter wheat II[f]	Peas (*Pisum sativum*)	Peas
		Stubble crop[c]		
6	2003, 2009, 2015	Winter rye (*Secale cereale*)	Winter rye	Winter rye
		Undersown crop[b]	Stubble crop[c]	Stubble crop[c]
Cropland share / %				
	Cereals	50.0	50.0	50.0
	Root crops	16.7	16.7	16.7
	Fodder legumes	33.3		
	Ley		16.7	
	Grain legumes		16.7	33.3
Catch crops / %				
	Undersown crops	16.7	16.7	16.7
	Stubble crops	33.3	33.3	50.0
	Total	50.0	50.0	66.7
Organic fertilization				
		Rotted manure (10 Mg DM ha^{-1} annual mean[e])	Green fallow mulched	
		No straw fertilization	Straw fertilization (50.0% of cropland)	Straw fertilization (83.3% of cropland)

[a]Removal of oat straw because of undersown crops.
[b]*Medicago sativa*, *Trifolium pratense*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*.
[c]Growth of stubble crops in every system as green manure. Crops were usually mixtures of *Raphanus sativus* and *Vicia sativa*. Details are given in Schulz (2012), p. 195, Table A7: http://geb.unigiessen.de/geb/volltexte/2012/9058/.
[d]Grass only (*Lolium multiflorum*).
[e]45 Mg FM ha^{-1} with potatoes, 15 Mg FM ha^{-1} with winter rye (deep-litter stable manure according to stock density of approximately 1 LU ha^{-1} arable land).
[f]Fodder mixture (*Pisum sativum*, *Avena sativa*) instead of winter wheat (*Triticum aestivum*) in 2002.

Table 4 Experimental set-up of the Organic Arable Farming Experiment Gladbacherhof (OAFEG), minor factor 'tillage intensity' according to Schulz *et al*. (2014); treatment names modified

Minor factor: tillage intensity				
	FIT	TLP	RIT	NIT
Stubble cultivation in summer	Cultivator depth 15 cm	Two-layer plough disruption down to 30 cm, inversion down to 15 cm	Plough or cultivator, 15 cm	Cultivator and rotary harrow disruption down to 30 cm, mixing down to 15 cm
Autumn cultivation before winter and summer crops	Plough (depth of 30 cm)	Plough (depth of 15 cm)	Plough (depth of 15 cm)	Without plough (depth of 15 cm)

FIT, full inversion tillage; NIT, non-inversion tillage; RIT, reduced inversion tillage; TLP, two-layer ploughing.

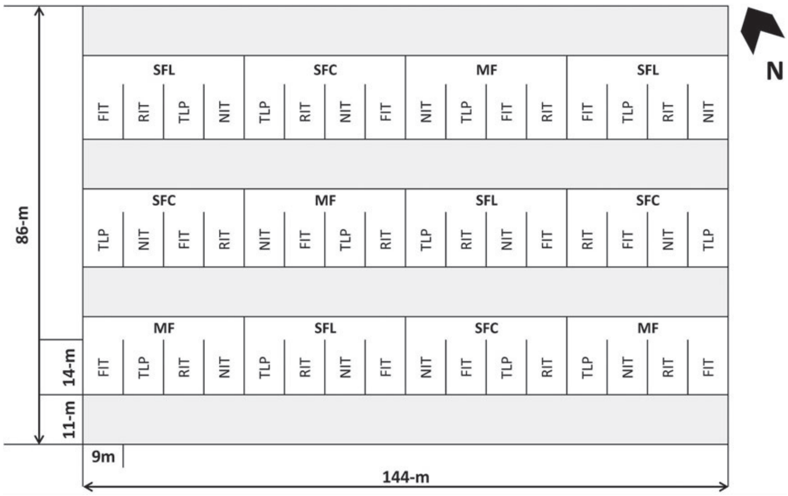

Figure 1 Experimental design of the OAFEG (Organic Arable Field Experiment Gladbacherhof). With mixed farming (MF), stockless cash crop farming with rotational ley (SFL) and stockless cash crop farming (SFC) as major factors, and full inversion tillage (FIT), two-layer plough (TLP), reduced inversion tillage (RIT) and non-inversion tillage (NIT) as minor factors.

we do not refer to equivalent soil mass, but calculated SOC and STN masses based on soil mass in each plot. Differences in the contents of SOC and STN between treatments at the start of the experiment in 1998 were accounted for in the assessment of SOC and STN masses after two (2009) and three (2015) crop rotation cycles. By processing the data according to Equations (2) and (3) below, we refer to homogenous initial SOC and STN contents over the experimental site in 1998:

$$SOC_t\_corrected = SOC_{tn} - SOC_{t0} + SOC_{t0}\_mean, \quad (2)$$

$$STN_t\_corrected = STN_{tn} - STN_{t0} + STN_{t0}\_mean, \quad (3)$$

where corrected SOC or STN masses are based on subtracting calculated initial contents of SOC or STN (t0) in 1998 from SOC and STN masses at tn (2009 and 2015). Following this, the mean SOC or STN mass in 1998 (t0_mean) was calculated and added to obtain an impression of the total SOC and STN masses in 2009 and 2015 rather than mass changes over the respective survey period (SOC and STN masses were calculated for all years according to Equation (1)).

Our aim was to evaluate the effect of taking into account the subsoil on the assessment of treatments in the OAFEG; therefore, we decided not to analyse the years 2009 and 2015 separately, but to calculate mean values for the treatments from the 2009 and 2015 data.

Statistics

Differences in corrected SOC and STN masses between treatments at different soil depths were evaluated by applying a linear mixed model:

$$Y_{ijk} = \mu + \alpha_i + \beta_i + (\alpha\beta)_{ij} + r_k + b_{ik} + e_{ijk}, \quad (4)$$

where Y_{ijk} is SOC or STN content of *i*th farming type and *j*th tillage treatment in the *k*th block, μ is the general mean, α_i is the main effect of *i*th farming type, β_i is the main effect of *j*th tillage treatment, $(\alpha\beta)_{ij}$ is the interaction between farming type and tillage treatment, r_k is the effect of *k*th block, b_{ik} is the error of *i*th main plot within the *k*th block and e_{ijk} is the sub-plot error (residual) (notation follows that of Piepho *et al*., 2003). Differences between treatments were determined by Fisher's LSD test. Residuals showed a near-normal distribution (Table S1 in Supporting Information), indicating that the linear model was suitable without transformation of the data.

Calculations were carried out separately for the soil layers 0–30 (corresponds to the topsoil horizon in the experiment), 30–60, 60–90 and 0–90 cm.

The sampling design in the experiment was stratified random sampling. Statistical analysis was carried out with STATISTICA 13 (Dell Inc., Tulsa, OK, USA).

Results

Table 5 lists the treatment means for SOC and STN at the level of the two factors 'farming type' and tillage intensity (ANOVA results are available in Table S2 of the Supporting Information). The results are shown separately for each variable (SOC and STN) and soil layer (0–30, 30–60, 60–90 and 0–90 cm).

Farming type

At the common sampling depth in most field experiments, which comprises only the topsoil (here 0–30 cm), there is a significant difference ($P < 0.01$) between 'farming type' treatments in the OAFEG (Table 5). Both SOC and STN masses are largest for the MF treatment, where straw and fodder crops were exported, but cattle manure was returned. The stockless farming system with cash crops in all years (SFC) has the smallest values for both SOC and STN, despite a considerable return of straw and green manure. The difference between the two treatments is 8400 kg SOC ha^{-1} and 800 kg STN ha^{-1}, respectively, corresponding to an SOC mass that is 18% larger in this soil layer under MF and a 15% larger STN mass.

There are no differences in SOC and STN masses between the treatments in the upper (30–60 cm) and lower (60–90 cm) subsoil because of the large variation in the data. Variation of data in these layers even affected the comparison between treatments with regard to differences in SOC and STN at 0–90 cm. Effects of 'farming type', as observed in the comparison of topsoil layers (0–30 cm), are not evident statistically for the whole profile (0–90 cm), despite considerable differences between treatment means (Table 5).

Tillage intensity

Table 5 shows that the tillage treatments have not resulted in any clear differences in either SOC or STN mass in the topsoil (0–30 cm). Treatment effects, however, are evident in the upper subsoil (30–60 cm) where the full-inversion tillage treatment, FIT, has resulted in significantly larger SOC ($P < 0.01$) and STN masses ($P < 0.001$) than the reduced tillage intensity treatments with a two-layer plough (TLP), reduced tillage depth (RIT) and non-inversion tillage (NIT). The TLP treatment has the second largest SOC and STN masses, but the difference is significant for the system with reduced tillage depth only ($P < 0.05$). Similar to the effects of 'farming type', there is no evidence of an effect of tillage treatments on SOC and STN masses in the lower subsoil (60–90 cm). If the effect of tillage on SOC and STN masses is evaluated for the whole soil profile (0–90-cm soil depth), FIT has significantly larger SOC and STN masses ($P < 0.05$ to 0.001, respectively) than all other treatments (TLP, RIT and NIT). This situation results from the differences in SOC and STN masses between treatments in the upper subsoil (30–60 cm), and cannot be detected from the evaluation of the topsoil layer (0–30 cm).

Discussion

The most interesting finding in our survey was that taking into consideration soil depths below the tilled horizon changed the assessment of the effect of different tillage treatments on the total masses of SOC and STN over a 0–90-cm soil profile depth. We found significantly larger SOC and STN masses in the soil profile under full inversion tillage than with reduced tillage treatments. This is in contrast to observations that reduced tillage can increase SOC and STN (e.g. Berner *et al*., 2008; Cooper *et al*., 2016), or will at least not reduce SOC and STN in the soil compared to full inversion tillage (Baker *et al*., 2007). Nevertheless, these surveys do not include subsoil layers, which was also identified as a potential bias by Baker *et al*. (2007). In our survey, the observed effect was a result of larger masses of both SOC and STN in the upper subsoil (30–60 cm) under full inversion tillage, which conforms to the results of a meta-analysis by Angers & Erikson-Hamel (2008). The latter authors discussed the effect of carbon inputs, spatial distribution and turnover of SOC in the soil, and translocation of dissolved SOC, on SOC stocks under full-inversion and no-till systems. Furthermore, Kautz *et al*. (2013) suggested that the allocation of nutrients in the subsoil might derive from root residues, root exudates and earthworm faeces, whereas depletion of nutrients is caused by root uptake. Even though the authors referred to nutrients and not to SOC and STN as indicators of OM, the mechanisms can be applied to these target variables also.

