Master thesis

Tim Zierfuss – July 2013

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Title:	Soil Organic Carbon sequestration in agricultural soils: the combined impact of no tillage continuous cropping and no tillage crop-pasture rotation on the level of soil organic carbon in the unfractionated soil and three size fractions and on the C:N ratio in an Uruguayan Agriudoll.

Soil Organic Carbon sequestration in agricultural soils

The combined impact of no tillage continuous cropping and no tillage crop-pasture rotation on the level of soil organic carbon in the unfractionated soil and three size fractions the C:N ratio in an Uruguayan Agriudoll.

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Preface

This thesis is the final product of a research project which I, as a Sustainable Development (Global Change and Ecosystems track) student of Utrecht University (the Netherlands), was allowed to perform at long term experimental site designed by the agronomy faculty of the Universidad de la República (Uruguay). The research proposal was set up in the Netherlands in coordination with both Universities. The field work, sample preparation and initial analysis of the data and statistics were performed in Uruguay. The carbon and nitrogen values were determined by the lab technicians of the Geolab in Utrecht. The discussion and conclusion were written the Netherlands. I would like to thank all those who were involved in this process and the Universidad de la República for providing me with this great opportunity.

Prior to this research there were no connections between Utrecht University and the Universidad de la República. This research not only provided me with an interesting topic for sustainability science and international experience, but also created a new academic link. This also provided an extra challenge with setting up and planning the research. The time required to perform the research was much more than anticipated. Finishing the research proposal within a strict time planning before traveling abroad is one of the requirements of the master thesis research program of Utrecht University. This is a very prudent requirement, but also increases the chance that the plans have to be adjusted along the way. The practice might be very different from theory. In the case of this research, this resulted in two months instead of two weeks of lab work due to the very labor intensive sieving methods. Moreover, high variability of the data, difficulties with the theory behind the laboratory methods and strange nitrogen values also required extra time for analysis. There is probably much more knowledge to be gained from this data set but due to strict deadlines, I had to focus only on the data which was essential to answer the research questions. Apart from these difficulties the time I spend in Uruguay and the process of conducting the research and writing the master thesis proved to be a lot of fun and very educational!

Special thanks to Aat and Jorge, who supervised and helped me during the sometimes difficult master thesis process, and to all the people in and around the soil lab in Montevideo, who looked after me and ensured I had a great time!

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Contents

1. Abstract

Sequestration of atmospheric carbon in agricultural soils in the form of soil organic carbon (SOC) can help fight climate change and improve soil fertility. This can be achieved by changing current agricultural management practices to new practices that store more carbon in the soil. Two promising practices are no-tillage and crop pasture rotation. This thesis evaluated the combined effect of these two practices by comparing no-tillage continuous cropping, no-tillage crop pasture rotation with a short (two year) pasture phase and no-tillage crop pasture rotation with a long (four year) pasture phase and their on the level of soil organic carbon in the unfractionated soil and the soil fractions coarse particulate organic matter (coarse POM), fine particulate organic matter (fine POM) and mineral associated organic matter (MAOM). Additionally it tried to measure the C:N (carbon to nitrogen) ratios to confirm and explain possible enhanced humification under no-tillage short and long crop pasture rotation (from here on all mentioned treatments were performed without tillage unless stated otherwise). The samples were taken at 0-5 cm, 5-10 cm and 10-20 cm depth at an eighteen year old experimental site at Paysandu (Uruguay) on a Typic Agriudoll. The carbon and nitrogen measurements were performed with dry combustion and factorial mixed ANOVA was used for the statistical analysis.

Significant differences between the three agricultural practices were only found in the 5-10 cm layer in the coarse and total POM-C ('-C' refers to the carbon in the fraction) fractions. This confirms that total POM-C and especially coarse POM-C are sensitive to changes in agricultural management. Crop pasture rotation with a four year pasture phase had the highest coarse POM-C concentration (0.34 g/kg), followed by the short crop pasture rotation with a two year pasture phase (0.26 g/kg) and continuous cropping with the lowest carbon level (0.20 g/kg). For total POM-C the concentrations were 0.90 g/kg for long crop pasture rotation, 0.62 g/kg for short crop pasture rotation and 0.58 g/kg for continuous cropping. So in the sensitive SOM fractions in the 5-10 cm layer long crop pasture rotation had the highest SOC level, followed by short crop pasture rotation and continuous cropping with the lowest SOC level.

There were differences between the total SOC concentrations of the three rotation systems, but they were not significant. The experiment needs more time for the effects to accumulate. That total SOC concentrations will keep accumulating in the coming years is indicated by the significant changes in the sensitive coarse and total POM-C fractions. Based on comparable scientific literature, it is estimated that sequestration at the experimental site will take place for about 22 more years and that the current changes will double.

The pasture phases of short and long crop pasture rotation had several significantly higher SOC concentrations in the soil fractions than the cropping phases. This supports that under no-tillage pastures have a positive effect on the carbon level. Furthermore long crop pasture rotation had higher coarse and total POM-C concentrations than short crop pasture rotation and the fourth year of pasture also had significantly higher concentrations than the second year of pasture of long crop pasture rotation. So a long (four year) pasture phase included in a crop rotation system is thus better regarding coarse and total POM-C concentrations than a short (two year) pasture phase.

It was not possible to confirm the occurrence of enhanced humification under short and long crop pasture rotation because the nitrogen measurement failed.

The recommendations for future research are measuring nitrogen to confirm enhanced humification under the crop pasture rotation systems, measuring the carbon saturation deficit to know more about the future development of the SOC level, using the fractionation method again, because it yielded significant results and using at least three replications to get significant result from the 0-5 cm layer and fine POM-C.

2. Introduction

An important sustainable challenge of this time is global warming caused by the greenhouse effect. Greenhouse gasses such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxides (N_2O) trap heat on earth by reflecting long wave radiation emitted by earth. Anthropogenic emissions have led to an increase of these gasses in the earth's atmosphere. Carbon dioxide levels have risen from around 280 ppm in pre-industrial times to 379 ppm in 2005, methane levels from 715 to 1732 ppb and nitrous oxide levels from 270 to 319 ppb (IPCC, 2007). According to the IPCC climate change will likely result in:

- fewer and warmer cold days and nights and warmer more frequent hot days and nights on land
- an increase of the frequency of heat waves and spells
- an increase of the frequency of heavy precipitation
- an increase of the areas affected by droughts
- an increase of intense tropical cyclone activity
- an increase of the incidence of extreme high sea level

This will especially affect ecosystems such as tundra, coral reefs and mangroves, agriculture at low latitudes, water resources in dry regions at mid-latitudes and dry tropics, low lying coastal regions and human health in populations with low adaptive capacity. It is thus very important to act and counter climate change.

One of the tools that could help to fight climate change is capturing and sequestering atmospheric carbon as organic carbon in agricultural soils. This could be done by land use change, because the type of agricultural practice influences the level of soil organic carbon (SOC). Changing from one practice to another can increase or decrease the SOC level. Research into recommended management practices (RMPs) for agriculture is thus a crucial scientific field (Christopher and Lal, 2007; Johnson et al., 2007; Lal, 2008). Even more when considering that high soil organic carbon levels are also related to high soil fertility: aggregate stability, the water retention capacity, the buffering of soil pH and the cation exchange capacity (CEC) (Essington, 2004). And by improving soil fertility carbon sequestration in soils is also beneficial for agriculture.

The improvement of agricultural soils is also related to another sustainability challenge. In 2050 the global food system will have to feed 9 billion people, many of which will be wealthier and wanting a high value diet, requiring more recourses for food production (Foresight, 2011). Moreover, our food system will face stresses from climate change, depleting fossil fuels (increasing their price), land degradation and agriculture related pollution (Post Carbon Institute, 2009).

Research into carbon sequestration in agricultural soils is thus both related to sustainable agriculture and climate change mitigation and would fit perfectly within the Global Change and Ecosystems track of the Sustainable Development program (Website Sustainable Development program, 2011).

For easy reading a short outlay of this chapter will be given. The chapter will start with an introduction into soils as a carbon pool. Then some important definitions will be given and it will be explained how certain carbon pools play a part in storing atmospheric carbon in the soil. After this there will be a short paragraph about the soil residence time of soil organic carbon. Then the chapter will continue on soil carbon saturation and the soil mechanisms that protect soil organic carbon from decomposition. Ensuingly the chapter will elaborate on a promising combination of two improved agricultural management practices: no tillage and crop pasture rotation. Thereafter this chapter will review some recent research in Uruguay related to no tillage crop pasture rotation and finally it will come to the problem definition, research aim, questions and hypothesis.

2.1. Scientific background

Soils as carbon pool

The world's soils are a major carbon pool. Batjes (1996) and Eswaran et al. (1993) estimate that just the upper meter of soils worldwide contain a total of around 1550 Pg (or 1.55 * 10^{15} Kg) of organic carbon. That is around three times the amount of carbon stored in aboveground biomass and twice the amount of carbon in the atmosphere (Eswaran et al., 1993; Lal, 2008). Release of soil carbon into the atmosphere can have serious climatic consequences. A good example is the release of organic carbon from permafrost soils. Due to global warming, previously frozen carbon will thaw and be released for microbial decomposition (Schuur et al., 2008). The released carbon enters the atmosphere and causes a positive feedback between temperature rise and an enhanced soil carbon release. This is further enhanced by the fact that the temperature increase at high northern latitudes is almost twice as high as the global average (IPCC, 2007). Permafrost could be a large source of carbon in a warmer world (Schuur et al., 2009). However, soils can also act as sinks and can thus help to mitigate climate change. For instance, Sauerbeck (2001) estimates that around 20 to 30 Pg of carbon could be stored in degraded mineral soils.

What is soil organic carbon and how does it enter the soil?

What exactly is soil organic carbon? It's the carbon in the soil that is derived from organic sources. This is opposed to inorganic carbons which are derived from geologic or soil parent material sources. Most soil inorganic carbons are carbonates. Soil organic and inorganic carbon together are known as total carbon. For further reading it is good to note that from here on, all carbon mentioned is organic, unless stated otherwise. According to Essington (2004), soil organic carbon occurs in three soil pools: as the carbon incorporated in the living ***biomass***, ***organic residues*** and ***soil organic matter*** (see figure 2.1.1). So all of these pools contain soil organic carbon. Note that carbon is not the only atom in these pools. Carbon is one of the constituent of these 'tissues'. Soil organic matter for instance also contains hydrogen and oxygen atoms in phenol or carboxyl groups or nutrients such as nitrogen and phosphorous.

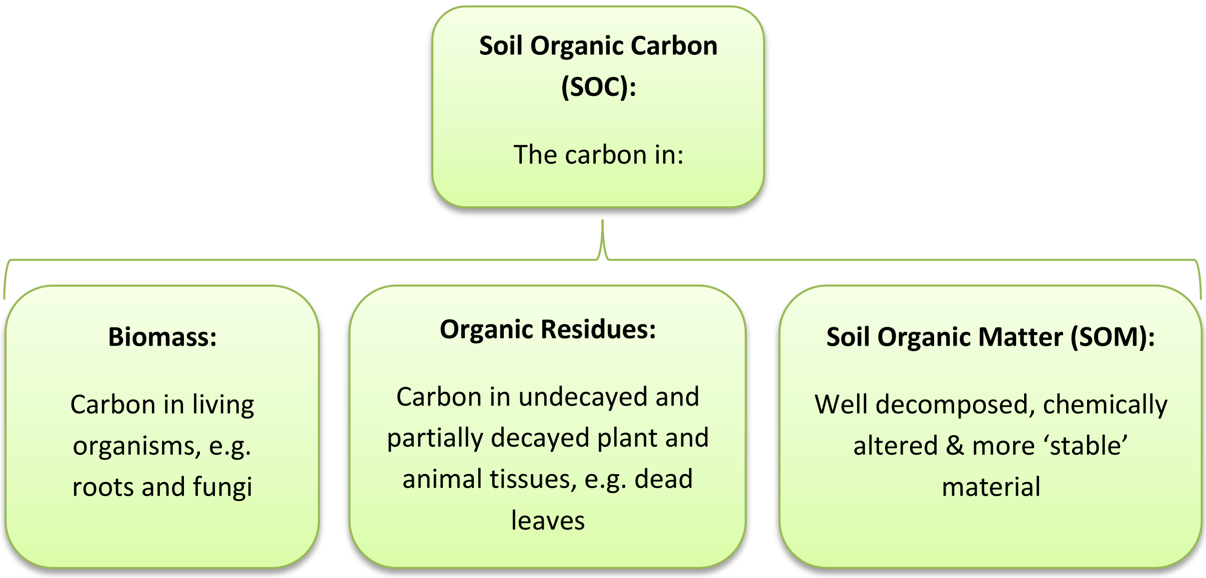

Figure 2.1.1: Pools of organic carbon in soils according to Essington (2004).

As can be seen in figure 2.1.1. biomass contains all living organisms and their compounds in the soil. Some examples are living roots, microbes and nematodes. Organic residues are the non-decayed and partially decayed plant and animal remains. Examples are leaf litter, dead roots and animal carcasses. Soil organic matter (SOM) is well decomposed, chemically altered and more stable over time compared to the original organic residues. SOM has very diverse and complex characteristics and even very well decomposed SOM can still have small microbial or plant remains. So the boundary between organic residues and SOM can be vague (Ashman and Puri, 2002).

There are also other possible definitions of SOM. According to Baldock and Skjemstad (1999) SOM consists of all the organic materials found in soils regardless of their origin or state of decomposition. This means SOM also contains the living biomass and organic residue pools from Essington (2004). This is a more practical definition. Scientist often use the term soil organic matter, but actually measure soil organic carbon. This is because in the reality of soil science a) the theoretical boundaries are vague, b) while large roots and the surface litter layer can be kept out of a sample, it is impractical to separate very tiny roots and micro-organisms from the soil sample and c) the inseparable parts hardly contribute to the total level of soil organic carbon. Therefore, when soil scientist want to measure SOM they are pragmatic and remove the litter layer (the layer of plant litter on top of the soil) and large roots from the sample and consider the rest of the soil organic carbon as being part of soil organic matter. The Essington (2004) definition is more interesting analytical, because it helps to understand the theory behind how these pools play a role in soil processes and thus carbon sequestration. Regardless of the definition, SOM is by far the largest and most stable of the SOC pools. The goal of this research is not to know exactly where the boundaries between the pools of SOC lay. When talking about the theory and the processes behind carbon sequestration, the term SOM will be used as defined by Essington (2004), but in the practice of sampling the Baldock and Skjemstad (1999) definition is more accurate.

Essington's pools: biomass, organic residues and SOM, are all interconnected and play a part in the pathway from atmospheric carbon to stored soil organic carbon. Plants are at the basis of carbon input into the soil. During their lives, plants take up atmospheric carbon through photosynthesis. Among other things, plants use this to form biomass above and below ground. Organic residues are formed when these plants or parts of them die. The plant biomass is deposited in a litter layer on top of the soil or in the case of roots is already in the soil. Additionally large herbivores can eat the plants and deposit the organic residue in the form manure. The residues are broken down by soil organisms, which are also part of the living biomass and upon death become organic residues. The residues that are the most easy to decompose are broken down first (Ashman and Puri, 2002). Eventually soil organic matter (SOM) is formed from microbial metabolites and resistant plant residues. SOM can be divided into nonhumic and humic substances.

Nonhumic substances are organic substances that belong to recognizable classes in biochemistry. Examples are simple carbohydrates, amino sugars and lignin. Generally the nonhumic substances are easily utilized by microorganisms and decomposed into its base components carbon dioxide (CO_2) and water (H_2O). In this process nutrients are released which can once again be used by plants. On this 'degradative journey' some organic substances may become recalcitrant (more resistant to composition). These are known as humic substances and do not belong to any discrete biochemical category (Ashman and Puri, 2002; Essington, 2004).

The process in which nonhumic substances are transformed into humic substances is called humification. The micro-organisms that take part in this humification use the carbon enclosed in the SOM as energy source and the nitrogen, which is also a component of SOM, for e.g. enzymes and amino acids. Nitrogen is often in short supply and thus limiting for humification. The amount of nitrogen contained in litter influences its quality. The better the litter quality the faster the speed at which SOM can be humified. This last property is often expressed as the carbon to nitrogen (C:N) ratio (Ashman and Puri, 2002).

So in short ***soil organic carbon*** is a constituent of the living biomass, organic residues and soil organic matter. A soil receives ***carbon input*** through the growth and death of plants and animals, which form ***organic residues***. These residues are broken down by soil organisms and stored in the soil as ***soil organic matter*** in a process called humification. The C:N ratio influences the humification speed.

Residence time

All soil organic carbon which is stored in the soil will be decomposed eventually. The time the soil organic carbon storage lasts can differ greatly. Table 2.1.1 shows the mean soil residence time of plant biomass and soil organic carbon in three soil fractions (Sauerbeck, 2001). Plant biomass and litter (organic residues) are transitory and do not store organic carbon for a long time. The 'active' SOM fraction which is more associated with soil fertility is decomposed within ten years on average. However the stable SOM fraction can stay in the soil for an average of a thousand years. SOM is thus the most interesting fraction for soil organic carbon sequestration.

Table 2.1.1: Mean residence time of soil organic carbon in several pools (from Sauerbeck, 2001, p. 255).

Organic carbon in	Examples	Mean residence time (years)
Plant biomass	Non-woody	$<10^0$
	Woody	10^1-10^2
Litter	Surface litter and crop residues	$5*10^0$
Active SOM	Partially decomposed litter and SOM protected in macro aggregates	10^0-10^1
Stable SOM	Stabilized by clay, chemically recalcitrant SOM, charcoal	10^2-10^3

Soil organic carbon storage and carbon saturation

The storage of soil organic carbon is influenced by climate, biota (vegetation and soil organisms), parent material, topography (Essington, 2004) and land use/management (West and Six, 2007). For a given agricultural soil at a specific location the average soil organic carbon level is a balance between carbon input through organic residues such as plant litter and manure, and output through decomposition and erosion (West and Six, 2007). With a change in agricultural management the SOC level can be increased by either enhancing the residue input or decreasing the decomposition. Examples of such management changes are leaving more residue on the fields after harvest and reducing tillage intensity. However, if the new management is not sustained the sequestered carbon will be released again (Sauerbeck, 2001).

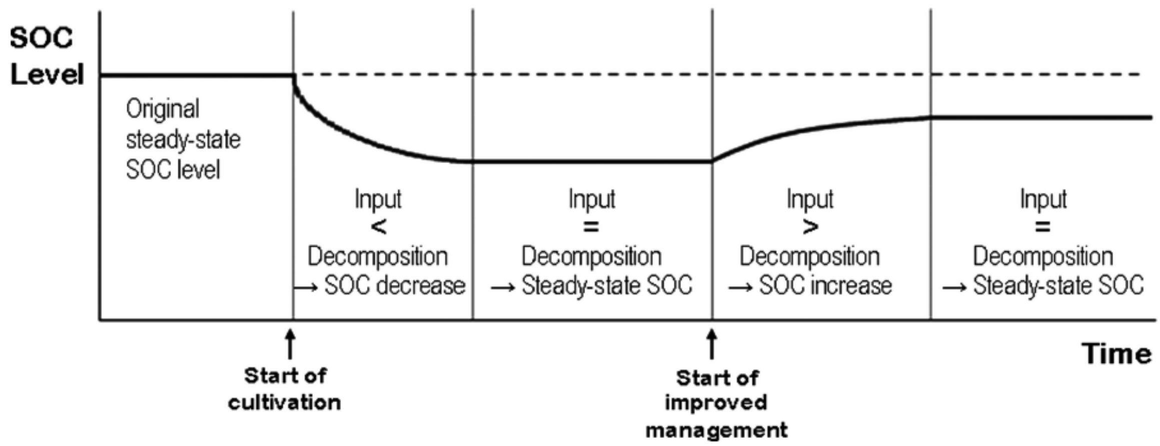

Figure 2.1.2: Soil organic carbon development caused by changes in carbon input or a decomposition (from Govaerts et al., 2009, p. 101).

Figure 2.1.2. which shows what happens to a steady state SOC level when a previously untilled soil is cultivated. The balance between input and decomposition is offset: the input becomes smaller

than decomposition. This results in a 20 to 40 percent decrease of soil organic carbon, mostly in the first few years after cultivation (Davidson and Ackerman, 1993). After this the rate of decrease lessens and eventually a new steady state SOC level is reached when input meets output (Sauerbeck, 2001). This is supported by West and Six (2007, p. 36) who state that 'sequestration rates are higher in the first half of the sequestration duration period and then decline as soil reaches a new steady state'. Next to this, differences in decomposition result in a change in the SOC level faster than changes in input (West and Six, 2007). Note that in practice this steady state SOC level will still fluctuate (around an average level) with other factors such as seasonal changes, land productivity and changes in mean annual temperature. So the steady state does necessarily have to be an equilibrium (West and Six, 2007). The time it takes for the SOC level to reach a new steady state can vary, but is no more than 50 to 100 years (Sauerbeck, 2001).

It used to be assumed that the relation between increased carbon input (into the soil) and the SOC level was linear. Several experiments support this. However, at some soils which already had a high SOC level, there was hardly an increase in SOC after a large increase in carbon input (Campbell et al. 1991; Solberg et al, 1997). Therefore some authors have suggested that soils (with the exception of wetland soils[1]) can become carbon saturated (Sauerbeck, 2001; Six et al., 2002; Stewart et al., 2008; West and Six, 2007). This means that a soil cannot store extra carbon regardless of the changes in inputs or management (West and Six, 2007). This is visualized in figure 1.1.3. These authors argue that the duration of individual experiments is often too short or the C input too low to show a saturation trend. To overcome this, Six et al. (2002) and West and Six (2007) compiled data from several experiments and found indications of an asymptotic (saturation) relation between increased C inputs and the SOC level.

