Influence of Zea mays in rotations on isotopic composition of soil organic matter

Wissenschaftliche Arbeit zur Erlangung des Grades
Master of Science (M.Sc.)
an der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München.

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Eingereicht am 26.07.2019 in Freising

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Index

1 Abstract

Soil organic carbon (SOC) is essential for ensuring important soil functions but is also a potential source or sink of CO_2 and thus important for climate change. The focus of this thesis was to determine the contribution of maize harvest residues (roots and stubble) to the SOC by isotope analyses. It was expected that maize cropping led to a measurable increase in $\delta^{13}C$ of SOC in former C3 plant rotations.

A long-term field trial in Viehhausen (Bavaria, Germany) with 10 different crop rotations covering various proportions of *Zea mays* (0, 25 or 50 %), and organic fertilization was investigated to determine the contribution of maize harvest residues to the SOC. Analysis of the SOC distribution on the trial site in different years were used to identify soil heterogeneity. The proportion of SOC derived from silage maize cropping, were only the roots and stubble remain on the field, was calculated using the natural ^{13}C abundance measurement technique and regression analyses. The past, present and future isotopic composition of the trial site was modelled and compared with measured data assuming a first order decomposition kinetic and by using a C balance model (CANDY) integrating yield data and organic fertilization as well as soil and fertilizer analyses.

Soil heterogeneity was mainly caused by a former road crossing the trial site until 1980. The SOC content of the trial site rose from 1.10 % in 2009 to 1.31 % in 2017. The SOC increase of individual crop rotations correlated significantly with the C input amounts from harvest residues and organic fertilization calculated in the C balance model. Different SOC contents as well as the variation in SOC increase between individual plots with the same management indicated non-states-steady conditions. The assumption of an exclusively first order decomposition was not justified but the increase in SOC content had to be considered in $\delta^{13}C$ modeling. On average 16.9 % C were replaced by maize roots and stubble in the topsoil (30 cm) of the trial site after 12 years of cropping (assuming 100 % maize in rotation). This yielded a total C input of 8450 kg ha^{-1} by maize harvest residues during 12 years. Calculating the amount of maize harvest residues with CANDY indicated that approximately 50 % of the maize C harvest residues entered SOC while the other 50 % were mineralized. The $\delta^{13}C$ turnover since 1961 was successfully modelled consistent with the measured data of soil samples from 2009 and 2017. The modelling revealed an unexpectedly high influence of previous grain maize cropping (1961 – 1995) on the present isotopic composition of SOC. From this followed that $\delta^{13}C$ of SOC should not change with a medium maize fraction in the rotation, while a slight decrease should be the result of a pure C3 rotation and a slight increase should result from a 50 % maize rotation.

In consequence, only slight changes in $\delta^{13}C$ (in opposite directions) can be expected from the given crop rotations. In combination with the given SOC turnover, $\delta^{13}C$ changes in a range of less than 2 ‰ between 2004 and 2017. Thus, the measured changes in $\delta^{13}C$ between 2009 and 2017 were insignificant although they followed the predictions. There was a significant difference in $\delta^{13}C$ between plots with 0 and 50 % maize in rotation in 2017, which confirmed the influence of maize roots and stubble on $\delta^{13}C$ turnover.

Due to the complex trial, the turnover could not only be calculated and modelled based on a specific scenario, but also for a variety of crop rotations with different fertilization. The results are therefore more general than from comparable investigations where e.g. only a maize monoculture was investigated. Since the complexity of the experimental setup had to be captured in the model as well, the model has gained in reliability and may also be suitable for the calculation of the $\delta^{13}C$ turnover of other field trials.

Modelling was a valuable tool to quantify the interacting effects of residue input varying in amount and isotopic composition, the varying net SOC sequestration and the SOC turnover. This facilitated to interpret the results and to disentangle the complex influences on SOC.

2 Index of abbreviations

C .... *carbon*
cdf .... *cumulative density distribution function*
CH_4 .... *methane*
CO_2 .... *carbon dioxide*
CR .... *crop rotation*
DM .... *dry matter*
DOC .... *dissolved organic matter*
EA .... *elemental analyzer*
F_{CR} .... *yield dependent factor (CANDY)*
FM .... *fresh matter*
IAEA .... *International Atomic Energy Agency*
IPCC .... *Intergovernmental Panel on Climate Change*
IRMS .... *isotope ratio mass spectrometry*
K_{CR} .... *yield independent factor (CANDY)*
MRT .... *mean residents time*
PEP .... *phosphoenolpyruvate*
Rubisco .... *Ribulose-1,5-bisphosphate carboxylase/oxygenase*
SD .... *standard deviation*
SILS .... *solid internal lab standard*
SOC .... *soil organic carbon*
TUM .... *Technical University of Munich*
VPDB .... Vienna *Pee Dee Belemnite*
WGS .... *Wideband Global SATCOM*

3 Introduction

Organic carbon is important for the maintenance of many soil functions and a “fundamental requirement for sustainable development in agriculture” (Franko and Merbach 2017). Arable soils provide an important source and sink for organic carbon and therefore play a pivotal role in climate change (Gleixner et al. 1999). Using isotope ratio mass spectrometry (IRMS), it is possible to determine the carbon turnover of arable soils under the influence of specific crops to derive effects on soil state and climate change given that a change in the isotopic composition of the source material had occurred at a known time.

The global amount of organic carbon in soils is approx. 2344 Gigatons. It is the largest terrestrial organic carbon pool on earth (Stockmann et al. 2013).

Global climate change has been scientifically proven to be caused by man-made increases in greenhouse gases, particularly by carbon dioxide (CO_2). Land use change, mainly deforestation for arable farming, is the second largest source with approx. 1.6 Gigatons carbon (C) per year (Dreves 2008).

Guo and Gifford (2002) estimated a reduction of 42 % and 59 % of total C stock by land use change from native forest to crop and pasture to crop respectively. This loss of C into the atmosphere is due to a sharp increase in mineralization. However, long-term studies show a possible increase of soil organic carbon (SOC) by adapted farming systems several decades after land use change and the potential to reduce greenhouse gases by C sequestration (Smith et al. 2008; Minasny et al. 2011). Arable soils contain around 1-3 % of SOC (Jenkinson 1968), where a higher content in soils is desired for several benefits such as a higher retainability for moisture, stabilization of soil structure, biological activity and availability and storage of nutrients for plant growth (Blume et al. 2010).

Due to these advantages and the importance for climate change, carbon-turnover of agricultural soils is the focus of several long-term studies and models, describing the processes leading to C input and C output (Jenkinson 1968; Gregorich et al. 1995; Gleixner et al. 1999; Schneckenberger and Kuzyakov 2007; Flessa et al. 2008; Helfrich et al. 2010; Novara et al. 2013; Coleman 2014; Franko and Merbach 2017). It is already known that appropriate crop management practices, such as organic fertilization, mulch-till and special crop rotations, can increase SOC (VELA 2014).

While elemental analyses are used to investigate the development of the total organic C content of a soil, it is particularly interesting to determine the turnover and residence time of SOC contributed by individual crops of a crop rotation. The measurement of stable isotopes is a useful and accurate tool to measure this contribution.

During photosynthesis, CO_2 containing the heavy stable carbon isotope ^{13}C is fractionated, i.e. CO_2 containing ^{12}C is preferentially assimilated. Due to different fractionation in the photosynthetic pathway of C3 and C4 plants, there is a higher natural abundance of ^{13}C in plant tissue of C4 plants (Schneckenberger and Kuzyakov 2007; Werth and Kuzyakov 2008).

This enables the calculation of SOC turnover following the input of C4 plant material into a soil with SOC predominantly derived from C3 plants (Gleixner et al. 1999).

The carbon turnover of roots has been little studied due to methodological difficulties in measuring root production and root degradation, but carbon isotopes may provide valuable insights in cases where the input of above-ground material remains small. The contribution of maize (*Zea mays L.*) roots and stubble to the SOC pool are determined under such conditions.

Maize is a widespread and frequently cultivated C4 crop for livestock farming and biogas plants. In Bavaria, more than 25 % (547100 ha) of total arable land is cultivated with maize, where 78 % is used as silage maize (Bayerisches Landesamt für Statistik 2017).

This thesis will make use of a long-term field experiment with varying proportions of maize in the rotations. The almost complete removal of above-ground biomass for biogas production in this experiment enables to quantify the contribution of roots and stubble to SOC. This experimental approach will be complemented by a modelling exercise that allows simulating the previous but also the future development of $\delta^{13}C$ turnover. It is expected that SOC increasingly acquires the isotopic composition of maize with an increasing amount of maize in the crop rotation and with increasing duration of the experiment. This should be measurable by an increase in the abundance of ^{13}C.

4 Material and Methods

4.1 Behavior and abundance of C isotopes in plants, soil and digestate

4.1.1 Characteristics of carbon isotopes

Isotopes are elements that differ in their number of neutrons and thus have different mass numbers, while the number of protons remains the same. The atomic number does not change. However, the respective elements are described more precisely by specifying the neutron number as an isotope. Carbon exists as two stable isotopes. It has either 12 or 13 neutrons and is then termed ^{12}C or ^{13}C.

Both carbon isotopes are present in organic material of animals and plants. The quantitative ratio of ^{12}C and ^{13}C is not random but depends on physical and chemical properties of the isotopes, on conversion processes in the organisms and on environmental influences.

The behavior of ^{12}C and ^{13}C in chemical processes can generally be described as follows (Fry 2008):

1) In kinetic reactions, ^{12}C reacts faster.
2) In exchange reactions, ^{13}C concentrates where bonds are strongest.

The ratio of two isotopes of an element is also called isotopic composition and usually expressed as so-called δ notation. The isotopic composition of carbon isotopes is calculated as follows:

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}} - 1 \right) \times 1000\ ‰ \qquad (1)$$

with

$\delta^{13}C$	ratio of ^{13}C and ^{12}C in a sample relative to the ratio in a standard [‰]
^{13}C	abundance of ^{13}C atoms in a substance
^{12}C	abundance of ^{12}C atoms in a substance
VPDB	Vienna Pee Dee Belemnite is the standard substance for carbon

The standard value is obtained from Vienna Pee Dee Belemnite (VPDB) as defined by the International Atomic Energy Agency (IAEA) in Vienna. The Pee Dee is a marine fossil from the Cretaceous period. Its ^{13}C to ^{12}C ratio is 0,0111802 (Zhang et al. 1990).

For many groups of organisms, representative ranges and average values for $\delta^{13}C$ have been determined by several scientific studies. Between C3 and C4 plants, there is a clear difference in $\delta^{13}C$ of approx. 14 ‰ and there are no overlapping ranges. Most C3 plants have a $\delta^{13}C$ of -29 ‰ to -26 ‰, C4 plants range from -14 ‰ to -12 ‰ (Smith and Epstein 1971; Gregorich et al. 1995; Finlay and Kendall 2007). Both have negative values due to the stronger fraction during CO_2 uptake by terrestrial plants than by marine carbonates.

4.1.2 Fractionation of carbon isotopes in C3 and C4 plants

Plants consume atmospheric CO_2 to build up their biomass. The $\delta^{13}C$ of CO_2 is about -8 ‰ (lit.), but $\delta^{13}C$ of plants is more negative due to fractionation processes during the uptake and fixation of CO_2 (Farquhar et al. 1989). In plants there are two major reasons contributing to the overall fractionation:

1) The different diffusivities of ^{12}C-CO_2 and ^{13}C-CO_2 from the free atmosphere into the intercellular pore space of a leaf across the stomatal pathway.
2) The distinct fractionation of ^{12}C and ^{13}C by the enzyme Rubisco (Farquhar et al. 1989).

Due to the poorer reactivity and inertia of heavy ^{13}C in contrast to ^{12}C, a reduction of ^{13}C content by 4.4 ‰ occurs in plants during the diffusion of atmospheric CO_2 through the stomata. However, the greatest discrimination occurs during the fixation of CO_2 in the Calvin Cycle of C3 plant cells. The CO_2-C attaches itself to ribulose-1,5-biphosphate. The enzyme Rubisco (Ribulose-1,5-bisphosphat-carboxylase/-oxygenase) catalyzes the reaction. The binding affinity of ^{12}C atoms is significantly higher and ^{13}C is rarely bound if enough ^{12}C is available via an atmospheric exchange. Sugar produced during C fixation is the basis for the formation of all organic compounds of a plant, hence the isotopic composition of plant material is determined by its sugar formation.

The CO_2 fixation of C4 plants works differently. The C fixation in C4 plants is spatially separated in two different cell types. Primary CO_2 is bound to phosphoenolpyruvate (PEP) in mesophyll cells by the enzyme PEP-carboxylase and is then transferred to the bundle sheath cells, in which the Calvin Cycle as above takes place. The affinity to bind CO_2 is considerably higher by PEP-carboxylase than by Rubisco, which is why a discrimination of ^{13}C and a relative enrichment of ^{12}C is low in the first step of fixation. There is still a higher affinity to fix ^{12}C through the Calvin Cycle in the bundle sheath cells, but there is almost no gas exchange between cells and atmosphere, leading to a high concentration of CO_2. Then, the ^{13}C fixation rate increases due to the high concentration and a lack of alternative biochemical processing pathways. As a result, more ^{13}C is fixed by C4 plants compared to C3 plants leading to a less negative $\delta^{13}C$ (Farquhar et al. 1989; Campbell et al. 2003).

4.1.3 Influence of the Suess effect on $\delta^{13}C$ of plants and SOC

The $\delta^{13}C$ of CO_2 has decreased by about 1.5 ‰ over the last 60 years, also described as the Suess effect, according to the findings by Suess (1955) and Keeling (1979) (Figure 1). The reason for this is man-made emissions of fossil C, which is depleted in ^{13}C. The isotopic composition of plants depends on the isotopic composition of atmospheric CO_2, as shown above. Thus, also $\delta^{13}C$ of plant material decreases by 1.5 ‰ simultaneously. The isotopic composition of inert SOC influenced by plant material changes with delay, depending on the C turnover rate.

So, $\delta^{13}C$ of SOC may be less negative than today's $\delta^{13}C$ of plant material. With increasing soil depth, the SOC turnover rate decreases due to lower C inputs and a reduced microbial activity, thus the isotopic composition may also increase with increasing soil depth (Flessa et al. 2000; Ludwig et al. 2005). Especially in long-term studies and models, the Suess effect has a distinct influence on the $\delta^{13}C$ turnover of soil and should be considered.

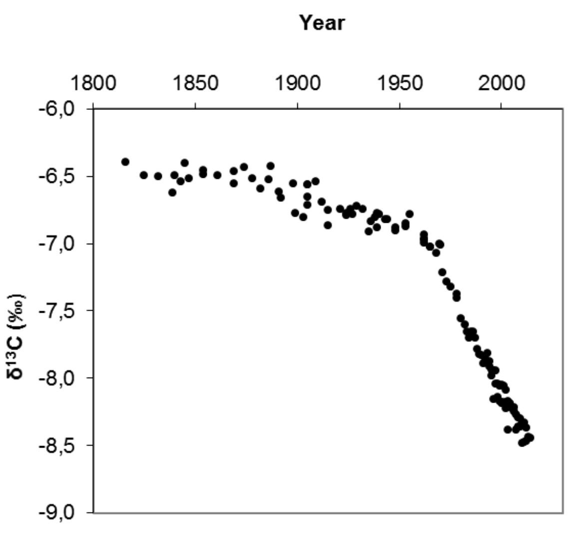

Figure 1: Isotopic composition of atmospheric CO_2 since 1800 (Source: Auerswald, K., pers. comm.).

4.1.4 Variation of $\delta^{13}C$ among plant tissue

In most investigations, the isotopic composition of above-ground plant material was measured. For investigations in this paper, it is important to know whether the composition varies among individual plant organs and whether the measured values of above-ground material can be used as a basis for the calculations of root-derived SOC. Shoot biomass of sunflower *(Helianthus annuus* L.), alfalfa *(Medicago sativa* L.), and perennial ryegrass *(Lolium perenne* L.) was always ^{13}C-depleted relative to root biomass (Klumpp et al. 2005). Reviews of several scientific papers show that C3 plant roots are enriched relative to leaves by 1 – 3 ‰, whereas C4 plant roots are nearly similar or slightly lower in $\delta^{13}C$ relative to leaves (Hobbie and Werner 2004; Cernusak et al. 2009).

According to Badeck et al. (2005) the following reasons contribute to the differences between the isotopic composition of plant organs:

1) Discrimination occurs during the transport of assimilates.
2) The metabolites used for export are enriched in ^{13}C (e.g. sucrose) with respect to the photosynthetic products.
3) Respiratory processes discriminate against ^{13}C at different degrees in different organs along with different types of metabolic pathways.
4) The biochemical composition varies from one organ to another along with characteristic signatures of metabolites.
5) Different rates of carboxylation for replenishment of Krebs Cycle intermediates (via PEP-carboxylase) lead to varying rates of incorporation of relatively heavy carbon.

Qian et al. (1997) have shown that $\delta^{13}C$ between maize roots and leaves differ by -1.4 ‰, meaning a lower ^{13}C content in roots compared to leaves. Badeck et al. (2005), however, did not found significant differences between the isotopic composition of roots and leaves of C4 plants (Figure 2) and this is the presently accepted view (Cernusak et al. 2009).

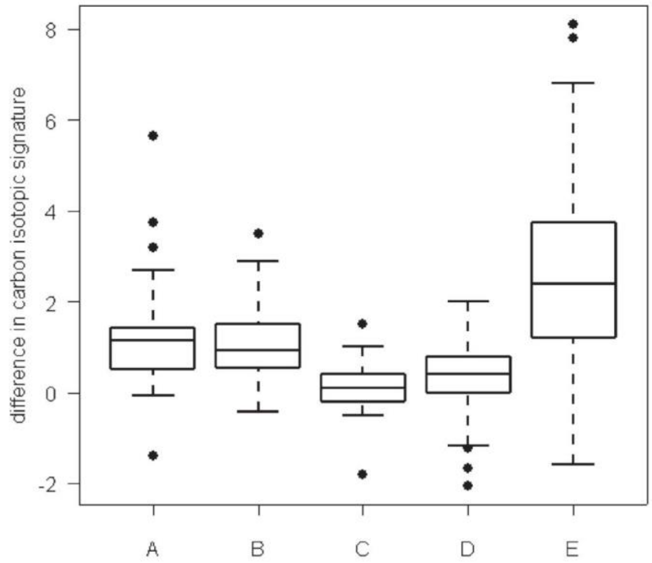

Figure 2: Differences between isotopic carbon signatures of plant organs: A) below-ground to above-ground; B) roots to leaves, C3 plants; C) roots to leaves, C4 plants; D) non-woody stems to leaves; E) woody stems to leaves. The line in the middle of each box represents the median (Badeck et al. 2005).

4.1.5 Fractionation of carbon isotopes through metanogenesis

During the ruminant metabolism and the biogas production through metanogenesis of plant material, residues (slurry or digestate) remain. The residues are used as organic fertilizer in agriculture. During the metabolism of sugar by methanogenic bacteria an isotope fractionation of the final products methane (CH_4) and CO_2 occurs. The ^{13}C abundance of CH_4 decreases significantly compared to sugar, while CO_2 becomes enriched in ^{13}C (Balabane et al. 1987). Thus, slurry and digestate may deviate in their isotopic composition, compared to the initial plant material and influence the isotopic composition of SOC.

The anaerobic digestion process of a biogas plant using grass silage was simulated in an experimental setup. Measurements of the isotopic composition of CH_4 and CO_2 resulted in values of 40.1 ‰ and 12.6 ‰ respectively (Lv et al. 2019). The equation of CH_4 production shows that the number of products CH_4 and CO_2 is the same:

$$C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4 \tag{2}$$

Based on the measured values of the research paper, the combined isotopic composition of CH_4 and CO_2 is then -27.4 ‰.

The fractionation effects thus negate each other and $\delta^{13}C$ of digestate remains almost the same compared to initial C3 plant material (-29 to -26 ‰). The same principles apply during methane production in marine sediments (Balabane et al. 1987).

4.1.6 Fractionation of carbon isotopes through soil respiration

During mineralization, senescent or dead plant material is introduced into the soil substrate. Conversion processes take place breaking down the material and transfer it to the SOC pool. This involves mechanical processes such as cutting or tearing through soil management as well as destroying plant cells through weather-related influences such as freezing and thawing. On the other hand, biochemical degradation of plant material takes place through decomposers such as earthworms, horn mites, springtails bacteria and fungi. During the mechanical comminution and biochemical decomposition, fractionation effects are conceivable regarding different physical and chemical properties of ^{13}C and ^{12}C. In addition, different plant components (e.g. lignin, cellulose, lipids etc.), which differ in their isotopic composition, are depleted at different speeds, regarding chemical properties. Both effects may lead to a difference in $\delta^{13}C$ between SOC and the plant material.

Usually there is negligible fractionation of ^{13}C during the transformation of plant residues into the SOC (Stout et al. 1981; Balesdent et al. 1993), although the microbial biomass, which forms only part of SOC, may become enriched by 2 ‰ compared to SOC while CO_2 becomes depleted (Santruckova et al. 2000). Even higher differences up to 3 ‰ were found between $\delta^{13}C$ of plant material and SOC, which was addressed to microbial respiration (Flessa et al. 2000). However, the Suess effect (see above) may also contribute to such large differences between fresh plant material and SOC.

4.2 Prerequisites to quantify the carbon turnover by the $\delta^{13}C$ turnover

The C turnover describes the input and output fluxes of SOC. The most important input source is plant material. The amount of C inputs through plant material is determined by plant growth, which depends on environmental factors such as climate and soil properties (Blume et al. 2010). In agriculture, the harvest residues determine the amount of C inputs. In addition, organic fertilizer (e.g. slurry, digestate) can be brought in. The most significant C output is caused by CO_2 which is produced during microbial respiration of organic material and then released into the atmosphere. A smaller fraction can also be discharged by leaching dissolved organic matter (DOC).

The final remaining products of mineralization are inorganic compounds, which in turn are available nutrients for plants. The duration of organic C in soil, prior to complete mineralization, depends on the stability of C compounds, physical soil properties (texture, C content, pH, soil type), environmental conditions (water and oxygen availability, temperature) and the associated activity of microorganisms (Blume et al. 2010). A distinction is made between a stable and a labile C-pool. There are three mechanisms leading to stabilization (Sollins et al. 1996; Lützow et al. 2008):

1) Recalcitrance describes the reduced microbial degradation of organic matter due to its stable molecular properties.
2) By the inclusion of organic substances in aggregates, a spatial separation to possible decomposers occurs whereby no decomposition can take place.
3) Decomposition can be prevented by binding the organic substance to the mineral fraction via molecular interactions.

While the stable pool is usually only poorly available for conversion processes, the labile pool is actively involved in the C turnover. The ratio of C input and C output determines whether there is an increase (input > output), a decrease (input < output) or a constant C concentration (steady-state; input = output) in soil. At a constant soil management and unchanged C input amounts, a steady-state occurs over time as further C losses or increases in soil are no longer possible. The reason for a finite C increase is the limited sequestration capacity of the soil which leads to a disproportionally increasing mineralization rate with an increasing C input (Sollins et al. 1996). However, even during a steady-state, the loss of old SOC with simultaneous input of new C leads to a quantifiable exchange of the specific isotopic composition, which can be used to calculate the C turnover given that:

1) Initial SOC is mineralized and discharged in the same way as newly entered material.
2) The initial isotopic composition of SOC is known.
3) The isotopic composition of newly entered material is known.
4) Newly entered material has a considerably different isotopic composition compared to the initial SOC.

