Effects of fertilizer type and rate on partitioning of soil organic matter into pools of different stability

Dissertation

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Preface

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The cumulative dissertation is based on three papers as first author, which are published in or submitted to international refereed journals. The manuscripts are included in chapters 2, 3 and 4.

The focus of the general introduction (chapter 1) is on theoretical and methodological issues, whereas specific introductions on the effect of fertilizer type and rate on soil are given in the following manuscripts (chapters 2, 3 and 4).

Chapter 2:
Heitkamp F, Raupp J and Ludwig B (2009): Impact of fertilizer type and rate on carbon and nitrogen pools in a sandy Cambisol. Plant and Soil 319: 259-275.

Chapter 3:
Heitkamp F, Raupp J and Ludwig B: Soil organic matter pools and crop yields as affected by the rate of farmyard manure and use of biodynamic preparations in a sandy Cambisol. Journal of Sustainable Agriculture (submitted).

Chapter 4:

Heitkamp F, Raupp J and Ludwig B: Effects of fertiliser type and rate on soil fractions of a sandy Cambisol – long-term and short-term dynamics. Journal of Plant Nutrition and Soil Science (accepted).

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List of abbreviations

BD	Biodynamic
C_{mic}	Microbial biomass carbon
C_{org}	Soil organic carbon
DOM; DOC	Dissolved organic matter or carbon
DYN	Farmyard manure with use of biodynamic preaparations
DYN_H	Biodynamically prepared farmyard manure at high rate (140 - 150 kg N ha^{-1} year^{-1})
DYN_L	Biodynamically prepared farmyard manure at low rate (50 - 60 kg N ha^{-1} year^{-1})
DYN_M	Biodynamically prepared farmyard manure at medium rate (140 - 150 kg N ha^{-1} year^{-1})
HF	Heavy fraction ($\rho > 2.0$ g cm^{-3})
HFOC	Heavy fraction organic carbon
IBDF	Institut für Biodynamische Forschung (Institute for Biodynamic Research)
FYM	Farmyard manure
FYM_H	farmyard manure at high rate (140 - 150 kg N ha^{-1} year^{-1})
FYM_L	farmyard manure at low rate (50 - 60 kg N ha^{-1} year^{-1})
FYM_M	farmyard manure at medium rate (100 kg N ha^{-1} year^{-1})
LF	Light fraction ($\rho \leq 2.0$ g cm^{-3})
LFOC, LFON	Light fraction organic carbon or nitrogen
MSI	Straw incorporation plus mineral fertilization
MSI_H	Straw incorporation plus mineral fertilization at high rate (140 - 150 kg N ha^{-1} year^{-1})
MSI_L	Straw incorporation plus mineral fertilization at low rate (50 - 60 kg N ha^{-1} year^{-1})
MSI_M	Straw incorporation plus mineral fertilization at medium rate (100 kg N ha^{-1} year^{-1})
NHC	Non-hydrolysable carbon
N_{mic}	Microbial biomass nitrogen
N_t	Total nitrogen
OBS	Organische Bodensubstanz
OM	Organic matter
SOM	Soil organic matter
WFPS	Water filled pore space

Summary

Type and rate of fertilizers influence the level of soil organic carbon (C_{org}) and total nitrogen (N_t) markedly, but the effect on C and N partitioning into different pools is open to question. The objectives of the present work were to:

(i) quantify the impact of fertilizer type and rate on labile, intermediate and passive C and N pools by using a combination of biological, chemical and mathematical methods;

(ii) explain previously reported differences in the soil organic matter (SOM) levels between soils receiving farmyard manure with or without biodynamic preparations by using C_{org} time series and information on SOM partitioning; and

(iii) quantify the long-term and short-term dynamics of SOM in density fractions and microbial biomass as affected by fertilizer type and rate and determine the incorporation of crop residues into labile SOM fractions.

Samples were taken from a sandy Cambisol from the long-term fertilization trial in Darmstadt, Germany, founded in 1980. The nine treatments (four field replicates) were: straw incorporation plus application of mineral fertilizer (MSI) and application of rotted farmyard manure with (DYN) or without (FYM) addition of biodynamic preparations, each at high (140 – 150 kg N ha^{-1} $year^{-1}$; MSI_H, DYN_H, FYM_H), medium (100 kg N ha^{-1} $year^{-1}$; MSI_M, DYN_M, FYM_M) and low (50 – 60 kg N ha^{-1} $year^{-1}$; MSI_L, DYN_L, FYM_L) rates.

The main findings were:

(i) The stocks of C_{org} (t ha^{-1}) were affected by fertilizer type and rate and increased in the order MSI_L (23.6), MSI_M (23.7), MSI_H (24.2) < FYM_L (25.3) < FYM_M (28.1), FYM_H (28.1). Stocks of N_t were affected in the same way (C/N ratio: 11). Storage of C and N in the modelled labile pools (turnover times: 462 and 153 days for C and N, respectively) were not influenced by the type of fertilizer (FYM and MSI) but depended significantly ($p \leq 0.05$) on the application rate and ranged from 1.8 to 3.2 t C ha^{-1} (7 – 13% of C_{org}) and from 90 to 140 kg N ha^{-1} (4-5% of N_t). In the calculated intermediate pool (C/N ratio 7), stocks of C were markedly higher in FYM treatments (15-18 t ha^{-1}) compared to MSI treatments (12-14 t ha^{-1}). This showed that differences in SOM stocks in the sandy Cambisol induced by fertilizer rate may be

short-lived in case of changing management, but differences induced by fertilizer type may persist for decades.

(ii) Crop yields, estimated C inputs (1.5 t ha^{-1} $year^{-1}$) with crop residue, microbial biomass C (C_{mic}, 118 – 150 mg kg^{-1}), microbial biomass N (17 – 20 mg kg^{-1}) and labile C and N pools did not differ significantly between FYM and DYN treatments. However, labile C increased linearly with application rate ($R^2 = 0.53$) from 7 to 11% of C_{org}. This also applied for labile N (3.5 to 4.9% of N_t). The higher contents of C_{org} in DYN treatments existed since 1982, when the first sampling was conducted for all individual treatments. Contents of C_{org} between DYN and FYM treatments converged slightly since then. Furthermore, at least 30% of the difference in C_{org} was located in the passive pool where a treatment effect could be excluded. Therefore, the reported differences in C_{org} contents existed most likely since the beginning of the experiment and, as a single factor of biodynamic agriculture, application of biodynamic preparations had no effect on SOM stocks.

(iii) Stocks of SOM, light fraction organic C (LFOC, $\rho \leq 2.0$ g cm^{-3}), light fraction organic N and C_{mic} decreased in the order $FYM_H > FYM_L > MSI_H, MSI_L$ for all sampling dates in 2008 (March, May, September, December). However, statistical significance of treatment effects differed between the dates, probably due to differences in the spatial variation throughout the year. The high proportion of LFOC on total C_{org} stocks (45 – 55%) highlighted the importance of selective preservation of OM as a stabilization mechanism in this sandy Cambisol. The apparent turnover time of LFOC was between 21 and 32 years, which agreed very well with studies with substantially longer vegetation change compared to our study.

Overall, both approaches; (I) the combination of incubation, chemical fractionation and simple modelling and (II) the density fractionation; provided complementary information on the partitioning of SOM into pools of different stability. The density fractionation showed that differences in C_{org} stocks between FYM and MSI treatments were mainly located in the light fraction, i.e. induced by higher recalcitrance of the organic input in the FYM treatments. Moreover, the use of the combination of biological, chemical and mathematical methods indicated that effects of fertilizer rate on total C_{org} and N_t stocks may be short-lived, but that the effect of fertilizer type may persist for longer time spans in the sandy Cambisol.

Zusammenfassung

Düngerart und Düngermenge beeinflussen stark die Vorräte an organischem Kohlenstoff (C_{org}) und Gesamt-Stickstoff (N_t) im Boden, der Effekt auf die Partitionierung von C und N in Pools unterschiedlicher Stabilität ist allerdings noch nicht vollständig geklärt. Daher wurden in dieser Arbeit folgende Ziele verfolgt:

(i) die Quantifizierung des Einflusses von Düngertyp und Düngerrate auf labile, intermediäre und passive C und N Pools mit Hilfe der Kombination biologischer, chemischer und mathematischer Methoden;

(ii) die Erklärung von in früheren Arbeiten berichteten Unterschieden in der Speicherung organischer Bodensubstanz (OBS) in Böden die mit Stallmist entweder mit (DYN) oder ohne Zusatz (FYM) biodynamischer Präparate gedüngt wurden; und

(iii) die Quantifizierung von Langzeit- und Kurzzeit-Dynamik von OBS in Dichte-Fraktionen und mikrobieller Biomasse unter dem Einfluss von Düngerart und Düngerrate, sowie die Inkorporation von Ernteresten in labile OBS Fraktionen.

Die untersuchten Bodenproben (sandige Braunerde) stammten von dem Langzeit-Düngungsversuch in Darmstadt, Deutschland (1980 gegründet). Die neun untersuchten Varianten (je vier Feldwiederholungen) waren: Stroheinarbeitung und Applikation von Mineraldünger (MSI), sowie Ausbringung von gerottetem Stallmist mit (DYN), bzw. ohne Anwendung (FYM) biologisch-dynamischer Präparate, jeweils bei hoher (140 – 150 kg N ha^{-1} $Jahr^{-1}$; MSI_H, DYN_H, FYM_H), mittlerer (100 kg N ha^{-1} $Jahr^{-1}$; MSI_M, DYN_M, FYM_M) und niedriger (50 – 60 kg N ha^{-1} $Jahr^{-1}$; MSI_L, DYN_L, FYM_L) Düngungsrate.

Die wichtigsten Ergebnisse waren:

(i) Die Vorräte an C_{org} (t ha^{-1}) waren signifikant von Düngerart und Düngerrate beeinflußt und stiegen in der Reihenfolge MSI_L (23.6), MSI_M (23.7), MSI_H (24.2) < FYM_L (25.3) < FYM_M (28.1), FYM_H (28.1). Die Vorräte an N_t waren auf die gleiche Weise beeinflußt (C/N ratio: 11). Speicherung von C und N in den modellierten labilen Pools (mittlere Umsatzzeiten: 462 und 153 Tage für C und N) waren nicht von der Düngerart (FYM und MSI) beeinflusst, sondern hingen signifikant ($p \leq 0.05$) von der Düngerrate ab. Die Vorräte reichten von 1.8 bis 3.2 t C ha^{-1} (7 – 13% von C_{org}) und von 90 bis 140 kg N ha^{-1} (3.5 – 4.9% von N_t). Im berechneten intermediären Pool (C/N-Verhältnis: 7) waren die C-Vorräte in den Böden der FYM Varianten merklich

höher (15 – 18 t ha^{-1}) als in den Böden der MSI Varianten (12 – 14 t ha^{-1}). Dies zeigte, dass die durch die Düngerraten induzierten Unterschiede in den OBS Vorräten in der sandigen Braunerde im Falle einer Bewirtschaftsänderung keinen längerfristigen Effekt aufweisen. Im Gegensatz dazu können sich die durch die Düngerart hervorgerufenen Unterschiede langfristig auf die OBS-Vorräte im Boden auswirken.

(ii) Erträge, geschätzte C-Einträge durch Erntereste (1.5 t ha^{-1} $Jahr^{-1}$), mikrobiell gebundener C (C_{mic}, 118 – 150 mg kg^{-1}), mikrobiell gebundener N (17 – 20 mg kg^{-1}), sowie labile C und N Pools zeigten keine signifikanten Unterschiede zwischen FYM und DYN Varianten. Allerdings stiegen die Anteile an labilem C linear mit der Düngerrate ($R^2 = 0.53$) von 7 auf 11% von C_{org}. Dies galt ähnlich für die Anteile an labilem N (3.5 bis 4.9% von N_t). Die höheren Gehalte an C_{org} in den DYN Varianten existierten bereits 1982 zum Zeitpunkt der ersten nach Düngerart und Düngerrate getrennten Beprobung. Seitdem konvergierten die C_{org} Gehalte der DYN und FYM Behandlungen leicht. Weiterhin sind mindestens 30% des Unterschiedes im C_{org} Gehalt zwischen DYN und FYM Varianten im passiven Pool lokalisiert und damit nicht durch die Bewirtschaftung zu erklären. Daher ist davon auszugehen, dass die Unterschiede im C_{org} Gehalt von Anfang an existierten. Die Anwendung biodynamischer Präparate, als ein einzelnes Element der biodynamischen Landwirtschaft, hatte keine Erhöhung der OBS-Vorräte zur Folge.

(iii) Die Vorräte an OBS, organischem C in der leichten Fraktion (LFOC, $\rho \leq 2.0$ g cm^{-3}), organischem N in der leichten Fraktion und C_{mic} sanken in der Reihenfolge $FYM_H > FYM_L > MSI_H, MSI_L$ an allen Probenahmeterminen in 2008 (März, Mai, September, Dezember). Die statistische Signifikanz der Varianteneffekte war je nach Probenahme-Datum unterschiedlich. Ein Grund dafür war vermutlich die unterschiedlich hohe räumliche Variation der Meßgrößen an den verschiedenen Probenahme-Terminen. Der hohe Anteil von LFOC an den C_{org}-Vorräten (45 – 55%) zeigte die hohe Bedeutung von selektiver relativer Anreicherung der OBS als Stabilisierungsmechanismus in der sandigen Braunerde. Die scheinbare Umsatzzeit des LFOC lag bei 21 bis 32 Jahren. Dies stimmte sehr gut mit anderen Studien überein, die nach wesentlich längeren Vegetationswechseln durchgeführt wurden.

Insgesamt brachten die beiden angewendeten Verfahren zur Pool-Unterteilung; (I) Kombination von Inkubation, chemischer Fraktionierung und einfacher Modellierung, sowie (II)

Dichtefraktionierung; komplementäre Erkenntnisse über die Partitionierung von OBS in Pools unterschiedlicher Stabilität. Die Dichtefraktionierung zeigte, dass Unterschiede der C_{org} Vorräte der MSI und FYM Varianten hauptsächlich durch Speicherung von C in der leichten Fraktion erklärt werden konnten. Das heißt, die Unterschiede im C_{org} Vorrat wurden durch höhere Rekalzitranz des organischen Inputs in den FYM Varianten hervorgerufen. Weiterhin brachte die Anwendung biologischer, chemischer und mathematischer Methoden die Erkenntnis, dass Effekte der Rate auf die gesamten C_{org} und N_t Vorräte bei Änderung der Bewirtschaftung schnell nachlassen. Der Effekt der Düngerart hingegen kann über längere Zeitspannen in der sandigen Braunerde überdauern.

1 Introduction

The regulation of the budget of soil organic matter (SOM) and nutrients by management is a central focus in agriculture, especially in organic agriculture (Fließbach et al. 2007). The budgets of SOM and nutrients determine soil fertility, i.e. the long-term productivity of soils. Furthermore, soil can act as a source or sink for carbon, thus affecting the atmospheric concentration of greenhouse gases (Paustian et al. 2000; Sauerbeck 2001).

In agro-ecosystems, three management options, namely crop rotation, tillage and fertilization, mainly affect the levels of SOM stocks (Paustian et al. 2000). However, stocks of SOM react slowly to management changes, and reach steady state conditions only after several decades (West and Post 2002). Thus, long-term field experiments are of outstanding importance in order to evaluate management effects on SOM stocks properly (Steiner 1995).

The focus in this dissertation is on the effect of fertilizer type and rate on SOM stocks.The effects of FYM and mineral fertilizer on levels of total SOM have been reported from some long-term experiments (Weigel et al. 1998; Alvarez 2005; Sleutel et al. 2006; Khan et al. 2007). However, most agricultural long-term experiments across the world are located on loamy soils (Debreczeni and Körschens 2003) and little information is available on the effect of fertilization on sandy soils (Christensen 1996a). Moreover, the combined effect of fertilizer type and rate at standardised application levels of N has seldom been reported (Edmeades 2003) and information on partitioning of OM into pools of different stability, as affected by fertilizer type and rate, is required (Loveland and Webb 2003).

This dissertation is based on results from the long-term fertilization trial in Darmstadt, Germany. The experiment was founded in 1980 by the Institute of Biodynamic Research (IBDF). The soil unit is a Cambisol with sandy texture (86% sand, 9% silt, 5% clay). Crops are grown in a four-year rotation and managed according to the standards for organic agriculture. Nine treatments are implemented in the field experiment, the factors being type and rate of fertilizer. Two types of FYM, one with and one without use of biodynamic preparations were applied at high, medium and low rates. The third type of fertilizer, also applied at high, medium and low rates, is applied in mineral form, but includes the incorporation of cereal straw. Detailed descriptions are provided in chapters 2.2.1and 3.2.1.

The following chapters of this introduction will focus on topics which are not, or less, covered by the introductions of the different manuscripts (chapters 2.1, 3.1 and 4.1). Therefore, this introduction will mainly deal with background knowledge as well as with theoretical and methodological considerations on concepts of soil organic matter turnover and its measurability. The introductions of the manuscripts will focus on the state of knowledge specific to effects of fertilizer type and rate on soil organic matter storage and turnover.

1.1 Turnover of soil organic matter in ecosystems

Ecosystems function in different subsystems, with the fluxes of matter and energy determining the integrity of the ecosystem. The gain of nutrients and energy by annual net primary production of the plant subsystem may be distributed in three different ways (Swift et al. 1979):

(i) storage in plant biomass, which is mostly relevant in forest stands, where net growth occurres,

(ii) export by herbivory or human activity (agriculture and forestry) beyond ecosystem boundaries or

(iii) transfer to the decomposition subsystem.

In the decomposition subsystem two processes, which are mainly mediated by microbial activity, are of outstanding importance for the functioning of any ecosystem. On the one hand, mineralization of organic matter (OM) provides nutrients in a plant-available form. On the other hand, humification of OM provides the built-up of SOM (Swift et al. 1979; Stevenson 1994). Under steady state conditions, the amount of input to the soil equals the amount of output within a given time and the turnover of SOM can be expressed by assuming a simple first-order decay model (e.g. Stewart et al. 2007). Under otherwise equal conditions, the steady-state level of SOM generally increases linearly with the steady-state level of OM input. However, this is only valid under the assumption of an infinite capacity of soil to store OM. In reality, increasing OM inputs may result in low, curvilinear increases of SOM, when approximating against a maximum level (Stewart et al. 2007).

Carbon is the major constituent of SOM and therefore I will refer to soil organic carbon (C_{org}) for the sake of simplicity. In agro-ecosystems, storage of C_{org} can be influenced via management by affecting processes of input and output from soil. The major part of C enters the arable soil as crop residues and/or organic fertilizers. Losses of C_{org} occur due to decomposition and subsequent mineralization to CO_2 by heterotrophic organisms. Losses of dissolved organic matter are assumed to be minor in agricultural soils (Paustian et al. 1997). Turnover times of C_{org} in arable surface soils range from decades to few centuries, and may increase considerably with depth (Flessa et al. 2008). The amount of C input can be affected by cropping system (e.g. crop type, cropping frequency), fertilization (crop productivity), residue management and application of organic fertilizers. The effects on crop management on decomposition and C mineralization are more complex. Residue decomposition may be affected by crop type, residue size (incorporation technique) or nutrient content (fertilization). Soil temperature, moisture and aeration may be affected by crop type (shading, use of water) soil structure (tillage), mulching, irrigation or drainage. Nutrient availability may be managed by mineral fertilization, manure addition, liming or N-fixation by legumes. These are just examples, taken from Paustian et al. (1997). However, several processes interact with each other and the final outcome, i.e. the amount of C_{org} stored, is further affeccted by factors such as soil type, texture, mineralogy and climate. Moreover, not only the amount, but also the quality of OM input may influence the level of SOM (Persson and Kirchmann 1994). Furthermore, OM input may also be partitioned into pools of different stability (e.g. Parton et al. 1988).

1.2 Division of SOM into compartments

Soil organic matter is a heterogeneous mixture of compounds differing in their chemistry and turnover (Baldock and Skjemstad 2000; Helfrich et al. 2006). SOM is therefore assumed to be a continuum of organic molecules, each with a distinct turnover time (Baldock and Skjemstad 2000). However, as an abstraction and simplification, almost all recent models simulating SOM turnover divide SOM into a small number of compartments or pools (Smith et al. 1997). These pools are assumed to have a distinct composition and, most frequently, are supposed to decay by first order kinetics. The decay constant of each distinct pool varies with its stability and environmental conditions, such as temperature, moisture or soil texture. In the SOM submodel of CENTURY, for example, turnover times of C pools range from < 1 year to 1000 years (Parton et al. 1988). A disadvantage of most models is that most of the conceptual pools (except e.g. microbial biomass or litter input) are not directly measurable and are therefore not experimentally verifiable (Christensen

1996b). However, there is no consensus for an “ultimate” method (see also chapter 1.4) to determine the size and turnover of SOM pools (Elliott et al. 1996). Approaches using the isotopic signature of carbon (^{14}C, ^{13}C) are extremely useful in this context, but require conditions (e.g. C_3/C_4 vegetation change, backup samples from long-term field experiments), which are seldom achieved (Wang and Hsieh 2002). Notwithstanding these problems, there is consensus about the usefulness of compartment models to describe, predict and increase the understanding of SOM dynamics (Balesdent 1996; Smith et al. 1997). Nevertheless, in current quantitative models only few processes and mechanisms of SOM stabilization are considered (Ludwig et al. 2008). The current state of knowledge on processes and mechanisms affecting the size and turnover of SOM pools are summarized in a recent conceptual model by von Lützow et al. (2008).

1.3 Concepts of SOM stabilization

In their review, von Lützow et al. (2006) outlined three major mechanisms of SOM stabilization, namely (i) selective preservation due to different recalcitrance of compounds, (ii) spatial inaccessibility to decomposer organisms and (iii) interactions of SOM with soil minerals and metal ions. Selective preservation is defined as the relative accumulation of recalcitrant molecules (e.g. lignin, lipids) and is further divided into primary and secondary recalcitrance. Primary recalcitrance is a function of molecular characteristics inherent to unaltered OM, whereas secondary recalcitrance includes recalcitrance of microbial products, humic polymers and charred material. Spatial inaccessibility is caused by occlusion of SOM in aggregates, intercalation into phyllosilicates, hydrophobicity and encapsulation into organic macromolecules. Organo-mineral interactions include e.g. processes of ligand exchange, polyvalent cation bridges and complexation of SOM with metal ions (von Lützow et al. 2006).

The three major mechanisms of SOM stabilization outlined above contribute to different extents to the formation of three conceptual pools described by von Lützow et al (2008), namely (I) an active pool with turnover times of up to 10 years, (II) an intermediate pool with turnover times between 10 and 100 years and (III) a passive pool with slower turnover times (>100 years). The size and turnover of the active pool is determined by the amount and composition of plant, microbial and faunal biomass and their respective residues. Thus, selective preservation is of crucial importance for the formation of the active pool. Selective preservation is also highly relevant for stabilization of SOM in the intermediate pool, although the importance of secondary recalcitrance is increased compared to the active pool. Furthermore, spatial inaccessibility via biogenic aggregation and organo-mineral interactions contributes to stabilization of SOM within the intermediate pool. While stabilization mechanisms and the corresponding processes leading to the formation of active and intermediate pools are more or less well understood, there is still little empirical evidence for some mechanisms of stabilization in the passive pool. Important processes may be abiotic microaggragation, formation of hydrophobic surfaces, various forms of organo-mineral interactions as well as formation of pyrogenic C. The formation of the passive pool is regarded as the result of various processes, depending on specific pedogenetic conditions (von Lützow et al. 2008). Therefore, the passive pool is independent of recent management (Helfrich et al. 2006).

A major drawback of the conceptual model presented by von Lützow et al. (2008) is that pools, specific to the described mechanisms, are not yet quantifiable, especially when referring to the passive pool (Ludwig et al. 2008). Therefore, it remains a matter of ongoing research, whether the implementation of our current understanding of stabilization mechanisms into models will increase their ability to describe and predict SOM dynamics. However, the implementation of measurable soil fractions as model pools may improve our understanding of SOM dynamics (Christensen 1996b; Elliott et al. 1996).

1.4 Methods for determination of pools or fractions

As already mentioned above (chapter 1.2), there is still no “ultimate” method to determine conceptually defined pools. Nevertheless, different methods focus on some of the above mentioned processes and mechanisms of SOM stabilization. Generally, three different categories of methods for fractionation of total SOM exist: physical, chemical and biological methods (Khanna et al. 2001). In the following sections, the basic principles of some methods will be described briefly.

1.4.1 Physical fractions

Physical fractionation methods are based on the premise, that the structure of soil regulates the dynamics of SOM turnover (Christensen 2001). For primary particle size and density fractionations, the dispersion of the soil is a crucial step of the method (Christensen 1992). Ultrasonication and prolonged shaking, which are the most widely used methods, have both been criticised: the former due to possible redistribution of SOM among fractions

(Balesdent 1996) and the latter due to more incomplete dispersion, compared to sonication (Christensen 1992).