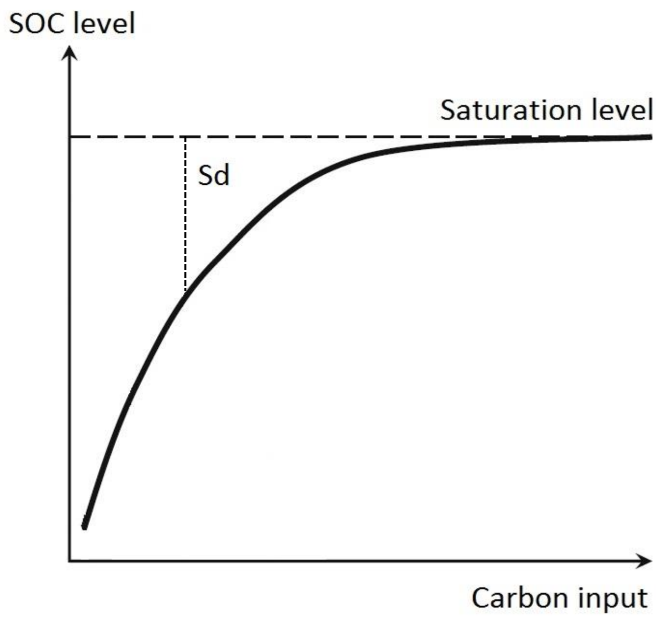

Figure 2.1.3: Soil organic carbon level as a function of carbon input. Sd is the saturation deficit: the difference between the actual SOC level and the saturation level (adapted from West and Six (2007)). This figure differs from 1.1.2., because carbon input is on the x-axis instead of time.

Figure 2.1.3. also shows that the further a soil is from its saturation level (saturation deficit) the higher the C stabilization efficiency (or the sequestered C per unit of added C to the soil: ΔSOC/ΔC input) will be. On the left side of the figure, the saturation deficit (Sd) and stabilization efficiency are higher than on the right side where the SOC level approaches the saturation level. Stewart et al. (2008) found evidence for this relation. They compared the carbon stabilization efficiency in the A and C horizons[2] at seven research sites. SOC levels are much lower in the C horizon than the A horizon. If the soil could be carbon saturated, than the saturation deficit would be larger in the C horizon than in the A horizon, as can be seen in figure 2.1.3. If this is the case, a similar carbon input should result in more carbon being stabilized in the C horizon than in the A horizon. And this is exactly what Stewart et al. (2008) found. A higher saturation deficit also causes a higher

[1] In wetland soils peat formation takes place which results in a soil consisting fully of organic residues protected from decomposition by water.

[2] Soil horizons are soil layers. The letters A and C refer to their classification. So 'C' in this case is not an abbreviation of carbon. The A horizon is the top soil, which is often rich in organic carbon and C horizon a (lower) layer which is largely unaffected by soil formation.

sequestration rate (ΔSOC/time) and a longer sequestration duration (the time sequestration takes place) (West and Six, 2007).

But what causes this saturation? Six et al. (2002) suggest that the maximum amount of carbon that can be sequestered in the soil is determined by the soils maximum protective capacity for SOM (in which the organic carbon is incorporated). They distinguish between four pools based on their specific protection/stabilization mechanism.

1. The chemical stabilization pool: SOM which is protected because it's bound to soil minerals such as clay and silt particles. Hassink (1997) found a direct relation between the silt and clay content and the amount of SOM bounded to it. This indicates that stabilization is limited to the silt and clay content.
2. The physical protection pool: SOM protected in macro and micro aggregates. This type of protection is sensitive to disturbance by cultivation and tillage. The maximum protection capacity is determined by the maximum aggregation, which in turn is determined by clay content and clay type.
3. The biochemical stabilization pool: SOM protected through its complex chemical composition. Six et al. (2002) do not present any actual mechanism which would explain that there is a maximum amount of carbon that can be stored by biochemical stabilization. They do however write that the size of this pool is probably limited because its age is much younger than the age of the soil (pedogenic age).
4. The unprotected SOM pool. This pool is not protected and thus very labile. Six et al. do not present any mechanism behind saturation for this pool either. They did however find indications that this pool can also be saturated. Solberg et al. (1997) and Six et al (using data from Campbell et al. (1991) and Janzen et al. (1992)) found no increase in (the light fraction part of) the unprotected pool with enhanced C input.

Although data suggests that soils can be carbon saturated, not all mechanisms behind it are well understood. Stewart et al. (2009) found evidence for the saturation of the chemical and biochemical pools. The physical pool showed contradicting results and the unprotected pool showed no saturation behavior. So some pools could become saturated even if the whole soil did not. Furthermore, they found that once the chemical pool was filled added C seemed to accumulate in physically protected and unprotected pool, which are less stable and more sensitive to cultivation and land use change. This behavior might be explained by the fact that the different soil processes that influence the protection of SOM have different stabilization speeds. Hence not all SOM fractions will achieve steady state at the same time (Six et al., 2002).

Promising agricultural management

One promising type of sustainable agricultural management related to carbon sequestration is the use of crop-pasture rotation (CPR) combined with no-tillage.

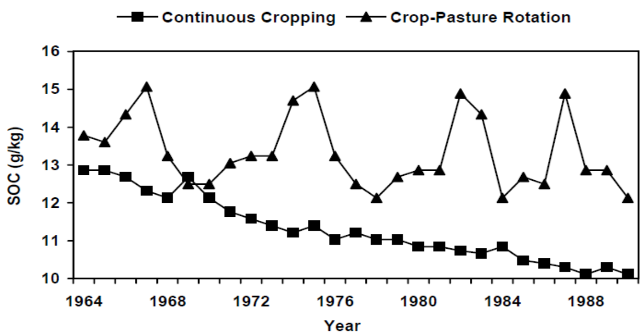

Figure 2.1.4: SOC concentration at 0-20 cm depth with CC and CPR with tillage (from Garcia-Préchac et al. (2004, p. 3) who adapted it from Díaz-Roselló (1992))

Pastures are associated with high levels of soil organic matter and carbon. According to Tisdall and Oades (1982) pastures feature an increase of soil organic matter (and thus SOC) because of the annual accumulation of phytomass, the increase of water stable aggregates and they refer to Führ and Sauerback (1968) who state that growing plants retard organic matter decomposition. The amount of root biomass is the main cause of the change in the quantity and distribution of carbon input (in the form of organic residues) in pasture systems (Haynes et al., 1991;

Kazyakov and Domanski, 2000; Bolinder et al., 2007). Because of fine roots and rhizodeposits (input of C into the soil through the root system) the carbon input from perennial pasture are greater than would be expected from the standing biomass (Trujillo et al., 2006). The incorporation of pastures into a cropping system can thus help to restore or improve SOC during the cropping period. This is shown in figure 2.1.4 on page eight, where the triangle line shows an increase in SOC in the pasture phase and a decrease in the cropping phase. Although the pasture phase restored the soil organic carbon levels after the cropping phase, 80 kg ha^{-1} was still lost annually.

Tillage can destroy soil aggregate structures. As was mentioned earlier, soil organic matter can be physically protected by soil aggregates. These aggregates reduce the oxygen availability for microbes, compartmentalize substrate and microbial biomass and compartmentalize microbial biomass and microbial grazers (Six et al., 2002). The destruction of soil aggregates results in carbon loss from the soil through oxidation and erosion of soil organic matter (Franzluebbers, 2005). Díaz-Roselló (1992) found a 25 percent loss of soil organic carbon during 28 years of continuous cropping with conventional tillage.

Together, no-tillage and the incorporation of pastures in a land use scheme could help inhibit and recover the loss of soil organic carbon and hence offer a promising subject for research. While crop pasture rotation (CPR) systems used to be more common, they have largely disappeared due to the specialization and intensification of agricultural systems (Salvo et al., 2010). Uruguay and some parts of Argentina form an exception. There CPRs are even more common than continuous cropping (Garcia-Préchac et al., 2004). Additionally much research into the combined effect of 'no tillage' and 'crop pasture rotations' is being conducted in Uruguay. Therefore this chapter will now zoom in on this research.

'No tillage' and 'crop pasture rotation' research in Uruguay

Garcia-Préchac et al. (2004) synthesized research studies into 'no-tillage' and 'crop pasture rotation' from 1960 on. They described that crop pasture rotations were adopted in the 1960s in Uruguay. From 1990 on no-tillage was increasingly applied in Uruguay. When comparing the influence of no-tillage (NT) versus conventional tillage (CT) and continuous cropping (CC) versus crop pasture rotation (CPR) Garcia-Préchac et al. (2004) found that the NT-CPR combination had the lowest erosion rate, which was the same as natural pasture, and that the soil organic carbon content was stable or increased compared with the original values after 6 years of treatment. Additionally, they found NT-CPR to be more sustainable because it was a more economically and climatically buffered system compared to NT-CC due to higher diversity and 50% less fuel and agrochemicals use.

Ernst and Siri-Prieto (2009) investigated the influence of CPR-NT on, inter alia, soil organic carbon (g kg^{-1}), soil organic carbon stock (Mg ha^{-1}) and total nitrogen (g kg^{-1}). They compared four practices: continuous cropping with conventional tillage and without tillage and crop pasture rotation (with a 3.5 year cropping phase and a 2.5 year pasture phase) with conventional tillage and without tillage. All plots at the experimental site had the same agricultural management until 1993. Sampling took place after twelve years of different agricultural practices. Ernst and Siri-Prieto (2009) found that the no-tillage treatments had a seven percent higher SOC level than tillage treatments. However, the inclusions of pastures did not result in enhanced SOC levels. All treatment plots lost nitrogen since the start of the experiment, but crop pasture rotations still had a higher N level than continuous cropping. So Ernst and Siri-Prieto did not find that the combination of no-tillage and crop pasture rotations sequestered carbon. But because the effects of land use change slowly accumulate in time they expected that the benefits would likely require more time to be detectable.

Ten years after agricultural management change Salvo et al. (2010) compared the effects of continuous cropping (CC) and crop pasture rotation (CPR) (with a three year cropping phase and a three year pasture phase) both with conventional tillage (CT) and no-tillage (NT)[3]. They measured SOC in three size fraction of SOM and the unfractionated soil. The size fractions were coarse particulate organic matter (POM[4]), fine POM and mineral associated organic matter (MAOM[5]). They fractionated the soil samples because POM (especially coarse POM) is more sensitive to agricultural management change and can serve as an early indication of a changing SOC level (see chapter three: methodology). Salvo et al. (2010) found that in the 0-3 cm layer, no-tillage had a significantly higher total SOC concentration (g kg^{-1}) than conventional tillage and that under conventional tillage, crop pasture rotation had a higher C stock (Mg ha^{-1}) than continuous cropping. However they did not find significant differences between the total SOC level of no-tillage crop pasture rotation and no-tillage continuous cropping. The differences in total SOC in the upper 18 cm, could mainly be attributed to changes in POM carbon (POM-C). Like Ernst & Siri-Prieto (2009), Salvo et al. (2010) concluded that more time was probably needed to show the combined effect of crop pasture rotations and no tillage. Furthermore, Salvo et al. (2010) found that in the upper 3 cm of the soil, no-tillage crop pasture rotation had a 12.5% higher MAOM-C level, while having (insignificantly) a lower POM-C level and the same total SOC level. They speculated that this could be explained by enhanced humification (transformation of POM-C into MAOM-C) under pastures, which are associated with higher nitrogen levels. Not only might no-tillage crop pasture rotation restore or increase SOM quantity it may also improve SOM 'quality'. This is important for carbon sequestration because MAOM is more stable over time. The carbon it contains is thus sequestered for a longer period of time. That crop pasture rotation had a higher nitrogen level than continuous cropping was corroborated by Ernst and Siri-Prieto (2009). However they did not measure nitrogen and carbon in the SOM fractions. So it is not possible to say if the same transformation of POM to MAOM took place.

2.2. Research overview

Problem definition

Improved knowledge about the combined effect of 'no-tillage' 'crop pasture rotation' on the level of soil organic carbon can help sequester atmospheric carbon in agricultural soils and improve soil fertility. This would help with fighting climate change and improving food production. Uruguay still features crop pasture rotations on a large scale and recent research on no-tillage crop pasture rotation has been done there. Most recently Ernst & Siri-Prieto (2009) and Salvo et al. (2010) tried to quantify the combined effect of several forms of no-tillage crop-pasture rotation on the total SOC level but found no significant differences. However, the change in agricultural management practice was quite recent (only 10 and 12 years ago) so it could be that the changes in SOC did not have enough time to accumulate. Moreover, it could be that important information was missed because, Salvo et al. (2010) did not measure nitrogen and Ernst and Siri-Prieto (2009) did not measure SOC in the SOM fractions. These fractions can provide early indications of future change and the nitrogen measurements (expressed as the C:N ratio) can shed light on possibly enhanced humification processes, which lead to changes within the fractions. That is why it was important to conduct research which included the fractionation method and nitrogen sampling at a site with a longer experimental running time. Additionally it could be that a longer pasture phase will result in higher SOC levels, because the benefits of the pasture phase will have more time to accumulate. Ernst and Siri-Prieto (2009) investigated a crop pasture rotation with a two and a half year pasture phase and Salvo et al. (2010) with a three year pasture phase. Therefore a comparison between a crop pasture rotation with a short (two year) and long (four year) pasture phase should be made.

[3] They also compared C_3 and C_4 summer crops, which is not relevant for this research.
[4] POM is SOM smaller than 2 mm and larger than 53 µm (Cambardella and Elliot, 1992).
[5] MAOM is the size fraction of SOM which is smaller than 53 µm.

Aim

The aim of this research was to quantify the difference in soil organic carbon in the unfractionated (or total) soil and its distribution in the fractions coarse particulate organic matter (POM), fine POM and mineral associated matter (MAOM) between continuous cropping (CC) and crop pasture rotations (CPR) with short and long pasture durations and no tillage (NT), 18 years after agricultural management change. Additionally, this research aimed to explain changes within the fractions by relating them to differences in the C:N ratios of the soil organic matter fractions.

Main research question

How do 'no-tillage continuous cropping' and 'no-tillage crop pasture rotation' with a pasture duration of two and four years affect the level of soil organic carbon and the C:N ratio in the unfractionated soil and in the SOM fractions coarse POM, fine POM and MAOM fractions in an Uruguayan Agriudoll?

Sub questions

The main research question can be divided into three sub questions. The first question is about the influence of the combination of no-tillage and crop pasture rotation on the total SOC level, which Ernst & Siri-Prieto (2009) and Salvo et al. (2010) did not find because the effects probably did not have enough time to accumulate. The second question is about the influence on the SOC level in the soil fractions coarse POM, fine POM and MAOM. Changes in these fractions indicate future change (see chapter 3: Methodology). The third question is about the influence of the added pasture phase on the C:N ratio of the SOM fractions, which could cause enhanced humification. In the top three centimeter of the soil Salvo et al. (2010) found that no-tillage crop pasture rotation had a higher level of MAOM-C ('-C' refers to the SOC in the SOM fraction), while having (insignificantly) a lower POM-C and the same total SOC level. As stated at the top paragraph of page ten, they speculated that this could be explained by enhanced humification (transformation of the SOC in POM to MAOM) under pastures which are associated with higher N levels.

SQ1: How much do no-tillage continuous cropping and no-tillage crop-pasture rotation with long (four year) and short (two year) pastures influence the level of soil organic carbon in the unfractionated soil?

SQ2: How much do no-tillage continuous cropping and no-tillage crop-pasture rotation with long (four year) and short (two year) pastures influence the level of soil organic carbon in the soil fractions coarse POM, fine POM and MAOM?

SQ3: How does crop pasture rotation influence the C:N ratios of the soil organic matter fractions compared to continuous cropping?

Hypotheses

Crop pasture rotation will have a higher SOC level than continuous cropping mainly because pasture feature a higher organic residue input through rhizodeposition (Haynes et al., 1991; Kazyakov and Domanski, 2000; Bolinder et al., 2007). The effect of adding a pasture phase to a cropping phase will be largest for the long pasture phase because the pasture benefits will have more time to accumulate.
Therefore the hypothesis for the first sub question (How much do no-tillage continuous cropping and no-tillage crop-pasture rotation with long (four year) and short (two year) pastures influence the level of soil organic carbon in the unfractionated soil?) is:

H1: No tillage crop pasture rotation has a higher level of soil organic carbon compared with continuous cropping and long crop pasture rotation will have a higher concentration than short crop pasture rotation.

The SOM fractions coarse POM, fine POM and MAOM are indicators of future change in the total SOC level, even when the change is small. Coarse POM is most sensitive to change in agricultural management followed by fine POM while MAOM hardly changes (Cambardella and Elliot, 1992; Morón and Sawchik, 2003). Therefore the general trends for the fractions will be the same as for total SOC, but it will be most strong for the most sensitive fraction coarse POM-C, followed by fine POM and much weaker for the stable fraction MAOM-C. Therefore the hypothesis for the second sub question (How much do no-tillage continuous cropping and no-tillage crop-pasture rotation with long (four year) and short (two year) pastures influence the level of soil organic carbon in the soil fractions coarse POM, fine POM and MAOM?) is:

H2: No tillage crop pasture rotation has a higher level of soil organic carbon in the three soil fractions compared with continuous cropping and long crop pasture rotation will have a higher concentration than short crop pasture rotation. The difference between the SOC levels in the fractions are the largest for POM-C, which is most sensitive to land use change, followed fine POM-C while MAOM-C hardly changes.

Ernst and Siri-Prieto (2009) found that crop pasture rotations had a higher N level than continuous cropping. Salvo et al. (2010) found indications for enhanced humification from the POM fraction to the MAOM fraction and speculated that this was caused by the higher nitrogen associated with pastures. Therefore the hypothesis for the second sub question (How does crop pasture rotation influence the C:N ratios of the soil organic matter fractions compared to continuous cropping?) is:

H3: No tillage crop pasture rotation will have higher nitrogen levels (and thus lower C:N ratios) than no tillage continuous cropping and this will lead to enhanced humification (a higher transformation of POM-C into MAOM-C).

3. Methodology

The sampling took place near Paysandú at one of several sites at the experimental station EEMAC (Estación Experimental 'Dr. Mario A. Cassinoni') of the Agronomy Faculty of the Universidad de la Republica. The experimental site was established in 1994. It has a sub-humid climate with an average temperature of 17 ^{o}C annually, 24 ^{o}C in summer and 12 ^{o}C in winter. The soil consists of a fine, mixed, active, termic Typic Argiudoll on a less than 1% slope. The A horizon of 18 cm has a pH of 5,7 and a clay, silt, sand content of respectively 289, 437 and 273 g kg^{-1} (Salvo et al., 2010).

The experimental site features four main land use types with 18 cyclic treatments and a control pasture. So in total there are five land uses. Each treatment is situated on 50 by 10 meter plots and all treatments have completed at least two cycles. The winter crops are wheat and barley and the summer crops are soybeans and sorghum. Soybeans are planted twice: once as a second crop after wheat and once as the first (and prime) crop after a fall period. The four year pastures consist of tall fescue (*Festuca arundinacea*), birdsfoot trefoil (*Lotus corniculatus*) and white clover (*Trifolium repens*) and the two year pastures of red clover (Trifolium pratense). Because sampling took place in summer, the summer crops soybeans (as first and second crop) and sorghum were be sampled; and because SOM (and thus SOC) changes slowly only the second and fourth years of pasture were sampled.

Below follows an overview five land use types and the plots that were sampled in 2012. The cropping and pasture phases are described in table 3.1.

1. Three year cropping phase followed by a 4-year pasture phase without tillage (long rotation no tillage: LR-NT). The following plots have been sampled: soybean (second crop of the year), sorghum, soybean (first crop of the year) and second and fourth year of pasture.
2. Three year cropping phase followed by a 2-year pasture phase without tillage (short rotation no tillage: SR-NT). The following plots have been sampled: soybean (second crop of the year), sorghum, soybean (first crop of the year) and second year of pasture.
3. Continuous cropping phase without tillage (continuous cropping no tillage: CC-NT). The following plots have been sampled: soybean (second crop of the year), sorghum and soybean (first crop of the year).
4. Three year cropping phase followed by a 4-year pasture phase with conventional tillage (long rotation conventional tillage: LR-CT). The crops were seeded with conventional tillage. This represents the traditional soil management system, which is included as a control check. The following plots have been sampled: soybean (second crop of the year) and second year of pasture.
5. No treatment: pasture since 1994 (Control), this pasture has been sampled.

Table 3.1: Rotation cycle with cropping and pasture phases, with specific crops and pasture plants. When second year of pasture is mentioned this refers to the fifth year in the whole rotation cycle.

	Cropping phase					
	Year 1		*Year 2*		*Year 3*	
Season:	Winter	Summer	Winter	Summer	Winter	Summer
Crop:	Wheat	Soybean (2nd crop)	Barley	Sorghum	Fall	Soybean (1st crop)

	Pasture phase			
	Year 4	*Year 5*	*Year 6*	*Year 7*
Short rotation	Wheat & red clover	Red clover	-	-
Long rotation	Wheat, White clover, Birdfoot trefoil & Fescue	White clover, Birdfoot trefoil & Fescue	White clover, Birdfoot trefoil & Fescue	White clover, Birdfoot trefoil & Fescue

By comparing the treatments with each other, their influence on SOC and N could be identified, because in 1994 they all shared the same land use history and conditions where the same. All differences are thus caused by the land use after 1994. The control pasture has been pasture since 1994. Before 1994 the entire site was used for cropping.

