Effect of crop residue incorporation on soil organic carbon dynamics

– Changes in carbon stocks and carbon fractions in an Italian long-term field experiment

Lisa Reiter

Effect of crop residue incorporation on soil organic carbon dynamics

Lisa Reiter

Abstract

Increasing the organic matter content in soil improves the soil productivity, its ability to hold water, deliver nutrients and it protects against erosion. It is also a way to sequester carbon (C) as a strategy to mitigate climate change. Straw incorporation is a widespread management practice that can be used for this purpose. However, straw can also be used for other purposes such as feed or bioenergy. The effect of straw incorporation on soil organic carbon (SOC) varies between studies and we need to improve our understanding of the effect of straw incorporation on C dynamics in order to use the resource in the best possible way. This study investigates the effect of straw incorporation on SOC stocks as well as C fractions under different nitrogen (N) fertilization levels. By studying different C fractions, and not only the bulk soil, more detailed knowledge is obtained about the C dynamics. For this study, soil samples were taken in 2006 from a long-term field trial in a clay soil in Italy. The results showed that straw incorporation significantly increased the SOC stock with 3.15 Mg ha^{-1} in the bulk soil. The effect of straw incorporation did decrease over the years in comparison with earlier results from the long-term experiment. The earlier observed effect of N on SOC was no longer present in the bulk soil. After 40 years of treatment, 3.3% of the total C input was retained in the soil. Two fractions were significantly affected by the straw incorporation, the non-resistant C associated with clay and silt particles (SC-rSOC) and the dissolved organic carbon. No significant effect due to residue treatment was found in the other fractions and N fertilization had no significant effect in any of the fractions. The C fraction varying most was the particulate organic matter followed by the fraction of C attached to sand particles and stable aggregates. The resistant fraction did not change with straw incorporation and seems to be insensitive to the treatments in a decadal perspective. Most C was stored in the SC-rSOC fraction and this was also the fraction that showed the highest significant increase after straw incorporation. Since this is believed to be one of the most stable fractions, straw incorporation might lead to a long-term C sequestration even though the absolute amount C sequestered was small.

Keywords: straw incorporation, carbon sequestration, fractionation, climate mitigation, agriculture

Table of contents

1   Introduction

Soil organic matter (SOM) can be defined as material produced by living organisms that is returned to the soil and undergoes decomposition processes (Bot & Benites, 2005). It is an important component for improving the physical, chemical and biological properties of the soil. It acts as a source of plant nutrients and a water absorbent (Lal, 2004) and is also an important factor when estimating the effect of fertilization, application of crop residues (Christensen, 1992) and the transport of substances such as pesticides (Blanco-Canqui & Lal, 2009). Many arable soils are depleted in SOM (Smith, 2004). An increase in quality and quantity of soil organic carbon (SOC), which constitute the major part of SOM, could improve the soil productivity (Lal, 2004). Moreover, to increase the SOC content in soils does not only bring advantages from an agricultural point of view, it is also seen as a way to mitigate climate change (IPCC, 2014a).

Soil carbon sequestration means removing C from the atmosphere in the form of CO_2 and subsequently storing it in soils as SOC (Lal, 2004). The anthropogenic greenhouse gas (GHG) emissions continue to increase in the world and the agricultural sector contributes with a substantial part. According to IPCC (2014a), agriculture, forestry and other land-use emitted 24% of the total emissions in 2010 (counted in CO_2-equivalents), with most of these emissions being caused by land-use change and drainage of wetlands (IPCC, 2007). Therefore the agricultural sector also has a responsibility to reduce its emissions where possible. Since many of the arable soils have lost much C, they also have a great potential to sequester more C than they do today (Freibauer *et al.*, 2004; Smith, 2004). One Recommended Management Practice for C sequestration in agriculture is straw incorporation (Freibauer *et al.*, 2004). The effect of straw management on the SOC dynamics is however not yet fully understood. Straw incorporation has been found to increase the SOC concentration in many cases, but the results vary both in magnitude and direction (Liu *et al.*, 2014; Lemke *et al.*, 2010). Several studies show an increase in SOC of up to 13% in average (Lehtinen *et al.*, 2014; Liu *et al.*, 2014)

whilst others find little difference or even decreasing SOC stocks after straw incorporation (Lemke *et al.*, 2010; Campbell *et al.*, 2001).

At the same time, IPCC (2014a) mentions other ways for mitigation of climate change within the agricultural sector. Instead of increasing SOC stocks by straw incorporation, straw can be used for bioenergy production to replace fossil fuels. This goes along with the goal of the European Union to increase the renewable energy from 9% in 2010 to 20% of the total energy consumption by 2020 (EU, 2009). However, it is important to consider the consequences of straw removal on the SOC stock and on the GHG emissions.

It can thus be understood that straw can be used for many different purposes. We need to improve our understanding of the effects of straw management and the dynamics of SOC in order to know how to handle this resource in the best way. Much of the realized work has studied the impact of straw incorporation on the bulk soil. However, one way to gain more knowledge about the effect is to look not only at the total SOC stock but at different C pools in the soil representing different stabilization mechanisms (von Lützow *et al.*, 2007).

In this thesis, a fractionation method is used with the aim to answer how the SOC stock is affected by straw incorporation and if the sequestration leads to a long-term stabilization. The effect of nitrogen (N) on these dynamics is also investigated. By this, light will be shed on the stabilization pathways of the residue-derived carbon and its potential as a climate sequestration option. The thesis does not focus on the GHG emissions from straw incorporation and only briefly discusses other possible usage of the straw. Straw residues and crop residues are used as synonyms in this report, unless stated otherwise.

2 Aim

2.1 Aim

The aim of this thesis is to get a better quantitative and qualitative understanding of the effect of straw incorporation on soil organic carbon (SOC). Therefore, soil samples from an Italian long-term field trial with straw incorporation at different nitrogen mineral fertilizer levels are analyzed. This is done by using a fractionation method with the purpose to quantify the effect of residues and nitrogen not only on the total carbon stock but also on different SOC fractions. This will give an idea of the stabilization pathways of the residue-derived carbon. In this way, the sustainability of this management practice with regard to carbon sequestration can be assessed.

2.2 Research questions

In the beginning of this thesis some quantitative and qualitative research questions were formed. The quantitative questions concern the total organic carbon stock and are as follows:

1. Is there a significant effect of straw incorporation on soil organic carbon stock? It is hypothesized that it is, and that this effect does increase significantly with nitrogen level.

2. What is the continuous pattern of the SOC stock at the Italian field trial? Does the trend in SOC stock seen earlier continue also at this new point in time?

3. Carbon stabilization efficiency (CSE) can be used as a way to calculate the C stabilization. It can be estimated by the retention coefficient, which is calculated as: the difference in SOC stock between treatments, divided by C input differ-

ences. Does the CSE (measured by the retention coefficient) increase with higher nitrogen level?

There were also some qualitative questions asked, focusing on the distribution of soil organic carbon in different fractions.

4. Is there a significant effect of straw incorporation on the different fractions? Which fraction responds most to straw incorporation? Can straw incorporation lead to carbon sequestration in a stable form?

5. Does the nitrogen level have any effect on the distribution of soil organic carbon in different fractions? Does an increased amount of nitrogen affect the carbon stabilization differently? I.e., does the nitrogen level affect not only the amount of organic carbon being sequestered, but also in which fraction it is found?

3 Background

3.1 Global carbon cycle

The C stock is determined by the balance of C inputs and outputs to the soil (Liu *et al.*, 2014). In order to assess the potential benefit of a C sequestration management practice it is therefore important to understand the total C fluxes between the different C pools. Carbon circulates between oceans, the atmosphere and the terrestrial biosphere (IPCC, 2007). It can be described as moving between five different C pools; the oceanic pool, the atmospheric, the geologic, the pedologic (soil), and the biotic pool (Lal, 2004).

Carbon moves from the atmosphere as CO_2 in to the oceanic pool and its surface and intermediate layers (IPCC, 2007). There it reacts with water and forms carbonic acid (H_2CO_3), which can dissociate to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), releasing H^+ ions. As the CO_2 concentration increases, this process causes ocean acidification (IPCC, 2007). Some of the dissolved CO_2 is stored in the marine biota through photosynthesis, like in phytoplankton. Most of this C is returned to the atmosphere by respiration but a small part of the phytoplankton sinks, meaning that the C becomes incorporated in sediments on the ocean floor. IPCC (2007) describes how the sediments might be transformed into coal, gas and oil after millions of years. These C sources constitute the geologic pool, which is one of the largest (Lal, 2004). The burning of oil, gas and coal as fossil fuels releases a large amount of long-term stored CO_2 and increases its concentration in the atmosphere. This human activity is the major cause of global warming (IPCC, 2014b).

The pedologic pool is another large C pool (Lal, 2004). Losses of C from this pool can occur by changes in land-use, which is the second largest source of anthropogenic GHG emissions after burning fossil fuels (IPCC, 2007). The main land use changes responsible for these emissions are deforestation and the conversion of natural vegetation to arable land in tropical regions (Don *et al.*, 2011). The

most important GHG is CO_2 which accounts for 76% of the total anthropogenic GHG emissions in the world (IPCC, 2014a). Methane (CH_4) is released in lower amounts than CO_2 but has a 24 times higher global warming potential (GWP) calculated on a time horizon of 100 years (IPCC, 2007). Converted to CO_2-equivalents it is the second most important GHG, accounting for 16% of the total amounts according to IPCC (2014a). Nitrous oxide (N_2O) is formed during nitrification and denitrification among others due to agricultural activities such as N fertilization (Don *et al.*, 2012). It is the third most important GHG and with a global warming potential 298 times higher than CO_2 it accounts for 6.2% of the total anthropogenic GHG emissions (IPCC, 2014a).

3.2   Carbon sequestration in agriculture

Agricultural soils have potential to sequester C and are thus important as CO_2 sinks (Smith *et al.*, 2000). When sequestering C in arable soils, the aim is to increase the SOC density, obtain a better distribution in depth and stabilize the SOC through different processes (Lal, 2004). An increase in SOC can be obtained either by increasing the carbon input, by decreasing the output or by combining both strategies (Freibauer *et al.*, 2004). This can be done directly by adding organic amendments to the soil or indirectly by increasing the crop productivity due to irrigation or fertilization (Stewart *et al.*, 2007). There are several Recommended Management Practices (RMPs) for carbon sequestration in agriculture. Some examples are no-till farming, incorporation of straw residues, manure or sewage sludge and cultivation of permanent crops or bioenergy crops (Freibauer *et al.*, 2004; Lal, 2004; Smith *et al.*, 2000).

Several studies have been made regarding the SOC sequestration potential of different management practices (Freibauer *et al.*, 2004; Smith, 2004; Smith *et al.*, 2000). When it comes to crop residues they can either be completely removed, which would result in a C loss, they can be directly incorporated into the field or they can be temporary removed and transformed into for example manure or sludge and thereafter be returned to the field (Kätterer *et al.*, 2012). Different treatments result in different chemical properties of the final soil amendment and will thus affect the SOC content to different extents. In the case of manure, most of this resource is already spread on arable land today. It does therefore not involve any net C sequestration on a regional scale but gives only a redistribution of C between fields (Kätterer *et al.*, 2012).

One management practice for SOC sequestration is converting cropland to grassland, and most studies find that this practice has the highest sequestration potential (1.2-1.9 Mg C ha^{-1} $year^{-1}$), even though it has been reported to be as low as 0.3-0.6 Mg C ha^{-1} $year^{-1}$ in some cases (Freibauer *et al.*, 2004). The sequestra-

tion potential for bioenergy crops and permanent crops is considerably lower (about 0.6 Mg C ha^{-1} year^{-1}) according to Freibauer *et al.* (2004), but it is still higher than for compost, no-till farming and sludge incorporation at the studied amounts. When it comes to the sequestration potential for straw incorporation, it differs between different studies. Freibauer *et al.* (2004) used numbers from Smith *et al.* (2000) to calculate the sequestration potential, which they found to be 0.7 Mg C ha^{-1} year^{-1}, the second highest potential after converting cropland to grassland. Contradictory, Vleeshouwers & Verhagen (2002) estimate the potential for straw incorporation to be only 0.2 Mg C ha^{-1} year^{-1} which is one of the lowest potentials reported for all cropland management practices according to them. The effect of manure incorporation also differs between studies. Freibauer *et al.* (2004) calculate a sequestration potential of 0.4 Mg C ha^{-1} year^{-1} whilst Vleeshouwers & Verhagen (2002) report it to be much higher, 1.5 Mg C ha^{-1} year^{-1}. Both studies use the same input amount and similar assumptions regarding management practice. Freibauer *et al.* (2004) discuss that it is probably due to the CESAR model used by Vleeshouwers & Verhagen (2002) for calculating the change in SOC and the effect of different land-uses. Vleeshouwers & Verhagen (2002) stated that the model overestimates the sequestration rate under certain conditions, such as those for manure application. It is worth to mention that the numbers from Smith *et al.* (2000), being the base of the calculations of Freibauer *et al.* (2004), have a high estimated uncertainty (>50%). However, several other studies have also found manure application having a larger effect on SOC sequestration than straw incorporation (Kätterer *et al.*, 2011; Cvetkov *et al.*, 2010; Thomsen & Christensen, 2010). Kätterer *et al.* (2011) studied for example the effect of different soil amendments on SOC concentration in the Ultuna long-term soil organic matter experiment. It was found that farmyard manure resulted in a slightly higher increase than straw incorporation after more than 50 years of treatment. The highest effect was however seen after incorporation of peat.