Aggregates are isolated from bulk soil mostly by slaking dried soil on sieves of different mesh sizes. Generally, aggregates have been classified as macro- (> 250 µm) and microaggregates (< 250 µm). In addition, subclasses, based on primary particle size classes, were also introduced (John et al. 2005). Using of the $\delta^{13}C$ signature after a C_3-C_4 vegetation change, several studies showed that OM in macroaggregates has a faster turnover (turnover time: 15-50 years) compared to microaggregates (turnover time: 100-300 years) (von Lützow et al. 2007). However, aggregates are not a homogenous fraction and can be further fractionated into free OM and mineral associated OM (Yamashita et al. 2006). Therefore, the mechanisms of selective preservation and spatial inaccessibility simultaneously affect the turnover of OM within aggregates (von Lützow et al. 2007). Comprehensive discussions on the issue of aggregate fractions can be found e.g. in Six et al. (2000; 2002).

Size fractionation of primary particles relies on the assumption that the spatial accessibility of OM to decomposers decreases with decreasing size of primary particles (sand > silt > clay). With decreasing size of particles, the specific surface area and the number of reactive sites increases, causing an increasing importance of organo-mineral complexes (von Lützow et al. 2007). In fact, 50-75% of C_{org} are generally found in the clay fraction of soils (Christensen 2001). Furthermore, Balesdent (1996) showed, that the turnover time of particle size fractions decreased with size from 0.5 years (> 2000 µm) to 63 years (< 50 µm). There is also a gradient in the chemical composition of OM, with least decomposed OM in the sand-sized fraction. Therefore, a combination of selective preservation and organo-mineral interactions determines the turnover times of size fractions (von Lützow et al. 2007). Comprehensive reviews on particle size fractionations can be found in Christensen (1992; 2001).

The concept of density fractionation is based on the assumption that SOM can be divided into a light fraction (LF), which consists of decomposing plant residues with a rapid turnover and a heavy fraction (HF), which consists of more decomposed OM associated with soil minerals with a slower turnover (Christensen 1992). Golchin et al. (1994) extended the method by a “free” light fraction and a light fraction occluded within aggregates, recognizing the importance of spatial inaccessibility of OM during decomposition. In general, the turnover of density fractions decreases in the order free LF > occluded LF >

HF (von Lützow et al. 2007). However, if the light fractions contain coal or soot, the apparent turnover of their OM is markedly slowed down (Flessa et al. 2008). Moreover, there is no consensus about which density is appropriate for a proper fractionation of SOM (Gregorich et al. 2006). Most often densities between 1.4 and 2.0 g cm^{-3} have been used (Gregorich et al. 2006), the "optimal" density perhaps depending on the specific soil in study (Marriott and Wander 2006). In the absence of highly recalcitrant material such as coal or soot, the fLF has been described as a good measure for the active pool (von Lützow et al. 2007). In contrast, the HF might be too heterogeneous in terms of turnover (Swanston et al. 2002) and further division, e.g. via chemical fractionation, might be useful (Helfrich et al. 2007).

1.4.2 Chemical fractions

A wide range of chemical fractionation procedures has been used, based on extractability by aqueous solvents, hydrolysability or oxidizability of SOM (von Lützow et al. 2007). Here I will refer to some of the most common used methods.

Extraction methods include the extraction of dissolved organic matter (DOM) as well as the classical fractionation into humin, fulcic acid and humic acid. Compared to forest soils, the quantitative importance of DOM in arable ecosystems is low (Chantigny 2003). Furthermore, DOM is not homogenous in terms of turnover times (Kalbitz et al. 2003) and represent a mixture of "old" and "new" C (Flessa et al. 2000). Therefore, albeit important in transport processes and pedogenesis (Zsolnay 1996), it is difficult to use DOM as a functional fraction (von Lützow et al. 2007). For the classical fractionation into humin and acids, Balesdent (1996) reported a similar distribution of "new C" in all fractions. Therefore, he concluded that humin, fulvic and humic acids were not useful as functional fractions in studies on SOM turnover.

Acid hydrolysis with hot 6 M HCl has been widely used to fractionate SOM into a labile, hydrolysable C fraction and a stable, non-hydrolysable C fraction (NHC). The NHC is much older (up to 1200 years in surface soils) than bulk C_{org}, as shown by ^{14}C analysis (Paul et al. 2006) and therefore NHC represent a stable pool of SOM. However, NHC can rapidly react to changes in landuse (Paul et al. 2006) and represents a relatively constant proportion of C_{org} (Helfrich et al. 2007). Therefore, application to bulk C_{org} cannot be recommended (Balesdent 1996).

Two oxidative treatments were most successful in isolating a stable pool of C_{org}. Namely, the methods were fractionation with hydrogen peroxide (H_2O_2) and disodium peroxodisulfate ($Na_2S_2O_8$) (Helfrich et al. 2007). The apparent ^{14}C ages of the residues were around 10000 years and "new C" was very effectively removed. However, Mikutta et al. (2005) reported that H_2O_2 may dissolve some soil minerals. Furthermore, the separation of LF material was necessary before successfully applying oxidation with H_2O_2 to soils (von Lützow et al. 2007). Oxidation with persulfate did not result in dissolution of soil minerals. The residue after oxidative treatment represent SOM preserved via organo-mineral associations, as well as some hydrophobic and highly recalcitrant organic compounds (Cuypers et al. 2002; Kögel-Knabner et al. 2008). A disadvantage of $Na_2S_2O_8$ oxidation may probably be an interference of the $NaHCO_3$ buffer with ^{14}C analysis (von Lützow et al. 2007). However, after removing the buffer by 0.01 M HCl, there was no evidence for interference, as shown by an unbiased $\delta^{13}C$ signal (Helfrich et al. 2007). Overall, both methods seem to be suitable for isolating a very old, thus passive, pool of SOM.

1.4.3 Biological and kinetically defined fractions

The size of biological fractions, i.e. microbial biomass and SOM mineralization, is determined by the integration of environmental effects, including climate, pH, quality of organic input or SOM, microbial community and the combination of specific stabilization mechanisms active in soil (Baldock and Skjemstad 2000). Therefore, specific mechanisms cannot directly be determined with biological fractions. However, for practical issues the integrative result may be of high interest (Paul et al. 2006).

Most commonly, the contents of C (Vance et al. 1987) and N (Brookes et al. 1985) in microbial biomass are determined by chloroform fumigation extraction. The method is based on the different extractability of C or N of fumigated and non-fumugated samples. During fumigation of soil with chloroform ($CHCl_3$), a part of the microbial biomass is killed and the cells lysed, increasing the extractable C and N in the sample. The difference between fumigated and non-fumigated samples is regarded as derived from microbial biomass. However, not all microbes are killed by fumigation and correction factors for calculation of microbial biomass C (C_{mic}) and N (N_{mic}) are used (Brookes et al. 1985; Vance et al. 1987). Although usually accounting for only 1-3% of bulk C_{org} in arable soils (Anderson and Domsch 1989), the microbial biomass reacts very early and sensitively to management (Powlson et al. 1987). The major part of decomposition and mineralization of

OM is mediated by microbes. Therefore, the microbial biomass is an important fraction of the active SOM (Khanna et al. 2001).

Aerobic long-term incubation studies, based on the study of Stanford and Smith (1972), have been used to determine the microbial respiration and net-N mineralization rates of soil samples. During the incubation microorganisms produce enzymes, which fractionate SOM while implicitly integrating the effects of substrate availability and physical protection by the soil matrix (Paul et al. 2006). The amount of used substrate can be measured as CO_2 or mineral N. However, during the decomposition of organic N considerable amounts may be fixed in the microbial biomass, especially under conditions of low N availability (Benbi and Richter 2002). Mineralization strongly depends on temperature, moisture and soil texture (Wang et al. 2004). While moisture is often held around the optimum of 50% water filled pore space (Baldock and Skjemstad 2000), there is still no consensus on the choice of incubation temperature and duration.

A mathematical description of mineralization data can be obtained by using simple models of, mostly, first-order kinetics (Equation 4) with one or two pools (Benbi and Richter 2002; Paul et al. 2006). This method has been criticized because the parameters of the equation may be interdependent (Sierra 1990). Nevertheless, constraining the model by either fixing the decay constants (Mallory and Griffin 2007) or experimental determination of one pool size (Paul et al. 2006) resulted in reasonable descriptions of C and N mineralization. A crucial advantage of this method is that an estimate for turnover times of active SOM is obtained without using isotopic markers.

1.5 Objectives

Summarising the findings above, there exists no single method for experimental verification of conceptual pools with homogenous composition and turnover. However, some authors in recent reviews suggest that combinations of different methods may be most successful in isolating meaningful functional pools of SOM (Gregorich et al. 2006; Paul et al. 2006; von Lützow et al. 2007). Furthermore, there is a lack of knowledge on the combined effect of fertilizer type and rate, especially in sandy soils at standardized application rates and under organic management conditions (Edmeades 2003). To the best of my knowledge, no study has ever investigated the effect of fertilizer type and rate on partitioning of C and N into pools of different stability under the previously stated conditions. A more comprehensive overview of the state of knowledge leading to objective 1 can be found in chapter 2.1.

<u>Objective 1:</u> **Quantification of the impact of fertilizer type and rate on labile, intermediate and passive C and N pools in a sandy Cambisol at Darmstadt by using a combination of biological, chemical and mathematical methods (chapter 2).**

Biodynamic agriculture is a special form of organic agriculture (Koepf et al. 1996). The biodynamic management (as a whole-farm approach) may lead to increased stocks of C_{org} and higher microbial activity in the soil (Fließbach et al. 2007). However, the usefulness of biodynamic preparations, in terms of C and N sequestration in the soil, is still subject to debate (Turinek et al. 2009). In the Darmstadt experiment, higher levels of C_{org} has been reported in treatments receiving biodynamic preparations (Raupp and Oltmanns 2006). However, initial conditions of the field experiment were not reported properly, and therefore, the higher stocks found under treatments receiving biodynamic preparations may already have existed before the start of the field experiment (Bachinger 1996). A more comprehensive overview of the state of knowledge leading to objective 2 can be found in chapter 3.1.

<u>Objective 2:</u> **Explanation of previously reported differences in the soil organic matter levels between soils receiving farmyard manure with or without biodynamic preparations by using C_{org} time series and information on SOM partitioning (chapter 3).**

As stated in the above (chapter 1.4.1), the LF is regarded to be a good measure of active SOM. It reacts more rapidly and sensitively to treatment effects than total SOM and thus, LF has been suggested being a good indicator for management effects, such as fertilizer type and rate, on SOM storage (Gregorich et al. 2006). Furthermore, the LF is formed by one specific process, namely selective preservation (von Lützow et al. 2007) However, properties which are sensitive to management may also be susceptible to environmental conditions (Campbell et al. 1999), which potentially may alter treatment effects. A more comprehensive state of knowledge leading to objective 3 can be found in chapter 4.1.

Objective 3: Quantification of the long-term and short-term dynamics of SOM fractions as affected by fertilizer type and rate and determination of the incorporation of crop residues into labile SOM fractions (chapter 4).

Overall, the aim of the dissertation was to deepen the understanding of the effect of fertilizer type and rate on SOM dynamics in an organically managed crop rotation on a sandy Cambisol.

2 Impact of fertilizer type and rate on carbon and nitrogen pools in a sandy Cambisol

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Abstract

Type and rate of fertilizers influence the level of soil organic carbon (C_{org}) and total nitrogen (N_t) markedly, but the effect on partitioning of C and N into different pools is open to question. Objectives were to investigate the impact of fertilizer type and rate on labile, intermediate and passive C and N pools in a sandy Cambisol at Darmstadt, Germany, after 27 years of different fertilization treatments. The six treatments were: straw incorporation plus application of mineral fertilizer (MSI) and application of farmyard manure (FYM) each at high (140 – 150 kg N ha^{-1} year^{-1}), medium (100 kg N ha^{-1} year^{-1}) and low (50 – 60 kg N ha^{-1} year^{-1}) rates. Soil microbial biomass C (C_{mic}) and N (N_{mic}) and C and net N mineralization (266 days incubation at 10°C and 50% waterfilled pore space) were determined. Soils (0 – 25 cm) of MSI treatments had significantly ($p \leq 0.05$) lower C_{mic} stocks (308 – 361 kg ha^{-1}) than soils of FYM treatments (404 – 520 kg ha^{-1}). Differences in N_{mic} stocks were less pronounced. After 266 days, mineralized C (1130 – 1820 kg ha^{-1}) and N (90 – 125 kg ha^{-1}) had significantly increased with fertilizer rate. The application of an exponential two-pool model showed that very labile pools (turnover times: 17 and 9 days for C and N, respectively) were small (1.3 – 1.8% of C_{org} and 0.5 – 1.0% of N_t) and not influenced by type or rate of fertilizer. Stocks of the modeled labile C and N pools (turnover times: 462 and 153 days for C and N, respectively) were not influenced by the type of fertilizer but depended significantly on the application rate and ranged from 7 to 13% of C_{org} and from 4 to 5% of N_t. In contrast, the size of the calculated intermediate C pool was greater for the FYM treatments, and depended significantly on the interaction of fertilizer type and rate. The intermediate N pool was unaffected by fertilizer type or rate. Passive C and N pools, as experimentally revealed by oxidation with disodium

peroxodisulfate ($Na_2S_2O_8$), were independent of the treatments. Overall, labile and intermediate pools were affected differently by the fertilizer type and the application rate.

2.1 Introduction

Knowledge about partitioning of soil organic matter (SOM) into pools of different stability is important for understanding dynamics of organic carbon (C_{org}) and total nitrogen (N_t) in the soil. Information on labile SOM pools will help optimizing fertilizer doses (Beraud et al. 2005). It is also crucial for modeling purposes (Fang et al. 2005) and to predict the sensitivity of SOM to global warming (Knorr et al. 2005). Although SOM has long been recognized as a key attribute of fertility in agro-ecosystems (Manlay et al. 2007), there is a gap of knowledge in how the type and rate of fertilizers affect the partitioning of C_{org} and N_t into different pools in the soil.

It is now well established that the type of fertilizer influences the level of SOM markedly. Mineral fertilizers stimulate plant growth, thus increasing the quantity of organic matter input to the soil (Paustian et al. 1997). In addition to stimulated plant growth, manured soils receive large quantities of organic matter and therefore have higher contents of SOM, regardless of soil type and texture (Weigel et al. 1998; Loveland and Webb 2003). However, less information is available for the effect of fertilizer type and rate on labile pools of SOM (Loveland and Webb 2003).

The microbial biomass is assumed to be a labile pool of nutrients (Khanna et al. 2001). For a wide range of soil types and textures, there is evidence that soils receiving farmyard manure (FYM) contain a higher microbial biomass than mineral fertilized soils (Witter et al. 1993; Weigel et al. 1998). Moreover, increasing additions of FYM (10 - 60 t ha^{-1} $year^{-1}$) led to increasing contents of microbial biomass carbon (C_{mic}) in soils of varying type and texture (Weigel et al. 1998). The effect of increasing additions of mineral fertilizer on microbial biomass stocks is variable: increases, but also no effects and decreases have been reported (Dick 1992; Vanotti et al. 1997; Jacinthe et al. 2002). In arable soils C_{mic} averages 2 – 3% of total C_{org} (Anderson and Domsch 1989), while the contribution of C_{mic} to potentially mineralizable C comprises between 4 and 40% (Khanna et al. 2001). Thus, a considerable part of labile SOM is derived from organic forms other than microbial biomass.

Laboratory long-term incubation experiments provide a reliable measurement of labile SOM. Incubation approaches are sensitive to management and, unlike in physical fractionation methods, charcoal does not interfere with the measurements (Khanna et al. 2001). Compared to mineral fertilization, several loamy soils showed a 70 to 100% higher net N mineralization when receiving the same amount of N with FYM (Bonde et al. 1988; Mallory and Griffin 2007). Several studies showed that net N mineralization increased with the application rates of either mineral fertilizer (El-Haris et al. 1983; Vanotti et al. 1997) or manure (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007). The combined effect of fertilizer type and rate was investigated only for one soil type: Habteselassie et al. (2006) reported for a coarse-silty, carbonatic Haploxeroll that C and net N mineralization were higher in the FYM treatments, but, contradictory to the results reported above, effects of application rates (100 and 200 kg N ha^{-1} year^{-1}) were insignificant.

Fitting simple models on the mineralization data, incubation studies also provide information on pool sizes of labile substrates and their turnover (Stanford and Smith 1972; Khanna et al. 2001; Benbi and Richter 2002). Reported turnover times of the labile pools were mostly between 180 and 320 days (Mikha et al. 2006; Mallory and Griffin 2007), but also substantially faster turnover (between 10 – 15 days) has been reported (El-Haris et al. 1983; Habteselassie et al. 2006).

However, although of major importance for nutrition of plants and microorganisms, the labile pool only comprises about 10% of total C_{org} (Khanna et al. 2001) and the non-labile pools have not always received sufficient consideration. As proposed by a process-oriented conceptual model, organic matter can be classified into a labile (turnover time: < 10 years), an intermediate (turnover time: 10 - 100 years) and a passive (turnover time: > 100 years) pool (von Lützow et al. 2008). Different mechanisms are responsible for stabilization: selective preservation due to chemical recalcitrance (labile pool), recalcitrance of strongly decomposed organic matter, aggregation and complexation of SOM with metal ions (intermediate pool) and organo-mineral interactions, hydrophobicity and formation of charred material (passive pool).

Summarizing the findings above, the literature reveals a gap in knowledge concerning the combined effect of type and rate of fertilizer on partitioning of C and N into pools of different stability in the soil. Our approach combines a medium-term field study (duration of 27 years) with biological and chemical fractionation methods (Figure 1). This aims to help fill this gap and thus contribute to a better understanding of SOM dynamics and soil fertility in agro-ecosystems. Objectives were to investigate the combined effect of fertilizer type and rate on labile, intermediate and passive C and N pools in a sandy Cambisol of the Darmstadt fertilization experiment.

2.2 Materials and methods

2.2.1 Study site

The experimental trial is situated near Darmstadt, Germany (49° 50’ N, 8° 34’ E), with an elevation of 100 m a.s.l. The mean temperature is 9.5°C and the mean annual precipitation is 590 mm. The soil is a Haplic Cambisol (WRB 2006) which developed on alluvial fine sands of the river Neckar (Bachinger 1996). For soil properties see Table 1.

Information from historical maps indicates that the experimental site has been converted from a mixed forest stand (presumably oak-pine forest which is the actual surrounding forest vegetation) to arable land between 1904 and 1920. Before the trial started, the site was managed as a biodynamic farm for at least 20 years (Abele 1987). In 1980, nine treatments (four replicates each) were arranged in a strip design, with the factors being type of fertilizer and its application rate. Besides fertilization, all management actions were the same. The crop rotation consisted of legumes (mainly red clover, *Trifolium pratense* L. or lucerne, *Medicago sativa* L.), spring wheat (*Triticum aestivum* L.), root crops (mainly potatoes, *Solanum tuberosum* L.) and winter rye (*Secale cereale* L.). Residues of potatoes were incorporated into the soil. Mean yields from 2003 to 2006 are provided in Table 2.

Table 1: Characteristics of the soils (0 – 25 cm) of the different fertilization treatments at the Darmstadt field trial in spring 2007 and C_{org} and N_t stocks in autumn 1982. Mean values and standard errors in parentheses (n = 4). Different letters indicate significant differences between individual treatments ($p \leq 0.05$)

Treatment[a]	pH	Bulk density (g cm^{-3})	C_{org} (t ha^{-1})	N_t (t ha^{-1})	C_{org}, 1982[b] (t ha^{-1})	N_t, 1982[b] (t ha^{-1})	Clay (%)	Silt (%)	Sand (%)
MSI$_H$	6.0 (0.4)	1.35 (0.03)	24.2 (0.5)a	2.3 (0.1)a	30.8	2.6	4.6 (0.3)	9.0 (0.5)	86.5 (0.3)
MSI$_M$	6.1 (0.3)	1.37 (0.04)	23.7 (0.9)a	2.2 (0.1)a	29.1	2.7	4.9 (0.3)	9.2 (0.4)	85.9 (0.5)
MSI$_L$	6.0 (0.3)	1.41 (0.02)	23.6 (0.8)a	2.2 (0.1)a	29.8	2.7	4.7 (0.2)	10.5 (0.9)	84.8 (0.9)
FYM$_H$	6.3 (0.2)	1.38 (0.03)	28.1 (0.9)b	2.8 (0.1)b	30.5	2.8	4.9 (0.4)	8.9 (0.3)	86.2 (0.3)
FYM$_M$	6.3 (0.2)	1.38 (0.01)	28.1 (1.0)b	2.7 (0.1)b	34.3	2.7	5.8 (0.1)	8.5(0.2)	85.7 (0.4)
FYM$_L$	6.4 (0.3)	1.35 (0.01)	25.3 (1.2)a	2.5 (0.1)c	29.8	2.9	5.6 (0.2)	9.0 (0.4)	85.4 (0.5)
Source of variation[c]	Type	ns	Type × rate	Type × rate	nd	nd	ns	ns	ns

[a] FYM$_H$, FYM$_M$, FYM$_L$: farmyard manure application at high, medium and low rate; MSI$_H$, MSI$_M$, MSI$_L$: straw incorporation plus mineral fertilizer application at high, medium and low rate; different letters indicate significant differences between means of treatments

[b] measured C_{org} or N_t stocks from the inventory in autumn 1982, the first date when all treatments were sampled

[c] significant effects at $p \leq 0.05$; ns: no significant effects; nd: not determined because of lack of data

As winter cover crop cultivated radish (*Raphanus sativus spp. oleiformis* L.) or white mustard (*Sinapis alba* L.) were sown. The choice of the cover crop did not depend on the adjacent or preceding main crop. From 1981 to 1984 fertilizer application was done with the aim of obtaining equal yields for the respective FYM and mineral fertilizer treatments discussed below. Six treatments were considered here. They have been treated since 1985 as follows:

(i) MSI_H: high application rate of mineral fertilizer (150 kg N ha^{-1} to root crops or 100 kg N ha^{-1} plus 40 kg N ha^{-1} as second application to cereals) plus straw incorporation (Figure 2).

(ii) MSI_M: medium application rate of mineral fertilizer (100 kg N ha^{-1} to root crops or 80 kg N ha^{-1} plus 20 kg N ha^{-1} as second application to cereals) plus straw incorporation.

(iii) MSI_L: low application rate of mineral fertilizer (50 kg N ha^{-1} to root crops or 60 kg N ha^{-1} to cereals) plus straw incorporation.

(iv) FYM_H: high application rate of rotted farmyard manure: 27 t fresh weight ha^{-1} as manure to root crops or 16 t fresh weight ha^{-1} plus 40 kg N ha^{-1} with urine (second application) to cereals. The total N input corresponded to the treatment MSI_H.

(v) FYM_M: medium application rate of rotted farmyard manure: 18 t fresh weight ha^{-1} as manure to root crops or 12 t fresh weight ha^{-1} plus 20 kg N ha^{-1} with urine (second application) to cereals. The total N input corresponded to the treatment MSI_M.

(vi) FYM_L: low application rate of rotted farmyard manure: 9 t fresh weight ha^{-1} as manure to root crops or cereals. The total N input corresponded to the treatment MSI_L.

The mineral fertilizer consists of calcium ammonium nitrate, super phosphate and potassium magnesia. Farmyard cattle manure stem from deep litter housing and therefore contained considerable amounts of straw. It is left to rot for three (winter rye) or six months (spring wheat and root crops) before application. When legumes were planted, no fertilizers were applied. Cumulative fertilizer N inputs since 1980 were: 2890, 2000 and 1110 kg N ha^{-1} for the treatments MSI_H, MSI_M and MSI_L and 3055, 2110 and 1165 kg N ha^{-1} for FYM_H, FYM_M and FYM_L treatments, respectively. The differences in N input between the respective MSI and FYM treatments are due to the different fertilization concept between 1980 and 1984. Previous results of the field experiment were given by Raupp and Oltmanns (2006).

The crop in 2006 was berseem clover (*Trifolium alexandrinum* L.) and the cover crop in winter was cultivated radish. In the FYM treatments, the last application of manure was in September 2004 and urine was applied in April 2005. In the MSI treatments, mineral fertilizer was applied in April and May (second application) 2005. After harvest in July 2005, rye straw was incorporated into the soil of the MSI plots.

At the beginning of the experiment in 1980, one bulked soil sample was taken from the experimental area (C_{org}: 1.09%, N_t: 0.08%). However, from 1982 onwards, results are present for all treatments (only means without standard errors until 1989, Table 1, Figure 3).

2.2.2 C and N input

The C and N input of the manure was calculated from annual measurements of dry matter, ash and N content. The mean values and standard errors were 25.5 ± 2.0% ash, 35.4 ± 1.6% dry matter and 0.67 ± 0.04% N in fresh matter. We assumed 52% of ash free matter as C (Larney et al. 2005). For the wheat and rye straw input yields of dry matter were determined and we assumed a C content of 45%.