At the same experimental station but on a different site, Salvo et al. (2010) found that changes in soil organic carbon levels were restricted to the arable layer (0-20 cm) and that the main changes occurred in the layer at 0-3 cm depth. At the experimental site used for this research the layers at 0-5, 5-10 and 10-20 cm depth were sampled in 2010 and the layers at 0-10 and 10-20 cm depth were sampled in 2011. By also sampling the layers at 0-5, 5-10 and 10-20 cm depth in 2012 it was possible to use the samples from the previous years as replications in time. Replications in space could not be taken. The samples from 2010 and 2011 could be used as replicates, because soil organic carbon changes very slowly. The sample of the same treatment 'moment' was used as replicate, e.g. the 'short rotation with no tillage second year pasture' plot samples from 2010 and 2011 were replications of the 'short rotation with no tillage second year pasture' plot sample of 2012. This means that the replicate will not come from the exactly the same treatment field (which would be pseudo replication). That the samples were not taken from the same field enhanced the validity as a substitution for a replication in space. Sampling at multiple depths also increased the chance that significant changes were detected, because if only samples from the 0-20 cm layer would have been taken, the changes occurring in the top 5 cm would have been diluted.

Sampling took place at the 14th and 15th of February. To reduce spatial variability, each layer sample was composed of 20 soil cores taken randomly throughout the treatment plot (USDA, 2004) with a soil probe (see figure 3.1). These soil cores were mixed into one sample. This mixed sample represents the average situation for a specific layer at a specific treatment.

Figure 3.1: Sample extracted with an auger. The core was cut at 5, 10 and 20 cm and separated into 0-5, 5-10 and 10-20 cm depth buckets with pieces of the other 19 cores of the plot.

The samples were stored refrigerated and afterwards dried at 50 °C in an air forced oven for 48 hours to stop microbial decomposition (USDA, 2004). In the laboratory they were crushed to 2 mm, because this was also done for the replicate samples of 2010 and 2011. The crushing also mixed the sample, but to assure homogeneous samples they were also mixed with a spoon afterwards.

All samples taken in 2010, 2011 and 2012 were checked for the presence of inorganic carbonates with 4.0 M HCl. Carbonates could offset the dry combustion measurements, none were found.

The samples taken from the layers at 0-5, 5-10 and 0-10 cm depth were physically fractionated in accordance with Cambardella and Elliott (1992). The three separated size fractions were: coarse particulate organic matter (POM) which is smaller than 2 mm and larger than 200 μm, fine POM which has a size between 200 μm and 50 μm and mineral associated organic matter (MAOM) which is smaller than 50 μm. The fractionation took place with meshes.

This fractionation was done to distinguish between more and less stable (or labile) pools. According to Haynes (2000) labile fractions are suitable to be used as indicators of changes in soil carbon, because over time parts of these labile fractions be transformed through humification into more stable fractions and will be sequestered in the soil for a longer period. So changes in labile pools can be considered as indicators of future changes in stable pools. A useful labile fraction would be

POM[6], which represents the carbon in the dynamic part of SOM and is much more associated with nutrient availability, while MAOM would be a good stable fraction because it is the more stable over time, difficult to degrade and represents the passive part (Galantini et al., 2004; Six et al., 2002). In 2003 Morón and Sawchik further divided POM into a coarse and fine fraction because they found that POM larger than 212 μm was more sensitive to changes in land use practices than POM that was smaller. It would thus be expected that the level of coarse POM would be affected by land use change earliest followed by the level of fine POM and that the level of MOAM would react latest.

The layer at 10-20 cm depth was not fractionated because the input from POM mainly occurs in the upper layer at 0-10 cm depth. So if changes occurred in this layer, they should already become apparent from the total SOC and N sample.

The carbon in the 2010 and 2011 samples was already measured with wet combustion with the addition of external heat. For this research dry combustion at high temperature was used to measure carbon in the samples. Therefore the 2010-2011 samples had to be analyzed again to yield comparable data. A benefit of dry combustion was that the total nitrogen fraction could be analyzed at the same time in a CN analyzer and this saved time. Furthermore a CN analyzer is very accurate and easy to use. With the nitrogen fractions the C:N ratio could be calculated, which was necessary to properly interpret the data. The added benefit of analyzing the samples from 2010 and 2011 again with dry combustion was that the accuracy of the results from wet combustion could be checked. This was a request from the involved Uruguayan scientists.

The carbon and nitrogen values of the MAOM fraction were calculated by subtracting the values of the coarse and fine POM fraction from the total values. This is a widely applied method because it is difficult and time consuming to measure the carbon and nitrogen in MAOM itself. This is explained in more detail in appendix B.

Three bulk density samples were taken with the 'cylinder method' from all sampling depths of each plot to yield an average value per layer per plot for 2012. The cylinder method works as follows. A ring with known dimensions was hammered into the soil. The ring samples were placed in a layer of water so they would become saturated to make them comparable. Any soil that extended out of the ring after saturation was removed. Next the samples were dried at 105 ^{o}C. and weighted with the ring. Finally the ring was cleaned and weighted. The soil weight was calculated by subtracting the ring weight from the 'soil with ring' weight. Because the dimensions of the ring were known (5.4 by 3 cm's or 68,71 cm^3) the weight of the soil could be expressed per volume. The bulk density ($g*cm^{-3}$) could be calculated by dividing the soil weight (g) by the volume in the ring. The results were compared with the average bulk density values of 2010 and 2011.

The bulk density data was used to calculate the soil carbon stock ($Mg*ha^{-1}$). For this the fixed depth method was used with the average bulk density values for each layer, because there were no significant differences between the plots. This is explained in more detail in appendix C.

Statistics

To check if the differences between the treatments were significant the PROC MIXED procedure (factorial mixed ANOVA) of SAS was used. The experiment was considered a factorial design with 13 treatments (fixed effects) and three replications in time (random effects). The years were considered as blocks and assumed to be independent. The 13 treatments excluded the control pasture and the crop years and the fourth year of pasture with conventional tillage, because they had only one replication. The control pasture was not sampled in previous years and long rotation with conventional tillage had only three plots at the experimental site, while it has seven years in a cycle. Thus not all cycle years could be present in each year. Of LR-CT, the second year of pasture was the only cycle year present in more than one year: 2011 and 2012. For the statistical analysis at least two measurements were needed.

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[6] POM is SOM smaller than 2 mm and larger than 53 μm (Cambardella and Elliot, 1992).

Orthogonal contrasts and contrast of least square means were performed using Tuckey's test. Because of the usually high variability of soil nitrogen and carbon measurements at agricultural sites P-values equal or smaller than 0.10 were considered significant.

The following treatments were compared with orthogonal contrasts:

- No tillage vs tillage
- Continuous cropping vs long and short pasture rotation
- Short rotation pasture vs short rotation crops
- Long rotation pasture vs long rotation crops

And for all four land use types:

- Soybean vs sorghum
- Soybean as second crop vs soybean as first crop

As is normal in this type of agricultural soil experiment there was a high variability in the data. Random events such as weather and pests might have had a different short term influence on the crop and pasture yields. For instance, 2011 might have been a very good year for the sorghum yield, a bad year for the soybean yield and a mediocre year for the pasture yield. This would lead to short term variability in the amount of carbon and nitrogen input into the soil, because for instance a higher crop yield also leaves more crop residues and roots on and in the soil for decomposition. On the long term this variability is diminished since all types of vegetation will have had good and bad years. But in the sampling period (2010-2012) this probably created noise. That is why a covariable, which reflected the carbon input through the dry matter yield of 2010, 2011 and 2012, was incorporated into the statistical model. So if there is an exceptionally low or high harvest the short term effect of the carbon input can be corrected. This resulted in a statistical procedure were it was first checked if the dry matter yield was significant as a covariable. Then if this was the case the model with the covariable was used and if it was not the model without the covariable was used. The covariable model is discussed in more detail in appendix D.

4. Results

This chapter will give an overview of the results of the 2010 to 2012 measurements. First it will review the results of soil organic carbon (SOC) then of the bulk density, the carbon stock and nitrogen (N) and finally the carbon to nitrogen (C:N) ratio. Although a lot of data for the individual crop and pasture plots was gathered, this research will focus on the three main rotation systems: Continuous Cropping with No Tillage (CC-NT), Short Rotation with No Tillage (SR-NT) and Long Rotation with No Tillage (LR-NT). The individual crops will only be mentioned when they are important to answer the research questions. To make the text, tables and figures more readable the '-NT's from the treatments have been left out except when comparisons with conventional tillage were made, e.g. in figure 4.1.1 and table 4.1.4. So all discussed treatments are 'no-tillage' unless mentioned otherwise. The treatments Long Rotation with Conventional Tillage (LR-CT) and the control pasture will be used as reference values in the carbon stock section. Note that a fraction followed by '–C' refers to the organic carbon in the fraction and '-N' to the nitrogen. So a MAOM-C concentration reflects the concentration of carbon from the MAOM fraction in the soil. In most tables POM is mentioned. This is the total particulate organic matter, so the sum of coarse and fine POM. A list of abbreviations can be consulted in appendix A. The accuracy of the measurements are shown in appendix F.

4.1. Carbon

In this section an overall description of the results will be presented first, followed by the specific significant differences in the orthogonal contrasts.

Overall results

To explore and describe the relations between the land use treatments and the organic carbon variables, a principle component analysis was performed. The result is shown in figure 4.1.1.

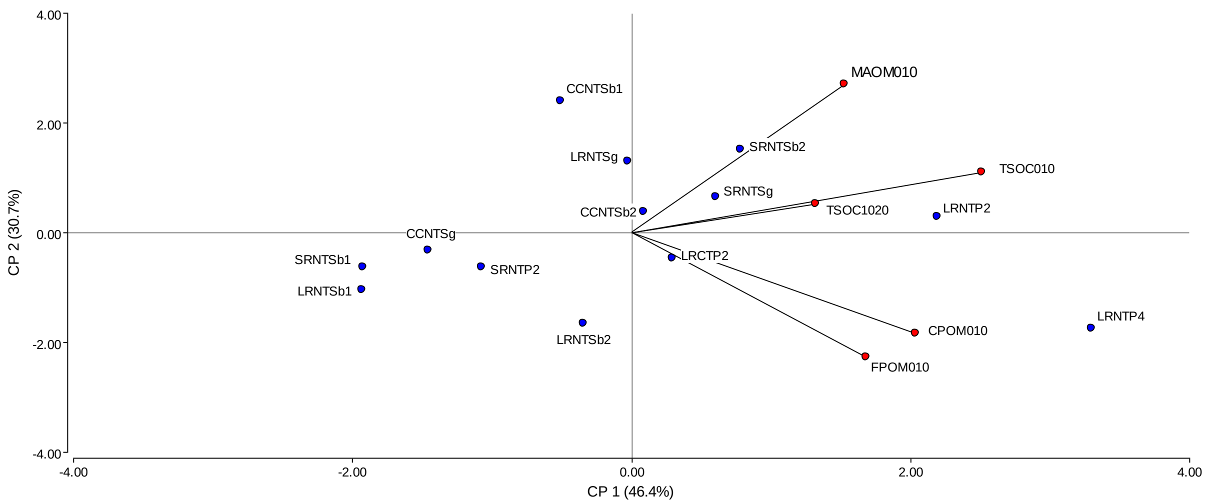

Figure 4.1.1: Principle component analysis of the 13 treatments (blue dots) and the main variables of organic carbon (red dots) in the 0-10 and 10-20 cm layers. The names of the variables are composed of two parts: the first part reflects the fraction in which the carbon was measured. So total soil organic carbon (TSOC) for the unfractionated soil and coarse particulate organic matter (CPOM), fine POM (FPOM) and mineral associated organic matter (MAOM) for the fractions. The second part '010' and '1020' reflects two sampling depths. The names of the treatments are composed of the main treatment, e.g. CCNT for continuous cropping no tillage and the specific crop: soybean as first crop (Sb1), soybean as second crop after wheat (Sb2), sorghum (Sg) second year of pasture (P2) and fourth year of pasture (P4).

In table 4.1.1 the red dots (below) are the organic carbon variables: total soil organic carbon in the unfractionated sample (TSOC), coarse particulate organic matter (CPOM), fine POM (FPOM) and mineral associated organic matter (MAOM) in the 0-10 (010) and 10-20 cm (1020) layers. The blue dots are the land use treatments with specific crops, CCNTSg which is the sorghum plot of continuous cropping with no tillage. The 0-5 and 5-10 cm layers were left out to give the figure a better overview. The associations between the variables from these layers and the treatments were similar to the ones in figure 4.1.1.

All carbon variables in figure 4.1.1 are directed towards the right side under the influence of mainly the second and fourth year of pasture of long rotation without tillage (LRNTP2 and LRNTP4). The other treatments are more clustered around the center and have little influence. On the negative side of principle component one (x-axis) are all but two of the crop plots and only one pasture plot of short rotation. Probably principle component one described POM carbon and TSOC which was higher under pasture than under crops. There is much less dispersion associated with principle component two (y-axis). No clear relation between the carbon variables and the treatments are visible. However, MAOM is situated at the top of the figure and the coarse and fine POM at the bottom. So the second principle component is related to a factor which influences MAOM and POM. This is probably a confounding factor. More details of the principle component analysis are reviewed in appendix E.

Table 4.1.1 shows the average organic carbon concentration (g/kg) of the fractions and whole soil per main treatment in the 0-10 and 10-20 cm layers. The sample standard deviation (S) was relatively high compared to the averages, especially for the coarse and fine POM. Furthermore the differences between treatments were about the same size as the S-values. The total SOC S-values were similar in the two layers. None of the differences shown in table 4.1.1 were significant. However the averages did give an indication of the expected changes.

Table 4.1.1: The average soil organic carbon concentration (SOC) in g/kg and their sample standard deviation (S) for continuous cropping (CC), short rotation (SR) and long rotation (LR) treatments (all no-tillage) in the 0-10 and 10-20 cm layers. All measurements had three replications. POM-C is coarse and fine POM-C combined.

Layer	Fraction	CC		SR		LR	
		g/kg	S	g/kg	S	g/kg	S
0-10	TSOC	21.1	1.50	21.0	2.02	22.1	1.74
	CPOM-C	0.61	0.27	0.71	0.48	0.92	0.31
	FPOM-C	0.83	0.34	0.93	0.29	1.13	0.20
	POM-C	1.44	0.51	1.65	0.39	2.04	0.56
	MAOM-C	19.7	1.35	19.3	1.76	20.0	1.65
10-20	TSOC	17.5	2.02	17.6	1.70	17.9	1.82

For all treatments the total SOC concentration was 22.3 percent higher in the 0-10 cm layer than in the 10-20 cm layer. Compared to the other treatments, long rotation had the highest organic carbon concentrations in both layers and in all fractions. The total SOC concentration of long rotation was 4.7 percent higher in the 0-10 cm layer and 2.3 percent in the 10-20 cm layer than the total SOC concentration of continuous cropping. The coarse and fine POM-C concentrations of long rotation were respectively 50.8 and 36.1 percent higher than that of continuous cropping. Compared to continuous cropping, short rotation had higher coarse and fine POM-C concentrations and lower MAOM-C concentrations. The total SOC concentrations in both layers showed little difference between short rotation and continuous cropping.

In 2010 and 2012 the 0-5 and 5-10 cm layers were sampled. The results are shown in table 4.1.2. The 0-5 and 5-10 cm layers showed the same SOC distribution as in the 0-10 and 10-20 cm layers: higher levels closer to the surface and the highest concentration with long rotation followed by short rotation and then continuous cropping. An exception was MAOM-C in the 0-5, where continuous cropping had the highest values and long rotation the lowest. The S values were much lower in the 5-10 cm layer than in the 0-5 cm layer.

Table 4.1.2: The average SOC concentrations in g/kg and their sample standard deviation (S) for continuous cropping (CC), short rotation (SR) and long rotation (LR) treatments (all no-tillage) in the 0-5 and 5-10 cm layers. All measurements had two replications.

Layer	Fraction	CC		SR		LR	
		g/kg	S	g/kg	S	g/kg	S
0-5	TSOC	24.2	2.90	24.5	2.62	25.3	2.86
	CPOM-C	1.07	0.39	1.34	0.62	1.83	1.12
	FPOM-C	1.16	0.57	1.49	0.42	1.86	0.54
	POM-C	2.23	0.66	2.83	0.90	3.69	1.13
	MAOM-C	22.0	2.91	21.7	2.48	21.6	2.37
5-10	TSOC	18.1	1.44	18.7	1.99	19.9	2.74
	CPOM-C	0.20	0.09	0.26	0.07	0.34	0.16
	FPOM-C	0.38	0.17	0.35	0.19	0.56	0.18
	POM-C	0.58	0.18	0.62	0.16	0.90	0.29
	MAOM-C	17.5	1.40	18.1	1.90	19.0	2.54

Significant differences

There were three fraction in which significant differences ($p \leq 0,10$) between the main treatments have been found: MAOM-C in the 0-5 cm layer and coarse POM-C and POM-C in the 5-10 cm layer. POM-C ($p=0.16$) in the 0-10 layer and TSOC ($p=0.12$) in the 0-5 cm layer were close to the $p \leq 0,10$ significance condition (see table 4.1.3). In the case of coarse POM-C in the 5-10 cm layer the significant differences occurred with and without the covariable model which used dry matter yields to correct for noise in the SOC changes caused by changes in carbon input. It was decided to use the covariable model for POM-C because it had a much lower p-value and more significant contrasts.

Table 4.1.3: P-values of SOC in the fractions and whole soil samples in all sampled soil layers. A p-value equal or lower than 0.10 indicates significance and is depicted in bold letters. The left p-value column shows the significance of dry matter production as a covariable of soil organic carbon change. If dry matter production is a valid covariable the 'covariable model' can be used. The second p-value column shows the p-value for the covariable model. A significant value means that significant differences occur within the contrasts. The third p-value column shows the p-values for the regular statistical model without the coviariable. The statistical models are explained in more detail in appendix D.

Fraction Variable	Layer	p value for covariable	p-value for model with covariable	p-value for model without covariable
TSOC	0-5	**0.06**	0.12	0.33
CPOM-C		0.51	0.73	0.70
FPOM-C		0.16	0.19	0.36
POM-C		0.87	0.61	0.52
MAOM-C		**0.06**	**0.097**	0.22
TSOC	5-10	0.87	0.51	0.45
CPOM-C		**0.04**	**0.002**	**0.03**
FPOM-C		0.49	0.96	0.41
POM-C		0.50	0.20	**0.03**
MAOM-C		0.79	0.54	0.50
TSOC	0-10	0.13	0.32	0.52
CPOM-C		0.48	0.54	0.34
FPOM-C		0.14	0.25	0.40
POM-C		0.12	0.17	0.16
MAOM-C		0.25	0.39	0.57
TSOC	10-20	0.37	0.22	0.39

Four orthogonal contrasts showed significant differences in POM-C between treatments in the 5-10 cm layer (table 4.1.4 and figure 4.1.2). However, the difference between conventional tillage (CT) and no-tillage (NT) was statistically unsound because there was only one sampled CT plot in the 5-10 cm layer with no replications. Additionally, the sampled CT plot was the second year of pasture (long rotation) in 2012. Since pasture plots scored higher than crop plots on average (see last contrast) the comparison with average values from the other treatments including crop plots was biased.

Table 4.1.4: Significant contrasts between the compared treatments for POM-C in the 5-10 cm layer without the covariable model (DF: 11). The first column shows the two treatments that were compared, for instance no-tillage versus conventional tillage (NT vs CT). The second column shows the p-value for the contrast. The following two columns show the average concentration and sample standard deviation of the left treatment in the comparison (e.g. NT) and the last two columns show the same for the right treatment in the comparison (e.g. CT). CT refers to treatment with conventional tillage, NT to treatment without tillage, CC refers to continuous cropping, LR to long rotation and SR to short rotation.

Compared treatments	p	Left treatment		versus	Right treatment	
		g/kg	S		g/kg	S
NT vs. CT	0.0092	0.70	0.25		1.31	.
CC-NT vs. LR-NT & SR-NT	0.0658	0.58	0.18		0.74	0.26
LR-NT vs. SR-NT	0.0223	0.83	0.29		0.61	0.16
LR-NT: pasture vs. crops	0.0159	1.06	0.28		0.68	0.18

The other results from table 4.1.4 are displayed in figure 3.1.3. The no tillage systems with pasture: long and short rotation together had a 27,6 percent higher POM-C concentration than no-tillage continuous cropping (without pasture). When long and short rotation were compared the long rotation POM-C concentration was 36,1 percent higher. Short rotation and continuous cropping did not differ much (0.61 and 0.59 g/kg) so the difference in the CC vs LR & SR contrast was probably caused by the long rotation system. Within long rotation the pasture phase had a 55,9 percent higher POM-C concentration compared to the cropping phase.