The studies on C sequestration from Freibauer *et al.* (2004) and Vleeshouwers & Verhagen (2002) have both been realized on a large scale, for the whole of Europe. However, Lal (2004) points out that it is very important to adapt the management practices to the site-specific conditions before implementation. Except the management practices, the SOC sink capacity is also determined by the antecedent SOM level, the climate and the soil profile characteristics. Kätterer *et al.* (2012) have found that the principal factors influencing the site-specific SOC dynamics under northern European conditions are the amount and quality of biomass being incorporated into the soil, the residue management and the organic amendments. According to their study, the most effective mitigation strategy is increasing the net primary production on a global scale, binding most possible carbon per

unit area. This implies that intensification of crop production would be one of the best options for sequestering carbon.

3.3 Straw incorporation as a carbon sequestration option

Incorporating straw in fields is a well-known agricultural management practice which gives several positive effects such as improved nutrient availability, better soil structure, improved water retention and less risk for erosion (Blanco-Canqui & Lal, 2009; Lal, 2005). However, there are also drawbacks with too much straw residues left in the field. It can act as a bridge between crops for diseases and if direct sowing is applied it might complicate the establishment of the seed. The straw can also be useful for the farmer for other purposes, such as livestock bedding material or for energy generation within the farm (Lehtinen *et al.*, 2014).

Several studies have also investigated the possibility to use straw incorporation as a C sequestration option. The results among studies differ and some have reported negligible or even negative effects. Campbell *et al.* (2001) did for example not see any significant effect on SOC when removing straw residues over a 10 year period. Likewise, Curtin & Fraser (2003) observed that straw management treatments (incorporation and removal, among others) did not affect the total soil C significantly and Poeplau *et al.* (2015) also observed little or no positive effect of straw incorporation in most of the six Swedish long-term experiments studied. Steinbeiss *et al.* (2008) found that incorporation of aboveground biomass led to an increase in SOC in the upper 20 cm but a decrease in the depth of 20-30 cm. Lemke *et al.* (2010) studied the effect of removing only parts of the straw residues and found that removing 22% did not cause any significant reduction of SOC over 50 years. However, the simulated removal of 50% suggested a decline in SOC whilst a removal of 95% showed a clear decline.

Many other studies do consider straw incorporation as a management practice that increase SOC stocks (Powlson *et al.*, 2008; Lal, 2005). Triberti *et al.* (2008) find it to be one of the most sustainable and economical C sequestration methods. Lehtinen *et al.* (2014) reviewed 50 long-term experiments in Europe and found that SOC concentration increased with 7% due to straw incorporation. Liu *et al.* (2014) did a similar meta-analysis, including 176 studies from all over the world and both short- and long-term experiments. According to their findings, straw incorporation increased SOC with 12.8% in average and Liu *et al.* (2014) concluded that it is an effective measure to increase SOC stocks in arable soils.

It is however clear that straw incorporation has different effect on SOC stocks under different conditions. One important factor is the clay content of the soil, since many studies have shown a positive linear relationship between clay content and SOC stock (Poeplau *et al.*, 2015; Lehtinen *et al.*, 2014; Liu *et al.*, 2014; Six *et*

al., 2002). Several of these studies found a larger effect on soils with clay content above 30-35%. Both Lehtinen *et al.* (2014) and Liu *et al.* (2014) conclude that the duration of the experiment is another important factor. Furthermore, the effect of straw incorporation is positively related to the straw C input rate and negatively related to a high initial C content (Liu *et al.*, 2014). The SOC content is also affected by both the quantity and quality of the substrate (Lal, 2004). The quality of the substrate is partly determined by its C:N ratio, which affects how easily it is decomposed by soil microbes (Blanco-Canqui & Lal, 2009). Residues that have a low C:N ratio is decomposed faster than those with high C:N ratio. Blanco-Canqui & Lal (2009) found for example that soybean decomposes more rapidly than maize residues due to a lower C:N ratio, which in turn affects the SOC content. Khan *et al.* (2007) also found that the type of crop residues affected the SOC stock. When comparing two contrasting crop rotations, it was seen that continuous maize maintained a higher SOC content over the years whilst a varied crop rotation including among others soybean decreased more in SOC content. Khan *et al.* (2007) concluded that it was probably explained by the higher C input from the maize residues.

When studying straw incorporation as a C sequestration option it is also important to consider fluxes of other greenhouse gases. Lehtinen *et al.* (2014) and Liu *et al.* (2014) both studied the GHG emissions of straw incorporation and found that CO_2 and N_2O emissions did increase due to straw incorporation but that CH_4 emission decreased in upland (non-paddy) soils (Liu *et al.*, 2014). However, considering all fluxes, Liu *et al.* (2014) concluded that straw incorporation still leads to a C sink in upland soils.

Some authors argue that the straw could contribute more to climate mitigation by being used as bioenergy instead of incorporating it to the field. Powlson *et al.* (2008) compared the climate change mitigation potential between incorporating cereal straw into the soil and using it for electricity generation. They found that using the straw for combustion to generate electricity saved seven times more CO_2 than when incorporating the residues in the soil. Poeplau *et al.* (2015) found that the effect on sandy soils is small and under such circumstances it might be better to use the straw for bioenergy generation. They also reason that it could be used as fodder for animals and then be returned to the field as manure, since this have been found to give a higher C sequestration than straw incorporation (for example Thomsen & Christensen, 2010). Wilhelm *et al.* (2004) and Blanco-Canqui & Lal (2009) reason that part of the straw residues could be used for bioenergy generation in some soils but that guidelines need to be developed for how much and on which soils this could be done.

3.4 The effect of nitrogen fertilization

N fertilization has been reported to influence the carbon sequestration but the effect differs between studies. Kätterer *et al.* (2012) studied for example the effect of N on SOC concentration in a series of long-term field trials at ten sites in southern and central Sweden. They found a positive effect of N since it increased the SOC concentration with 7% in average over all sites. Other studies have also showed that N fertilization increases the C sequestration (Kätterer *et al.*, 2011; Christopher & Lal, 2007). The main reason for this increased sequestration is the increased net primary production (Kätterer *et al.*, 2012). The SOC stock is namely directly related to the amount of crop residues incorporated and that is in turn related to the amount of N applied, as also reported by Christopher & Lal (2007). Adding N has also been found to increase the proportion of added C that is stabilized in the soil (Poeplau *et al.*, 2015; Kirkby *et al.*, 2014). This is explained by increased substrate use efficiency when N is added together with straw.

A few studies have found that N fertilization instead has caused a decrease in SOC concentration (Khan *et al.*, 2007). This was observed after about 50 years of cultivation and mineral N fertilization in a field trial in Illinois, USA. However, the unfertilized control did also lose SOC why the observed decline could not be entirely explained by the N effect. Kätterer *et al.* (2012) emphasize that it is important to evaluate the results of the N fertilization on SOC concentration carefully since there are many factors that influence the result. The trend in the reference plot is stated as one of the main aspects to consider, together with the initial organic matter content and possible changes in bulk density in long-term experiments when converting C concentration to mass.

One concept that is currently discussed when it comes to C sequestration is priming. It can be defined as an increased decomposition of old stable organic C due to the addition of new easily-decomposable organic compounds (Kuzyakov *et al.*, 2000). Results supporting this theory have been found in the laboratory study by Fontaine *et al.* (2007), where it was seen that the addition of fresh crop residues increased the decomposition of old C in the subsoil. The study of Kirkby *et al.* (2014) also supports the theory of priming since they observed that the loss of old, stable carbon was increased with straw incorporation. Kirkby *et al.* (2014) discussed that this could explain why the effect of residue incorporation is sometimes smaller than expected and argue that C sequestration needs additional nutrient application. However, Kätterer *et al.* (2012) did not find any evidence of priming in the long-term experiments analyzed in their study. The effect of N fertilization on C sequestration seems to be a complex matter that needs to be further investigated.

3.5 Soil carbon stabilization

Carbon stabilization is one of the aims with C sequestration (Lal, 2004). Stabilized carbon refers to carbon that is protected from decomposition and thus has a slower turnover rate than unstabilized organic matter (von Lützow *et al.*, 2006; Six *et al.*, 2002). The age of SOC can range between a few years up to thousands of years (von Lützow *et al.*, 2007). There are several mechanisms behind C stabilization and they are all not yet fully understood (von Lützow *et al.*, 2006). Over time, these stabilization mechanisms have been classified in different ways. Six *et al.* (2002) identify the mechanisms physical protection, chemical stabilization and biochemical stabilization. However, von Lützow *et al.* (2006) suggest that this description should be avoided since these different mechanisms are often used in a confusing and inconsistent way between authors. Von Lützow *et al.* (2006) instead classify the stabilization mechanisms as (i) selective preservation (ii) spatial inaccessibility and (iii) stabilization by interaction with surfaces and metal ions.

The selective preservation mechanism is explained by von Lützow *et al.* (2006) as recalcitrant molecules being accumulated relatively to others. It comprises among others plant litter, rhizodeposits and microbial products. The spatial inaccessibility refers to C being unavailable for decomposition due to for example occlusion in aggregates and encapsulation of C within networks of recalcitrant molecules. The last mechanism is described by von Lützow *et al.* (2006) as stabilization of C through inter-molecular interactions between organic matter and mineral surfaces and metal ions. This can occur by ligand exchange, polyvalent cations bridges and weak interactions such as H-bonding and van der Waals forces.

Earlier, the term humification has been widely used when discussing C stabilization. For example, Lal (2004) identifies the soil C stabilization processes aggregation, humification and translocation into sub-soil. However, von Lützow *et al.* (2006) advice against using the term humification since it does not actually conform to C stabilization. It has been believed that humification occurs through extracellular processes and that the humus is stabilized due to its chemical state, but newer findings indicate that this is not very likely (von Lützow *et al.*, 2006; Hedges *et al.*, 2000). Von Lützow *et al.* (2006) write in their literature review that most evidence indicates that stable compounds are formed by intracellular microbial processes, not extracellular, and that stabilization is not decided only by the chemical form but also by the other stabilization mechanisms. These findings are supported by the study by Bol *et al.* (2009) who also found that there is no selective preservation based on chemical recalcitrance. Von Lützow *et al.* (2006) further conclude that microbes can decompose any naturally originated organic matter. The recalcitrance of a compound is only important in the initial stage of decomposition, then spatial inaccessibility and organo-mineral interactions become increasingly important.

The stabilization mechanisms can impact different fractions of the soil differently (Stewart *et al.*, 2009; von Lützow *et al.*, 2007). In general it is believed that the turnover rate increases as the particle size decreases (von Lützow *et al.*, 2007; Derrien *et al.*, 2006). Since the turnover rates for SOM are influenced by environmental conditions and soil properties, no absolute values of turnover rates can be given (von Lützow *et al.*, 2006). However, estimations can be obtained by using stable isotope (^{13}C) tracers as a natural label of SOM, for example when changing land-use from C3 plants to C4 plants since they have different ratios of ^{13}C/^{12}C (von Lützow *et al.*, 2007). This method will reflect the SOM turnover ranging from just a few years up to hundreds of years. It is useful when it comes to evaluate how well a fractionation method has separated young and old organic matter from each other (von Lützow *et al.*, 2007).

The fraction that has the fastest turnover is the particulate organic matter (POM) (Poeplau & Don, 2014). It decomposes within a few years since it is not associated to any minerals (von Lützow *et al.*, 2006). It is less stable than C stored in both the sand and the silt and clay-fraction. The turnover times of different fractions have been reviewed by von Lützow *et al.* (2007). Studies of ^{13}C tracers have shown that the sand fraction contains the youngest SOC of the mineral fractions, ranging from 0.5 to 374 years (von Lützow *et al.*, 2007). The SOC in the clay fraction is the second youngest, having turnover times between 76 to 190 years. The oldest SOC is found in the silt fraction, having an age of 115 to 676 years. The material in the silt and clay fraction is not directly derived from fresh plant material (as it is in the coarser fractions) but reworked by soil fauna and microorganisms (Bol *et al.*, 2009). Von Lützow *et al.* (2007) conclude that SOC in the finest clay fraction is younger than coarse clay and silt. They discuss that this might be explained by incomplete disaggregation of aggregated clay particles in the coarser fractions that contain protected SOC. Not only the amount, but also the type of clay mineral matters for C stabilization (Paustian, 2014; von Lützow *et al.*, 2007, 2006). Six *et al.* (2002) found that soils dominated by 1:1 clay minerals stabilized less C. This is because such clay minerals have no layer charge and therefore only weak bonding affinities to SOC (von Lützow *et al.*, 2006).

Carbon can also exist in soils as dissolved organic carbon (DOC). This C fraction consists of many different organic compounds, from fresh litter and to recalcitrant material (Haynes, 2005). Its stability thus differs depending on which material is dominating the fraction (von Lützow *et al.*, 2007). For example, Kalbitz *et al.* (2003) found in their incubation experiment that 55-85% of the easily biodegradable material (like litter and maize straw) in DOC was decomposed within 1-2 weeks. However, for the material in the DOC with low biodegradability not even 10% of the initial content was degraded after three months.

16

It is believed that a small fraction of the SOC is biochemically recalcitrant and remains in the soil as an inert fraction (von Lützow *et al.*, 2007). That is because very old carbon has been found when studying the natural ^{14}C isotope (O'Brien *et al.*, 2013; Eusterhues *et al.*, 2005). This method detects decomposition on a time scale of 200-40,000 years. Eusterhues *et al.* (2005) found the resistant fraction being 500-3900 years older than the bulk soil when studying acid forest soils. They also showed that the age increases with soil depth since the subsoil contained resistant SOC almost 6000 years old. In grassland, the resistant fraction had a turnover time of 1200-3000 years (O'Brien *et al.*, 2013).