A major limitation of most field experiments is the lack of input data (Steiner 1995). The Darmstadt experiment provides good measurements for the inputs mentioned above, but quantification of inputs from crop residues and roots are lacking. Therefore they were estimated as a linear regression of the measured yields (Table 2) with the amount of crop residues and roots for each year and crop as included in the CANDY model and described by Franko (1997):

$$C_{input} = K + yield \times F \tag{1}$$

where C_{input} is the C in the residues of the crop and roots retained in the soil (dt C ha^{-1}), *yield* refers to the amount (dt ha^{-1}) of the harvested main product of potatoes (fresh matter), grains (including 14 % water content) or clover (dry matter, 105°C). *K* (intercept) and *F* (slope) are crop specific constants (Franko 1997). The values for *K* (dt C ha^{-1}) are 23.9 (clover), 3.1 (spring wheat), 0.8 (potatoes) and 4.0 (winter rye) The values for *F* are 0.014 (clover), 0.078 (spring wheat) and 0.080 (winter rye) dt C (dt dry matter $yield)^{-1}$ or 0.016 (potatoes) dt C (dt fresh matter $yield)^{-1}$. We made a rough estimate of the C input of rhizodeposition by multiplying the amount of C input with 1.5 (cereals and clover) or 1.35 (potatoes) as outlined by Ludwig et al. (2007) and Y. Kuzyakov (personal communication).

For the N input we considered N-fertilization and symbiontic N_2-fixation. We estimated N input via fixation as a function of the yield according to Bachinger and Zander (2004):

$$Nfix = Pfix \times yield \times (Nyield + Ryr \times Nres) \times Nred \tag{2}$$

where N_{fix} is the amount of fixed N (kg N ha^{-1} $year^{-1}$), P_{fix} is the crop specific portion of N in the plant derived from fixation, *yield* refers to the yield of dry matter from three cuts (dt ha^{-1} $year^{-1}$), N_{yield} is the N content of the yield (% N), R_{yr} is the ratio of yield and residues plus roots, N_{res} is the N content of residues and roots (% N) and N_{red} is a reduction factor depending on the N content of the residues from the previous crop. We set P_{fix} to 0.8 (Russelle and Birr 2004), N_{res} to 1.6 and N_{red} to 0.85 for rye as the preceding crop (Bachinger and Zander 2004). N_{yield} was measured (2.0 to 2.3%) and R_{yr} was calculated as:

$$Ryr = \frac{yield \times 0.45}{Cinput} \tag{3}$$

where *yield* is the dry matter yield (dt ha^{-1}), 0.45 is the relative C content of the yield and C_{input} is the estimation for C input (dt ha^{-1}) of clover as revealed by equation (1).

2.2.3 Sampling and soil characterization

Field sampling was conducted in February 2007 before fertilizer application and sowing. For every fertilizer type and application rate (four replicates each) 25 soil samples were taken with a core sampler (Ø 4 cm) from the plow layer (25 cm). The samples were combined and mixed thoroughly. Soil samples for the time course were taken from the plow layer every year in autumn after harvest with four repetitions per plot. The soil samples were sieved (≤ 2 mm) and any recognizable plant material was removed. Soil samples were dried at 40°C except for those samples used for the analysis of microbial biomass and the mineralization experiment. Subsamples of dry soil (40°C) were furthermore dried at 105°C for determination of residual moisture. Results are expressed on an oven-dry base (105°C). Bulk density was determined gravimetrically with cylinders. The pH (soil-solution ratio 2.5 ml g^{-1}) was determined in 0.01 m $CaCl_2$ solution. Organic carbon and N_t were measured by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany). In three samples, containing carbonate, $CaCO_3$ was destroyed by pre-treatment with HCl (10%). Texture was determined by sieving and sedimentation according to DIN ISO 11277 (2002).

Table 2: Average yields of main products and cereal straw of the crops grown in the Darmstadt field trial from 2003 (spring wheat), 2004 (potatoes), 2005 (winter rye) and 2006 (berseem clover). Means and standard errors in parentheses (n = 4). Different letters indicate significant differences between means of individual treatments ($p \leq 0.05$)

Treatment[a]	Spring wheat grain	Spring wheat straw	Potatoes (t ha^{-1})[b]	Winter rye grain	Winter rye straw	Berseem clover
MSI_H	4.1 (0.2)	4.4 (0.2)	26.1 (0.9)a	3.9 (0.2)	6.4 (0.2)	3.1 (0.3)ab
MSI_M	4.1 (0.2)	4.2 (0.1)	25.8 (0.5)a	4.0 (0.2)	5.8 (0.2)	3.0 (0.2)ab
MSI_L	3.8 (0.1)	3.7 (0.1)	19.1 (0.9)bc	3.8 (0.3)	5.3 (0.1)	2.8 (0.3)a
FYM_H	3.4 (0.2)	3.3 (0.0)	21.3 (1.0)b	4.1 (0.2)	5.5 (0.1)	5.1 (0.2)c
FYM_M	3.2 (0.1)	2.8 (0.0)	19.7 (1.6)bc	3.7 (0.2)	5.2 (0.2)	4.6 (0.0)c
FYM_L	2.8 (0.2)	2.4 (0.1)	17.8 (0.3)c	3.3 (0.4)	4.3 (0.5)	3.3 (0.2)b
Source of variation[c]	Type	Type, rate	Type, rate, type × rate	ns	Type, rate	Type, rate, type × rate

[a] For treatment abbreviations see Table 1

[b] Amounts are given in dry matter for straw and clover hay, in fresh matter for potatoes and include 14% water content for grain

[c] significant effects at $p \leq 0.05$; ns: no significant effects

2.2.4 Microbial biomass

Microbial biomass nitrogen (N_{mic}) and carbon were analyzed by chloroform fumigation extraction (Vance et al. 1987). Soils were preincubated for 10 days at 50% waterfilled pore space (WFPS, equals 17% gravimetric water content) at 20°C. Two portions of soil (10 g) were taken from the sample. One portion was fumigated with $CHCl_3$ for 24 h at 25 °C. Both subsamples were extracted with 0.5 m K_2SO_4. The extracts were analyzed for organic carbon by infrared detection of CO_2 (Dimatoc, Dimatec, Essen, Germany) and for total N by chemoluminescence detection (DimaN, Dimatec, Essen, Germany) after combustion at 850°C. Microbial biomass was calculated as the difference between fumigated and unfumigated samples with conversion factors of 0.45 and 0.54 for C_{mic} and N_{mic}, respectively (Brookes et al. 1985; Wu et al. 1990).

2.2.5 Mineralization experiment

Triplicates of (60 g dry matter equivalent) fresh, sieved soil samples were filled into plastic syringes with a volume of 110 ml. In the columns the samples had a bulk density of 1.4 g cm^{-3}. The soil samples were brought to 50% WFPS, then covered with punctured PE-foil to prevent loss of water and ensure gas exchange. Incubation temperature was 10°C which was nearly the annual mean temperature (9.5°C). After one week of pre-incubation, measurement was conducted at different intervals. Carbon and nitrogen mineralization were measured in the same samples. Böttcher (2004) outlined that the length of incubation is essential for parameter estimations (see below). He suggested continuing incubation until 50 - 80% of t_{90} is reached, where t_{90} is 90% of the time which will be needed to reach the amount of maximal mineralizable substrate. Shorter or longer duration times will increase uncertainties in parameter estimation. After 266 days 74% of t_{90} were reached for net N mineralization. Since respiration was also very low at this point, we decided to terminate the incubation, although for C mineralization only 25% of t_{90} were reached.

Respiration was measured directly via CO_2 evolution in the headspace of the syringes with a photoaccustic measurement device (INNOVA 1312 AirTech Instruments, LumaSense Technologies AS, Ballerup, Denmark). Prior tests assured the 1:1 comparability with a gas chromatograph ($R^2 = 0.96$). The respiration rate was calculated by linear regression of 5 to 8 measurement points, depending on the respiration rate and time of gas enrichment in the headspace. Measurement was conducted weekly until day 35, thereafter biweekly until day 91 and then on day 119, 147 and 266.

Measurement of net N mineralization was conducted by leaching according to Stanford and Smith (1972). Briefly, the volume of leaching solution (10 mm $CaCl_2$) was 800 ml, corresponding to a soil:solution ratio of 13 ml g^{-1}. As the soil had high sand content (Table 1), aeration was ensured. Thus, we did not add sand as proposed by Stanford and Smith (1972). After leaching, the extracts were filtered (0.45 µm) and frozen. NO_3^- and NH_4^+ concentrations in the extracts were measured with a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Cergy-Pontoise, France). Before application of suction to recover the columns to 50% WFPS, N-free nutrient solution (25 ml) was added (Stanford and Smith 1972). After one week of pre-incubation, the columns were leached to remove all mineral N before the incubation period. Measurement was conducted weekly until day 35, thereafter on day 63, 91, 119 and 266. The greater intervals in comparison to CO_2 evolution were chosen to provide a sufficient N concentration for measurement in the leachate.

Parfitt et al. (2001) showed that C mineralization can be underestimated in some soils, when incubated for longer times without leaching. However, to account for losses of soluble compounds which could contribute to C mineralization, we measured the amount of dissolved organic carbon (DOC) in the leachates.

2.2.6 Chemical fractionation

Several chemical fractionation methods exist to isolate SOM fractions with slow turnover. In a systematic comparison, treatment with hydrogen peroxide (H_2O_2) or sodium peroxodisulfate ($Na_2S_2O_8$) were most effective in isolating SOM with low apparent turnover times (Helfrich et al. 2007). Since H_2O_2 can disintegrate some minerals (Mikutta et al. 2005), $Na_2S_2O_8$ seems to be the most effective method for isolating mineral protected SOM. Analysis of persulfate treated samples showed that the residual was enriched in long-chain aliphatics (hydrophobic), highly condensed lignin polymers and pyrogenic (“black C”) material (Cuypers et al. 2002). Therefore, the fraction isolated with $Na_2S_2O_8$, represents mineral protected and highly biochemically recalcitrant SOM and has been suggested as a good measure for the passive pool (Helfrich et al. 2007).

To estimate the size of the passive C and N pools ($C_{passive}$, $N_{passive}$) we carried out an oxidation with $Na_2S_2O_8$ (Helfrich et al. 2007). Half a gram of soil was dispersed in 250 ml distilled water by ultrasound (440 J ml^{-1}). Twenty gram of $Na_2S_2O_8$ and 22 g $NaHCO_3$ were added. The samples were heated to 80°C on a magnetic stirrer. To avoid C contamination, glass coated stir-bars were used. After 48 hours the sample was washed twice with 40 ml water. Remaining carbonate traces were removed by adding 40 ml 5 mm HCl. Then the residual sample was washed three times until neutral pH, freeze-dried and analyzed for the C and N content as described above. The content of C and N in the passive pool was calculated on base of the initial weight.

2.2.7 Parameter estimation

A two-pool model with first-order kinetics has been found to provide the best description of C and N mineralization in laboratory experiments with constant temperature and moisture conditions (Benbi and Richter 2002; Wang et al. 2004):

$$Y_{m}(t) = \sum_{i=1}^{2} Y_i \times (1 - \exp(-k_i \times t)) \quad (4)$$

where $Y_m(t)$ is C or N mineralized (kg ha^{-1}) at time t (days), Y_i is the i-th C or N pool (kg ha^{-1}), k_i the i-th decay constant (day^{-1}). The subdivision in two pools (very labile and labile, Figure 1) does not imply the absolute amount of mineralizable SOM in every pool but the ability of the soil microorganisms for SOM mineralization at different time scales. The very labile pool indicates the flush at initial stages and is represented by readily mineralizable SOM present in the soil. The size of the labile pool is determined by the replenishment of bio-available substrate (Wang et al. 2004).

There are concerns that estimates of four unknown parameters are highly sensitive to incubation conditions (particularly duration) and that the parameters Y_i and k_i are interdependent (Böttcher 2004; Wang et al. 2004). To overcome the interdependency and sensitivity to duration, some authors suggested fixing the decay constants (Wang et al. 2004; Mallory and Griffin 2007). We followed the approach of Mallory and Griffin (2007) and fitted equation (4) to the combined dataset of all treatments and fixed the decay constants to the obtained values. The obtained decay constants for C mineralization were: $k_1 = 0.0589\ d^{-1}$ and $k_2 = 0.0022\ d^{-1}$, for net N mineralization the decay constants were $k_1 = 0.1075\ d^{-1}$ and $k_2 = 0.0065\ d^{-1}$. Then we fitted the models obtained in this way to the data points of every field replicate. Model parameter estimation of the stocks of very labile and labile pools was conducted with SigmaPlot 10.0, using the Marquard-Levenberg algorithm. Intermediate C and N pools ($C_{intermediate}$, $N_{intermediate}$) were calculated as the difference between C_{org} or N_t and the sum of the very labile, labile and passive pools (Figure 1).

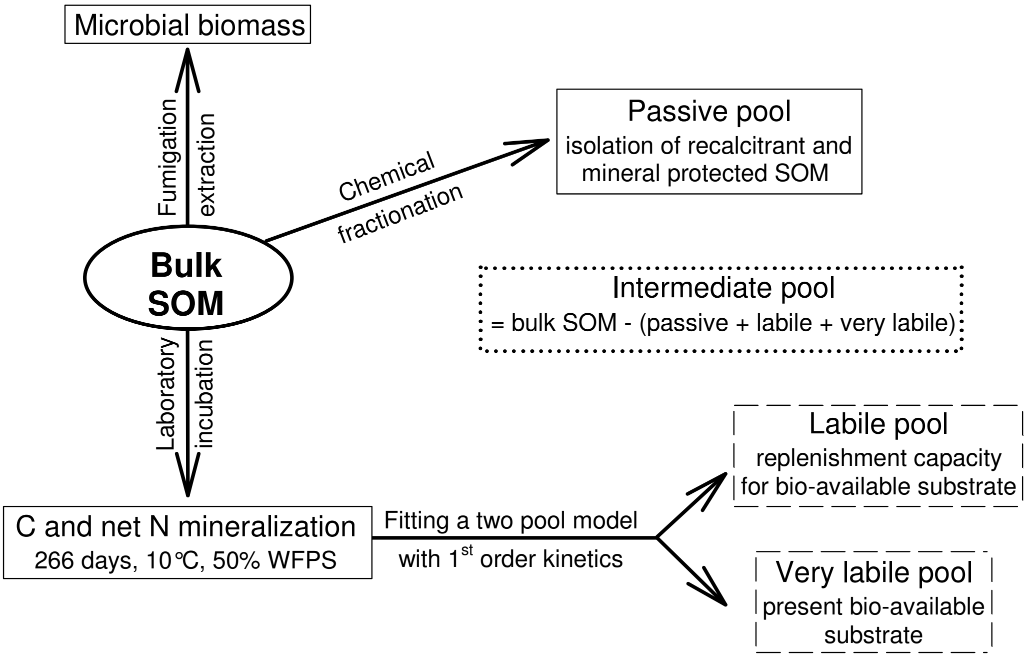

Figure 1: Scheme of the pools mentioned in this manuscript and the ways how they were obtained. Frames indicate whether pools were measured (solid), modeled (dashed) or calculated (stippled). Text on the arrows shows the methodological approach.

2.2.8 Statistics

To account for the structure of the strip design we applied a mixed model in order to test for significant ($p \leq 0.05$) effects. The field trial was divided into main rows and main columns. Thus, the following statistical model with the notation proposed by Piepho et al. (2003) was used:

$$R_k + C_l + RC_{kl} + T_i + A_j + TA_{ij} : \underline{TR_{ik} + ARC_{jkl} + TARC_{ijkl}} \qquad (5)$$

where R_k is the effect of the k-th main row, C_l is the effect of the l-th main column, RC_{kl} is the effect of the kl-th main row and main column interaction, T_i is the effect of the i-th fertilizer type, A_j is the effect of the j-th fertilizer rate, TA_{ij} is the effect of the ij-th fertilizer type and rate interaction, TR_{jk} is the error of the *jk*-th fertilizer type and main row interaction, ARC_{jkl} is the error of the *jkl*-th fertilizer rate and main row and main column interaction and $TARC_{ijkl}$ is the error of the *ijkl*-th subplot. Effects listed before the colon are fixed whereas those after the colon are random, the residual terms are underlined. Differences between means were tested using least significant differences (LSD) at $p \leq 0.05$.

In order to quantify the effect of the application of fertilizer or manure N, we applied a linear regression of cumulative N applications since 1980 with measured or modeled C and N pools. The regression was calculated for the datasets of MSI and FYM treatments separately (n = 12). When $R^2 < 0.33$, the regression was not significant ($p \leq 0.05$). Statistics were calculated with SAS 9.1 (SAS Institute Inc., Cary, USA).

2.3 Results

2.3.1 C and N input

Estimated mean annual C inputs (Figure 2) of crop residues and roots (2003 – 2006) were almost equal between MSI treatments (1.5 -1.6 t ha^{-1} $year^{-1}$) and FYM treatments (1.5 t ha^{-1} $year^{-1}$). In the particular year of cultivation, carbon input (t ha^{-1}) was highest from clover (3.6) and declined in the order winter rye (1.0 – 1.0) > spring wheat (0.8 – 0.9) > potatoes (0.5 – 0.7). Significant effects are the same as for the yields (Table 2). In the four-year course of the crop rotation, measured mean annual C inputs (t ha^{-1} $year^{-1}$) with straw were between 1.0 and 1.2 in the MSI treatments, whereas measured differences in C inputs from manure were more pronounced and ranged from 0.6 to 1.3 in the FYM treatments (Figure 1). Therefore, the estimated input of C from crop residues and roots contributed to more than 50% of the total annual mean input in the FYM treatments. Overall, cumulative estimated and measured C inputs (t ha^{-1}) since 1980 decreased in the order FYM_H (77) > FYM_M (64) > MSI_H (62) > MSI_M (61) > MSI_L (58) > FYM_L (51).

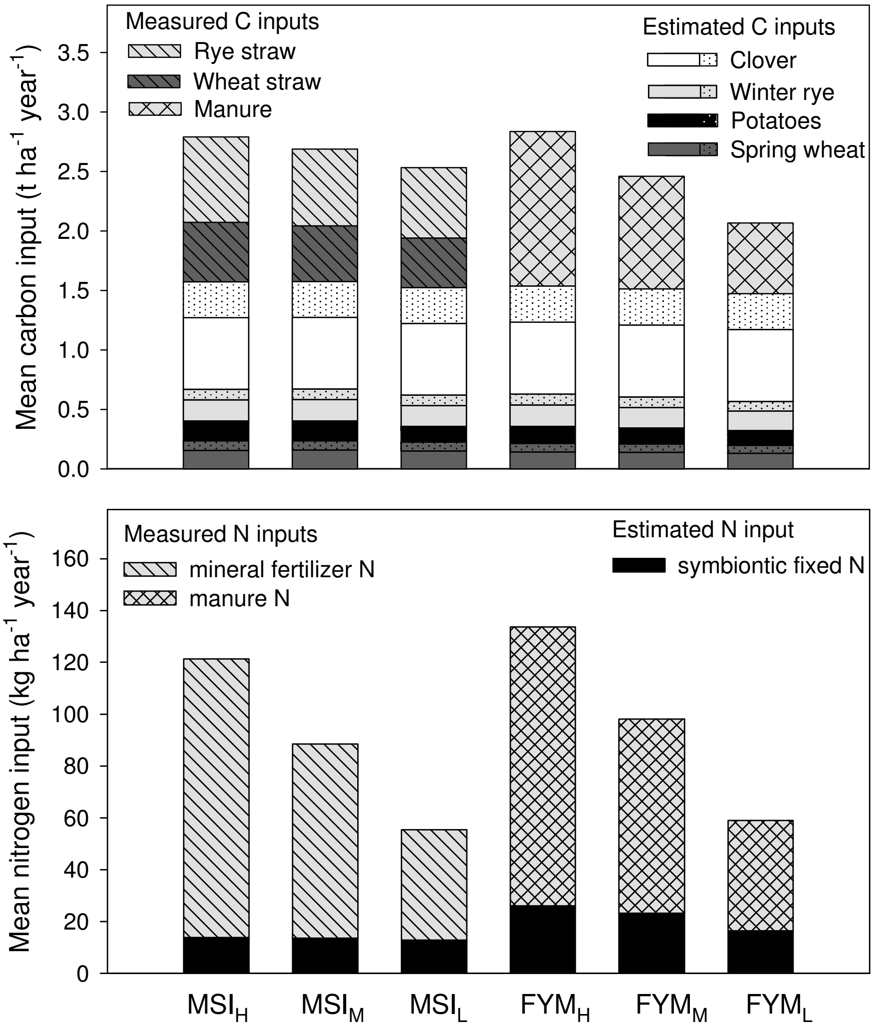

Figure 2: Measured (shaded: fertilizer, manure and straw) and estimated (non-shaded: root- and harvest residues, N_2-fixation) C and N inputs to the soils of the different fertilization treatments. Estimated portion of C input by rhizodeposition is stippled. Values are the fouryear means of the last crop rotation period (2003 to 2006). For treatment abbreviations see Table 1.

Estimated symbiontic N_2-fixation in 2005 (Figure 2) was smaller in MSI treatments (51 – 55 kg ha^{-1}) than in FYM treatments (66 – 104 kg ha^{-1}). Therefore, 10 to 20% (MSI treatments) and 20 to 30% (FYM treatments) of the mean annual N input were estimated to be derived from N_2-fixation. Cumulative N inputs (t ha^{-1}) since 1980 decreased in the order FYM_H (4.1) > MSI_H (3.7) > FYM_M (3.0) > MSI_M (2.8) > FYM_L (2.0) > MSI_L (1.9).

The marked gradient in C and N inputs at the different rates and the consideration of two different fertilizers indicate that this field experiment is well suited for studying the effects of the type and rates of fertilizer application on C and N pools in the soil.

2.3.2 Total C_{org} and N_t stocks

The stocks of C_{org} in February 2007 showed no significant difference between the treatments FYM_L and MSI_L, but higher FYM rates gave higher C_{org} and N_t figures as compared to the corresponding MSI treatments (Table 1). Stocks of C_{org} and N_t were linearly related to the application rate only in the FYM treatments ($R^2 = 0.27$ for C_{org} and 0.30 for N_t, Table 4).

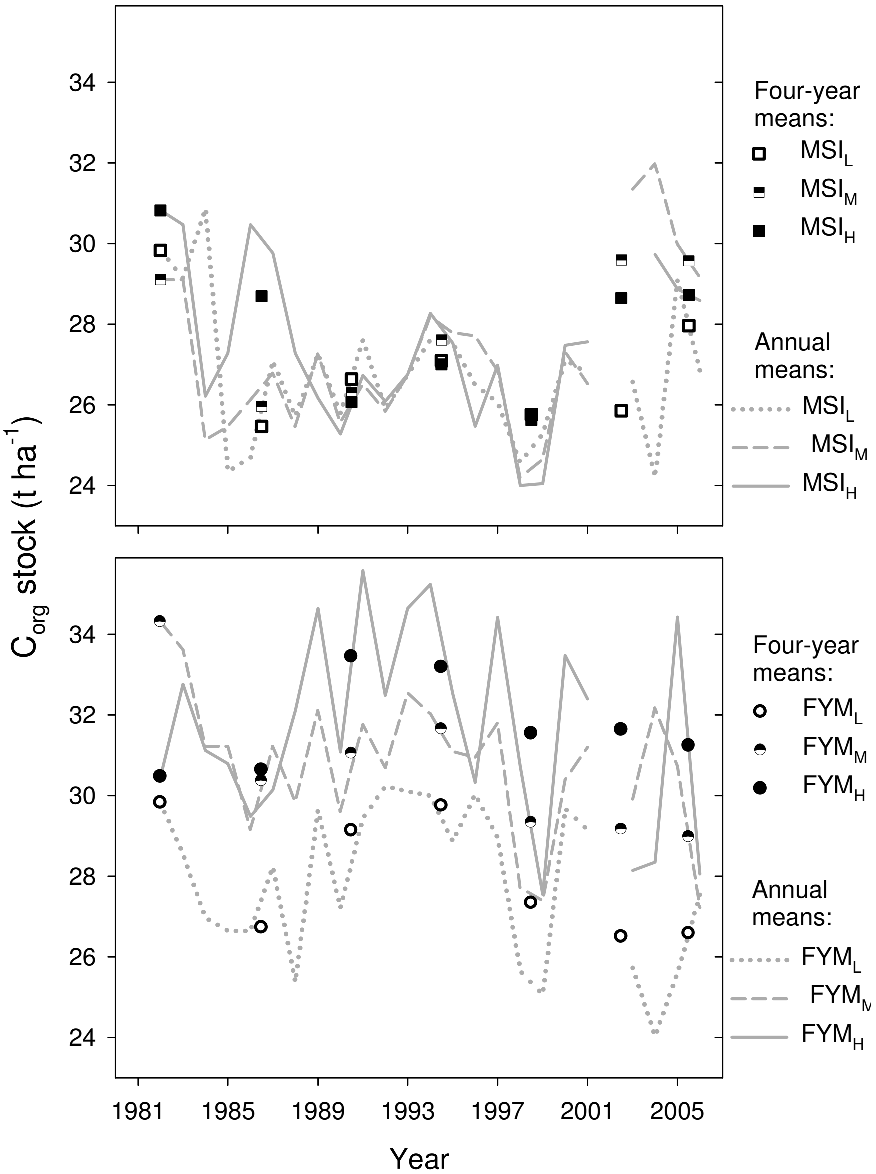

Figure 3: Changes in the stocks of organic carbon of the fertilizer treatments since 1982. Data points are the mean values of one crop rotation period (4 years and 4 replicates) except for the first (1982, starting value) and last data set (two-year mean of 2005/2006). Lines show the results of the annual measurements (n = 4). For treatment abbreviations see Table 1.