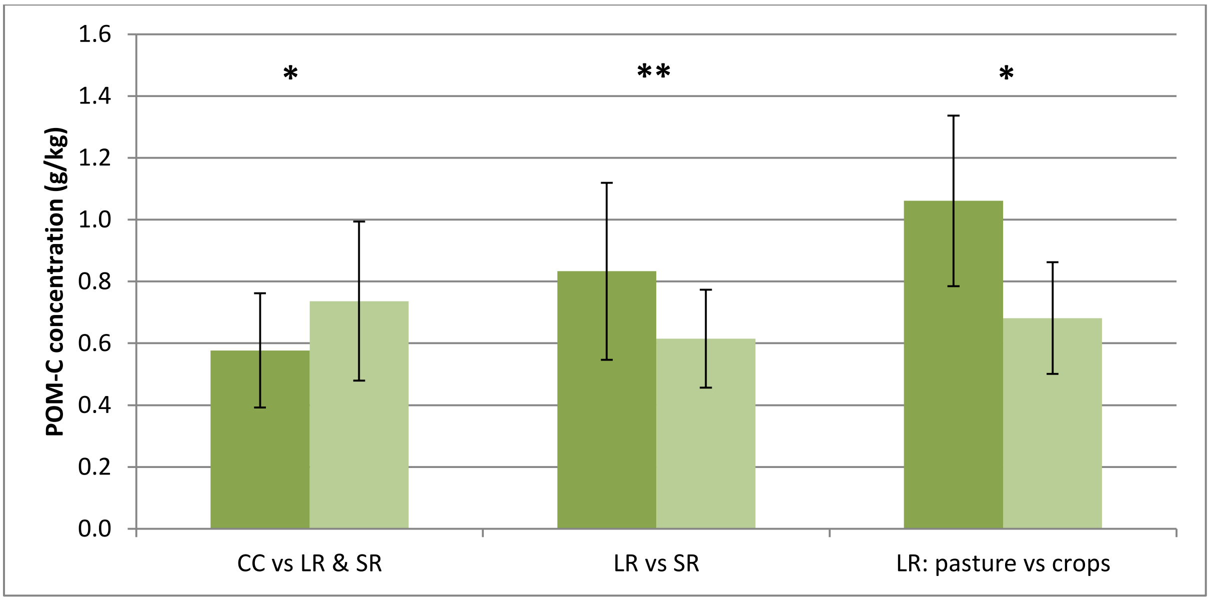

Figure 4.1.2: Significant differences between the POM-C concentrations [g/kg] of treatments in the 5-10 cm layer without the dry matter covariable model. The pairs of bars depict the two average concentrations of the compared treatments with their sample standard deviation. In the case of LR versus SR the left bar belongs to LR and the right bar to SR. All treatments in this figure were performed without tillage and CC refers to continuous cropping, LR to long rotation and SR to short rotation. The asterisks (*) stand for the level of significance (* stands for P≤0.1 while P>0.05 and ** for P≤ 0.05 while P>0.01).

Seven orthogonal contrasts showed significant differences for coarse POM in the 5-10 cm layer with the covariable model (table 4.1.5). The first four compared treatments are displayed in figure 4.1.3a and the last three in figure 4.1.3b).

Long and short rotation had on average a 40 percent higher coarse POM-C concentration than continuous cropping. As opposed to the POM-C values in the 5-10 cm layer, the differences between the rotation systems and continuous cropping could not be attributed to the long rotation system alone. Both rotation systems had higher concentrations: long rotation had a 50 percent and short rotation a 30 percent higher concentration. With long rotation the pasture phase has a 120 percent higher concentration than the cropping phase. For short rotation, on the other hand, the phases are almost equal.

Table 4.1.5: Significant differences between the coarse POM-C concentrations of treatments in the 5-10 cm layer with the covariable model. The first column shows the two treatments that were compared. The second column shows the p-value for the contrast. Then the average concentrations and sample standard deviations of the left and right compared treatments are displayed. CC refers to continuous cropping, LR to long rotation and SR to short rotation. All treatments were performed without tillage.

Compared treatments	p	Left treatment		versus	Right treatment	
		g/kg	S		g/kg	S
CC vs. LR & SR	0.0018	0.20	0.09		0.28	0.13
LR vs. SR	0.0883	0.30	0.16		0.26	0.07
SR: pasture vs. crops	0.0407	0,27	0,02		0,26	0,08
SR: soybean 1st vs. soybean 2nd	0.0158	0,22	0,10		0,31	0,12
LR: pasture vs. crops	0.0001	0,44	0,15		0,20	0,07
LR: 2nd vs. 4th year pasture	0.0021	0.31	0.01		0.57	0.08
LR: soybean 1st vs. soybean 2nd	0.0582	0.23	0.00		0.24	0.06

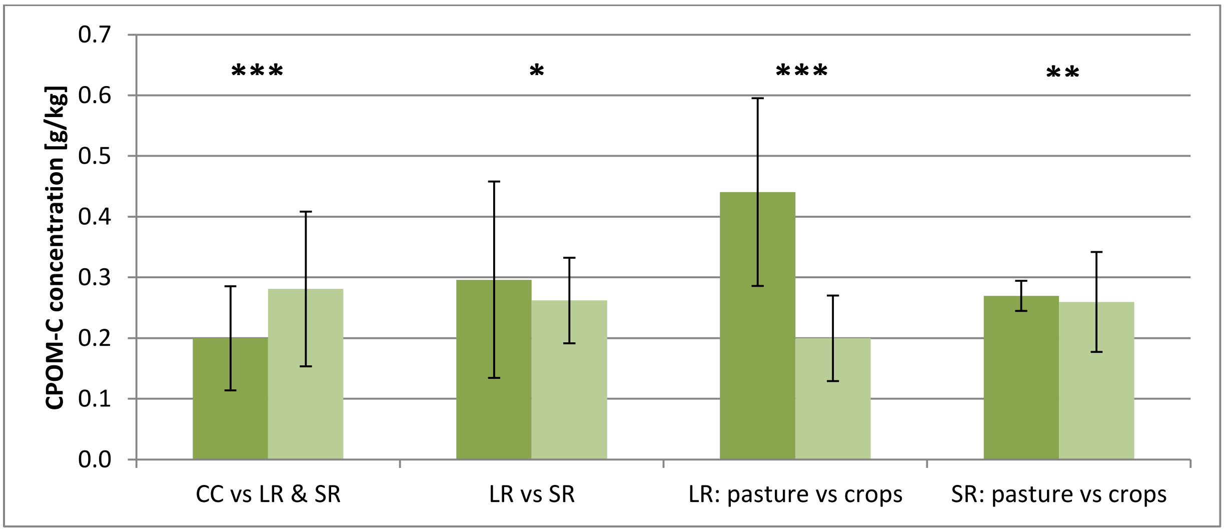

Figure 4.1.3a: Significant differences between coarse POM-C concentrations [g/kg] of treatments in the 5-10 cm layer with the dry matter covariable model. The pairs of bars depict the two average concentrations of the compared treatments with their sample standard deviation. In the case of LR versus SR the left bar belongs to LR and the right bar to SR. All treatments in this figure were performed without tillage and CC refers to continuous cropping, LR to long rotation and SR to short rotation. The asterisks (*) stand for the level of significance (* stands for P≤0.1 while P>0.05, ** for P≤ 0.05 while P>0.01 and *** for P≤0.01).

For both long and short rotation the treatment with soybean as the second crop (after winter crop wheat) had a higher carbon concentration than soybean as the first crop (see figure 3.1.3b). The difference was more pronounced in short rotation where the difference was 40.9 percent than long rotation where it was 4.3 percent. In the main treatment cycles, soybean as the second crop is planted in the year after the pasture phase and soybean as the first (and only) crop occurs at the end of the cropping phase.

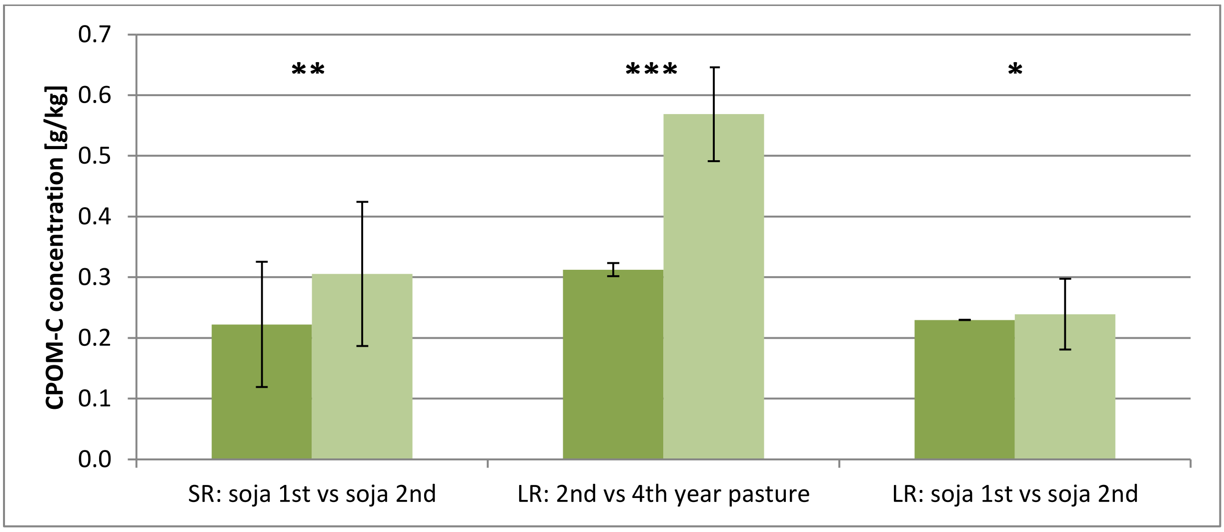

Figure 4.1.3b: Significant differences between coarse POM-C concentrations [g/kg] of treatments in the 5-10 cm layer (with the dry matter covariable model). The pairs of bars depict the two average concentrations of the compared treatments with their sample standard deviation. In the case of LR:2nd versus 4th year pasture the left bar belongs to LR second year pasture and the right bar to LR fourth year pasture. All treatments in this figure were performed without tillage and LR refers to long rotation and SR to short rotation. The asterisks (*) stand for the level of significance (* stands for P≤0.1 while P>0.05, ** for P≤ 0.05 while P>0.01 and *** for P≤0.01).

Next to this for long rotation the fourth year of pasture had 83.9 percent higher CPOM-C concentration than the second year.

Table 4.1.6: Significant contrasts MAOM 0-5 with the covariable. The first column shows the two compared treatments. The second column shows the p-value for the contrast. Then the average concentrations and sample standard deviations of the left and right compared treatments are displayed. CC refers to continuous cropping, LR to long rotation and SR to short rotation. All treatments were performed without tillage.

Compared treatments	p	Left treatment		versus	Right treatment	
		g/kg	S		g/kg	S
SR: pasture vs. crops	0.0793	22.67	3.17		21.03	2.40
SR: soybean 1st vs. soybean 2nd	0.0092	18.38	0.99		23.36	1.44
LR: pasture vs. crops	0.0480	22.36	2.18		20.65	2.43
LR: soybeans vs. sorghum	0.0194	20.87	3.05		21.35	0.84

The model with the covariable showed also some significant differences between the MAOM-C concentrations of the compared treatments in the 0-5 cm layer. These are displayed in table 4.1.6 and figure 4.1.4. The MAOM-C concentration was 8.3 percent higher in the long rotation pasture phase than in the long rotation cropping phase. For short rotation the pasture phase MAOM-C concentration was 7.8 percent higher. This did not result in any significant differences between the three main treatments. The continuous cropping even had a slightly higher MAOM-C concentration in this layer but this was not significant. For short rotation soybean as the second crop had a 27,1 percent higher concentration than soybean as the first and prime crop. Also the combined soybean concentrations had a slightly lower (2.3 percent) than the sorghum concentrations for long rotation. However, the sample standard variation for the soybean concentration is relatively high.

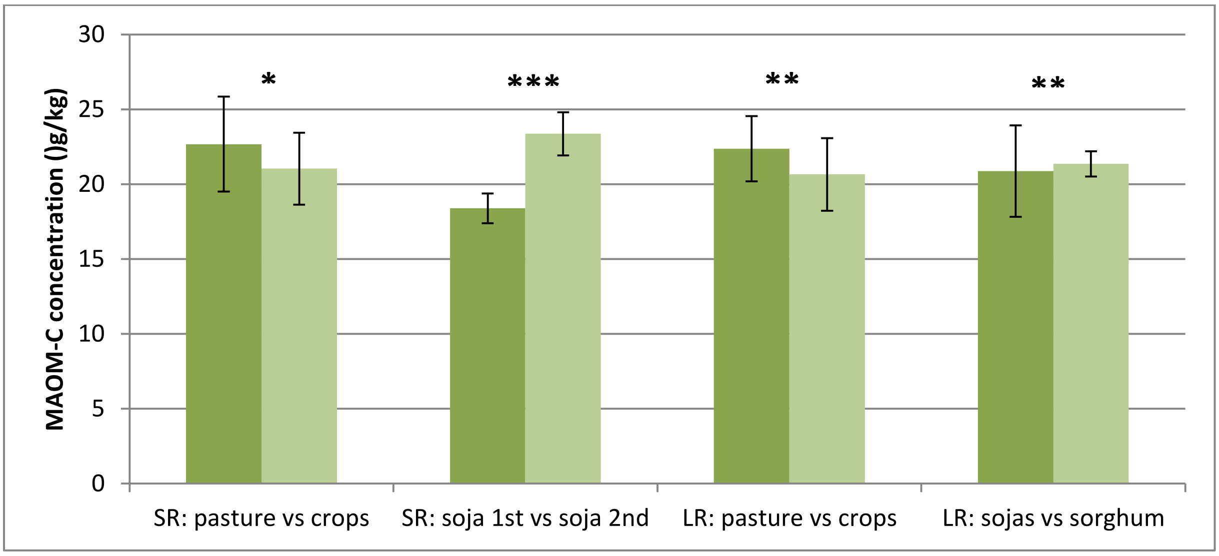

Figure 4.1.4: Significant differences between the MAOM-C concentrations [g/kg] in the 0-5 cm layer with the dry matter covariable model. The pairs of bars depict the two average concentrations of the compared treatments with their sample standard deviation. In the case of LR: pasture versus crops the left bar belongs to the LR pasture phase and the right bar to the LR cropping phase. All treatments in this figure were performed without tillage, LR refers to long rotation and SR to short rotation. The asterisks (*) stand for the level of significance (* stands for P≤0.1 while P>0.05, ** for P≤ 0.05 while P>0.01 and *** for P≤0.01).

4.2. Soil bulk density

No significant differences between the soil bulk densities of the treatments were found. Not only the regular orthogonal contrasts were used for the statistical analysis, but also new contrast which compared the bulk density of each crop separately. The bulk density measurements were necessary to calculate the carbon stock. For the fixed depth method it should be multiplied with the carbon concentration and layer thickness. For all treatments (including the separate cycle years) three bulk density samples were at each depth in 2010, 2011 and 2012. So nine samples per cycle year of the treatments where taken in total. The values range from about 1,0 to 1,5 g/cm^3. Using these individual bulk density values to calculate the carbon stock would have incorporated this variation because bulk density is a multiplier in the calculation, while the bulk density did not show significant differences. The carbon stock could also be calculated with the average value for each layer shown in the table 4.2.1. but the differences are small and smaller than the sample standard deviation.

Table 4.2.1: Average bulk density values (g/cm3) and their sample standard deviation (S) for each sampled layer. The 0-5 and 5-10 cm layers have two replicates (2010-2012) and the 0-10 and 10-20 cm layer three (also 2011).

	0-5 cm	5-10 cm	0-10 cm	10-20 cm
Average (g/cm^3)	1.180	1.231	1.193	1.246
S	0.142	0.105	0.117	0.102

Even the total average (0-20 cm layer) for no tillage treatments (1.213 g/cm^3 with a sample standard deviation of 0.118) did not differ much from the average for tillage treatments (1.238 g/cm^3 with a sample standard deviation of 0.122). That is why the overall bulk density (1.214 g/cm^3) was used to calculate the carbon stock.

4.3. Carbon Stock

The average carbon stock was calculated using the 1.214 g/cm^3 bulk density for all treatments. Since only half the pasture years were measured they were 'weighted' twice as heavy in the average as the cropping years.

Often the carbon stock differences between continuous cropping and short or long pasture rotation will be explained as an increase for long rotation compared to continuous cropping. What is meant with this is that there is a relative increase between treatments but not necessarily an absolute increase in time for that treatment. For example, if a farmer with a continuous cropping field with a current carbon stock of 57 Mg/ha would have switched that field to a long rotation system in 1994 the carbon stock would currently be 59 Mg/ha. So it would relatively increase with 2 Mg/ha. However the initial carbon stock in 1994 was not measured and might have been higher that it currently is, 60 Mg/ha for instance.

The results for the three main treatments and the reference pasture are displayed in table 4.3.1.

Table 4.3.1: Overview of the carbon stock results for the three main treatments continuous cropping (CC), short rotation (SR) and long rotation (LR) and the control pasture (all no tillage).

	Mg/ha	CC	SR	LR	Pasture
0-5 cm	TSOC	8.93	9.05	9.35	10.04
	CPOM-C	0.40	0.49	0.68	1.14
	FPOM-C	0.43	0.55	0.69	0.93
	POM-C	0.82	1.04	1.36	2.07
	MAOM-C	8.10	8.00	7.98	7.97
5-10 cm	TSOC	6.67	6.89	7.34	6.66
	CPOM-C	0.07	0.10	0.12	0.29
	FPOM-C	0.14	0.13	0.21	0.23
	POM-C	0.21	0.23	0.33	0.51
	MAOM-C	6.46	6.66	7.01	6.15
0-10 cm	TSOC	31.15	30.93	32.56	33.40
	CPOM-C	0.90	1.06	1.36	2.67
	FPOM-C	1.23	1.37	1.66	2.28
	POM-C	2.13	2.43	3.02	4.95
	MAOM-C	29.02	28.50	29.54	28.45
10-20 cm	TSOC	25.87	25.99	26.44	22.36
0-20 cm	TSOC	57.02	56.92	59.00	55.76

In the 0-5 cm layer the TSOC increased a little with the inclusion of pasture. A longer pasture phase led to a greater increase with the control pasture having the highest carbon stock. The TSOC is the sum of its fractions. Considering this, the differences in TSOC were caused by a higher coarse and fine POM-C stock, while the MAOM-C stock stayed almost the same.

Although the control pasture has the highest coarse and fine POM-C values in the 5-10 cm layer, it had the lowest MAOM-C and TSOC values. Short rotation had values a little higher than continuous cropping for TSOC and MAOM-C and coarse POM-C. Long rotation showed higher values. Where the MAOM-C stock did not differ much between treatments in the 0-5 cm layer, in the 5-10 cm layer it increased from continuous cropping to short rotation and then long rotation.

The combined values for the 0-10 cm layers (in which the 2011 samples were also included) showed that compared to continuous cropping, short rotation had a higher coarse and fine POM-C stock but a lower value for MAOM-C. This resulted in a slightly lower TSOC stock. Compared to short rotation and continuous cropping, long rotation had the highest values. The control pasture had the highest coarse and fine POM-values and the lowest MAOM-C value, similar to short rotation.

In the 10-20 cm layer the control pasture showed the lowest values. Because the control pasture did have the highest values for the 0-10 cm layer this could possibly indicating shallow rooting of the pasture grasses. Overall there was a small increase from continuous cropping to short rotation to long rotation in the 10-20 cm layer.

Overall long rotation had the highest carbon stock in the 0-20 cm layer. Short rotation and continuous cropping had a similar carbon stock and the control pasture had the lowest. There was also an overall decrease of the carbon stock with depth.

The other reference treatment was long rotation with conventional tillage. Only the second year of pasture was sampled twice. Table 4.3.2 shows the values for the second year of pasture for long rotation with conventional tillage and without it.

Table 4.3.2: A comparison between the carbon stocks (Mg/ha) of second year of pasture of long rotation without tillage and long rotation with conventional tillage.

		Long rotation No tillage Second pasture year	Long rotation Conventional tillage Second pasture year
0-5 cm	TSOC	9.86	8.23
	CPOM-C	0.60	0.65
	FPOM-C	0.95	0.60
	POM-C	1.55	1.25
	MAOM-C	8.31	6.98
5-10 cm	TSOC	7.16	7.08
	CPOM-C	0.12	0.11
	FPOM-C	0.21	0.38
	POM-C	0.32	0.48
	MAOM-C	6.83	6.60
0-10 cm	TSOC	32.82	30.90
	CPOM-C	1.25	1.43
	FPOM-C	1.89	1.42
	POM-C	3.14	2.85
	MAOM-C	29.68	28.05
10-20 cm	TSOC	28.80	28.92
0-20 cm	TSOC	61.61	59.82

The TOC stock was larger for the no-tillage treatment in the 0-5 cm layer. This was mainly caused by a higher fine POM-C (and total POM-C) and MAOM-C stock. Coarse POM-C was slightly higher for the conventional tillage treatment. In the 5-10 cm layer the TSOC stocks are almost similar. The treatment with tillage had a higher fine POM-C stock and the treatment without tillage had a higher MAOM-C stock. For the 0-10 cm layer the no-tillage treatment had the highest TSOC stock. The tillage treatment had a higher coarse POM-C stock, but the no tillage treatment a higher fine and total POM-C stock. The MAOM-C stock was also larger for the no tillage treatment. Both treatments had about the same carbon stock in the 10-20 cm layer. In the total arable layer (0-20 cm) no-tillage had 1.79 Mg more organic carbon per hectare. 1.33 Mg/ha could be explained by the MAOM-C difference in the 0-5 cm layer.

Table 4.3.3 on the next page gives an overview of the carbon stock changes between short and long rotation compared to continuous cropping in percentages. Although some of the differences in table 4.3.1 and 4.3.2 were small in absolute numbers, especially for coarse and fine POM, the percentual increase of these fractions was high. Overall coarse POM changed the most, followed by fine POM and the total SOC. MAOM changed the least. Changes decreased with increasing depth except for MAOM-C and TSOC stocks which increased more in the 5-10 cm layer than in the 0-5 cm layer.