The SOC and STN inputs to subsoil with plant roots

Roots are likely to be the most important source of SOC and STN in the subsoil (Rumpel *et al*., 2002). Root growth in the soil is strongly affected by soil structure and the physical accessibility of the soil (Ehlers *et al*., 1983, Hamblin, 1986; Pabin *et al*., 1998). Unfortunately, root growth and the distribution of roots in the soil profile have not been assessed in the OAFEG during the survey period under study. Rather, the assessment of BD and penetration resistance associated with different tillage systems might indicate possible effects on root growth. We found that BD was not affected by tillage in the topsoil (0–30 cm) and upper subsoil (30–60 cm) layers (Table S2 in Supporting Information). Similar results have been reported by Angers *et al*. (1997), who did not observe any differences in the effects of tillage treatments on BD after 11 years of parallel mouldboard ploughing, chisel ploughing and no-till. However, the results of Schulz (2012) on penetration resistance showed that a compaction zone occurred in the soil under all tillage treatments in the OAFEG, but the location of the zone in the profile differed between the treatments. According to Schulz (2012), the compaction zone under FIT was at 30–40-cm soil depth, which relates to the 30–60-cm layer assessed in our survey. For the other treatments, the compaction zone was within the 0–30-cm soil layer. Therefore, the easily accessible soil layer was deeper under FIT than under the reduced tillage treatments, which might have

Table 5 Mean corrected SOC and STN masses (kg ha^{-1}) (average from 2009 and 2015), and C:N ratios in the Organic Arable Farming Experiment Gladbacherhof (OAFEG) long-term field experiment at different soil depths depending on 'farming type' and tillage intensity

		SOC / kg ha^{-1}			STN / kg ha^{-1}			
Treatment	Soil depth/ cm	Mean	SE	LSD	Mean	SE	LSD	C:N
Farming treatments								
MF	0–30	54 600	1271	3266	6100	92	237	9.0
SFL	0–30	49 800			5700			8.7
SFC	0–30	46 200			5300			8.7
MF	30–60	21 600	2226	–	2500	155	–	8.6
SFL	30–60	23 200			2800			7.9
SFC	30–60	20 400			2600			8.2
MF	60–90	18 900	2293	–	2000	137	–	9.4
SFL	60–90	20 400			2100			9.3
SFC	60–90	19 600			2100			9.7
MF	0–90	95 100	4599	–	10 600	290	–	9.0
SFL	0–90	93 400			10 700			8.6
SFC	0–90	86 100			10 000			8.8
Tillage treatments								
FIT	0–30	49 900	1192	–	5700	105	–	8.7
TLP	0–30	49 400			5600			8.8
RIT	0–30	50 500			5700			8.9
NIT	0–30	50 900			5800			8.9
FIT	30–60	27 000	1706	3508	3100	130	267	8.8
TLP	30–60	21 400			2700			7.9
RIT	30–60	18 800			2400			8.0
NIT	30–60	19 900			2400			8.1
FIT	60–90	21 000	1051	–	2200	100	–	9.7
TLP	60–90	18 800			2000			9.4
RIT	60–90	19 200			2200			8.8
NIT	60–90	19 600			2000			9.8
FIT	0–90	97 900	2762	5679	11 000	219	450	8.9
TLP	0–90	89 500			10 300			8.7
RIT	0–90	88 400			10 200			8.7
NIT	0–90	90 500			10 200			8.9

SOC, soil organic carbon; STN, soil total nitrogen.
'Farming type' treatments: MF, mixed farming; SFL, stockless cash crop farming with rotational ley; SFC, stockless farming with cash crops in all years. Tillage intensity treatments: FIT, full inversion tillage; NIT, non-inversion tillage; RIT, reduced inversion tillage; TLP, two-layer plough.

promoted root growth in the upper subsoil above the compacted zone (30–40-cm soil depth). Furthermore, the inversion of soil by tillage might lead to the translocation of organic matter (OM) into deeper levels of the soil (see below).

In addition to this process, Kautz *et al.* (2013) stressed the relevance of macropores for root growth and the resulting effect on microbial activity and nutrient uptake by roots in deeper soil layers. According to these authors, roots grow preferentially through the soil profile in macropores, and their results showed an increase in microbial activity in the rhizosphere, and increased mobilization and uptake of bound $NH4^{+}$. Even though SOC and STN values and the C:N ratio (Table 5) at 30–60-cm depth in the OAFEG do not indicate a larger root biomass or greater microbial activity under reduced tillage in this soil layer, the processes described by Kautz *et al.* (2013) could result in a depletion of OM at 30–60-cm depth, at least with regard to STN.

Translocation of dissolved SOC and STN to the subsoil

Dissolved organic matter (DOM) is known to include a large share of easily decomposable direct microbial metabolites (Guggenberger *et al*., 1994). The effect of management on the condition of soil water plays a key role in the distribution of nutrients or OM, or both, within the soil profile. Kuipers (1984), for example, had already stated that the effect of tillage on crops is mainly associated with the effect of water in the soil or on the surface. In addition, as shown by Kalbitz *et al*. (2000), the distributions of SOC and STN in the soil profile are also affected by the vertical transport of dissolved material. This process could also add to the explanation of the effect of tillage on SOC and STN in the subsoil in the OAFEG. However, it should be kept in mind that assessment of the effect of tillage on solute transport in soil is complex (Besson *et al*., 2011). The vertical movement of DOM in soil depends on soil water content, pore size and pore distribution within the profile (Unger & Cassel, 1991). Changes in soil physical properties induced by tillage are affected by soil texture, clay mineralogy, water content, depth and the concentration of OM (Unger & Cassel, 1991). With regard to tillage effects in the OAFEG, we have to consider vertical distribution of OM in the soil and the different depths of the compaction zones. With FIT, soil inversion (and, thus, incorporation of OM) occurred at 0–30-cm depth; with TLP and RIT it was at 0–15-cm depth. There was no inversion of the soil in the NIT treatment. The depth of the compaction zone corresponded with the inversion depth of the different types of tillage. The amount of DOM and the content of SOM (thus SOC and STN) are both closely related to how the different tillage treatments affect the vertical movement of water. The compaction zone is assumed to have an effect on the movement of water through the soil (Richard *et al*., 2001). Consequently, the translocation of dissolved SOC and STN by water transport might have led to their accumulation within the upper boundary of the compaction zone. With the reduced tillage treatments (TLP, RIT and NIT) this accumulation would have occurred within the 0–30-cm soil layer, whereas it would be in the 30–60-cm layer with FIT.

Microbial activity and OM turnover

A reduction in both microbial biomass and microbial activity has been indicated by some authors for the upper 0–30-cm soil depth with reduced tillage systems (Ahl *et al*., 1997; Vian *et al*., 2009). Ahl *et al*. (1997) also reported more microbial activity and larger contents of SOC and STN at 30–50-cm depth with full inversion tillage than with reduced inversion tillage. Their experiment was carried out under warmer climatic conditions, but it still conforms to the observations under FIT in the OAFEG, at least for SOC and STN masses. Even though microbial biomass was not assessed in the OAFEG, we assume that the values would be larger for FIT than for the reduced tillage treatments as a consequence of the relation between MB and SOC (Kaiser *et al*., 1992). The possibly larger MB for the FIT treatment did not lead to a depletion of SOC and STN masses at the 30–60-cm depth. A possible explanation might be a larger input of OM by roots and DOM (see above) that could have offset possible losses with the turnover of the soil. Larger crop yields with FIT compared to NIT (Schulz, 2011) support this assumption. Furthermore, a reduced input of OM to the subsoil in the reduced tillage treatments might have led to a depletion of SOC and STN masses compared to FIT.

Our analysis has shown that consideration of the subsoil further changed the assessment of types of farming in relation to their effect on SOC and STN masses. Differences between the treatments that were evident in the topsoil were not observed when they were evaluated for the soil profile to 90 cm. However, the effect of SFL on SOC and STN masses at the 30–60-cm depth appears to offset the more positive effect on SOC and STN in the drilosphere with MF. This might be a statistical issue because the large variation in the data of the subsoil probably affected the possibility of detecting differences between treatments, but it could also be a management effect. A possible explanation could be that non-leguminous crops with MF are provided with sufficient amounts of readily available nutrients (see Table 3). In this case, the need for an extended rooting depth for nutrient acquisition would be less pronounced than with SFL and SFC. This is supported by results on the dependence of root growth on nutrient supply by Chirinda *et al.* (2012). We assume that the apparent increase in SOC and STN at the 30–60-cm depth with SFL is the result of enhanced root growth within that soil layer. The missing effect of (assumed) increased root growth under SFC, compared to both MF and SFL, on SOC and STN at 30–60-cm depth could result from the less favourable crop rotation without any perennial legume fodder crops (Leithold *et al*., 2015). Smaller yields with SFC for all crops (Schulz, 2012) support this assumption.

Conclusions

Our results from the OAFEG long-term field experiment indicate the importance of including soil layers below the topsoil (>30 cm) in evaluation of the effect of management on SOC and STN masses in arable soil. Consideration of SOC and STN masses below the topsoil, in particular with regard to tillage, significantly changed the assessment of the effects of different tillage treatments: there were no marked differences between full-inversion tillage (FIT) and the three reduced tillage treatments (TLP, RIT and NIT) in the topsoil layer (0–30 cm), whereas the amounts of SOC and STN under FIT at 30–60-cm depth were significantly larger for this treatment than all reduced tillage treatments, resulting overall in larger SOC and STN masses under this tillage treatment.

Even though different masses and a different spatial distribution of SOC and STN in the topsoil are relevant for several ecological functions, a reliable quantification of the effect of management on SOC and STN amounts in arable soil requires an extension of sampling depth below the topsoil. The required sampling depth may depend on site factors. In our survey, significant treatment effects on SOC and STN masses were observed in the upper subsoil (30–60 cm), whereas further extension of the sampling depth (60–90 cm) had little effect on our assessment of treatment effects.

Supporting Information

The following supporting information is available in the online version of this article:

Table S1. Skewness coefficients for soil organic carbon (SOC) and soil total nitrogen (STN) data subsets used in the analyses.