To allow an easier interpretation, we averaged the C_{org} stocks from the time course over the four-year period of the crop rotation. In the first crop rotation period (1985 - 1988) C_{org} stocks of all treatments declined, with exception of the stocks in the FYM_H treatment, which remained at the level of 1982 (Figure 3). This treatment was the only one, where the C_{org} stocks were consistently higher or around the starting value until the beginning of the latest rotation (2005/2006). Over the whole course, differences in C_{org} stocks between the fertilizer rates were much more obvious and consistent in the soils of the FYM than in those of the MSI treatments. Net C_{org} changes in the soil (t ha^{-1}) between 1982 and 2005/2006 were -1.9 (MSI_L), 0.5 (MSI_M), -2.1 (MSI_H), -3.2 (FYM_L), -5.3 (FYM_M) and 0.8 (FYM_H), whereas the high loss in the FYM_M treatment with an exceptional high starting value can be ascribed to losses within the first rotation period.

2.3.3 Microbial biomass

The interaction of fertilizer type and rate had a significant effect on the stocks of C_{mic} (Table 3). Soils of the FYM treatments had higher C_{mic} stocks (404 – 520 kg ha^{-1}) than those of the MSI treatments (308 – 361 kg ha^{-1}). The C_{mic} stocks in the FYM treatments rose slightly ($R^2 = 0.12$, Table 4) and those in the MSI treatments declined slightly ($R^2 = 0.16$) with the application rate. Stocks of N_{mic} were also significantly lower in MSI than in FYM treatments but showed no significant relation to the fertilizer application rate (Table 3). The C_{mic}/C_{org} ratio was only higher in the FYM_H compared to the MSI_H treatment.

Table 3: Stocks of microbial biomass C and N and the Cmic/Corg ratio in soils (0 – 25 cm) of the different fertilization treatments. Mean values and standard errors in parentheses (n = 4). Different letters indicate significant differences between means of individual treatments (p ≤ 0.05)

Treatment[a]	C_{mic} (kg ha^{-1})	N_{mic} (kg ha^{-1})	C_{mic}/C_{org} (%)
MSI_H	308 (27)a	42 (10)	1.27 (0.10)a
MSI_M	339 (19)a	45 (5)	1.44 (0.10)ab
MSI_L	361 (37)a	48 (6)	1.53 (0.15)ab
FYM_H	520 (33)b	68 (10)	1.85 (0.09)b
FYM_M	404 (27)c	67 (9)	1.44 (0.05)ab
FYM_L	423 (63)c	60 (13)	1.68 (0.26)ab
Source of variation[b]	Type × rate	Type	Type × rate

[a] For treatment abbreviations see Table 1
[b] Significant effects at p ≤ 0.05

2.3.4 C and net N mineralization

Cumulative C mineralization after 266 days ranged from 1130 kg ha^{-1} (MSI$_L$) to 1820 kg ha^{-1} (FYM$_H$). For both fertilizer types increasing application rates led to significantly increasing C mineralization (R^2 = 0.64 for MSI and 0.55 for FYM treatments, LSD: 261 kg ha^{-1}, Table 4 and Figure 4).

Cumulative net mineralized N depended significantly on the rate of fertilizer (LSD: 17 kg ha^{-1}, Figure 4). At the high rate, cumulative mineralization was markedly lower in the soils of the MSI treatments (90 - 110 kg ha^{-1} 266 days^{-1}) than in the soils of the FYM treatments (95 – 125 kg ha^{-1} 266 days^{-1}). The steeper slope of the regression in the soils of the FYM treatments (R^2 = 0.65) compared to the soils of the MSI treatments (R^2 = 0.27, Table 4, Figure 4) indicated a stronger effect of the rate of FYM application on net N mineralization.

2.3.5 C pools

Cumulative C mineralization was well described (R^2 > 0.99) by the applied two-pool model. The modeled carbon storage in the very labile C pool (turnover time: 17 days) ranged from 330 to 430 kg ha^{-1} (1.3 to 1.8% of C_{org}, C/N ratio: 23) and was independent of treatments (Figure 5). Modeled stocks of the labile C pool (1790 – 3210 kg ha^{-1} or 7 – 13% of C_{org}, C/N ratio: 22) with a turnover time of 462 days were significantly influenced by the application rate for both MSI and FYM treatments (R^2 = 0.58 and 0.59, LSD: 0.45 t ha^{-1}, Table 4 and Figure 5).

Calculated stocks of $C_{intermediate}$ (C/N ratio: 7) were significantly influenced by the interaction of fertilizer type and rate (Figure 5), and were lower for soils of the MSI (12 – 14 t ha^{-1} or 52 – 60% of C_{org}) than for those of the FYM treatments (15 – 18 t ha^{-1} or 60 – 62% of C_{org}). Least significant difference within type and between rates was 2.4 t ha^{-1}, LSD between types and within rates was 10.3 t ha^{-1}and LSD between types and rates was 9.4 t ha^{-1}. However, from the regression analysis no linear quantitative influence of the rate was revealed (R^2 = 0.05 and 0.09 for MSI and FYM treatments respectively, Table 4). In the experimentally determined pool $C_{passive}$ between 7 and 9 t C ha^{-1} (27 – 38% of C_{org}) was stored (Figure 5). It was not influenced by fertilizer type or rate. The C/N ratio in the passive pool was 82.

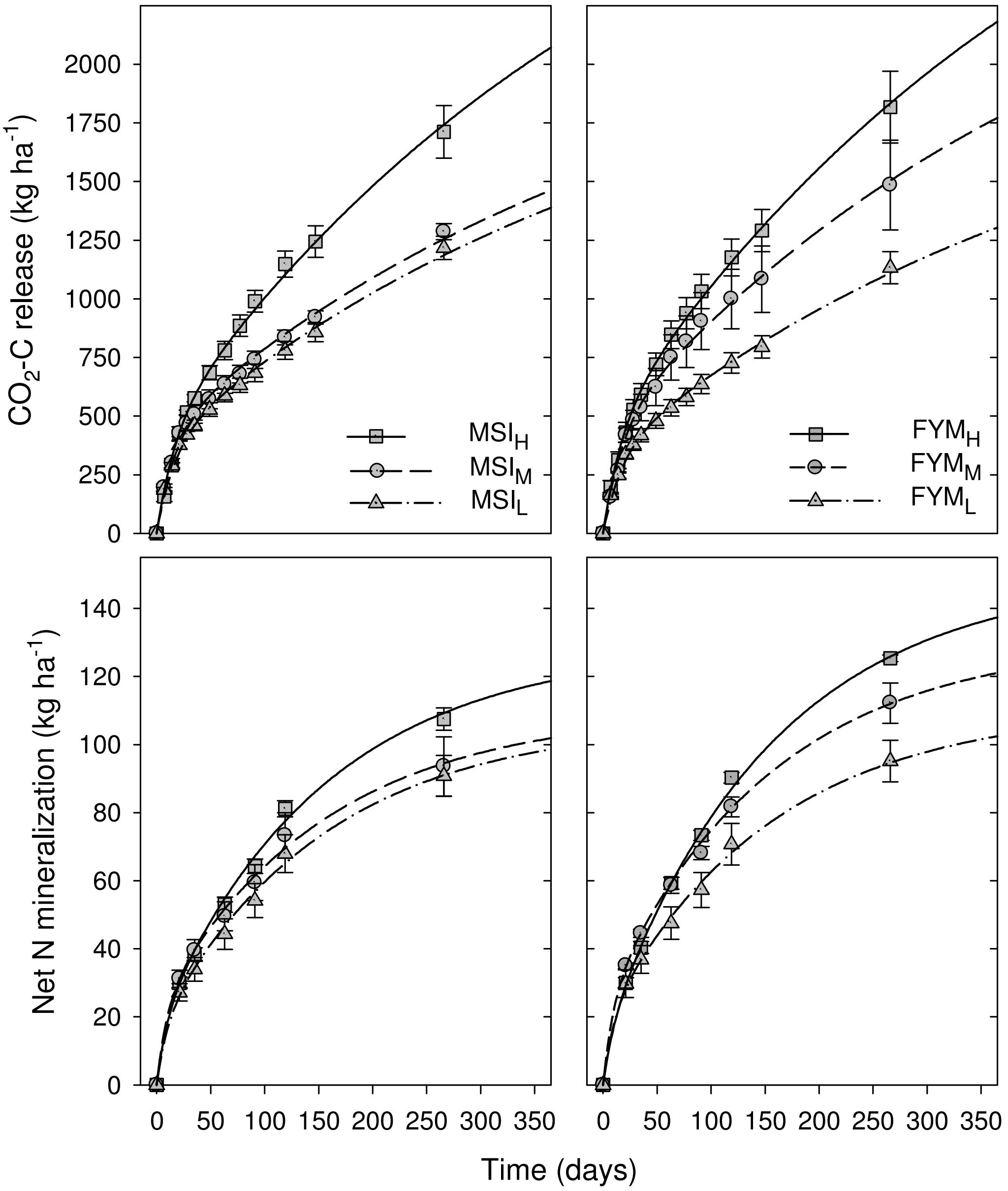

Figure 4: Carbon and net nitrogen mineralization of the soils of the fertilizer treatments from the Darmstadt field trial (0 – 25 cm). Datapoints are mean values of field replicates (n = 4) with standard errors, lines were interpolated with an exponential two-pool model. For treatment abbreviations see Table 1.

2.3.6 N pools

Cumulative net N mineralization was well described ($R^2 > 0.99$) by the applied two-pool model. The modeled very labile N pool (10 – 20 kg ha^{-1} or 0.5 – 1.0% of N_t) with a turnover time of 9 days was unaffected by type or rate of fertilizer (Figure 5, Table 4). Nitrogen storage in the modeled labile pool (90 – 140 kg ha^{-1} or 4 – 5% of N_t, turnover time: 153 days) was significantly affected by the rate of fertilizer (LSD: 0.02 t ha^{-1}). The relationship between application rate and the labile N pool was stronger in the soils of the FYM treatments ($R^2 = 0.74$, Table 4) than in the soils of the MSI treatments ($R^2 = 0.26$).

Stocks of the calculated pool $N_{intermediate}$ were higher in soils of FYM (2.3 – 2.6 t ha^{-1} or 88% of N_t) than in those of MSI (1.9 – 2.0 t ha^{-1} or 92% of N_t) treatments, but no significant effects were revealed (Figure 5). Oxidative fractionation resulted in very low and variable (high standard errors) stocks in the pool $N_{passive}$ (Figure 5). It ranged from 70 to 160 kg N ha^{-1} (3 – 7% of N_t) and was not affected by fertilizer rate or type.

2.4 Discussion

2.4.1 C and N input

Estimated mean annual C inputs (Figure 2) of crop residues and roots (2003 – 2006) were only slightly higher for MSI treatments than for FYM treatments (Figure 2) because of slightly higher crop yields in the MSI treatments (Table 2). Overall, our data of estimated (C of roots and crop residues) and measured (straw and manure) C inputs indicated that root and crop residue inputs contributed to more than 50% of the total C inputs, when calculated on average of the four-year crop rotation. The estimates of C input according to Franko (1997), slightly modified by Ludwig et al. (2007) are in a range reported by others. For instance, in the year of cultivation we estimated C input of spring wheat residues to be 0.8 – 0.9 t ha^{-1}, including rhizodeposits. This is near the lower range (0.9 – 3.0 t ha^{-1}) of values for wheat (revealed by ^{14}C labeling in pot experiments) summarized by van Veen et al. (1991). The low estimated C input can be attributed to the low soil fertility of the sandy Cambisol. For Germany, mean yield of spring wheat was 5.3 t ha^{-1} in 2003 (Statistisches Bundesamt Deutschland 2008), while the yields in the Darmstadt experiment were between 2.8 and 4.1 t ha^{-1} (Table 2). The estimation of C input of clover roots and residues was 3.6 t ha^{-1} in the year 2006. This was the same amount reported by Aeschlimann et al. (2005) for C inputs of white clover as revealed by a CO_2-exchange balance.

The estimated amounts of fixed N are in the lower range reviewed by Ledgard and Steele (1992) for red clover (49 – 373 kg ha^{-1} year^{-1}). Our estimates suggested that less than 30% of the mean annual N input were derived from N_2-fixation.

2.4.2 Total C_{org} and N stocks

Twenty-seven years of different fertilization regimes resulted in significantly higher stocks of C_{org} and N_t in the soils with FYM additions compared to those with additions of straw and mineral fertilizer (February 2007, Table 1) and increasing applications of manure tended to increase C_{org} and N_t stocks slightly (Table 4). Our findings are in accordance with those of others for several soil types with loamy and sandy textures (Christensen 1996; Weigel et al. 1998).

The effect of the rate in the FYM treatments appeared in the second rotation period (1988 – 1991) and was, with few exceptions in individual years, consistently present from then on (Figure 3). Only the FYM_H treatment provided a sufficient balance between C input and mineralization to maintain consistently the level of C_{org} stocks measured in 1982. In the soils of the MSI treatments the rate of fertilizer did not affect the stocks of C_{org} consistently (Figure 3), despite of increasing C input of straw with increasing rate (Figure 2). The C losses (1982 compared to means of crop rotation) in the soils of the MSI_L and MSI_H treatments were in the same magnitude (2 t ha^{-1}) and the smallest loss appeared in the soil of the MSI_M treatment. This may indicate an optimal balance for build up of C stocks between C input and mineralization in the MSI_M treatment. On the one hand, the C input in the MSI_L treatment may be too low to maintain the starting level of C_{org}. On the other hand, the activity of microorganisms is enhanced by a high N supply and could hence lead to decomposition rates that exceed the fertilizer enhanced C input (Khan et al. 2007).

2.4.3 Microbial biomass

The higher C_{mic} stocks in the soils of the FYM treatments are in accordance with other findings for several soils and textures (Weigel et al. 1998). Contradictory to our results, Witter et al. (1993) reported similar C_{mic} contents for FYM and MSI treatments in a loamy Cambisol. The stocks of C_{mic} were significantly correlated with C mineralization and the stocks in the modeled labile C pool (r = 0.66 resp. 0.64) in the soils of the FYM treatments only, indicating a decoupling of mass and activity of microbes in the MSI treatments.

From the regression analysis a very weak effect of the application rates on the size of C_{mic} was revealed for the FYM treatments (Table 4). Microbial biomass N was not affected by the rate. This was surprising, as we expected increases in the stocks of microbial biomass with increasing FYM additions (Weigel et al. 1998). The previous crop was clover, where the C input to the soil was estimated to be similar between treatments (Figure 2). This short-term effect was probably stronger than the effect of the application rate of FYM on the stocks of C_{mic} and N_{mic}.

In the MSI treatments C_{mic} declined slightly with increasing amount of fertilizer and straw input. In a loamy Luvisol Jacinthe et al. (2002) showed that an increasing amount of straw addition (8 or 16 t straw ha^{-1} $year^{-1}$) increased C_{mic} gradually. Application of mineral fertilizer (244 kg N ha^{-1} $year^{-1}$) led to a constant decrease in C_{mic} contents. Our results suggest that suppression of C_{mic} stocks may increase with mineral fertilizer rate. This effect may even be more pronounced than the effect of increasing C input from root and crop residues and straw incorporation which may increase the stocks of C_{mic} in the long-term.

Table 4: Quantitative impact of fertilizer application on different C and N stocks or pools, analyzed by linear regression of cumulative N inputs (kg ha^{-1}) from fertilizer or manure (1980 – 2007) with respective C and N stocks or pools of the soils in MSI and FYM treatments (n = 12). Regressions with $R^2 > 0.32$ are significant ($p \leq 0.05$).

	MSI[a]			FYM[a]		
	slope ($\times 10^{-3}$)	intercept (kg ha^{-1})	R²	slope ($\times 10^{-3}$)	intercept (kg ha^{-1})	R²
C_{org}	310	2.3×10^4	0.03	1.5×10^3	2.4×10^4	0.27
C_{mic}	-30	395	0.16	13	104	0.12
mineralized C in 266 days	278	849	0.64	362	714	0.55
very labile C	25	350	0.09	55	277	0.31
labile C	656	959	0.58	749	906	0.59
$C_{intermediate}$	-545	1.4×10^4	0.05	879	1.5×10^4	0.09
N_t	30	2.2×10^3	0.02	170	2.3×10^3	0.30
N_{mic}	-3.9	52.7	0.04	3.8	56.9	0.02
mineralized N in 266 days	9.4	78.5	0.27	15.6	77.8	0.65
very labile N	0.6	16.8	0.05	-2.4	23.1	0.09
labile N	11.4	74.5	0.26	23.1	64.0	0.74
$N_{intermediate}$	30	1.9×10^3	0.01	145	2.2×10^3	0.38

[a] FYM: rotted cattle farmyard manure, MSI: mineral fertilizer plus straw incorporation

2.4.4 C mineralization

Potential losses of mineralizable C with DOC due to the leaching were between 50 and 200 kg ha^{-1} (not shown). This amount was in the magnitude of the standard errors for CO_2-C release (Figure 4). Furthermore, Qualls (2005) showed that only about 40% of DOC from litter was decomposable in the soil in one year.

Cumulative CO_2-C release after 266 days was of similar magnitude in FYM and MSI treatments and increased significantly with increasing application rate (Figure 4, Table 4). Our findings are in line with other studies, where increasing C mineralization was reported with increasing addition of mineral fertilizer (Vanotti et al. 1997) or straw (Jacinthe et al. 2002), indicating that increasing C additions (FYM treatments) and/or higher C inputs with roots and crop residues are able to increase C mineralization. However, in a calcareous loam Habteselassie et al. (2006) did not find any effect of the rate (100 or 200 kg N ha^{-1} year^{-1}) of FYM and mineral fertilizer on C mineralization, which suggests that the partitioning of C (as FYM or roots and residues) to C sequestration and C mineralization differs considerably between different soils.

2.4.5 Net N mineralization

Net N mineralization after 266 days in the soils of the MSI treatments differed markedly from those of the FYM treatments. However, only the effect of the fertilizer rate was significant. Increasing net N mineralization with increasing rates of manure (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007) or mineral fertilizer (El-Haris et al. 1983; Vanotti et al. 1997) has been described for various loamy soils and is consistent with our results. Compared to the soils of the MSI treatments, the steeper slope of the regression between fertilizer N input and net N mineralization in the soils of the FYM treatments (Table 4) suggests that differences between the fertilizer types may develop with increasing rates.

2.4.6 C pools

In the soils of all treatments the modeled very labile C pool (turnover time: 17 days) was small. The C stored in the very labile pool is assumed to stem from less decomposed plant material of the previous crop (Benbi and Richter 2002) and is readily bio-available (Wang et al. 2004). This makes this modeled pool sensitive to sampling time. Presumably because the crop and its estimated crop residue-C and root-C inputs were the same (Figure 2), no differences between the treatments emerged (Figure 5).

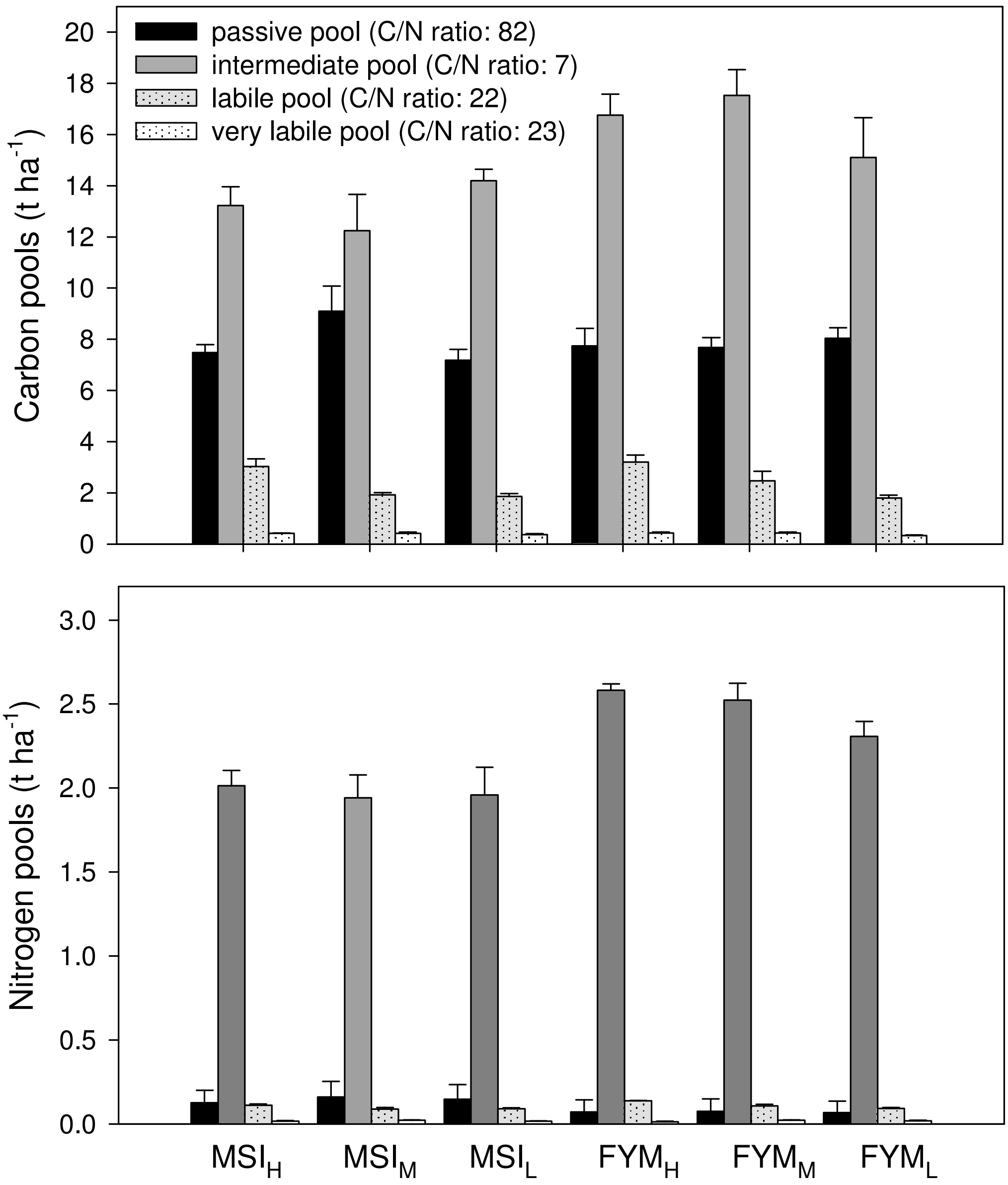

Figure 5: Carbon and nitrogen storage in different pools in the soils (0 - 25 cm) of the fertilization treatments. Mean values with standard errors (n = 4). For treatment abbreviations see Table 1.

The size of the modeled labile C pool (turnover time: 462 days) was significantly related to the rate of fertilizer but not affected by the type (Figure 5, Table 4). Our finding that the labile C pool increased with increasing input of mineral fertilizer is consistent with results reported before for various loamy soils and this has been attributed to increasing root and crop residue C inputs with increasing fertilization (Vanotti et al. 1997). In our study, the lack of difference between the FYM and MSI treatments was unexpected. Perhaps this can be attributed to the presumption that the previous crop (clover) and its estimated C inputs were the same. The high C input (estimated to 3.6 t ha^{-1}) could have diminished the effect of the fertilizer types. Nevertheless, this would be contradictory to the revealed rate effect, since the C input from clover was estimated to be equal between rate treatments. Additionally, the lack of an effect of the fertilizer type is due to the long time span since the last straw incorporation (July 2005, afterwards cultivation of clover) or manure application (September 2004 before sowing of winter rye), which exceeded the modeled turnover time of the labile pool. In the later stages of decomposition, the characteristics of organic materials are known to approximate to each other (Randall et al. 1995; Yang and Janssen 2002). Therefore, mineralization of straw was presumably faster at the initial stages in 2005/2006, which led to a depletion of labile straw-C and an approximation of the size of labile C from manure.

There was a marked difference in the amount of the calculated pool $C_{intermediate}$ in the soils of the FYM and MSI treatments and the effect of the interaction of fertilizer type and rate was significant (Figure 5). A higher chemical recalcitrance of FYM in relation to straw could explain the difference in the amount of $C_{intermediate}$ between FYM and MSI treatments. However, as outlined by the regression analysis, the quantitative effect of the fertilizer application rate was small (Table 4). This was surprising and may be explained by interactions of chemical recalcitrance and physico-chemical stabilization mechanisms (Marschner et al. 2008). Additionally, we estimated that more than 50% of the C inputs were derived from roots and crop residues (Figure 2), which could have diminished the effect of the rate of FYM applications.

The size of the experimentally determined pool $C_{passive}$ was not influenced by type or rate of fertilizer, which is consistent with the concept that the passive pool should not be influenced by recent management (Helfrich et al. 2007).