Table 4.3.3: Carbon sequestration of short and long rotation compared to continuous cropping in percentages.

	%	SR	LR
0-5 cm	TSOC	1.35	4.69
	CPOM-C	24.48	71.07
	FPOM-C	28.87	60.56
	POM-C	26.76	65.62
	MAOM-C	-1.23	-1.50
5-10 cm	TSOC	3.31	10.10
	CPOM-C	31.91	68.85
	FPOM-C	-5.92	48.78
	POM-C	7.18	55.73
	MAOM-C	3.18	8.59
10-20 cm	TSOC	0.48	2.23

Most changes between treatments were not significant when compared with mixed ANOVA. Table 4.3.4 shows the significantly increase in sequestered coarse and total POM-C with short and long rotation compared to continuous cropping in the 0-20 cm layer. The increase is shown in kilogram per hectare and as a percentage of the total continuous cropping stock. The MAOM differences are not presented because they reflect differences within treatments and not between. Total POM-C should be regarded as the net difference because coarse POM-C is a part of total POM-C.

Table 4.3.4: The total significantly sequestered C in the 0-20 cm layer in kilogram per hectare compared to continuous cropping and as a percentage of total continuous cropping carbon stock.

	Coarse POM-C	POM-C	Increase of POM-C compared to continuous cropping TSOC stock in the 0-20 cm layer
	kg/ha	kg/ha	%
Short rotation	23.5	15.3	0.027
Long rotation	50.8	118.6	0.208

Short rotation significantly sequestered an extra 15.3 kg of organic carbon per hectare compared to continuous cropping. This was a 7.18 percent increase in POM-C but only a 0.03 percent increase when compared to the total SOC of continuous cropping. The coarse POM-C stock increase was higher but this increase was reduced because the fine POM-C stock decreased.

The increase in sequestered carbon with long rotation was 118.6 kg/ha, almost eight times higher than for short rotation. The increase was 55.7 percent compared to the POM-C stock but only 0.21 percent compared to the total SOC stock of continuous cropping.

The total (but not significant) increase in the 0-20 cm layer is shown in table 4.3.5. There was a 3.5 percent increase with long rotation but a -0.2 percent decrease for short rotation.

Table 4.3.5: Carbon sequestration of short and long rotation compared to continuous cropping in the 0-20 cm layer.

	Carbon stock	Carbon sequestered	Carbon sequestered
	Mg/ha	Mg/ha	%
CC	57.02	.	.
SR	56.92	-0.10	-0.17
LR	59.00	1.98	3.48

4.4. Nitrogen

The nitrogen values in all samples were also measured to yield C:N ratio data. Unfortunately the nitrogen results showed some unexpected values especially in the coarse and fine POM fractions and in the 5-10 cm layer.

The average concentration of POM nitrogen is shown in table 3.4.1 with its sample standard deviation (S). There was a clear increase of nitrogen from 2010 to 2012. This was most pronounced in the 5-10 cm layer were the 2012 concentration was twice as high as the 2010 concentration. The standard deviation was higher too in 2011 and 2012 than in 2010, especially in the 5-10 cm layer.

Table 4.4.1: average POM nitrogen concentrations (g/kg) found in the sampling layers from 2010 to 2012 with sample standard deviation (S).

Sampling year	POM-N 0-5 cm		POM-N 5-10 cm		POM-N 0-10 cm	
	g/kg	S	g/kg	S	g/kg	S
2010	0.21	0.10	0.07	0.02	0.14	0.05
2011					0.18	0.06
2012	0.36	0.12	0.14	0.05	0.24	0.06

The total nitrogen samples (see appendix H) were more consistent over the years but the statistical analysis (only the model without the covariable was used) showed that only the replications were significantly different in the 0-5 ($p=0.089$) and 5-10 cm ($p=0.032$) layers. The coefficients of variance were 11,9 and 10,1 respectively.

If the years had significantly different nitrogen concentrations, it might still be possible to compare the percentual differences of the main treatments, because the actual levels of the concentrations are then left out. This comparison was performed and showed an overall increase in POM-N but decrease in total and MAOM nitrogen for short and long rotation compared to continuous cropping. However there was a very high variability within the data.

4.5. C:N ratio

The average C:N ratios for each year and layer are displayed in table 3.5.1 with their sample standard deviation. The C:N ratios decrease from 2010 to 2012. This effect was caused by the nitrogen concentrations. The C:N ratio is calculated by dividing the carbon by the nitrogen concentration. The carbon concentration did not differ significantly between the replication years so the higher nitrogen concentrations led to lower

C:N ratios. That the nitrogen concentration rose from 2010-2012 resulted in a decreasing C:N ratio in these years.

4.5.1: Average POM CN ratio of all treatments and the sample standard deviation in the three fractionated layers. Note that a there is a decrease in CN ratio from 2010 to 2012 (caused by a rise in N concentration).

Sampling	0-5 cm		5-10 cm		0-10 cm	
year	Average	S	Average	S	Average	S
2010	14.40	3.25	10.34	1.74	13.18	2.33
2011	.	.	.	.	9.65	4.38
2012	9.38	3.00	6.62	3.78	8.22	2.50

5. Discussion

This chapter will start discussing the differences in the concentrations soil organic carbon (SOC) in the whole soil and in the soil fractions. Several significant differences for SOC in the soil fractions were mentioned in chapter 4 (results), but only the differences which are directly related to the research questions will be discussed in this chapter. After this the chapter will continue with carbon stock and carbon sequestration and then the nitrogen and C:N ratio data. The chapter will conclude with discussing the future development of the SOC levels at the experimental site and recommendations future research.

H1: No tillage crop pasture rotation has a higher level of soil organic carbon compared with continuous cropping and long crop pasture rotation will have a higher concentration than short crop pasture rotation.

H2: No tillage crop pasture rotation has a higher level of soil organic carbon in the three soil fractions compared with continuous cropping and long crop pasture rotation will have a higher concentration than short crop pasture rotation. The difference between the SOC levels in the fractions are the largest for POM-C, which is most sensitive to land use change, followed fine POM-C while MAOM-C hardly changes.

H3: No tillage crop pasture rotation will have higher nitrogen levels (and thus lower C:N ratios) than no tillage continuous cropping and this will lead to enhanced humification (a higher transformation of POM-C into MAOM-C).

To make this chapter more readable, short and long crop pasture rotation will be referred to as short and long rotation and all mentioned treatments were performed without tillage unless stated otherwise.

5.1. carbon

The total SOC concentrations for continuous cropping and short rotation were almost similar in the 0-10 and 10-20 cm layers. Only long rotation is lightly higher, but insignificantly. This is similar to the findings of Ernst & Siri-Prieto (2009) and Salvo et al. (2010), but contrary to hypothesis one. For the 10-20 cm layer the soil was not fractionated, so there was no data for the fractions.

The main differences in soil organic carbon concentration between the three main treatments for the fractionated layers are summarized in figure 5.1.1 on the next page. Total soil organic carbon is divided into particulate organic matter carbon (POM-C) and mineral associated organic matter carbon (MAOM-C) in 5.1.1a-c. POM-C from 5.1.1a-c is further divided in its coarse and fine fraction in figure 5.1.1d-f. Note that the y-axes have different ranges.

Significant results

The differences between the continuous cropping, short rotation and long rotation systems were only significant for total POM-C and coarse POM-C in the 5-10 cm layer. Long rotation had a 54.7 percent higher total POM-C concentration and a 50.0 percent higher coarse POM-C concentration compared to continuous cropping. Short rotation had an almost similar total POM-C concentration and a 30.0 percent higher coarse POM-C concentration compared to continuous cropping. This shows the positive effect of the inclusion of pastures in a cropping system and that total POM-C and especially coarse POM-C are more sensitive to the land use change than MAOM-C and total SOC.

What further indicates that pastures have a positive effect on the SOC concentration is that the pasture phase often had a significant higher coarse and total POM-C concentration. In the 5-10 cm layer the pasture phase of long rotation had a 55.9 percent higher total POM-C concentration and a 40.9 percent coarse POM-C concentration than the cropping phase. Next to this, short rotation had a 4.3 percent higher coarse POM-C concentration than the cropping phase and no significantly higher total POM-C level. Note that the long (four year) pasture phase has much higher coarse and total POM-C concentrations than the short (two year) pasture phase. So a longer pasture phase positively influences the coarse and total POM-C level. This was already indicated by the principle component analysis which showed that the pasture phase of long rotation and high soil organic carbon levels were related. Moreover it is corroborated by the fact that the fourth year pasture plot of long rotation significantly had a 120 percent higher coarse POM-C concentration than the second year pasture plot of long rotation and that in the 0-5 cm layer the MAOM-C concentrations of the pasture phase of long and short rotation was 8.3 and 7.8 percent higher than the cropping phase respectively.

That a short and especially a long pasture phase have a positive effect on the concentrations of coarse and total POM-C, which are the fractions most sensitive to changes in agricultural practice (Cambardella and Elliot, 1992; Morón and Sawchik, 2003; Salvo et al., 2010), supports hypothesis two. The changes in the sensitive fractions are indications of future change in the more stable fractions and the total level of SOC (Haynes, 2000). At this moment the changes in total SOC are not yet significant. Hence, hypothesis one will probably be validated in the future.

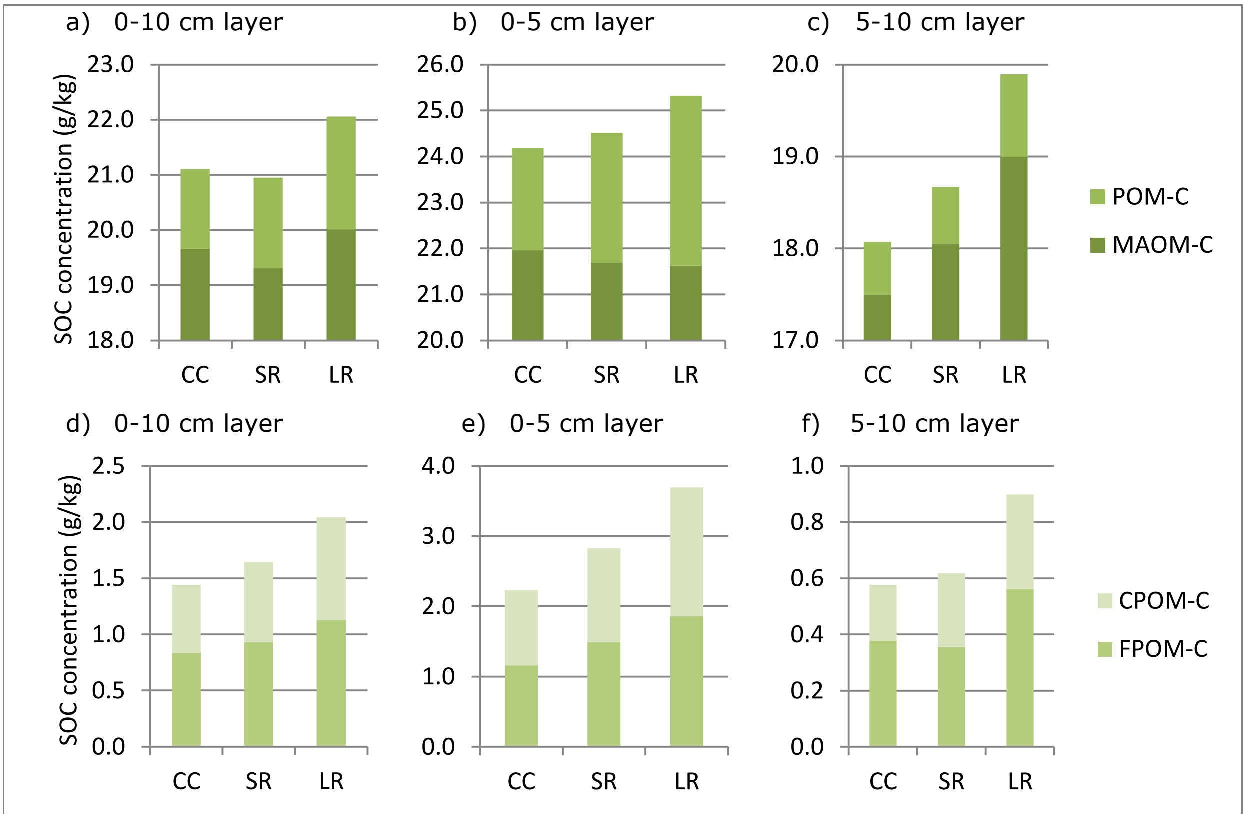

Figure 5.1.1: (a-c) Total SOC concentration divided in POM-C and MAOM-C and (d-f) the POM-C of a-c divided in its coarse and fine fractions (CPOM-C and FPOM-C) for the (a & d) the 0-10 layer, (b & e) the 0-5 cm layer (c & g) the 5-10 cm layer for continuous cropping (CC), short rotation (SR) and long rotation (LR) (all no tillage).

Fine POM-C

The concentrations of coarse POM-C and total POM-C showed significant differences in the 5-10 cm layer but fine POM-C concentrations did not. There were differences in the fine POM-C concentrations which had about the same size and trends as the coarse POM-C concentrations (see fig. 5.1.1d-f) but these were not significant. Coarse and fine POM-C together form total POM-C which also showed significant differences, so it could be expected that if total POM-C shows significant differences fine POM-C should too. That this is not the case might be explained by the fact that the significant differences of coarse POM-C could have caused the total POM-C to be significant. The differences were ‘diluted’ by the insignificant differences of the fine POM-C concentrations. This is supported by the fact that all the contrasts that were significant for total

POM-C were also significant for coarse POM-C but that coarse POM-C had more significant contrasts. Moreover, the p-values of the 'continuous cropping versus long and short rotation' and 'long rotation pasture versus crop phase' contrasts were much lower for coarse POM-C than for total POM-C.

The 0-5 cm layer

Significant differences between the three treatment systems did not occur in the 0-5 cm layer but did in the in the 5-10 cm layer. This is contrary to the findings of Salvo et al. (2010): that the main changes in SOC level occurred in the 0-3 cm layer. This could have two reasons.

First, the high standard deviation in the 0-5 cm layer could have caused the results to be insignificant. The standard deviation of the coarse POM-C concentrations was higher in the 0-5 cm layer than in the 5-10 cm layer (table 5.1.1). Especially compared with the actual difference in concentration. In both depths the standard deviation was higher than the actual change in concentration, but for the 5-10 cm depth the change and standard deviation differed much less.

Table 5.1.1: The difference in coarse POM-C concentration of short and long rotation compared to continuous cropping in g/kg and percent and the sample standard deviation (SD).

		Short rotation	Long rotation
0-5 cm layer	Difference g/kg	0.26	0.76
	Sample SD	0.62	1.12
	Percentual difference	24%	71%
5-10 cm layer	Difference g/kg	0.06	0.14
	Sample SD	0.07	0.16
	Percentual differnce	32%	69%

Secondly, as mentioned above pastures have a higher below ground soil organic carbon input than crops in the form of rhizodeposition which originates from the root biomass. The differences pasture rhizodeposition could result in would be less pronounced in the 0-5 cm layer, because this layer also gets relatively large quantities of SOC input from surface residues. In the 5-10 cm layer, rhizodeposition is the main form of SOC input. So this could result in smaller percentual differences and thus less significance. However table 5.1.1 shows comparable percentual differences between the layers. Thus the lack of significance is probably caused by a higher variation in the 0-5 cm layer.

Opposing MAOM-C trends

In the 0-10 cm layer the MAOM-C concentration was highest for long rotation followed by continuous cropping and then short rotation (figure 5.1.1a and 5.1.1d). Figure 5.1.1b and c show that the 'low' MAOM-C concentration for short rotation in 5.1.1a was an average of two opposite 'trends' for MAOM-C in the 0-5 and 5-10 cm layers. In the 0-5 cm layer continuous cropping had the highest MAOM-C concentration, followed by short rotation and long rotation had the lowest concentration, but in the 5-10 cm layer it was the other way around. These trends were not significant but could hint to underlying soil processes, which might be found in other comparable research. Therefore these trends will be discussed in the following section.

It was hypothesized that the MAOM-C concentrations of long rotation would be highest followed by short rotation and then continuous cropping and that MAOM-C would be the fraction which would be least sensitive to land use change (H2). So it was unexpected that continuous cropping had the highest MAOM-C concentration followed by short and then long rotation in the 0-5 cm layer. Especially since in the 5-10 cm layer it was the other way around. It was also unexpected that in the 5-10 cm layer, the MAOM-C was (insignificantly) the main contributor to the absolute increase in the total SOC. There are two soil processes which could account for this: SOC transport from the 0-5 to the 5-10 cm layer and by enhanced humification under pasture rotation (H3) in the 5-10 cm layer.

The opposing MAOM-C trends could be caused by the transport of the very fine MAOM-C (<50 μm) particles deeper into the soil by infiltrating precipitation. However the soil of this site has a medium to heavy texture and according to Six et al., (2002) this enhances the capacity of the soil to protect MAOM-C. Moreover the inclusion of pastures into a crop rotation should improve soil structure and wet aggregate stability which would inhibit transport of soil material. If MAOM-C is being transported from the 0-5 to the 5-10 cm layer the same trend should be visible for the clay and silt particles to which it is associated. However, while in the 5-10 cm layer MAOM-C increased with 3.4 percent for short rotation and 8.6 percent for long rotation compared to continuous cropping, the total MAOM soil fraction weight decreased with 2.8 percent for short rotation and increased with 0.4 percent for long rotation compared to continuous cropping. So transport of the MAOM-C particles is unlikely.

Enhanced input and humification in the root zone of the pasture grasses could also be an explanation for the enhanced MAOM-C levels in the 5-10 cm layer of short and long rotation. Compared to continuous cropping, pastures have a higher root biomass and associated rhizodeposition[7] (Rees et al., 2005). According to Franzluebbers and Stuedemann (2002) who compared (inter alia) pastures with conservation cropland in Southern Piedmont (USA), POM-C in the 0-5 cm layer originates from surface plant residues and animal manures while in lower layers it originates from plant roots. Below the 0-5 cm layer the effect of higher root biomass under pastures should thus be more pronounced. Ernst et al. (2009) found an indication of enhanced input from below ground biomass at a crop pasture rotation site after twelve years of experimental time. Measuring total SOC they found no significant differences between continuous cropping and crop pasture rotation (both no tillage). However they did find a 'consistent trend' towards a higher total SOC level under continuous cropping than under the crop pasture rotation in the 0-6 cm layer, but a higher level for the crop pasture rotation than the continuous cropping in the 6-12 cm layer. Ernst and Siri-Prieto (2009) did not fractionate the soil samples so it is not known if these changes were caused by POM-C or MAOM-C. But since MAOM-C changed most in the 5-10 cm layer of this research, the results from Ernst and Siri-Prieto (2009) could corroborate the MAOM-C results found with this research. It should however be kept in mind that for both the values from Ernst and Siri-Prieto (2009) and this research were insignificant and moreover that the layers were not exactly similar. The reason that MAOM-C and not POM-C was the main contributor to the increase in SOC might be that higher nitrogen levels under pastures enhance humification (POM-C conversion to MAOM-C). Also Rees et al. (2005) describe exudation which is diffusion of mucilage and compounds with a low weight (mainly sugars, amino acids and organic acids) from roots. According to them the factors that affect the balance between soil inputs from rhizodeposition (through exudation and root death) and the following decay, may also decide the subsequent fate of the organic matter that is deposited. Exudates are readily decomposable while dying roots contain materials such as lignin or cellulose which has a much lower decomposition rate. Also exudation influences the microbial community and vice versa (Rees et al., 2005). This could influence the MAOM-C concentration in the 5-10 cm layer which originates from roots but not the 0-5 cm layer which originates from surface plant litter. Salvo et al. (2010) found indications of enhanced humification under no tillage crop pasture rotations compared to no tillage continuous cropping in the 0-3 cm layer. This was attributed to higher nitrogen levels. However with this research the indication of enhanced humification occurred in the 5-10 cm layer and not in the 0-5 cm layer. Moreover the C:N ratio's (an often used measure of decomposability) did not differ between continuous cropping, short rotation and long pasture rotation in the 5-10 cm layer but they did in the 0-5 cm layer (see table 5.3.2 in the nitrogen and C:N ratio section). Unfortunately, the nitrogen data which was supposed to shed light on these processes was inconclusive (see 5.3).

The variability within the data from the 0-5 cm layer probably causes the MAOM-C concentration of continuous cropping to seem higher than the concentrations of short and long rotation. The differences were very small (see table 4.1.2), the standard deviation was high and the differences between continuous cropping, short rotation and long rotation were insignificant. Furthermore the

[7] Rhizodeposition refers to deposition from roots.

mixed ANOVA showed that in the 0-5 cm layer, the pasture phase of short and long rotation significantly had a 1.64 and 1.71 g/kg higher MAOM-C concentration than the cropping phase. It is not likely that including pastures into a cropping systems would result in lower average MAOM-C concentrations while the pasture has a significantly positive influence on the MAOM-C concentration. Moreover, table 5.1.2. shows that the average MAOM-C concentrations of the pasture phases of short and long rotation were higher than the cropping phase of continuous cropping. So it were the cropping phases of the short and long rotations that caused the average short and long MAOM-C concentrations to be lower than the concentration for continuous cropping. What is more likely is that the lower average MAOM-C concentrations for short and long rotation were caused by experimental noise. For the MAOM-C concentrations in the 5-10 cm layer this is less likely to be the case because the differences were larger, the standard deviations were smaller, and the trend was in line with the general effect of pastures.

Table 5.1.2. overview MAOM-C concentrations (g/kg) of the cropping and pasture phases of continuous cropping and short and long rotation in the 0-5 cm layer.