Only a small amount of the added plant residues is stabilized in soil (Wilhelm *et al.*, 2004). It can be difficult to quantify that amount due to the complex structure of SOC and the soil system (Menichetti, 2014). One strategy is to use data from long-term field trials to examine the effect of residue incorporation (Lugato *et al.,* 2006). By subtracting the SOC stock of residue incorporation with that of residue removal, a net change in SOC stock after long-term treatment can be calculated. Dividing that net change with the difference in C input gives a SOC retention co-efficient. This coefficient represents the fraction of the residue-derived carbon that actually has become stabilized. This coefficient may have many different names (conversion efficiency, Lugato *et al.* (2006); net humification efficiency, Kirkby *et al.* (2014) and humification coefficient, Kätterer *et al.* (2011)). It has been found to vary greatly, between -16% to 41% depending on the type of organic amendment and nutrient levels (Kirkby *et al.*, 2014; Kätterer *et al.*, 2011; Lugato *et al.*, 2006). Kätterer *et al.* (2011) also found that root-derived C contributed 2.3 times more to C stabilization than above-ground biomass.

3.5.1 Carbon saturation

When estimating SOC sequestration potential using models, it is often assumed that the C stock is linearly related to the C input (Stewart *et al.*, 2007). This implies that C stocks would increase infinitely with increasing C inputs. Some long-term experiments indeed support such a first-order decomposition process (Stewart *et al.*, 2007) whilst other studies show signs of no or little effect of increased C input (Powlson *et al.*, 2011; Reicosky *et al.*, 2002; Campbell *et al.*, 2001). This has raised the question about soil carbon saturation.

If the amount of C input to a soil changes, the C content will increase or decrease for some time (Stewart *et al.*, 2007). After decades, a new equilibrium will be reached depending on environmental conditions and soil properties (von Lützow *et al.*, 2006). A dynamic equilibrium is obtained when the C input equals the C losses. The theory of soil C saturation means that there is a maximum equilibrium C level that can be reached when C input is maximized (Stewart *et al.*, 2007). It is an upper limit to how much the C level can increase. As the soil approaches

saturation level, the increase in SOC stock becomes smaller and smaller with increased C input rate. As the storage efficiency approaches zero, the soil will have less ability to sequester additional C.

Stewart *et al.* (2007) used data from a number of long-term field experiments in models comparing SOC saturation and no saturation. They found that SOC saturation does occur but that it is not always seen in agricultural field experiments since the range of C input levels is too small for the saturation tendency to show. Several other studies also support the theory that soils can become C saturated (Chung *et al.*, 2008; Six *et al.*, 2002). Stewart *et al.* (2007) further conclude that the greatest efficiency of C sequestration will be found in soils that are far from saturation.

Just as for C stabilization, the saturation processes act differently in different fractions (Stewart *et al.*, 2009). Stewart *et al.* (2009) saw for example that unaggregated fractions reached C saturation faster than aggregated. They also investigated the effect of saturation deficit versus the texture of soil on the accumulation of residues. In a model exercise they found that saturation deficit was more important, since two soils with very different texture but similar C saturation deficits accumulated a similar amount of residue-derived C.

The time for reaching a new SOC equilibrium differs between soils. Paustian (2014) writes that after a change in C input rate, it can take several decades to reach a new equilibrium depending on the mean residence time of the SOC. After a land-use change (such as afforestation of cropland) it takes more than 120 years to reach a new, higher equilibrium level after increasing the C input rate (Poeplau *et al.*, 2011). However, if decreasing the C input rate, a new lower equilibrium is established quite quickly (17-23 years) according to Poeplau *et al.* (2011).

3.5.2   Carbonates in soil

Soil carbon can also be found in inorganic forms, typically present as carbonates. The most common types of carbonates are calcite ($CaCO_3$) and the more recalcitrant dolomite, [$CaMg(CO_3)_2$] (Schumacher, 2002). These inorganic forms of C are generally derived from the soil parent material but can also be found in agricultural soils due to liming. Dolomite, which can be present in coastal sediment, has a low solubility and can be difficult to dissolve (Komada *et al.*, 2008). Carbonates are usually removed with acid treatment and Komada *et al.* (2008) summarize the most common acids used; hydrochloric (HCl), sulfurous (H_2SO_3) and phosphoric (H_3PO_4) acid. One method for destroying carbonates is suggested by Steinbeiss *et al.* (2008), using 120 µl sulfurous acid (5-6% SO_2). However, the effect of this method may vary since sulfurous acid has been found to be insufficient for removal of more recalcitrant carbonates (Kennedy *et al.*, 2005). In such a case, Kennedy *et al.* (2005) suggest using HCl instead.

18

3.6   Soil carbon fractionation

The aim with dividing the soil organic matter (SOM) of the bulk soil into fractions is often to obtain different functional pools (von Lützow *et al.*, 2007). These SOM pools differ from each other in their size, function, composition and thus turnover rates and are often used for studying SOM dynamics in SOM turnover models. Von Lützow *et al.* (2007) however concluded that this is difficult to achieve and few of the reviewed fractionation methods did actually identify functional pools. Since then, Zimmermann *et al.* (2007) have proposed a new fractionation method which was found to fit the model pools of the Rothamsted carbon model (RothC). That gives the advantage that the fractions of the Zimmermann method can be used for modelling studies.

Different types of fractionation methods are in use; physical fractionation, chemical fractionation and a combination of both (von Lützow *et al.*, 2007). Each method is based on a theory about the main mechanism behind SOM stabilization (Christensen, 1992). The mechanisms are often described as chemical stabilization, physical protection and biochemical stabilization; thus, many fractionation methods are classified in a similar way. It is common to combine physical and chemical fractionation methods, for example, by first performing some physical fractionation steps and then continue with chemical methods (von Lützow *et al.*, 2007).

3.6.1   Physical fractionation

The theory behind physical fractionation methods is that bioaccessibility is crucial for degradation of SOM (von Lützow *et al.*, 2007). By separating particles based on their size and density, different functional SOM pools are believed to be identified. Only primary organo-mineral complexes are considered in physical fractionation not aggregates which are disrupted during the procedure. The primary organo-mineral complexes are released after breaking all aggregates by soil dispersion and this step is therefore a very important part of physical fractionation (Christensen, 1992). Through dispersion the particles are separated due to acoustic cavitation. It is important to find the right treatment time, since too short treatment leads to incompletely dispersed soils and excessive treatment can result in disruption of sand and silt sized aggregates. The importance of soil dispersion is showed clearly by Jastrow *et al.* (1996) who found that almost 90% of the SOC in surface soils is found within aggregates. However, many studies have failed to perform a complete dispersion of soils according to Christensen (1992), even though it is not reasonable to brake all microaggregates (<2μm). The microaggregates in the clay fraction are namely very stable and do not even brake after 'complete' dispersion (Chenu & Plante, 2006).

Some other methods used in physical fractionations besides dispersion are density separation and particle size fractionation (Christensen, 1992). The assumption behind particle size fractionation is that particles with different size have different mineralogical composition. The SOM bound to these different particle sizes will therefore have different structure, function and thus degradation properties. By distinguishing different size fractions, different SOM pools will also be separated. For example the sand fraction (>63 μm) binds little SOM, since it consists mainly of quarts, which has weak bonding affinities to SOM (von Lützow et al., 2007). The clay fraction (<2 μm) on the other hand binds a large part of the SOM since such particles have a large specific surface area and many reactive sites. According to the literature review by von Lützow et al. (2007), 50-70% of the SOC is usually found in the clay fraction whilst 20-40% is found associated with silt-sized particles and less than 10% in the sand fraction.

Not all SOC is bound to mineral particles in the soil, some also exists freely (von Lützow et al., 2007). This material is sometimes called the light fraction and can be separated by a density fractionation. A density fraction is a physical fractionation method for SOM that is not tightly adsorbed to mineral fractions of the soil (Christensen, 1992). The light fraction consists mostly of relatively fresh plant residues, also called particulate organic material (POM), and generally has a density less than 1.6-2.2 g ml^{-1}. The heavy fraction consists of SOM bound to minerals and is more processed and stabilized organic material (Christensen, 1992). The heavy fraction has a high specific density since it is closely associated with soil minerals and thus sinks to the bottom during the separation. Earlier it was common to use organic liquids for the density fractionation but nowadays a solution of inorganic salt is more widely used. One of the most common salts is sodium polytungstate $Na_6(H_2W_{12}O_{40})$ since it is less toxic than the former organic liquids and since it can be mixed to a wide range of densities. Christensen (1992) writes that when separating the light fraction it is very important to maintain the density of the fractionation liquid. The most important factors to control are the choice of density, the exact density adjustment and the temperature control during the separation treatment. According to von Lützow et al. (2007) the POM fraction can constitute 10-70% of the total SOC. The fraction is usually smaller in arable soils than in forest soils since they usually have less litter input and higher degradation rate (Haynes, 2005).

3.6.2 Chemical fractionation

Besides the physical fractionation methods, chemical methods are also used (von Lützow et al., 2007). Historically, these methods have dominated the studies of SOM (Oades, 1994). Earlier, SOM was divided by scientists in humic and non-humic substances (Brady & Well, 2002). The humic substances were classified

20

into different chemical groupings: humic acids, fulvic acids and humin. These were separated through solubility and were thought to be stabilized to different degrees. However, studies of the tracer ^{14}C showed that this separation of SOM did not give homogeneous functional fractions and they did not represent the humification process (Oades, 1994). Therefore the research around SOM began to focus more on biological and physical approaches after the 70's. Today the discussion has moved away from humic substances but there are still chemical fractionation methods that are used for isolating other fractions. On example is the method of extracting SOM in an aqueous solution, isolating the dissolved organic matter (DOM). DOM is a mix of component (Haynes, 2005). The fraction is defined by its size, <0.45 μm and is the most mobile fraction of all (von Lützow *et al.*, 2007). By diffusion and convection it can enter almost all soil compartments. Just as with POM, the fraction is in general larger in forest soils than in arable soils (Poeplau & Don, 2013). In arable soils it usually constitutes a small part of all SOM; about 0.05-0.4% according to Haynes (2005). Since DOC constitutes only a small part of the total SOC and since it is a non-homogeneous fraction it is difficult to use this fraction as a functional pool for modelling.

Another chemical fractionation method consists of separating the SOM according to its resistance to oxidation (von Lützow *et al.*, 2007). There are several oxidizing agent, but one that is commonly used is sodium hypochlorite, NaOCl (Siregar *et al.*, 2005). After treatment, a non-oxidized residue is left which has been reported to constitute 28% to 88% of SOM by Siregar *et al.* (2005). The residual fraction is believed to be stable and resistant due to its content of very old carbon (Eusterhues *et al.*, 2005)**.** However, it has been found that this resistant fraction is sensitive to land-use change and it is thus discussed if the fraction really is inert (Poeplau & Don, 2013).

4 Materials and Methods

4.1 Experimental site and sampling

The study site is a long-term field trial located in Padova in the north-east of Italy at 6 m above sea level. The soil is a clay loam, classed as a fluvi-calcaric cambisol according to FAO-UNESCO (1990). At the start of the experiment in 1966 the carbonate content in the upper soil layer (0-30 cm) was 331 g kg^{-1}, bulk density was 1.44 g cm^{-3}, pH was 7.8 and the organic matter content was 1.81% (Lugato *et al.*, 2006). For more details on the site and its climate see Lugato *et al.* (2006).

The experiment includes two different treatments: residues incorporated (RI) and residues removed (RR). The two treatments are factorially combined with five different N mineral fertilization rates (0, 60, 120, 180 and 240 kg N ha^{-1}) in four replicates. The nitrogen was first applied as ammonium-nitrate but since 1981 it has been applied in form of urea, distributed as top dressing in two applications. The experimental design is shown in Figure 1.

Maize (*Zea mais* L.) has historically been grown at the location for a long time before the experiment started. Since 1966, maize was cultivated every year but in 1985 a crop rotation started with maize every second to third year alternated most frequently with sugarbeet (*Beta vulgaris* L.) but also soybean (*Glycine max* (L.) Merr.), potato (*Solanum tuberosum* L.) and winter wheat (*Triticum aestivum* L.). Standard seedbed preparation is performed every year at the corresponding time for each crop and is preceded by ploughing to 30 cm depth in the autumn.