2.4.7 N pools

The modeled very labile N pool (turnover time: 9 days) was small in all treatments and not affected by rate or type of fertilizer (Figure 5, Table 4). As with the very labile C pool, we assume a compensating effect of the previous crop on this N pool. Storage of N in the modeled labile pool (turnover time: 153 days) was significantly affected by the rate of fertilizer. An increasing size of the modeled labile N pool (Figure 5, Table 4) with increasing rate of mineral fertilizer (El-Haris et al. 1983; Vanotti et al. 1997) or manure (Whalen et al. 2001) has been reported for several loamy soils. In contrast to our results, higher modeled pools of labile N for the soils of the FYM treatment were obtained when comparing manure and mineral fertilization in loamy soils (Mikha et al. 2006; Mallory and Griffin 2007). In the latter studies, straw was not incorporated in mineral fertilized soils. Due to the wide C/N ratio of cereal straw N may be immobilized and adds up to the labile pool. This presumption is corroborated by the study of Bonde et al. (1988) who did not find differences in the modeled labile N pool between FYM and MSI treatments in a loamy Cambisol. It is also possible that the previous cultivation of clover masked fertilizer induced differences in the modeled stock of labile N as discussed above. However, the regression analysis revealed a stronger influence of the rate on labile N in the soils of the FYM treatments (Table 4), indicating that the differences in the size of the labile N pool may develop with increasing rates.

The stocks of the calculated pool $N_{intermediate}$ were markedly higher in the soils of the FYM treatments (Figure 5) but the difference was statistically insignificant. Storage in the pool $N_{passive}$, as revealed by chemical fractionation, was very variable as indicated by the high standard errors. The wide C/N ratio (82) indicates the low ability of the sandy Cambisol for N stabilization in the passive pool, either by preferential removal of N or selective preservation of C. Helfrich et al. (2007) observed a slight selective removal of N, compared to other chemical fractionation methods. However, not to the extent we observed. Therefore we suggest the possible involvement of charred material in the formation of passive SOM. Knicker et al. (2005) showed, that charred plant material in a pine stand contains very little N (C/N ratio: 100 – 150). Furthermore, charred material is resistant to oxidative treatments (Kögel-Knabner et al. 2008) and was found by Cuypers et al. (2002) in the residual of peroxodisulfate treated soils. Since the former vegetation was a mixed forest, historical fire events are likely in the warm and dry climate.

2.5 Conclusions

Applying a combination of chemical and biological fractionations, enhanced by a simple modeling approach, on soil samples of a medium-term field trial gave us gainful insights in SOM dynamics under different fertilizer managements.

We could show that mineralization and modeled labile pools were influenced by the rate under both MSI and FYM treatments. Accounting only total C_{org} and N stocks the effect of the rate was small (FYM) or even absent (MSI). Since the approach of fitting a simple model to mineralization data provided an approximation of turnover times in the labile pool without the need of using isotopic signatures, we can judge the rate of both fertilization types as a short time impact. From the division of an intermediate and passive pool we could ascribe the difference in C_{org} and N_t stocks between fertilizer types to the intermediate pool with theoretical turnover times of decades to centuries.

Although time consuming, incubation approaches provide the advantage of specific substrate utilization by microbes. Nevertheless, incubation temperature and duration are not standardized and may strongly affect the result. Furthermore, the usefulness of chemical fractionation methods as a measure for the passive pool, especially for N, has to be further elaborated. Our combination of approaches could be improved by ^{14}C dating of the soil and the chemical fraction.

3 Soil organic matter pools and crop yields as affected by the rate of farmyard manure and use of biodynamic preparations in a sandy Cambisol

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ABSTRACT. One aim of biodynamic agriculture is to improve soil fertility. Our objectives were to (i) quantify the effect of three levels of farmyard manure (FYM) applications on microbial biomass and pools with different stability; and (ii) explain previously reported differences in the soil organic matter levels between soils receiving FYM with (DYN) or without (FYM) biodynamic preparations. Yield of crops (potatoes, winter rye and clover) significantly increased in proportion to the application rate of FYM and did not differ between DYN and FYM fields. Contents of labile C (70 – 114 g [kg $C_{org}]^{-1}$, turnover time 462 days) and labile N (35 – 49 g [kg $N_t]^{-1}$, turnover time of 153 days) were strongly related to the application rate. Soils of the DYN treatments had higher C_{org} and N_t contents compared to soils of the FYM treatments, but initial spatial variation of C stocks was not studied adequately in the beginning of the experiment in 1980. About 30% of the difference could be ascribed to storage in the passive pool (turnover time >> 600 years) and thus not to treatment effects. Overall, the study showed that increasing rates of FYM increased C and N availability in both DYN and FYM treatments. Nevertheless, efficiency of C sequestration in a more stable form (intermediate pool) decreased with increasing rate.

3.1 Introduction

Fertilization with farmyard manure (FYM) has many beneficial effects on soil. For instance, fertilization with FYM leads generally to higher levels of organic carbon (C_{org}) and total nitrogen (N_t) than the application of mineral fertilizers (Christensen 1996; Weigel et al. 1998). Similarly, increasing rates of FYM increase the stocks of C_{org} and N_t (Christensen 1996; Weigel et al. 1998), the content of microbial biomass (Weigel et al. 1998) and the rate of net N mineralization (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007). Less information is available about the impact of FYM with biodynamic (BD) preparations on the soil (Turinek et al., 2009).

Biodynamic preparations consist of plant parts or extracts treated with animal tissues, water and/or soil (Koepf et al. 1996; Turinek et al. 2009). When added to composting dairy manure piles, it has been shown by Carpenter-Boggs et al. (2000b) that BD-treated compost piles maintained a higher temperature and had 65% more nitrate at the end of the composting process. Furthermore, the phospholipid fatty acid profile of the microbial community differed. Variable results exist on the effect of BD-FYM on soil properties: Compared to fertilization with FYM, higher C_{org} contents and enzyme activities but similar microbial biomass contents were observed in a sandy Cambisol fertilized with BD-FYM (Bachinger 1996; Raupp and Oltmanns 2006) at the field experiment of the current study (Darmstadt, Germany). In a loamy Fluvisol, treated for 9 years with FYM and BD-FYM, a faster decomposition and lower metabolic quotient were reported in the BD treatments, but C_{mic} contents did not differ (Zaller and Koepke 2004). In a loamy Haploxeroll, no significant effects on soil biological properties were reported after two years of application of FYM and BD-FYM (Carpenter-Boggs et al. 2000a).

Soil organic matter (SOM) is operationally divided into different pools with distinct turnover times. For example, a classification into three C and N pools in the soil was proposed by Parton et al. (1988): a labile (turnover time 1.5 years), an intermediate (turnover time: 25 years) and a passive pool (turnover time: 1000 years) were described. Because labile and intermediate pools provide a supplement of nutrients in addition to direct fertilization, a special aim of BD agriculture is to achieve a high amount of SOM stored in these pools (Koepf et al. 1996). For determination of the passive pool, Helfrich et al. (2007) evaluated several chemical fractionation methods. They concluded that fractionation with sodium peroxodisulfate ($Na_2S_2O_8$) removed preferably young C. The

^{14}C-age of the remaining C fraction was more than 10000 years and apparent turnover time (derived from ^{13}C measurements) was much more than 600 years. Furthermore, land-use and initial C content did not influence the C content of the remaining fraction. Thus, fractionation of SOM with $Na_2S_2O_8$ can be used as a measure for the passive pool.

Quantifying the partitioning of C and N into labile, intermediate and passive pools is important for our understanding of soil fertility and SOM stabilization. In a previous study, we compared the effect of mineral fertilizer and manure application (without BD-preparations) on the distribution of C and N in several SOM pools in soils of the Darmstadt experiment (Heitkamp et al. 2009). Here, we report the effects of BD-FYM additions on total C and N stocks and different pools. Until now, little information has been available on how the rate of BD-FYM application influences partitioning of organic matter into the above-mentioned pools. Our objectives were to (i) explain previously reported (Bachinger 1996; Raupp and Oltmanns 2006) differences in the C_{org} content between manure and BD-manure treatments and (ii) compare the effects of the application rates of both types of manure on microbial biomass and SOM pools in a sandy Cambisol at the long-term experiment site in Darmstadt, Germany.

3.2 Material & methods

3.2.1 Site description

The long-term experiment is situated near Darmstadt, Germany (49° 50’ N, 8° 34’ E, mean annual temperature: 9.5°C, mean precipitation: 590 mm) and focuses on organic, mineral and biodynamic treatments. The soil is a Haplic Cambisol with sandy texture (5% clay, 10% silt and 85% sand). Historical maps show that the experimental area was converted from a mixed forest stand to arable land between 1904 and 1920. The experiment started in 1980 with nine treatments. Crop rotation (since 1985) have consisted of spring wheat, potatoes, winter rye and legumes (clover, *Trifolium* spp. or Lucerne, *Medicago sativa* L.). Yields are given in Table 5.

Soil (A horizon, 0-25 cm) was sampled from plots (25 m^2) receiving BD field sprays plus BD treated manure at three different rates. Application rates were high (DYN_H), medium (DYN_M) and low (DYN_L). Biodynamic compost preparations (herbal extracts, 502 – 507) were applied at rates of 2-4 mg kg^{-1} to FYM. Preparations 500 (horn manure) and 501 (horn silica) were sprayed on the field at rates of 200-300 and 4 g ha^{-1}, respectively. More information about biodynamic preparations can be found in the literature (Koepf et al. 1996; Carpenter-Boggs et al. 2000a; Zaller and Koepke 2004; Turinek et al. 2009). Three treatments using unprepared FYM at corresponding rates served as control (FYM$_H$, FYM$_M$, FYM$_L$). Since 1985, the FYM and BD-FYM applications have corresponded for one crop rotation (four years) on average to 108, 75 or 43 kg N$_t$ ha^{-1} year^{-1}. Legumes were not fertilized; application rates for the other crops are as follows:

High: 27 t fresh weight ha^{-1} (150 kg N$_t$ ha^{-1}) to potatoes or 16 t fresh weight plus 40 kg N$_t$ ha^{-1}as urine to cereals (140 kg N$_t$ ha^{-1})

Medium: 18 t fresh weight ha^{-1} to potatoes or 12 t fresh weight ha^{-1} plus 20 kg N$_t$ ha^{-1} with urine to cereals (both 100 kg N$_t$ ha^{-1})

Low: 9 t fresh weight ha^{-1} to potatoes (50 kg N$_t$ ha^{-1}) or cereals (60 kg N$_t$ ha^{-1}).

Farmyard manure was rotted for three to six months before application. All management actions were the same apart from the application rate and use of preparations. In 1980, at the commencement of the field study, baseline soil data were only collected on a single bulked sample (C$_{org}$: 10.9 g kg^{-1}, N$_t$: 0.8 g kg^{-1}). Since 1982, all treatments were analyzed for C$_{org}$ and N$_t$, but for the period prior to 1988 only mean values without standard errors are available. A detailed description of the field experiment is given by Raupp and Oltmanns (2006).

3.2.2 C and N inputs

The C inputs with harvest residues and roots were estimated as a linear function of the crop yields (Franko 1997) (Table 5) by additionally accounting for rhizodeposition (Ludwig et al. 2007) (Equation 5).

$$Cinput = K + yield \times F \tag{5}$$

C_{input} is the input of C by crop residues and roots (dt C ha^{-1}), *yield* refers to the crop yield (dt ha^{-1}) and *K* (intercept) and *F* (slope) are crop specific constants. The values used for *K* (dt C ha^{-1}) were 23.9 (berseem clover), 3.1 (spring wheat), 0.8 (potatoes) and 4.0 (winter rye) and for *F* 0.014 (berseem clover), 0.078 (spring wheat) and 0.080 (winter rye) dt C (dt dry matter yield)$^{-1}$ or 0.016 (potatoes) dt C (dt fresh matter yield)$^{-1}$ (Franko, 1997). *R* accounts for the portion of the C input by rhizodeposition (potatoes: 1.35, other crops 1.50) (Ludwig et al. 2007).

Additionally to the FYM-N_t input we considered symbiotic N_2-fixation of berseem clover (Bachinger and Zander 2004):

$$Nfix = Pfix \times yield \times (Nyield + Ryr \times Nres) \times Nred \tag{6}$$

3.2.3 Yields, soil sampling and characterization

Crops were harvested from the core of the plots (18.75 m^2), cleaned and weighed fresh (potatoes), air dried (grains, 14% water content) or oven-dried (legumes, 105°C).

Soil samples were taken in February 2007 after berseem clover (*Trifolium alexandrinum* L.) cultivation. Twenty-five soil samples (A-horizon, 0–25 cm) were bulked for each field replicate. Samples were sieved (< 2 mm) and dried (40°C) or stored moist at 4°C. The pH (soil:solution [w/v] ratio 1:2.5) was determined in 0.01 M $CaCl_2$ solution. Organic carbon and N_t were measured by dry combustion (Elementar Vario El, Heraeus, Hanau, Germany). If present, $CaCO_3$ was destroyed by pre-treatment with HCl (10%). Microbial biomass C (C_{mic}) and N (N_{mic}) were analyzed by chloroform fumigation extraction (CFE) (Vance et al. 1987) at 55% water holding capacity (WHC) with conversion factors of 0.45 for calculation of C_{mic} and 0.54 for N_{mic} (Brookes et al. 1985; Wu et al. 1990).

Table 5: Yields (t ha-1) of the main products (grain, tubers, forage) of the crops at the Darmstadt long term experiment (2003-2006). Yield of grain contains 14% water, yield of tubers is given in fresh weight and yield of clover aboveground biomass is given in dry weight. Mean values with standard errors (n = 4).

Treatment[§]	Spring wheat	Potatoes	Winter rye	Clover forage
DYN_H	3.2 (0.3)	21.7 (2.4)	4.3 (0.3)	4.8 (0.6)
DYN_M	3.1 (0.2)	19.6 (2.4)	3.8 (0.4)	4.1 (0.4)
DYN_L	2.7 (0.3)	17.6 (0.9)	3.2 (0.7)	3.5 (0.5)
FYM_H	3.4 (0.2)	21.3 (1.0)	4.1 (0.2)	5.1 (0.2)
FYM_M	3.2 (0.1)	19.7 (1.6)	3.7 (0.2)	4.6 (0.0)
FYM_L	2.8 (0.2)	17.8 (0.3)	3.3 (0.4)	3.3 (0.2)
Source of variation	ns	Rate	Rate	Rate

[§] DYN_H, DYN_M, DYN_L: farmyard manure application plus biodynamic preparations at high, medium and low rate. FYM_H, FYM_M, FYM_L: farmyard manure application at high, medium and low rate.

3.2.4 Incubation experiment

For the mineralization experiment, we incubated 60 g of soil in leaching columns (three replicates per soil sample) at 10°C for 266 days at 55% WHC. Concentrations of CO_2 were measured with a photoaccustic measurement device (INNOVA 1312 AirTech Instruments, LumaSense Technologies AS, Ballerup, Denmark). Measurement of net N mineralization was conducted by leaching (Michel et al. 2006). The volume of leaching solution (10 mM $CaCl_2$) was 800 ml. Extracts were filtered (0.45 µm) and analyzed for NO_3^- and NH_4^+ with a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Cergy-Pontoise, France) and for DOC (Dimatoc, Dimatech, Essen, Germany).

3.2.5 Passive pool

To estimate the size of the passive pool ($C_{passive}$, $N_{passive}$) we carried out an oxidation with disodium peroxodisulphate ($Na_2S_2O_8$) (Helfrich et al. 2007). Briefly, 0.5 g soil was dispersed in 250 ml distilled water by ultrasound and 20 g NaS_2O_8 and 22 g $NaHCO_3$ were added. After stirring (48 hours, 80°C), the soil was washed and carbonate traces removed with HCl. The remaining fraction was analyzed for C and N.

3.2.6 Data processing

For determination of the very labile and the labile pools we fitted a two-pool double exponential model to the data of every plot. The equation is:

$$Ym(t) = \sum_{i=1}^{2} Yi \times (1 - \exp(-ki \times t)) \qquad (7)$$

where $Y_m(t)$ is C or N mineralized (mg kg^{-1}) at time t (days), Y_i is the i-th C or N pool (mg kg^{-1}), k_i the i-th decay constant (day^{-1}).

Because of uncertainties in estimation of four unknown parameters (Böttcher, 2004), we followed the approach of Mallory and Griffin (2007) and fitted equation (7) to the combined dataset of all plots (treatments and replicates) and fixed the decay constants to the obtained values. Briefly, decay constants were 0.0589 day^{-1} (very labile C pool), 0.0022 day^{-1} (labile C pool), 0.1075 day^{-1} (very labile N pool) and 0.0065 day^{-1} (labile N pool).

Intermediate pools ($C_{intermediate}$, $N_{intermediate}$) were calculated as the difference between C_{org} resp. N_t and the sum of the very labile, labile and passive pools.

Statistical analysis was performed in SAS (SAS Institute Inc., Cary, USA) using a two-factorial (type and rate of manure) mixed model which accounted for the randomization structure of the field experiment. Effects were regarded as significant if $p \leq 0.05$. The quantitative effect of the cumulative applied N on C and N pools was analyzed by linear regression (Webster 2007).

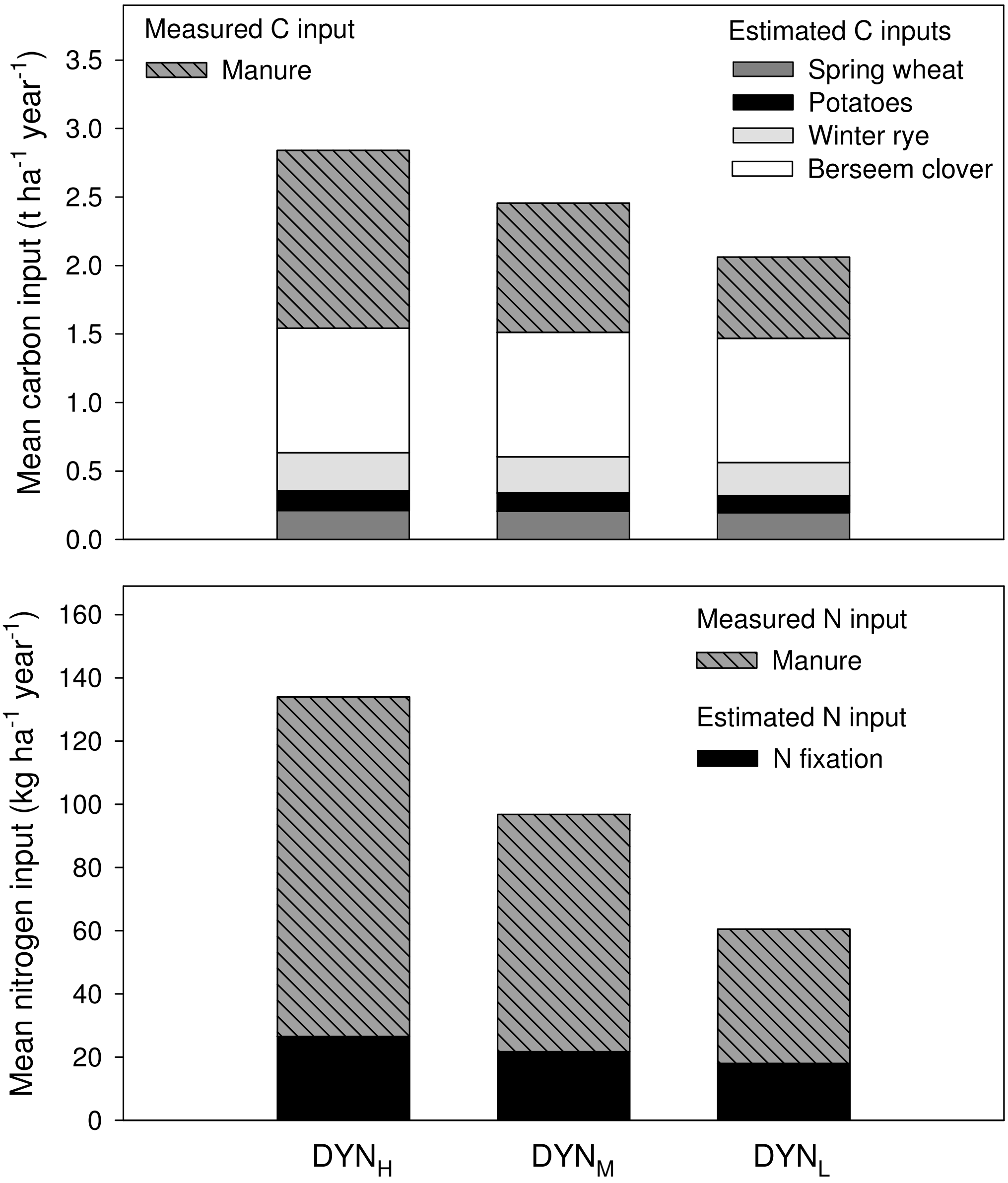

Figure 6: Measured (manure, shaded) and estimated (harvest residues and root C, symbiotic fixed N) C and N inputs of the last crop rotation period (2003-2006). For N fixation a constant efficiency of 0.68 was assumed across treatments. For Treatment abbreviations see Table 5.

3.3 Results & discussion

3.3.1 Crop yields

Yields for potatoes, winter rye and clover increased significantly with the application rate, but were unaffected by the FYM type (DYN: FYM with biodynamic preparations; FYM: FYM without biodynamic preparations) (Table 5). For spring wheat, we observed a trend ($p = 0.10$) of an effect of the rate. Compared to average yields of Germany, the yields are relatively low. For instance, mean yields (for years given in Table 5, t ha^{-1}) of spring wheat (5.7), potatoes (41.6), rye (5.3) and clover/grass-clover (8.8) (Statistisches Bundesamt Deutschland 2008) were markedly higher than in the Darmstadt experiment. In Darmstadt, the crops yielded between 40 and 70% of the German average, due to the relatively dry site conditions and the low soil fertility of the sandy Cambisol. Only few long-term experiments in Europe exist on low fertility sandy soils. In the fertilization experiment in Thyrow, Germany (15 t FYM ha^{-1} year^{-1}) potato yields were comparable (20 t ha^{-1}) with those of Darmstadt. The winter rye yield was remarkably higher than the yield at the Sandmarken site at Askov, Denmark (1.7 t ha^{-1}) (Edmeades 2003).

Despite the relatively high range of the FYM rate, the increase in yield was relatively low. A regression analysis (not shown) revealed an increase of yield by 20% (spring wheat, potatoes, clover) to 30% (winter rye) when N application increased by 100%. Therefore, efficiency of FYM application decreased with increasing yield.

3.3.2 C and N inputs to the soil

The measured and estimated mean annual C inputs (Figure 6) between 2003 and 2006 increased in the order low rate (2.1 t ha^{-1} year^{-1}) < medium rate (2.5 t ha^{-1} year^{-1}) < high rate (2.8 t ha^{-1} year^{-1}). Estimates and measurements for DYN and FYM treatments at same rates were identical. Less than 50% of the total C input was derived from manure and more than 30% was estimated to stem from clover (Figure 6). Our estimates of C inputs from roots and crop residues were within the range reported by others (van Veen et al. 1991; Aeschlimann et al. 2005).

Estimated N fixation ranged from 70 to 105 kg ha^{-1} in the year of clover cultivation (Figure 1). Because of higher yields, the amount of fixed N increased with application rate. Therefore, between 20% (FYM_H and DYN_H) and 30% (FYM_L and DYN_L) of the mean annual N inputs (2003 – 2006) were derived from fixation. The amount of N fixation in the above given scenarios were well in the range (49 – 373 kg ha^{-1} year^{1}) reviewed by Ledgard and Steele (1992).

Table 6: Absolute and relative changes of the Corg contents (ΔCorg) between 1982 (first sampling of individual treatments) and 2007 (Figure 7). Data of microbial biomass C, microbial biomass N, cumulative (266 days) mineralized C and net mineralized N of the soils (0–25 cm) of the different fertilization treatments sampled in 2007. Mean values and standard errors in parentheses (n = 4).

Treatment[§]	ΔC_{org} (g kg^{-1})	ΔC_{org} (%)	C_{mic} (mg kg^{-1})	N_{mic} (mg kg^{-1})	Mineralized C (mg kg^{-1})	Net-mineralized N (mg kg^{-1})
DYN_H	-1.7	-16	125 (6)^{ab}	18 (2)	543 (32)	38 (1)
DYN_M	-1.8	-17	134 (16)^{ab}	20 (4)	529 (48)	33 (1)
DYN_L	-1.8	-18	124 (14)^{ab}	17 (2)	395 (17)	29 (1)
FYM_H	-1.2	-13	151 (9)^{a}	20 (3)	527 (52)	36 (1)
FYM_M	-1.3	-14	118 (8)^{b}	19 (3)	432 (56)	33 (2)
FYM_L	-1.6	-18	126 (22)^{ab}	18 (5)	336 (28)	28 (2)
Source of variation[#]	nd	nd	Type×rate	ns	Rate	Rate

Means followed by different letters are significantly different ($p \leq 0.05$)

[§] For treatment abbreviations see Table 5; values of FYM treatments were recalculated from Heitkamp et al. (2009)

[#] ns denotes not significant, nd denotes not determined

3.3.3 Microbial biomass

The C_{mic} contents ranged from 124 (DYN_L) to 134 mg kg^{-1} (DYN_M) and from 118 to 151 mg kg^{-1} in the FYM treatments (Table 6). Only C_{mic} contents in soils of FYM_H and FYM_M treatments were significantly different. Contents of N_{mic} (17 – 20 mg kg^{-1}) were not significantly affected (Table 6). The regression analysis revealed no quantitative effects of the application rates (Table 7). Low C_{mic} contents can be explained by the sandy texture and low C_{org} content of the soil (Machulla et al. 2001). In the soil (sand-loam) of the long-term experiment in Skiernewiece, Poland, C_{mic} contents (70 to 150 mg kg^{-1}) were well comparable to the values obtained in this study (Weigel et al. 1998). Other studies reported increasing contents of C_{mic} with increasing FYM additions for loamy and sandy soils (Dick 1992; Weigel et al. 1998), therefore the lack of an increase with the rate in our study was unexpected. The last FYM applications occurred 29 months before sampling and the C input from berseem clover (previous crop) was estimated to be equal in all treatments (Figure 6). This probably superimposed the effect of the application rate on microbial biomass in our study, suggesting that the reported (Dick 1992; Weigel et al. 1998) response may have been only short.