CC	SR		LR	
Pasture phase	Cropping phase	Pasture phase	Cropping phase	Pasture phase
22.00	21.03	22.67	20.65	22.36

So the two opposing MAOM-C trends which can be seen in 5.1.1 are probably noise, especially in the 0-5 cm layer. However the MAOM-C differences in the 5-10 cm layer could also indicate enhanced humification in the 5-10 cm layers.

5.2. Carbon sequestration and climate change

Since the start of the experiment in 1994, the soil has been adapting to a new dynamic equilibrium for the organic carbon and nitrogen content. As mentioned earlier, soils can act as sinks (and sources) of atmospheric carbon en thus help to reduce the increase in atmospheric carbon dioxide (CO_2) levels. In this way it can help to mitigate climate change.

Uruguay has an agricultural land area of 14.8 million hectares which can be divided into 13.2 million hectares of permanent pasture and 1.6 million hectares of arable land (FAOSTAT, 2012). Table 5.2.1 shows the division of arable land into temporary pastures (less than five years), temporary crop area (used for crops that have a growing cycle of less than one year) and fallow land (at least one growing season but less than five years). This arable land category should coincide with the long rotation, short rotation and continuous cropping areas combined. It is hard to separate the data between the three treatments. The temporary crop area could for instance belong to continuous cropping but also the cropping phases of both pasture rotations. No tillage was performed at 655.1 $*10^3$ hectares in Uruguay in 2008, which was about 52,8 percent of the temporary crop area (FAO AQUASTAT, 2012).

Table 5.2.1: overview of land use in Uruguay: the temporary crops area consists of all land used for crops with a less than one-year growing cycle which are harvested each year. Arable land consists of temporary crops but also temporary pastures (less than 5 years), fallow land. As a reference value the permanent meadow and pasture area is also displayed. The data comes from FAOSTAT (2012).

Year	Arable land area (10^3 ha)	Temporary pastures (>5 years) (10^3 ha)	Temporary crop area (10^3 ha)	Fallow land (10^3 ha)
2008	1661	321	1240	100

If the results from this research would be used to determine the best cropping system to use in Uruguay with respect to SOC levels, this would consider the whole arable land area. That is why the differences in carbon stock were calculated for this area. The results are displayed in table 5.2.2. Note that the unit kiloton or gigagram equals 10^9 gram.

Table 5.2.2: Theoretical significant carbon sequestration in kiloton or gigagram after 18 years if the whole arable land area of Uruguay was converted from continuous cropping (no tillage) to short or long pasture rotation.

	Coarse POM-C Sequestration	POM-C sequestration
Short rotation	39.0	25.4
Long rotation	84.4	197.0

The change in carbon stock displayed in table 5.2.2 is not high compared to the values in Petagram (10^{15} gram) mentioned in the introduction. Hence it will not matter much for climate change. POM-C is however closely associated with soil fertility. The enhanced coarse POM-C and total POM-C stock will thus increase soil fertility and be beneficial for agriculture. Furthermore the actual amount of change in the experimental agricultural soils of this research was probably higher than the significant change due to higher variation. So this might mean more sequestration.

5.3. Nitrogen and C:N ratio

The nitrogen results were not reliable. The replication in time was the only factor which significantly influenced the nitrogen levels. This was either caused by something related to the field conditions or to the laboratory analysis. It was not possible to find a valid explanation for these values. If there would be extra time and money available, the best course of action would be to reanalyze some of the most outlying samples with possibly a cheaper laboratory method to verify that at least the measurements were correct. If this is the case a sample could be re-fractionated to check if the results are similar. If this is the case than new samples should be taken for nitrogen, in 2013. For now it was only possible to extract some indications from the current C:N ratios which could be calculated from the data.

The articles of Ernst & Siri-Prieto (2009) and Salvo et al. (2010) can only partly assist in ascertaining what the nitrogen data should have shown. Ernst et al. (2009) found 11% higher total nitrogen content for crop pasture rotation than for continuous cropping. Unfortunately the samples were not fractionated. For the Salvo et al. (2010) research fractionation took place but nitrogen was not sampled.

Table 5.3.1: Overview of several C:N values found in comparable scientific articles. The Cambardella and Elliot data was sampled after 20 years of no tillage wheat cultivation and the Fabrizzi et al. data on a 4 year no tillage corn-wheat rotation.

		Total soil	Coarse POM	Fine POM	Total POM	MAOM
0-7.5 cm	Fabrizzi et al. (2003)	11.1	18.0	11.4	13.9	10.8
7.5-15 cm	Fabrizzi et al. (2003)	10.3	15.9	9.74	11.1	10.2
0-10 cm	Fabrizzi et al. (2003)	10.9	17.5	11.0	13.2	10.7
0-20 cm	Cambardella and Elliott (1992)	11.4			20.1	10.0

Table 5.3.1 shows an overview of C:N values found in comparable scientific articles. The C:N ratios found in Uruguay for the unfractionated soil are about 10-11. Fabrizzi et al. (2003) measured C:N ratios at 0-7.5 and 7.5-15 cm depth at a continuous cropping system without tillage on a Typic Agriudoll[8] at Tandil, Argentina. Converted to a 0-10 cm layer this would be a C:N ratio of 10.9 for the unfractionated soil, 17.8 for coarse POM, 11.1 for fine POM and 10.7 for MAOM. In Sidney

[8] which was the same soil as was used for this experiment

(Nebraska) Cambardella and Elliott (1992) found a C:N ratio of 11.4 for the unfractionated soil, 20.1 for POM and 10.0 for MAOM for the 0-20 cm layer.

The C:N ratios found for this research have a high variability and are often very low when compared to the ratios found in literature. This is the case for all years but especially 2012 and 2011, all fractions but especially coarse and fine POM and all depths but especially the 5-10 cm layer. The C:N ratios found for 2010 are comparable to the ratios found in literature. It had 5 values below 7.5 cm while the 2011 and 2012 had much more of these low values. These four low values were removed and this resulted in the C:N ratios in table 5.3.2 on the next page. The data from Cambardella and Elliot (1992) and Fabrizzi et al. 2003 (table 5.3.1) were taken at treatments comparable to the no tillage continuous cropping treatment of this research (table 5.3.2.). On average coarse POM had the highest C:N ratio followed by fine POM, the total soil and finally MAOM. This is roughly the same as the Fabrizzi et al. (2003) data. The 5-10 cm layer does not show this trend. The total soil C:N values showed no clear trend but at all depths short rotation had a slightly lower C:N ratio and the long rotation C:N ratio was almost the same as continuous cropping. The same was the case for MAOM. The only real differences occurred in coarse and fine POM. Except for the 5-10 layer, short and long rotation show a lower C:N ratio than continuous cropping. When considering that POM is the most susceptible to changes in land use and showed the highest organic carbon concentrations for short and long rotation, the lower C:N ratio could indicate that the no tillage crop pasture treatments not only have more carbon stored in soil organic matter but that this organic matter also has a higher quality.

Table 5.3.2: C:N ratios calculated with the 2010 data

		Total soil	Coarse POM	Fine POM	MAOM
0-5 cm	CC	10.78	17.77	16.32	10.34
	SR	10.30	15.24	14.11	10.00
	LR	10.87	15.59	12.79	10.68
5-10 cm	CC	10.16	9.20	10.14	9.68
	SR	9.88	11.13	11.54	8.49
	LR	10.10	9.92	11.23	9.18
0-10 cm	CC	10.50	15.51	13.70	9.48
	SR	10.11	14.15	13.32	9.24
	LR	10.53	14.11	12.27	9.77
10-20 cm	CC	10.64	.	.	.
	SR	10.03	.	.	.
	LR	11.12	.	.	.

5.4. Future SOC development and research recommendations?

Eighteen year after the experiment started, significant differences between no-tillage continuous cropping, short rotation and long rotation were restricted to the 5-10 cm layer in the coarse POM-C and total POM-C fractions that are most sensitive to land use change. Over time these changes in the sensitive fraction will proliferate into the other more stable fractions through humification and increase the total amount of SOC (Haynes, 2000). However, at this moment no significant differences were found between the total SOC concentrations. As was the case for Ernst & Siri-Prieto (2009) and Salvo et al. (2010), the effect of no-tillage crop-pasture rotation probably still need more time to accumulate. The next section will try to say something about the future development of SOC at the Paysandu experimental site.

Future SOC development

As was written in the introduction (chapter 2) a soil which is closer to its saturation level will have a lower carbon stabilization efficiency (ΔSOC / ΔC input) and shorter sequestration duration (the time from the start of sequestration to when the new steady state level is reached). The soils at the experimental site are rich in carbon and are thus likely to be close to their saturation level. So it could be that a large increase in C input is needed to gain a small increase in SOC.

It is also possible to estimate how long sequestration will take place based on comparable research. The combined effect of no-tillage crop pasture rotation has not yet been quantified, but the effect of switching from conventional tillage to no-tillage and from cropping to pasture has been investigated.

West and Post (2002) quantified potential C sequestration rates for the conversion of conventional tillage (CT) to no-tillage (NT) and enhancing crop rotation complexity. They did this using a global data set of 67 long term agricultural experiments consisting of 276 paired treatments. They found that carbon sequestration after a conversion from CT to NT is likely to peak after 5 to 10 years with SOC attaining a new steady state in 15 to 20 years. On average this conversion can sequester 0.57 ± 0.14 Mg C ha^{-1} y^{-1}. Because the experiment at Paysandu started 18 years ago, little further increase caused by the conversion of conventional tillage to no-tillage can be expected. The data for enhanced rotation complexity were more variable and showed no sequestration peak, but on average a new steady state SOC level was reached in about 40 to 60 years and 0.20 ± 0.12 Mg C ha^{-1} was sequestered annually. The enhanced rotation complexity concerned conversion of a for instance monoculture to rotation cropping or increasing the number of crops in the cropping cycle. No research West and Post (2002) used concerned the incorporation of a pasture phase.

Conant et al. (2001) reviewed 115 studies with over 300 data points on the effects on soil carbon of grassland management and land conversion into grassland. According to them sequestration rates for such conversions are highest in the first 40 years after treatment begins and can persist to a lesser extend afterwards. Conversion of cultivation to pasture could sequester 1.01 Mg C ha^{-1} y^{-1}. This was one the largest sequestration rates they found which was probably due to the previous C depletion caused by cultivation. For this research the soil at the experimental site was not depleted and the saturation deficit was most likely smaller. So the sequestration rate should also be lower. Moreover, the incorporation of a pasture phase to a cropping cycle is not the same as switching from a cropping cycle to a continuous pasture. The actual difference in total SOC between no-tillage continuous cropping and crop pasture rotation measured after 18 years at Paysandu was 1.98 Mg C ha^{-1}, which equals 0.11 Mg C ha^{-1} $year^{-1}$. This value seems realistic considering the Conant et al. (2001) research.

Both West and Post (2002) and Conant et al (2001) arrive at a sequestration duration of the same magnitude: 40-60 years until a new steady state after enhancement of rotation complexity and 40 years for the main sequestration after conversion of cultivated land into pastures. This is also in line with Sauerbeck (2001) who states that a new steady state is reached not more than 50 to 100 years after the improvement of agricultural management. Due to the carbon rich soil at Paysandu it is reasonable to estimate that the sequestration duration will not take longer than 40 years. This is 2.22 times the current experimental time. Because the sequestration rate (Sauerbeck, 2001; West and Six, 2007) is usually higher at the start of the sequestration period, no more than twice the current increase in C stock should be expected: 3.96 Mg C ha^{-1}. Note however that the difference in total SOC on which this is based was not significant. However, this is best possible estimation which could be made with the current data.

Future research recommendations

For future research it would is useful to measure the saturation level at the field site. As was explained above this influences sequestration duration and the carbon stabilization efficiency. This could help to project the future development of the SOC level. This could be done with the method from Stewart et al. (2008).

It would be prudent to keep sampling the 0-5 and 5-10 layers as was done in 2010 and 2012, because the significant changes were found in these upper layers of the soil. However, the results for the 0-5 and 5-10 cm layers were only based on two sample replications, because no more were available. As was discussed earlier in this chapter, more replications could probably have yielded better results for (all) the POM-C concentrations in the 0-5 cm layer and fine POM-C concentrations in the 5-10 cm layer. For future research three replications should be the minimum.

There is probably more to be gained from this data set. The discussion left out the differences between individual crops, but some significant differences were found. The data could therefore be used for other research too.

The failure of the nitrogen measurements made it impossible to make answer research sub question three about the probably enhanced nitrogen levels under crop pasture rotation and its influence on enhanced humification of POM-C into MAOM-C. However, similar to Salvo et al. (2010) indications of enhanced humification under pastures were found. This offers a good subject for future research.

6. Conclusions

The aim of this research was to quantify the difference in soil organic carbon in the unfractionated (or total) soil and its distribution in the fractions coarse particulate organic matter (POM), fine POM and mineral associated matter (MAOM) between continuous cropping (CC) and crop pasture rotations (CPR) with short (two year) and long (four year) pasture durations and no tillage (NT), 18 years after the experiment started (from here on all mentioned treatments were performed without tillage). Additionally, this research aimed to explain changes within the fractions by relating them to differences in the C:N ratios of the soil organic matter fractions. As will be explained below, this aim could only partly be realized.

The differences between continuous cropping, short crop pasture rotation and long crop pasture rotation were only significant for total POM-C ('-C' refers to the carbon in the soil fraction) and coarse POM-C in the 5-10 cm layer. Long crop pasture rotation had the highest coarse POM-C concentration (0.34 g/kg), followed short crop pasture rotation (0.26 g/kg) and continuous cropping (0.20 g/kg). For total POM-C the concentrations were 0.90 g/kg for long crop pasture rotation, 0.62 g/kg for short crop pasture rotation and 0.58 g/kg for continuous cropping. That only the sensitive POM-C fractions showed significant differences and had the highest concentrations under long crop pasture rotation, followed by short crop pasture rotation supported hypothesis two.

Hypothesis one about changes in the SOC concentrations of the unfractionated soil could not be validated. Differences were found but they were not significant. More time is required for these changes to accumulate. The significant differences in the sensitive fractions coarse and total POM-C indicate that changes in the more stable MAOM-C fraction and total SOC will keep accumulating in the coming years. For future research it is important to measure the carbon saturation deficit of the soils at the experimental site at Paysandu in order to know how much more SOC could be sequestered and how much longer further sequestration will take place. Based on comparable scientific literature, it is estimated that sequestration at the experimental site will take place for about 22 more years and that the current changes will double.

The pasture phases of short and long crop pasture rotation had several significantly higher SOC levels in the soil fractions than the cropping phases. In the 0-5 cm layer short crop pasture rotation had a MAOM-C concentration of 22.67 g/kg under pasture and 21.03 g/kg under cropping and long crop pasture rotation had a MAOM-C concentration of 22.36 g/kg under pasture and 21.35 g/kg under cropping. In the 5-10 cm layer long crop pasture rotation had a total POM-C concentration of 1.06 g/kg under pasture and 0.68 g/kg under cropping, short crop pasture rotation had a coarse POM-C concentration of 0.27 g/kg under pasture and 0.26 g/kg under cropping and long crop pasture rotation had a coarse POM-C concentration of 0.44 g/kg under pasture and 0.20 g/kg under cropping. This supports that pastures have a positive effect on the carbon level. This is corroborated by the principle component analysis which showed that the pasture phase of long crop pasture rotation and high soil organic carbon levels were related. Furthermore, in the 5-10 cm layer long crop pasture rotation had a coarse POM-C concentration of 0.57 g/kg at the fourth year pasture plot and a concentration of 0.20 g/kg at the second year pasture plot. So not only did long crop pasture rotation have higher coarse and total POM-C concentrations than short crop pasture rotation, the fourth year of pasture also had significantly higher concentrations than the second year of pasture of long crop pasture rotation. So a long (four year) pasture phase included in a crop rotation system is thus better regarding coarse and total POM-C concentrations than a short (two year) pasture phase.

That significant differences between the three systems were only found in the 5-10 cm layer and not the 0-5 cm layer was probably caused by a higher variability. This was also the case for the fine POM-C concentrations, which had no significant differences, while coarse and total POM-C did. For this research only two replications could be taken from the 0-5 and 5-10 cm layers. To get more significant results at least three replications should be taken.

There were indications that MAOM-C could play a more important role in the layers below 5 cm where rhizodeposition is more important than surface residue deposition. The rhizodeposits could have had a different composition which could have caused more humification. This would oppose the notion that only coarse POM-C is sensitive to land use change. However, it was not possible to give a conclusive answer with the gathered data, so this will have to be done with further research.

The nitrogen data was unfortunately not reliable and the only significant difference it showed was caused by the replications in time. This also caused some of the C:N values to be unrealistically low. However, the 2010 data only had a couple of clearly unreliable C:N ratios and seemed to be relatively accurate compared to C:N ratios found in literature. This data 'indicated' that in the 0-5 and 0-10 cm layers that pasture rotations (especially with a four year pasture phase) not only had higher levels of soil organic carbon (mainly as coarse POM), but that the quality of soil organic matter was also higher because it had lower C:N ratios. Because the nitrogen data was unreliable, it was not possible to validate the third hypothesis about possible enhanced humification from POM-C to MAOM-C under crop pasture duration.

It was possible to extrapolate the significant differences between the total POM-C concentrations from the experimental site to the whole of Uruguay. Hypothetically, if all arable land in Uruguay ($1.7*10^6$ ha)[9] would have been changed from no tillage continuous cropping to short or long rotation in 1994, 25.4 Gg. would be significantly sequestered with short rotation and 197.0 Gg would be sequestered with long rotation. This amount of carbon is not much at the climate change scale but is important to sustain the fertility of Uruguayan agricultural soils.

[9] To make this calculation possible it is assumed that all arable land was used for continuous cropping in 1994, which it was not.

References:

Ashman, M. R., Puri, G., (2002): *Essential soil science: a clear and concise introduction to soil science.* Blackwell Science Ltd, Malden, Massachusetts, USA.

Batjes, N. H., (1996): Total carbon and nitrogen in the soils of the world. European Journal of Soil Science, 47: 151-163.

Baldock, J. A., Skjemstad, J. O., (1999): Organic soil carbon/soil organic matter. In K. I., Peverill, L. A., Sparrow, D. J., Reuter (Eds), *Soil analysis: An interpretation manual*., 159-170, CSIRO Publishing, Collingwood, Victoria, Australia.

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 and Environment, 118: 29-42.

Cambardella, C. A., Elliott, E. T., (1992): Particulate soil organic matter changes across a grassland cultivation sequence. Soil Science Society of America Journal, 56: 777-783.

Campbell, C. A., Bowren, K. E., Schnitzer, M., Zentner, R. P., Townley-Smith, L., (1991): Effect of crop rotations and fertilization on soil biochemical properties in a thick Black Chernozem. Canadian Journal of Soil Science. 71: 337-387.

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

Conant, R.T., Paustian, K., Elliot, E.T., (2001): Grassland Management and Conversion into Grassland: Effects on Soil Carbon. Ecological Applications, 11(2): 343-355.

Davidson, E. A., Ackerman, I. L., (1993): Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry, 20: 161-193.

Díaz-Roselló, R. M., (1992): Evolución de la material orgánica en rotaciones de cultivos con pasturas (Organic matter evolution in crops and pastures rotations). Revista INIA-Uruguay Inv. Agr. 1: 103-110.

Duiker, S. W., Lal, R., (1999): Crop residue and tillage effects on carbon sequestration in a luvisol in central Ohio. Soil & Tillage Research, 52: 73-81.

Ellert, B., H., Bettany, J., R., (1995): Calculation of organic matter and nutrients stored in soils under different contrasting management regimes. Canadian Journal of Soil Science, 75: 529-538.

Ernst, O., Siri-Prieto, G., (2009): Impact of perennial pasture and tillage systems on carbon input and soil quality indicators. Soil & Tillage Research, 105: 260-268.

Essington, M. E., (2004): *Soil and water chemistry: an integrative approach*. CRC Press LCC, Boca Raton, Florida, USA.

Eswaran, H., van den Berg, E., Reich, P., (1993): Organic carbon in soils of the world. Soil Science Society of America Journal, 57: 192-194.

Fabrizzi, K., P., Morón, A., García, F., O., (2003): Soil Carbon and Nitrogen Organic Fractions in Degraded vs. Non-Degraded Mollisols in Argentina. Soil Science Society of America Journal, 67: 1831-1841.

FAOSTAT, 2012: http://faostat.fao.org/site/377/DesktopDefault.aspx?PageID=377#ancor. FAOSTAT, © FAO Statistics Division 2012, accessed on 30 June 2012,

FAO AQUASTAT, 2012: http://www.fao.org/ag/ca/6c.html. CA Adoption Worldwide, AQUASTAT data query, ©2012 FAO of the UN, Commissioned for the exclusive use of FAO – Conservation Agriculture.

Foresight (2011): *The Future of Food and Farming: Challenges and choices for global sustainability: Executive Summary*. The Government Office for Science, London, England.

Franzluebbers, A., J., (2002): Soil organic matter stratification ratio as an indicator of soil quality. Soil Tillage Research, 66: 95-106.

Franzluebbers, A., J., Stuedemann, J., A., (2002): Particulate and non-particulate fractions of soil organic carbon under pastures in the Southern Piedmont USA. Environmental Pollution, 116: 53-62.

Franzluebbers, A. J., (2005): Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. Soil and Tillage Research, 83: 120-147.

Führ, F., Sauerbeck, D., (1968): Decomposition of wheat straw in the field as influenced by cropping and rotation. Isotopes and Radiation in Soil Organic-Matter Studies, 1:241-250. (from Tisdall & Oades, 1982).