Condition 1 usually cannot be proven but must be assumed to be true in a simple experiment. The isotopic composition of SOC and the newly introduced material is measured by mass spectrometry (Condition 2, 3). Condition 4 is true if initial SOC originates from C3 plants and newly introduced C contains C4 plant material (or vice versa) were a significant difference in the isotopic composition of both sources exists. The initial soil derived from C3 vegetation is then progressively replaced by C4 plant material causing a change in $\delta^{13}C$ of SOC over time (Gleixner et al. 1999).

The experimental setup in this thesis met the mentioned requirements and is described below in more detail.

4.3 Experimental setup

4.3.1 Trial site

The trial site for investigations in this paper was at the experimental station of the Technical University of Munich (TUM) in Viehhausen, in the Tertiary Hill Country, 30 km north-east of Munich, 480 m above sea level, with 797 mm mean annual precipitation and a mean temperature of 7.5 °C (Reents et al. 2015). The GPS coordinates were 48.39633, 11.65050 (WGS 84 (lat/lon) (BayernAtlas 2018).

Since 1953, practice-oriented research has been carried out at the experimental station in Viehhausen. In 1961 the experimental station was converted to a pig fattening system based on grain maize. In 1995 a conversion to organic farming took place and maize cultivation was completely stopped. Based on farm records, it was possible to reconstruct the land use, the crop rotations and yields of individual fields of Viehhausen up to 1997 (Rintelen diverse years). The trial site examined for this survey was not continuously under cultivation by the TUM (1961 - 1967; 1986 - present) due to a land consolidation in 1968 and later land acquisitions. Therefore, the present trial site was comprised by different former fields and there were some gaps in data, regarding crop rotations and yields (Appendix; Figure 37, Figure 38, Figure 39). The estimated proportions of former fields of the present trial site as well as annually cultivated crops and yields were collected (Appendix, Table 16). Data showing a frequent grain maize cropping until 1992 which was considered in further investigations.

The soil of the trial site was a Parabraunerde, partly a Braunerde with an appearing Pseudovergleyung (endo-aquatic attributes) according to the German classification system (Ad-hoc-Arbeitsgruppe Boden der Staatlichen Geologischen Dienste und der Bundesanstalt für Geowissenschaften und Rohstoffe and Bundesanstalt für Geowissenschaften und Rohstoffe

2005) from loess or loess loam, see Table 1 (Obermeier 1998; Reents et al. 2015). Using the US soil taxonomy (Soil Survey Staff 2015) the soil was categorized as a Hapludalf derived from loess with silty loam texture down to at least 1 m. Using the World Reference Base (IUSS Working Group WRB 2015) the soil was a Haplic Luvisol (Manganiferric, Siltic).

The four benchmark soils indicated rather homogenous conditions between sites but also between depths. For instance, soil pH was always neutral varying between 6.3 and 7.0, with slightly higher values in the plow horizon (mean 6.9) than below (mean 6.5). Also clay content was rather homogenous (varying between 20 and 29 %). Silt and sand varied more in the subsoil, while their contents were similar in the top soil (on average 63.5 % silt and 13.5 % sand). The SOC exhibited a pronounced variation over depth, with in average 1.24 % in the plow horizon, a sharp decrease to the next horizon (on average 0.39 %) and a more gradual decrease further downward (on average 0.26 % in the lowest horizon).

Table 1: Soil characterization of the trial site. Location of position numbers see Appendix, Figure 35. Soil type, horizon (German denomination), depth [cm], soil texture (German classification), clay (< 2 µm), silt 2 – 63 µm), sand (63 – 2000 µm), pH (in 0.02 M $CaCl_2$), organic carbon = SOC, erodibility = K-Factor (according to "Bodenkundliche Kartierungsanleitung, AG Boden 1996") (Obermeier 1998).

No.	Soiltype	Horizon	Depth [cm]	Soiltexture	Clay [%]	Silt [%]	Sand [%]	pH	SOC [%]	K-Factor
81	sLL	Ap	0-25	Ut4	20	68	12	7	1.15	>0.5
		Bt	25-45	Lu	28	63	9	6.7	0.49	
		BgtI	45-90	Lu	27	63	10	6.7	0.26	
		llBgv2	60-90	Lu	24	55	21	6.6	0.22	
82	LL	Ap	0-23	Lu	23	63	10	7	1.28	0.3-0.5
		Bg t	23-57	Lt2	28	49	23	6.4	0.36	
		llBv	57-95	St3	22	14	64	6.4	0.24	
		lllBv	70-95	Ts4	27	13	60	6.4	0.24	
83	sLL	Ap	0-30	Lu	20	63	17	6.9	1.17	0.3-0.5
		Bgl	30-77	Lu	29	62	9	6.3	0.34	
		llBvg	77-95	Lu	25	56	19	6.4	0.30	
84	sBB	Ap	0-32	Lu	25	60	15	6.7	1.36	0.3-0.5
		Bgv	32-70	Lu	27	60	13	6.3	0.35	
		llBvg	70-95	Ls4	24	20	56	6.6	0.27	

The trial site was partly inclined to north-east. On old maps up to the year 1980, a road (probably gravel, no tar), which had been connecting Viehhausen and Sünzhausen for more than 100 years, crossed the trial site (Figure 3). The exact position was unclear because due to different map shapes and reference systems, a deviation of about 2-4 meters of the course may have occurred. The former road likely was about 4-5 meters wide. Until the land consolidation in

1968, two fields were separated from each other by the former road and only the north-eastern field was cultivated by the TUM. On current aerial photographs (2018), the original course of the road was still partly visible. The culture growing on it stands out from surrounding plants in a lighter or darker green (BayernAtlas 2018). In 38 years of soil cultivation material from field and road was mixed and the borders are blurred. Possible effects on yield and soil properties were considered.

Figure 3: Field layout of the experiment (oriented north). The total size is 255 m in length and 155 m in width (approx. 4 ha). The four blocks consisting of one year of the rotation are arranged from left to right (delineated by a light blue line). The ten rotations including the three replicates of rotation 1 are arranged up and down. Eight plots (four rows within a block and two rows within a rotation have the same rotation and the same crop; four of them are fertilized with digestate, four do not receive fertilizer). A former road (red line) crosses the trial site (Source: Stefan Kimmelmann).

The experiment, denoted 'Biomasseversuch Viehhausen', which was examined in this thesis, was established in 2004 to evaluate the C cycle of an organic farm with integrated biogas plant and its effects on soil properties. During harvest, almost the entire plant (except roots and stubble) was removed from the field. Digestate was returned to the field as organic fertilizer. As no biogas plant was operated at the experimental station in Viehhausen, digestate of a nearby biogas plant (Eichethof, Hohenkammern) was used. The fermentation substrate of the biogas plant was mainly plant material, which was produced on the trial site.

Due to a high lignin content and a low nitrogen content of digestate (compared to fresh plant material), changes in soil properties (aggregate stability, C content, nitrogen content, catalase activity) were expected.

To investigate these changes, different crop rotations with different amounts of digestate fertilization were established (Table 2).

There were ten crop rotations denoted CR1 to CR10. Crop rotation 1 was replicated three times (CR1a, CR1b, and CR1c), once in both side columns and once in the middle column of the experiment. This was intended to capture any trends in yield. Each year of a crop rotation formed one block (block 1-4) divided into 8 plots, 4 plots with and the others without digestate fertilization. The plots (6 m width, 12 m long) were not randomized but arranged in a repetitive pattern. In two years, one year of clover grass and one year of winter wheat was grown uniformly on all crop rotations (Table 2). After wheat, cover crops were cultivated which were either mulched or integrated into the crop of the third year as nurse crops after harvest. The cover crops were not considered in this thesis. In the third and fourth crop year, the crop rotations differed. The following crops were cultivated in different combinations: Maize, broad bean, soybean, clover-grass, triticale, winter-wheat and sunflower. Due to the crop combinations, the rotations differed in their proportion of silage maize by 0 %, 25 % and 50 %.

Table 2: Sequence of crops in the crop rotations; digestate amounts applied to the fertilized plots are given in parentheses, (m^3 ha^{-1} a^{-1}). Cover crops are not included. CG denotes clover grass. [1]Straw was not removed from the trial site after harvest for broad bean and soybean but in all other cases.

Crop rotation	Year 1	Year 2	Year 3	Year 4	Maize [%]
CR1a	CG	Wheat (40)	Maize (60)	Triticale (50)	25
CR2	CG	Wheat (30)	Maize (60)	Wheat (30)	25
CR3	CG	Wheat (20)	Broad bean[1]	Wheat (30)	0
CR4	CG	Wheat (20)	Soybean[1]	Wheat (30)	0
CR5	CG	Wheat (50)	Maize & CG (40)	Triticale (50)	25
CR6	CG	Wheat (50)	Maize & CG (40)	Maize & CG (50)	50
CR1b	CG	Wheat (40)	Maize (60)	Triticale (50)	25
CR7	CG	Wheat (40)	Maize (45)	Triticale (50)	25
CR8	CG	Wheat (50)	Maize (45)	Sunflower & CG (40)	25
CR9	CG	Wheat (70)	CG	Triticale (50)	0
CR10	CG (60)	Wheat (80)	CG	CG (30)	0
CR1c	CG (60)	Wheat (40)	Maize (60)	Triticale (50)	25

4.3.2 Sampling method

To quantify a possible change of the isotopic composition of SOC, altered by maize plant residues, the different proportions of maize in the crop rotations had to be related to $\delta^{13}C$ of SOC in linear regressions. For this purpose, suitable plots with different maize proportions were investigated. The SOC content of all plots was investigated to evaluate possible soil heterogeneities due to the former road and the topography of the trial site, which may have influenced the results and interpretation of the isotope measurements. Samples from several years were investigated to capture temporal changes in the isotopic composition and SOC content. Only the topsoil (30 cm) was sampled as the main root biomass of agricultural crops is found in the upper 30 cm (about 90 % of the total root biomass) according to Klimanek (1997). Through tillage, new plant material mainly was mixed in the plow horizon (30 cm) and thus remained in the topsoil. Hence the major part of conversion processes and changes of soil properties took place in this horizon.

All plots were already sampled and measured by the Lehrstuhl für Ökolandbau in 2009, 2013 and 2017 regarding SOC content, total N and pH.

Additionally, all fertilized plots of CR1b (25 % maize in crop rotation) were sampled in 2018.

All samples were taken between October and November to avoid possible seasonal variations of the isotopic composition and the C of soil. For each plot, five samples were taken using a 30 cm Pürckhauer drill. To avoid edge effects, the samples were taken at a minimum distance of 75 cm from the edge of the plots (Figure 7). Samples of each plot where homogenized, sieved with 2.0 mm mesh width, air dried for two weeks and then stored in plastic cans. Visible organic matter such as root pieces was removed.

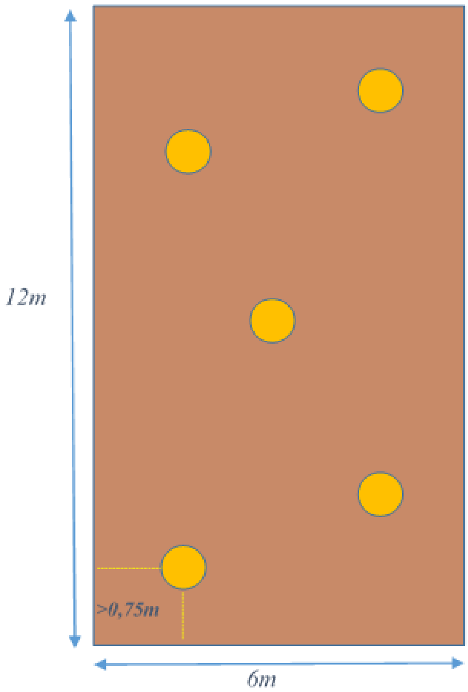

Figure 4: Sampling pattern of a plot. Five drill holes (yellow circles) with a minimum distance of 75 cm from the edge of the plots.

Only a selected part of the available soil samples was used for further isotope analyses (Appendix, Figure 36), since the sample processing and measurement was time consuming and costly. Among the samples taken 2009, 4 plots of CR1a (25 % maize in rotation) on the northern margin of the trial site and 4 plots of CR1b (25 % maize) in the middle of the trial site as well as all plots of CR6 (50 % maize) and CR10 (0 %) were examined in block 3. Thus, all maize proportions and possible site effects on the trial site were recorded. Among the samples of block 3 taken 2017, 50 % have already been prepared and measured in former investigations. To completely record the crop rotations CR6, CR9 and CR10, the missing plots were supplemented. Thus, also in 2017, enough plots with 0, 25 and 50 % maize content were recorded, which were compared with the samples from 2009. All plots sampled in 2018 were used to quantify possible site effects of the trial site and the influence of a single maize year within a four-year crop rotation regarding possible short-term changes in the isotopic composition of SOC.

The $\delta^{13}C$ of digestate and its influence on the isotopic composition of the SOC were analyzed to model the $\delta^{13}C$ turnover. In the years 2014 and 2015, liquid digestate (approx. 1 liter per sample) was taken right before fertilization, filled into plastic cans and stored in a freezer at -20 °C. In 2014 one sample was taken, denoted G14, in 2015 a sample was taken in May (G15 I) and in July (G15 II), respectively. In 2016, one part of sample G15 I was separated, thawed and frozen again denoted G15 III. The samples from 2015 were already measured in 2016. To obtain a higher reliability, the samples were reprocessed and measured again for this thesis.

4.3.3 Sample processing

About 1 ml of each soil sample was filled in 2 ml Eppendorfer tubes, dried in a compartment drier (4 hours, 60 °C) and then grounded with a ball mill (Retsch Mixer Mill MM 301) for 20 seconds at a frequency of 30 [$1s^{-1}$]. To prevent the samples from regaining moisture, they were kept in closed Eppendorfer tubes in a desiccator until analysis.

About 1.5 ml of each liquid digestate sample was put into Falcon tubes and dried in a compartment drier for two weeks and then processed as the soil samples.

4.3.4 Decarbonization of inorganic carbon

Especially on plots situated on the former road, soil may contain inorganic C as carbonates (e.g. $CaCO_3$) from gravel. Carbonates differ isotopically from organic C and had to be removed following Harris et al. (2001) by acid fumigation. Carbonate removal was applied for all soil samples to be measured in this thesis. For samples taken 2017 already measured in previous investigations, carbonate removal was applied only on samples of plots crossed by the former road (319, 321, 323, 325, 327, 329, 333, 337, 338) To this end, 10 ± 0.05 mg of milled samples were weighed into 3.3 x 5 mm silver cups and moistened with 15 µl of de-ionized water and placed above a 12 N HCl solution in a desiccator (without silica gel) for 24 h. Afterwards samples were dried at 30 °C for 24 h and then enclosed in bigger silver capsules of 5 x 9 mm. The digestate samples where not HCl treated. Only 0.7 mg were weighed out in 3.3 x 5 mm tin capsules due to an expected high C content, enough for measurements in EA and IRMS.

4.3.5 Isotope and SOC analysis

The samples were measured at the Lehrstuhl für Grünlandlehre of the TUM, Weihenstephan. The SOC content and $\delta^{13}C$ were determined with an elemental analyzer (EA) (NA 1110; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Each sample was measured against a laboratory working standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06 ‰ standard deviation (SD)). After every tenth sample a solid internal lab standard (SILS; fine ground wheat flour “Rosenmehl”) was run as a blind control. The SILS amount was adjusted to deliver about the same amount of C as

the soil or digestate samples. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision for wheat flour repeats was 0.11 ‰ (SD).
All measured values were recorded, sorted and transferred to an Excel sheet. In addition, drifts that can occur during the measurement where corrected.

4.4 Statistical analysis

Datasets of samples from 2009, 2017 and 2018 were summarized in Excel, where all statistics were done. Predicted and observed values were compared with linear regression analysis to determine whether the model represents reality. If necessary, model parameters were modified to improve the correlations.
Linear and multiple regressions were used to calculate the relationship between different variables (e.g. maize content, digestate amount, SOC content) and the measured $\delta^{13}C$ values of the soil samples. Means (e.g. treatments or years) were compared by one-way ANOVA. Significance was assumed for $p < 0.05$ if not otherwise specified.
To examine whether an increase on SOC content was influenced by the initial SOC content, SOC contents of 2009 were ranked leading to a cumulative density distribution function (cdf) (Hedderich and Sachs 2018). The SOC contents of the samples of 2017 were then plotted against the rank of samples of 2009 and the deviation to the cdf was visually inspected.

4.5 Analysis of influencing factors on SOC

The $\delta^{13}C$ turnover was directly linked to the C turnover. To improve the interpretation of the measured $\delta^{13}C$ of plots, the SOC contents of the plots were set in relation to individual possible influencing factors (fertilizer, crops, soil properties) on the SOC. For the calculation of C inputs by fertilizers and plants, the calculations according to Franko (1997) were used. Possible temporal changes of the SOC content between 2009 and 2017 were also investigated. Using a map on which the individual plots could be differentiated by color (color scale) according to their SOC concentration, potential soil heterogeneities due to variable SOC contents that were not caused by C inputs via fertilization and harvest residues should be identified. For this investigation, the effect of fertilization and the influence of harvest residues in the crop rotations were deducted from the SOC of the plots.
The impact of digestate fertilization on the SOC was quantified by a direct comparison of SOC derived by fertilized and unfertilized plots individually for each crop rotation. The difference between the mean values of the SOC contents of the fertilized and unfertilized plots in a crop

rotation was subtracted from the fertilized plots. The effect of the former road on SOC was considered to enable removing the effect of the harvest residues in the crop rotations on the SOC. To remove the effect of the road, the difference between the mean value of the road plots and the mean value of the surrounding plots was added to the SOC of the road plots. To remove the effect of the crop rotations, the difference between the mean value of all plots and the mean value of the individual crop rotations was subtracted from the SOC content of the individual plots of a crop rotation.

The topography was also determined to show possible correlations with the SOC and the isotopic composition of individual plots. Using *Google Earth Pro*, the meters of height of each two adjacent plots of a crop rotation were determined. The differences in height were transferred to a map and linked to a color scale to visualize the topography.

4.6 Modelling the δ^{13}C turnover

4.6.1 General calculations

A model of the δ^{13}C turnover was set up in Excel. The predicted δ^{13}C values were used as a reference in the later statistical evaluation. The development of δ^{13}C under former soil use was modeled. The future δ^{13}C course was predicted and the change under different scenarios was simulated.

The δ^{13}C turnover model was based on a C turnover model that calculates the C input and output sources of a homogeneously assumed C pool (SOC) based on first order decomposition kinetic (Jenkinson and Rayner 1977; Parton et al. 1987; Paustian et al. 1992; Smith et al. 1997). The different isotopic compositions of C3 plants, C4 plants and digestate were related to the C turnover model and its C sources, respectively. The δ^{13}C turnover of the SOC was then calculated from a given initial δ^{13}C value.

The initial δ^{13}C value of SOC (SOC_0) has been derived from C3 plant material without any fractionation:

$$\delta^{13}C\ (SOC_0)\ =\ \delta^{13}C\ (C3\, plant) \tag{3}$$

The initial amount of SOC within a given ploughing depth (SOC_0) was calculated by the ploughing depth (m), the dry bulk density of soil (t m^{-3}) and the SOC content of soil (%) given:

$$SOC_a\ = PD \times BD_s \times SOC_c \tag{4}$$

with

SOC_a soil organic carbon amount [kg ha^{-1}]

PD ploughing depth [m]

BD_s bulk density of soil [t m^{-3}]

SOC_c C content of soil [%]

The annual C input from maize roots and stubble and the C input from C_3 plants was calculated. There were two methods to calculate the C input:

Method I:

Specific parameters for arable crops that allow a yield-dependent calculation of the C input using Table 3 and Formula (5) (Franko 1997).

Table 3: Crop specific parameters to calculate the yield dependent C input (Franko 1997).

Culture	**K_{CR} [dt $ha^{-1}a^{-1}$]**	**F_{CR}**	**C_{SC}**
Sugar beet	1.6	0.008	0.35
Potato	0.8	0.016	0.45
Spring barley	3.1	0.078	0.55
Winter cereal	4.0	0.080	0.55
Sunflower	12.0	0.168	0.50
Silage maize	10.4	0.005	0.45
Grain maize	13.5	0.060	0.45
Lucerne	20.0	0.014	0.35
Pea	17.5	0.100	0.55
Rye	4.5	0.004	0.45
Broad bean	10.0	N/A	N/A

$$C_{CR} = K_{CR} + F_{CR} \times \text{Yield} \quad (5)$$

with

C_{CR} C input through crop residues [dt ha^{-1} a^{-1}]

K_{CR} yield independent factor [dt ha^{-1} a^{-1}]

F_{CR} yield dependent factor

Yield fresh matter (FM) [dt ha^{-1} a^{-1}]

Only a certain proportion of plant material is effectively introduced into soil, the other part is prematurely respired by microorganisms. The effective C input was therefore calculated using a synthesis coefficient (C_{SC}) which was developed for several crops (Table 3):

$$C_{eff} = C_{CR} \times C_{SC} \tag{6}$$

with

C_{eff} effective C input [dt ha^{-1} a^{-1}]

C_{CR} C input through crop residues [dt ha^{-1} a^{-1}]

C_{SC} synthesis coefficient

Method II:

The C input by silage maize was estimated based on yield multiplied by the ratio of below-ground and above-ground plant material (Flessa et al. 2000). The ratio of roots and stubble to harvested above-ground material of maize was estimated at 0.20 according to Klimanek (1997). The yields were corrected for moisture content. The C content of plant material was set to a constant factor for all crops of 0.45, which was similar to the factor recommended by the IPCC for herbaceous biomass from grassland and cropland (0.47) (Verchot et al. 2006).

$$C_{CR} = R_{below/above} \times P_C \times Yield \tag{7}$$

with

C_{CR} C input through crop residues [dt ha^{-1} a^{-1}]

$R_{below/above}$ ratio of below-ground and above-ground plant material

P_C carbon content of plant material

Yield dry matter (DM) [dt ha^{-1} a^{-1}]

An example demonstrating the calculation of C_{CR} by silage maize using both methods shows the different results according to average yields in Viehhausen. (Table 4):

Table 4: Two methods to calculate the C input through plant material. Calculations are made regarding average yield data of Viehhausen (384 dt ha^{-1} a^{-1} FM equals 128 dt ha^{-1} a^{-1} DM).