3.3.4 C and net N mineralization

The mean of cumulative C mineralization after 266 days (Table 6) was of similar magnitude in the soils of the DYN (395 – 543 mg kg^{-1}) and FYM (336 – 527 mg kg^{-1}) treatments. Carbon mineralization was significantly affected by the rate but not by the type of fertilizer.

Cumulative net N mineralization after 266 days (Table 6) increased significantly with application rate in DYN and FYM treatments (Table 7). The general increase of net N mineralization with the rate of FYM is consistent with the results of others, reported for clayey loamy soils (Haploboroll, Haplargid) of temperate and subtropical climates (Whalen et al. 2001; Khorsandi and Nourbakhsh 2007). This suggests that increasing FYM additions increase the potential of SOM for net N mineralization across various soil types, textures and climates.

3.3.5 Total C_{org} and N_t contents

The C_{org} contents in 2007 (Figure 7, g kg^{-1}) decreased in the order DYN_H (9.2) > DYN_M (8.8) > DYN_L (8.3) > FYM_M (8.2) > FYM_H (8.1) > FYM_L (7.5). Type as well as rate had a significant effect on the C_{org} contents. Total N contents showed a similar pattern (C/N ratio: 10). The effect of FYM to increase C_{org} levels (Table 7) with increasing rate has been reported for several soil types and textures (Christensen 1996; Weigel et al. 1998). Nevertheless, the quantitative effect of the FYM rates in this study was low (Table 7). A similar result was also reported by Christensen (1996) for a sand loam. The less distinct rate effects on C_{org} levels of coarse textured soils indicate the importance of clay and silt particles for C stabilization in soils (von Lützow et al. 2008). Once the “capacity” of clay and silt particles approaches saturation, C input will be partitioned to a greater extent to a relatively labile pool (Stewart et al. 2008).

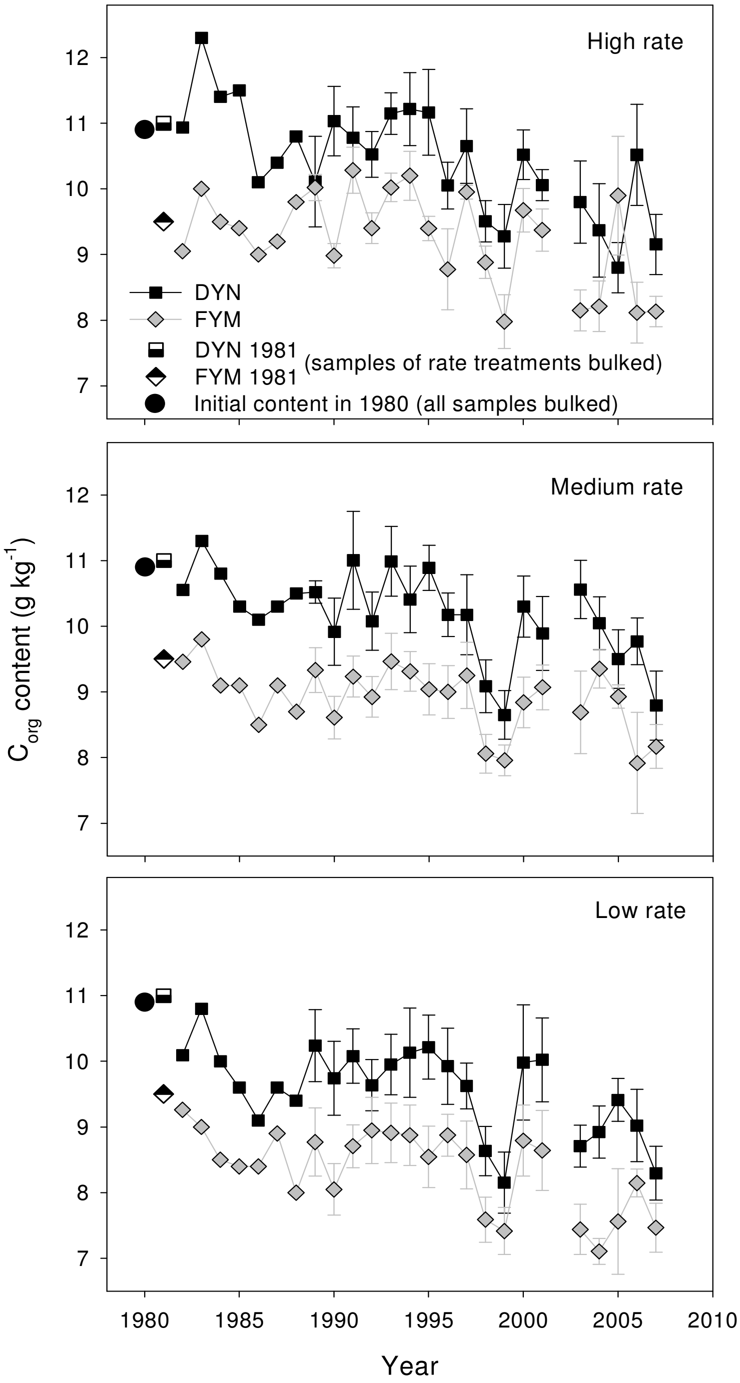

Figure 7: Time course of C_{org} contents (0-25 cm) in soils of DYN and FYM treatments. The initial value was measured for a bulked sample; measurements in 1981 were differentiated according to type but bulked across rate treatments. Until 1988 only means without errors were available, since 1988 mean values (n = 4) with standard errors. Values of FYM treatments were recalculated from Heitkamp et al. (2009). For treatment abbreviations see Table 5.

Differences in the results between DYN and FYM treatments in our study already appeared in 1982, two years after commencement of the field experiment (Figure 7). Two possible explanations for the results could be:

Explanation 1: Existing spatial variations were not recognized due to the bulking of soil samples at the commencement of the field experiment and observed effect of fertilizer type was not induced by treatments.

Explanation 2: Applications of BD-field preparations and BD-FYM resulted in an immediate effect on C and N storage and the preserving effect of DYN treatments on C_{org} and N_t contents has lasted ever since.

The high spatial and temporal variation in the C_{org} data (Figure 7) suggests that rapid changes might be possible in this sandy Cambisol. However, relative losses since the 1982 baseline value were higher in the DYN_H and DYN_M treatments. This suggests a slow convergence between FYM and DYN treatments, indicating that explanation 1 is more likely than explanation 2.

3.3.6 C pools

In the pool $C_{passive}$ 300 g (kg C_{org})$^{-1}$ were stored, regardless of the application rate (Figure **8**). Our finding is consistent with the concept that the passive pool (turnover time >> 600 years) is not influenced by recent management (Helfrich et al. 2007). Although the absolute values did not differ significantly between treatments, the amount of C in the passive pool was higher in the DYN treatments and accounted for 30% of the higher C_{org} values compared to the FYM treatments. Thus, at least one third of the differences in C_{org} contents cannot be ascribed to treatment effects (Helfrich et al. 2007).

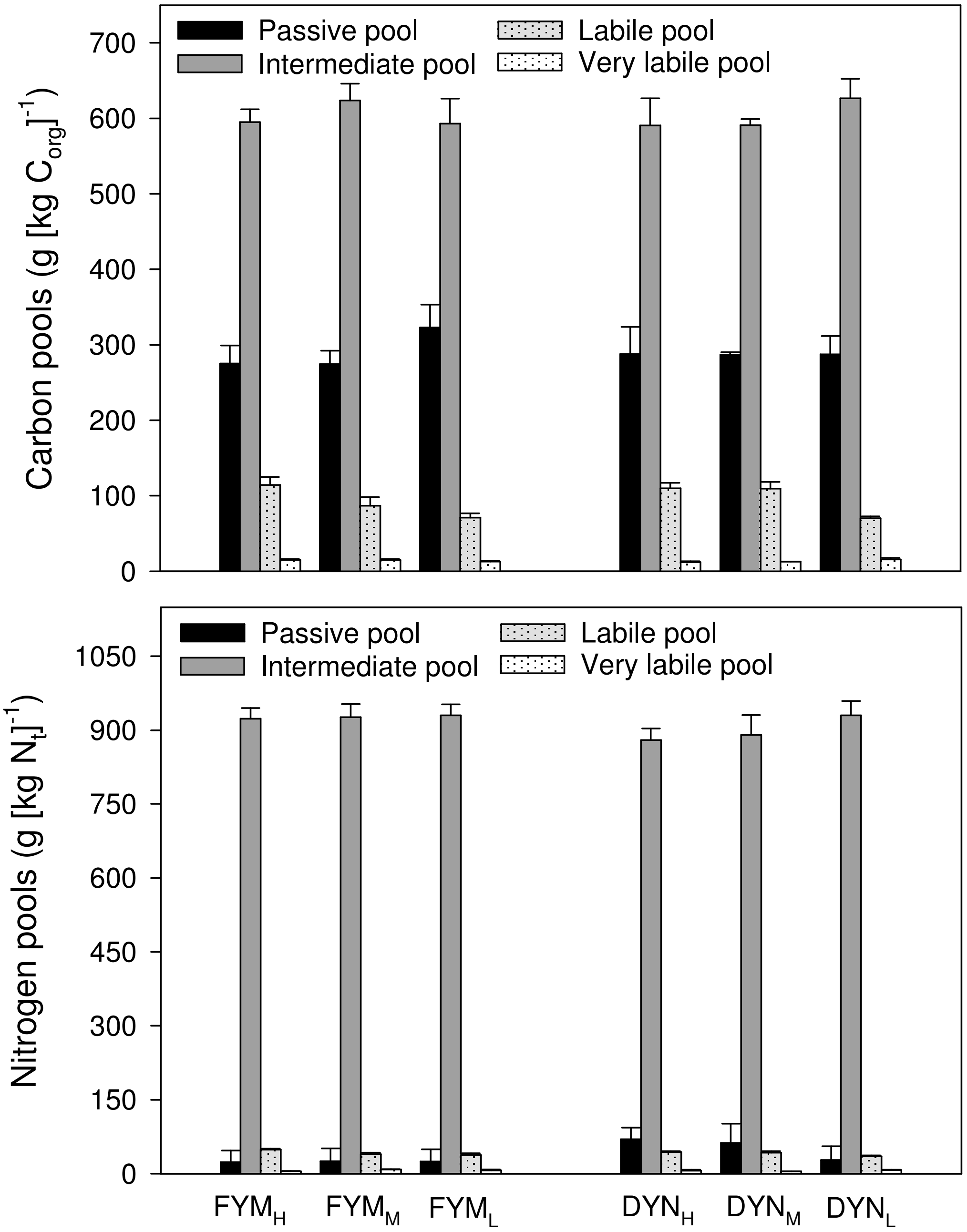

Figure 8: Proportion of carbon and nitrogen pools to Corg and total N contents of the soils (0-25 cm) of the different fertilization treatments. Mean values with standard errors (n = 4). Values of FYM treatments were recalculated from Heitkamp et al. (2009). For treatment abbreviations see Table 5.

The relative pool size of $C_{intermediate}$ comprised 600 g (kg C_{org})$^{-1}$ and was not influenced by the application rate (Table 7, Figure 8). The absolute amount (g kg^{-1}) of C stored in the intermediate pool was significantly affected by FYM type and comprised between 4.5 and 5.1 in the soils of the FYM and between 5.2 and 5.4 in soils of the DYN treatments (not shown). Therefore, ca. 55% of the observed difference in C_{org} between FYM and DYN treatments were located in $C_{intermediate}$. We assume a turnover time of decades for this pool (Parton et al. 1988; von Lützow et al. 2008). Thus, the marked losses of C_{org} contents between might be well explained by losses in the pool $C_{intermediate}$. Furthermore, the labile C pool at the same rate is not significantly different in FYM and DYN treatments (Figure 8). Assuming the same percentage of pool transfer ("humification") from labile to intermediate (Parton et al. 1988), this might explain the higher relative net loss of C_{org} in the DYN treatments compared to the FYM treatments (Table 6).

Table 7: Regression analysis of measured and modeled C and N pools of the soil (0–25 cm) of the DYN treatments with cumulative fertilizer Nt input (t ha^{-1}) from 1980 to 2007 (n = 12).

Properties	Intercept$^{\$}$	Slope§	R²
C_{org} (g kg^{-1})	7.8	0.5	0.16
C_{mic} (mg kg^{-1})	126	1	0.00
very labile C (g [kg C_{org}]$^{-1}$)	18	-2	0.32
labile C (g [kg C_{org}]$^{-1}$)	53	21	0.53
$C_{intermediate}$ (g [kg C_{org}]$^{-1}$)	643	-19	0.09
$C_{passive}$ (g [kg C_{org}]$^{-1}$)	287	0	0.00
N_t (g kg^{-1})	0.75	0.05	0.14
N_{mic} (mg kg^{-1})	18	0	0.00
very labile N (g [kg N_t]$^{-1}$)	7	-1	0.05
labile N (g [kg N_t]$^{-1}$)	30	5	0.43
$N_{intermediate}$ (g [kg N_t]$^{-1}$)	956	-27	0.12
$N_{passive}$ (g [kg N_t]$^{-1}$)	6	22	0.09

$^{\$}$The unit is given within the first column
§The unit is: (unit of intercept) (t ha^{-1})$^{-1}$

Between 70 and 114 g C (kg C_{org})$^{-1}$ was located in the labile pool (turnover time: 462 days, Figure 8). Increasing proportions of labile C were significantly related to the amount of FYM-N_t input ($R^2 = 0.53$, Table 7) and cumulative input of 1 t N_t ha^{-1} with FYM or BD-FYM (C/N-ratio: 13) led to an increase of labile C by 21-23 g (kg C_{org})$^{-1}$ (Table 7). This shows that increasing C input may shift relative C partitioning in favor of labile pools (Stewart et al. 2008).

The very labile C pool (turnover time: 17 days) comprised between 12 and 16 g (kg C_{org})$^{-1}$ and the proportion decreased slightly with the application rate (Figure 8, Table 7), but was unaffected by FYM type.

3.3.7 N pools

In the pool $N_{passive}$ only 25 to 70 g (kg N_t)$^{-1}$ were stored (Figure 8) and no type or rate effect occurred. With increasing decomposition the C/N ratio generally declines (Swift et al. 1979). Thus, the C/N ratio of the passive pool should be narrower than that of the bulk soil (Helfrich et al. 2007). This is, however, not the case for highly recalcitrant material such as charcoal or soot, which can have wide C/N ratios. The high C/N ratio (70) of passive SOM revealed in this study suggests the involvement of charred material in the formation of the passive pool.

The highest proportion of N (880 – 930 g [kg N_t]$^{-1}$) was stored in the pool $N_{intermediate}$ regardless of treatment (Figure 8). Similarly to $C_{intermediate}$ an effect of the rate was absent (Table 7) and we assume the same mechanisms were involved.

The proportion of labile N (turnover time 153 days) ranged from 35 to 49 g (kg N_t)$^{-1}$ and was related to the application rate, only ($R^2 = 0.43$, Table 7, Figure 8). Therefore, increasing FYM applications led to an accumulation of labile N in SOM. In our study, we found an increase of 5-6 g (kg N_t)$^{-1}$ per ton applied FYM-N_t in the labile N pool (corresponding to an increase of 6.0 mg (kg soil)$^{-1}$ (FYM-N_t)$^{-1}$). Increasing amounts of labile N with increasing FYM application rates have been reported for a loamy Chernozem by Whalen et al. (2001). They reported after 25 years of FYM applications (30 to 180 t FYM ha^{-1}, corresponding to ca. 320 to 1200 kg N_t ha^{-1}) an increase (mg (kg soil)$^{-1}$ (t FYM-N_t)$^{-1}$) of 2.2. Thus, the increase found in our study after two decades at comparably low rates of FYM application is relatively high.

The very labile N pool (turnover time: 9 days) comprised between 5 and 7 g (kg N_t)$^{-1}$ and was not affected by treatment.

3.4 Conclusions

Comparing the application of farmyard manure with and without biodynamic preparations revealed only minor, insignificant differences in the crop yields, content of microbial biomass, labile soil organic matter, and amount of mineralized carbon or nitrogen.

Total organic carbon and nitrogen contents were significantly higher in soils receiving BD preparations. Effects of fertilizer treatments could be excluded for about 30% of the differences in organic carbon and total nitrogen contents, due to storage in the passive pool (turnover >> 600 years). The major part (> 55%) of the differences in the contents of organic carbon and total nitrogen were located in the intermediate pool. The greater storage in the case of the DYN treatment in the slow pools (85%) and the C_{org} studies with time suggested that the observed differences between DYN and FYM treatments were likely to have existed since the beginning of the experiment.

Increasing amounts of farmyard manure led to increasing yields and increasing potential for C and N supply to microbes and plants from SOM in addition to direct fertilization. In contrast, the intermediate pools were not influenced by the FYM rate. Overall, management of sandy soils via manure application rates does not offer the option of large sequestration of carbon and thus reduction of greenhouse gases, since higher application rates resulted in higher sequestration of labile C only.

4 Effects of fertiliser type and rate on soil fractions of a sandy Cambisol – long-term and short-term dynamics

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Abstract

The application of density fractionation is an established technique, but studies on short term dynamics of labile soil fractions are scarce. Objectives were (i) to quantify the long-term and short-term dynamics of C and N in light fraction (LFOC, LFON, $\rho \leq 2.0$ g cm^{-3}) and soil microbial biomass C (C_{mic}) in a sandy Cambisol as affected by 28 years of different fertilization and (ii) to determine the incorporation of C_4-C into these labile fractions during one growing season of amaranth. The treatments were: straw incorporation plus application of mineral fertilizer (MSI) and application of farmyard manure (FYM) each at high (MSI_H, FYM_H, 140–150 kg N ha^{-1} year^{-1}) and low (MSI_L, FYM_L, 50–60 kg N ha^{-1} year^{-1}) rates at four field replicates. For all three sampling dates in 2008 (March, May and September), stocks of LFOC, LFON and C_{mic} decreased in the order $FYM_H > FYM_L > MSI_H, MSI_L$. However, statistical significance varied markedly among the sampling dates, e.g., with LFOC being significantly different ($p \leq 0.05$) in the order given above (sampling date in March), significantly different depending on the fertilizer type (May) or non-significant (September). The high proportion of LFOC on the stocks of soil organic C (45 to 55%) indicated the low capacity of soil organic matter stabilization on mineral surfaces in the sandy Cambisol. The incorporation of C_4-C in the LFOC during one growing season of amaranth was small in all four treatments with C_4-LFOC ranging from 2.1 to 3.0% of total LFOC in March 2009, and apparent turnover times of C_3-derived LFOC ranged from 21 to 32 years for the sandy soils studied. Overall, our study indicates that stocks of LFOC and LFON in a sandy arable soil are temporarily too variable to obtain robust significant treatment effects of fertilizer type and rate at common agricultural practices within a

season, despite the use of bulked six individual cores per plot, a common number of field replicates of four and a length of treatments (28 years) in the order of the turnover time (21 - 32 years) of C_3-derived LFOC.

4.1 Introduction

Density fractionations and the determination of microbial biomass are well established techniques to trace management effects on labile soil fractions (*Campbell* et al., 1999a,b, for reviews see *Smith* and *Paul* (1990) and *Gregorich* et al. (2006)). For instance, there is general evidence that various soils receiving farmyard manure (FYM) show higher levels of microbial biomass C (C_{mic}) compared to soils receiving mineral fertilizer only. Moreover, increasing additions of FYM led to increasing stocks of C_{mic} (*Weigel* et al., 1998). In contrast, effects of mineral fertilizer rates on C_{mic} levels depended on interactions with crop or soil type (*Vanotti* et al., 1997) and/or amount of crop residues returned (*Jacinthe* et al., 2002). Similar to C_{mic}, light fraction organic C (LFOC) is also generally considered as a suitable indicator which reacts to changes in management (*Gregorich* et al., 2006). For instance, increasing additions of FYM increased LFOC stocks in various loamy soils (*Liang* et al., 1998; *Carter* et al., 2003; *Gulde* et al., 2008). However, in loamy soils receiving mineral fertiliser, LFOC did not always increase and sometimes even decreased at high (up to 400 kg N ha^{-1} $year^{-1}$) application rates (*Liang* et al., 1998).

A large number of studies relied on one sampling date for the determination of labile soil fractions (e.g., *Haynes* 2000, *Carter* et al. 2003, *Malhi* et al. 2003, *Mariott* and *Wander* 2006, *Sleutel* et al. 2006, *Gulde* et al. 2008). This is surprising, since the few studies on seasonal dynamics available indicated that labile soil attributes had markedly temporal dynamics. For instance, in agro-ecosystems of moist temperate regions, stocks of C_{mic} and microbial biomass N (N_{mic}) tend to increase during the growing season (*Nieder* et al., 2008), perhaps caused by increasing temperatures or substrate availability due to plant development. Similarly, LFOC showed a marked variability among sampling dates in crop rotation (*Campbell* et al., 1999a,b) or tillage experiments (*Wander* and *Yang*, 2000; *Yoo* and *Wander*, 2008). This high dynamic may be the reason for different results from the same sampling site: *Liang* et al. (1998) sampled a site in Canada in spring 1995 and *Carter* et al. (2003) in autumn 1993 or 1994. One study reported significant increases in LFOC stocks with fertilizer N application rate, but the other did not. This suggests that labile soil fractions, which are sensitive to management, also exhibit a high temporal variability. Therefore, it is important to recognize short-term dynamics of labile soil fractions in order to evaluate their reliability to indicate long-term trends in C and N storage in the soil.

Objectives were (i) to quantify the long-term and short-term dynamics of soil organic carbon (C_{org}), C_{mic}, LFOC and light fraction organic N (LFON) in a sandy Cambisol as affected by 28 years of different fertilization and (ii) to determine the incorporation of C_4-C into the labile fractions during one growing season of amaranth.

4.2 Materials and methods

4.2.1 Site description

The field trial is situated near Darmstadt, Germany (49° 50’ N, 8° 34’ E, 100 above sea level). Annual mean temperature is 9.5°C and annual precipitation is 590 mm. The soil, a Haplic Cambisol, has developed on alluvial fine sands (Table 8). In 1980, the experiment was set up as a strip design with four replicates, the treatments being type and rate of fertilizer. Since 1985, the crop rotation consists of legume (clover, *Trifolium ssp*, or alfalfa, *Medicago sativa* L.) spring wheat (*Triticum aestivum* L.), potatoes (*Solanum tuberosum* L.) and winter rye (*Secale cereale* L.). Winter cover crops were mainly white mustard (*Sinapis alba* L.) and oil radish (*Raphanus sativa* L. var. *oleiformis* Pers.). The trial was managed organically (except for the application of mineral fertilizer in the some of the treatments described below) and weeding has been done mechanically. Four of nine fertilization treatments were considered here:

(i) MSI_H: high application rate of mineral fertilizer (150 kg N ha^{-1} to root crops or 100 kg N ha^{-1} plus 40 kg N ha^{-1} as second application to cereals) plus cereal straw incorporation.

(ii) MSI_L: low application rate of mineral fertilizer (50 kg N ha^{-1} to root crops or 60 kg N ha^{-1} to cereals) plus cereal straw incorporation.

(iii) FYM_H: high application rate of rotted farmyard manure: 27 Mg fresh weight ha^{-1} as manure to root crops or 16 Mg fresh weight ha^{-1} plus 40 kg N ha^{-1} with urine (second application) to cereals. The total N input corresponded to the mineral fertilizer input in treatment MSI_H.

(iv) FYM$_{L}$: low application rate of rotted farmyard manure: 9 Mg fresh weight ha^{-1} as manure to root crops or cereals. The total N input corresponded to the mineral fertilizer input in treatment MSI$_{L}$.

Legumes were not fertilized. Further details are given by *Raupp* and *Oltmanns* (2006) and *Heitkamp* et al. (2009). The management from March 2008 to March 2009 is given inTable 9. In 2008, the C$_4$-plant amaranth (*Amaranthus hypochondriacus* L.) was grown, making it possible to trace the incorporation of C$_4$-C in the labile soil fractions. Amaranth was fertilized according to the scheme of cereals described above. Due to the incorporation of amaranth straw in MSI and FYM treatments (1st of October 2008), the experimental setup has been markedly changed. Therefore, we present the data before (Figure 9 and Figure 10) and after the change (Table 8) separately.