Disturbed soil samples were taken to a depth of 20 cm in spring (late March) 2006 after one soil cultivation and just before seed-bed preparation. Samples of 400 g were gathered from four different places in the center of each plot and were subsequently mixed together. The samples were left to air-dry and thereafter sieved over a 2 mm sieve in order to remove large crop residues and roots.
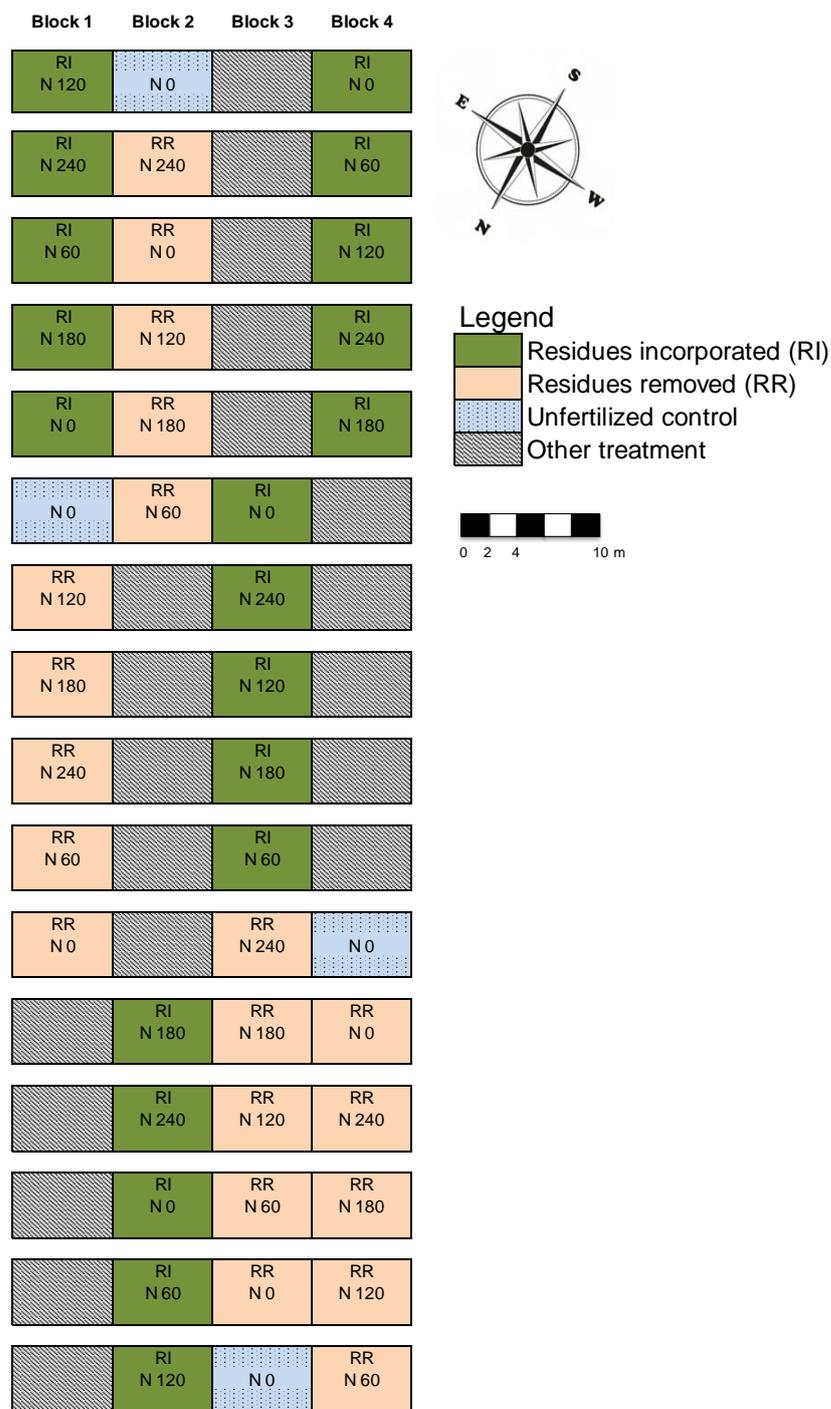
Figure 1. The experimental design of the Padova long-term field trial. Dark green = residue incorporated (RI), pink = residue removed (RR), blue with dots = unfertilized control, grey striped = other treatment not considered in this study. The two treatments RI and RR are factorially combined with five different N mineral fertilization levels (N0-240) in four replicates. Figure adapted after drawing from Professor Antonio Berti, Padova University.

4.2   Fractionation

The work followed the fractionation method described by Zimmermann *et al.* (2007) and is based on both physical and chemical soil C properties. The C pools can later be connected to the Rothamsted Carbon (RothC) model. The improvements of the method suggested by Poeplau *et al.* (2013) are also implemented.

Before starting the fractionation procedure, subsamples had to be taken from the main sample. This was done by spreading out the main sample on a plate and dividing it into four equally sized parts, where soil was taken from the two opposite parts in order to get a representative subsample. In total 30 grams of the fine soil (<2 mm) was weighed in for further fractionation. Additionally, two grams were taken for analysis of the bulk soil for total C, total N and organic C by making three strikes in the spread out main sample. The analyses were done with an elemental analyzer (LECA, St Joseph, Michigan, USA).

Figure 2 shows the main steps of the fractionation procedure. The 30 grams of fine bulk soil were added to 150 ml deionized water and were then dispersed for 3 minutes at 29% amplitude by a calibrated ultrasonic probe (Sonics & Materials Inc, Newtown, USA). An output power of 20 W was used as suggested by Poeplau *et al.* (2013). Since the soil has a clay content of 35% and particles settled on the bottom quickly, the suspension was dispersed for 1.5 minutes, stirred and then dispersed again for another 1.5 minutes. The mix was thereafter wet sieved with 2000 ml deionized water over a 63 μm sieve. In this way, the fine fraction was separated from the coarse fraction.

The suspension containing the fine fraction was centrifuged at 4000 rpm for 15 minutes in order to separate the particles from the water. The particles were decanted, dried at 60°C, grinded with a mortar into a fine homogeneous powder and weighed. Thereafter, the fine fraction was separated into two different fractions: silt and clay (SC) and resistant organic carbon (rSOC). The rSOC fraction was isolated by taking one gram of the fine fraction in the same way as when starting the fractionation. Subsequently, 45 ml of 6% sodium hypochlorite, NaOCl adjusted to pH 8 with concentrated HCl was added, following a modified method of Kaiser & Guggenberger (2003). The suspension was stirred in a vortex mixer for 30 seconds and then left to oxidize for 18 hours in 20°C. The solution was centrifuged at 4000 rpm for 15 minutes, the residues decanted and washed with 45 ml deionized water. Then, the suspension was stirred in the vortex mixer and centrifuged again. The whole oxidation step was repeated twice. During the last repetition the samples were washed with deionized water two times to make sure that they were completely clean. Finally, the rSOC fraction was dried at 60°C, weighed and milled into a homogeneous powder.

In order to separate the dissolved organic carbon (DOC) fraction from the fine solid fraction, 200 ml of the suspension that was left after the centrifugation was

filtered through a 45 µm nylon mesh using a vacuum pump. The DOC fraction was then frozen and analyzed for concentration of DOC by thermal oxidation with a liquid analyzer (TOC-VCPH, Shimadzu).

The coarse fraction separated by wet sieving was dried at 60°C and weighed. It consists of two fractions; sand and stabile aggregates (SA) and particulate organic matter (POM). The POM was separated from the SA by using the high density liquid sodium polytungstate, $Na_6(H_2W_{12}O_{40})$ with the density of 2.0 g cm^{-3} (Sometu, Berlin). Poeplau *et al.* (2013) suggested this change instead of the density 1.8 g cm^{-3} used by Zimmermann *et al.* (2007) in order to get a better isolation of the POM. After stirring the mixture it was centrifuged at 3000 rpm for 15 minutes and the light fraction POM, which floated up, was separated from the SA. Both the POM and the SA were put in sieve bags, washed with deionized water, dried at 60°C and the POM fraction was weighed. The weight of SA was given by the difference between the weight of the total coarse fraction and the POM.
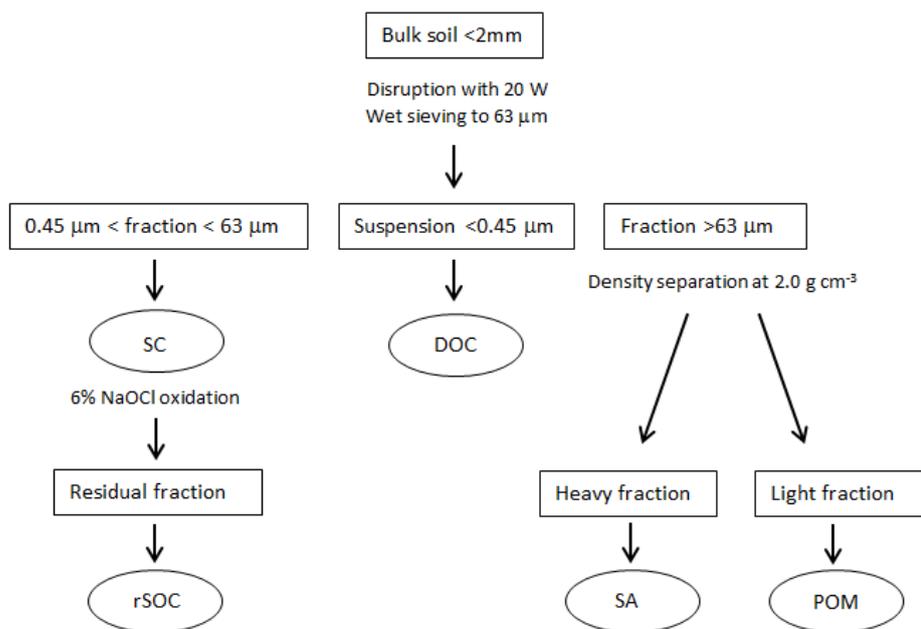

Figure 2. Illustration of the fractionation procedure: SC = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, SA = sand and stable aggregates, POM = particulate organic matter. Figure adapted after Zimmermann *et al.* (2007) including suggested method improvements by Poeplau *et al.* (2013).

4.3 Determination of the natural ^{13}C isotope

The original idea was to measure the abundance of the natural isotope ^{13}C in order to trace the maize-derived C and obtain detailed information about the C dynamics. A requirement for this analysis method is to remove all carbon sources from the samples except the organic carbon by acid treatment before mass-spectrometric analysis. However, the Padova soil contains carbonates which were proven to be very difficult to eliminate completely. Even though the acid treatment of Steinbeiss *et al.* (2008) was followed, there were still carbonates left in the soil samples. Hence, the only fraction to be analyzed for ^{13}C abundance was the POM, which only consists of particulate organic matter. This was done by ball milling the fraction and weighing in 0.5 g of the homogeneous material in tin capsules. The weight was chosen based on the POM's estimated C concentration. Subsequently, the POM was analyzed for C- and N-concentration as well as the abundance of ^{13}C with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific). The ^{13}C data were however not used in this work.

4.4 Data preparation

Organic carbon concentrations were converted to SOC stocks by multiplying the concentration with the weight of each fraction, the sampling depth and the bulk density (1.44 g cm^{-3}). In order to compare the results with previous findings from the same field trial, the sampling depth of 30 cm instead of 20 cm was used. This was done by assuming that the C concentration is homogeneous throughout the first 30 cm, which is the ploughing depth. The same procedure was used by Lugato *et al.* (2006). The C input was calculated based on the harvest data. The harvested biomass was converted to C input by assuming a C content of 45% in crop residues (Blanco-Canqui & Lal, 2009). It was also assumed that 10% of the harvested biomass was incorporated in the treatment Residues removed.

The retention coefficient of residue-derived carbon after 40 years of treatment was obtained by first calculating the difference in SOC stock between Residues incorporated (RI) and Residues removed (RR). Then the total C input was given by the sum of above- and below-ground biomass (litter plus roots and rhizodeposits), which had been calculated based on yield data by the use of C allocation coefficients as described by Bolinder *et al.* (2007). The difference in total input (RI-RR) finally gives the straw derived input since that is the only parameter that differs between the two treatments. The retention coefficient was calculated by dividing the change in SOC (ΔSOC) stock by the treatment difference in C input, using Equation 1:

$$\frac{SOC\ stock_{RI} - SOC\ stock_{RR}}{Difference\ in\ C\ input\ between\ RI\ and\ RR} \qquad \text{Equation 1}$$

The original archived soil samples were air dried prior to the experiment started but drying during the fractionation work of the thesis was conducted at 60°C. Therefore the original samples lost water during the fractionation procedure which had to be corrected for. A water loss of 1.5% was measured and the initial sample weight of 30 g was thus corrected to 29.5 g.

4.5 Statistical analysis

Data were analyzed using R Studio (RStudio, 2014) and tested for normality with the Shapiro-Wilk's W-test, critical level P < 0.05. Since the test showed that the original data were not normally distributed but right-skewed, it was transformed using the reciprocal transformation, i.e. 1/x. The normalized data were then tested for significance using one-way analysis of variance (ANOVA) and the post-hoc test Tukey's honest significant difference (HSD) test, whilst correlation was tested by using Spearman rank-order correlation. Critical level was set to $p<0.05$ in all tests.

5   Results

5.1   Response of the total SOC stock on straw incorporation

The results show that there is a strongly significant effect of straw incorporation on the bulk soil in the Padova long-term field trial (Figure 3). Straw incorporation sequestered 3.15 Mg SOC per hectare compared with residue removal ($p = 0.001$) in average over all N fertilization levels. However, when grouping the SOC stocks according to the N levels there was no significant effect of straw incorporation ($p=0.97$), see further down in Figure 8. As shown in Figure 8, the mean SOC stock was still higher for RI than for RR at each N level, but since the spatial variability within each sample group was higher than the mean difference between treatments the effect was not significant. This clearly indicates that the treatment effect on SOC was too small to overcome the spatial variability with four replicates (n=4). Figure 8 furthermore shows that the N fertilization level did not affect the results ($p=0.99$). Figure 3 and the first part of Figure 8 answer research question 1 (see page 7) by illustrating that there is a significant effect of straw incorporation but the effect is small and cannot be detected in a group of four replicates due to spatial variation. The effect of straw incorporation is not influenced by N fertilization.
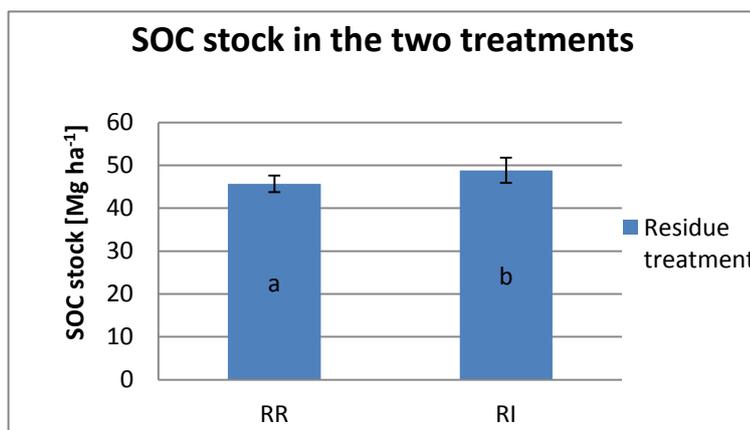

Figure 3. The SOC stock for Residues incorporated (RI) and Residues removed (RR), n=20. Error bars show the standard deviation over the five different N levels. RI is significantly higher than RR at $p=0.05$.