Table S2. Overview of ANOVA tables for two-factor split-plot design: soil organic carbon (SOC) and soil total nitrogen (STN) in the Organic Arable Farming Experiment Gladbacherhof (OAFEG) at 0–30-, 30–60-, 60–90- and 0–90-cm depths and bulk density (BD) at 0–30-, 30–60- and 60–90-cm soil depths.

Acknowledgements

The authors acknowledge the great work of the team at Gladbacherhof experimental station and of the laboratory team at the Centre for Organic Farming at Giessen University. In addition, we acknowledge the support of Dr Klemens Ekschmitt (Giessen University). We thank the anonymous reviewers for their valuable comments that helped to finalize this paper, and acknowledge the patient support of the editors of EJSS.

References

Ahl, C., Jörgensen, R.G., Kandeler, E., Meyer, B. & Wöhler, V. 1997. Microbial biomass and activity in silt and sand loams after long–term shallow tillage in central Germany. *Soil & Tillage Research*, **49**, 93–104.

Angers, D.A. & Eriksen-Hamel, N.S. 2008. Full-inversion tillage and organic carbon distribution in soil profiles: a meta-analysis. *Soil Science Society of America Journal*, **72**, 1370–1374.

Angers, D.A., Bolinder, M.A., Carter, M.R., Gregorich, E.G., Drury, C.F., Liang, B.C. *et al.* 1997. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. *Soil & Tillage Research*, **41**, 191–201.

Baker, J.M., Ochsner, T.E., Ventera, R.T. & Griffis, T.J. 2007. Tillage and soil carbon sequestration–What do we really know? *Agriculture, Ecosystems & Environment*, **118**, 1–5.

Berner, A., Hildemann, I., Fließbach, A., Pfiffner, L., Niggli, U. & Mäder, P. 2008. Crop yield and soil fertility response to reduced tillage under organic management. *Soil & Tillage Research*, **101**, 89–96.

Besson, A., Javaux, M., Bielders, C.L. & Vanclooster, M. 2011. Impact of tillage on solute transport in a loamy soil from leaching experiments. *Soil & Tillage Research*, **112**, 47–57.

Chirinda, N., Olesen, J.E. & Porter, J.R. 2012. Root carbon input in organic and inorganic fertilizer-based systems. *Plant and Soil*, **359**, 321–333.

Cooper, J., Baranski, M., Stewart, G., Nobel-de Lange, M., Bàrberi, P., Fließbach, A. *et al.* 2016. Shallow non–inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. *Agronomy for Sustainable Development*, **36**, 22.

Dolan, M.S., Clapp, C.E., Allmaras, R.R., Baker, J.M. & Molina, J.A.E. 2006. Soil organic nitrogen in a Minnesota soil as related to tillage, residue, and nitrogen management. *Soil & Tillage Research*, **89**, 221–231.

Ehlers, W., Köpke, U., Hesse, F. & Böhm, W. 1983. Penetration resistance and root growth of oats in tilled and untilled loess soil. *Soil & Tillage Research*, **3**, 261–275.

Food and Agriculture Organization of the United Nations (FAO) 2014. *World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*. Update 2015. World Soil Ressources Report No 106, FAO Sales and Marketing Group, Rome.

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

Hamblin, A.P., 1986. The influence of soil structure on water movement, crop root growth, and water uptake. *Advances in Agronomy*, **38**, 95–158.

International Standards Organization (ISO) 1996. ISO 10694: 1996–08. *SI Units and Recommendations for the Use of their Multiples and of Certain Other Units. Soil Quality–Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis)*. Beuth-Verlag GmbH, Berlin.

International Standards Organization (ISO) 1998. ISO 13878:1998. *SI Units and Recommendations for the Use of their Multiples and of Certain Other Units. Soil Quality–Determination of Total Nitrogen Content by Dry Combustion (Elementary Analysis)*. Beuth-Verlag GmbH, Berlin.

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

Kaiser, E.A., Mueller, T., Joergensen, R.G., Insam, H. & Heinemeyer, O. 1992. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. *Soil Biology & Biochemistry*, **24**, 675–683.

Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R. *et al.* 2013. Nutrient acquisition from arable subsoils in temperate climates: a review. *Soil Biology & Biochemistry*, **57**, 1003–1022.

Kuipers, K. 1984. The challenge of soil cultivation and soil water problems. *Journal of Agricultural Engineering Research*, **29**, 177–190.

Leithold, G., Hülsbergen, K.-J. & Brock, C. 2015. Organic matter returns to soils must be higher under organic compared to conventional farming. *Journal of Plant Nutrition and Soil Science*, **178**, 4–12.

Machado, P.L.O.A., Sohi, S.P. & Gaunt, J.L. 2003. Effect of no-tillage on turnover of organic matter in a Rhodic Ferralsol. *Soil Use and Management*, **19**, 250–256.

Olson, K.R. & Al-Kaisi, M.M. 2015. The importance of soil sampling depth for accurate account of soil organic carbon sequestration, storage, retention and loss. *Catena*, **125**, 33–37.

Pabin, J., Lipiec, J., Wøodek, S., Biskupski, A. & Kaus, A. 1998. Critical soil bulk density and strength for pea seedling root growth as related to other soil factors. *Soil & Tillage Research*, **46**, 203–208.

Piepho, H.P., Büchse, A. & Emrich, K. 2003. A hitchhikers's guide to mixed models for randomized experiments. *Journal of Agronomy and Crop Science*, **189**, 310–322.

Richard, G., Cousin, I., Sillon, J.F., Bruand, A. & Guérif, J. 2001. Effect of compaction on the porosity of a silty soil: influence on unsaturated hydraulic properties. *European Journal of Soil Science*, **52**, 49–58.

Rumpel, C., Kögel-Knabner, I. & Bruhn, F. 2002. Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. *Organic Geochemistry*, **33**, 1131–1142.

Sanchez, J.E., Paul, E.A., Willson, T.C., Smeenk, J. & Harwood, R.R. 2002. Corn root effects on the nitrogen supplying capacity of a conditioned soil. *Agronomy Journal*, **94**, 391–396.

Schmidt, H., Schulz, F. & Leithold, G. 2006. Organic farming trial Gladbacherhof. Effects of different crop rotations and tillage systems. In: *Long-term Field Experiments in Organic Farming*, ISOFAR Scientific Series 1 (eds J. Raupp, C. Pekrun, M. Oltmanns & U. Köpke), pp. 165–182. Verlag Dr. Köster, Berlin.

Schulz, F. 2011. Einfluss reduzierter grundbodenbearbeitung auf erträge und bodenparameter im dauerfeldversuch Gladbacherhof. In: *Es geht ums Ganze: Forschen im Dialog von Wissenschaft und Praxis Beiträge zur 11. Wissenschaftstagung Ökologischer* Landbau, Justus-Liebig-Universität Gießen, 15–18 März 20 (eds G. Leithold, K. Becker, C. Brock, S. Fischinger, A.-K. Spiegel, K. Spory *et al.*). Verlag Dr. Köster, Berlin.

Schulz, F. 2012. *Vergleich ökologischer Betriebssysteme mit und ohne Viehhaltung bei unterschiedlicher Intensität der Grundbodenbearbeitung*. Dissertationsschrift (Doktorarbeit) Justus-Liebig-Universität Gießen, Giessener Schriften zum Ökologischen Landbau 5, Verlag Dr. Köster, Berlin. [WWW document]. URL http://geb.uni–giessen.de/geb/volltexte/2012/9058/ [accessed on 8 August 2017].

Schulz, F., Brock, C., Schmidt, H., Franz, K.–.P. & Leithold, G. 2014. Development of soil organic matter stocks under different 'farming types' and tillage systems in the organic arable farming experiment Gladbacherhof. *Archives of Agronomy and Soil Science*, **60**, 313–326.

Unger, P.W. & Cassel, D.K. 1991. Tillage implement disturbance effects on soil properties related to soil and water conservation: a literature review. *Soil & Tillage Research*, **19**, 363–382.

West, T.O. & Post, W.M. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Science Society of America Journal*, **66**, 1930–1946.

Wilts, A.R., Reicosky, D.C., Allmaras, R.R. & Clapp, C.E. 2004. Long-term corn residue effects. *Soil Science Society of America Journal*, **68**, 1342–1351.

Van den Bygaart, A.J., Gregorich, E.G. & Angers, D.A. 2003. Influence of agricultural management on soil organic carbon: a compendium and assessment of Canadian studies. *Canadian Journal of Soil Science*, **83**, 363–380.

Vian, J.F., Peigne, J., Chaussod, R. & Roger-Estrade, J. 2009. Effects of four tillage systems on soil structure and soil microbial biomass in organic farming. *Soil Use and Management*, **25**, 1–10.

Chapter 5: Discussion, constraints and perspectives

5.1 The assessment of N-pathways in greenhouse experiments

As already explained in the introduction of the thesis, nitrogen pathways are an important element to evaluate SOM dynamics. Against this background and also against the background of plant nutrition and fertilizer management, it is important to be able to estimate the NUE and the complementary uptake of soil N and fertilizer N. Other important N pathways are N leachate, but above all gaseous N losses. The first question of this thesis was: *What is the share of nitrogen from SOM-mineralisation taken up by a crop that is fertilised with mineral nitrogen?* In chapter 2, this question was answered for sorghum-sudangrass compared to maize in a bag experiment conducted in a greenhouse. Results showed that it is possible to detect differences between the plants regarding STN uptake and fertilizer N uptake with such an experimental setup. However, the method has some constraints, and the results should be interpreted with care. On the one hand, no complete recovery of applied ^{15}N could be realized. Possible ^{15}N losses due to emission or leachate were not recorded, as a complete ^{15}N fertilizer balance was not the subject of the investigation. However, the assessment of these pathways could be a valuable addition to future experiments. On the other hand, growing conditions of the plants in the experiment differed considerably from those at field level. Soil properties and climate, for example, are not comparable to natural conditions. Not only did the plants in the experiment grow in a considerably disturbed soil substrate without natural structuring, but the roots of the plants also had only limited growing space, and standard plant spacing was difficult to implement in the relatively small bags. The question arises as to whether an investigation of the complete fertilizer N-balance under natural field conditions would not achieve better results. However, there are other obstacles to this approach. For example, the spatial delimitation of the analysed soil volume is just as difficult as the complete recording of N leachate. In addition, the measurement of N_2 emissions, as already mentioned, proves to be difficult against large atmospheric background masses. The result of the respective uncertainties in the recording of individual N pathways is an incomplete ^{15}N fertilizer balance. Against all these backgrounds, the use of lysimeters could be a solution. Lysimeters represent a spatially defined system that offers plants growing conditions that are more similar to natural conditions, e.g. with regard to soil depths, plant density and climatic conditions. In addition, more natural soil conditions can be achieved, e.g. by filling largely intact soil monoliths into the lysimeters, as done in the studies of Yankelzon et al. (2024) and Dowdell & Webster (1980), or the lysimeters regain their natural aggregation over years of use, as in the study by Portela et al. (2006). Various authors have already carried out ^{15}N labelled fertilizer studies in lysimeters. Specific scopes included the distribution of N fertilizer in the plant-soil system (e.g. Lindberg et al., 1989), the complementary uptake of fertilizer N and STN by plants (e.g. Dowdell & Webster, 1980; Dressel & Jung, 1990), as well as fertilizer N losses through leachates (e.g. Portela et al. 2006, Sørensen et al., 2023) and through gaseous emissions (e.g. Yankelzon et al., 2024). The latter and most recent study attempted to evaluate the total ^{15}N fertilizer balance under winter wheat and included the measurement of N_2 using the ^{15}NGF method. In their study, Yankelzon et al. (2024) measured N_2 emissions (in closed chambers, covering the whole lysimeter), these correlated positively with the amounts of unrecovered ^{15}N. The authors conclude that the ^{15}NGF method provides realistic estimates of field N_2 emissions and that lysimeter studies are a suitable approach for a complete ^{15}N fertilizer balance. However, standards for such lysimeter-studies should be developed, e.g. for fertilizer application methods, lysimeter dimensions and soil sampling design as well as for analytical approaches, to improve comparability of results.