Method I (Franko 1997)	**Method II (Flessa et al. 2000)**
Calculation:	Calculation:
10.4+0.005*384 dt ha^{-1}	0.2*0.45*128 dt ha^{-1}
10.4+1.92 dt ha^{-1}	11.52 dt ha^{-1}
C_{CR} = 1232 kg ha^{-1} a^{-1}	C_{CR} = 1152 kg ha^{-1} a^{-1}

The C input by digestate was calculated by the amount of digestate (dt ha^{-1} a^{-1}), the dry matter content and the C content of dry matter:

$$\mathrm{C}_d = \mathrm{FM}_d \times \mathrm{DM}_d \times \mathrm{C}_{\mathrm{DM}} \tag{8}$$

with

C_d C input through digestate [dt ha^{-1} a^{-1}]

FM_d fresh matter of digestate [dt ha^{-1} a^{-1}]

DM_d dry matter content of digestate [%]

C_{DM} C content of DM [%]

Via the annual C input by digestate fertilization (from C_3 plant material), crop residues from C_3 plants and the additionally integrated C_4 plant material, the total annual supply of $\delta^{13}C$ was calculated with:

$$\delta^{13}\mathrm{C}\,(C\ input_t) = C_{CR}(C4)\ \times \delta^{13}\mathrm{C}\,(C4) + C_{CR}(C3) \times \delta^{13}\mathrm{C}\,(C3) + C_d \times \delta^{13}\mathrm{C}\,(C_d) \tag{9}$$

with

$\delta^{13}C$ (C input $_t$)	total annual supply of $\delta^{13}C$ through C3 and C4 material [‰]
C_{CR} (C4)	proportion of C input through C4 plant material in rotation [%]
$\delta^{13}C$ (C4)	$\delta^{13}C$ of C4 plant material [‰]
C_{CR} (C3)	proportion of C input through C3 plant material in rotation [%]
$\delta^{13}C$ (C3)	$\delta^{13}C$ of C3 plant material [‰]
C_d	proportion of C input through digestate in rotation [%]
$\delta^{13}C$ (C_d)	$\delta^{13}C$ of C_d [‰]

The annual C output resulting from microbial respiration as CO_2 and leaching as DOC was estimated as well. In a simple version of the model the annual C output equals the C input, hence a change in SOC content was not considered in the model. The recent $\delta^{13}C$ of SOC was then calculated by:

$$\delta^{13}\mathrm{C}_{(t)} = \frac{SOC_{(t-1)} \times \delta^{13}\mathrm{C}\left(\mathrm{SOC}_{(t-1)}\right) + C\ input_t \times \delta^{13}\mathrm{C}\,(C\ input_t)}{SOC_{(t)}} \tag{10}$$

with

$\delta^{13}C_{(t)}$	$\delta^{13}C$ of current SOC [‰]
$SOC_{(t-1)}$	previous SOC amount [kg ha^{-1}]
$\delta^{13}C$ ($SOC_{(t-1)}$)	$\delta^{13}C$ of previous SOC [‰]
C input $_t$	total annual C input [kg ha^{-1} a^{-1}]
$\delta^{13}C$ (C input $_t$)	total annual supply of $\delta^{13}C$ through C3 and C4 material [‰]
$SOC_{(t)}$	$SOC_{(t-1)}$ + C input $_t$ [kg ha^{-1}]

4.6.2 Uniform and differentiated model types (Model I + II)

Three fundamental changes in farming management from 1961 until today have influenced the isotopic composition of the SOC at the trial site. While the trial site was considered as uniformly cultivated until 2004, it was divided into plots afterward. Depending on the C3 and C4 input quantities, individual $\delta^{13}C$ turnover rates on the plots resulted, which could not be represented in a uniform model. Hence, two models were necessary. A uniform model (Model I) that simulated and visualized the $\delta^{13}C$ turnover with a certain configuration from 1961 onwards, and a differentiated model (Model II) that simultaneously captured the different management configurations existing on the trial site since 2005 to calculate the present $\delta^{13}C$ of soil (Table 5). Model II and possible model adaptations were then compared and evaluated with the measured $\delta^{13}C$ of the plots via a regression.

Table 5: Fundamental changes in farming management at the trial site and the required models to simulate the $\delta^{13}C$ turnover.

Model	Time	Management
I (part 1)	1961-1995	grain maize; inorganic fertilization, uniform management
I (part 2)	1996-2004	no maize; unknown organic fertilization, uniform management
II	Since 2005	known proportions of maize; known amount of organic fertilization, plot-specific management

4.6.2.1 Model I (part 1)

Annual yield data from 1961 to 2004 were partly available to calculate the C input and $\delta^{13}C$ supply of the crops (Rintelen diverse years). Missing yield years were supplemented by estimated average values. Between 1968 and 1985 there was no yield data available, hence a silage maize content of 30 % in crop rotation was assumed regarding former yield data. The distribution of farmland in Viehhausen has changed over the years due to land consolidations. Former fields overlapped partially with the current trial site (Appendix, Figure 37, Figure 38, and Figure 39). The proportion, the former fields overlapped the trial site between 1961 and

1997 were estimated visually (Appendix, Table 17). The total annual C input on the trial site and its $\delta^{13}C$ turnover was thus calculated by the sum of the C input amounts of the overlapping fields.

The ploughing depth was assumed to be 0.3 m since ploughing was also carried out at this depth in 2018. The dry bulk density was estimated at 1.45 t m^{-3} and thus corresponded to common values of Viehhausen (Obermeier 1998). The C content of soil was calculated at 1.0 %. The $\delta^{13}C$ of maize was set to -13.22 ‰, that of C3 plants to -28 ‰. Between 1961 and 2004, the annually varying yields of C3 and C4 plants were calculated gradually because there was no uniform repetitive crop rotation. Hence the visualized $\delta^{13}C$ turnover was presented in more detail. The annual C input by grain maize was calculated according to Method II, Chapter 4.6.1. To calculate the total yield of above-ground plant material based on the grain maize yields, a grain-straw ratio of 1:1.3 was assumed. The above-ground C input resulted from the DM yield multiplied by 0.47 (according to IPCC), the below-ground C input was derived by multiplying the ratio of below-ground to above-ground plant material (0.2). For C3 crops, a simplified uniform total C input of 20 dt ha^{-1} was assumed based on the results of Klimanek (1997). The effective portion of C input (C_{eff}) was calculated using the total C input of harvest residues multiplied with synthesis coefficient C_{SC} (0.45) according to Franko (1997).

4.6.2.2 Model I (part 2)

Since 2005, Model I (part 2) was used to simulate the past, present and future turnover with any constant parameters in a continuous model. The effective C input by plant material was now calculated with Method I, as it was assumed to be more accurate then Method II. Missing factors (K_{CR}, F_{CR}) of crops not listed in Table 3 were derived from other crops. No value for F_{CR} was given for the broad bean and was therefore adopted from the potato, assuming similar growing characteristics. The soybean was calculated like the broad bean and clover grass like lucerne. The C input from sunflowers was specified higher in comparison to other crops, since, only the seeds were harvested, and the rest of the plant remained on the field (denoted as ‘seed sunflower’ according to grain maize) (Table 3). At the trial site, the entire plant was harvested except for roots and stubble (denoted as ‘silage sunflower’ according to silage maize). Hence, K_{CR} and F_{CR} were adapted to calculate the C input more accurate. The K_{CR} and F_{CR} was derived approximating the ratio of K_{CR} and F_{CR} of grain maize to silage maize:

$$K_{CR\ \text{silage sunflower}} = K_{CR\ \text{grain maize}} / K_{CR\ \text{silage maize}} * K_{CR\ \text{seed sunflower}} = 9.2$$

$F_{CR\ silage\ sunflower} = F_{CR\ grain\ maize} / F_{CR\ silage\ maize} * F_{CR\ seed\ sunflower} = 0.014$

For calculations of maize induced $\delta^{13}C$ turnover, a new initial isotopic composition of SOC was considered. The initial assumption, $\delta^{13}C$ of SOC equals $\delta^{13}C$ of C3 plant material, changed at the beginning of Model I (part 2) since soil has already been influenced by C4 material. The new initial value corresponded to $\delta^{13}C$ of Model I (part 1) in 2004. In addition, the SOC content was set to 1.1 % according to samples measured in 2009.
According to measurements by the Lehrstuhl für Ökolandbau, the SOC at the trial site has increased from 2009 to 2017 due to organic farming and the associated increase in C inputs through crops and digestate fertilization. Since the $\delta^{13}C$ turnover depends on the SOC quantities, an increase in C was integrated. The C output, however, should increase with increasing SOC to limit an infinite sequestration of C. A finite sequestration then leads to a new C steady-state in soil. Therefore, mineralization factor F_m was integrated into the model which was calculated from the quotient of the initial C output$_0$ (equals C input) and SOC_0 (Formula (11)). In addition, F_m was corrected by factor F_A to simulate the approximate measured C increase of the trial site:

$$F_m = C\ output_0 \ / \ SOC_0 \text{ x } F_A \tag{11}$$

with

F_m	mineralization factor to limit C output
C output$_0$	initial C output in the model equals C input [kg ha^{-1}]
SOC_0	initial SOC amount [kg ha^{-1}]
F_A	correction factor [%]

As a result, the annually increasing C output was:

$$SOC_{(t)} \times F_m \tag{12}$$

with

$SOC_{(t)}$	SOC at time t [kg ha^{-1}]
F_m	mineralization factor to limit C output

4.6.2.3 Model II

In Model II the known proportions of maize in rotations and the plot specific C input amounts due to digestate fertilization and harvest residues were used to calculate the isotopic composition of SOC. A digestate analysis yielded a C content of dry matter of 40.3 (Table 6). The dry matter content was set to 5.8 according to Reinhold et al. (2012) (Appendix, Table 15). The different crops and the amount of digestate for each crop rotation (Table 2) were used to calculate the annual C input amounts by digestate for each plot (Formula (8)).

Table 6: Ingredients of digestate. SD = Standard Deviation

Variable	Content (%)	SD
Loss on ignition	7.4	2.4
Total nitrogen	6.4	0.7
Ammonium nitrogen	3.7	0.7
Phosphate (as P_2O_5)	2.1	0.1
Potassium (as K_2O)	9.0	0.8
Carbon	40.3	2.2
Sulphur	0.5	0.04

The proportion of maize and C3 plants in a crop rotation were considered to calculate the annual C input amounts by harvest residues for each plot. After a complete four-year crop rotation, the maize proportion corresponds to 0, 25 or 50 % depending on the crop rotation. For intermediate years, the proportion varies, hence an excel sheet was created in which the annual crop rotations since 2004 were listed for each plot to calculate the exact proportion. The annual maize proportion then resulted from the previous maize years divided by the total amount of all crop years. For all other crops, the proportion was determined in the same way. Since 2004, yield data from the trial site of individual crops were documented. The yield differences between fertilized and unfertilized crops were significant in most cases and thus considered in the model (Table 7).

Table 7: Mean annual yields (dt ha^{-1} a^{-1}) of fertilized and unfertilized plots on the trial site. WW = Winter Wheat, SM = Silage Maize, BB = Broad Bean, T = Triticale, R = Rye, SF = Sunflower, S = Sorghum, SB = Soybean, BB = Broad Bean. Water content of fresh matter of BB, SB and WW = 14 %.

		BB	R	S	CG	SM	SB	SF	T	WW
fertilized	fresh matter (FM)	31	229	296	566	384	28	423	233	60
	dry matter (DM)	27	47	83	105	128	24	95	97	51
unfertilized	fresh matter (FM)	26	155	193	499	231	27	300	121	39
	dry matter DM	22	34	51	94	77	23	66	52	34

The yields were used to calculate the annual C input amounts of the plots through harvest residues, according to Franko (1997), multiplied by their proportion in the crop rotation. Crops that were not listed in Table 7 were derived as in Model I by known factors from Table 3. The synthesis coefficient C_{SC} was set to 0.45 for all crops and for digestate to calculate the effective C input amount. The effective C input amount was also calculated with an alternative method by multiplying the SOC amount of a plot with the C turnover rate k (see below, Chapter 4.7).
The proportion of digestate-C, C3-C and C4-C inputs on individual plots and their isotopic composition were used to calculate the $\delta^{13}C$ supply for each plot (Formula (9)).
The SOC amount (kg ha^{-1}) was calculated for each plot using the specific SOC content of each plot (Formula (4)). For soil depth and bulk density, the same values as in the previous models were valid, using 0.3 m and 1.45 respectively (Formula (4)). The expected $\delta^{13}C$ of measured samples of individual years were then calculated using the $\delta^{13}C$ supply, the total effective C input amount (crops and digestate) and the initial SOC amount and its isotopic composition (Formula (10)).

4.6.3 Quantification of model parameters

To obtain an accurate model, the isotopic composition of C3 and C4 plants, digestate and the initial SOC were required. For plant material, values from the literature were used. For digestate and initial SOC, values were derived from available data of the trial site.

It could be proven that negligible fractionation takes place during digestate production in biogas plants (Chapter 4.1.5). The $\delta^{13}C$ of digestate was thus calculated by Formula (13) and by the digestate substrate composition documented since 2009 (Table 8). The isotopic composition of cattle manure, also used as a substrate, was unknown. The cattle where fed with C3 plants, hence it was assumed that the isotopic composition of cattle manure was -27 ‰.

Table 8: Dry matter amount (t) of substrates used for biogas production at Eichethof (Hohenkammern). Source of digestate for fertilization of the trial site since 2009. Cattle were fed with C3 plants.

Substrate	2009	2010	2011	2012	2013	2014	2015	2016	2017	Mean
Silage maize	546	349	166	61	45	99	0	69	125	162
Corncop	42	0	0	0	0	0	0	0	0	5
Grass silage	544	1001	1240	1567	1843	1869	1975	1924	1883	1538
Whole grain	50	174	43	324	456	268	259	266	255	233
Straw	0	18	0	0	0	0	0	0	0	2
Manure (cattle)	13	226	574	708	837	869	852	852	854	643
Total	1195	1769	2024	2660	3181	3104	3086	3111	3117	2583

$$\delta^{13}C_D = \frac{C_{C4}}{C_{Ct}} \times \delta^{13}C_{C4} + (1 - \frac{C_{C4}}{C_{Ct}}) \times \delta^{13}C_{C3} \tag{13}$$

with

$\delta^{13}C_D$ — $\delta^{13}C$ of digestate [‰]

C_{C4} — C amount of C4 plant material [t DM]

C_{Ct} — total amount of C [t DM]

$\delta^{13}C_{C4}$ — $\delta^{13}C$ of C4 plant material [‰]

$\delta^{13}C_{C3}$ — $\delta^{13}C$ of C3 plant material [‰]

For Model II the initial $\delta^{13}C$ of SOC in 2004 was required. However, the earliest samples were taken in 2009 and have already been affected by the crop rotations. Therefore, the mean $\delta^{13}C$ of measured plots without maize in the crop rotation and without digestate fertilization from the years 2009 and 2017 were used to approximate the value in 2004.

4.7 Calculation of the C turnover by a linear regression

The calculation of the C turnover was based on the natural ^{13}C abundance measurement technique developed by Balesdent et al. (1987). In a modified form, a linear regression of measured $\delta^{13}C$ and the proportion of maize harvest residues of individual plots was used to calculate the proportion of SOC derived from C4 plant material in a rotation with 100 % maize. The proportion of SOC derived from maize was then used to calculate the C turnover rate and the amount of the annual effective C input due to maize harvest residues.

The proportion of SOC derived from C4 plant material can be calculated if conditions of Chapter 4.2 are true (Balesdent and Mariotti 1996):

$$f = (\delta - \delta_{ref})/(\delta_{maize} - \delta_{C3}) \quad (14)$$

with

f	SOC derived from C4 plant material [%]
δ	measured $\delta^{13}C$ of soil sample [‰]
δ_{ref}	$\delta^{13}C$ of soil from C3 cropping [‰]
δ_{maize}	$\delta^{13}C$ of maize plant material [‰]
δ_{C3}	$\delta^{13}C$ of C3 plant material [‰]

The C turnover of an initial SOC pool C_0 follows a classical decay function. The carbon C_t that has not been turned over after a certain time *t* depends on the turnover rate *k*:

$$C_t = C_0 \times e^{kxt} \quad (15)$$

with

C_0	initial SOC content [%]
C_t	initial SOC content after time t [%]
k	turnover rate
t	time

Rearranging yields:

$$\ln\left(\frac{C_t}{C_0}\right) = k \times t \quad (16)$$

The C_t from Formula (16) corresponds to the remaining old C (C_{old}) of the initial SOC pool (C_0) and can thus be described as a proportion of C_0. The proportion of newly added C by maize (C_{new}) derived from Formula (14) corresponds to $\delta^{13}C$ of the slope (S_r) of the linear regression of $\delta^{13}C$ of SOC and the C input proportion of maize at 100 %, divided by the difference of $\delta^{13}C$ of C3 and C4 plants:

$$C_{new} = S_r \times 100/(\delta_{C4} - \delta_{C3}) \quad (17)$$

with

C_{new}	new C added to SOC
S_r	slope of the regression of $\delta^{13}C$ and the proportion of C input of maize
$(\delta_{C4} - \delta_{C3})$	difference of $\delta^{13}C$ of C3 and C4 plants

Thus, *k* can be calculated as follows:

$$k = \frac{\ln\left(\frac{C_{old}}{C_0}\right)}{t} \tag{18}$$

with

C_{old} 1 - C_{new} (%)

C_0 initial SOC pool = 100 %

The half-life-time, after which 50 % of the SOC is turned over is calculated by:

$$t_{1/2} = \frac{\ln(0{,}5)}{k} \tag{19}$$

The mean residence time (MRT) is the mean length of time C has spent in the soil, calculated by:

$$MRT = -\frac{1}{k} \tag{20}$$

The total amount of effective C input (C_{eff_t}) added to the soil every year is calculated by:

$$C_{eff_t} = -k \times SOC \tag{21}$$

4.8 Calculation of the $\delta^{13}C$ and C turnover by a multiple regression

A multiple regression of the measured $\delta^{13}C$ and the following variables of individual plots were used to calculate the C turnover:

V1) SOC [%]

V2) C input through maize [%]

V3) C input through digestate [%]

The multiple regression yielded the coefficients of the intersect, V1, V2 and V3 which were used to calculate the expected $\delta^{13}C$ of individual plots by:

$\delta^{13}C$ (SOC) = intersect + V1*coefficient (V1) + V2*coefficient (V2) + V3*coefficient (V3)

The coefficient of V2, equal to the slope of the linear regression (see above), was used to calculate the proportion of SOC derived by maize followed by the calculation of the C turnover.

4.9 Analysing the short-term $\delta^{13}C$ turnover during a four-year crop rotation

To analyse possible short-term changes of the isotopic composition of a four-year crop rotation influenced by one year of maize cropping, the isotopic composition of Block 1 - 4 of the fertilized plots of CR1b (25 % maize in rotation), sampled in October 2018, were analysed. Maize was grown on block 3 in 2018. In 2017, maize was grown in block 4. It was assumed that harvest residues of 2018 were not recorded during measurements because they have not yet been decomposed and were thus filtered out during sample preparation. Maize grown in 2018 was thus assumed to have no influence on the measurable $\delta^{13}C$ of SOC, thus the blocks were ranked by the years after the last maize was grown as follows:

1. block 4 (2017)
2. block 1 (2016)
3. block 2 (2015)
4. block 3 (2018)

5 Results

5.1 Influencing factors of SOC and $\delta^{13}C$ turnover

The $\delta^{13}C$ and SOC of all samples measured 2017 correlated significantly (Figure 5). The slope indicated an increase of $\delta^{13}C$ of approx. 1.5 ‰ when SOC changed by 1 %. The SOC varied by up to 0.62 % among plots indicating a possible variation of measured $\delta^{13}C$ due to SOC content of 0.95 ‰ (Figure 5).

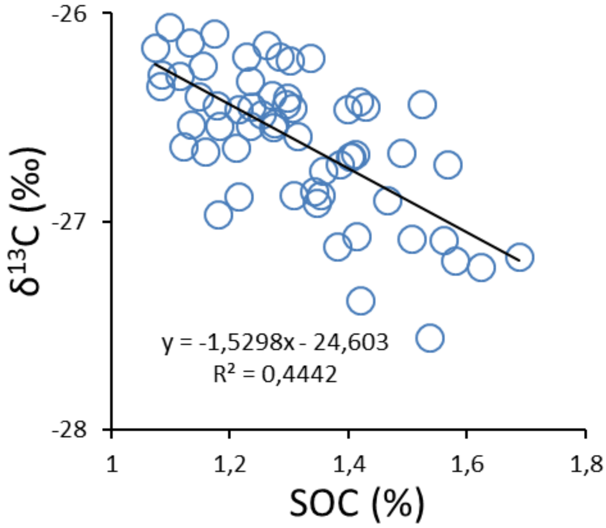

Figure 5: Correlation between $\delta^{13}C$ and SOC content of all samples measured 2017. ($n = 58$, $p < 0.05$)

Within crop rotations with identical fertilization there was variation in $\delta^{13}C$ of up to 0.7 ‰ (CR10). The maximum variation of all data was 1.48 ‰. There was a visible trend in data showing a decreasing isotopic composition with increasing crop rotation number. The $\delta^{13}C$ of fertilized plots were slightly more negative compared to unfertilized ones (Figure 6).

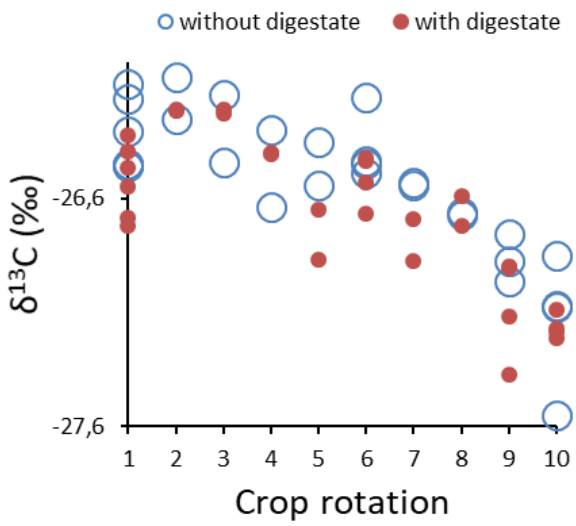

Figure 6: Isotopic composition of sampled fertilized (red circles) and unfertilized (blue circles) plots of the crop rotations (1 - 10) in 2017. CR1a, CR1b and CR1c are grouped together. Highest variation between unfertilized plots of CR10 (0.7 ‰). Maximum variation of all data is 1.48 ‰.

5.1.1 Crop rotation

There was a significant positive relationship between the calculated C input by harvest residues and digestate according to Franko (1997) and the mean SOC content of individual crop rotations measured in 2017 (Figure 7).

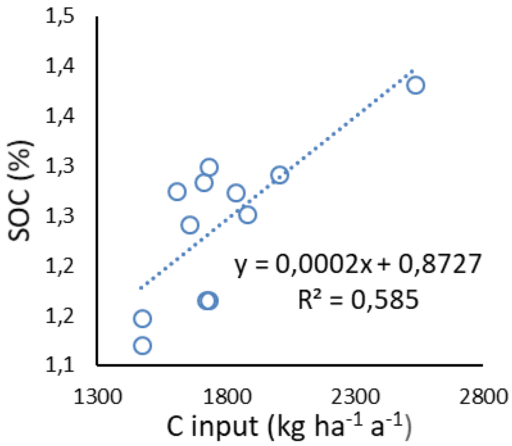

Figure 7: Correlation between C input (kg ha^{-1} a^{-1}) by plant material and digestate in individual crop rotations according to Franko (1997) and the mean SOC content of individual crop rotations measured by the Lehrstuhl für Ökolandbau 2017. (n = 12, $p < 0.05$)

The SOC change between 2009 and 2017 (Figure 8) excluded the influence of former various SOC contents. Hence its correlation with the estimated C input per crop rotation became closer than the correlation between the calculated C inputs and the measured mean SOC content of individual crop rotations sampled in 2017 (Figure 7).