The effect of straw incorporation on the bulk SOC stock in the Padova long-term field trial has earlier been investigated by Lugato *et al.* (2006). They observed that the SOC content decreased for both treatments Residues incorporated (RI) and Residues removed (RR) between the start in 1966 and their last measurement point in 1993, but the SOC decreased more when residues were removed. Lugato *et al.* (2006) also observed that the decrease was more pronounced in the beginning of the experiment, and that the soil then seemed to approach a new SOC equilibrium.

After adding the new point in time of 2006, it can be seen that the effect of straw incorporation was highest in the beginning of the experiment and that it was decreasing during the recent two decades (Figure 4). The difference between treatments was highest in 1986 (4.56 Mg ha^{-1}) and decreased to 3.15 Mg ha^{-1} in 2006. The SOC value in 2006 was similar to the value in 1993 (3.43 Mg ha^{-1}) and thus supports the theory of Lugato *et al.* (2006) that a new equilibrium is approaching.

When studying the effect of each N level over time separately, a similar pattern was observed as for the total average effect (Figure 5). From 1982 to 2006, the effect of straw incorporation decreased at each N level, although not significantly according to the Spearman rank-order correlation. In 1993, after 27 years of straw incorporation, there was a big difference in SOC stock between the different N fertilization levels, ranging from 2.1 Mg ha^{-1} (N=0) to 5.7 Mg ha^{-1} (N=240). However, between 1993 and 2006, the effect of N fertilization has decreased and the results thus indicate that the N fertilization had largest effect in the beginning of the experiment. In 2006, the different N fertilization levels resulted in relatively similar SOC stocks (ranging from 2.3 to 3.9 Mg ha^{-1}). It can be seen from Figure 5 that the effect of straw incorporation at the highest N fertilization levels (180 and 240 kg N ha^{-1}) has decreased and the effect at the lower levels (0, 60 and 120 kg N ha^{-1}) has increased slightly, so they almost meet at an intermediate SOC level. The lowest effect of straw incorporation in 2006 was found at N=60 and the highest at N=180, whilst N=0 resulted in an intermediate effect. Figure 4 and Figure 5 answers research question 2 by showing that the SOC stock is not increasing any more with residue incorporation but it rather seems to have reached a new equilibrium.
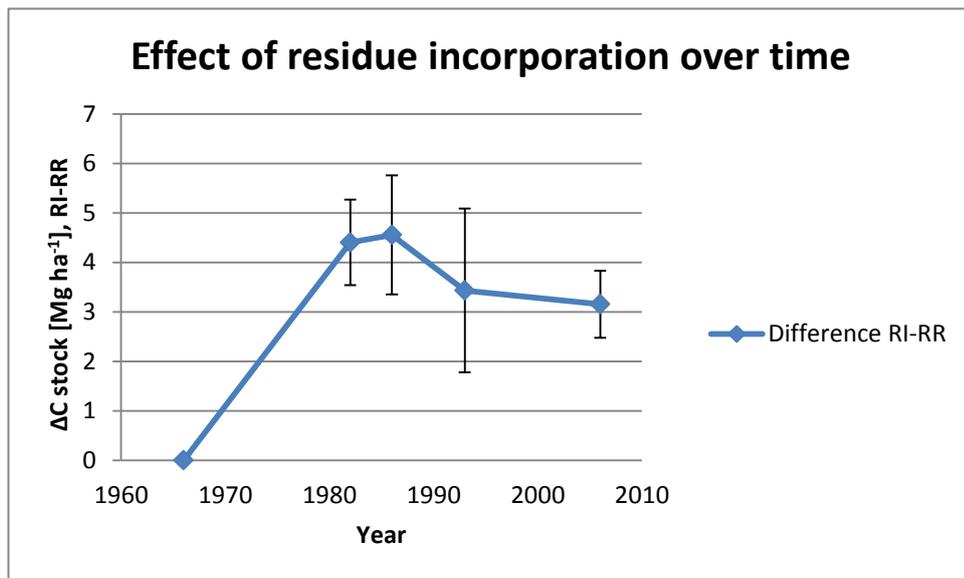
Figure 4. The temporal evolution of the overall effect of residue incorporation between 1966 and 2006. The effect is averaged over all five N levels (standard deviation shown as error bars) and calculated as the difference in bulk SOC stock between Residues incorporated (RI) and Residues removed (RR).
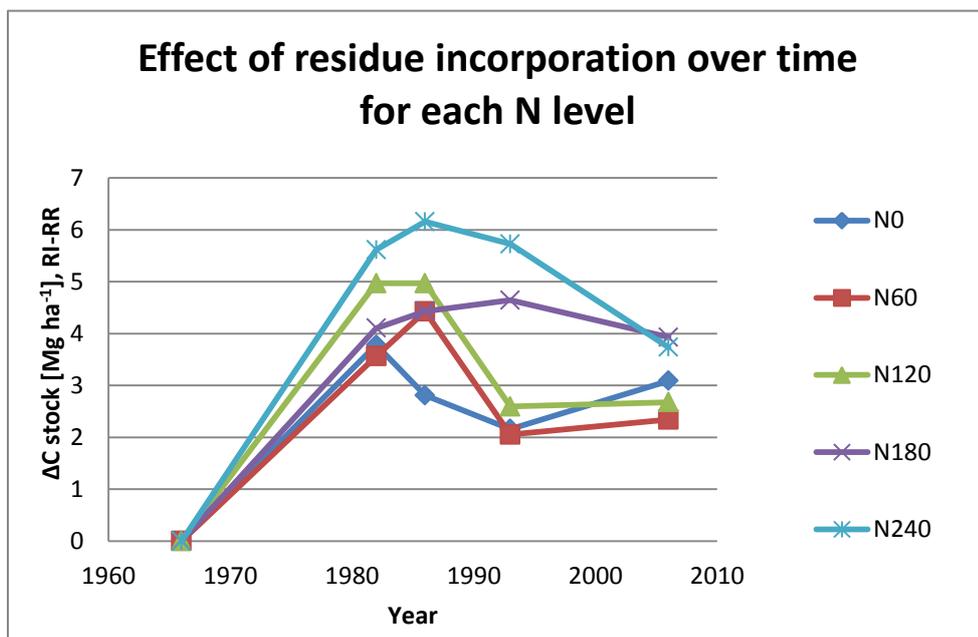

Figure 5. The temporal evolution of the effect of residue incorporation between 1966 and 2006 for each nitrogen level (0-240 kg ha^{-1}). The effect is calculated as the difference in bulk SOC stock between Residues incorporated (RI) and Residues removed (RR).

In the beginning of the field experiment, maize was the only crop grown until 1984. From 1985, a varied crop rotation started consisting of maize every second to third year and between that, different crops like soybean, sugarbeet and winter wheat. These different crops have different biomass production which lead to a clear difference in C input between the period with only maize (1966-1984) and that with crop rotation (1985-2007), see Figure 6. The ΔC input was higher during the years with maize cultivation (in average 2.3 Mg ha^{-1}) than during crop rotation (in average 2.0 Mg ha^{-1}). The difference was however most pronounced at low N levels, at higher N levels the two crop rotations had almost the same ΔC input.
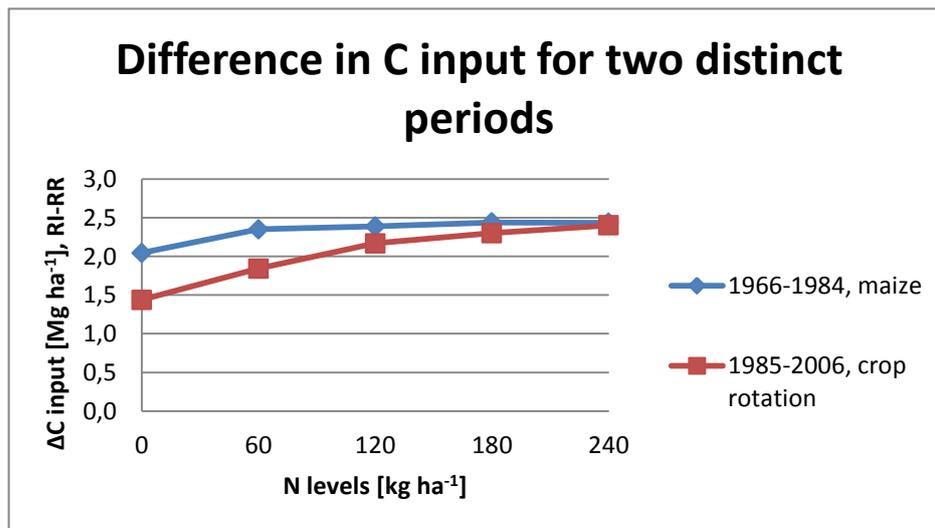

Figure 6. The difference in C input between Residues incorporated (RI) and Residues removed (RR) over N fertilization levels for two different time periods; 1966-1984 when only maize was grown and 1985-2006 when a varied crop rotation was applied.

The retention coefficient for the straw derived C was calculated as the difference in SOC stocks between treatments divided by the difference in total C input between treatments. This is an estimate of the C stabilization efficiency and the result is presented in Figure 7. Residues incorporated (RI) received between 132 and 191 Mg straw derived C ha^{-1}, depending on the N fertilization level since the start in 1966 and Residues removed (RR) received between 52 and 86 Mg straw derived C ha^{-1}. The difference in SOC stock between treatments varied between 2.7 and 3.9 Mg straw derived C ha^{-1}. This gives retention coefficients ranging between 2.5-3.9%, in average 3.3%. After 40 years, the lowest SOC retention was obtained when fertilizing with 60 kg N ha^{-1} (2.5%) and the highest with 0 kg N ha^{-1} (3.9%). This means that 2.5-3.9% of the C input is still left in the soil after 40 years of straw incorporation. The retention coefficients at the highest N fertilization levels (N=180 and N=240) were slightly lower than the retention coefficient at N=0,

which was surprising. These results answer research question 3, namely that the SOC retention coefficient did not increase with higher N fertilization levels. This means that higher N fertilization rate did not lead to increased C stabilization efficiency.
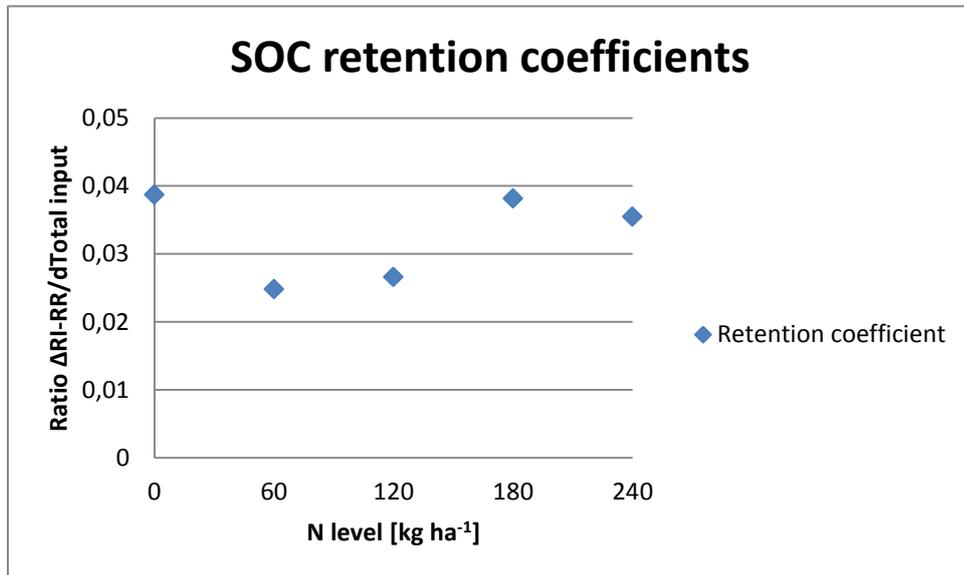

Figure 7. The SOC retention coefficients for each N fertilization level after 40 years of treatment. The retention coefficient is calculated as the difference in final SOC stock between the treatments Residues incorporated (RI) and Residues removed (RR) after 40 years of treatment divided by the total C input difference between treatments during that period. The SOC retention coefficient is used as an estimation of the C stabilization efficiency.

5.2   Response of the fractions on straw incorporation

The different fractions investigated are: rSOC (resistant organic carbon), SC-rSOC (carbon adsorbed to the silt and clay particles that is not resistant), SA (particulate organic matter adsorbed to sand particles or incorporated in stabile aggregates), DOC (dissolved organic carbon) and POM (particulate organic matter).