García-Préchac, F., Ernst, O., Siri-Prieto, G., Terra, J. A., (2004): Integrating no-till crop pasture rotations in Uruguay. Soil and Tillage Research, 77: 1-13.

Galantini, J. A., Iglesias, J. O., Rossell, R. A., (2004): Calidad y dynámica de las fracciones orgánicas en la region semiárida pampeana. XIX Congresso Argentino de la Ciencia del Suelo. II Symposio Nacional sobre Suelos Vertisólicos, Paraná Argentina CD-rom.

Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K. D., Dixon, J., Dendooven, L., (2009): Conservation Agriculture ans Soil Carbon Sequestration: Between Myth and Farmer Reality. Critical Reviews in Plant Science, 28: 97-122.

Hassink, J., (1997): The capacity of soils to preserve organic C and N by there association with clay and silt particles. Plant Soil, 191: 77-87.

Haynes, R. J., Swift, R. S., Stephen, R. C., (1991): Influence of mixed cropping rotations (pasture arable) on organic matter content, water stable aggregations and clod porosity in a group of soils. Soil and Tillage Research, 19: 77-87.

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

IPCC, (2007): *Climate Change 2007 – Synthesis Report*. IPCC, Geneva, Switzerland.

Jagadamma, S. Lal, R., (2010): Distribution of organic carbon in physical fractions of soils as affected by agricultural management. Biology and Fertility of Soils, 46: 543-554.

Janzen, H. H., Campbell, C. A., Brandt, S. A., Lafond, G. P., Townly-Smith, L., (1992): Light fraction organic matter in soils from long-term crop rotations. Soil Science Society of America Journal, 56: 1799-1806.

Johnson, J. M. F., Franzluebbers, A. J., Lachnicht Weyers, S., Reicosky, D. C., (2007): Agricultural opportunities to mitigate greenhouse gas emissions. Environmental Pollution, 150: 107-124.

Kuzyakov, Y., Domanski, G., (2000): Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science, 163: 421-431.

Lal, R., (2008): Carbon sequestration. Philosophical Transactions of the Royal Society B, 363: 815-830.

Lee, J., Hopmans, J., W., Rolston, D., E., Baer, S., G., Six, J., (2009): Determining soil carbon stock changes: Simple bulk density corrections fail. Agriculture, Ecosystems and Environment, 134: 251-256.

Morón, A., Sawchik, J., (2003): Soil quality indicators in a long-term crop-pasture rotation experiment in Uruguay. 40 años de rotaciones agrícolas-ganaderas, 134: 67-76.

Perdomo, C., Irisarri, P., Ernst, O., (2009): Nitrous oxide emissions from an Uruguayan argiudoll under different tillage and rotation treatments. Nutrient Cycling in Agroecosystems, 84: 119-128.

Post Carbon Institute, (2009): *The Food and Farming Transition.* Post Carbon Institute, Sebastopol, USA.

Rees, R. M., Bingham, I. J., Baddeley, J. A., Watson, C. A., (2005): The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. Geoderma, 128: 130-154.

Salvo, L., Hernandez, J., Ernst, O., (2010): Distribution of soil organic carbon in different size fractions, under pasture and crop rotations with conventional tillage and no-till systems. Soil & Tillage Research, 109: 116-122.

Sauerbeck, D, R., (2001): CO2 emissions and C sequestration by agriculture – perspectives and limitations. Nutrient Cycling in Agroecosystems, 60: 253–266.

Schumacher, B. A., (2002): *Methods for the determination of total organic carbon (TOC) in soils and sediments*. United States Environmental Protection Agenacy, Las Vegas, USA.

Schuur, E. A. G., Bockheim, J., Canadell, J. G., Euskirchen, E., Field, C. B., Goryachkin, S. V., Hagemann, S., Kuhry, P., Lafleur, P. M., Lee, H., Mazhitova, G., Nelson, F. E., Rinke, A., Romanovsky, V. E., Shiklomanov, N., Tarnocai, C., Venevsky, S, Vogel, J. G., Zimov, S. A., (2008): Vulnerability of Permafrost Carbon to Climate Change: Implications for the Global Carbon Cycle. BioScience, 58: 701-714.

Schuur, E. A. G., Vogel, J. G., Crummer, K. G., Lee, H., Sickman, J. O., Osterkamp, T. E., (2009): The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature, 459: 556-559.

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: 155-176.

Soil Science Society of America, (1996): *Methods of Soil Analysis: Part 3 Chemical Methods*, Soil Science Society of America Book Series, Madison, Wisconsin, USA.

Solberg, E. D., Nyborg, M., Izaurralde R. C., Malhi, S. S., Janzen, H. H., Molina-Ayala, M., (1997): Carbon storage in soils under continuous cereal grain cropping: N fertilizers and straw. In R., Lal, J. M., Kimble, R. F., Follet, B. A., Stewart (eds.), *Management of Carbon Sequestration in Soil*., 235-254, CRC Press, Boca Raton, Florida, USA.

Stewart, C. E., Paustian, K., Conant, R. T., Plante, A. F., Six, J., (2008): Soil carbon saturation: Evaluation and corroboration by long-term incubations. Soil Biology & Biochemistry, 40: 1741-1750.

Sustainable Development program website of Utrecht University: http://www.uu.nl/university/masters/nl/susdev/studyprogramme/Pages/default.aspx

Tisdall, J. M., Oades, J. M., (1982): Organic matter and water stable aggregates. Journal of Soil Science, 33: 141-163.

Trujillo, W., Fisher, M. J., Lal, R., (2006): Root dynamics of native savanna and introduced pastures in the Eastern Plains of Colombia, Soil & Tillage Research, 87: 28-38.

United States Department of Agriculture - Natural Resources Conservation Service, 2004: *Soil Survey Laboratory Methods Manual: Soil Survey Investigations Report*, No 42, version 4.0, USDA-NRCS, Lincoln, Nebraska, USA.

West, T.O., Post, W.M., (2002): Soil Organic Carbon Sequestration Rates by Tillage and Crop Rotation: A Global Data Analysis. Soil Science Society of America Journal, 66: 1930-1946.

West, T. O., Six, J., (2007): Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. Climatic Change, 80: 25-41.

Appendices

A: List of abbreviations

B: MAOM calculation

C: Carbon stock calculation

D: Statistics

E: Principle component analysis

F: Accuracy of the CN measurement

G: Carbon data

H: Nitrogen data

I: C:N ratios

Appendix A: List of abbreviations

BD: Soil bulk density

C: Carbon

CC-NT: Continuous cropping no tillage

MAOM: Mineral associated organic matter

LR-CT: Long rotation with conventional tillage

LR-NT: Long rotation no tillage

N: Nitrogen

P2: Second year of pasture

P4: Fourth year of pasture

POM: Particulate organic matter, can be divided into coarse (CPOM) and fine POM (POM)

CPOM: Coarse particulate organic matter

FPOM: Fine particulate organic matter

Sb1: Soybean as first and only crop after a fall period. In the cropping cycle this crop is grown two years after soybean as a second crop.

Sb2: Soybean as second crop (cultivated after wheat). In the cropping cycle this crop is grown two years before soybean as a first crop.

Sd: Saturation deficit

Sg: Sorghum

SOC: Soil organic carbon

SOM: Soil organic matter

SR-NT: Short rotation no tillage

TSOC: Total soil organic carbon: used in relation with the fractions of soil organic carbon. The SOC fractions coarse POM, fine POM and MAOM together form TSOC.

Appendix B: MAOM calculations

The alternative for measuring the organic carbon and nitrogen in MAOM is to calculate their values by subtracting the coarse and fine POM values from the total soil values, since coarse POM + fine POM + MAOM = TSOC/N. To calculate and not measure MAOM is a widely applied procedure because it is far easier than to prepare and analyze the MAOM fractions for all samples. That is why for this research the unfractionated soil was analyzed with dry combustion to measure TSOC and calculate MAOM.

However, Lucía Salvo[10] found that when comparing the total amount of soil organic carbon (SOC) found in the soil with the summed amount in the fractions, an average of 10 percent of the total SOC was missing (unpublished data). It was assumed that this missing 10 percent belonged to the MAOM fraction. Ten percent is a lot especially compared to the amount of coarse and fine POM which Salvo found to be around 4 and 6 percent of the total SOC respectively. It was not possible to obtain data about the variability of this 10 percent.

Finding out exactly where the 10 percent of carbon belonged to is a whole separate research project. To get an insight in the variability and possibly the source of the missing carbon it was decided to analyze 20 MAOM samples and compare them with the calculated MAOM values.

The results of this analysis are shown in table B.1. The summed fractions contained 9.55% less SOC than the unfractionated soil. The sample standard deviation was 15.02%. The missing 9.55% was in line with Salvo's findings, but the sample standard deviation was high. Also for some samples more SOC was found in the summed fractions than in the total soil. There is less nitrogen missing but the variability is still high.

Table B.1: Average percentage of missing soil, soil organic carbon and nitrogen between the unfractionated soil and the summed fractions coarse POM, fine POM and MAOM. Positive values reflect missing amounts. Note that in the process of fractionation half a gram of hexamethaphosphate (in a solution) was added to disperse the sample.

	MAOM soil weight	MAOM carbon	MAOM nitrogen
Average amount in whole sample (30 grams soil)	30 g	0.6 g	0.06 g
Percentage missing	-0.77 %	9.55 %	1.38 %
Sample std	3.28 %	15.02 %	15.49 %

What could explain the carbon and nitrogen loss?

1. *Lost during the fractionation:* There should not be any soil lost or gained during fractionation. The 'system' was closed and the fractionation was performed with great care to ensure that no residues remained on the meshes. Some small sand and silt grains were always stuck in the mesh, because it was very fine. However the amount stayed the same and was negligible. The initial 30 grams were weighed on a balance with an accuracy of 0.01 gram accuracy. The summed fraction soil weight did not attain this level of accuracy. On average its weight was 0.77% higher than the initial 30 grams (0.233 g.). So the samples gained weight. This can partly be explained by the fact that during dispersion a solution of hexametaphosphate was used. The water was evaporated but the 100 ml added solution should add 0.5 gram. The sample standard deviation of 3.28% (0.99 gram) was also higher than expected. However this does not explain the missing carbon and nitrogen.
2. *Left in the evaporation beaker:* The MAOM residue was very hard to remove from the 2 liter beakers. Figure B.1. (on the next page) shows one of the beakers with residue (left and center) and the same beaker after about one hour of scraping (right). The residue in the beakers could be divided into three parts. At the bottom of the beaker was a hard and carbon and nitrogen rich[11] (black) layer topped with sedimented mineral material (whitish). These two layers were fairly easy

[10] During her research which resulted in the published paper from Salvo et al .(2010).

[11] That this layer was carbon and nitrogen rich could be deduced from the fact that is was black and thus had a high level of soil organic matter which contained the carbon and nitrogen.

to remove. The third part consisted of carbon and nitrogen rich material bound to the sides of the beaker. It was impossible to completely remove this part of the residue. On average 0.16 grams of relatively carbon rich material remained in the beaker. So this was not in the measurement and might explain the difference.

3. *The MAOM sample not homogeneous:* After the removal of the residue the recovered MAOM fraction was put into two grinding flasks, because it did not fit into one. Care was taken to divide the carbon rich fraction equally between the two flask. But this was difficult. So the concentration in the two flasks after grinding might have differed. The contents of the two flasks were mixed after grinding. But they had the same color so it was hard to see when sample was well mixed. Also because the carbon layer at the bottom formed a hard crust, there might have been very small carbon chips left after grinding. This was of course checked and none were found.
4. *Biochemical processes during evaporation of the water in the MAOM sample:* Drying the MAOM samples after fractionation in an air forced oven took several days at best. During this time biochemical processes concerning carbon and nitrogen could have taken place. However the soil microbes were removed with the initial drying, so this also seems unlikely.

Figure B.1: Beakers used to dry the >50 µm (MAOM) fractions.

From which fraction does the missing carbon and nitrogen originate?

The missing carbon and nitrogen could have belonged to the POM fraction. It could have been lost in the process of fractionation if either a) not all coarse and fine POM material was transferred from the meshes to the petri dishes or b) if decomposition took place especially while the sample came in contact with water. The first was checked by sight, any POM remaining on the mesh should have been visible by its black color. This was checked and if any POM was found it was transferred to the petri dish. The second is unlikely since the soil microbes were removed during the initial drying and the drying of POM after fractionation took only half a day at 60^{o} C. So contact with water was limited. It is more probable that the missing substances were in the residue that was left in the evaporation beaker and that they were part of the MAOM. What also could be possible is that the summed fractions were measured correctly but that the total sample measurement was not. However it is more likely that the error originated from the fractions since they were fractionated. The more complex the processing the higher the chance of errors. Furthermore all samples were treated the same except for the fractionation. It is also possible that both the summed fractions and the total sample were measured correctly but that the sample bag (with the twenty crushed soil cores) was not well mixed. Soil carbon is distributed heterogeneously in soils. The sample that fractionated could have had an inherently different carbon and nitrogen level than the sample that was not fractionated. However great care was taken to mix the soil in the sample bag. So it is unlikely that this is the explanation.

After taking this all into account it can be concluded that it is the most likely that the carbon and nitrogen were lost during the drying and processing of the MAOM sample and it is thus justifiable to calculate MAOM by subtracting coarse and fine POM from the total organic carbon and nitrogen content of the soil sample. In the few cases where the value of summed fractions exceeded the whole sample value, there might have been an inherent difference in the sample that was fractionated and the one that was not.

Appendix C: Carbon stock calculations

There are two main ways of calculating the carbon stock. The fixed depth method (FD) which uses the bulk density and the concentration of a fixed depth layer to calculate the carbon stock in that layer.

$$C_{i,fixed} = conc_i * \rho_i * T_i * 10^4$$

Where C is the carbon stock (kg C ha^{-1}), ρ is the bulk density (Mg m^{-3}), $conc_i$ is the carbon concentration (kg C Mg^{-1}) and 10^4 is a unit conversion factor (m^2 ha^{-1}).

However bulk density has a high spatial and temporal variability. Using the fixed depth method can lead to a comparison of unequal soil masses (Ellart & Bettany, 1995). A soil with a lower bulk density has less soil mass in a layer of fixed thickness. So if one would calculate the carbon stock right before and after tillage for the 0-20 cm layer. The resulting lower bulk density would show an decrease in carbon stock, while the amount of carbon did not change at all. The equivalent soil mass method (ESM) compares the carbon stock not between layers with a fixed size but with an equivalent soil mass.

$$M_i = \rho_i * T_i * 10^4$$

Where M_i is dry soil mass (Mg ha^{-1})

$$M_{i,add} = M_{i,equiv} - M_i$$

$$C_{i,equiv} = C_{i,fixed} - conc_{top} * M_{i-1,add} + conc_{bottom} * (M_{i,add} - M_{i-1,add})$$

Where $C_{i,equiv}$ is the equivalent soil carbon (Mg C ha^{-1}), $M_{i-1,add}$ is the mass subtracted from this layer to match the upper layer to its equivalent soil mass and $M_{i,add}$ is the mass of the layer added to make it match the equivalent soil mass.

For the equivalent soil mass (M_{equiv}) method a reference soil mass is selected for each soil layer. This can be the highest soil mass, the lowest or the original soil mass which was sampled before the treatment. Then the soil mass (M_i) is calculated for the layers (n=i) of a soil. A lower bulk density will result in a lower soil mass. The difference between $M_{i,equiv}$ and M_i is the mass that must be added from the layer below.

Lee et al. (2009) used a simulation and field observations to compare the FD method and equivalent soil mass method using the minimum, maximum and original soil mass as a reference. They concluded that the fixed depth method resulted in biased carbon stocks and that for soils with a relatively uniform distribution of carbon concentration, the maximum ESM method was appropriate when bulk density decreased and the minimum ESM method was appropriate when bulk density increased. For soils with a non-uniform carbon distribution the minimum ESM was best. The original ESM method is best under all circumstances. When bulk density has a large spatial and temporal variation or has a great uncertainty, carbon concentrations could describe the changes more accurately than the fixed depth method.

In the case of this research, no original bulk density data was available, no change caused by the land use was expected, no significant differences were found and the variability was high. Using the bulk density for each plot would only incorporate its variability into the carbon stock and if the average is used then all the data have the same multiplier so it would not change anything. That was why it was decided to use the carbon concentrations to describe the changes in soil carbon and to use an average bulk density only for the fixed depth method to reflect changes in carbon stock for the purpose of calculation carbon sequestration.

Appendix D: Statistics

A standard model for factorial mixed ANOVA was constructed.

Standard model: $y_{ijk} = \mu + \gamma_j + \tau_i + \varepsilon_{ijk}$

Where y_{ijk} is the outcome value of dependent variable, μ is the mean value, γ is the random effect of the years, τ is the fixed effect of the treatments and ε is the experimental error.

Using SAS GLM it was checked that the years were independent. As explained in the methodology chapter, a covariable model was also constructed to reduce the noise of related to different carbon inputs. Aboveground dry matter yield was measured at the experimental site and could be used as a covariable. In this way it was possible to get more significant results from the data.

Covariable model: $y_{ijk} = \mu + \gamma_j + \tau_i + \beta(x_i - \bar{x}) + \varepsilon_{ijk}$

Where y_{ijk} is the outcome value of dependent variable, μ is the mean value, y is the random effect of year, *τ is the* fixed effect of the treatment, $\beta(x_i - \bar{x})$ is the covariable with regression coefficient *β* and *ε:* experimental error.

The covariable model assumes that:

1) Dry matter yield has a linear relation with soil organic carbon input (the amount of residues and roots that stay behind at the plot to be incorporated into the soil). Duiker and Lal (1999) showed a linear relation between residue application and SOC sequestration with plow tillage and no tillage in Ohio.
2) Dry matter contains about the same amount of carbon for all types of crops and pasture. This is true for the carbon content per grams of dry matter. However, in the case of pasture, the same amount of dry matter yield from grass reflects more input of carbon than crops. Grasses have a larger root-shoot ratio compared to crops (Trujillo et al; 2006). So for the same amount of dry matter yield above ground there is a higher carbon input below ground from the root zone. This would suggest that the covariate model is more appropriate for the top layers and less for the lower layer where roots become a more important input factor.
3) There is an average yield for all the plots. However, since carbon is a fertility indicator it will influence the crop yield in the long run for instance 50 years when differences in carbon concentrations become more pronounced. The different dry matter yields are then not only caused by weather or pests but also by the carbon concentration. This would make the covariable model counterproductive.

The covariate model is not a perfect tool and will have to be improved. Other factors such as the weather or fertilizer input could be added. Also the way of calculating carbon input should be more refined. The most important results are also significant without the covariable model, but the covariable also points out to some other effects that might be significant.

Other statistical options were also considered but in the end not used for this report:

- 3 main treatments (LRNT SRNT and CCNT) with the indivual years as nested effects, but this was not possible because the nested effects were unbalanced: LRNT has seven years, SRNT has five and CCNT has three.
- If the principle component analysis would describe several factors a multivariate model would have been run.
- A linear regression model between the years (ΔSOC=carbon stock + input crops). The years were however not significantly different (over a longer period this might be possible).

Appendix E: Principle component analysis

A principle component analysis was performed to describe the relations between the treatments and the variables. Table E.1 shows the factors and their Eigenvalues and proportion of variance which are explained by the factors. The variables from the 0-5 and 5-10 cm layers were omitted to simplify the PCA. This omission resulted in a five percent increase in the accounted variance by the second factor. Factor one (CP1) described 46,4 percent of the variance and factor two 30,7 percent. Together they accounted for 77,1 percent of the variance. The other factors all had an eigenvalue value less than one and accounted for little variance. They were thus not meaningful and could be dismissed.

Table E.1: Overview of the SOC principle component analysis.

Factor	Eigenvalue	Proportion	Cumulative proportion
1	2.32	0.46	0.46
2	2.53	0.31	0.77*
3	0.86	0.17	0.94
4	0.29	0.06	1.00
5	0.00	0.00	1.00

Table E.2. Shows that total soil organic carbon (TSOC) at 0-10 and 10-20 centimeter depth was mainly influenced by principle component one while and POM and MAOM were influenced by both components. Component one had a positive relation with all variables while principle component two had a negative relation with coarse (C) and fine (F) particulate organic matter (POM) and a positive relation with mineral associated organic matter (MAOM).

Table E.2: Amount of variance described by the first and second principle component. Note that the cophenetic correlation coefficient = *0.935* which shows that the dimension reduction has a low distortion.

Variables	Principle component 1	Principle component 2
TSOC010	0.92	0.33
TSOC1020	0.48	0.16
CPOM010	0.75	-0.55
FPOM010	0.62	-0.68
MAOM010	0.56	0.80

Appendix F: Weighting procedure and accuracy of the CN measurement

The samples were weighted in cups in Montevideo (Uruguay) and analyzed in Utrecht (the Netherlands). The cups were made of delicate tin foil. The weighting proceeded as follows. A cup were placed on a microgram balance which was calibrated to zero µg with the cup on it. Then about 10 mg of soil was put into the cup. It was closed by folding it into a ball and the exact weight with a µg precision was noted. Just before the samples were sent to the Netherlands it was discovered that some of the cups were damaged and some of the contents had spread through three of the boxes that contained the samples. It was not possible to see which cups where damaged only that soil material had spread throughout the boxes. The boxes had to be cleaned and most samples had to be weighted again. Next to soil samples, standard samples containing acetanilide and atropine of which the carbon and nitrogen content were known were weighted to calibrate the CN-analyzer. Nicotinamide of which the carbon and nitrogen content was also known was used as a control sample to check the accuracy of the measurements. The results are presented in table F.1. Between 2.5 and 0.5 µg of acetanilide, atropine and nicotinamide was weighted for the control and calibration samples.