5.2 The importance of soil sampling procedures

One problem already mentioned in connection with SOM models is their sometimes poor validation. Against this background, the aspect of soil sampling plays an important role. So far, there is a lack of standards regarding the sampling procedure concerning e.g. sampling frequency, sampling grid, bulk density and soil depth. Especially the latter has to receive more focus in future research efforts. Therefore, the second question of this thesis was whether *it is necessary to assess SOM quantity changes in the subsoil in order to estimate and validate C and N balances in the soil*. Up to now, many of the long-term field experiments used for model calibration/validation have focused on SOM dynamics within the upper soil layers. As reported in Smith et al. (1997), only in two out of seven long-term field experiments, namely “Rothamsted Geescroft Wilderness” and the “Waite Permanent Rotation Trial”, SOM was assessed in soil layers down to or below 45 cm at least at one point of time. However, in their comparison of models Smith et al. (1997) only used SOM contents of the upper 15 to 30 cm soil depth. In their review, Poeplau et al. (2011) also reported that only a few studies (< 20 %) included measurements of subsoil SOC stocks even though the SOC dynamic of topsoils accounts for only 75 % of total changes. Against the background of modelling, Rumpel & Kögel-Knabner (2011) state that it is necessary to investigate subsoil OM dynamics at the field level and to find out which C sources are important, how these are controlled, what contribution microbial-derived OM has compared to plant-derived OM and how subsoil C fluxes are influenced by C-inputs, -outputs and -stabilization processes. However, these aspects have not yet been sufficiently clarified, which is why Hicks Pries et al. (2023) also call on the scientific community to investigate land-use change and climate change effects on subsoil OM and integrate them into SOM models.

Chapter 4 of this thesis on the role of subsoil in the assessment of management induced effects on SOC and STN provides evidence that SOM in deeper soil layers can affect model performance validation. Taking subsoil SOC and STN dynamics into account, it was possible to detect the effects of tillage intensity in a long-term field experiment that were not significant with view of the topsoil only. The obtained results therefore are suitable to improve the validation of SOM balance models. The cause of these effects could not be clarified, though, and the results therefore cannot be used to generalize tillage effects on subsoil SOM stocks of comparable sites. It is merely a hint towards the need of “digging deeper” as Lal (2018) suggests. However, the positive effect of conventional tillage for SOC in soil layers > 30 cm is in line with findings of Gross & Glaser (2021). The authors conducted a meta-analysis on the effect of manure application on SOM storage. Out of 101 studies with a total of 592 treatments they reviewed, 103 treatments included measurements on SOM below 30 cm soil depth. In studies investigating subsoil SOC responses to manure application in combination with different tillage intensities, the authors found that conventional tillage (CT) and reduced tillage (RT) had no overall difference in their effect on total SOC sequestration. However, CT on average led to higher SOC sequestration at soil depths ≤ 15 cm and > 30 cm compared to RT. SOC accumulations in soil layers > 30 cm with RT were small but also significant. These observations are contradicted e.g. by the study by Krauss et al. (2022). The authors investigated the SOC storage down to a soil depth of 100 cm, in organic field experiments on tillage, with a duration of eight and 21 years. They found that RT in this soil horizon led to an overall higher SOC accumulation than CT (3.6% higher, respectively 4.0 Mg ha^{-1} yr^{-1}). The SOC enrichment occurred with RT both in the upper soil layers (0 - 10/15 cm) and in deeper layers (70 - 100 cm). The SOC accumulation was accompanied by comparatively lower yields, which they attribute to higher weed growth under RT compared to CT. With regards to OM sources for subsoil SOM, Rumpel & Kögel-Knabner (2011) suggest that dissolved organic matter (DOM), root biomass and POM (particulate organic matter, physically or biologically transported) are the main sources of SOM in subsoils. The authors also conclude that microbial-derived OM plays a greater role in this context than plant-derived OM and they identify mineral interactions with OM as important stabilization processes of SOM in subsoils. For example, OM can be protected from decomposition by physical protection in soil aggregates. However, specific OM-sources and -stabilization mechanisms are ultimately dependent on climate, site properties and land-use (Rumpel & Kögel-Knabner, 2011). For example, in the study by Leuther et al. (2022) on subsoil OM after 36 years of high-rate farm yard manure (FYM) application on a ploughed soil, DOM played a subordinate role as a source of subsoil SOM. Their results showed that most of the observed SOM accumulation in the subsoil came from preferential fresh, labile POM likely due to stimulation of root growth and bioturbation. The authors attributed the small contribution of DOM to the rather dry climate of the experimental site. However, only high application- rates of FYM (at a minimum of 100 Mg FM ha^{-1} a^{-1}), which are not common in agricultural practice, led to the observed SOM increase in subsoil layers, and the authors suggest that they came with significant C and N losses.

In addition to the sources of OM inputs to the subsoil, mechanisms must be discussed that lead to a shift of OM into subsoil and/or to its stabilization. On the one hand, OM inputs from roots and DOM can lead to an increase in SOM content in subsoils, while at the same time a positive priming effect (PE) can promote the decomposition of old SOM through the input of fresh SOM (Hicks Pries et al., 2023). Which of these effects are predominant depends on various factors. Management practices that have a positive effect on aggregate formation can be favourable for SOM storage in subsoils. Aggregates can provide a physical protection for OM against decomposition, at least until the natural capacity of the soil for such protection is reached (Hassink & Whitmore, 1997). On the other hand, C and N availability play an important role, as increased mineralization rates can occur after the input of fresh OM if C and N limitation is present. Fontaine et al. (2007) state that fresh organic carbon (OC) in subsoils represents a new energy source for microbes, which ultimately also stimulates the decomposition of the old SOM stored in depth. If N limitation is predominant, the input of fresh OM and thus C into subsoils can also stimulate so-called "microbial nitrogen mining", which in turn leads to increased N mineralization (Schimel & Weintraub, 2003; Craine et al., 2007). Although the input of fresh OM into subsoils by roots is obvious, the role of roots with regards to soil aggregation is discussed contradictorily. According to Dijkstra et al. (2021), roots are both attributed with forming aggregates, as well as with breaking bonds of OM to minerals, metals and calcium via rhizodeposition. The former leads to protection of OM in aggregates against microbial decomposition, the latter promotes microbial access to OM as nutrient source.

For the long-term experiment, which was evaluated in chapter 4, it would have been necessary, against this background, to carry out a series of further investigations at the beginning and during the experiment. For example, the development of soil aggregation at different soil depths over time, the vertical distribution of roots, the fluxes of DOM and the microbial activity in different soil layers would probably have led to an improved basis for discussion of the results. This would have required further perturbation of the soil. The investigation also highlighted the importance of plot-wise assessing SOM content and bulk densities of the respective soil layers at the start of an experiment. Determining parameters in a composite sample, even if it is done block-wise, makes it difficult to evaluate the results afterwards. The importance of SOM dynamics in deeper soil layers becomes especially evident if the scope of modelling is to predict carbon sequestration in the context of which total quantities are decisive. Our suggestion to at least consider SOM dynamics down to 60 cm soil depth, if not further, is in accordance with the study of Lal (2018) who states that the soil layer of 0-100 cm is crucial in the assessment of SOC changes and the effects of land use change, soil management and other perturbations, since it contains ~55 % of the total SOC stock. However, short-term SOM changes are not easily assessed, due to reasons described in the introduction. The third question of this thesis was to determine whether *short-term field experiments can be used to assess SOM change under cropping* *systems as a basis for the validation of SOM balances and other models.* Several studies estimated the yearly potential of SOM sequestration due to land management (table 2). Next to the predicted mean annual SOC changes, the table shows how many years it would need to significantly detect the respective effect. This was done first with proposed average minimum detectable differences (MDD) of 2 and 5 Mg C ha^{-1} (Conant et al., 2003; Garten & Wullschleger, 1999 respectively) and in addition with an MDD of 1.77 Mg C ha^{-1} calculated for the short-term field experiment described in chapter 3. As can be seen in the table, all annual SOC sequestration rates are well below 1.77 Mg C ha^{-1}.

Table 2: Management practise, potential SOC sequestration and observation period needed for detection.