The highest SOC content was found in the SC-rSOC fraction, ranging between 20 and 30 Mg SOC ha^{-1} whilst the smallest fractions were POM and DOC, both containing about 0.5-1 Mg SOC ha^{-1} (Figure 8 and Figure 9). In comparison, the total SOC stock of the bulk soil contained about 45-50 Mg C ha^{-1}. The second largest fraction was clearly the rSOC, containing about 17 Mg ha^{-1} and thereafter the SA fraction (1-2 Mg ha^{-1}). For several of the fractions, above all those containing the lowest amount of SOC, there was a large variation between the samples. Straw incorporation had no significant effect on the SOC stock in the fractions rSOC, SA and POM (p=0.99, 0.20 and 0.76 respectively) when averaged over all

N levels. For the fractions SC-rSOC and DOC on the other hand there was a strong significant effect of straw incorporation ($p=0.001$ and $p=0.01$ respectively). However, just as for the bulk soil, the effect was not significant when grouping the treatments by N levels due to high spatial variability.
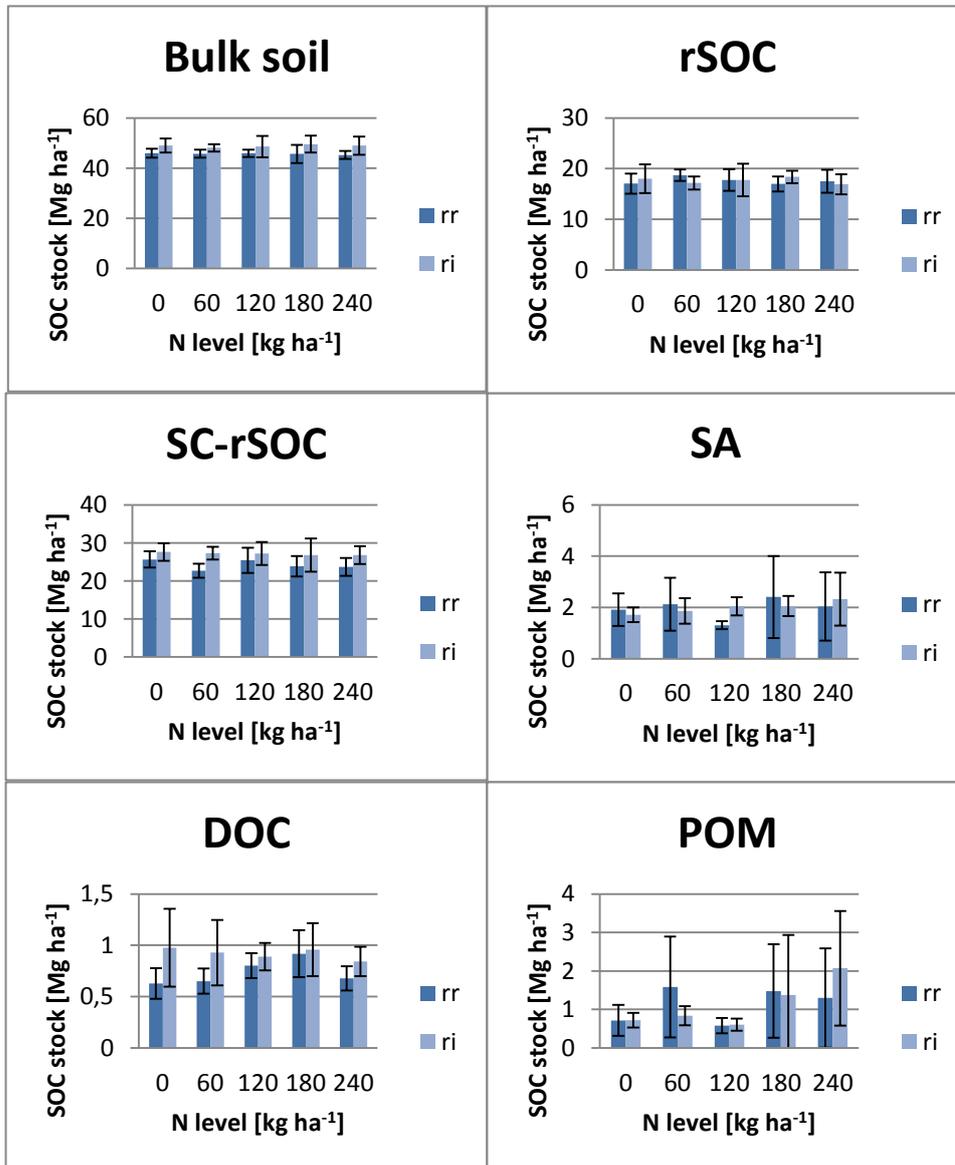

Figure 8. The SOC stocks of the bulk soil and that of each fraction at each N fertilization level, n=4. The error bars show the standard deviation over the four replicates. No treatment group is significantly different from the other at $p=0.05$. The scale on the y-axis differs according to the size of the SOC stock in the bulk soil and in each fraction.
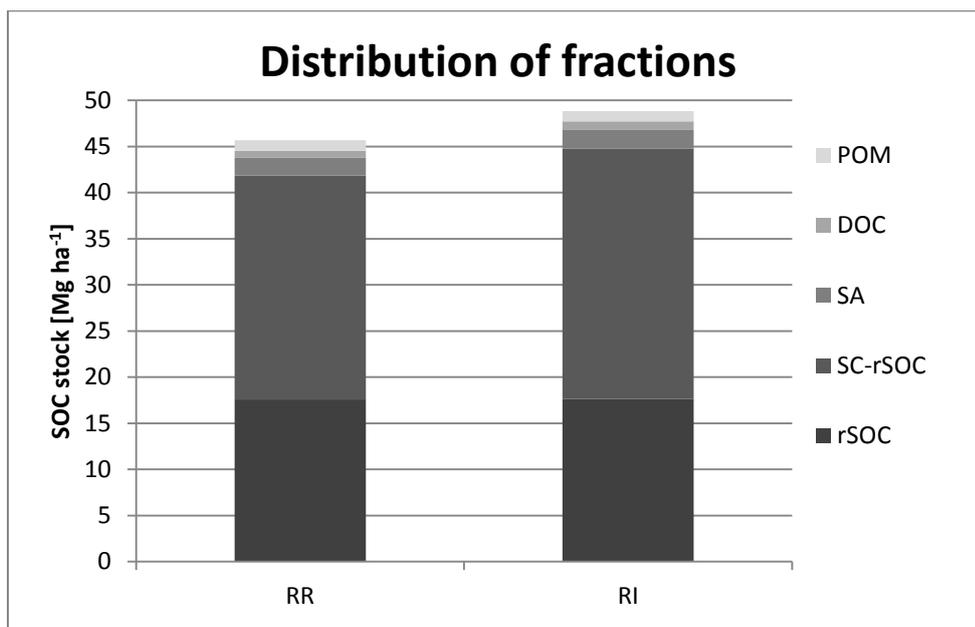
Figure 9. The distribution of fractions in the treatments Residues incorporated (RR) and Residues removed (RI). The values are averaged over all N levels (n=20).

Table 1 shows the absolute and relative effect of straw incorporation on the SOC fractions at different N levels. It was clear that the POM varied most between different N levels. This is not surprising since POM is the most sensitive fraction to management practices. In relative numbers, the effect of straw incorporation on POM varied between a decrease of 53% and an increase of 159%. Also the DOC varied a lot in relative terms, between 104% and 156%. It is however important to remember that these two fractions contained very little SOC, why even a small change in SOC stock results in a large relative difference. The relative differences are hence a bit misleading for POM and DOC. The fraction that varied least in relative numbers is rSOC (92-108%, in average 100%, thus zero change).

The fraction that responded most to straw incorporation in relative numbers is SA, which shows an average increase of 128%. In absolute numbers, the SC-rSOC fraction responded most. This fraction contained most of the straw derived C (Figure 8). Since the silt-and-clay fraction in general is considered to be one of the most stable fractions, straw incorporation seemed to lead to a long-term SOC sequestration in the Padova soil.

These results answer research questions 4 and 5; SA responded most to straw incorporation in relative terms but was not significantly affected by the treatment. SC-rSOC responded most in absolute terms and was, together with DOC, the only fraction significantly affected by straw incorporation. The significant increase in the SC-rSOC fraction implies that straw incorporation can lead to a long-term C

sequestration. N fertilization did not affect the C stabilization significantly in any of the fractions. Just as for the bulk soil, it is clear that the spatial variation within each sample group was larger than the difference between treatments when using four replicates (n=4).

Table 1. The absolute (Mg ha^{-1}) and relative (%) effects of straw incorporation on SOC in the separate fractions and the bulk soil at each N level as well as the average effect

Treatment	rSOC	SC-rSOC	SA	DOC	POM	Bulk
N=0, Absolute effect (Mg ha^{-1})	0,97	1,96	-0,20	0,35	0,003	3,09
N=0, Relative effect	106%	108%	90%	156%	100%	107%
N=60, Absolute effect (Mg ha^{-1})	-1,51	4,58	-0,26	0,28	-0,75	2,34
N=60, Relative effect	92%	120%	88%	143%	53%	105%
N=120, Absolute effect (Mg ha^{-1})	-0,01	1,83	0,74	0,09	0,03	2,68
N=120, Relative effect	100%	107%	156%	111%	104%	106%
N=180, Absolute effect (Mg ha^{-1})	1,39	2,96	-0,35	0,04	-0,10	3,93
N=180, Relative effect	108%	112%	85%	104%	93%	109%
N=240, Absolute effect (Mg ha^{-1})	-0,59	3,10	0,28	0,17	0,77	3,74
N=240, Relative effect	97%	113%	114%	124%	159%	108%
Average, Absolute effect (Mg ha^{-1})	0,051	2,89	0,041	0,18	-0,01	3,15
Average, Relative effect	100%	112%	107%	128%	102%	107%

6   Discussion

6.1   Carbon in bulk soil

6.1.1   Response of the total carbon stock

The results showed that straw incorporation significantly increased the SOC stock in the bulk soil with 3.15 Mg ha^{-1} after 40 years of treatment, averaged over all N levels (see Figure 3). This corresponds to an increase of 6.4% and is in the range of what other studies have found (Lehtinen *et al.*, 2014; Liu *et al.*, 2014). As Liu *et al.* (2014) stated, the effect depends on many variables, both natural conditions and management practices. The duration of the experiment is one important factor for the magnitude of the observed effect. The Padova field-trial is a long-term experiment that has been running since 1966 which increases the chances for observing an effect of the straw incorporation compared with more short-term studies (Lehtinen *et al.*, 2014). Several other studies have been performed under a relative short time period (Curtin & Fraser, 2003; Campbell *et al.*, 2001), which could be one explanation to the low response of straw incorporation sometimes observed.

However, not only the duration is important for the C sequestration from straw incorporation. Many studies have concluded that the effect of straw incorporation also depends on clay content (Lehtinen *et al.*, 2014; Liu *et al.*, 2014; Wiesmeier *et al.*, 2014) and has less impact on sandy soils (Poeplau *et al.*, 2015). This can be due to the spatial inaccessibility of organic material that is occluded in clay aggregates. The Padova soil has high clay content (35%) and thus it was not surprising to observe a positive effect of straw incorporation. However, the increase in SOC of 6.4% for the Padova soil is in the lower range of what has been found at other sites. Lehtinen *et al.* (2014) reported a European average increase of 7% and Liu *et al.* (2014) found a global average of 12%. This could depend on other factors such as straw C input rate, initial SOC stock, saturation deficit and mean annual temperature (e.g. Wiesmeier *et al.*, 2014; Poeplau *et al.*, 2011; Stewart *et al.*, 2009).

For example, soils in warmer climate generally accumulate C slower than those in colder climate due to a higher rate of microbial processes and thus higher decomposition (Lal, 2004).

Even though many studies have reported an increase in SOC stock due to straw incorporation, the effect seems to be quite small in comparison to other management practices. Several studies have shown a higher SOC sequestration after manure incorporation, for example Kätterer *et al.* (2011) and Thomsen & Christensen (2010). Other management practices have also been suggested to have a higher potential for sequestering C, above all converting cropland to grassland (Poeplau *et al.*, 2011; Freibauer *et al.*, 2004; Vleeshouwers & Verhagen, 2002). This would be beneficial from a C sequestration point of view but would bring drawbacks such as less agricultural land for producing food and feed. Also manure incorporation has disadvantages such as GHG losses during storing, why Triberti *et al.* (2008) in a comparison conclude that straw incorporation is the best way to reach a significant C sequestration. Another important aspect is how much of the sequestered SOC that is really long-term stabilized by for example spatial inaccessibility and selective preservation. Menichetti (2014) stated that it is challenging to quantify the proportion of any C input that is actually stabilized in the soil.

The effect of residue incorporation can also be related to the amount of C input, giving a retention coefficient that indicates the C stabilization efficiency, i.e. how much of the added C that really is recovered in the soil after a certain time. This work showed that the retention coefficient for the Padova soil ranged between 2.5% and 3.9% after 40 years of straw incorporation, depending on the N level (3.3% in average). That means that the retention coefficient has decreased over time, since Lugato *et al.* (2006) found it to be 5.6% on average after 28 years of residue incorporation. It is clear that the amount of straw derived C retained in soil was quite small in comparison to other soil amendments, just as for the effect on total C stock discussed above. Kätterer *et al.* (2011) studied the stabilization of several different soil amendments and found that the fraction of aboveground crop residues retained in the soil after 50 years was 0.17 (17%) which was considerably lower than retention of both farmyard manure (0.32) and sewage sludge (0.54). This was probably due to the fact that crop residues are fresh material not processed yet. They are less recalcitrant than for example manure, and since recalcitrance is important in the beginning of decomposition (von Lützow *et al.*, 2006) this enhances the stabilization of processed compounds more than of easily available crop residues.

6.1.2 Evaluating the temporal trend

The temporal trend of the C stocks in the Padova field trial showed less response to straw incorporation over time, especially after 1989. Stewart *et al.* (2007) ar-

gued that the C sequestration may decrease as the soil reaches C saturation (its maximum C storage capacity), and an initial thought was therefore that the decreasing treatment effect at Padova could have been due to the fact that the soil was reaching its saturation level. However, Stewart *et al.* (2007) concluded that most field trials do not reach C saturation due to too low C input. In their study, the long-term field experiments had C inputs ranging from 0.17 to 7.42 Mg ha^{-1} for between 12 to 96 years. The Padova field experiment is comparable to the sites presented by Stewart *et al.* (2007) in terms of C input and duration, i.e. 4.80 Mg ha^{-1} and 40 years, respectively. Thus, it is unlikely that the Padova soil has reached C saturation during the studied period. This is further strengthened by the fact that the original SOC levels when the experiment started were higher than the present SOC levels, showing that the soil has the potential to sequester more SOC. Therefore, C saturation cannot be the explanation to the decreased SOC sequestration rate in the Padova soil.