Directly after tillage in May 08, the depth of the Ap horizon was 33 cm and bulk density was small (1.13 g cm^{-3}). For the other sampling dates, bulk density was greater (1.26 to 1.44 g cm^{-3}) and depth of the Ap horizon was 25 to 29 cm.

Table 8: Site characteristics of the Ap horizon (0 27 cm) of the soil of the Darmstadt experiment before introduction of amaranth into the crop rotation (March 2008). Means (n = 4) with standard errors in parentheses.

	pH	**Bulk density** / g cm^{-3}	**C$_{org}$** / Mg ha^{-1}	**N$_{t}$** / Mg ha^{-1}	**Particle size (µm) distribution**		
					< 2 / %	2 – 63 / %	63-2000 / %
MSI$_{L}$	6.0 (0.4)^{a}	1.37 (0.05)	27.1 (0.7)^{a}	2.3 (0.1)^{a}	4.7 (0.2)	10.5 (0.9)	84.8 (0.9)
MSI$_{H}$	6.1 (0.2)^{a}	1.42 (0.02)	26.8 (0.9)^{a}	2.3 (0.1)^{a}	4.6 (0.3)	9.0 (0.5)	86.5 (0.3)
FYM$_{L}$	6.6 (0.1)^{b}	1.38 (0.04)	30.1 (1.4)^{b}	2.6 (0.1)^{b}	5.6 (0.2)	9.0 (0.4)	85.4 (0.5)
FYM$_{H}$	6.5 (0.2)^{b}	1.37 (0.04)	32.2 (1.0)^{b}	2.8 (0.1)^{c}	4.9 (0.1)	8.9 (0.2)	86.2 (0.3)

Letters indicate comparison of means among fertilization treatments; exclusively those values, which are significantly different (LSD, $p \leq 0.05$) are followed by different letters.

4.2.2 Sampling and characterization

Soil sampling took place on 10th of March, 19th of May, 12th of September, 10th of December 2008 and 9th of March 2009 (Table 9). On every plot (25 m^2), six samples of the whole Ap-horizon (down to 25-33 cm depth) were bulked. The border to the B-horizon was clearly recognizable by change of color. Stocks were calculated based on an equivalent soil mass in every plot in the Ap-horizon (3660 Mg ha^{-1}), thus sampling depth varied for each sampling date between 25 and 33 cm due to varying bulk density. The soil was sieved (< 2 mm), and one part of the samples was stored field moist at 4°C and another part was dried (48 h at 40°C). Results are expressed on an oven-dry base (105°C). Bulk density was determined gravimetrically from soil cores, the pH (soil:solution ratio 1 : 2.5) was determined in 0.01 M $CaCl_2$. C_{org} and total N (N_t) were measured by dry combustion (Vario El, Elementar, Hanau, Germany).

4.2.3 Microbial biomass

Microbial biomass was analyzed by chloroform fumigation extraction (*Vance* et al., 1987) at 50% water holding capacity. Two portions of moist soil (10 g) were taken from the sample and pre-incubated for a week. One portion was fumigated with $CHCl_3$ for 24 h at 25°C. Both subsamples were extracted with 0.5 M K_2SO_4. The extracts were analyzed for organic C by infrared detection of CO_2 and for total N by chemo luminescence detection after combustion at 850°C (Dimatoc, Dimatec, Essen, Germany). Microbial biomass was calculated as the difference between fumigated and unfumigated samples with conversion factors of 0.45 and 0.54 for C and N respectively (*Brookes* et al., 1985; *Wu* et al., 1990).

4.2.4 Soil fractionation

The soil was fractionated by density according to *John* et al. (2005) with slight modifications. Briefly, 10 g of moist soil were placed in a centrifugation tube together with 40 ml sodiumpolytungstate (SPT). The SPT was adjusted to a cut-off density of $\rho = 2.0$ g cm^{-3} (*John* et al., 2005; *Marriott* and *Wander*, 2006). Due to the high sand content, separation of aggregate-occluded and free LFOC was not reasonable. The suspension was shaken overnight (18 h) together with 8 glass beads before centrifugation (30 minutes at 4000 *g*). After centrifugation, the supernatant (LFOC, LFON) was vacuum-filtered (0.45 µm) and washed with 2 l of distilled water to remove the SPT salt. The heavy fraction (>2 g cm^{-3}) was washed with distilled water and particles were precipitated with a few drops of $AlCl_3$.

4.2.5 Isotopic analysis

Bulk soil, isolated soil fractions, plant parts and salt extracts were finely ground for analysis of $\delta^{13}C$ values by an isotope ratio mass spectrometer (Finnigan MAT, DELTA^{plus}) coupled with an elementary analyzer (Fisons EA11081). Measurements were performed at the Center for Stable Isotope Research and Analysis, University of Göttingen, Germany. The C isotope ratio of the samples were expressed as δ^{13}C values (‰ V-PDB):

$$\delta^{13}C = ((R_{sam} / R_{std}) - 1) \times 10^3, \tag{8}$$

Where R_{sam} is the $^{13}C/^{12}C$ ratio for the sample and R_{std} is the $^{13}C/^{12}C$ratio of the reference standard.

Samples for determination of the isotopic signature of C_{mic} were extracted with 0.05 M K_2SO_4, because higher salt concentrations could hamper the analysis (*Potthoff* et al., 2003). The extracts were freeze dried. The δ^{13}C value of C_{mic} ($\delta_{13}C_{MB}$) was calculated by a mass balance equation:

$$\delta^{13}C_{MB} = (\delta^{13}C_F \times C_F - \delta^{13}C_{NF} \times C_{NF}) / (C_F - C_{NF}), \tag{9}$$

where $\delta_{13}C_F$ is the δ^{13}C value (‰ V-PDB) of the fumigated extract, $\delta_{13}C_{NF}$ is the δ^{13}C value (‰ V-PDB) of the unfumigated sample, C_F is the concentration (µg g^{-1}) of extractable C from the fumigated and C_{NF} from the unfumigated soil samples.

Proportions of amaranth-derived C were calculated as:

$$f = (\delta_{sample} - \delta_{ref}) / (\delta_{amaranth} - \delta_{wheat}), \tag{10}$$

where δ_{sample} is the δ^{13}C value (‰ V-PDB) of the sample under C_4 vegetation or with C_4-derived residues, δ_{ref} is the value of the sample before amaranth was planted, $\delta_{amaranth}$ is the signature of amaranth biomass and δ_{wheat} is the value of winter wheat. As δ_{ref}, we used material from the first two sampling dates before amaranth was sown. The mean value (± standard error, n = 16) of δ_{wheat} was -28.11 ± 0.04‰ V-PDB. After harvest and before straw incorporation, we used the δ^{13}C values of amaranth roots and after incorporation a weighted mean of root and straw signature for $\delta_{amaranth}$. The mean values (± standard error, n = 16) were -12.24 ± 0.05‰ V-PDB for roots and -13.21 ± 0.04‰ V-PDB for above ground biomass, the latter accounting for 62±1% of the amaranth-derived C-input after straw and residue incorporation. The apparent turnover time *T* of LFOC was calculated assuming a single pool with kinetics of first order decay:

$$T = 1/k = -(t - t_0) / \ln(C_t / C_{t0}), \tag{11}$$

Where k is the decay constant, t the time of sampling, t_0 the time of vegetation change (germination of amaranth on the 30th of May), C_t is the remaining portion of C_3-C (%) at sampling time and Ct_0 is the C_3-C at t_0 (100%).

4.2.6 Statistics

All data were normally distributed (Lilliefors-test, $p \leq 0.05$). Significance of effects ($p \leq 0.05$) was tested with a two-factorial (fertilizer type and rate) linear mixed model (SAS 9.1, SAS Institute, Cary, USA) according to the randomization structure (strip design) of the field experiment (for details see *Heitkamp* et al., 2009). Statistics were calculated separately for every sampling date.

Table 9: Dates of management and soil sampling.

Date	Event
10/03/2008	1st soil sampling and fractionation
17/04/2008	FYM application (60 and 100 kg N ha^{-1} to FYM$_L$ and FYM$_H$, respectively) Ploughing of all plots
19/05/2008	2nd soil sampling and fractionation
23/05/2008	Application of mineral fertilizer (60 and 100 kg N ha^{-1} to MSI$_L$ and MSI$_H$, respectively) Sowing of amaranth (germination after 1 week)
30/06/2008	2nd application of N to MSI$_H$ (40 kg N ha^{-1})
04/07/2008	2nd application of N to FYM$_H$ (40 kg N ha^{-1} as urine)
12/09/2008	Harvest of amaranth 3rd soil sampling and fractionation, and root sampling
01/10/2008	Incorporation (15 cm) of harvest residues and 3.6 Mg fresh mass ha^{-1} straw
20/10/2008	Sowing of oil radish (emerged until germinal sheet stage, perished by frost in winter)
10/12/2008	4th soil sampling and fractionation
09/03/2009	5th soil sampling and fractionation

4.3 Results and discussion

4.3.1 Long-term effects on C_{org} and N_t stocks

All three sampling dates in 2008 (March, May and September) indicated that stocks of C_{org} decreased in the order FYM$_H$ > FYM$_L$ > MSI$_H$, MSI$_L$ (Figure 9). This effect of fertilizer type is consistent with results of others for soils of varying type and texture (*Persson* and *Kirchmann*, 1994; *Weigel* et al., 1998).

Differences in C_{org} stocks between the treatments, however, were significant only for the first two sampling dates, whereas in September 08 no significant effects occurred (Figure 9). Spatial variation of C_{org} stocks was highest in September (FYM_L, FYM_H, MSI_H) or May (MSI_L), suggesting that an increased spatial variation of C_{org} stocks in the Ap horizon after the growing season in September at least partly contributed to the absence of significant results between the treatments. Similarly, N stocks decreased in the same order as C_{org} stocks and significant differences depended on the sampling date (Figure 9).

4.3.2 Dynamics of microbial biomass

Microbial biomass C and N decreased in the order $FYM_H > FYM_L > MSI_H$, MSI_L in 3 of 5 sampling dates, but significant treatment effects were found only in May, eight weeks after FYM application (Figure 10, Table 10). Low stocks are typical for sandy arable soils (*Weigel* et al., 1998). Likewise, higher stocks of C_{mic} were reported for soils receiving FYM compared to soils receiving mineral fertilizer (*Liang* et al., 1998; *Weigel* et al., 1998). However, the small difference between MSI and FYM treatments in our study might be explained by the straw incorporation in the MSI treatments, as also shown by *Witter* et al. (1993) in a clayey Cambisol in the Ultuna experiment, Sweden, where straw incorporation completely evened out the different size of C_{mic} between FYM and mineral fertilization.

Stocks of C_{mic} (Figure 10) increased continuously (by 55 to 150 kg C ha^{-1}) from March 2008 to December for all treatments investigated. This was in the range of increases (mean of 120 kg ha^{-1}) reviewed by *Nieder* et al. (2008) and was induced by increasing substrate availability from rhizodeposits and crop litter (Table 10). After winter, the levels of C_{mic} stocks decreased to the levels observed in March 2008. Earlier investigations (1991/1992) in the same experiment also revealed increasing C_{mic} stocks from spring to summer. In summer, C_{mic} stock of the FYM_H treatment was significantly higher than that of MSI_H. After a decline in autumn C_{mic} stocks rose again in January 1992 (*Bachinger*, 1996). Overall, the concern, that C_{mic} might be too sensitive to environmental conditions for an indication of management effects (*Geisseler* and *Horwath*, 2009) is supported by the dependence of treatment effects on sampling dates (Figure 10).

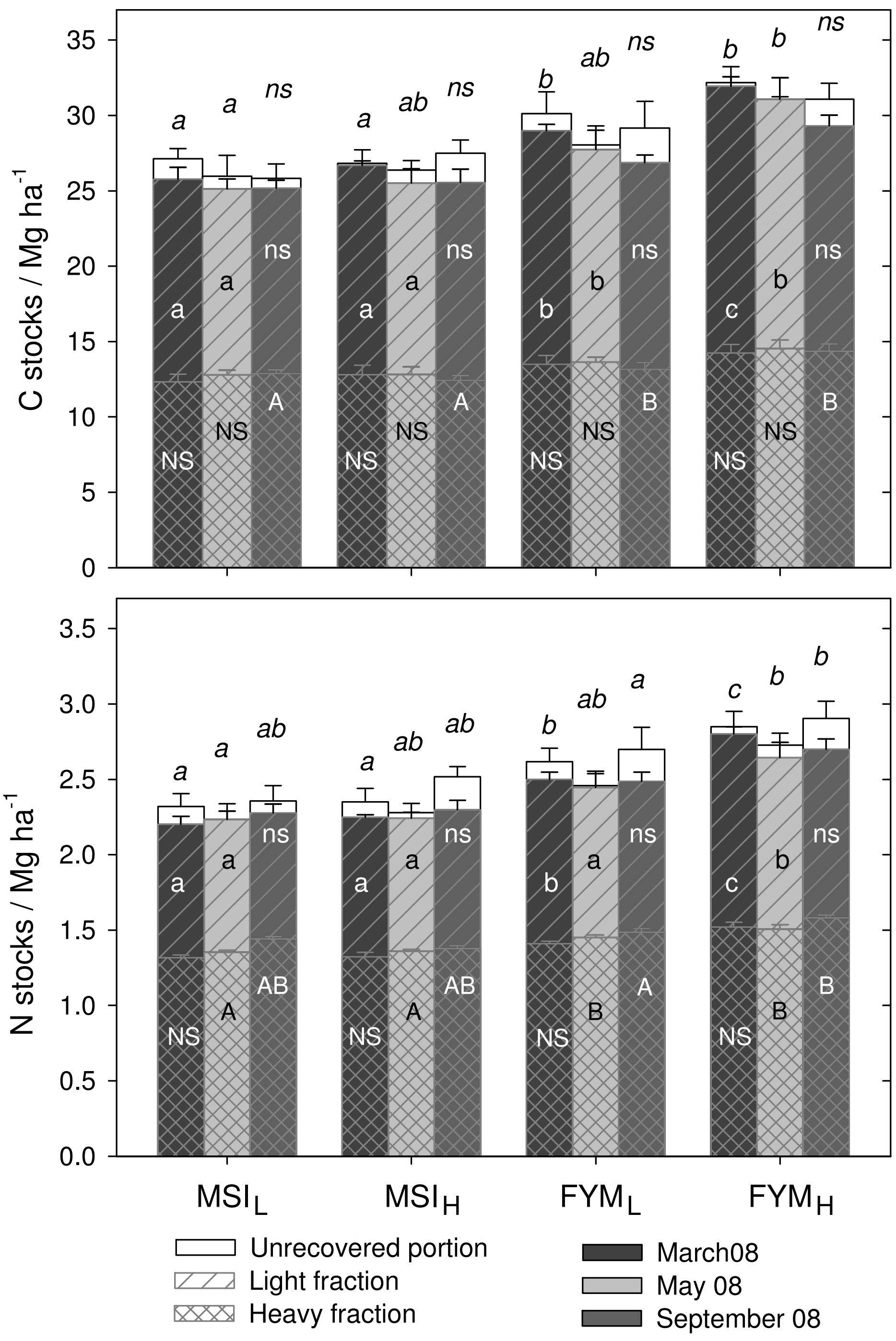

Figure 9: Seasonal course of C and N stocks (A-horizon) in whole soil (fractions plus unrecovered portion) and soil fractions of the fertilizer treatments using an equivalent mass approach. Different letters indicate significant differences at one single sampling date in the total stock (italic letters), in the light fraction (lower case letters) or in the heavy fraction (upper case letters). Non-significant effects are designated by ns. Means and standard errors.

Microbial biomass N showed a similar temporal course to C_{mic} (Figure 10). However, for C_{mic}, differences between treatments were significant for the second sampling date, whereas for N_{mic}, differences were significant for the first and third sampling date (Figure 10).

With 12.2 to 24.9% C_4-derived C_{mic} of the total C_{mic} (Table 10), C_4-derived C_{mic} responded fast to the cultivation of amaranth due to the small size of C_{mic}. *Rochette* and G*regorich* (1998) reported 9% of C_{mic} was derived from maize (input to soil: 4.6 Mg C ha^{-1}) in a loamy Dystrochrept. Therefore, the proportion of active microbial biomass was high in the sandy Cambisol. Nevertheless, even after 40 years of maize cultivation *John* et al (2003) reported that only 45% of C_{mic} were derived from maize, indicating an effective recycling of "old" C. Consequently, the high rate of "new" C incorporation into C_{mic} is not likely to persist.

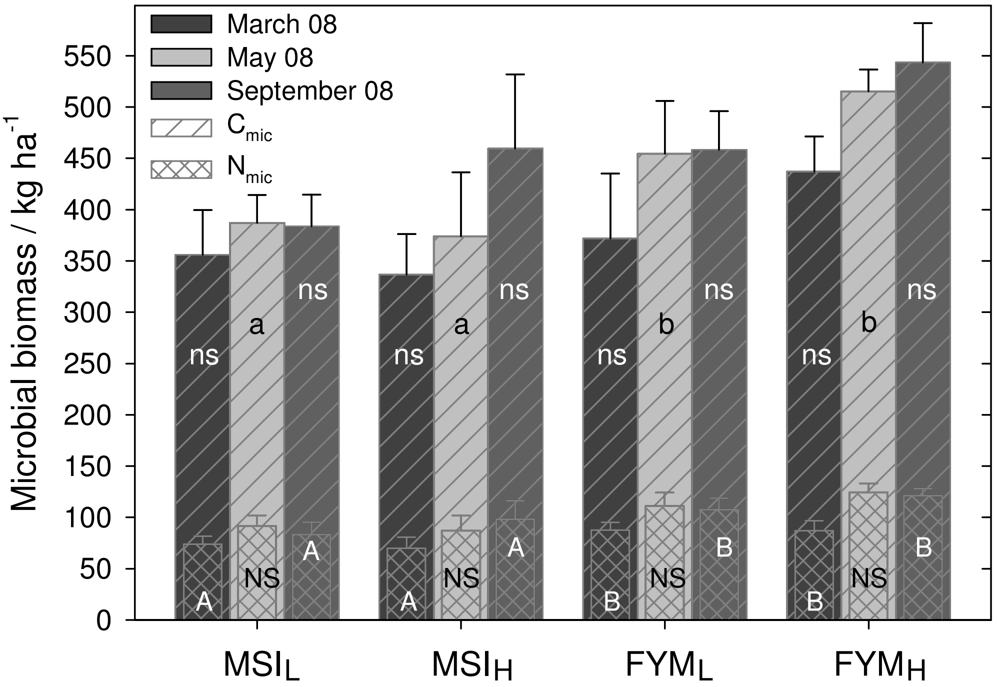

Figure 10: Stocks of microbial biomass C and N in the soil (A-horizon) of the fertilizer treatments using an equivalent soil mass approach. Different letters indicate significant differences between treatments at one single sampling date in C_{mic} (lower case letters) or in N_{mic} stocks (upper case letters). Non-significant effects are designated by ns. Means and standard errors (n = 4).s

4.3.3 Dynamics of density fractions

The light fraction comprised between 1.1 to 1.8% of soil mass (data not shown). This is in accordance with the 0.2 to 2.4% reported by *Janzen* et al. (1992) for clayey and loamy Chernozems. However, the proportion of LFOC on total C_{org} in our study was much higher (45 to 55%) than previously reported (1 to 35%) for loamy arable soils (*Gregorich* et al., 2006). The main reason, for the higher proportion reported here is most probably the low clay (5%) and silt (9%) content of the Cambisol (Table 8), leading to a proportional small capacity for C stabilization on mineral surfaces (*Baldock* and *Skjemstad*, 2000).

For all three sampling dates in 2008 (March, May and September) stocks of LFOC (Mg ha^{-1}) decreased in the order FYM_H (17.7-15.0 > FYM_L (15.5-13.7 > MSI_H (13.9-12.7) ≥ MSI_L (13.4-12.3) (Figure 9). Stocks of LFON were in the same order (C/N ratio: 14). The order of stocks of LFOC in the treatments agree with those reported for loamy soils comparing the application of mineral fertilizer (only stubble and root incorporation) and FYM (*Liang* et al., 1998; *Carter* et al., 2003).

Statistical significance for treatment effects on LFOC varied markedly among the sampling dates: stocks of LFOC were significantly different ($p \leq 0.05$) in the order given above (sampling date in March), significantly different depending on the fertilizer type (May) or non-significant (September). Thus, 28 years of continuous treatments were not sufficient to obtain robust significant treatments effects of fertilizer type and rate at common agricultural practices on stocks of LFOC. For two Canadian long-term crop rotations on a silt loam, *Campbell* et al. (1999a) also reported a marked seasonal variation of LFOC and explained 29% of variability for LFOC by using multiple regressions which included temperature, moisture and precipitation. They suggested that the assessment of labile soil attributes should be done several times during a season or at the same time in subsequent years.

The cultivation of amaranth from May until September resulted in only slight stocks of C_4-derived LFOC. C_4-derived stocks ranged from 65 to 152 kg C ha^{-1}, which accounted for 0.6 to 1.2 % of the total LFOC (Table 8). Incorporation of amaranth straw in October resulted in further increases of C_4-derived LFOC, but contribution of C_4-derived LFOC to the total LFOC ranged only from 1.8 to 3.8% in December 2008 and from 2.1 to 3.0% in March 2009 (Table 8), indicating that many years are required for a built-up of LFOC due to changed management. The small incorporation of amaranth-C into LFOC may be explained by a fast mineralization (approximately 40 to 60%, unpublished data) compared to humification of amaranth. Using equation 4 resulted in apparent turnover times between 21 and 32 years (Table 8). This apparent turnover time consisted very well with those (22 to 36 years) reviewed by Gregorich et al. (2006) after vegetation changes of 12 to 30 years. However, one should keep in mind that the assumption of a first order kinetics for the dynamics of LFOC is only a rough approximation, since besides other factors, the presence of coal or soot in the LFOC pool has a significant effect on the turnover time of LFOC (*Flessa* et al., 2008).

Table 10:Proportion of LFOC and Cmic derived from amaranth and calculated apparent turnover time of C_3-derived LFOC as a mean of three sampling dates. Means and standard errors (n = 4).

	C_4-derived proportion / %			**Apparent turnover time /** years
	September 08	December 08	March 09	
LFOC				
MSI_L	1.2 (0.1)	3.8 (0.5)	3.0 (0.5)	21
MSI_H	0.6 (0.1)	2.4 (0.5)	2.9 (0.3)	32
FYM_L	0.8 (0.4)	2.7 (0.7)	2.1 (0.4)	31
FYM_H	1.1 (0.3)	1.8 (0.5)	2.4 (0.6)	29
C_{mic}				
MSI_L	6.1 (1.4)a	21.0 (2.7)	12.2 (0.9)a	
MSI_H	12.7 (2.4)b	28.0 (2.5)	24.9 (3.0)b	
FYM_L	5.7 (1.6)a	18.0 (5.4)	14.3 (1.1)a	
FYM_H	10.2 (1.6)b	25.4 (2.4)	21.4 (2.0)b	

Letters indicate comparison of means among fertilization treatments; exclusively those values, which are significantly different (LSD, $p \leq 0.05$) are followed by different letters.

Considering the turnover time (21 - 32 years) of C_3-derived LFOC in our study, the duration of 28 years of fertilizer type and rate treatments at common agricultural practices was expected to be sufficient for a detection of significant effects on LFOC and LFON stocks. The absence of robust significant treatments effects may be due to the large spatial variability of these stocks. Alluvial sands exhibit a high spatial heterogeneity of pH values, influencing soil properties such as C_{org} contents, microbial biomass and respiration (*Heinze* et al., 2010). Although we bulked six individual cores per plot (25 m^2) and had four field replicates, intra-plot variation still could have diminished the power of our statistical tests (*Webster*, 2007). A larger number of soil cores per plot and more field replicates may be helpful for the assessment of statistically significant treatment effects, but the large costs of maintaining field experiments often result in a replicate number of three or four.

The stocks of heavy fraction organic C (HFOC, Figure 9) were similar for all treatments and four sampling dates, indicating that 28 years of contrasting fertilization did not induce marked differences in HFOC stocks. However, since September 2008, significant differences between the FYM and the MSI treatments occurred, but absolute differences were only small (Figure 9). For marked changes in HFOC stocks, much larger FYM inputs are required. For instance, *Gulde* et al. (2008) reported significant increases in mineral associated C with annual application of FYM at rates of 7 to 13 Mg C ha^{-1}.

Only for the last sampling date, the data indicated that significant amounts (1.7 to 2.0 Mg ha^{-1} in MSI and 1.0 Mg ha^{-1} in FYM treatments) of LFOC were apparently net-transferred into HFOC between December 2008 and March 2009 (not shown). Similarly, *Wander* and *Yang* (2000) observed an increase in the heavy fraction during winter after straw incorporation in autumn. Since it has been shown that freeze-thaw cycles reduce fungal biomass, increase leaching of dissolved organic carbon and induce changes in the molecular structure of soil organic matter, especially of labile components like free lipids and sugars (*Feng* et al., 2007; *Schmitt* et al., 2008), we speculate that freeze-thaw cycles were involved in the net transfer from LFOC to HFOC, because we observed at least three periods of frost down to 20 cm between December 2008 and March 2009.