Management practise	SOC sequestration	Years until detection of sequestration is possible		
	Mg C ha^{-1} yr^{-1}	with MDD = 5 Mg C ha^{-1}	with MDD = 2 Mg C ha^{-1}	with MDD = 1.77 Mg C ha^{-1}
Conversion of cropland to grassland [1]	0.40-0.80	6.3-12.5	2.5-5.0	2.2-4.4
Conversion to organic farming [2]	0.45-0.55	9.0-11.1	3.6-4.4	3.2-3.9
Introduction of cover crops [3]	0.24-0.4	12.5-20.8	5.0-8.3	4.4-7.4
Enhancing crop rotation [4]	0.08-0.32	15.6-62.5	6.3-25.0	5.5-22.1
Conversion from conventional to no-tillage [4]	0.36-0.64	7.8-13.9	3.1-5.5	2.7-4.9

[1]*Lugato et al. (2014);* [2]*Gattinger et al. (2012);* [3]*Poeplau & Don (2015);* [4]*West & Post (2002).*

Therefore, the method proposed in chapter 3 would not lead to a detection of these sequestered SOC amounts. However, the required observation period could be reduced considerably (table 2). Moreover, SOC sequestration does not proceed evenly until it has reached its equilibrium. Rather, there is a peak in sequestration, followed by decreasing annual rates. With changes in tillage, for example West & Post (2002) report SOC sequestration to peak within the first 5-10 years with small effects until an equilibrium at 15-20 years. Management practices with smaller effects on SOC stock and therefore longer time periods before an equilibrium is reached, will have different time spans for such a peak. Nevertheless, higher initial SOC increase (or decrease) can be expected after the implementation of a management practice, so that the method from chapter 3 could possibly enable the detection of all management effects listed in table 2 within a period of 3-4 years, which is a common project duration nowadays. However, this would have to be verified in further investigations. An additional gradual extension of the sampling depth (up to 100 cm) would be useful in this context. Nevertheless, the method presented in chapter 3 is suitable for improving the calibration of the HU-MOD balance model and is therefore proposed as a method in field trials that are specifically aimed at developing SOM balance methods or at studying novel approaches in crop management (e.g. new fertilizers, crops or tillage equipment). As a standard for all field experiments, the method is probably too time-consuming and cost-intensive. At the time of writing this thesis, no comparable study is known.

Chapter 6: Conclusions

In the following, the conclusions of this thesis are drawn based on the questions posed at the beginning:

What is the share of nitrogen from SOM-mineralisation taken up by a crop that is fertilised with mineral nitrogen?

It was shown in chapter 2 that, to a certain extent, a bag experiment is suitable to advance the development of SOM balance models. In the bag experiment conducted to compare the mineral fertilizer use efficiency (FNU) and complementary uptake of fertilizer N and STN by sorghum-sudangrass versus maize, it was shown that both crops used considerable amounts of N mineralized from SOM. However, significant differences were observed between the crops. It is therefore not advisable to use parameters of maize as a proxy for balancing the effects of sorghum-sudangrass in the SOM balance. However, the experiment had limitations in the recovery of the total fertilizer applied in the form of ^{15}N-labelled ammonium nitrate. N losses could have been caused by emissions and leachate, which were not measured in the experiment. A direct transfer of the results to field level is not recommended though, since the natural conditions in the field differ from the conditions in a bag experiment (soil and water conditions, climate, etc.). Yet, the data available on sorghum-sudangrass is currently still limited. Therefore, the parameters on nitrogen utilization and FNU presented in chapter 2, if used instead of proxies, could improve the validation performance of SOM balance models. The latter could not be verified, as no long-term field experiments on sorghum-sudangrass are available to date that could have been used for a model validation. Such field trials on sorghum-sudangrass, ideally in comparison to other well-studied crops, are urgently needed and should, in addition to recording SOC and STN dynamic (up to 100 cm), investigate nitrogen utilization, N gas emission and N leachate. N emission and N leachate should also be assessed in future bag experiments.

Can short-term field experiments be used to assess SOM change under cropping systems as a basis for the validation of SOM balances and other models?

The data collection in a short-term field experiment through an adapted sampling design and data treatment (chapter 3) resulted in the detection of quantity changes between 3.7 % of background SOC and 2.6 % of background STN levels after one year observation period. This corresponds to 1.77 Mg SOC ha^{-1} and 0.14 Mg STN ha^{-1}. Compared to detectable amounts of 2 Mg SOC change ha^{-1} with n=5 samples and 5 Mg SOC change ha^{-1} with n=16 samples, reported by Conant et al. (2003) and Garten & Wullschleger (1999), respectively, this result is considerable, and no comparable study is available so far. The conducted data collection proved to be suitable for the validation of crop production systems in SOM balance models like HU-MOD. It is therefore recommended that this method is used in short-term field trials aimed at developing SOM balance models or investigating new management practices, fertilizers or crops. The time and cost of this sampling method should not be underestimated. The consideration of the subsoil can also provide further valuable information for SOM balance models, if they relate to mass fluxes in the soil and not to a fixed soil layer, as shown in chapter 4. Further improvements to the sampling design could include additional series, consideration of variable spatial impacts on the soil in crop stands and bulk density estimation parallel to each sampling procedure.

Is it necessary to assess SOM quantity changes in the subsoil in order to estimate and validate C and N balances in the soil?

The results of chapter 4 emphasize the importance of subsoil SOC and STN dynamics in the evaluation of tillage effects. In the survey, significant tillage effects could be observed in the upper subsoil (30-60 cm). Above and below that layer (0-30 cm and 60-90 cm) no significant effects occurred. The reason for the observation could only be discussed, though. The required sampling depth may depend on site factors as well as the management practice, fertilizer or the crop that is studied. The extension of sampling depth is required to collect reliable data on SOC and STN masses needed for SOM model validation. Future long-term field experiments should therefore include a plot-wise assessment of SOC and STN, as well as bulk density in soil layers down to a soil depth of 100 cm.

References

Bandilla, Karl W. „Carbon Capture and Storage“. In *Future Energy*, 669–92. Elsevier, 2020. https://doi.org/10.1016/B978-0-08-102886-5.00031-1.

Bashir, O., Ali, T., Baba, Z.A., Rather, G.H., Bangroo, S.A., Mukhtar, S.D., Naik, N., Mohiuddin, R., Bharati, V., Bhat, R.A., 2021. Soil Organic Matter and Its Impact on Soil Properties and Nutrient Status, in: Dar, G.H., Bhat, R.A., Mehmood, M.A., Hakeem, K.R. (Eds.), Microbiota and Biofertilizers, Vol 2. Springer International Publishing, Cham, pp. 129–159. https://doi.org/10.1007/978-3-030-61010-4_7

Bot, A., Benites, J., 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production, FAO soils bulletin 80. Food and Agriculture Organization of the United Nations, Rome.

Bremner, J.M., 2018. Nitrogen-Total, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), SSSA Book Series. Soil Science Society of America, American Society of Agronomy, Madison, WI, USA, pp. 1085–1121. https://doi.org/10.2136/sssabookser5.3.c37

Brock, C., Franko, U., Oberholzer, H., Kuka, K., Leithold, G., Kolbe, H., Reinhold, J., 2013. Humus balancing in Central Europe—concepts, state of the art, and further challenges. J. Plant Nutr. Soil Sci. 176, 3–11. https://doi.org/10.1002/jpln.201200137

Brock, C., Franko, U., Wiesmeier, M., 2023. Soil management for carbon sequestration, in: Encyclopedia of Soils in the Environment. Elsevier, 424–432. https://doi.org/10.1016/B978-0-12-822974-3.00124-5

Brock, C., Hoyer, U., Leithold, G., Hülsbergen, K.-J., 2012. The humus balance model (HU-MOD): a simple tool for the assessment of management change impact on soil organic matter levels in arable soils. Nutr. Cycl. Agroecosystems 92, 239–254. https://doi.org/10.1007/s10705-012-9487-z

Brock, C., Knies-Deventer, H., Leithold, G., 2011. Assessment of cropping-system impact on soil organic matter levels in short-term field experiments. Zeitschrift für Pflanzenernährung und Bodenkunde 174, 867–870. https://doi.org/10.1002/jpln.201100147

Brock, C., Oberholzer, H. -R., Franko, U., 2017. Soil organic matter balance as a practical tool for environmental impact assessment and management support in arable farming. Eur. J. Soil Sci. 68, 951–952. https://doi.org/10.1111/ejss.12495

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Ann. Appl. Biol. 162, 145–173. https://doi.org/10.1111/aab.12014

Campbell, E.E., Paustian, K., 2015. Current developments in soil organic matter modeling and the expansion of model applications: a review. Environ. Res. Lett. 10, 123004. https://doi.org/10.1088/1748-9326/10/12/123004

Carter, M.R., 2002. Soil Quality for Sustainable Land Management: Organic Matter and Aggregation Interactions that Maintain Soil Functions. Agron. J.94, 38–47. https://doi.org/10.2134/agronj2002.3800

Christensen, B.T., 1987. Decomposability of organic matter in particle size fractions from field soils with straw incorporation. Soil Biol. Biochem. 19, 429–435. https://doi.org/10.1016/0038-0717(87)90034-4

Christensen, B.T., 1992. Physical Fractionation of Soil and Organic Matter in Primary Particle Size and Density Separates, in: Lal, R., Stewart, B.A. (Eds.), Soil Restoration, Advances in Soil Science. Springer New York, New York, NY, pp. 1–90. https://doi.org/10.1007/978-1-4612-2930-8_1

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European J. Soil Science 52, 345–353. https://doi.org/10.1046/j.1365-2389.2001.00417.x

Conant, R.T., Smith, G.R., Paustian, K., 2003. Spatial Variability of Soil Carbon in Forested and Cultivated Sites: Implications for Change Detection. J. of Env. Quality 32, 278–286. https://doi.org/10.2134/jeq2003.2780

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1

De La Cruz, V.Y.V., Tantriani, Cheng, W., Tawaraya, K., 2023. Yield gap between organic and conventional farming systems across climate types and sub-types: A meta-analysis. Agric. Syst. 211, 103732. https://doi.org/10.1016/j.agsy.2023.103732

Dick, W.A., Gregorich, E.G., 2004. Developing and maintaining soil organic matter levels., in: Schjønning, P., Elmholt, S., Christensen, B.T. (Eds.), Managing Soil Quality: Challenges in Modern Agriculture. CABI Publishing, UK, pp. 103–120. https://doi.org/10.1079/9780851996714.0103

Dijkstra, F.A., Zhu, B., Cheng, W., 2021. Root effects on soil organic carbon: a double-edged sword. New Phytol. 230, 60–65. https://doi.org/10.1111/nph.17082

Dowdell, R.J., Webster, C.P., 1980. A lysimeter study using nitrogen-15 on the uptake of fertilizer nitrogen by perennial ryegrass swards and losses by leaching. J. soil sci. 31, 65–75. https://doi.org/10.1111/j.1365-2389.1980.tb02065.x