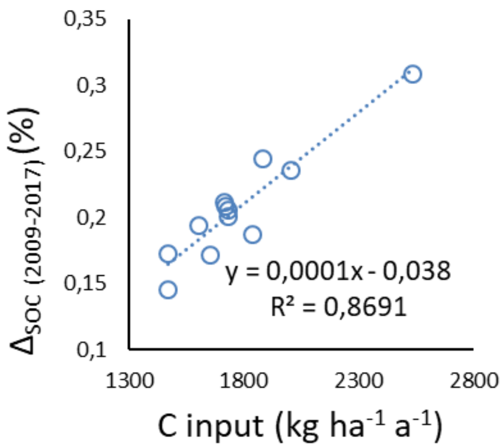

Figure 8: Correlation of C input (kg ha^{-1} a^{-1}) by plant material and digestate in individual crop rotations calculated according to Franko (1997) and the SOC change between 2009 and 2017 in this crop rotations. (n = 12 and $p < 0.05$)

There was a slightly positive and significant correlation between the SOC content and the proportion of clover-grass in the crop rotation of plots sampled in 2017 (Figure 9).

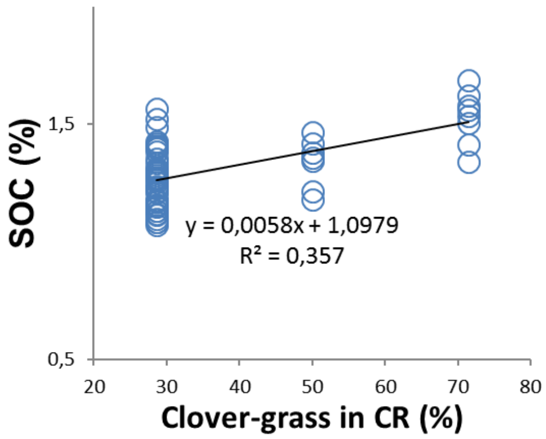

Figure 9: Significant correlation between the SOC content and the proportion of clover-grass in the crop rotation of samples taken 2017. ($n = 58$, $p < 0.05$)

5.1.2 Organic fertilization

There was a positive but not significant correlation of SOC change between fertilized and unfertilized plots of a crop rotation and the amount of digestate fertilization (m^3 ha^{-1}) per crop rotation (Figure 10). Four outliers were evident. The upper two overestimated the relationship and originated from CR9 and CR10 having a high clover grass content of 50 and 75 % respectively.

The other two outliers originated from CR6 and CR8 which were the only once having 50 % row crops in the crop rotation (maize and sunflower).

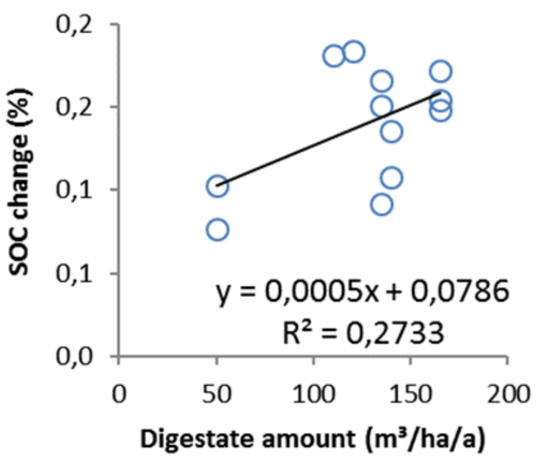

Figure 10: Correlation of SOC change and digestate (m^3 ha^{-1}) for each crop rotation.

5.1.3 Heterogeneous soil properties

A SOC increase from 2009 to 2017 was observed for almost all samples (Figure 11). The regression lines of 2009 and 2017 converge to each other with increasing SOC contents in 2009, hence the C enrichment decreased the higher the former C content was in 2009. The regression lines would cross each other at a SOC content of 1.55 %.

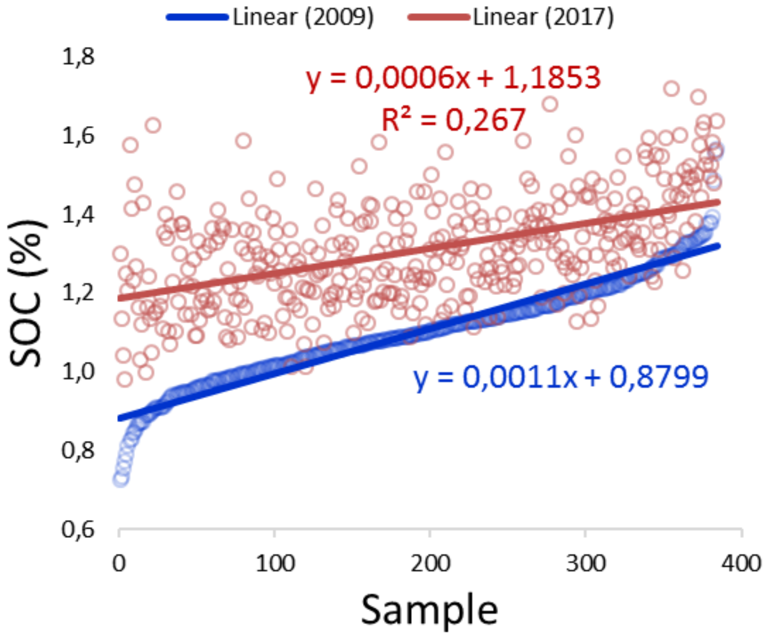

Figure 11: The SOC content of all plots sampled and measured 2009 (blue points) and 2017 (red circles) by the Lehrstuhl of Ökolandbau. The data of 2009 are ranked to a cdf (Chapter 4.4). The regression lines of 2009 (blue) and 2017 (red) intersect at a SOC content of 1.55 %.

The variance of SOC between 2009 and 2017 within plots of a crop rotation ranged from 0.006 to 0.024 (Figure 12). There was a significant difference in the variance between some crop rotations. Within block 3 the variance was highest in crop rotation CR10 with 0.032, followed by CR3 with 0.022. In all other crop rotations, the average variance was significantly lower at 0.006 compared to CR10 and CR3.

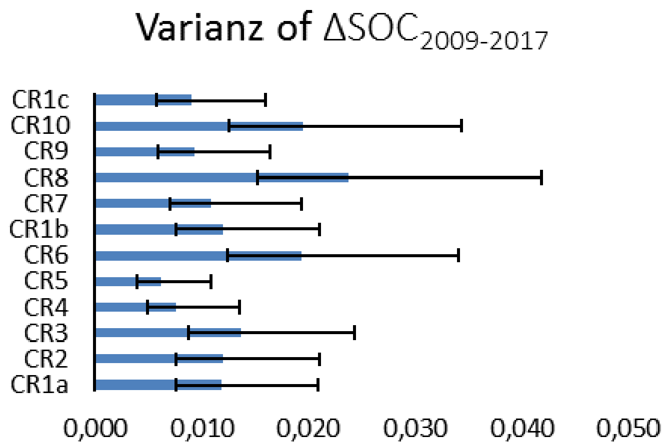

Figure 12: Variances of SOC enrichment between 2009 and 2017 within plots of a crop rotation. Error bars show SE. ($n = 32$, $p < 0.05$)

The intercept of the regression lines of SOC contents of 2009 and 2017 of samples taken from crop rotation CR3 and CR4 was at a SOC content of approx. 1.29 % (Figure 13). In these crop rotations the estimated C input by digestate and plant material was lowest (1651 kg ha^{-1} a^{-1}). The intercept of samples taken of CR8, CR9 and CR10 was higher with an SOC content of approx. 1.68 % (Figure 14). In these crop rotations the estimated C input was highest compared to all other crop rotations (av. 2500 kg ha^{-1} a^{-1}).

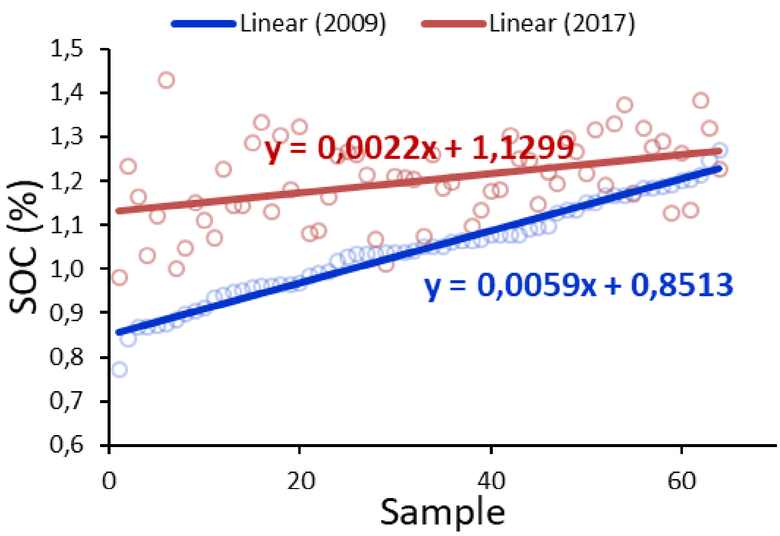

Figure 13: The SOC content of all plots of crop rotations CR3 and CR4 of 2009 (blue line) and 2017 (red line) sampled and measured by the Lehrstuhl of Ökolandbau with the lowest C input of all crop rotations (av. 1651 kg ha^{-1} a^{-1}). The data of 2009 are ranked to a cdf (Chapter 4.4). The regression lines of 2009 (blue) and 2017 (red) intersect at a SOC content of 1.29 %.

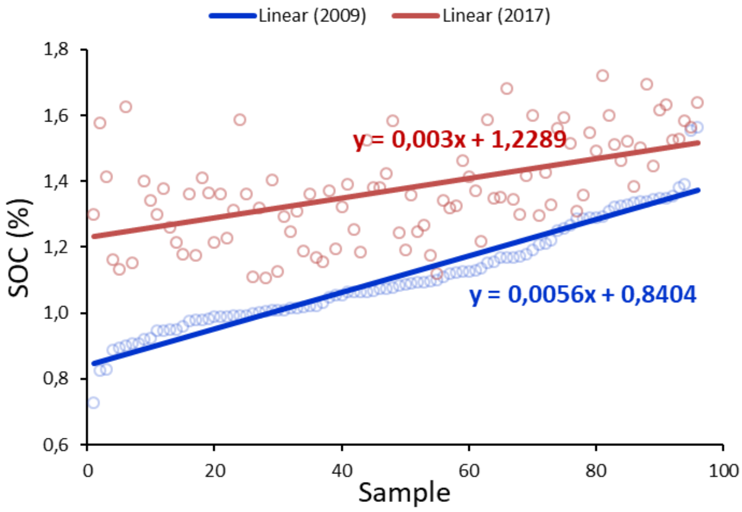

Figure 14: The SOC content of all plots of crop rotations CR8, CR9 and CR10 of 2009 (blue line) and 2017 (red line) sampled and measured by the Lehrstuhl of Ökolandbau with the highest C input of all crop rotations (av. 2500 kg ha^{-1} a^{-1}). The data of 2009 are ranked to a cdf (Chapter 4.4). The regression lines of 2009 (blue) and 2017 (red) intersect at a SOC content of 1.68 %.

Average SOC contents of block 1 - 4 in the years 2009 and 2017 indicated a site effect with increasing C from block 4 to block 1 (Figure 15). Block 1 was located at the lower edge of the trial site and might be enriched in C and loess by erosion. However, there was no significant difference between SOC of block 1 and 4 in both years. There was a highly significant increase of SOC from 2009 to 2017. The average SOC content from all blocks in 2009 was 1.1 % and raised to 1.31 % in 2017.

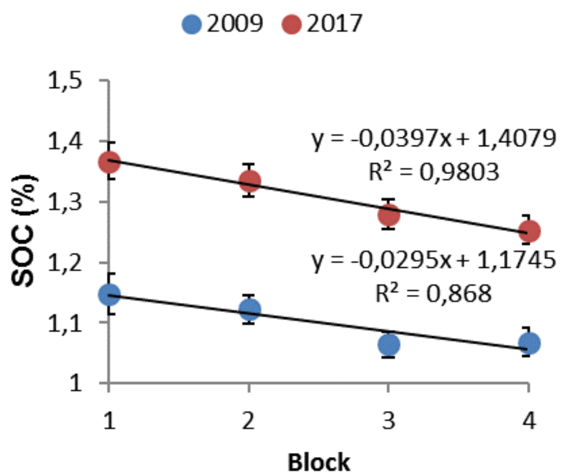

Figure 15: Average SOC contents of block 1 - 4 in 2009 (blue points) and 2017 (red points). SOC contents of block 1 and 4 in the years 2009 and 2017 increase from block 4 to block 1 but there is no significant difference ($n = 192$, $p < 0.05$). Highly significant increase of SOC from 2009 to 2017 ($n = 763$, $p < 0.001$). The average SOC content from all blocks in 2009 amounts 1.10 % and rises to 1.31 in 2017. Error bars show SE.

There was variation in SOC in the fertilized plots of block 1 - 4 of CR1b sampled in 2018 of 0.3 % (Figure 16). Only fertilized plots where measured, hence variation caused by differences in the amount of digestate were excluded. There was no significant drift in SOC through the blocks. The SD of SOC of block 1 (0.02) was lower than of the other blocks (block 2: 0.07; block 3: 0.06; block 4: 0.08). Block 1 was ploughed and prepared for wheat sowing a few days before sampling. The SD of plots sampled in 2009, showed similar variation in block 3, which were also sampled in October but after wheat.

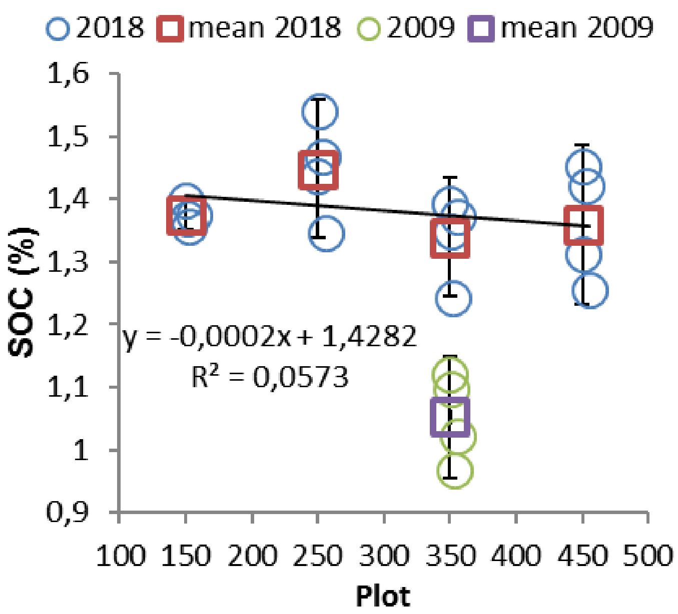

Figure 16: The SOC content of fertilized plots of CR1b, block 1 - 4, 2018 (blue circles) and block 3, 2009 (green circles). Squares indicate means, error bars show SD.

The plots of the trial site sampled in 2017 were heterogenous regarding SOC (Figure 17). Red labeled plots contained less C than green ones. The fertilized plots partly had visible higher SOC contents than the unfertilized plots. For example, in CR10, a pattern was clearly visible.

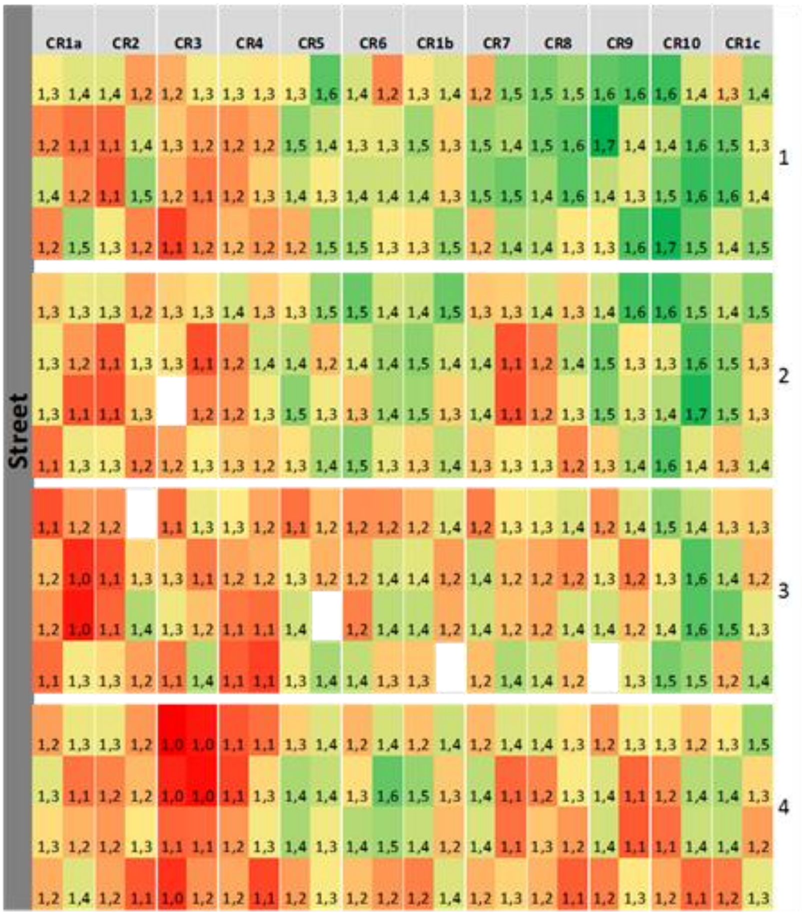

Figure 17: The SOC content of all plots sampled in 2017. Color gradation illustrates SOC content (intense red are lowest values; intense green are highest values. Missing values are white. Top row indicates the crop rotation. Numbers at the right denote blocks.

The SOC content of fertilized plots corrected by the effect of digestate on SOC led to a more homogenous SOC distribution (Figure 18). However, there was still variation in SOC. The SOC contents of plots of CR10 were remarkably higher. These plots contained a high proportion of clover grass in the crop rotation (75 %).

The former road has influenced the affected plots, which contained less SOC. The difference between the SOC content of plots on the former road and on surrounding plots was highly significant. Block 3, was affected in CR3, CR4 and CR5. The SOC contents of plots of the same crop rotation and fertilization also differed. The highest variance was in CR10 (0.018), CR9 (0.016), CR8 (0.014) and CR3 (0.010) with differences in SOC up to approx. 0.5 %.

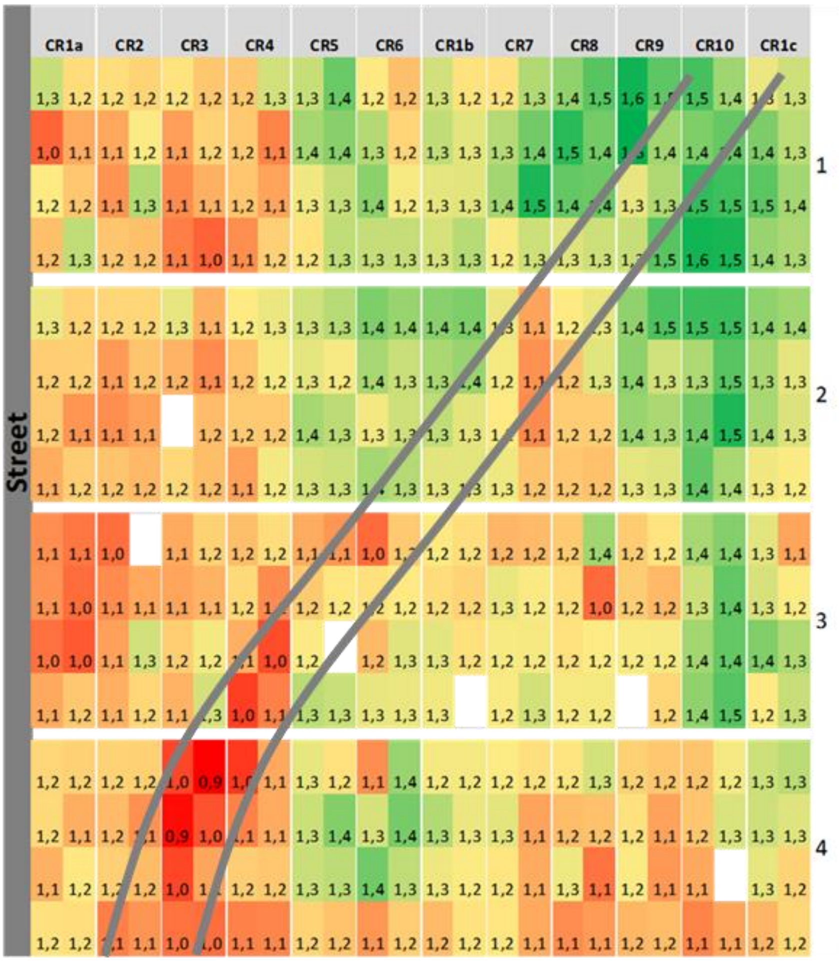

Figure 18: The SOC content of all plots measured in 2017. Color gradation illustrates SOC content (intense red are lowest values; intense green are highest values. Missing values are white. Top row indicates the crop rotation. Numbers at the right denote blocks. Fertilized plots are corrected by the effect of digestate. Two grey lines border the former road crossing the trial site. The difference between the SOC content between the former road and the surrounding plots is highly significant. (two-sample t-test; $p < 0.001$; $n = 82$)

The highest point of the trial site was in the southern part of block 4 (Figure 19). The lowest point was in the middle of the outer edge of block 1. The maximum difference in altitude was 12 meters. The slope from the highest to the lowest point of the trial site was about 7 % and within crop rotations at a maximum of 3 %.

The plots were still heterogenous regarding SOC after the impact of digestate, the former road and the crop rotation were deducted (Figure 20). The former road could not be removed completely and was still visible on the map as red plots with low SOC contents surrounded by yellow plots with mean SOC contents in block 3 and 4 and as yellow plots with mean SOC contents surrounded by green colored plots with high SOC contents in block 1.

The highest SOC content was not at the lowest point of the trial site but lies in the southern part of block 1 (Figure 19, Figure 20). The crop rotations CR1a to CR4 hold lower SOC contents although they were located at a lower point of the trial site than crop rotations with higher SOC contents. At the highest point of the trial site in block 4 the SOC content seemed to be lower.

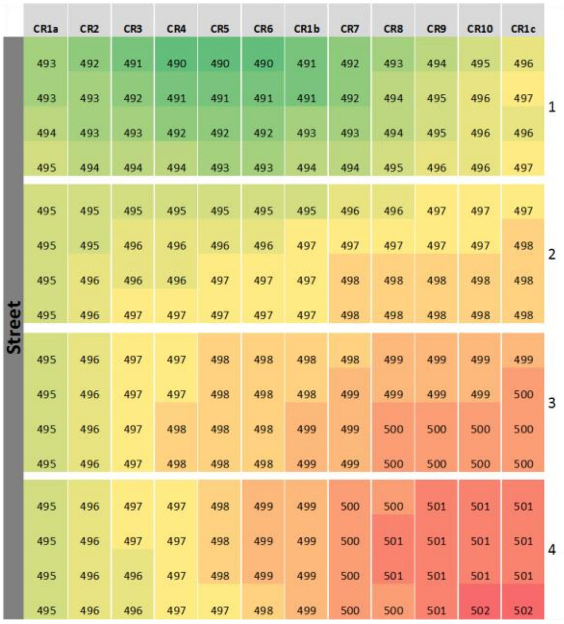

Figure 19: Topography of the trial site plotted in a color scale (intense red are lowest values, intense green are highest values, numbered in meters of height.