Table F.1: Measurement accuracy: Nicotinamide was used to check the accuracy of the measurements. One nicotinamide sample was analyzed after 12 soil samples. The average C and N content of the samples is expressed as weight percentage of the sample.

	N measured	N real	C measured	C real	CN measured	CN real
Average (%)	22,981	22,956	58,823	58,989	2,560	2,570
Sample std.	0,816	0,028	1,786	0,056	0,056	.
Coeff Var	3,550	0,122	3,037	0,095	2,180	.
Min % off	-8,926	.	-10,057	.	-4,051	.
Max % off	6,093	.	2,274	.	3,871	.

In table F.1. the measured C and N content are expressed as weight percentages of the control samples. The sample standard deviations were small but the minimum percentage of error for nitrogen and carbon were relatively high: 9 and 10 percent, respectively. This might have been caused by spillage from damaged cups. The substances used for the calibration and control samples were very fine and white and a had very high carbon and nitrogen content. A very small amount of particles, which would be difficult to detect could have caused a contamination and offset the measurements. They could also have been damaged during the transport of the samples to the Netherlands. If such a thing occurred it might have offset the samples but nothing was detected, when the samples arrived in the Netherlands.

Appendix G: Carbon data

Carbon concentration data in g/kg soil. The treatment code consist of 'rotation type' 'tillage type' and 'crop'.

Plot	Treatment code	Year	0-5 cm layer					5-10 cm layer					0-10 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
17	CC NT Sb2	2010	26.17	1.65	1.59	3.24	22.94	18.56	0.35	0.29	0.64	17.93	22.37	1.07	0.89	1.96	20.41	16.97
09	CC NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	22.57	0.99	1.43	2.42	20.15	17.43
02	CC NT Sb2	2012	22.55	1.04	0.37	1.41	21.14	16.40	0.15	0.37	0.52	15.88	19.47	0.56	0.37	0.93	18.55	16.75
02	CC NT Sg	2010	22.06	0.66	1.95	2.61	19.45	16.74	0.10	0.44	0.54	16.21	19.40	0.38	1.19	1.56	17.84	17.80
17	CC NT Sg	2011	.	.	.	.	.	.	.	.	.	.	21.37	0.41	0.89	1.30	20.08	19.13
09	CC NT Sg	2012	21.56	1.39	1.05	2.44	19.12	19.67	0.23	0.67	0.90	18.78	20.62	0.78	0.86	1.64	18.98	14.03
09	CC NT Sb1	2010	23.73	0.98	0.73	1.71	22.01	19.66	0.17	0.16	0.33	19.32	21.69	0.53	0.44	0.98	20.72	19.79
02	CC NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	19.22	0.34	0.62	0.96	18.26	15.50
17	CC NT Sb1	2012	29.06	0.71	1.26	1.97	27.08	17.39	0.19	0.34	0.54	16.85	23.22	0.42	0.82	1.24	21.98	20.31
01	LR NT Sb2	2010	23.74	3.32	1.27	4.59	19.15	16.65	0.20	0.27	0.47	16.18	20.19	1.77	0.80	2.57	17.63	16.62
16	LR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	21.11	0.52	1.30	1.82	19.29	18.66
11	LR NT Sb2	2012	22.70	1.80	1.40	3.20	19.50	18.19	0.28	0.72	1.00	17.19	20.44	0.95	1.06	2.00	18.44	18.35
04	LR NT Sg	2010	23.78	0.42	1.54	1.97	21.81	19.44	0.07	0.50	0.58	18.86	21.61	0.23	1.01	1.24	20.37	16.91
08	LR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	19.45	0.30	0.56	0.87	18.59	15.20
01	LR NT Sg	2012	29.56	3.93	1.37	5.30	24.25	19.30	0.19	0.45	0.64	18.65	24.43	1.65	0.89	2.54	21.89	15.72
11	LR NT Sb1	2010	23.54	0.80	1.00	1.79	21.75	16.62	0.23	0.52	0.75	15.87	20.08	0.51	0.76	1.27	18.81	17.79
03	LR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	21.35	0.49	1.17	1.66	19.69	19.23
12	LR NT Sb1	2012	20.68	1.00	2.24	3.24	17.44	17.50	0.23	0.42	0.65	16.84	19.09	0.57	1.25	1.82	17.27	15.78
12	LR NT P2	2010	28.95	1.54	2.51	4.05	24.89	21.36	0.30	0.50	0.80	20.56	25.15	0.90	1.43	2.33	22.82	20.39
04	LR NT P2	2011	.	.	.	.	.	.	.	.	.	.	20.58	0.65	0.80	1.45	19.13	19.70
08	LR NT P2	2012	24.48	1.73	2.61	4.34	20.14	17.43	0.32	0.63	0.95	16.48	20.96	0.99	1.61	2.59	18.36	18.43
03	LR NT P4	2010	27.65	2.58	1.68	4.26	23.39	21.23	0.62	0.43	1.05	20.18	24.44	1.56	1.03	2.60	21.84	18.01
12	LR NT P4	2011	.	.	.	.	.	.	.	.	.	.	21.79	1.14	1.18	2.31	19.48	15.12
04	LR NT P4	2012	24.17	1.36	1.80	3.16	21.01	25.40	0.51	0.93	1.44	23.96	24.79	0.92	1.36	2.28	22.50	19.30

Plot	Treatment code	Year	0-5 cm layer					5-10 cm layer					0-10 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
14	SR NT Sb2	2010	23.77	0.72	0.71	1.43	22.34	20.91	0.22	0.71	0.93	19.99	22.34	0.46	0.72	1.18	21.16	17.67
13	SR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	20.94	0.54	1.07	1.61	19.33	20.15
10	SR NT Sb2	2012	27.49	1.42	1.69	3.12	24.37	18.20	0.39	0.14	0.53	17.67	22.85	0.83	1.13	1.96	20.89	15.25
10	SR NT Sg	2010	25.01	2.72	1.54	4.25	20.76	18.44	0.22	0.47	0.69	17.75	21.73	1.56	1.00	2.57	19.16	19.64
07	SR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	20.91	0.39	0.86	1.25	19.66	19.89
18	SR NT Sg	2012	24.49	1.29	1.26	2.54	21.95	19.07	0.28	0.14	0.43	18.64	21.78	0.82	0.66	1.48	20.29	17.59
18	SR NT Sb1	2010	20.78	0.72	0.98	1.70	19.08	16.50	0.15	0.35	0.50	16.01	18.64	0.43	0.67	1.09	17.55	15.85
14	SR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	21.21	0.53	1.03	1.56	19.65	18.16
13	SR NT Sb1	2012	20.79	1.26	1.85	3.11	17.68	20.53	0.30	0.29	0.58	19.95	20.66	0.80	1.03	1.84	18.83	16.06
07	SR NT P2	2010	27.78	1.31	1.56	2.87	24.92	20.89	0.25	0.47	0.72	20.17	24.34	0.73	1.05	1.78	22.56	18.88
18	SR NT P2	2011	.	.	.	.	.	.	.	.	.	.	17.65	0.66	0.72	1.38	16.27	15.24
14	SR NT P2	2012	23.62	1.31	1.88	3.19	20.43	15.63	0.29	0.26	0.55	15.08	19.63	0.78	1.12	1.91	17.72	17.81
x	LR CT P2	2010	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	LR CT P2	2011	.	.	.	.	.	.	.	.	.	.	21.11	1.03	0.62	1.65	19.47	20.11
05	LR CT P2	2012	22.30	1.77	1.62	3.39	18.90	19.20	0.29	1.02	1.31	17.89	20.75	0.90	1.31	2.21	18.53	19.08
C	Control Pasture	2012	27.20	3.09	2.52	5.61	21.60	18.05	0.77	0.61	1.39	16.66	22.63	1.81	1.55	3.36	19.27	15.15

Appendix H: Nitrogen data

Nitrogen concentration data in g/kg soil. The treatment code consist of 'rotation type' 'tillage type' and 'crop'.

Plot 2012	Treatment	Year	0-5 cm layer					5-10 cm layer					0-10 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
17	CC NT Sb2	2010	2.42	0.08	0.09	0.17	2.25	1.96	0.04	0.05	0.09	1.87	2.19	0.06	0.07	0.13	2.06	1.81
09	CC NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	1.78	0.06	0.10	0.15	1.63	1.25
02	CC NT Sb2	2012	2.28	0.08	0.04	0.12	2.17	1.70	0.02	0.04	0.05	1.65	1.99	0.04	0.04	0.08	1.91	1.58
02	CC NT Sg	2010	2.23	0.04	0.13	0.16	2.06	1.70	0.01	0.04	0.05	1.65	1.96	0.02	0.08	0.11	1.86	1.59
17	CC NT Sg	2011	.	.	.	.	.	.	.	.	.	.	2.11	0.07	0.14	0.21	1.90	1.71
09	CC NT Sg	2012	2.08	0.13	0.21	0.34	1.74	1.85	0.05	0.14	0.19	1.65	1.96	0.09	0.17	0.27	1.70	1.59
09	CC NT Sb1	2010	2.04	0.06	0.05	0.11	1.93	1.76	0.02	0.02	0.04	1.73	1.90	0.04	0.03	0.07	1.83	1.75
02	CC NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	1.90	0.08	0.14	0.22	1.68	1.56
17	CC NT Sb1	2012	2.40	0.38	0.18	0.57	1.83	1.33	0.05	0.03	0.08	1.25	1.86	0.19	0.11	0.30	1.56	1.77
01	LR NT Sb2	2010	2.42	0.15	0.09	0.24	2.18	1.80	0.02	0.03	0.05	1.75	2.11	0.09	0.06	0.15	1.96	1.59
16	LR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	2.12	0.09	0.19	0.28	1.84	1.52
11	LR NT Sb2	2012	2.02	0.10	0.20	0.30	1.72	1.33	0.06	0.06	0.12	1.21	1.67	0.08	0.13	0.21	1.46	1.21
04	LR NT Sg	2010	2.39	0.10	0.19	0.29	2.10	1.98	0.01	0.04	0.05	1.93	2.18	0.05	0.11	0.16	2.02	1.62
08	LR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	1.74	0.09	0.16	0.24	1.49	1.69
01	LR NT Sg	2012	2.59	0.28	0.09	0.37	2.22	1.49	0.02	0.14	0.15	1.33	2.04	0.12	0.12	0.23	1.80	1.61
11	LR NT Sb1	2010	2.18	0.05	0.07	0.12	2.06	1.74	0.02	0.05	0.08	1.67	1.96	0.03	0.06	0.10	1.86	1.40
03	LR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	2.19	0.09	0.16	0.25	1.93	1.65
12	LR NT Sb1	2012	1.87	0.10	0.27	0.37	1.50	1.66	0.06	0.04	0.10	1.56	1.77	0.08	0.14	0.22	1.54	1.51
12	LR NT P2	2010	2.46	0.12	0.18	0.30	2.16	2.00	0.05	0.05	0.10	1.90	2.23	0.08	0.11	0.19	2.04	1.73
04	LR NT P2	2011	.	.	.	.	.	.	.	.	.	.	1.53	0.04	0.06	0.10	1.43	1.83
08	LR NT P2	2012	1.92	0.10	0.27	0.37	1.55	1.58	0.02	0.06	0.08	1.50	1.75	0.06	0.16	0.22	1.53	1.69
03	LR NT P4	2010	2.30	0.26	0.11	0.38	1.92	1.90	0.05	0.03	0.08	1.81	2.10	0.15	0.07	0.22	1.87	1.77
12	LR NT P4	2011	.	.	.	.	.	.	.	.	.	.	1.75	0.07	0.09	0.16	1.59	1.57
04	LR NT P4	2012	1.37	0.27	0.29	0.56	0.80	2.16	0.07	0.08	0.15	2.02	1.77	0.17	0.18	0.35	1.42	1.64

Plot	Treatment	Year	0-5 cm layer					5-10 cm layer					0-10 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
14	SR NT Sb2	2010	2.28	0.04	0.06	0.10	2.18	1.91	0.02	0.06	0.08	1.83	2.09	0.03	0.06	0.09	2.00	1.68
13	SR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	1.91	0.04	0.17	0.21	1.71	1.36
10	SR NT Sb2	2012	2.35	0.11	0.17	0.28	2.07	1.76	0.06	0.12	0.18	1.58	2.06	0.08	0.15	0.24	1.82	1.77
10	SR NT Sg	2010	2.51	0.25	0.10	0.35	2.16	1.95	0.02	0.04	0.07	1.88	2.23	0.15	0.07	0.21	2.01	1.76
07	SR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	1.77	0.02	0.07	0.09	1.68	1.52
18	SR NT Sg	2012	2.43	0.14	0.20	0.34	2.09	1.88	0.07	0.11	0.18	1.71	2.16	0.11	0.15	0.26	1.90	1.41
18	SR NT Sb1	2010	2.13	0.05	0.07	0.11	2.02	1.85	0.02	0.03	0.05	1.80	1.99	0.03	0.05	0.08	1.91	1.85
14	SR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	1.75	0.04	0.08	0.11	1.64	1.45
13	SR NT Sb1	2012	2.25	0.14	0.25	0.38	1.86	1.93	0.07	0.15	0.22	1.72	2.09	0.10	0.20	0.30	1.79	1.67
07	SR NT P2	2010	2.52	0.07	0.12	0.19	2.33	2.05	0.02	0.04	0.06	1.99	2.29	0.04	0.08	0.12	2.16	1.91
18	SR NT P2	2011	.	.	.	.	.	.	.	.	.	.	1.81	0.04	0.16	0.20	1.61	1.16
14	SR NT P2	2012	1.92	0.07	0.23	0.30	1.61	1.72	0.06	0.14	0.21	1.51	1.82	0.07	0.19	0.26	1.56	1.30
x	LR CT P2	2010	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	LR CT P2	2011	.	.	.	.	.	.	.	.	.	.	1.95	0.09	0.05	0.14	1.81	1.56
05	LR CT P2	2012	1.85	0.11	0.23	0.33	1.51	1.99	0.02	0.08	0.10	1.51	1.92	0.06	0.15	0.20	1.71	1.73
C	Control Pasture	2012	2.38	0.20	0.27	0.47	1.91	1.96	0.07	0.16	0.24	1.91	2.17	0.13	0.21	0.35	1.91	1.82

Appendix I: C:N ratios

Sample C:N ratios. The treatment code consist of 'rotation type' 'tillage type' and 'crop'. C:N ratios below 7.5 were considered to be unrealistically low and depicted in bold letters.

Plot	Treatment code	Year	0-5 cm layer					5-10 cm layer					0-10 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
02	CC NT Sb2	2010	10.80	19.72	17.54	18.59	10.19	9.49	9.85	**5.48**	**7.25**	10.39	10.21	17.13	12.65	14.76	9.07	9.40
17	CC NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	12.67	17.77	14.90	15.95	12.36	13.96
09	CC NT Sb2	2012	9.87	13.49	9.76	12.27	9.74	9.65	10.01	10.39	10.28	**7.32**	9.77	12.80	10.06	11.55	8.55	10.61
09	CC NT Sg	2010	9.91	17.56	15.57	16.03	9.43	9.85	8.76	11.50	10.87	7.85	9.89	15.45	14.62	14.81	8.65	11.23
02	CC NT Sg	2011	.	.	.	.	.	.	.	.	.	.	10.11	**5.77**	**6.22**	**6.07**	10.57	11.19
17	CC NT Sg	2012	10.38	10.68	**4.99**	**7.17**	11.01	10.66	**4.21**	**4.79**	**4.62**	10.81	10.51	8.63	**4.91**	**6.18**	10.92	8.81
17	CC NT Sb1	2010	11.63	16.02	15.84	15.94	11.39	11.15	8.98	8.79	8.89	10.00	11.41	13.95	13.83	13.89	10.72	11.29
09	CC NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	10.10	**4.08**	**4.45**	**4.31**	10.87	9.94
02	CC NT Sb1	2012	12.12	**1.84**	**6.95**	**3.48**	14.79	13.07	**4.24**	9.97	**6.73**	9.20	12.46	**2.17**	**7.40**	**4.08**	12.00	11.49
12	LR NT Sb2	2010	9.82	22.12	13.80	18.95	8.80	9.25	8.73	9.98	9.41	11.77	9.57	20.38	13.00	17.31	8.10	10.46
04	LR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	9.97	**6.04**	**6.87**	**6.61**	10.48	12.29
08	LR NT Sb2	2012	11.24	17.88	**7.04**	10.67	11.34	13.69	**4.41**	12.03	8.10	10.00	12.21	11.67	8.21	9.55	10.72	15.13
03	LR NT Sg	2010	9.94	**4.39**	8.09	**6.85**	10.36	9.84	7.86	12.79	11.86	8.96	9.90	**4.75**	8.94	7.67	9.68	10.43
12	LR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	11.20	**3.52**	**3.58**	**3.56**	12.44	9.00
04	LR NT Sg	2012	11.42	14.27	15.29	14.52	10.91	12.98	11.51	**3.31**	**4.19**	8.39	11.99	14.00	7.68	10.88	9.85	9.74
11	LR NT Sb1	2010	10.80	17.42	13.32	14.88	10.56	9.54	10.52	9.75	9.97	7.71	10.24	15.13	11.87	12.98	9.13	12.71
03	LR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	9.75	**5.37**	**7.18**	**6.53**	10.18	11.63
12	LR NT Sb1	2012	11.06	10.06	8.32	8.79	11.62	10.53	**3.54**	11.03	**6.32**	11.22	10.81	**6.97**	8.73	8.09	11.51	10.44
01	LR NT P2	2010	11.76	13.05	13.81	13.51	11.52	10.68	**5.79**	10.33	7.96	9.51	11.28	10.68	12.99	11.99	10.56	11.78
16	LR NT P2	2011	.	.	.	.	.	.	.	.	.	.	13.45	16.04	12.56	13.92	13.41	10.75
11	LR NT P2	2012	12.76	17.50	9.72	11.81	12.99	11.01	13.60	11.21	11.92	10.63	11.97	16.67	9.98	11.78	11.84	10.91
04	LR NT P4	2010	12.03	9.78	14.93	11.32	12.17	11.20	12.57	13.31	12.86	10.50	11.66	10.26	14.55	11.62	11.37	10.20
08	LR NT P4	2011	.	.	.	.	.	.	.	.	.	.	12.46	16.97	13.23	14.83	12.22	9.63
01	LR NT P4	2012	17.66	**4.97**	**6.18**	**5.60**	26.14	11.75	**7.31**	12.27	9.89	29.81	14.04	**5.49**	**7.45**	**6.51**	28.00	11.77

Plot	Treatment code	Year	0-5 cm layer					5-10 cm layer					0-5 cm layer					10-20 cm layer
			Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total	Coarse POM	Fine POM	POM	MAOM	Total
18	SR NT Sb2	2010	10.44	16.56	12.95	14.53	10.26	10.96	11.96	11.57	11.67	9.18	10.68	15.11	12.30	13.26	9.72	10.49
14	SR NT Sb2	2011	.	.	.	.	.	.	.	.	.	.	10.94	14.17	**6.25**	7.69	11.34	14.77
13	SR NT Sb2	2012	11.71	13.32	10.06	11.33	11.76	10.32	**6.21**	**1.23**	**2.96**	8.52	11.11	9.74	**7.30**	8.16	10.08	8.60
07	SR NT Sg	2010	9.96	10.90	15.72	12.26	9.59	9.48	9.15	11.37	10.55	8.20	9.75	10.77	14.44	11.96	8.85	11.16
18	SR NT Sg	2011	.	.	.	.	.	.	.	.	.	.	11.82	16.11	12.56	13.49	11.73	13.06
14	SR NT Sg	2012	10.07	8.96	**6.35**	**7.45**	10.49	10.13	**4.25**	**1.32**	**2.43**	8.91	10.09	7.61	**4.37**	**5.72**	9.70	12.47
10	SR NT Sb1	2010	9.76	15.39	14.57	14.91	9.47	8.92	7.87	12.06	10.40	7.94	9.37	13.15	13.82	13.55	8.71	8.58
07	SR NT Sb1	2011	.	.	.	.	.	.	.	.	.	.	12.11	13.92	13.62	13.72	12.00	12.57
18	SR NT Sb1	2012	9.25	9.16	**7.48**	8.08	9.49	10.61	**4.37**	**1.95**	**2.71**	10.70	9.88	7.68	**5.29**	**6.12**	10.10	9.64
14	SR NT P2	2010	11.02	18.11	13.19	15.06	10.69	10.18	15.54	11.14	12.37	8.66	10.64	17.55	12.70	14.33	9.68	9.89
13	SR NT P2	2011	.	.	.	.	.	.	.	.	.	.	9.74	16.96	**4.41**	**6.83**	10.11	13.11
10	SR NT P2	2012	12.32	17.91	8.21	10.55	12.66	9.11	**4.51**	**1.80**	**2.63**	9.34	10.80	11.49	**5.91**	**7.39**	10.98	13.73
x	LR CT P2	2010	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	LR CT P2	2011	.	.	.	.	.	.	.	.	.	.	10.82	11.63	12.62	11.98	10.73	12.88
05	LR CT P2	2012	12.07	16.46	**7.20**	10.19	12.49	9.65	12.33	13.63	13.33	11.82	10.82	15.38	8.99	10.83	12.24	11.02
c	Control Pasture	2012	11.42	15.08	9.35	11.83	11.32	9.19	10.37	**3.78**	**5.86**	8.73	10.41	13.56	**7.20**	9.64	10.10	8.33