Dressel, J., Jung, J., 1990. ^{15}N Studies of the Behavior of Fertilizer Nitrogen in Three Different Soils (Lysimeter Trials). J. Agronomy Crop Science 164, 217–223. https://doi.org/10.1111/j.1439-037X.1990.tb00810.x

Elliott, E.T., Cambardella, C.A., 1991. Physical separation of soil organic matter. Agric. Ecosyst. Environ. 34, 407–419. https://doi.org/10.1016/0167-8809(91)90124-G

Fageria, N.K., 2012. Role of Soil Organic Matter in Maintaining Sustainability of Cropping Systems. Commun. Soil Sci. Plant Anal. 43, 2063–2113. https://doi.org/10.1080/00103624.2012.697234

FAO 2009. How to Feed the World in 2050. Rome, Italy, Food and Agriculture Organization of the United Nations, Rome, Italy. Online: https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf, [14.07.2024]

FAO, IFAD, UNICEF, WFP, WHO 2023. The State of Food Security and Nutrition in the World 2023, Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum. Rome, FAO. https://doi.org/10.4060/cc3017en

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280. https://doi.org/10.1038/nature06275

Franko, U., Kolbe, H., Thiel, E., Ließ, E., 2011. Multi-site validation of a soil organic matter model for arable fields based on generally available input data. Geoderma 166, 119–134. https://doi.org/10.1016/j.geoderma.2011.07.019

Friedl, J., Cardenas, L.M., Clough, T.J., Dannenmann, M., Hu, C., Scheer, C., 2020. Measuring denitrification and the N2O:(N2O + N2) emission ratio from terrestrial soils. Curr. Opin. Environ. Sustain. 47, 61–71. https://doi.org/10.1016/j.cosust.2020.08.006

Gabrielle, B., Mary, B., Roche, R., Smith, P., Gosse, G., 2002. Simulation of carbon and nitrogen dynamics in arable soils: a comparison of approaches. Eur. J. Agron. 18, 107–120. https://doi.org/10.1016/S1161-0301(02)00099-0

Garsia, A., Moinet, A., Vazquez, C., Creamer, R.E., Moinet, G.Y.K., 2023. The challenge of selecting an appropriate soil organic carbon simulation model: A comprehensive global review and validation assessment. Glob. Chang. Biol. 29, 5760–5774. https://doi.org/10.1111/gcb.16896

Garten, C.T., Wullschleger, S.D., 1999. Soil Carbon Inventories under a Bioenergy Crop (Switchgrass): Measurement Limitations. J. of Env. Quality 28, 1359–1365. https://doi.org/10.2134/jeq1999.00472425002800040041x

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N.E.-H., Niggli, U., 2012. Enhanced top soil carbon stocks under organic farming. Proc. Natl. Acad. Sci. U.S.A. 109, 18226–18231. https://doi.org/10.1073/pnas.1209429109

Gerke, J., 2022. The Central Role of Soil Organic Matter in Soil Fertility and Carbon Storage. Soil Syst. 6, 33. https://doi.org/10.3390/soilsystems6020033

Gregorich, E.G., Beare, M.H., McKim, U.F., Skjemstad, J.O., 2006. Chemical and Biological Characteristics of Physically Uncomplexed Organic Matter. Soil Science Soc. of Amer. J. 70, 975–985. https://doi.org/10.2136/sssaj2005.0116

Gross, A., Glaser, B., 2021. Meta-analysis on how manure application changes soil organic carbon storage. Sci. Rep. 11, 5516. https://doi.org/10.1038/s41598-021-82739-7

Hassink, J., Whitmore, A.P., 1997. A Model of the Physical Protection of Organic Matter in Soils. Soil Science Soc. of Amer. J. 61, 131–139. https://doi.org/10.2136/sssaj1997.03615995006100010020x

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon Sequestration in Ecosystems: the Role of Stoichiometry. Ecology 85, 1179–1192. https://doi.org/10.1890/02-0251

Hicks Pries, C.E., Ryals, R., Zhu, B., Min, K., Cooper, A., Goldsmith, S., Pett-Ridge, J., Torn, M., Berhe, A.A., 2023. The Deep Soil Organic Carbon Response to Global Change. Annu. Rev. Ecol. Evol. Syst. 54, 375–401. https://doi.org/10.1146/annurev-ecolsys-102320-085332

Hoffland, E., Kuyper, T.W., Comans, R.N.J., Creamer, R.E., 2020. Eco-functionality of organic matter in soils. Plant Soil 455, 1–22. https://doi.org/10.1007/s11104-020-04651-9

Hülsbergen, K.-J., 2003. Entwicklung und Anwendung eines Bilanzierungsmodells zur Bewertung der Nachhaltigkeit landwirtschaftlicher Systeme, 1. Aufl. ed, Berichte aus der Agrarwissenschaft. Shaker, Aachen.

IPCC, 2023. Summary for Policymakers. In: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)].https://doi.org/10.59327/IPCC/AR6-9789291691647

Janzen, H.H., 2004. Carbon cycling in earth systems—a soil science perspective. Agric. Ecosyst. Environ. 104, 399–417. https://doi.org/10.1016/j.agee.2004.01.040

Keating, B.A., Carberry, P.S., Bindraban, P.S., Asseng, S., Meinke, H., Dixon, J., 2010. Eco-efficient Agriculture: Concepts, Challenges, and Opportunities. Crop Sci. 50. https://doi.org/10.2135/cropsci2009.10.0594

Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C., Batten, G., 2011. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. Geoderma 163, 197–208. https://doi.org/10.1016/j.geoderma.2011.04.010

Kolbe, H., 2010. Site-adjusted organic matter–balance method for use in arable farming systems. Z. Pflanzenernähr. Bodenk. 173, 678–691. https://doi.org/10.1002/jpln.200900175

Körschens, M., 2006. The importance of long-term field experiments for soil science and environmental research–a review. Plant Soil Environ. 52(Special Issue), 1-8.

Körschens, M., Rogasik, J., Schulz, E., 2005. Balance and standard values of soil organic matter. Landbauforschung Volkenrode, 55(1), 1-10.

Krauss, M., Wiesmeier, M., Don, A., Cuperus, F., Gattinger, A., Gruber, S., Haagsma, W.K., Peigné, J., Palazzoli, M.C., Schulz, F., Van Der Heijden, M.G.A., Vincent-Caboud, L., Wittwer, R.A., Zikeli, S., Steffens, M., 2022. Reduced tillage in organic farming affects soil organic carbon stocks in temperate Europe. Soil Tillage Res 216, 105262. https://doi.org/10.1016/j.still.2021.105262

Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biol. 30, 207. https://doi.org/10.1071/FP02085

Lal, R., 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. Land Degrad. Dev. 17, 197–209. https://doi.org/10.1002/ldr.696

Lal, R., 2018. Digging deeper: A holistic perspective of factors affecting soil organic carbon sequestration in agroecosystems. Glob. Chang. Biol. 24, 3285–3301. https://doi.org/10.1111/gcb.14054

Lal, R., 2020. Soil organic matter and water retention. Agron. J. 112, 3265–3277. https://doi.org/10.1002/agj2.20282

Lamb, W.F., Wiedmann, T., Pongratz, J., Andrew, R., Crippa, M., Olivier, J.G.J., Wiedenhofer, D., Mattioli, G., Khourdajie, A.A., House, J., Pachauri, S., Figueroa, M., Saheb, Y., Slade, R., Hubacek, K., Sun, L., Ribeiro, S.K., Khennas, S., De La Rue Du Can, S., Chapungu, L., Davis, S.J., Bashmakov, I., Dai, H., Dhakal, S., Tan, X., Geng, Y., Gu, B., Minx, J., 2021. A review of trends and drivers of greenhouse gas emissions by sector from 1990 to 2018. Environ. Res. Lett. 16, 073005. https://doi.org/10.1088/1748-9326/abee4e

Le Noë, J., Manzoni, S., Abramoff, R., Bölscher, T., Bruni, E., Cardinael, R., Ciais, P., Chenu, C., Clivot, H., Derrien, D., Ferchaud, F., Garnier, P., Goll, D., Lashermes, G., Martin, M., Rasse, D., Rees, F., Sainte-Marie, J., Salmon, E., Schiedung, M., Schimel, J., Wieder, W., Abiven, S., Barré, P., Cécillon, L., Guenet, B., 2023. Soil organic carbon models need independent time-series validation for reliable prediction. Commun. Earth Environ. 4, 158. https://doi.org/10.1038/s43247-023-00830-5

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069

Leifeld, J., Kögel-Knabner, I., 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use? Geoderma 124, 143–155. https://doi.org/10.1016/j.geoderma.2004.04.009

Leithold, G., Hülsbergen, K.-J., Michel, D., Schönmeier, H., 1997. Humusbilanzierung – Methoden und Anwendung als Agrar-Umweltindikator. In: Deutsche Bundesstiftung Umwelt (ed.): Umweltverträgliche Pflanzenproduktion. Initiativen zum Umweltschutz 5. Zeller Verlag, Osnabrück, Germany, pp. 43–54.

Leuther, F., Wolff, M., Kaiser, K., Schumann, L., Merbach, I., Mikutta, R., Schlüter, S., 2022. Response of subsoil organic matter contents and physical properties to long-term, high-rate farmyard manure application. European J. Soil Science 73, e13233. https://doi.org/10.1111/ejss.13233

Lindberg, T., Bonde, T.A., Bergström, L., Pettersson, R., Rosswall, T., Schnürer, J., 1989. Distribution of15N in the soil-plant system during a four-year field lysimeter study with barley (Hordeum distichum L.) and perennial meadow fescue (Festuca pratensis Huds.). Plant Soil 119, 25–37. https://doi.org/10.1007/BF02370266

Lugato, E., Bampa, F., Panagos, P., Montanarella, L., Jones, A., 2014. Potential carbon sequestration of European arable soils estimated by modelling a comprehensive set of management practices. Glob. Chang. Biol. 20, 3557–3567. https://doi.org/10.1111/gcb.12551

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., Van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Muhie, S.H., 2022. Novel approaches and practices to sustainable agriculture. J. Agr. Food Res. 10, 100446. https://doi.org/10.1016/j.jafr.2022.100446

Myers, R.J.K. (1995): Modelling of soil organic matter dynamics. In: Lefroy, R.D.B., Blair, G.J., Craswell, E.T., 1995. Soil organic matter management for sustainable agriculture: a workshop held in Ubon, Thailand, 24-26 August 1994. Australian Centre for International Agricultural Research, Canberra.