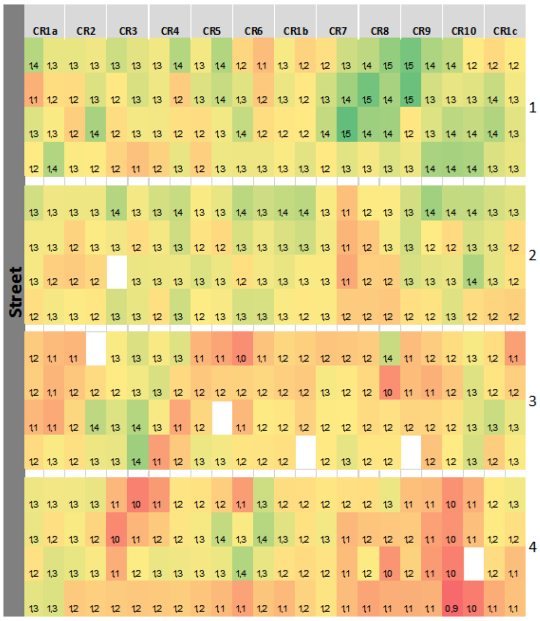

Figure 20: Fertilized plots are corrected by the effect of digestate also the effect of the former road and the crop rotations are partly removed. (Structure and colors as in Figure 17)

5.2 Model parameters

5.2.1 δ^{13}C of digestate

The measured δ^{13}C of digestate sampled in 2014 and 2015 varied between -28.20 ‰ and -22.98 ‰ (Figure 21). The N content varied between 2.27 % and 3.39 %. One sample deviated strongly from the others regarding N content and δ^{13}C. The reason of deviation was unknown. Replicated measurements showed that it was not a measurement error. Hence a calculated median of the δ^{13}C values (-26.77 ‰) was used as a robust estimator for further calculations.

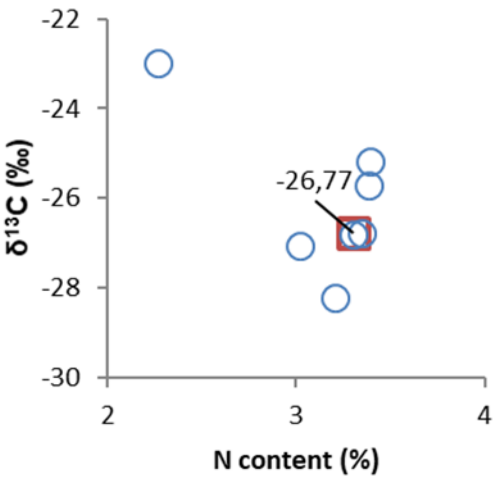

Figure 21: Correlation of the isotopic composition and nitrogen content of digestate samples measured in 2014 and 2015 (blue circles). Correlation of the medians of the isotopic composition and the nitrogen content of measured plots (red square).

The calculated average isotopic composition of 2014 and 2015 according to the substrate composition of digestate was -26.78 ‰ and corresponded to the above calculated median of the measured $\delta^{13}C$ values (-26.77 ‰). The calculated $\delta^{13}C$ of digestate in 2009 (-20.22 ‰) was significantly higher than in 2017 (-26.45 ‰) due to a high proportion of maize in the substrate composition in 2009 (49 %), compared to 2017 (4 %) (Table 9). The average $\delta^{13}C$ of digestate from 2009 to 2017 was calculated to -26.11 ‰ and used for further modelling.

Table 9: Calculated isotopic composition of digestate of individual years (2009 – 2017) according to the proportion of maize in the digestate substrate composition. Mean $\delta^{13}C$ of all years yields the digestate model parameter.

Year	**Maize (%)**	**$\delta^{13}C$ (‰)**
2009	49	-20.22
2010	20	-24.28
2011	8	-25.87
2012	2	-26.68
2013	1	-26.80
2014	3	-26.56
2015	0	-27.00
2016	2	-26.69
2017	4	-26.45
Mean	6	-26.11

5.2.2 $\delta^{13}C$ of initial SOC

The $\delta^{13}C$ of plots without maize and without fertilization in the crop rotation of samples taken in 2009 and 2017 varied between -27.55 ‰ and -26.14 ‰ (Figure 22). The SOC content varied between 0.95 % and 1.54 %. The mean $\delta^{13}C$ of all samples was -26.78 ‰, the mean SOC content was 1.22 %.

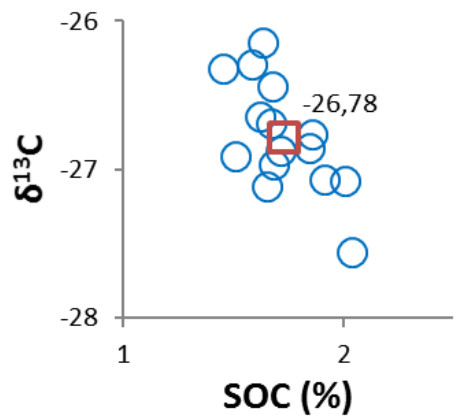

Figure 22: Correlation between $\delta^{13}C$ and the SOC content of individual unfertilized plots without maize in the crop rotation sampled in 2009 and 2017 (blue circles). Correlation of the mean $\delta^{13}C$ and SOC of all plots (red square).

5.3 Model I: $\delta^{13}C$ turnover since 1961

There was an increase in $\delta^{13}C$ from -28.0 ‰ to approx. -26.6 ‰ until 1995 (Figure 23). From 1995 to 2005, a clear decrease of $\delta^{13}C$ was apparent. Since 2005 $\delta^{13}C$ in soil increased again, although the slope is lower than between 1961 and 1995. In 2009 the modeled $\delta^{13}C$ was -26.6 ‰.

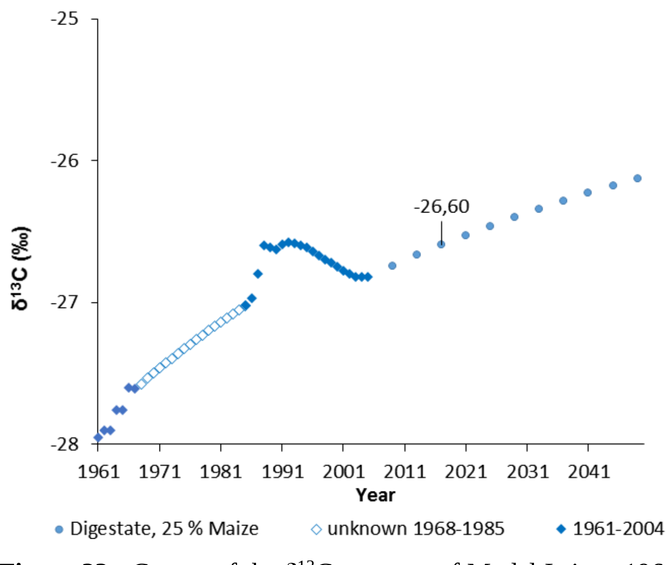

Figure 23: Course of the $\delta^{13}C$ turnover of Model I since 1961. Unfilled checks are unknown crop rotations and yields estimated to 30 % silage maize and 53 dt ha^{-1}. Since 2005 model parameters are set to digestate fertilization (35 m^3 ha^{-1} a^{-1}) and 25 % silage maize in the crop rotation.

The $\delta^{13}C$ turnover of five different model configurations since 2004, common on the trial site, varies dependent on the model parameters (maize content in the crop rotation and digestate fertilization) (Figure 24). In rotations without maize $\delta^{13}C$ decreases, in rotations with maize $\delta^{13}C$ increases. The effect of fertilization is stronger between the model configurations without maize in rotation, compared to rotations with 38 % maize. The $\delta^{13}C$ turnover rate of the model configurations declines by time until a new $\delta^{13}C$ steady-state is established were the isotopic composition of soil remains unchanged (Table 10).

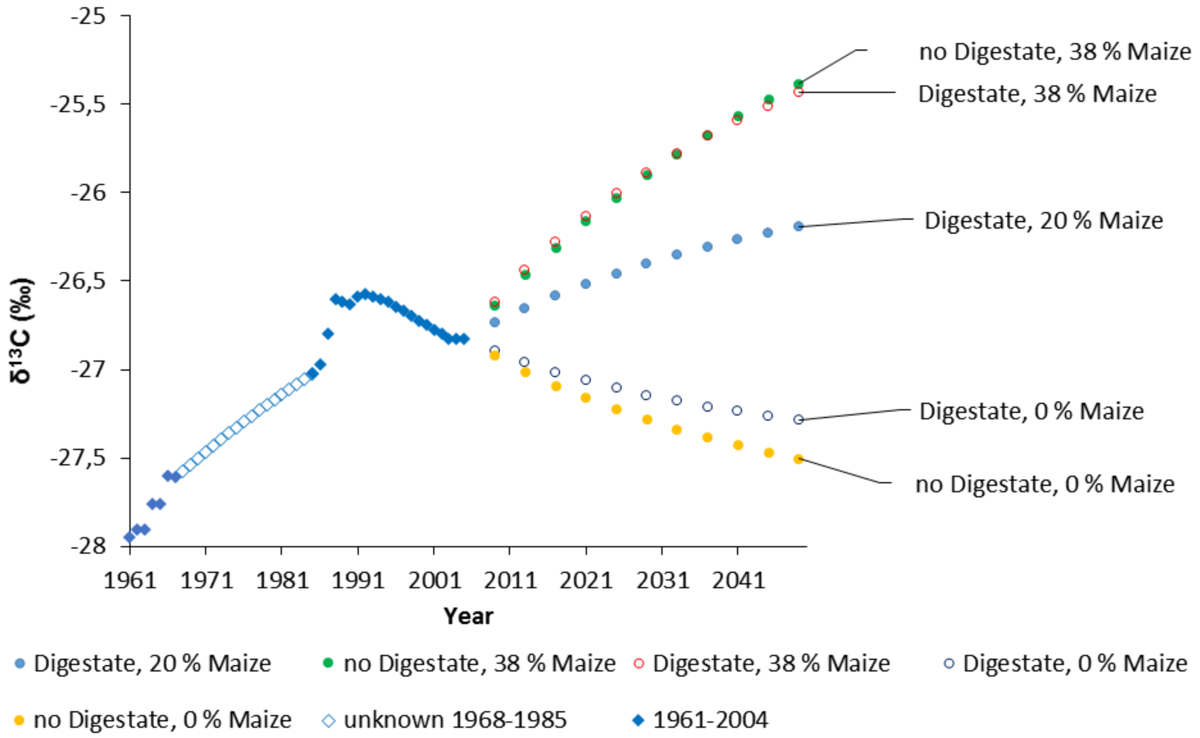

Figure 24: Course of the $\delta^{13}C$ turnover of Model I since 1961. Unfilled checks are unknown crop rotations and yields estimated to 30 % silage maize and 53 dt ha^{-1}. Five possible model configurations common on the trial site (fertilized or unfertilized plots with 0, 20 and 38 % maize in the crop rotation) from 2005 onwards show different $\delta^{13}C$ turnover courses (colored circles).

Table 10: Isotopic composition of SOC of five model configurations in the year in which the $\delta^{13}C$ steady-state is reached. Delta $\delta^{13}C$ shows the maximum change of the isotopic composition of SOC in the model configurations since 2005 (-26.8 ‰). Values rounded to one decimal place.

Model configuration	**Year**	**$\delta^{13}C$ steady-state (‰)**	**$\Delta\ \delta^{13}C_{(2005\text{-steady-state})}$ (‰)**
no digestate, 38 % maize	2517	-23.7	3.1
digestate, 38 % maize	2445	-24.4	2.4
digestate, 20 % maize	2233	-25.7	1.1
digestate, 0 % maize	2137	-27.5	0.7
no digestate 0 % maize	2269	-28.0	1.2

5.3.1 δ¹³C turnover of individual model configurations compared to observations

From 2009 to 2017 there were marginal changes in the isotopic composition of rotations (CR6, CR1b, CR10) and no clear relation between the maize content in the rotation and the $\delta^{13}C$ turnover (Figure 25). In plots without digestate fertilization and 33 % or 43 % (av. 38 %) maize in the crop rotation (denoted $CR6_{nd}$), an increase in $\delta^{13}C$ was visible. Also, in plots with digestate fertilization and 17 %, 20 % or 21 % (av. 20 %) maize (denoted $CR1b_d$). Whereas $\delta^{13}C$ of plots with digestate and 33 % and 43 % maize (denoted $CR6_d$) and the once with 0 % maize in the rotation (denoted $CR10_d$) decreased.

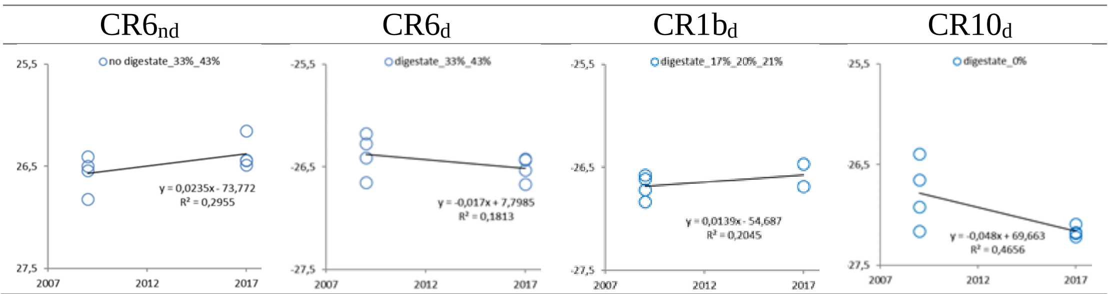

Figure 25: Changes in the isotopic composition between 2009 and 2017 of soil samples grouped by crop rotations with different maize content and digestate fertilization: CR6nd) on av. 38 % maize with digestate fertilization; CR6d) on av. 38 % maize without digestate fertilization; CR1bd) on av. 20 % maize with digestate fertilization; CR10d) 0 % maize with fertilization.

The $\delta^{13}C$ of individual rotations (fertilized or unfertilized) sampled in 2009 and 2017 did not differ significantly. The variability of $\delta^{13}C$ was higher in rotations without maize and digestate, respectively (0.79 ‰ in 2009 and 0.7 ‰ in 2017) (Figure 26).

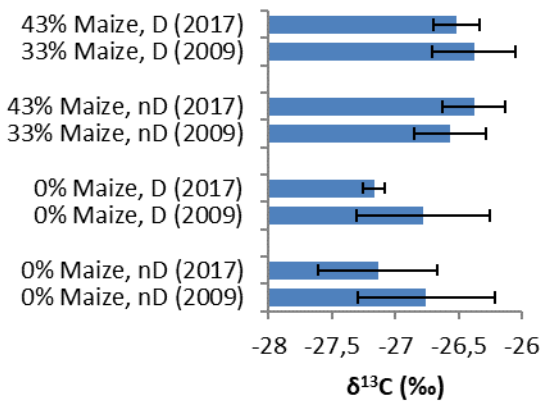

Figure 26: Comparison of the mean isotopic composition of individual rotations sampled 2009 and 2017. Measured $\delta^{13}C$ values do not differ significantly. Error bars show SE. (one-way ANOVA, df = 35, $p < 0.05$)

The $\delta^{13}C$ of different fertilized and unfertilized rotations measured in 2009 did not differ significantly regarding the proportion of maize in the rotations (Figure 27).

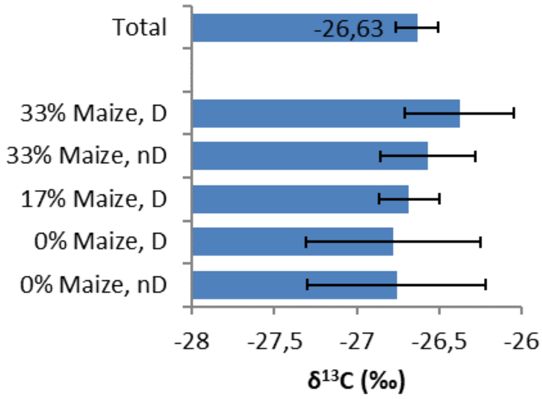

Figure 27: Mean $\delta^{13}C$ values of plots in rotations with different maize contents measured in 2009 do not differ significantly. Error bars show SE. (one-way ANOVA, df = 15, $p < 0.05$)

In 2017 there was a significant difference between plots with 43 % and 0 % maize in the rotation. The influence of maize on $\delta^{13}C$ after 12 years had thus a measurable effect. Whether the plots were fertilized or not had no significant effect (Figure 28). This insight is also evident regarding fertilized and unfertilized model configurations that have been shown above (Figure 24).

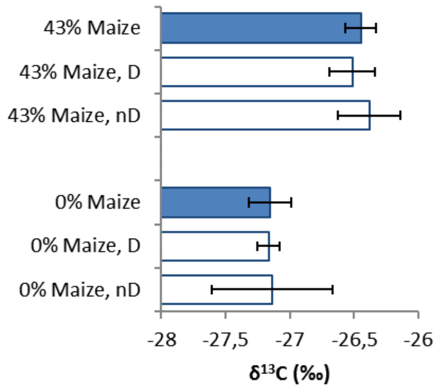

Figure 28: The $\delta^{13}C$ values between plots with 43 % maize and 0 % maize sampled 2017 differ significantly. Error bars show SE. (one-way ANOVA, df = 12, $p < 0.05$)

The $\delta^{13}C$ turnover of model configurations shown above (Figure 24) and the measured $\delta^{13}C$ turnover of $CR6_{nd}$, CR1b and CR10 from 2009 to 2017 overlapped adequately. The model configuration with digestate fertilization and 38 % maize did not match the measurement results of digestate and 33 % or 43 % maize ($CR6_d$).

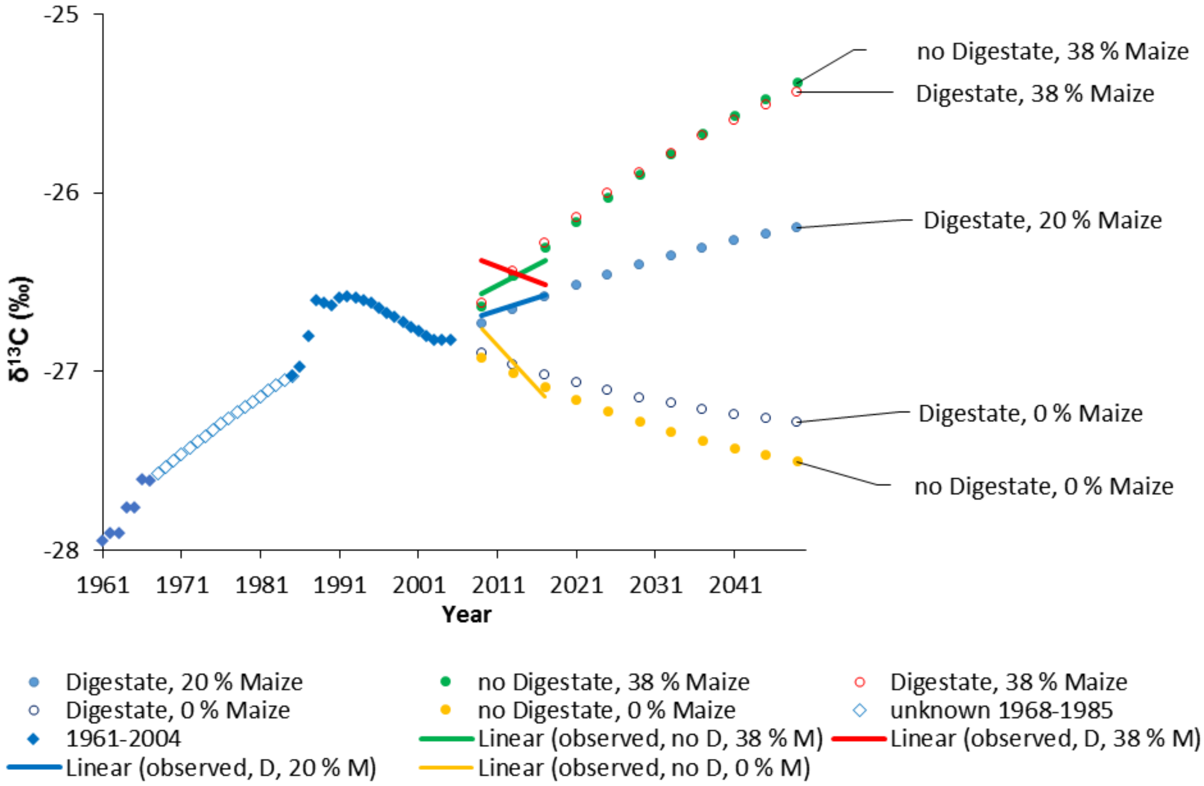

Figure 29: Course of the $\delta^{13}C$ turnover of Model I since 1961. Unfilled checks are unknown crop rotations and yields. Five possible model configurations common on the trial site (fertilized or unfertilized plots with 0, 20 and 38 % maize in the crop rotation) from 2005 onwards show different $\delta^{13}C$ turnover courses. The observed (linear) and the modeled $\delta^{13}C$ of the different management constellations (points, circles) overlay each other, except the graph with digestate fertilization and 38 % maize does not fit.

5.4 Model II: Comparison of expected and observed $\delta^{13}C$

There was a significant correlation between measured $\delta^{13}C$ of plots sampled in 2017 and the modeled $\delta^{13}C$ of these plots calculated by Model II and the model parameters of Table 11(Figure 30). Crop rotations CR3 and CR4 (8 samples) have been omitted as outliers as they were influenced by the former road in block 3 were the most measurements were done. The 1:1 regression overlaid with the regression line of observed and expected values. The range between the lowest and the highest $\delta^{13}C$ was about 0.95 ‰ for the expected values and 1.48 ‰ for the observed ones. By omitting two samples with the lowest measured $\delta^{13}C$ as outliers, the range was only 1.14 ‰.

Table 11: Parameters for Model II to calculate the isotopic composition of SOC for individual plots.

Parameter	**Value**
δ^{13}C SOC	-26.78 ‰
δ^{13}C maize plant material	-13.22 ‰
δ^{13}C digestate	-26.11 ‰
δ^{13}C C3 plant material	-28 ‰
Synthesis coefficient C_{SC}	0.45

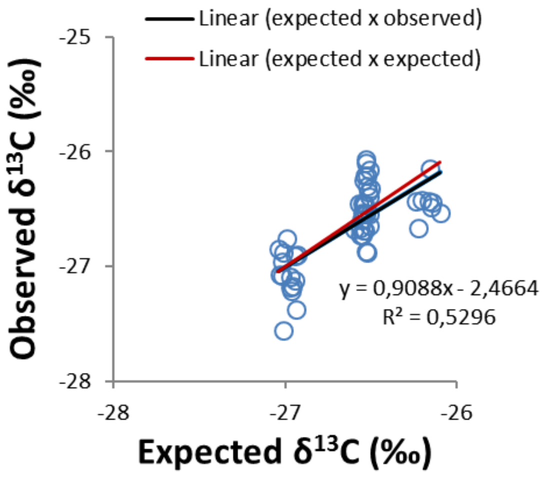

Figure 30: Correlation between measured (observed) and modeled (expected) δ^{13}C of plots sampled and measured 2017, without CR3 und CR4 (black line). The red line shows the ideal 1:1 regression. ($n = 50$, $p < 0.05$)

The correlation of measured and modeled δ^{13}C (Model II) was significant and improved (compared to Figure 32) by calculating the annual effective C input using a C turnover rate of -0.015. The slope of the regression line was then slightly 1.