A potential explanation for the observed trend in SOC over time can probably be related to the historical crop rotation. Figure 4 shows that there was a clear decrease in ΔSOC after 1986. This corresponded very well to the stop of continuous maize cultivation that took place in 1985 and which led to a clear decrease of C input during the period 1985-2006 (figure not shown). As von Lützow *et al.* (2006) described, after a change in C input a new SOC equilibrium will eventually be reached and this was probably what happened in the Padova soil. Microbes have been found to adapt relatively quickly to a decreased input, as new equilibrium after a land-use change may be reached after 17-23 years, depending on the type of land-use change (Poeplau *et al.*, 2011). Under continuous straw return (C losses equal C inputs), Liu *et al.* (2014) found that the increase in C sequestration stopped after 12 years. The decreased C input in 1985 thus may explain the decreased effect of straw incorporation in 2006.

Even though a new C equilibrium might be reached, the C sequestration potential of the Padova soil remains the same. It therefore has the potential to increase again. However, Poeplau *et al.* (2011) showed that the SOC stock decreases faster after a decrease in C input than it increases again after an increased input. It would thus probably take a long time to reach the initial C levels of 1966 in the Padova soil, even if the C input was increased again.

6.2 Carbon in fractions

The fractionation method showed that most C was located in the fraction SC-rSOC, i.e. the non-resistant C adsorbed to silt and clay particles (Figure 8 and Figure 9). It also showed that the C content in this fraction increased most with straw incorporation in absolute numbers (2.89 Mg ha^{-1}) and second most in relative

numbers (+12%), see Table 1. The average increase was significant. The SC-rSOC fraction is considered to be one of the more stable fractions. Since most of the total increase in SOC after straw incorporation accumulated in this fraction, the effect of straw incorporation in Padova can be considered to be long-lasting.

The SC-rSOC fraction contained 56% of the total SOC when residues were incorporated and 53% when residues were removed (Figure 9). The fraction with resistant C, rSOC contained the second largest C portion, 36-39%. Together, the C in these two fractions (C associated with clay and silt particles) thus constituted over 90% of the total SOC. Some studies reported lower proportions in the SC fraction (Poeplau & Don, 2013; Stewart *et al.*, 2009) but several other studies have reported similar numbers as for the Padova soil (Bol *et al.*, 2009). Von Lützow *et al.* (2007) presented similar numbers in their literature review, saying that 50-75% of the SOC is normally found in the clay fraction (<2 µm) and 20-40% in the silt fraction (2-63 µm) for temperate soils, summing up to around 90% in total. They further report that the sand fraction normally contain less than 10% of the total SOC which is also consistent with the results of the Padova soil, where the SA fraction contained just over 4% OC. Poeplau & Don (2013) reported the SA fraction to be the second most sensitive fraction to changes in C input which was in agreement with the findings by Stewart *et al.* (2009).

The results from this study showed that the response of POM to straw incorporation varied most (Table 1). The study also showed that POM in the Padova soil constituted only slightly more than 2% of total SOC, which is quite a low number. According to von Lützow *et al.* (2007), the POM fraction varies greatly but typically ranges between 10-70%. It is well known that the POM fraction is highly affected by land-use and shows seasonal variability in the field (Poeplau & Don, 2014; Chenu & Plante, 2006; Christensen, 1992). It is possible that the sampling time (early spring, i.e. March preceded by soil cultivation and ploughing) could have affected the amount of POM found in the field or it could be a result of the relatively warm and humid winter climate in Padova causing rapid decomposition. The results also show that the variation within the POM samples was very high in comparison to the other fractions and no significant effect of straw incorporation was observed (Figure 8). The high variation in POM values were probably caused by high spatial variability (Christensen, 1992) but also by their low amount, which means that even a small change made a large difference. The amount of POM could also have been affected by the sorting of the material before the fractionation method started, where all material >2mm was removed.

DOC constituted the smallest fraction in the Padova soil together with POM. DOC was the only fraction except SC-rSOC where the straw incorporation had a significant effect, if averaged over all N levels. Poeplau & Don (2013) reported that this fraction was more sensitive to changes in C input than the bulk soil which

was the case also in the Padova soil where DOC showed the largest average response to the straw incorporation (Table 1).

The rSOC fraction is assumed to be an inert fraction which is not expected to vary much with management practice (Zimmermann *et al.*, 2007). Some studies have however questioned this, since it has been shown that rSOC responds almost as much as the bulk soil to straw incorporation (Poeplau & Don, 2013). However, the results from Padova show that rSOC changed very little with different N levels and the average effect was zero (see Table 1). These results support the assumption that rSOC is an inert fraction.

The results of this study showed that N had no effect on C sequestration, neither in the bulk soil nor regarding the input-normalized effect. It did not affect the distribution of SOC between fractions either (Figure 8). It is surprising that there was no effect of N on the bulk soil, since Lugato *et al.* (2006) did observe an effect in Padova and several other studies reported the same pattern (Lemke *et al.*, 2010; Christopher & Lal, 2007). As Figure 5 shows, the effect of residue incorporation did not differ as much between different N fertilization levels in 2006 as it did in 1993. The reason for this decreased N effect cannot be explained entirely by the data from this study. One part of the explanation could be the average decreased effect of straw incorporation. It is possible that the SOC values from 2006 are a delayed response to the decreased C input starting from 1985. Another possible explanation could be the change in crop rotation. Different crop residues have different C:N ratios which affects decomposition (Blanco-Canqui & Lal, 2009). The crop rotation shifted from maize monoculture to a rotation with a high proportion of soybeans and sugarbeets which are known to have more N-rich crop residues than maize and may therefore decompose faster. The observed decrease in treatment differences during the recent decade (Figure 4 and Figure 5) may therefore be due to both decreasing treatment differences in C input and reduced C retention due to N-rich residues. Furthermore, soybean is an N fixating crop and does therefore not benefit from the N fertilization as much as the other crops. This could also have contributed to the decreased effect of N fertilization. However, soybean was only grown two years since the change of crop rotation why this is most likely not the main reason to the observed trends.

The results presented here are based on the topsoil only and nothing can be concluded about the pattern in the subsoil. Even though the largest SOC changes usually occur close to the soil surface, the subsoil can show SOC changes too (Menichetti, 2014; Poeplau *et al.*, 2011). In the study by Poeplau *et al.* (2011) for example, SOC changes in the subsoil followed the same trend as those observed in the topsoil but to a smaller extent. Since the subsoil contains a large part of the total SOC it could play a role when it comes to increasing the C sequestration, as discussed by Kaiser & Guggenberger (2003) and Menichetti (2014). Little is known

about the C dynamics in the subsoil (von Lützow *et al.*, 2006) and it would be interesting to get a better understanding of the processes governing subsoil SOC stabilization.

6.3   Alternative use of resources

This study showed that the effect of straw incorporation in the Padova long-term field trial was quite small and that is has decreased over time. However, most of the sequestered C was found in the silt and clay fraction and can thus be considered a long-term stabilization. This is positive from a climate change point of view since that C will not return to the atmosphere immediately, not even if residue incorporation were to cease. Straw incorporation also brings along other advantages, such as improved soil nutrient levels, better water retention capacity and less risk for erosion (Blanco-Canqui & Lal, 2009). Furthermore it results in higher yields (Liu *et al.*, 2014). Although there also are drawbacks, such as increased CO_2 and N_2O emissions (Lehtinen *et al.*, 2014; Liu *et al.*, 2014), straw incorporation has been suggested to lead to a C sink in upland soils (Liu *et al.*, 2014).

However, there are many options of how to handle the straw. Removing the straw as feed and then returning the processed product in form of farmyard manure has seen to give higher C sequestration, as mentioned before. Such handling does on the other hand also include gas losses before the processed product reaches the field again and Schlesinger (2000) questions its possibility to result in a C sink. The potential of such a management practice is also decided by other factors, for example how many animals there are in a region (Kätterer *et al.*, 2012). Other methods, such as focusing the C sequestration strategies to surplus arable land (Smith *et al.*, 2000) or aiming for an increased net primary production (Kätterer *et al.*, 2012) are other ways to increase the SOC sequestration. There are many different C sequestration options and it is difficult to say which one is the best alternative. It is also crucial to increase the use of renewable energy sources, where straw residues is discussed as one option.

In the Padova long-term field trial, close to all residues are removed in the Residues removed treatment. There are studies that have investigated the effect of only removing a certain fraction of the crop residues and leaving the rest on the field. That might be a good way to compromise between the different needs for the straw. It would be interesting to investigate if there is an optimal straw removal rate that permits maintaining SOC levels and soil function and at the same time biofuel production or feed production. However, such a determination must be adopted to regional or maybe even field specific values (Wilhelm *et al.*, 2004). Since the effect of straw incorporation at the Padova field trial is quite small it

could be possible to use some of the straw for other purposes. A quantification of that amount does however lie outside the scope of this thesis.

6.4 Evaluation of the method

For the Padova long-term field trial, this was the first time the soil was fractionated and the C dynamics investigated in detail. This has given information about the response of SOC on straw incorporation not only in the bulk soil but also in the different fractions. Since it is the first soil fractionation of the Padova field trial it is not possible to follow the change in SOC distribution in different fractions over time. It would be interesting to know, for example, if the largest fraction, the non-recalcitrant C occluded in the silt- and clay fraction (SC-rSOC) changed with the difference in C input that occurred 1985 and how fast such a change occurred in that case. In the future, it would be of great value to perform continuous fractionation studies at the same site (in Padova and other long-term field trials) in order to assess the temporal change also in SOC fractions and not just in the bulk soil, as mostly has been done so far. The Zimmermann method has also the advantage that the results can be used for SOC modelling using the RothC model, something that is valuable when it comes to investigating the future C sequestration, for example in a changing climate.

The drawbacks with fractionation studies in general are, however, that they are quite time-consuming and that it is difficult to identify functional C pools that are homogeneous. Von Lützow *et al.* (2007) found in their literature review very few fractionation methods that actually resulted in functional C pools. However, the Zimmermann method was not included in this review and it does incorporate the chemical oxidation step with NaOCl which was concluded to be promising by von Lützow *et al.* (2007). It is thus possible that this fractionation method is one of the best available at the moment, especially after incorporating the improvements by Poeplau *et al.* (2013) regarding among others the soil dispersion. Since that is one of the most crucial steps in physical soil fractionation it is of course important to continue to improve this step as much as possible.

During the fractionation procedure it is assumed that no material is lost, but in reality some material losses did occur in almost each fractionation step. It was probably due to transferring of the samples back and forth between sieve bags, beakers and so on. This is a drawback since it is not known exactly which material was lost and its C content. However, it seems like more or less the same amount was lost for each sample, why the relative difference between them should be the same.

Another way to obtain more detailed information about C dynamics is by studying C isotopes. The initial intention in this thesis was to also measure the abun-

dance of the natural isotope ^{13}C in order to trace the maize-derived C and its distribution between fractions. However, due to carbonates in the soil that was not possible. Even though the procedure by Steinbeiss *et al.* (2008) was followed, which has worked well in other studies like Poeplau & Don (2014), it did not remove the carbonates from the Padova soil. It seems very likely that this soil is derived from marine sediments, since it is located at only 6 m above sea level and shells were found in the soil. In this environment, poorly soluble dolomite can be present. This type of carbonate cannot be dissolved sufficiently by sulfurous acid (Kennedy *et al.*, 2005), which is what is used in the Steinbeiss method. This is probably the reason why the acid treatment was not successful in the Padova soil. Another explanation could be that the shells were not completely crushed during the ball-milling. Kennedy *et al.* (2005) recommend using a weak HCl solution (1-2 M) for removing such recalcitrant carbonates. The best results have been obtained when fumigating the samples with HCl but in order to do that, the exposure time should be adapted for each sample type (Komada *et al.*, 2008). Due to time constraints it was not possible to perform such acid tests during this work and no ^{13}C analyses could therefore be performed on the fractions containing carbonates.

7 Conclusions

Residue incorporation significantly increases the SOC stock (3.15 Mg ha^{-1}), but the effect in the Padova soil was quite small in comparison to previous studies. The effect decreased over time which was most likely due to the decrease in C input as a consequence of a change in crop rotation in 1985. Increasing mineral N fertilization did not affect the C sequestration, neither in the bulk soil nor in any of the SOC fractions. The fractionation study showed that most C was retained in the fraction with non-resistant C associated to clay and silt particles and this was also the fraction increasing most in absolute numbers after residue incorporation. Thus, this is probably the most important fraction for sequestering C in Padova. That means that even though quite a small amount of the C input was retained in the soil after 40 years (on average 3.3%), C accumulated in a fraction that is believed to be relatively stable. The resistant C fraction did not change with straw incorporation, supporting the hypothesis that an inert fraction was isolated.

Other management practices such as manure incorporation have often been found to sequester more C than straw incorporation. However, it is important to consider in which fraction that C is stored in order to assess the long-term benefit. It is also important study the net effect of any management practice on all GHG emissions when aiming for C sequestration. For future work it would be interesting to continue with fractionation studies and preferably continuously in order to assess if and how the C distribution between fractions changes over time. It would also be of great interest to investigate if there is room for removing part of the straw residues to use for other purposes without depleting SOC stocks and soil function.

Finally, straw incorporation seems to lead to a small but presumably rather stable C sequestration in clay soils and has probably a long-term sequestration effect even after an eventual change in management practices. Straw incorporation might therefore be a sustainable way to sequester C, irrespective of the level of nitrogen fertilization.

References

Blanco-Canqui, H. & Lal, R. (2009). Crop Residue Removal Impacts on Soil Productivity and Environmental Quality. *Critical Reviews in Plant Sciences*, 28, pp 139–163.

Bolinder, M. A., Janzen, H. H., Gregorich, E. G., Angers, D. A. & VandenBygaart, A. J. (2007). An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. *Agriculture, Ecosystems & Environment*, 118, pp 29–42.