Novoa, R., Loomis, R.S., 1981. Nitrogen and plant production. Plant Soil 58, 177–204. https://doi.org/10.1007/BF02180053

Oberholzer, H.-R., Weisskopf, P., Gaillard, G., Weiss, F., Knuchel, R.F., 2006. Methode zur Beurteilung der Wirkungen landwirtschaftlicher Bewirtschaftung auf die Bodenqualität in Ökobilanzen. Agroscope, Zürich, Switzerland.

Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. SOIL 5, 15–32. https://doi.org/10.5194/soil-5-15-2019

Oldfield, E.E., Wood, S.A., Bradford, M.A., 2018. Direct effects of soil organic matter on productivity mirror those observed with organic amendments. Plant Soil 423, 363–373. https://doi.org/10.1007/s11104-017-3513-5

Paul, C., Bartkowski, B., Dönmez, C., Don, A., Mayer, S., Steffens, M., Weigl, S., Wiesmeier, M., Wolf, A., Helming, K., 2023. Carbon farming: Are soil carbon certificates a suitable tool for climate change mitigation? J. Environ. Manage. 330, 117142. https://doi.org/10.1016/j.jenvman.2022.117142

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agric. Ecosyst. Environ. 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Poeplau, C., Don, A., Vesterdal, L., Leifeld, J., Van Wesemael, B., Schumacher, J., Gensior, A., 2011. Temporal dynamics of soil organic carbon after land-use change in the temperate zone - carbon response functions as a model approach: Soil Organic Carbon and Land-Use Change. Glob. Chang. Biol. 17, 2415–2427. https://doi.org/10.1111/j.1365-2486.2011.02408.x

Portela, S.I., Andriulo, A.E., Sasal, M.C., Mary, B., Jobbágy, E.G., 2006. Fertilizer vs. organic matter contributions to nitrogen leaching in cropping systems of the Pampas: 15N application in field lysimeters. Plant Soil 289, 265–277. https://doi.org/10.1007/s11104-006-9134-z

Praveen, B., Sharma, P., 2019. A review of literature on climate change and its impacts on agriculture productivity. J. Public Aff. 19, e1960. https://doi.org/10.1002/pa.1960

Richardson, K., Steffen, W., Lucht, W., Bendtsen, J., Cornell, S.E., Donges, J.F., Drüke, M., Fetzer, I., Bala, G., Von Bloh, W., Feulner, G., Fiedler, S., Gerten, D., Gleeson, T., Hofmann, M., Huiskamp, W., Kummu, M., Mohan, C., Nogués-Bravo, D., Petri, S., Porkka, M., Rahmstorf, S., Schaphoff, S., Thonicke, K., Tobian, A., Virkki, V., Wang-Erlandsson, L., Weber, L., Rockström, J., 2023. Earth beyond six of nine planetary boundaries. Sci. Adv. 9, eadh2458. https://doi.org/10.1126/sciadv.adh2458

Rumpel, C., Amiraslani, F., Chenu, C., Garcia Cardenas, M., Kaonga, M., Koutika, L.-S., Ladha, J., Madari, B., Shirato, Y., Smith, P., Soudi, B., Soussana, J.-F., Whitehead, D., Wollenberg, E., 2020. The 4p1000 initiative: Opportunities, limitations and challenges for implementing soil organic carbon sequestration as a sustainable development strategy. Ambio 49, 350–360. https://doi.org/10.1007/s13280-019-01165-2

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant Soil 338, 143–158. https://doi.org/10.1007/s11104-010-0391-5

Scheer, C., Fuchs, K., Pelster, D.E., Butterbach-Bahl, K., 2020. Estimating global terrestrial denitrification from measured N2O:(N2O + N2) product ratios. Curr. Opin. Environ. Sustain. 47, 72–80. https://doi.org/10.1016/j.cosust.2020.07.005

Schimel, J., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35, 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schlesinger, W.H., Amundson, R., 2019. Managing for soil carbon sequestration: Let's get realistic. Glob. Chang. Biol. 25, 386–389. https://doi.org/10.1111/gcb.14478

Schreefel, L., Schulte, R.P.O., De Boer, I.J.M., Schrijver, A.P., Van Zanten, H.H.E., 2020. Regenerative agriculture – the soil is the base. Glob. Food Sec. 26, 100404. https://doi.org/10.1016/j.gfs.2020.100404

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232. https://doi.org/10.1038/nature11069

Shamrikova, E.V., Kondratenok, B.M., Tumanova, E.A., Vanchikova, E.V., Lapteva, E.M., Zonova, T.V., Lu-Lyan-Min, E.I., Davydova, A.P., Libohova, Z., Suvannang, N., 2022. Transferability between soil organic matter measurement methods for database harmonization. Geoderma 412, 115547. https://doi.org/10.1016/j.geoderma.2021.115547

Shi, T., Cui, L., Wang, J., Fei, T., Chen, Y., Wu, G., 2013. Comparison of multivariate methods for estimating soil total nitrogen with visible/near-infrared spectroscopy. Plant Soil 366, 363–375. https://doi.org/10.1007/s11104-012-1436-8

Smith, P., 2004. How long before a change in soil organic carbon can be detected? Glob. Chang. Biol. 10, 1878–1883. https://doi.org/10.1111/j.1365-2486.2004.00854.x

Smith, P., Smith, J.U., Powlson, D.S., McGill, W.B., Arah, J.R.M., Chertov, O.G., Coleman, K., Franko, U., Frolking, S., Jenkinson, D.S., Jensen, L.S., Kelly, R.H., Klein-Gunnewiek, H., Komarov, A.S., Li, C., Molina, J.A.E., Mueller, T., Parton, W.J., Thornley, J.H.M., Whitmore, A.P., 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. Geoderma 81, 153–225. https://doi.org/10.1016/S0016-7061(97)00087-6

Smith, P., Soussana, J., Angers, D., Schipper, L., Chenu, C., Rasse, D.P., Batjes, N.H., Van Egmond, F., McNeill, S., Kuhnert, M., Arias-Navarro, C., Olesen, J.E., Chirinda, N., Fornara, D., Wollenberg, E., Álvaro-Fuentes, J., Sanz-Cobena, A., Klumpp, K., 2020. How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Glob Chang Biol 26, 219–241. https://doi.org/10.1111/gcb.14815

Sørensen, P., Pedersen, B.N., Thomsen, I.K., Eriksen, J., Christensen, B.T., 2023. Plant availability and leaching of ^{15}N-labelled mineral fertilizer residues retained in agricultural soil for 25 years: A lysimeter study. J. Plant Nutr. Soil Sci. 186, 441–450. https://doi.org/10.1002/jpln.202200288

Stevenson, F.J., Elliott, E.T., Cole, C.V., Ingram J., Oades, J.M., Preston, C., Sollins, P.J., 1989. Methologies For Assessing The Quantity and Quality of Soil Organic Matter. D.C. Coleman, J.M. Oades, and G. Urehara (Eds.), Dynamics of Soil Organic Matter in Tropical Ecosystems, 96822, NiFTAL, Honolulu. 1–249.

Tabatabai, M.A., 2015. Soil Organic Matter Testing: An Overview, in: Magdoff, F.R., Tabatabai, M.A., Hanlon, E.A. (Eds.), SSSA Special Publications. Soil Science Society of America, Madison, WI, USA, pp. 1–9. https://doi.org/10.2136/sssaspecpub46.c1

Thamo, T., Pannell, D.J., 2016. Challenges in developing effective policy for soil carbon sequestration: perspectives on additionality, leakage, and permanence. Clim. Policy 16, 973–992. https://doi.org/10.1080/14693062.2015.1075372

Trumbore, S.E., 1997. Potential responses of soil organic carbon to global environmental change. Proc. Natl. Acad. Sci. U.S.A. 94, 8284–8291. https://doi.org/10.1073/pnas.94.16.8284

Tubiello, F.N., Salvatore, M., Ferrara, A.F., House, J., Federici, S., Rossi, S., Biancalani, R., Condor Golec, R.D., Jacobs, H., Flammini, A., Prosperi, P., Cardenas-Galindo, P., Schmidhuber, J., Sanz Sanchez, M.J., Srivastava, N., Smith, P., 2015. The Contribution of Agriculture, Forestry and other Land Use activities to Global Warming, 1990–2012. Glob. Chang. Biol. 21, 2655–2660. https://doi.org/10.1111/gcb.12865

Tuomisto, H.L., Hodge, I.D., Riordan, P., Macdonald, D.W., 2012. Comparing energy balances, greenhouse gas balances and biodiversity impacts of contrasting farming systems with alternative land uses. Agric. Syst. 108, 42–49. https://doi.org/10.1016/j.agsy.2012.01.004

Van Dijk, M., Morley, T., Rau, M.L., Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nat. Food 2, 494–501. https://doi.org/10.1038/s43016-021-00322-9

Vandergeten, E., Azadi, H., Teklemariam, D., Nyssen, J., Witlox, F., Vanhaute, E., 2016. Agricultural outsourcing or land grabbing: a meta-analysis. Landscape Ecol. 31, 1395–1417. https://doi.org/10.1007/s10980-016-0365-y

Van Groenigen, J.W., Van Kessel, C., Hungate, B.A., Oenema, O., Powlson, D.S., Van Groenigen, K.J., 2017. Sequestering Soil Organic Carbon: A Nitrogen Dilemma. Environ. Sci. Technol. 51, 4738–4739. https://doi.org/10.1021/acs.est.7b01427

VDLUFA 2004. Körschens, M., Rogasik, J., Schulz, E., Böning, H., Eich, D., Ellerbrock, R. H., Franko, U., Hülsbergen, K.-J., Köppen, D., Kolbe, H., Leithold, G., Merbach, I., Peschke, H., Prystav, W., Reinhold, J., & Zimmer, J. (2004). Humusbilanzierung : Methode zur Beurteilung und Bemessung der Humusversorgung von Ackerland : Standpunkt / VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, ed.). Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten.

Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007

Waksman, S.A., 1936. Humus Origin, Chemical Composition, and Importance in Nature: Soil Sci. 41, 395. https://doi.org/10.1097/00010694-193605000-00010

Wander, M., 2004. Soil Organic Matter Fractions and Their Relevance to Soil Function, in: Magdoff, F., Weil, R. (Eds.), Soil Organic Matter in Sustainable Agriculture, Advances in Agroecology. CRC Press. https://doi.org/10.1201/9780203496374.ch3

Weiglein, T.L., Strahm, B.D., Bowman, M.M., Gallo, A.C., Hatten, J.A., Heckman, K.A., Matosziuk, L.M., Nave, L.E., Possinger, A.R., SanClements, M.D., Swanston, C.W., 2022. Key predictors of soil organic matter vulnerability to mineralization differ with depth at a continental scale. Biogeochemistry 157, 87–107. https://doi.org/10.1007/s10533-021-00856-x

West, T.O., Post, W.M., 2002. Soil Organic Carbon Sequestration Rates by Tillage and Crop Rotation: A Global Data Analysis. Soil Science Soc. of Amer. J. 66, 1930–1946. https://doi.org/10.2136/sssaj2002.1930

Wiesmeier, M., Mayer, S., Burmeister, J., Hübner, R., Kögel-Knabner, I., 2020. Feasibility of the 4 per 1000 initiative in Bavaria: A reality check of agricultural soil management and carbon sequestration scenarios. Geoderma 369, 114333. https://doi.org/10.1016/j.geoderma.2020.114333

Yang, P., Wang, Y., Hu, B., Li, S., Chen, S., Luo, D., Peng, J., 2024. Predicting soil organic carbon content using simulated insitu spectra and moisture correction algorithms in southern Xinjiang, China. Geoderma Regional 37, e00783. https://doi.org/10.1016/j.geodrs.2024.e00783

Yankelzon, I., Schilling, L., Butterbach-Bahl, K., Gasche, R., Han, J., Hartl, L., Kepp, J., Matson, A., Ostler, U., Scheer, C., Schneider, K., Tenspolde, A., Well, R., Wolf, B., Wrage-Moennig, N., Dannenmann, M., 2024. Lysimeter-based full fertilizer 15N balances corroborate direct dinitrogen emission measurements using the 15N gas flow method. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-024-01801-4

ANNEX

List of peer reviewed publications

Publications included in this thesis:

Knebl, L., Leithold, G., Brock, C., 2015. Improving minimum detectable differences in the assessment of soil organic matter change in short-term field experiments. J. Plant Nutr. Soil Sci. 178, 35–42. https://doi.org/10.1002/jpln.201400409

Author Contributions:
Conceptualization, Christopher Brock; methodology, Lucas Knebl and Christopher Brock; software, Lucas Knebl; validation, Lucas Knebl; formal analysis, Lucas Knebl; investigation, Lucas Knebl and Christopher Brock; resources, Christopher Brock and Günter Leithold; data curation, Lucas Knebl; writing—original draft preparation, Lucas Knebl; writing—review and editing, Christopher Brock, Günter Leithold; visualization, Lucas Knebl; supervision, Christopher Brock and Günter Leithold; project administration, Christopher Brock; funding acquisition, not applicable.

Knebl, L., Leithold, G., Schulz, F., Brock, C., 2017. The role of soil depth in the evaluation of management-induced effects on soil organic matter. Eur. J. Soil Sci. 68, 979–987. https://doi.org/10.1111/ejss.12492

Author Contributions:
Conceptualization, Lucas Knebl and Christopher Brock; methodology, Lucas Knebl and Christopher Brock; software, Lucas Knebl; validation, Lucas Knebl; formal analysis, Lucas Knebl; investigation, Lucas Knebl and Christopher; resources, Christopher Brock and Günter Leithold and Franz Schulz; data curation, Lucas Knebl; writing—original draft preparation, Lucas Knebl; writing—review and editing, Christopher Brock, Günter Leithold and Franz Schulz; visualization, Lucas Knebl; supervision, Christopher Brock and Günter Leithold; project administration, Christopher Brock; funding acquisition, not applicable.

Knebl, L., Gattinger, A., Niether, W., Brock, C., 2023. Uptake of Fertilizer Nitrogen and Soil Nitrogen by Sorghum Sudangrass (Sorghum bicolor × Sorghum sudanense) in a Greenhouse Experiment with 15N-Labelled Ammonium Nitrate. Soil Syst. 7, 71. https://doi.org/10.3390/soilsystems7030071

Author Contributions:
Conceptualization, Lucas Knebl and Christopher Brock; methodology, Lucas Knebl; software, Lucas Knebl; validation, Lucas Knebl, Christopher Brock and Andreas Gattinger; formal analysis, Lucas Knebl; investigation, Lucas Knebl; resources, Christopher Brock; data curation, Lucas Knebl; writing—original draft preparation, Lucas Knebl; writing—review and editing, Christopher Brock, Andreas Gattinger, Wiebke Niether; visualization, Lucas Knebl; supervision, Christopher Brock and Andreas Gattinger; project administration, Christopher Brock; funding acquisition, Christopher Brock.

Further peer reviewed publications:

Katsoulas, N., Løes, A.-K., Andrivon, D., Cirvilleri, G., De Cara, M., Kir, A., Knebl, L., Malińska, K., Oudshoorn, F.W., Willer, H., Schmutz, U., 2020. Current use of copper, mineral oils and sulphur for plant protection in organic horticultural crops across 10 European countries. Org. Agr. 10, 159–171. https://doi.org/10.1007/s13165-020-00330-2

Manuelian, C.L., Valleix, S., Bugaut, H., Fuerst-Waltl, B., Da Costa, L., Burbi, S., Schmutz, U., Evans, A., Katsoulas, N., Faliagka, S., Aksoy, U., Çiçekli, Ö., Dróżdż, D., Malińska, K., Whistance, L., Johnson, M., Knebl, L., Righi, F., De Marchi, M., 2023. Farmers concerns in relation to organic livestock production. Italian Journal of Animal Science 22, 1268–1282. https://doi.org/10.1080/1828051X.2023.2252005

Binacchi, F., Niether, W., Brock, C., Knebl, L., Brændholt, A., Wolf, B., Gattinger, A., 2023. Demystifying the agronomic and environmental N performance of grain legumes across contrasting soil textures of central Germany. Agriculture, Ecosystems & Environment 356, 108645. https://doi.org/10.1016/j.agee.2023.108645

Non peer reviewed publication:

Knebl L., Blumenstein B., Wufka A., Brock C., Möller D., Gattinger A., 2021. Humusersatz und Strohvergärung: Widerspruch oder Patentlösung? Biogasjournal 1/2021, 92-100.

Conference papers:

Knebl, L., Blumenstein, B., Möller, D., Wufka, A., Brock, C., Gattinger, A., 2019. Bodenumsatz von Getreidestroh und Gärresten unterschiedlicher Qualität aus der Strohvergärung. [Turnover of wheat straw and wheat straw digestates in arable soils.] In: Mühlrath, Daniel; Albrecht, Joana; Finckh, Maria R.; Hamm, Ulrich; Heß, Jürgen; Knierim, Ute und Möller, Detlev (Hrsg.) Innovatives Denken für eine nachhaltige Land- und Ernährungswirtschaft. Beiträge zur 15. Wissenschaftstagung Ökologischer Landbau, Kassel, 5. bis 8. März 2019, Verlag Dr. Köster, Berlin.

Blumenstein, B., Knebl, L., Gattinger, A., Brock, C., Wufka, A., & Möller, D., 2019. „Stroh macht Strecke": Zur Wirtschaftlichkeit der (über-) betrieblichen Logistik von Corg. Innovatives Denken für eine nachhaltige Land-und Ernährungswirtschaft. Beiträge zur 15. Wissenschaftstagung Ökologischer Landbau, Kassel, 5. bis 8. März 2019.

Schulz, F., Knebl, L., Gattinger, A., 2019. Reduzierte Bodenbearbeitung im Dauerfeldversuch Gladbacherhof - Einfluss auf Erträge und Bodenparameter in der 3. Rotation. [Reduced soil tillage in the long-term field experiment Gladbacherhof - Effects on yields and soil parameters in the 3rd rotation.] In: Mühlrath, Daniel; Albrecht, Joana; Finckh, Maria R.; Hamm, Ulrich; Heß, Jürgen; Knierim, Ute und Möller, Detlev (Hrsg.) Innovatives Denken für eine nachhaltige Land- und Ernährungswirtschaft. Beiträge zur 15. Wissenschaftstagung Ökologischer Landbau, Kassel, 5. bis 8. März 2019, Verlag Dr. Köster, Berlin.

Blumenstein, B., Knebl, L., Möller, D., Gattinger, A., Brock, C., 2021. Carbon Balance And Economics: Trade-Offs Or Synergies In The Case Of Anaerobic Digestion Of Cereal Straw? Vortrag at: Organic World Congress 2021, Science Forum: 6th ISOFAR Conference co-organised with INRA, FiBL, Agroecology Europe, TP Organics and ITAB, Rennes, France, 8 - 10 September, 2021.

Løes, A.-K., Schmutz, U., Katsoulas, N., Caceres, R., Manuel De Cara, F., Cirvillieri, G., Kir, A., Knebl, L., Malinska, K., Oudshoorn, F., Raskin, B., Rayns, F., Valleix, S., Conroy, J., 2021. Peat, Plastic And Fertilisers In Organic Growing Across Europe - Current Use And Future Options. Poster at: Organic World Congress 2021, Science Forum: 6th ISOFAR Conference co-organised with INRA, FiBL, Agroecology Europe, TP Organics and ITAB, Rennes, France, 8 - 10 September, 2021.

Acknowledgements

I would like to express my great gratitude to my professors Prof. Dr. Günter Leithold and Prof. Dr. Andreas Gattinger, who made it possible for me to complete this thesis and always had an open ear for me. I would also like to thank Prof. Dr. Jan Siemens, who agreed to be my second supervisor.

Special thanks also go to my co-authors, in particular to my mentor and friend Dr. Christopher Brock, from whom I was able to learn a lot about scientific work, the planning and implementation of projects and how to be patient and keep positive when things don't go as planned. I would also like to thank Dr. Wiebke Niether for her support, whose productive advice is always a benefit.

In addition, I would like to thank Maria Barfels and Pia Doernfeld for their commitment at the laboratory and for always thinking along with me, as well as Dr. Klemens Ekschmitt, who supported me in my struggle with the R. software.

Finally, I would like to thank my former teachers as well as my friends and family who believed in me, supported me and made sure that I never ran out of coffee.