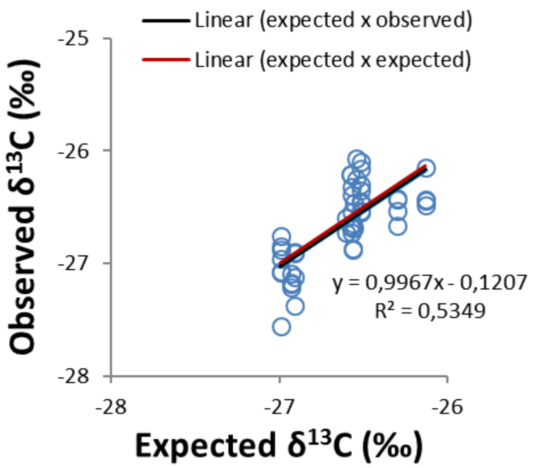

Figure 31: Correlation between measured (observed) and modeled (expected) δ^{13}C values of plots sampled and measured 2017, without CR3 und CR4 (black line). The red line is the ideal 1:1 regression. Turnover rate k (-0.015) is used to calculate the effective annual C input through plant material and digestate. ($n = 50$, $p < 0.05$)

5.5 C turnover (linear regression)

There was a significant correlation between the C input proportion by maize and $\delta^{13}C$ from samples of block 3 taken in 2017 and 2018 (Figure 32). The crop rotations CR3 and CR4 were omitted as outliers. The isotopic composition increased with increasing maize C input. There was scattering in $\delta^{13}C$ of plots between a crop rotation, especially with 0 % maize C input in rotation.

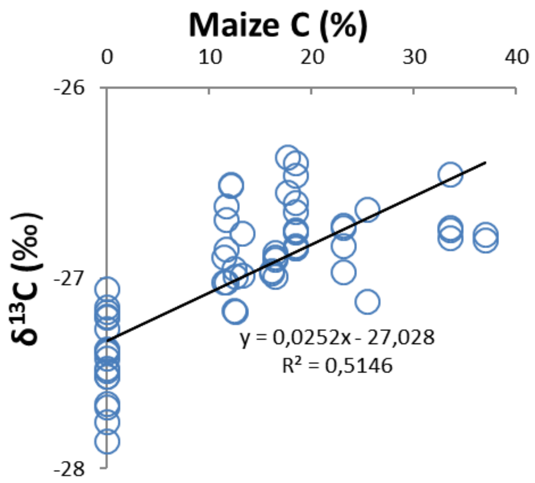

Figure 32: Correlation of the C input proportion by maize and $\delta^{13}C$ from samples of block 3 taken in 2017 and 2018, without CR3 and CR4. ($p < 0.05$, $n = 99$)

The slope of the regression of the proportion of maize C input and $\delta^{13}C$ was 0.0252. Hence, 100 % maize in crop rotation would increase $\delta^{13}C$ of SOC by 2.5 ‰. The difference of $\delta^{13}C$ between C3 (-28 ‰) and maize (-13.22 ‰) was calculated to -14.78 ‰. After 12 years of monoculture with maize 16.9 % of SOC would have been turned over and replaced, giving a turnover rate of -0.015 yr^{-1}. The half-life-time after which 50 % of SOC is turned over, was calculated to 45 years. The MRT was calculated to 65 years.

Based on an average SOC amount on the trial site of 50.000 kg ha^{-1}, the amount of new organic matter added to the topsoil (30 cm) every year was calculated to 772 kg ha^{-1} a^{-1}.

Table 12: Variables and results of the C turnover calculation according to the slope of the regression line of C input content through maize and $\delta^{13}C$ of SOC of all plots sampled and measured 2017 and 2018 without CR3 and CR4.

Years	12	
Slope S_r	0.025	
SOC	50000	kg ha^{-1}
C3-C4	14.78	‰
C_{new}	16.91	%
C_{old}	83	%
k	-0.015	yr^{-1}
$t_{1/2}$	45	Yr
MRT	65	Yr
C input	772	kg ha^{-1} a^{-1}

5.6 C turnover (multiple regression)

There was a significant correlation between measured $\delta^{13}C$ of plots sampled in 2017 without CR3 and CR4 and the calculated $\delta^{13}C$ of these plots according to the multiple regression (Figure 33). SOC had the greatest significant influence on $\delta^{13}C$ of soil (V1, coefficient: -1.077) followed by maize C % (V2, coefficient: 0.019). Digestate had no significant influence on $\delta^{13}C$ of soil (V3, coefficient: 0.003). The proportion of SOC derived by maize according to V2 was calculated to 12.86 %. The annual C input was calculated to 573 kg ha^{-1} a^{-1} (Table 13).

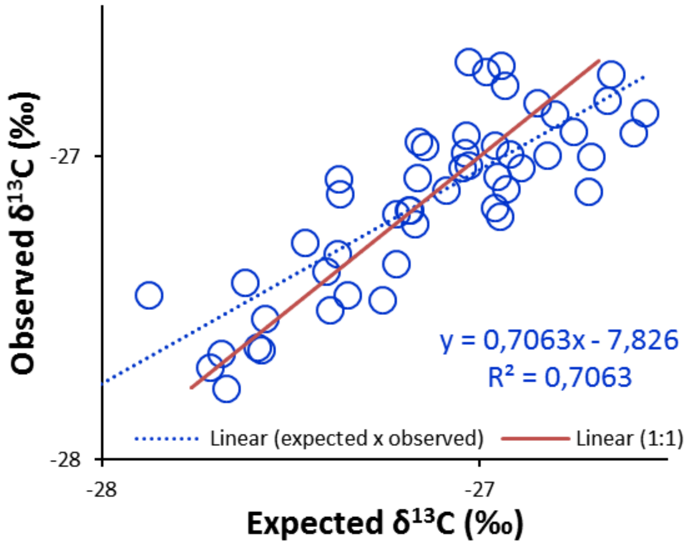

Figure 33: Correlation of $\delta^{13}C$ of plots measured in 2017 without CR3 and CR4 (observed) and the calculated (expected) $\delta^{13}C$ of these plots by the coefficient of the variable V2 (maize C input content) in a multiple regression (blue line) with the additional variables V1 (SOC content) and V3 (C input content through digestate). The red line shows the 1:1 regression. ($n = 50$, $p < 0.05$)

Table 13: Variables and results of the C turnover calculation according to the coefficient of the variable of C input through maize (V2, 0.019) of the multiple regression (Figure 33).

Years	12	
Slope S_r	0.019	
SOC	50000	kg ha^{-1}
C3-C4	14.78	‰
C_{new}	12.86	%
C_{old}	87	%
K	-0.011	yr^{-1}
$t_{1/2}$	60	yr
MRT	87	yr
C input	573	kg ha^{-1} a^{-1}

5.7 Short-term $\delta^{13}C$ turnover during a four-year crop rotation

There was no significant correlation between the years after the last maize cropping and $\delta^{13}C$ of SOC in the crop rotation CR1b (Figure 34). With increasing years after the last maize cropping on a block, $\delta^{13}C$ seemed to increase. The highest $\delta^{13}C$ was in in Block 3, four years after the last maize cropping. The lowest $\delta^{13}C$ was in block 4, one year after the last maize cropping. The variance of the SOC content of individual crop rotations sampled in 2017 was lowest in CR1b (Table 14).

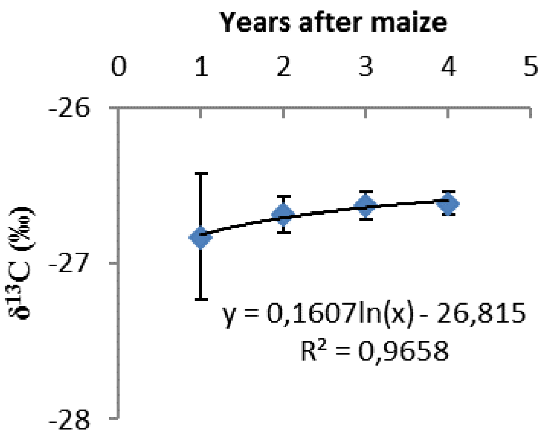

Figure 34: Logarithmic regression of years after maize and $\delta^{13}C$ of block 1 - 4 of the fertilized plots of CR1b measured in October 2018. No significant differences between the blocks. Error bars show SE.

Table 14: Variance of the SOC content of individual crop rotations sampled in 2017.

Rotation	CR1a	CR2	CR3	CR4	CR5	CR6	CR1b	CR7	CR8	CR9	CR10	CR1c
Variance	0.006	0.004	0.010	0.007	0.006	0.009	0.003	0.009	0.014	0.016	0.018	0.005

6 Discussion

6.1 Influencing factors on SOC and $\delta^{13}C$ on the trial site

There was a significant negative correlation between SOC and $\delta^{13}C$. Two theories can explain the correlation:

1) The SOC has been increased and turned over mainly by C3 C input then by maize leading to a more negative $\delta^{13}C$ with increasing SOC. This presupposes specific C input amounts and associated isotopic compositions of the C sources involved in the turnover.
2) Plots that have had higher SOC concentrations since the beginning of maize cultivation in 1961 are turned over more slowly, leading to a slower increase of $\delta^{13}C$ through maize C input. This presupposes that the trial site is heterogeneous regarding soil properties.

The SOC content of almost all plots had increased since the beginning of the Biomasseversuch, probably due to the change of conventional agriculture to organic farming, associated with higher C inputs from organic fertilizers and higher amounts of crop residues. The correlation between the SOC content and SOC change (increase) between 2009 and 2017 and the estimated total annual C input by digestate and plant material according to Franko (1997) was significant. This method for calculating C input quantities seems thus suitable for C balancing and for modelling the $\delta^{13}C$ turnover. The C input amounts through digestate and plant material differed between the crop rotations. With increasing C input amounts, the SOC content also had increased, consistent to several studies (Franko et al. 1995; Franko 1997; Coleman 2014).
Crop rotations with a high clover grass content (> 50 %) increased SOC more strongly compared to rotations with low clover grass contents (25 %), consistent with higher C input amounts according to Franko (1997). Clover grass was cut several times a year forming a sward consisting of a dense root system. The inherent carbon was not removed from the field and may thus contributed to a relatively high accumulation of SOC (Acharya et al. 2012). Especially in crop rotations with perennial clover grass, the soil was not mixed for a longer period by tillage, which may slow down mineralization by microorganisms. This resulted in a higher SOC accumulation. However, in contrast to this explanation, the relationship between SOC and clover grass content was weak. There was a positive but not significant correlation of SOC change between fertilized and unfertilized plots of a crop rotation and the amount of digestate fertilization ($m^3\ ha^{-1}$) per crop rotation. The crop rotations were fertilized with different amounts of digestate, which also have contributed to the increase of SOC. In addition, the fertilization

increased plant growth and thus the C input by plant material. In consequence, the fertilized plots had a higher SOC content than the unfertilized plots. This also implies that crop rotations with a low clover grass content can also have a high SOC content if they receive high amounts of digestate. For example, CR6 had a maize content of 50 % and a clover grass content of 25 %, the annual calculated C input due to harvest residues and digestate fertilization was as high as in CR9 with 0 % maize and 50 % clover grass (2238 and 2355 kg ha^{-1} a^{-1} respectively). The average C input by maize of all sampled plots was 20 % of the total C input, consequently, the C input by digestate and C3 plant material was 80 %. Hence, maize had a significantly lower influence on the turnover and mainly the C3-C inputs and their isotopic composition led to rising SOC contents and the observed decline in $\delta^{13}C$ of SOC, supporting the first theory (see above). The decline in $\delta^{13}C$ also indicated a former higher $\delta^{13}C$ of soil on the trial site which is discussed later in more detail. Only in rare cases the $\delta^{13}C$ of plots increased with increasing SOC content. In these plots the maize content was high, and no fertilization had been applied.

The C sequestration of plots decreased with increasing SOC content also indicating a limited C sequestration capacity of soil and the formation of a new C steady-state on the trial site consistent to McFee et al. (1995). A possible C steady-state on the trial site was reached earlier and at a lower level on plots with a lower C input but steady-state has not yet been achieved in 2018.

Deviant soil properties on the plots may led to different water, air and nutrient supply for plants and thus may have influenced plant growth and C inputs through harvest residues (Franko 1997; Klimanek 1997). Hence, an enrichment of SOC of varying intensity within plots of a crop rotation indicated soil heterogeneity and associated varying $\delta^{13}C$ turnover rates.

Part of this soil heterogeneity was evident from the variation in SOC contents increasing from block 4 to block 1 indicating a site effect. Block 1 was located at the lower edge of the trial site. It is a common phenomenon that SOC contents increase downslope. In particular, footslope and toeslope positions, which are relatively more moist (Walker et al. 1968), also have larger amounts of organic matter and fine particles (Malo et al. 1974), while upslope positions loose topsoil material mainly due to tillage erosion (Zhao et al. 2018).

The variation in SOC of plots of block 3 in crop rotation CR1b were similar in 2009 and 2018 but differed compared to the other blocks. This indicated that the variation was not made by sampling and measurement errors but by outlasting site effects. Thus, a long-lasting soil heterogeneity cannot be excluded that was already established before 1961 and still affects the C and $\delta^{13}C$ turnover in the present.

Summarized, the negative correlation between SOC and $\delta^{13}C$ may also be explained by the second theory (see above). Therefore, the trial site was further analyzed regarding soil heterogeneity.

The effect of digestate fertilization, the harvest residues and the former road on the SOC was deducted to analyze site effects that have not been caused by farming management (crop rotation and digestate fertilization) and the road. The deduction referred to a more homogeneous SOC pattern but there was still 46 % of total variation left. The remaining variation of SOC content on the plots was likely due to unknown site effects and measurement errors including errors in farming management (e.g. higher or lower digestate application as intended)

There was no clear visible correlation between SOC content and topography regarding water erosion. Only at the upslope the SOC content appeared to be lower consistent to Malo et al. (1974). This could also indicate a higher amount of fine particle and a higher water content of soil in the footslope compared to the upslope according to Walker et al. (1968) and associated differences in plant growth, C stabilization (Klimanek 1997) and $\delta^{13}C$ turnover rates between plots. However, the trial site was structurally diverse. The individual plots were cultivated at different times of the year and rarely left fallow at the same time. In addition, the cultivation of cover crops largely maintained the soil cover. A water-induced erosion was therefore unlikely since the beginning of the long-term trial. During conventional agriculture before 1995, a water-induced erosion at a slope gradient of 3-7 % could have been conceivable under the given conditions (slope length, soil texture, precipitation) and could have influenced the soil properties of individual parts of the trial site. More likely, tillage erosion may had a stronger influence on soil heterogeneity consistent to Zhao et al. (2018). For example, tillage could have shifted the topsoil of crop rotations from block 4 to 1. Especially in CR9, CR10 and CR1c the differences in meters of height between block 4 and 1 were largest and SOC increased with decreasing meters of height.

Although the former road has disappeared 40 years ago, its influence was still present in 2018 and interfered the evaluation of data. The road had a significant influence on the SOC content of affected plots. The SOC was generally lower in these plots and the C enrichment by time was also lower. Hence also the $\delta^{13}C$ turnover was influenced on these plots. Possible reasons were remaining mineral residues of the road (e.g. gravel) and soil compaction, which may have impaired the conditions for plant growth and C storage.

In adjacent plots of a crop rotation where one plot was influenced by the road and the other was not influenced, the differences in SOC were 0.1 to 0.3 % (without the effect of digestate). In Model I (50 % maize in rotation, no fertilization, initial $\delta^{13}C$ = -28 ‰) the calculated turnover on the road is faster due to the lower SOC content. The difference in $\delta^{13}C$ between plots with 1 % and 1.3 % SOC would be about 0.4 ‰ after 40 years. The real average difference was probably smaller as in most crop rotations only 25 % maize were grown. In addition, the differences between road plots and surrounding plots were only 0.1% in most cases. In these plots the difference in $\delta^{13}C$ turnover would only amount 0.1 ‰ after 40 years.
To avoid biases in the modeling of the $\delta^{13}C$ turnover and the calculation of the C turnover, selected plots were thus omitted as outliers (8 plots of CR3, CR4 in block 3). This adaption has significantly improved the correlation between the observed and expected $\delta^{13}C$ in Model II. The R^2 has increased from previously 0.23 to 0.53, the slope increased from 0.59 to 0.92.

Other, small-scaled soil heterogeneities between single plots are not probable for the trial site according to its geology derived by a homogenous loess horizon (> 25 cm). The remaining variation of SOC contents are thus caused by measurement errors and unintended deviations in the management which have not been further investigated.
According to Körschens (2010), SOC contents are subject to large fluctuations during the vegetation period as well as between the years. A fluctuation of 0.2 % C was observed on arable land during the vegetation period (Körschens 1982). Similarly, high fluctuations could also be observed in other long-term experiments over 40 years (Rogasik et al. 2008; Baumecker et al. 2009). Hence, only long-term experiments over several decades can reliably prove stable changes in SOC (Rogasik et al. 2008). The short observation time on the trial site in Viehhausen thus limited a reliable interpretation of shown results regarding SOC-dependent $\delta^{13}C$ turnover rates. The reliability of data would increase with increasing duration of the experiment thus future measurements are required.

6.2 Evaluation of model parameters

6.2.1 $\delta^{13}C$ of digestate

The comparison of measured digestate samples with calculated $\delta^{13}C$ values of these samples proved that the isotopic composition of digestate can be calculated via the substrate composition of the digestate components (plant material, cattle manure etc.). The findings are in agreement

to Balabane et al. (1987) and Lv et al. (2019) who showed that there is no fractionation of C isotopes during metanogenesis.
The average $\delta^{13}C$ of digestate as a parameter for the model was calculated to be -26.11 ‰ according to data of substrate composition from 2009 until 2017. The substrate composition of digestate from 2004 to 2008 was unknown and digestate originated from another organic farm. Biogas production without maize is unusual in the region of the trial site due to economic reasons (Herrmann 2013). It is not unlikely that digestate in the first few years has been more influenced by maize than in the last years. The true average value of $\delta^{13}C$ in digestate may even be somewhat higher than calculated and would change the calculated $\delta^{13}C$ turnover of fertilized plots in the model. The rotations in the experiment indicate that the maize content in the biogas source cannot be higher than 50 % because clover-grass is required to fix atmospheric nitrogen. Such a rotation is, however, extremely uncommon in organic farming and would produce no other farm product than biogas. It is more likely that the maize content in rotations of organic farming systems is not higher than 25 %. The mean maize content in digestate would increase from 6 % (mean of 2009 to 2017) to 13 % (mean of 2004 to 2017) if a maize content in digestate of 25 % is assumed during the questionable period, given that the questionable period is 5 years while digestate is known for 9 years. This would change $\delta^{13}C$ of calculated digestate from -26.11 ‰ to -25.23 ‰. The difference of 0.87 ‰ is hence the upper limit of the error, which may be caused by the lacking data during 2004 to 2008. Very likely, the true error is much lower, because the present calculation of a worst case is only true for the cropland while the grassland area of the organic farm has to be considered as well.
The influence of digestate from the first years on the calculated average $\delta^{13}C$ of digestate for the model also decreases with increasing test duration of the experiment. Since the future maize content in digestate remains at 6 % the calculated error decreases to 0.30 ‰ after 20 years. The assumed $\delta^{13}C$ for digestate was therefore well suited to model the long-term $\delta^{13}C$ turnover.

6.2.2 $\delta^{13}C$ of initial SOC

The $\delta^{13}C$ of most C3 plants varies between -26 ‰ and -29 ‰ (Smith and Epstein 1971; Gregorich et al. 1995; Finlay and Kendall 2007). Isotope measurements of different cattle diets cropped in 2003 including triticale (seeds), wheat (seeds) clover-grass (fresh, silage) and grassland (fresh, silage) showed average $\delta^{13}C$ values of -28.4 ‰ for roughage and -26.8 ‰ for concentrates (Schwertl et al. 2005). The measured plant material was cultivated in the region of Viehhausen and was thus representative for this trial. Concentrates from C3 grains were enriched by 1-4 ‰ relative to leaves (Hobbie and Werner 2004). This indicated that non-grain plant material of triticale and wheat compared to seeds measured by Schwertl et al. (2005) were probably depleted in ^{13}C and thus similar in $\delta^{13}C$ compared to roughage. Therefore, $\delta^{13}C$ of SOC was equally developed by harvest residues of common C3 crops with a $\delta^{13}C$ of approx. -28 ‰. In Addition, the entire variation in plants is only reflected in soil if plants of the same low or high values grow on a soil over centuries. This is highly unlikely because many different species will contribute to SOC and growing conditions will vary during different years. Hence the variation in soil should be much smaller than the variation in plants. There is one exception from this reasoning. Most of the variation of $\delta^{13}C$ in C3 plants is caused by variation in stomatal opening (Farquhar et al. 1989), which in turn is influenced by drought. Soils differing considerably in water storage capacity could hence also differ in $\delta^{13}C$ because drought will occur more often on soils with low storage capacity. Given that storage capacity on the trial site is high and variation is small, differences in soil induced drought should be marginal.

Considering the Suess effect, $\delta^{13}C$ of plant material in 2003 was probably 1.5 ‰ lower compared to 1961. Hence the initial $\delta^{13}C$ of SOC developed by C3 plant material used in Model I was perhaps -26.5 ‰ instead of -28 ‰ Nevertheless, with the parameters chosen, Model I was able to reproduce precisely the measured $\delta^{13}C$ of SOC in 2009 and 2017. The following possible reasons may explain this:

1) The Suess effect was lower than expected.
2) Other estimated parameters in Model I (e.g. the proportion of maize in the unknown crop years (1968-1986) have compensated the error. In a consequence they were estimated wrong.
3) The SOC turnover in the topsoil through younger, more negative plant material was stronger than estimated.

A combination of the reasons is plausible but cannot be quantified. Compared to Model I, the initial $\delta^{13}C$ of SOC could be determined more accurate in Model II.

The most reliable method to determine the initial $\delta^{13}C$ value for Model II is to measure soil samples. However, no samples were taken at the beginning of the experiment in 2004. Hence, the mean value of $\delta^{13}C$ of unfertilized plots without maize content from 2009 and 2017 were used yielding -26.78 ‰. A possible approach to verify the quality of this initial value was to compare it with the modelled $\delta^{13}C$ from Model I. The isotopic composition of the SOC of Model

I in 2009 corresponded to -26.76 ‰ on average of all simulated model configurations and thus coincided exactly to the mean of the measured $\delta^{13}C$ of 2009. The modeled $\delta^{13}C$ in 2004 was -26.82 ‰ and differed only slightly from the measurement results (0.06 ‰). The determined initial $\delta^{13}C$ value for Model II was therefore suitable for modeling.

6.3 Evaluation of the models

6.3.1 Model I

Model I was suited to describe the $\delta^{13}C$ turnover of the past decades, which has been strongly influenced by grain maize cultivation. The isotopic composition of SOC was still influenced by grain maize in 2017 (av. -26.6 ‰). It was less negative than a soil that has only been influenced by C3 plant material (approx. -28 ‰). This finding explains unexpected results in the measurement data, as a general increase in $\delta^{13}C$ of SOC through maize cultivation was expected in the first thoughts of this thesis. However, a constant or decreasing $\delta^{13}C$ from 2009 to 2017 was observed on certain plots congruent to the modeled $\delta^{13}C$ turnover of different model configurations in 2009 and 2017.