Bol, R., Poirier, N., Balesdent, J. & Gleixner, G. (2009). Molecular turnover time of soil organic matter in particle-size fractions of an arable soil. *Rapid Communications in Mass Spectrometry*, 23, pp 2551–2558.

Bot, A. & Benites, J. (2005). *The importance of soil organic matter: key to drought-resistant soil and sustained food production*. Rome: Food and Agriculture Organization of the United Nations. (FAO soils bulletin; 80). ISBN 9251053669.

Brady, N. C. & Well, R. R. (2002). Soil organic matter. Humus: Genesis and Nature. l: In: Stephen Helba (Ed) *The Nature and Properties of Soils*. 13. ed. New Jersey: Pearson Education.

Campbell, C. A., Selles, F., Lafond, G. P. & Zentner, R. P. (2001). Adopting zero tillage management: Impact on soil C and N under long-term crop rotations in a thin Black Chernozem. *Canadian Journal of Soil Science*, 81, pp 139–148.

Chenu, C. & Plante, A. F. (2006). Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the "primary organo-mineral complex". *European Journal of Soil Science*, 57, pp 596–607.

Christensen, B. T. (1992). Physical Fractionation of Soil and Organic Matter in Primary Particle Size and Density Separates. l: In: Stewart, B. A. (Ed) *Advances in Agronomy*. pp 1–90. Boca Raton: CRC Lewis Publishers.

Christopher, S. F. & Lal, R. (2007). Nitrogen Management Affects Carbon Sequestration in North American Cropland Soils. *Critical Reviews in Plant Sciences*, 26, pp 45–64.

Chung, H., Grove, J. H. & Six, J. (2008). Indications for Soil Carbon Saturation in a Temperate Agroecosystem. *Soil Science Society of America Journal*, 72, p 1132.

Curtin, D. & Fraser, P. M. (2003). Soil organic matter as influenced by straw management practices and inclusion of grass and clover seed crops in cereal rotations. *Australian Journal of Soil Research*, 41, pp 95–106.

Cvetkov, M., Santavec, I., Acko, D. K. & Tajnsek, A. (2010). Soil organic matter content according to different management system within long-term experiment. *Acta Agriculturae Slovenica*, 95, pp 79–88.

Derrien, D., Marol, C., Balabane, M. & Balesdent, J. (2006). The turnover of carbohydrate carbon in a cultivated soil estimated by 13C natural abundances. *European Journal of Soil Science*, 57, pp 547–557.

Don, A., Osborne, B., Hastings, A., Skiba, U., Carter, M. S., Drewer, J., Flessa, H., Freibauer, A., Hyvönen, N., Jones, M. B., Lanigan, G. J., Mander, Ü., Monti, A., Djomo, S. N., Valentine, J., Walter, K., Zegada-Lizarazu, W. & Zenone, T. (2012). Land-use change to bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy*, 4, pp 372–391.

Don, A., Schumacher, J. & Freibauer, A. (2011). Impact of tropical land-use change on soil organic carbon stocks - a meta-analysis: SOIL ORGANIC CARBON AND LAND-USE CHANGE. *Global Change Biology*, 17, pp 1658–1670.

EU (2009). *Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC* [online]. Brussels. (L 140/16).

Eusterhues, K., Rumpel, C. & Kögel-Knabner, I. (2005). Stabilization of soil organic matter isolated via oxidative degradation. *Organic Geochemistry*, 36, pp 1567–1575.

FAO-UNESCO (1990). *Soil Map of the World. Revised Legend.* Rome: FAO.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B. & Rumpel, C. (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, 450, pp 277–280.

Freibauer, A., Rounsevell, M. D. ., Smith, P. & Verhagen, J. (2004). Carbon sequestration in the agricultural soils of Europe. *Geoderma*, 122, pp 1–23.

Haynes, R. J. (2005). Labile organic matter fractions as central components of the quality of agricultural soils: an overview. *Advances in Agronomy*, 85, pp 221–268.

Hedges, J. I., Eglinton, G., Hatcher, P. G., Kirchman, D. L., Arnosti, C., Derenne, S., Evershed, R. P., Kögel-Knabner, I., De Leeuw, J. W., Littke, R. & others (2000). The molecularly-uncharacterized component of nonliving organic matter in natural environments. *Organic Geochemistry*, 31, pp 945–958.

IPCC (2007). *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

IPCC (2014a). *Climate Change 2014 - Synthesis Report*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

IPCC (2014b). *Technical Summary. In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Jastrow, J., Boutton, T. & Miller, R. (1996). Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. *Soil Science Society of America Journal*, 60, pp 801–807.

Kaiser, K. & Guggenberger, G. (2003). Mineral surfaces and soil organic matter. *European Journal of Soil Science*, 54, pp 219–236.

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

Kennedy, P., Kennedy, H. & Papadimitriou, S. (2005). The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. *Rapid Communications in Mass Spectrometry*, 19, pp 1063–1068.

Khan, S. A., Mulvaney, R. L., Ellsworth, T. R. & Boast, C. W. (2007). The Myth of Nitrogen Fertilization for Soil Carbon Sequestration. *Journal of Environment Quality*, 36, p 1821.

46

Kirkby, C. A., Richardson, A. E., Wade, L. J., Passioura, J. B., Batten, G. D., Blanchard, C. & Kirkegaard, J. A. (2014). Nutrient availability limits carbon sequestration in arable soils. *Soil Biology and Biochemistry*, 68, pp 402–409.

Komada, T., Anderson, M. R. & Dorfmeier, C. L. (2008). Carbonate removal from coastal sediments for the determination of organic carbon and its isotopic signatures, δ13C and Δ14C: comparison of fumigation and direct acidification by hydrochloric acid. *Limnology and Oceanography: Methods*, 6, pp 254–262.

Kuzyakov, Y., Friedel, J. K. & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. 32, pp 1485–1498.

Kätterer, T., Bolinder, M. A., Andrén, O., Kirchmann, H. & Menichetti, L. (2011). Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems & Environment*, 141, pp 184–192.

Kätterer, T., Bolinder, M. A., Berglund, K. & Kirchmann, H. (2012). Strategies for carbon sequestration in agricultural soils in northern Europe. *Acta Agriculturae Scandinavica, Section A - Animal Science*, 62, pp 181–198.

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma*, 123, pp 1–22.

Lal, R. (2005). World crop residues production and implications of its use as a biofuel. *Environment International*, 31, pp 575–584.

Lehtinen, T., Schlatter, N., Baumgarten, A., Bechini, L., Krüger, J., Grignani, C., Zavattaro, L., Costamagna, C. & Spiegel, H. (2014). Effect of crop residue incorporation on soil organic carbon and greenhouse gas emissions in European agricultural soils. *Soil Use and Management*, 30, pp 524–538.

Lemke, R. L., VandenBygaart, A. J., Campbell, C. A., Lafond, G. P. & Grant, B. (2010). Crop residue removal and fertilizer N: Effects on soil organic carbon in a long-term crop rotation experiment on a Udic Boroll. *Agriculture, Ecosystems & Environment*, 135, pp 42–51.

Liu, C., Lu, M., Cui, J., Li, B. & Fang, C. (2014). Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis. *Global Change Biology*, 20, pp 1366–1381.

Lugato, E., Berti, A. & Giardini, L. (2006). Soil organic carbon (SOC) dynamics with and without residue incorporation in relation to different nitrogen fertilisation rates. *Geoderma*, 135, pp 315–321.

Von Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. & Flessa, H. (2006). Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science*, 57, pp 426–445.

Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. & Marschner, B. (2007). SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry*, 39, pp 2183–2207.

Menichetti, L. (2014). *Improving our understanding of carbon cycling in agroecosystems by studying δ13C signatures in soil carbon stocks and fluxes* [online]. Uppsala. (2014:5).

Oades, J. M. (1994). Organic Matter: Chemical and Physical Fractions. l: In: Lefroy, R. D. B., Blair, G. J., & Craswell, E. T. (Eds), Ubon, Thailand, augusti 24 1994. p 163. Ubon, Thailand: Australian Centre for International Agricultural Research.

O'Brien, S. L., Jastrow, J. D., McFarlane, K. J., Guilderson, T. P. & Gonzalez-Meler, M. A. (2013). Decadal cycling within long-lived carbon pools revealed by dual isotopic analysis of mineral-associated soil organic matter. *Biogeochemistry*, 112, pp 111–125.

Paustian, K. (2014). Soil: Carbon Sequestration in Agricultural Systems. l: In: van Alfen, N. K. (Ed) *Encyclopedia of Agriculture and Food Systems*. pp 140–152. San Diego: Elsevier. ISBN 9780080931395.

Poeplau, C. & Don, A. (2013). Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. *Geoderma*, 192, pp 189–201.

Poeplau, C. & Don, A. (2014). Soil carbon changes under Miscanthus driven by C4 accumulation and C3 decompostion - toward a default sequestration function. *GCB Bioenergy*, 6, pp 327–338.

Poeplau, C., Don, A., Dondini, M., Leifeld, J., Nemo, R., Schumacher, J., Senapati, N. & Wiesmeier, M. (2013). Reproducibility of a soil organic carbon fractionation method to derive RothC carbon pools: Soil carbon fractionation ring trial. *European Journal of Soil Science*, 64, pp 735–746.

Poeplau, C., Don, A., Vesterdal, L., Leifeld, J., Van Wesemael, B., Schumacher, J. & Gensior, A. (2011). Temporal dynamics of soil organic carbon after land-use change in the temperate zone - carbon response functions as a model approach: SOIL ORGANIC CARBON AND LAND-USE CHANGE. *Global Change Biology*, 17, pp 2415–2427.

Poeplau, C., Kätterer, T., Bolinder, M. A., Börjesson, G., Berti, A. & Lugato, E. (2015). Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments. *Geoderma*, 237-238, pp 246–255.

Powlson, D. S., Glendining, M. J., Coleman, K. & Whitmore, A. P. (2011). Implications for Soil Properties of Removing Cereal Straw: Results from Long-Term Studies. *Agronomy Journal*, 103, p 279.

Powlson, D. S., Riche, A. B., Coleman, K., Glendining, M. J. & Whitmore, A. P. (2008). Carbon sequestration in European soils through straw incorporation: Limitations and alternatives. *Waste Management*, 28, pp 741–746.

Reicosky, D. C., Evans, S. D., Cambardella, C. A., Allmaras, R. R., Wilts, A. R. & Huggins, D. R. (2002). Continuous corn with moldboard tillage: Residue and fertility effects on soil carbon. *Jornal of soil and water conservation*, 57, pp 277–284.

RStudio (2014). *RStudio: Integrated development environment for R* [online]. Version: 0.98.1091. Boston, MA: RStudio. Available from: http://www.rstudio.org/. [Accessed 2014-12-10].

Schlesinger, W. (2000). Carbon sequestration in soils: some cautions amidst optimism. *Agriculture, Ecosystems and Environment*, 82, pp 121–127.

Schumacher, B. A. (2002). Methods for the determination of total organic carbon (TOC) in soils and sediments. United States Environmental Protection Agency. Available from: http://epa.gov/nerlesd1/cmb/research/papers/bs116.pdf. [Accessed 2015-02-28].

Siregar, A., Kleber, M., Mikutta, R. & Jahn, R. (2005). Sodium hypochlorite oxidation reduces soil organic matter concentrations without affecting inorganic soil constituents. *European Journal of Soil Science*, 56, pp 481–490.

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

Smith, P. (2004). Carbon sequestration in croplands: the potential in Europe and the global context. *European Journal of Agronomy*, 20, pp 229–236.

Smith, P., Powlson, D. S., Smith, J. U., Falloon, P. & Coleman, K. (2000). Meeting Europe's climate change commitments: quantitative estimates of the potential for carbon mitigation by agriculture. *Global Change Biology*, 6, pp 525–539.

Steinbeiss, S., Temperton, V. M. & Gleixner, G. (2008). Mechanisms of short-term soil carbon storage in experimental grasslands. *Soil Biology and Biochemistry*, 40, pp 2634–2642.

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

Stewart, C. E., Paustian, K., Conant, R. T., Plante, A. F. & Six, J. (2009). Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biology and Biochemistry*, 41, pp 357–366.

Thomsen, I. K. & Christensen, B. T. (2010). Carbon sequestration in soils with annual inputs of maize biomass and maize-derived animal manure: Evidence from 13C abundance. *Soil Biology and Biochemistry*, 42, pp 1643–1646.

Triberti, L., Nastri, A., Giordani, G., Comellini, F., Baldoni, G. & Toderi, G. (2008). Can mineral and organic fertilization help sequestrate carbon dioxide in cropland? *European Journal of Agronomy*, 29(1), pp 13–20.

Vleeshouwers, L. M. & Verhagen, A. (2002). Carbon emission and sequestration by agricultural land use: a model study for Europe. *Global Change Biology*, 8, pp 519–530.

Wiesmeier, M., Hübner, R., Spörlein, P., Geuß, U., Hangen, E., Reischl, A., Schilling, B., von Lützow, M. & Kögel-Knabner, I. (2014). Carbon sequestration potential of soils in southeast Germany derived from stable soil organic carbon saturation. *Global Change Biology*, 20, pp 653–665.

Wilhelm, W. W., Johnson, J. M. F., Hatfield, J. L., Voorhees, W. B. & Linden, D. R. (2004). Crop and soil productivity response to corn residue removal. *Agronomy Journal*, 96, pp 1–17.

Zimmermann, M., Leifeld, J., Schmidt, M. W. I., Smith, P. & Fuhrer, J. (2007). Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science*, 58, pp 658–667.

Acknowledgement

I would like to thank post-doc Christopher Poeplau, Department of Ecology, Swedish University of Agricultural Sciences who has been my supervisor during this thesis. He has contributed with guidance during the laboratory work and has been a good support during the whole process. I also want to thank Professor Antonio Berti, University of Padova for assisting with soil samples and information about the field trial.