4.4 Conclusions

In the duration of the field experiment (28 years), soils receiving rotted farmyard manure generally showed higher stocks of C_{org}, N_t, LFOC, LFON and C_{mic}, compared to application of mineral fertilizer with straw incorporation. The higher stocks of C_{org} and N_t in soils receiving FYM were mainly caused by storage of C and N in the light fraction. Because the proportion of LFOC on total C_{org} was very high in this sandy Cambisol. Moreover, management by fertilization practices induced only minor differences in C and N stocks, stabilized by soil minerals. Therefore, in the sandy Cambisol of our study, the relative importance of recalcitrance for stabilization of C and N stocks is high, since physical protection by aggregates or mineral associations are less relevant compared to loamy soils. The apparent turnover time of LFOC was between 21 and 32 years, which was in good agreement with estimates from studies with substantially longer vegetation changes compared to our study. Overall, long-term effects on SOM and its fractions were mainly governed by fertilizer type, but statistical significance of each treatment’s effect depended markedly on the sampling date.

5 Conclusions

The combination of different methods (incubation, simple modelling, chemical fractionation) applied to soil samples of the long-term (27 years) field experiment enhanced the understanding of SOM partitioning under different fertilizer management. More precisely, the modelled labile SOM pools were significantly affected by fertilizer rate and not by type. Therefore, additional plant nutrition from SOM mineralization might be similar between fertilizer types at the given application rate. The higher total SOM stocks in FYM treatments, compared to MSI treatments, were due to an increased storage of SOM in the intermediate pool. These results showed that differences in SOM stocks induced by fertilizer rate may be short-lived, while differences induced by fertilizer type may persist for decades after changing management in the sandy Cambisol investigated.

The combined methodological approach was also used for explanation of previously reported differences in the SOM content between DYN and FYM treatments. The approach revealed that about 30% of the differences in SOM contents between DYN and FYM treatments could not be ascribed to application of BD-preparations due to storage in the passive, management independent, pool. Together with large differences in C_{org} contents between fertilizer types already apparent two years after the start of the field experiment and a slow convergence of C_{org} contents with time, the observed differences were likely to have existed since the beginning of the experiment. Consequently, the use of BD-preparations as a single element of biodynamic agriculture had no effect on soil fertility or C sequestration. Overall, the application rate of FYM (with and without BD-preparations) led to increasing crop yields and an increasing potential for C and N supply to microbes and plants from SOM in addition to direct fertilization. However, increasing additions of FYM did not increase C sequestration in stable pools in the sandy Cambisol.

By using a density fractionation, differences in SOM stocks between fertilizer types and FYM rates were explained by storage of SOM in the light fraction ($\rho \leq 2.0$ g cm^{-3}). This, together with the high proportions of LFOC and LFON (45 - 55%), showed the crucial importance of selective preservation as a stabilization mechanism of OM in this sandy Cambisol. Short-term dynamics of light fraction and microbial biomass influenced markedly the statistical significance of treatment effects. However, LFOC and LFON stocks were always in the order $FYM_H > FYM_L > MSI_H, MSI_L$. Incorporation of crop residue C into the light fraction was quantitatively of minor importance, due to the small residue input compared to the large stock of LFOC. When measuring labile SOM fractions, such as light fraction and microbial biomass, several measurements throughout the year should be taken. However, variation was lowest in samples taken in early spring and was therefore the preferable “single sampling” date. It remains open to question if this result is transferable in general.

Overall, the quantitatively most important differences in the sandy Cambisol were found between fertilizer types (MSI and FYM) in non-passive pools or fractions (i.e. intermediate pool, light fraction). The information provided by the combined approach (incubation, chemical fractionation, simple modelling) and by density fractionation was complementary. More precisely, density fractionation indicated selective preservation as the major stabilisation mechanism in the sandy Cambisol, whereas using the combined approach allowed judging the fertilizer rates as short-lived effects. For the sandy Cambisol investigated, this indicated that switching fertilization from mineral to organic may increase C storage in the long-term, whereas increasing the rate of FYM or mineral fertilizer (including cereal straw incorporation) leads to additional nutrient release from SOM.

6 References

Abele U (1987) Produktqualität und Düngung - mineralisch, organisch, bio-dynamisch. Schriftenreihe des Bundesministers für Ernährung, Landwirtschaft und Forsten, 345. Landwirtschaftsverlag GmbH, Münster-Hiltrup

Aeschlimann U, Nosberger J, Edwards P J, Schneider M K, Richter M, Blum H (2005) Responses of net ecosystem CO_2 exchange in managed grassland to long-term CO_2 enrichment, N fertilization and plant species. Plant Cell Environ 28:823-833

Alvarez R (2005) A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use Manage 21:38-52

Anderson T-H, Domsch K H (1989) Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol Biochem 21:471-479

Bachinger J (1996) Der Einfluss unterschiedlicher Düngungsarten (mineralisch, organisch, biologisch-dynamisch) auf die zeitliche Dynamik und räumliche Verteilung von bodenchemischen und -mikrobiologischen Parametern der C- und N-Dynamik sowie auf das Pflanzenwachstum von Winterroggen. Ph.D.-thesis, Justus-Liebig-Universität, Gießen, Germany.

Bachinger J, Zander P (2004). Planungswerkzeuge zur Optimierung der Stickstoffversorgung in Anbausystemen des Ökologischen Landbaus - Standort- und Vorfruchtabhängige Kalkulation der N-Salden von Anbauverfahren. Ressortforschung für den ökologischen Landbau, Braunschweig, FAL Agricultural Research, pp 21-30

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

Balesdent J (1996) The significance of organic separates to carbon dynamics and its modelling in some cultivated soils. Eur J Soil Sci 47:485-493

Benbi D K, Richter J (2002) A critical review of some approaches to modelling nitrogen mineralization. Biol Fert Soils 35:168-183

Beraud J, Fine P, Yermiyahu U, Keinan M, Rosenberg R, Hadas A, Bar-Tal A (2005) Modeling carbon and nitrogen transformations for adjustment of compost application with nitrogen uptake by wheat. J Environ Qual 34:664-675

Bonde T A, Schnürer J, Rosswall T (1988) Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Biol Biochem 20:447-452

Böttcher J (2004) Uncertainties of nonlinearly estimated parameters from incubations of soil organic matter. J Plant Nutr Soil Sci 167:293-302

Brookes P C, Landman A, Pruden G, Jenkinson D S (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837-842

Campbell C A, Biederbeck V O, Wen G, Zentner R P, Schoenau J, Hahn D (1999) Seasonal trends in selected soil biochemical attributes: Effects of crop rotation in the semiarid prairie. Can J Soil Sci 79:73-84

Campbell C A, Lafond G P, Biederbeck V O, Wen G, Schoenau J, Hahn D (1999b) Seasonal trends in soil biochemical attributes: Effects of crop management on a Black Chernozem. Can J Soil Sci 79:85-97

Carpenter-Boggs L, Kennedy A C, Reganold J P (2000a) Organic and biodynamic management: Effects on soil biology. Soil Sci Soc Am J 64:1651-1659

Carpenter-Boggs L, Reganold J P, Kennedy A C (2000b) Effects of biodynamic preparations on compost development. Biol Agric Hort 17:313-328

Carter M R, Angers D A, Gregorich E G, Bolinder M A (2003) Characterizing organic matter retention for surface soils in eastern Canada using density and particle size fractions. Can J Soil Sci 83:11-23

Chantigny M H (2003) Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113:357-380

Christensen B T (1992) Physical fractionation of soil and organic matter in primary particle size and density seperates. Advances in Soil Science 20:1-90

Christensen B T (1996a) The Askov long-term experiments on animal manure and mineral fertilizers. In: Smith J U (eds) Evaluation of soil organic matter models using existing long-term datasets. Springer, Berlin, pp 301-312

Christensen B T (1996b) Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: revision of model structure. In: Smith J U (eds) Evaluation of soil organic matter models using existing long-term datasets. Springer, Berlin, pp 143-159

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

Cuypers C, Grotenhuis T, Nierop K G J, Franco E M, de Jager A, Rulkens W (2002) Amorphous and condensed organic matter domains: the effect of persulfate oxidation on the composition of soil/sediment organic matter. Chemosphere 48:919-931

Debreczeni K, Körschens M (2003) Long-term field experiments of the world. Archives of Agronomy and Soil Science 49:465-483

Dick R P (1992) A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agr Ecosyst Environ 40:25-36

DIN ISO 11277 (2002) Bodenbeschaffenheit – Bestimmung der Partikelgrößenverteilung in Mineralböden – Verfahren mittels Siebung und Sedimentation.

Edmeades D C (2003) The long-term effects of manures and fertilisers on soil productivity and quality: a review. Nutr Cycl Agroecosys 66:165-180

El-Haris M K, Cochran V L, Elliot L F, Bezdicek D F (1983) Effect of tillage, cropping and fertilizer management on soil nitrogen mineralization potential. Soil Sci Soc Am J 47:1157-1161

Elliott E T, Paustian K, Frey S D (1996) Modeling the measurable or measure the modelable: a hierarchical approach to isolate meaningful soil organic matter fractions. In: Powlson D S, Smith P, Smith J U (eds) Evaluation of soil organic matter models using existing long-term datasets. Springer, Berlin, pp 161-180

Fang C, Smith P, Smith J U (2005) A simple equation for simulating C decomposition in a multi-component pool of soil organic matter. Eur J Soil Sci 56:815-820

Feng X, Nielsen L L, Simpson M J (2007) Responses of soil organic matter and microorganisms to freeze-thaw cycles. Soil Biol Biochem 39: 2027-2038

Flessa H, Ludwig B, Heil B, Merbach W (2000) The origin of soil organic C, dissolved organic C and respiration in a long-term maize experiment in Halle, Germany, determined by C-13 natural abundance. J Plant Nutr Soil Sci 163:157-163

Flessa H, Amelung W, Helfrich M, Wiesenberg G L B, Gleixner G, Brodowski S, Rethemeyer J, Kramer C, Grootes P M (2008) Storage and stability of organic matter and fossil carbon in a Luvisol and Phaeozem with continuous maize cropping: A synthesis. J Plant Nutr Soil Sci 171:36-51

Fließbach A, Oberholzer H-R, Gunst L, Mäder P (2007) Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agr Ecosyst Environ 118:273-284

Franko U (1997) Modellierung des Umsatzes der organischen Bodensubstanz. Arch Acker- Pfl Boden 41:527-547

Geisseler D, Horwath W (2010) Short-term dynamics of soil carbon, microbial biomass, and soil enzyme activities as compared to longer-term effects of tillage in irrigated row crops. Biol Fert Soils in press

Golchin A, Oades J, Skjemstad J, Clarke P (1994) Study of free and occluded particulate organic matter in soils by solid state 13C Cp/MAS NMR spectroscopy and scanning electron microscopy. Aust J Soil Res 32:285-309

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

Gulde S, Chung H, Amelung W, Chang C, Six J (2008) Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Sci Soc Am J 72:605-612

Habteselassie M Y, Stark J M, Miller B E, Thacker S G, Norton J M (2006) Gross nitrogen transformations in an agricultural soil after repeated dairy-waste application. Soil Sci Soc Am J 70:1338-1348

Haynes R J (2000) Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. Soil Biol Biochem 32: 211-219

Heinze S, Raupp J, Joergensen R G (2010) Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant Soil in press

Heitkamp F, Raupp J, Ludwig B (2009) Impact of fertilizer type and rate on carbon and nitrogen pools in a sandy Cambisol. Plant Soil 319:259-275

Helfrich M, Ludwig B, Buurman P, Flessa H (2006) Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state C-13 NMR spectroscopy. Geoderma 136:331-341

Helfrich M, Flessa H, Mikutta R, Dreves A, Ludwig B (2007) Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. Eur J Soil Sci 58:1316-1329

Jacinthe P A, Lal R, Kimble J M (2002) Effects of wheat residue fertilization on accumulation and biochemical attributes of organic carbon in a central Ohio Luvisol. Soil Sci 167:750-758

Janzen H H, Campbell C A, Brandt S A, Lafond G P, Townley-Smith L (1992) Light-fraction organic matter in soils from long-term crop rotations. Soil Sci Soc Am J 56:1799-1806

John B, Ludwig B, Flessa H (2003) Carbon dynamics determined by natural C-13 abundance in microcosm experiments with soils from long-term maize and rye monocultures. Soil Biology & Biochemistry 35:1193-1202

John B, Yamashita T, Ludwig B, Flessa H (2005) Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128:63-79

Kalbitz K, Schmerwitz J, Schwesig D, Matzner E (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113:273-291

Khan S A, Mulvaney R L, Ellsworth T R, Boast C W (2007) The myth of nitrogen fertilization for soil carbon sequestration. J Environ Qual 36:1821-1832

Khanna P K, Ludwig B, Bauhus J, O´Hara C (2001) Assessment and significance of labile organic C pools in forest soils. In: Lal R, Kimble J M, Follett R F, Stewart B A (eds) Assessment methods for soil carbon. Lewis Publishers, Boca Raton, pp 167-182

Khorsandi N, Nourbakhsh F (2007) Effect of amendment of manure and corn residues on soil N mineralization and enzyme activity. Agron Sustain Dev 27:139-143

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

Koepf H H, Schaumann W, Haccius M (1996) Biologisch-dynamische Wirtschaftsweise: eine Einführung. Ulmer, Stuttgart

Knorr W, Prentice I C, House J I, Holland E A (2005) Long-term sensitivity of soil carbon turnover to warming. Nature 433:298-301

Koepf H H, Schaumann W, Haccius M (1996) Biologisch-dynamische Wirtschaftsweise: eine Einführung. Ulmer, Stuttgart

Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. J Plant Nutr Soil Sci 171:61-82

Larney F J, Ellert B H, Olson A F (2005) Carbon, ash and organic matter relationships for feedlot manures and composts. Can J Soil Sci 85:261-264

Ledgard S F, Steele K W (1992) Biological nitrogen fixation in mixed legume/grass pastures. Plant Soil 141:137-153

Liang B C, MacKenzie A F, Schnitzer M, Monreal C M, Voroney P R, Beyaert R P (1998) Management-induced change in labile soil organic matter under continuous corn in eastern Canadian soils. Biol Fert Soils 26:88-94

Loveland P, Webb J (2003) Is there a critical level of organic matter in the agricultural soils of temperate regions: a review. Soil Till Res 70:1-18

Ludwig B, Schulz E, Rethemeyer J, Merbach I, Flessa H (2007) Predictive modelling of C dynamics in the long-term fertilization experiment at Bad Lauchstädt with the Rothamsted Carbon Model. Eur J Soil Sci 58:1155-1163

Ludwig B, Kuka K, Franko U, Lützow M v (2008) Comparison of two quantitative soil organic carbon models with a conceptual model using data from an agricultural long-term experiment. J Plant Nutr Soil Sci 171:83-90

Machulla G, Blume H-P, Jahn R (2001) Schätzung der mikrobiellen Biomasse von Böden aus anthropogenen und natürlichen Substraten - ein Beitrag zur Standortbewertung. J Plant Nutr Soil Sci 164:547-554

Malhi S S, Gill K S, Harapiak J T, Nyborg M, Gregorich E G, Monreal C M (2003) Light fraction organic N, ammonium, nitrate and total N in a thin Black Chernozemic soil under bromegrass after 27 annual applications of different N rates. Nutr Cycl Agroecosys 38: 1527-1536

Mallory E B, Griffin T S (2007) Impacts of soil amendment history on nitrogen availability from manure and fertilizer. Soil Sci Soc Am J 71:964-973

Manlay R J, Feller C, Swift M J (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agr Ecosyst Environ 119:217-233

Marriott E E, Wander M (2006) Qualitative and quantitative differences in particulate organic matter fractions in organic and conventional farming systems. Soil Biol Biochem 38:1527-1536

Marschner B, Brodowski S, Dreves A, Gleixner G, Gude A, Grootes P M, Hamer U, Heim A, Jandl G, Ji R, Kaiser K, Kalbitz K, Kramer C, Leinweber P, Rethemeyer J, Schäffer A, Schmidt M W I, Schwark L, Wiesenberg G L B (2008) How relevant is recalcitrance for the stabilization of organic matter in soils? J Plant Nutr Soil Sci 171:91-110

Michel K, Matzner E, Dignac M-F, Kogel-Knabner I (2006) Properties of dissolved organic matter related to soil organic matter quality and nitrogen additions in Norway spruce forest floors. Geoderma 130:250-264

Mikha M M, Rice C W, Benjamin J G (2006) Estimating soil mineralizable nitrogen under different management practices. Soil Sci Soc Am J 70:1522-1531

Mikutta R, Kleber M, Kaiser K, Jahn R (2005) Review: Organic matter removal from soils using hydrogenperoxide, sodium hypochlorite, and disodium peroxodisulfate. Soil Sci Soc Am J 69:120-135

Nieder R, Harden T, Martens R, Benbi D K (2008) Microbial biomass in arable soils of Germany during the growth period of annual crops. J Plant Nutr Soil Sci 171:878-885

Parfitt R L, Salt G J, Saggar S (2001) Effect of leaching and clay content on carbon and nitrogen mineralisation in maize and pasture soils. Aust J Soil Res 39:535-542

Parton W J, Stewart J W B, Cole C V (1988) Dynamics of C, N, P and S in grassland soils: a model. Biogeochem 5:109-131

Paul E A, Morris S J, Conant R T, Plante A F (2006) Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? Soil Sci Soc Am J 70:1023-1035

Paustian K, Collins H P, Paul E A (1997) Managements controls on soil carbon. In: Paul E A, Elliott E T, Paustian K, Cole C V (eds) Soil organic matter in temperate agroecosystems: long-term experiments in North America. CRC Press, Boca Raton, pp 15-49

Paustian K, Six J, Elliott E T, Hunt H W (2000) Management options for reducing CO_2 emissions from agricultural soils. Biogeochem 48:147-163

Persson J, Kirchmann H (1994) Carbon and nitrogen in arable soils as affected by supply of N fertilizers and organic manures. Agr Ecosyst Environ 51:249-255

Piepho H P, Büchse A, Emrich K (2003) A hitchhiker´s guide to mixed models for randomized experiments. J Agron Crop Sci 189:310-322

Potthoff M, Loftfield N, Buegger F, Wick B, John B, Joergensen R G, Flessa H (2003) The determination of $\delta^{13}C$ in soil microbial biomass using fumigation-extraction. Soil Biol Biochem 35:947-954

Powlson D S, Brookes P C, Christensen B T (1987) Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol Biochem 19:159-164

Qualls R G (2005) Biodegradability of fractions of dissolved organic carbon leached from decomposing leaf litter. Environ Sci Technol 39:1616-1622

Randall E W, Mahieu N, Powlson D S, Christensen B T (1995) Fertilization effects on organic matter in physically fractionated soils as studied by ^{13}C NMR: Results from two long-term field experiments. Eur J Soil Sci 46:557-565

Raupp J, Oltmanns M (2006) Soil properties, crop yield and quality of farmyard manure with and without biodynamic preparations and with inorganic fertilizers. In: Raupp J, Pekrun C, Oltmanns M, Köpke U (eds) Long-term field experiments in organic farming. ISOFAR Scientific Series, Berlin, pp 135-155

Rochette P, Gregorich E G (1998) Dynamics of soil microbial biomass C, soluble organic C and CO2 evolution after three years of manure application. Can J Soil Sci 78:283-290

Russelle M P, Birr A S (2004) Large-scale assessment of symbiotic dinitrogen fixation by crops: soybean and alfalfa in the Mississippi river basin. Agron J 96:1754-1760

Sauerbeck D R (2001) CO_2 emissions and C sequestration by agriculture - perspectives and limitations. Nutr Cycl Agroecosys 60:253-266

Schmitt A, Glaser B, Borken W, Matzner F (2008) Repeated freeze-thaw cycles changed organic matter quality in a temperate forest soil. J Plant Nutr Soil Sci 171: 707-718

Sierra J (1990) Analysis of soil nitrogen mineralization as estimated by exponential models. Soil Biol Biochem 22:1151-1153

Six J, Elliott E T, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32:2099-2103

Six J, Conant R T, Paul E A, Paustian K (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155-176

Sleutel S, De Neve S, Nemeth T, Toth T, Hofman G (2006) Effect of manure and fertilizer application on the distribution of organic carbon in different soil fractions in long-term field experiments. Eur J Agr 25:280-288

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 (1997) A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. Geoderma 81:153-225

Stanford G, Smith S J (1972) Nitrogen mineralization potentials of soils. Soil Sci Soc Amer Proc 36:465-472

Statistisches Bundesamt Deutschland (2008): GENESIS-online. https://www-genesis.destatis.de/genesis/online/. Accessed 16 Sep 2008

Steiner R (1995) Long-term experiments and their choice for the research study. In: Barnett V, Payne R, Steiner R (eds) Agricultural sustainability - Economic, environmental and statistical considerations. John Wiley & Sons, Chichester, pp 15-21

Stevenson F J (1994) Humus chemistry. Genesis, composition, reactions. John Wiley & Sons, New York

Stewart C E, Paustian K, Conant R T, Plante A F, Six J (2007) Soil carbon saturation: concept, evidence and evaluation. Biogeochem 86:19-31

Stewart C E, Plante A F, Paustian K, Conant R T, Six J (2008) Soil carbon saturation: Linking concept and measurable carbon pools. Soil Sci Soc Am J 72:379-392

Swanston C W, Caldwell B A, Homann P S, Ganio L, Sollins P (2002) Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. Soil Biol Biochem 34:1121-1130

Swift M J, Heal O W, Anderson J M (1979) Decomposition in terrestial ecosystems. Studies in Ecology, 5. Blackwell Scientific Publications, Oxford

Turinek M, Grobelnik-Mlakar S, Bavec M, Bavec F (2009) Biodynamic agriculture research progress and priorities. Renewable Agriculture and Food Systems 24:146-154

van Veen J A, Liljeroth E, Lekkerkerk L J A (1991) Carbon fluxes in plant-soil systems at elevated atmaspheric CO_2 levels. Ecol Appl 1:175-181

Vanotti M B, Bundy L G, Peterson A E (1997) Nitrogen fertilizer and legume-cereal rotation effects on soil productivity and organic matter dynamics in Wisconsin. In: Paul E A, Elliott E T, Paustian K, Cole C V (eds) Soil organic matter in temperate agroecosystems: long-term experiments in North America. CRC Press, Boca Raton, pp 105-119

Vance E D, Brookes P C, Jenkinson D S (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703-707

von Lützow M, Kogel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur J Soil Sci 57:426-445

von Lützow M, Kogel-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

von Lützow M, Kögel-Knabner I, Ludwig B, Matzner E, Flessa H, Ekschmitt K, Guggenberger G, Marschner B, Kalbitz K (2008) Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. J Plant Nutr Soil Sci 171:111-124

Wander M M, Yang X (2000) Influence of tillage on the dynamics of loose- and occluded-particulate and humified organic matter fractions. Soil Biol Biochem 32:1151-1160

Wang W J, Smith C J, Chen D (2004) Predicting soil nitrogen mineralization dynamics with a modified double exponential model. Soil Sci Soc Am J 68:1256-1265

Wang Y, Hsieh Y-P (2002) Uncertainties and novel prospects in the study of the soil carbon dynamics. Chemosphere 49:791-804

Webster R (2007) Analysis of variance, inference, multiple comparisons and sampling effects in soil research. Eur J Soil Sci 58:74-82

Weigel A, Klimanek E-M, Körschens M, Mercik S (1998) Investigations of carbon and nitrogen dynamics in different long-term experiments by means of biological soil properties. In: Lal R, Kimble J M, Follett R F, Stewart B A (eds) Soil processes and the carbon cycle. CRC Press, Boca Raton, pp 335-344

West T O, Post W M (2002) Soil organic carbon sequestration rates by tillage and crop rotation: A global data analysis. Soil Sci Soc Am J 66:1930-1946

Whalen J, Chang C, Olson B (2001) Nitrogen and phosphorus mineralization potentials of soils receiving repeated annual cattle manure applications. Biol Fert Soils 34:334-341

Witter E, Martensson A M, Garcia F V (1993) Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biol Biochem 25:659-669

WRB (2006) World reference base for soil resources 2006. World Soil Resources Reports No 103, FAO, Rome

Wu J, Joergensen R G, Pommerening B, Chaussod R, Brookes P C (1990) Measurement of soil microbial biomass C by fumigation-extraction--an automated procedure. Soil Biol Biochem 22:1167-1169

Yamashita T, Flessa H, John B, Helfrich M, Ludwig B (2006) Organic matter in density fractions of water-stable aggregates in silty soils: Effect of land use. Soil Biol Biochem 38:3222-3234

Zaller J G, Koepke U (2004) Effects of traditional and biodynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long-term field experiment. Biol Fert Soils 40:222-229

Zsolnay A (1996) Dissolved humus in soil waters. In: Piccolo A (eds) Humic substances in terrestial ecosystems. Elsevier Science B.V., Amsterdam, pp 171-223

Yang H S, Janssen B H (2002) Relationship between substrate initial reactivity and residues ageing speed in carbon mineralization. Plant Soil 239:215-224

Yoo G, Wander M M (2008) Tillage effects on aggregate turnover and sequestration of particulate and humified soil organic carbon. Soil Sci Soc Am J 72:670-676