The plots concerned were mainly cultivated with C3 plants, whose more negative isotopic composition (compared to SOC) reduced the higher $\delta^{13}C$ of SOC. The influence of maize roots and stubble since 2004 was much smaller and difficult to quantify. Even on plots with higher proportions of maize in the crop rotation, only minor, non-significant changes in the isotopic composition between 2009 and 2017 were evident.

The $\delta^{13}C$ of a soil would remain unchanged if the annual $\delta^{13}C$ supply by C inputs corresponds to the $\delta^{13}C$ of the soil. Low C inputs by maize and correspondingly high inputs by C3 plants can lead to this equilibrium. If the C input by maize in a rotation would be 12 % (common on the trial site), a $\delta^{13}C$ supply of -25.6 ‰ is calculated in the model, which leads to a $\delta^{13}C$ turnover of 0.25 ‰ after 12 years (initial $\delta^{13}C$ was set to -26,78 ‰). Even in crop rotations with the highest C input through maize in 2017 (34 %) the $\delta^{13}C$ supply would only be -23.02 ‰. Under the given SOC turnover on the trial site, this leads to a $\delta^{13}C$ turnover of 0.6 ‰ in 12 years. Since sampling errors caused inaccuracies of about 0.5 ‰ (SD) and the measurement error of IRMS was 0.11 ‰ (SD), the total error is at least as large as the calculated $\delta^{13}C$ turnover after 12 years. This indicates that the $\delta^{13}C$ turnover through maize stubble and roots in rotations with C3 plants cannot be detected clearly after 12 years of cropping under the given conditions. Since the $\delta^{13}C$ of the SOC corresponds to the $\delta^{13}C$ supply in the long term, future measurements of plots with a maize C input of 12 % will not show any noticeable changes in $\delta^{13}C$. The maximum

$\delta^{13}C$ turnover will be about 3.8 ‰ in crop rotations with 50 % maize which is more likely to be detectable. If the trial site had not been affected by the cultivation of grain maize, higher changes of up to 5 ‰ would be measurable, more likely to show significant correlations. To determine the suitability of an experimental setup for investigations as described in this thesis, it is thus essential to consider the prehistory of soil.

Digestate had only a small impact on the turnover of crop rotations with 38 % maize until 2040 due to its isotopic composition similar to the common $\delta^{13}C$ supply of C3 plants and maize. When the $\delta^{13}C$ steady-state in rotations with 38 % maize was reached, a difference in $\delta^{13}C$ of soil of 0.7 ‰ between the fertilized and unfertilized model configuration appeared. In crop rotations without maize, the impact of digestate was higher until 2040 leading to a less negative $\delta^{13}C$ when plots were fertilized. This is reasonable because the isotopic composition of digestate was calculated to be less negative than C3 plant material. When the $\delta^{13}C$ steady-state in the rotations without maize was reached, a difference in $\delta^{13}C$ of soil of 0.5 ‰ between the fertilized and unfertilized model configuration appeared, which is less then between rotations with 38 % maize. Hence, the influence of digestate on the $\delta^{13}C$ turnover between different rotations changes by time, when the isotopic composition of soil changes.

The isotopic composition of the model parameters (initial SOC, C3 plant material, C4 plant material, digestate) and the calculation of the yield-dependent C inputs are decisive for an accurate calculation of the $\delta^{13}C$ turnover. Determining the initial $\delta^{13}C$ of soil influenced by former C3 crop rotations (varying by approx. 3 ‰) and the Suess effect (indicating a change of $\delta^{13}C$ of plant material by 1.5 ‰ in 60 years) had constitute the greatest uncertainty. The Suess effect also interfered the modeled $\delta^{13}C$ turnover, which was calculated with a constant parameter regarding $\delta^{13}C$ of plant material but did not account a change in $\delta^{13}C$ of plant material over the years. The calculations of the yield-dependent C inputs were also based on estimates that could not be verified. The model is therefore sensitive to biased assumptions. Comparing the modeled turnover with measured data cannot verify individual estimated variables if several other variables are also uncertain. Therefore, in Model II estimated variables were replaced by measured values to exclude sources of error.

6.3.2 Model II

The comparison of measured samples from 2017 with the modeled once showed an adequate correlation although there was only a marginal $\delta^{13}C$ turnover of SOC between 2004 and 2017 in a range of less than 2 ‰ due to minor C inputs through maize roots and stubble, high C3 plant C inputs and the previously discussed influence of grain maize.

In addition, the comparison of the model and measurement data was sensitive regarding measurement errors and heterogeneous site conditions leading to variation of $\delta^{13}C$ in a similar range. To detect time dependent model errors more accurately, a longer observation period is recommended.

In contrast to Model I, the C increase on the trial site was not calculated in Model II. This may have slightly underestimate the $\delta^{13}C$ turnover because:

1) A SOC increase indicates that more of the new plant material enters the SOC pool than old C is mineralized, hence there was no first order decomposition kinetic.
2) The turnover calculated by $\delta^{13}C$ supply and the total C input of all previous crop years was not calculated annually, but in a single step. The SOC content of the actual year was used to model the $\delta^{13}C$ turnover of several years, assuming the SOC amount in steady-state. However, the turnover decreases with increasing SOC content, hence the turnover in 2009 was faster than in 2017 which was not considered.

The slightly underestimated turnover may explain the higher range of observed $\delta^{13}C$ (1.14 ‰ when outliers were omitted) compared to the expected range (0.95 ‰).

The C enrichment decreased with increasing SOC content. A possible C steady-state on the trial site would be reached earlier and at a lower level on plots with a lower C input but has not yet been achieved. A site dependent varying C enrichment and the required time to reach a new steady-state was not considered when modelling the $\delta^{13}C$ turnover, leading to additional uncertainties.

The effective C input by harvest residues and digestate was previously calculated using the synthesis coefficient developed by Franko (1997). In a modified form of the model the turnover rate k was used which was calculated to determine the C turnover. The model has improved slightly. In contrast to the synthesis coefficient, which was developed according to trials in Bad Lauchstädt, Germany (Klimanek 1997), probably under different conditions, the turnover rate has a direct relation to the trial site in Viehhausen and may therefore be better suited for modelling. Since the turnover rate was calculated based on measured plots that have been affected by the SOC increase between 2009 and 2017, the SOC increase was partly considered in this model.

Possible interactions between plant growth, fertilization and cover crops could led to higher yields and C inputs on individual plots (Klimanek 1997). However, the average yields of all plots (divided into fertilized and unfertilized plots) were used in the model, and cover crops were not included, as difficult to be quantified and distinguished from the main crops. The average yields of individual plots could have been integrated into the model to improve it, but there were too little yield data available. In contrast to Model I, the influence of the Suess effect on the calculated $\delta^{13}C$ was negligible, since only a turnover period of 12 years was considered in which there was only a marginal change in the isotopic composition of the atmosphere (0.5 ‰) and even less change in soil. For a longer observation period, the Suess effect should be integrated into the model to avoid errors.

Due to the physical and chemical properties of the C isotopes and their resulting constant and predictable behavior in natural processes, the calculation of the $\delta^{13}C$ turnover is generally based on simple calculations. A precise $\delta^{13}C$ turnover modeling in Model II is thus mainly dependent on the accuracy of model parameters and the C turnover model. There are several studies and models that have dealt with the modelling of the C turnover (Smith et al. 1997; Stockmann et al. 2013), in particular the model CANDY (Franko et al. 1997). These models are more complex and accurate than the model used in this thesis. Hence, there is still potential to improve the $\delta^{13}C$ turnover model by implementing an existing C turnover model, e.g. the CANDY model. However, the combination of a C turnover model with a $\delta^{13}C$ turnover model to calculate the $\delta^{13}C$ of the SOC, as shown in Model II, has not yet been implemented in other investigations, according to the current knowledge.

6.4 Evaluation of the calculated C turnover

After 12 years, on average 16.9 % (1.4 % a^{-1}) C were replaced by maize harvest residues in the topsoil of the trial site in a 100 % maize rotation. Assuming a SOC content of 50000 kg ha^{-1} (common on the trial site) this yields a total C input of 8450 kg ha^{-1}.

The calculated C input by maize roots and stubble using CANDY was 14785 kg ha^{-1} after 12 years in a 100 % maize rotation. Accordingly, 57 % of the available maize C harvest residues were effectively added to SOC. According to the slope of the multiple regression (0.019), 43 % of the maize C harvest residues were effectively added to SOC.

The input amounts of the individual C sources have been calculated using the average yields and the assumptions of Franko (1997) and are therefore estimates. Hence, the accuracy of the C turnover calculation depends on the reliability of these estimates. The experimental setup in Viehhausen was originally not designed for the investigations of this thesis. Only three different proportions of maize were distinguished in the crop rotations (0, 25, 50 %). The yield data in Viehhausen indicated that maize yields decreased with an increasing proportion of maize in the crop rotation probably caused by a lack of nutrients, an increasing competition by weeds and due to possible increasing diseases and insect pests. An experimental setup that also contains crop rotations with higher proportions of maize (75 and 100 %) would probably change the results. One would then expect a flattening of the slope of the regression which is used for the calculation of the turnover leading to a higher MRT and a smaller amount of SOC derived by maize. If CR6 with 50 % maize in rotation is excluded in the liner regression, the slope rises by 65 % from 0.025 to 0.038 yielding a maize C content in soil of 25.7 % after 12 years, which is 8.8 % higher than calculated when CR6 is included in the regression. Assuming a flattening of the slope to 0.016 (65 % of 0.025) by additional rotations with 75 and 100 % maize, the calculated maize C in soil after 12 years would be 10.8 %.
According to Balesdent and Mariotti (1996), the greatest variation in the calculation of the C turnover is probably caused by site and its management. The C turnover rate was clearly dependent on soil properties and differs between the plots due to soil heterogeneity. The calculation of the C turnover of individual plots was not possible because of the experimental setup in Viehhausen, as C3 plants and organic fertilizer were integrated in the crop rotations next to maize. The C input by C3 plants and digestate were rarely determined variables that contributed to the $\delta^{13}C$ turnover but could not be distinguished from the influence of maize C input. Only by using a regression, it was possible to calculate the turnover of the total trial site. Nevertheless, the variation of C input amounts by C3 plants and digestate between individual crop rotations led to variation of $\delta^{13}C$. For example, the variation for plots with 0 or 21 % maize content was approx. 0.8 ‰. As a result, the regression and thus the turnover calculations became less accurate. Omitting three (of 51) measured plots with the lowest $\delta^{13}C$ of rotations without maize, the slope of the regression decreases from 0.025 to 0.021 which yields a C maize content of 14.8 % after 12 years. This is 2.1 % less than previously calculated (16.9 %).
In addition, the variation of $\delta^{13}C$ within a crop rotation due to soil heterogeneities of approx. 1 ‰ and a marginal mean difference of $\delta^{13}C$ between single crop rotations of less than 2 ‰ due to a marginal $\delta^{13}C$ turnover by maize roots and stubble may led to uncertainties in the

calculation of the maize induced C turnover. In other studies, the turnover was calculated by a direct comparison of plots where only C3 or C4 plants were cultivated for several decades. This provides significant higher differences in $\delta^{13}C$ of soil. In these studies, the C turnover was calculated by Formula (14).

A long-term trial in Halle (Germany) that started in 1878 was suitable to calculate the C turnover by Formula (14). At Halle, one part of a trial area with rye monoculture was replaced by a silage maize monoculture in 1961. The C input by maize roots and stubble and its influence on the C turnover was investigated comparable to this study. According to Flessa et al. (2000), after 37 years 15 % of SOC in the topsoil originated from maize C. A total possible C input of 2.94 kg m^{-2} by maize residues was calculated. The effective carbon input down to a depth of 35 cm caused by maize was calculated to 771 g m^{-2}. Hence, 26 % of the available C through plant residues was effectively stabilized in the SOC. Hence, the C content developed by maize plant material in Viehhausen was as high as in Halle but after a third of time. In Viehhausen approx. twice as much of the available C from harvest residues was effectively stabilized in the SOC.
The maize yields in Halle (88 dt ha^{-1}) were significantly lower than in Viehhausen (128 dt ha^{-1}). The soil in Halle was described as a sandy loess with a sand content of 70 %. In Viehhausen the soil was a loess with silty loam. The annual precipitation in Halle was 465 mm and therefore significantly lower than in Viehhausen (797 mm). The lower yields in Halle can thus be explained by the site conditions. The yields in Halle also lead to lower C inputs by harvest residues and my partly explain the lower turnover compared to Viehhausen. In addition, the C turnover was probably also different between the sites due to different clay and silt contents and associated C stabilization rates.
In Halle, the C input by maize harvest residues was calculated strongly dependent on the yields, according to Flessa et al. (2000) as described in Chapter 4.6.1 (Method II). According to Klimanek (1997), there was no significant correlation between increasing grain yields (in a range of 45 to 82 dt ha^{-1}) and the root biomass of wheat and barley. The above-ground crop residues, on the other hand, increased significantly with increasing yields. The increase was attributed to the increasing number of shoots per plant. In contrast to wheat and barley, maize has only one shoot regardless of yield. An increase in harvest and root residues due to increasing yields is thus only slightly noticeable in silage maize. This was considered when calculating the maize C Input by harvest residues according to Franko (1997) as described in Chapter 4.6.1 (Method I), were yield was almost neglected due to a low yield-dependent factor F_{CR} (0.005).

Using the yields of Halle (assuming DM = 37 %), Method I would yield a total amount of harvest residues of 4.28 kg m^{-2} in 37 years which is 45 % higher than calculated by Method II. According to Method I, only 18 % instead of 26 % of the available C harvest residues would have been calculated as effectively stabilized in the soil of Halle. This indicates that the results in both trials differ strongly due to the approached calculation methods. To better compare results, on method should be selected for both sites. According to the insights of Klimanek (1997), Method I may be better suited to compare different trial sites as different yields are almost neglected.

The amount of root exudates as an additional C source is still poorly quantified and was not considered in both methods, although it has already been shown that the C amount of root exudates of maize plants at the end of a cultivation period could be as large as the root biomass (Schulze 1993). Assuming that root exudates will increase the calculated C amounts by maize harvest residues by 80 % (20 % are assumed to refer to stubble), this yields a C input of 26613 kg ha^{-1} (instead of 14785 kg ha^{-1}) after 12 years. Hence the calculated effective C input would be 32 % (instead of 57 %). Hence, the amount of the C input through harvest residues is probably underestimated in both methods. Further studies on root exudates are thus necessary to quantify the amount of C harvest residues (Flessa et al. 2000).

In this paper a difference of -14.78 ‰ between C3 and C4 plant material was assumed to calculate the C turnover. In Halle, the difference between $\delta^{13}C$ of C3 and C4 plants was set to 16.8 ‰. According to Flessa et al. (2000), $\delta^{13}C$ of maize was measured yielding -11.6 ‰ which is relatively high in comparison to references in literature (-12 to -14 ‰). Using 16.8 ‰ for the calculations of the turnover in Viehhausen, the proportion of new C decreases from 16.9 to 14.9 %. Accordingly, only 30 % (compared to 35 %) of the available C by digestate and plant material (C3 + C4) would be calculated as the effective C input in soil. Only 50 % (compared to 57 %) would be calculated for maize plants, solely.

In a long-term trial in Woodslee, Ontario (Canada), the influence of grain maize on the SOC was investigated to determine the C turnover and the C content of the SOC derived by maize. After 32 years 22 – 30 % of the SOC in the topsoil was exchanged (Gregorich et al. 1996). This corresponds to an C exchange of 0.7 to 0.9 % per year which is lower than calculated in Viehhausen. The C input by grain maize harvest residues is significantly higher than from silage maize and should therefore led to a higher maize content in the SOC. On the other hand, the average yields of maize corn and straw in Ontario (66 dt ha^{-1} a^{-1} calculated by 50.9 dt ha^{-1} a^{-1}

corn yield and a corn-straw ratio of 1:1.3) were only half of the average yields of Viehhausen. In addition, Balesdent and Balabane (1996) showed significantly lower degradation rates of maize roots compared to the above-ground plant material. Accordingly, 60 % of the effectively added C into the soil originated from root residues. Similar findings could also be shown in a laboratory study by Klimanek (1997). Since in Viehhausen only roots and stubble remained on the field, the proportion of more stable C compounds increased in relation to the total C input by maize. Thus, the C input and turnover in Ontario probably was reduced by lower yields and higher C degradation rates of maize harvest residues. The remaining difference between Ontario and Viehhausen may also referred to the farming management and unknown site conditions.

Young, C4-derived carbon, showed a higher turnover than older, C3-derived carbon (John et al. 2003). The C turnover of older C3 plant material was slowed down due to stable bounds to the mineral fraction, recalcitrance and inclusions in aggregates which reduced the mineralization. Compared to the natural C3 vegetation which has existed for centuries and which was able to build up a stable C pool, the time in which C4 plant material was added to the soil was relatively short. Hence there might be a more stable SOC pool derived by C3 plant material, next to a more labile SOC pool derived by C4 plant material. The C turnover in soil would then lead to a preferential mineralization of new C4 plant material and a lower mineralization of old C3 plant material. In long-term experiments, as shown in the studies of Viehhausen, Halle and Ontario, the SOC enrichment by C4 plant material would thus decrease after the labile SOC pool is replaced by C4 plant material. Also, the calculated proportion of effective C input into the SOC by maize harvest residues would decrease by time. This may also partly explain the low differences in the proportion of effective C input derived by maize between Halle and Viehhausen, although the test durations varied by decades. Future calculations based on frequent isotopic measurements could examine whether the C4 content in soil increases steadily or decreases over time giving a better insight of possible stable and labile C pools in soil and their impact on the C turnover.

Summarizing the results of Viehhausen, Halle and Ontario, a high range of 10 to 50 % of the available harvest residues were effectively transferred to the SOC pool of the topsoil after several decades and more than 50 % were mineralized prematurely.

A comparison of the trials to evaluate the results is difficult for several reasons:

1) different amounts of yield.
2) different methods and assumptions to calculate the C input by plant residues.
3) different site conditions (soil properties, precipitation, etc.).
4) test duration of different lengths.

Although the trial in Halle provides a simple method to calculate the C turnover, a long-term maize monoculture is undesired in sustainable agriculture and thus does not supply representative results, comparable to Viehhausen. The experimental setup and the methods applied in Viehhausen captured the complex interrelationships of a common organic farming system with several crop rotations and organic fertilization and is thus as close to reality as possible.

6.5 Short-term $\delta^{13}C$ turnover during a four-year crop rotation

There was no significant change in $\delta^{13}C$ during a four-year crop rotation with 25 % maize in CR1b due to high variation in data compared to minor differences in $\delta^{13}C$ between the blocks. The increasing $\delta^{13}C$ with increasing years after the last maize cropping might indicate a subsequent delivery of C from maize plant material to the SOC through a gradual decomposition over time. However, the annual losses of C4 C due to microbial respiration and the simultaneous supply of C3 plant material were expected to decrease $\delta^{13}C$ with increasing years after the last maize cropping and thus contradict the findings made in CR1b. The plots of identical rotations had different SOC contents, $\delta^{13}C$ turnover rates and values. Thus, the differences in the $\delta^{13}C$ of the blocks of CR1b were not solely caused by the last maize cultivation. Compared to other crop rotations, CR1b had the lowest variance in SOC content and is therefore best suited to show unaffected short-term changes in $\delta^{13}C$ (Table 14). However, the $\delta^{13}C$ turnover on the trial site due to maize harvest residues was marginal and not significant after 12 years, hence a $\delta^{13}C$ turnover in 4 years is even less measurable.

7 Conclusions

Soil heterogeneities led to deviations of the C and $\delta^{13}C$ turnover on the plots. The course of a former road that disappeared 40 years ago still influenced the SOC and $\delta^{13}C$ turnover. Due to the detected soil heterogeneities, an unexpected high variation of $\delta^{13}C$ occurred and could be considered in the data analysis. Especially the crop rotations CR3 and CR4, crossed by the former road, were omitted as outliers leading to significant model improvements.

The modelled $\delta^{13}C$ time course since 1961 was consistent with the measured data of soil samples from 2009 and 2017. Modelling revealed an unexpected high influence of previous grain maize cropping on the isotopic composition of soil (leading to a rather high initial $\delta^{13}C$ at the start of the experiment in 2004). Crop rotations where the C input by maize harvest residues was about 20 % led to a $\delta^{13}C$ supply like the $\delta^{13}C$ of soil. Consistent with the expectation that $\delta^{13}C$ of SOC should not change over time under these conditions, no significant changes in $\delta^{13}C$ of SOC during the observation period (12 years) were observed. Measurement and modeling also agreed for those rotations with higher C inputs by maize residues (38 %) and indicated a significant increase in $\delta^{13}C$ of SOC while in pure C3 rotations $\delta^{13}C$ of SOC decreased. Hence all expectations were confirmed. In contrast to other similar experiments, which all aim to create a large effect by introducing maize on soils that formerly had not seen C4 plants, this experiment has two advantages: (i) it caused a smaller disturbance (especially with 20% maize in residues) and (ii) it caused deviations to both sides. This avoided artifacts by a sudden introduction of maize (e.g. due to differences in turnover time of C3 and C4 residue that cause imbalances until a new equilibrium is reached) and it proved that the change of $\delta^{13}C$ in SOC is reversibly reflecting cropping history.

On average 16.9 % (1.4 % a^{-1}) C would have been replaced by 100 % maize in rotation through roots and stubble in the topsoil (30 cm) yielding a total C input of 8450 kg ha^{-1}. The calculation of total residues according to the CANDY model considering measured yields generated twice this amount. This indicated that approx. 50 % of the harvest residues entered SOC while the other 50 % were mineralized if reliability of the CANDY model is assumed.

The former grain maize cropping, the low $\delta^{13}C$ turnover on the trial site, the soil heterogeneities and the SOC increase since the beginning of the Biomasseversuch were challenging but could be successfully considered by modelling. The experimental setup and the methods applied in Viehhausen captured the complex interrelationships of a common organic farming system with several crop rotations and organic fertilization and their influences on the SOC turnover, close to reality.

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9 Appendix

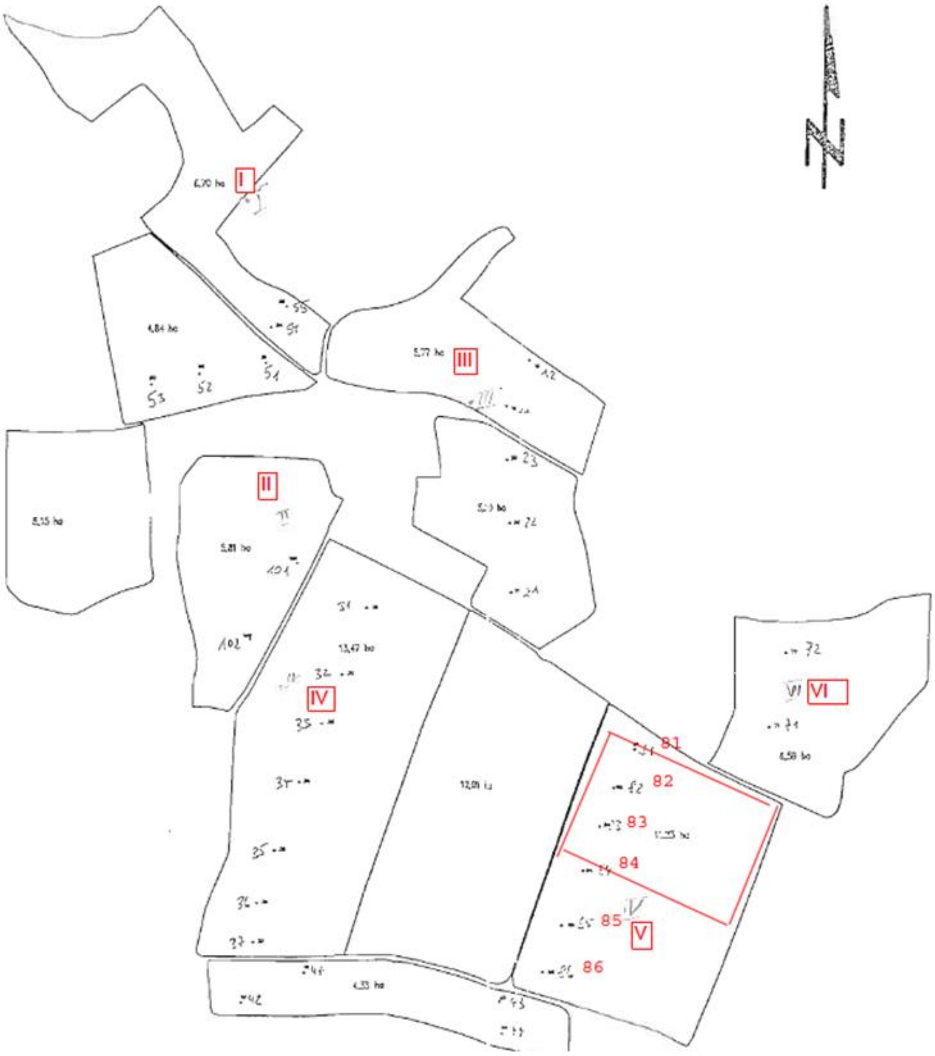

Figure 35: Position numbers for the assignment of the soil characteristics of the trial site according to Obermeier (1998). The northern part of field V is the trial site (labeled in red).

Table 15: Properties of digestate according to Monitoring Thüringer BGA 2004 to 2008, n = 125 (Reinhold et al. 2012). DM = dry matter, FM = fresh matter.

Parameter	Unit	Mean (Digestate)
DM	%	5.80
pH		7.71
Nt	% (FM)	0.43
NH_4-N	% (Nt)	68.9
organic C	% (DM)	39.2
C/N		5
P	% (FM)	0.8
K	% (FM)	3.04
Mg	% (FM)	0.06
S	mg kg^{-1} (DM)	7.638
S	kg m^{-3}	0.39
Zn	mg kg^{-1} (DM)	620
Cu	mg kg^{-1} (DM)	393

Table 16: Annual average crop yields (dt ha^{-1}) from the experimental station Viehhausen (1960 – 1997). WW = Winter Wheat, SW = Summer Wheat, SB = Summer Barley, WB = Winter Barley, O = Oat, GM = Grain Maize, BB = Broad Bean, WR = Winter Rape, WRe = Winter Rye, T = Triticale, P = Peas. Red marked values are estimates.

Year	WW	SW	SB	O	GM	WB	BB	WRa	WRe	T	P
1960	33.2	28.3	30.3	30.3	45.8	-	-	-	-	-	-
1961	31.3	33.8	32.7	-	46.8	-	-	-	-	-	-
1962	45.9	39.3	31.3	-	44.7	-	-	-	-	-	-
1963	45.5	33.65	38.8	48.3	53.0	-	-	-	-	-	-
1964	45.9	33.65	31.4	38.6	46.2	-	-	-	-	-	-
1965	36.2	28	23.7	29	21.5	-	-	-	-	-	-
1966	28.5	32.5	34	39.2	52.9	-	-	-	-	-	-
1967	36.2	31.5	29.3	40.3	42.1	-	-	-	-	-	-
1968	44.6	43.95	37.2	-	47.7	-	-	-	-	-	-
1969	43.2	56.4	-	-	54.1	-	-	-	-	-	-
1970	39.6	42.4	-	-	52.9	-	-	-	-	-	-
1971	44.3	42.4	-	-	55.5	-	-	-	-	-	-
1972	44.6	42.4	-	-	41.0	-	-	-	-	-	-
1973	39.7	43.3	-	34.5	55.6	-	-	-	-	-	-
1974	47.6	40.3	-	49.8	24.7	-	-	-	-	-	-
1975	47.6	34.2	-	41.3	39.2	-	39.2	-	-	-	-
1976	46.9	46.7	-	-	42.6	-	9.5	-	-	-	-
1977	53.3	48.3	-	36	63.4	-	42.1	-	-	-	-
1978	61.1	42.4	-	51	51.3	58	33.8	-	-	-	-
1979	43.3	-	-	41.3	61.2	44.8	34.4	-	-	-	-
1980	56.1	-	-	-	42.9	62.5	36.6	30.2	-	-	-
1981	70.5	-	-	-	55.7	62.9	33.7	30.9	-	-	-
1982	69.7	-	-	-	64.3	67.4	39.8	34.8	-	-	-
1983	52.2	-	-	-	56.8	69.7	31.6	22.4	-	-	-
1984	69.8	-	-	-	70.9	58.5	36	37.8	-	-	-
1985	67	-	-	-	70.9	66.1	41.2	37.7	-	-	-
1986	62.1	-	-	-	85.0	45.7	41.2	24.8	-	-	-
1987	61.5	-	-	-	71.3	48.9	26.8	28.6	-	-	-
1988	64	-	-	-	84.8	42.6	38.4	41.3	-	-	-
1989	76.3	-	-	-	68.8	69.7	37.4	35.6	-	-	-
1990	86.3	-	-	-	-	62.5	38.3	40.5	-	-	-
1991	66.5	-	-	-	-	67.4	43.7	34.3	-	-	-
1992	74.5	-	-	-	-	72.2	-	43.8	-	-	-
1993	69.9	-	-	-	-	42.5	-	27.4	-	-	-
1994	65.2	-	-	-	-	65	-	26.3	-	-	-
1995	68.5	-	-	-	-	48.9	-	37.7	-	-	-
1996	54.2	-	-	-	-	-	32.1	-	32	49.1	53.6
1997	55.1	-	-	-	-	-	-	-	25.9	59.4	45.6

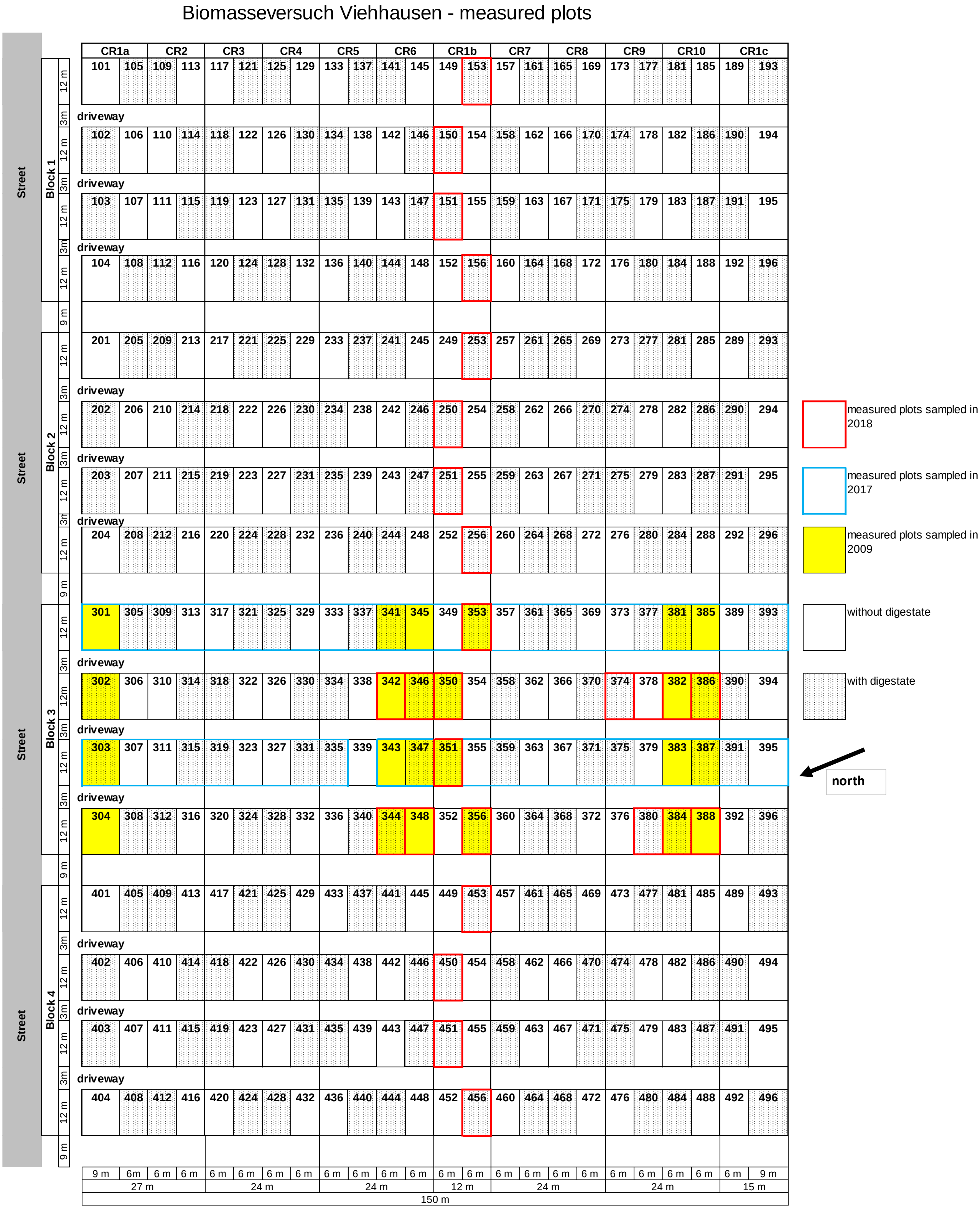

Figure 36: Overview of all plots of the trial site in Viehhausen. The trial site is divided into four blocks. Fertilized and unfertilized plots and measured plots, sampled in different years, are labeled.

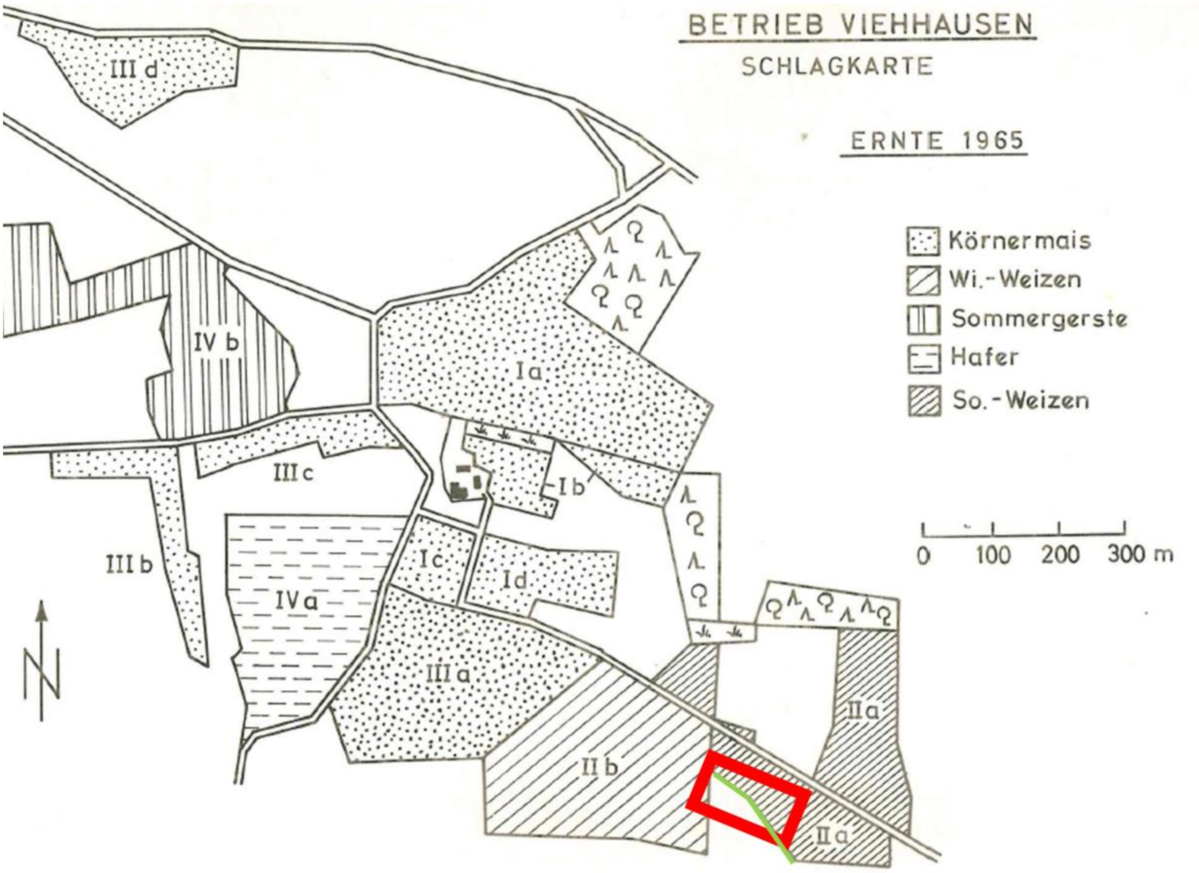

Figure 37: Map of the experimental station of Viehhausen in the harvest year 1965 (Rintelen diverse years). The trial site is labeled in red. The green line shows the former road which bordered two different fields until land consolidations in 1968. The southwestern part of the trial site was not owned by the TUM from 1961 to 1967.

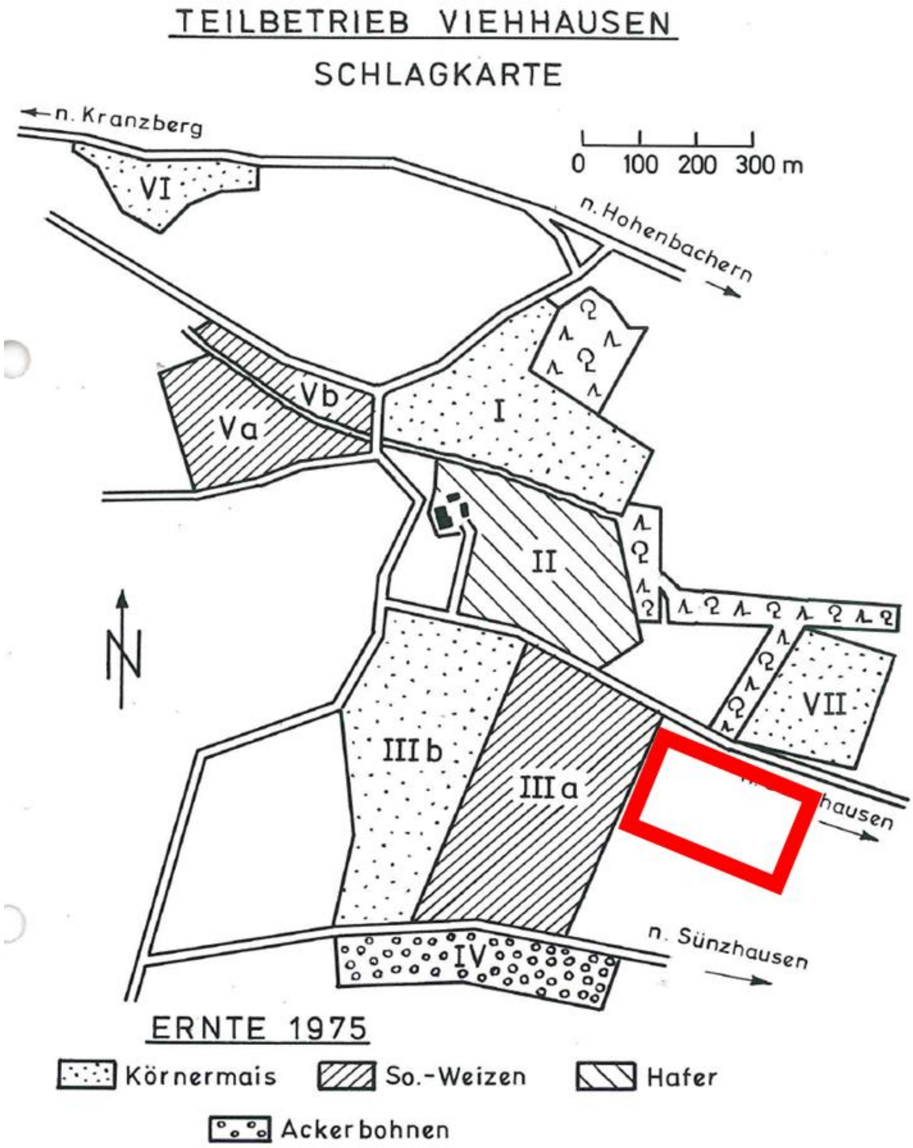

Figure 38: Map of the experimental station of Viehhausen in the harvest year 1975 (Rintelen diverse years). The trial site is labeled in red. The green line shows the former road which bordered two different fields until land consolidations in 1968. The complete trial site was not owned by the TUM from 1968 to 1985. Crop rotations and yields of this time are unknown.

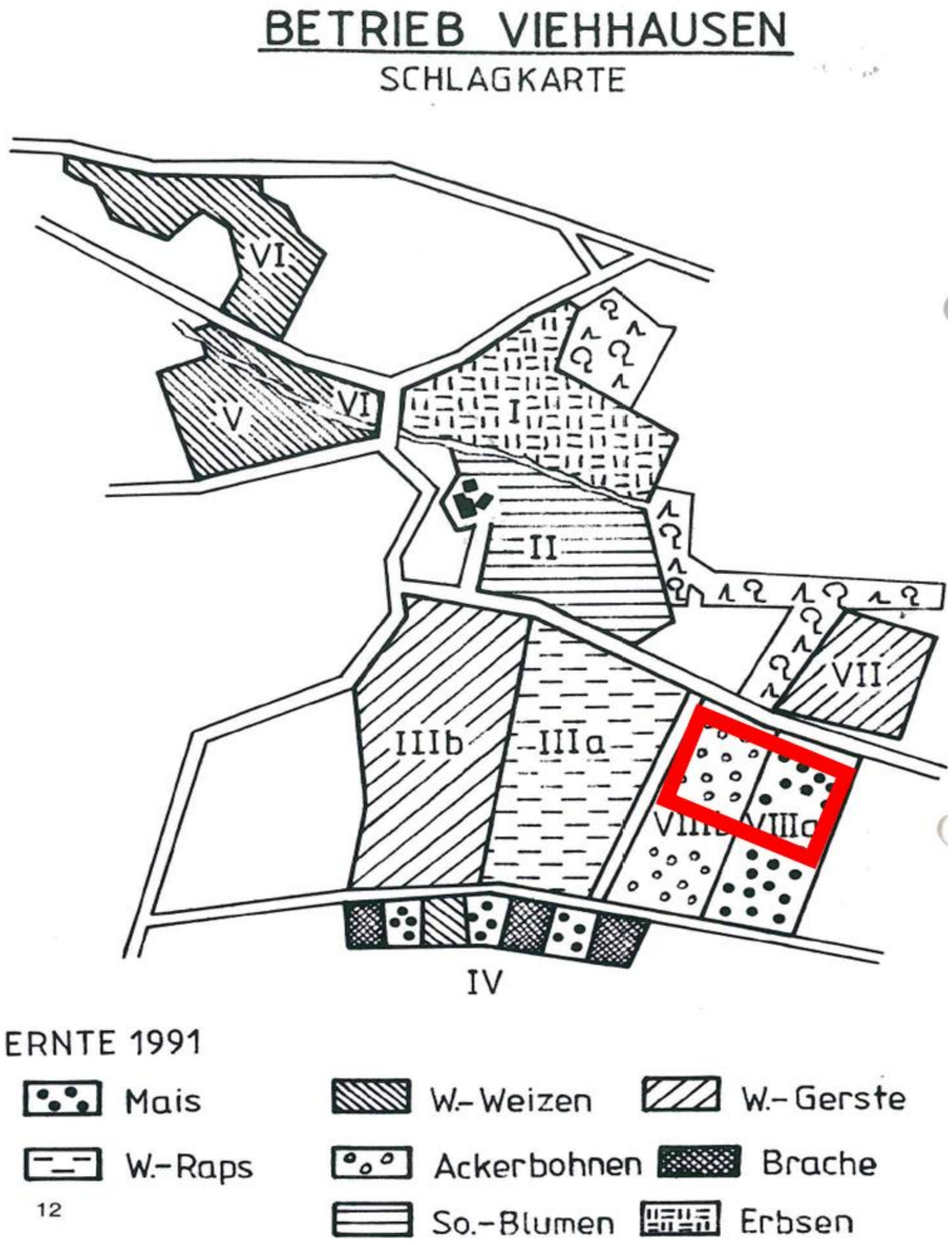

Figure 39: Map of the experimental station of Viehhausen in the harvest year 1991 (Rintelen diverse years). The trial site is labeled in red. The complete trial site was farmed by the TUM since 1985 but was divided into two arable fields with different cropping.

Table 17: Former fields (denoted by Figure 37, Figure 38, Figure 39) and the cultivated crops overlapping the trial site in different proportions between 1961 and 1997. WW = Winter Wheat, SW = Summer Wheat, SB = Summer Barley, WB = Winter Barley, O = Oat, GM = Grain Maize, BB = Broad Bean, WR = Winter Rape, WRe = Winter Rye, T = Triticale, P = Peas, S = Sunflower, G = unspecific Grain. Red marked values are estimates.

Year	affected Area	Proportion (%)	Culture	Year	affected Area	Proportion (%)	Culture
1961	N/A	100	G	1980	external	100	GM
1962	N/A	100	G	1981	external	100	G
1963	IIa	80	SB	1982	external	100	G
1963	IIb	5	SB	1983	external	100	G
1963	external	15	G	1984	external	100	GM
1964	IIa	80	GM	1985	external	100	G
1964	IIb	5	GM	1986	N/A	N/A	G
1964	external	15	G	1987	V IIIa	50	GM
1965	IIa	80	SW	1987	VIIIb	50	P
1965	IIb	5	WW	1988	V IIIa	50	WW
1965	external	15	G	1988	VIIIb	50	GM
1966	IIa	80	GM	1989	V IIIa	50	WW/P
1966	IIb	5	GM	1989	VIIIb	50	WW/P
1966	external	15	G	1990	V IIIa	50	WRa
1967	IIa	80	SB	1990	VIIIb	50	WRa
1967	IIb	5	O	1991	V IIIa	50	GM
1967	external	15	G	1992	V IIIa	45	S
1968	external	100	G	1992	VIIIb	50	GM
1969	external	100	G	1992	V IIIaa	5	S
1970	external	100	GM	1993	V IIIa	45	WW
1971	external	100	G	1993	VIIIb	50	WW
1972	external	100	G	1993	V IIIaa	5	land freeze
1973	external	100	GM	1994	VIIIa	50	WB
1974	external	100	G	1994	VIIIb	50	WB
1975	external	100	G	1995	VIIIa	50	WRa
1976	external	100	GM	1995	VIIIb	50	WRa
1977	external	100	G	1996	VIIIa,b	100	G
1978	external	100	G	1997	VIIIa,b	100	G
1979	external	100	G				