Soil carbon sequestration during the establishment-phase of *Miscanthus x giganteus*

A study on three spatial scales

PhD thesis

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

Parts of the work in Chapter 4 were done in collaboration with Dr. David Styles (School of Environment, Natural Resources and Geography, Bangor University, Bangor, United Kingdom) who helped with the economic modelling, and Dr. Astley Hastings (Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, United Kingdom) who performed the *Miscanthus* biomass production model.

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Dublin, May 2013

Jesko Zimmermann

Summary

In recent years the use of biomass for energy production has become an increasingly important measure for mitigating global change. While national and EU legislation strongly advocate the further development of the bioenergy sector, the scientific debate has been inconclusive. There is particular concern that land-use change to bioenergy production can lead to CO_2 emissions. These emissions result from the loss of vegetation and the soil disturbance when ploughing natural ecosystems and pastures as a preparation for planting bioenergy crops. A possible solution is to use perennial energy crops such as willow or *Miscanthus*. Recent research on experimental fields has shown a high soil carbon sequestration potential across Europe; however, it can be expected that sequestration rates will differ on commercial plantations.

The aim of this study was to assess the factors influencing soil carbon sequestration under commercial *Miscanthus* plantations. An initial survey was conducted on 16 farms in south-east Ireland planted in 2006/2007 using the ^{13}C natural abundance method to identify *Miscanthus*-derived carbon stocks. Annual carbon sequestration rates were 0.62 Mg ha^{-1}±0.59 SD and 0.90 Mg ha^{-1} ±0.53 SD on former tillage and former grassland, respectively, close to values reported in earlier literature. Mixed effects modelling identified former land-use (grassland or tillage), initial soil organic carbon content, and pH as main explanatory variables for variability in total soil organic and *Miscanthus*-derived carbon. A comparison with the adjacent former land-use also showed that soil organic carbon losses due to land-use change were not significant.

To analyse the fate of newly sequestered carbon a soil fractionation experiment was performed. The fraction with which the soil organic carbon is associated has a significant impact on decomposability and turn-over time. The results showed the freshly sequestered carbon is mainly found as particulate organic matter (76.9 %), and therefore is in a labile state with short turn-over times. The experiment furthermore shows no significant differences in the distribution of the different soil fractions and soil organic

carbon distribution between the *Miscanthus* and the control sites, representing the former land-use.

At the field scale, a significant number of commercial *Miscanthus* plantations showed a large number of open patches, possibly impacting crop yield and soil carbon sequestration. Significantly lower *Miscanthus*-derived carbon values were found in the open patches compared to adjacent high density *Miscanthus* patches (1.51 ±0.31 Mg ha^{-1} and 2.78 ±0.25 Mg ha^{-1}, respectively). Using satellite imagery, remote sensing analysis revealed an average loss of 13.69 % ±4.71 SD of the cropped area, leading to a reduction of 7.38 % ±7.34 SD in *Miscanthus*-derived carbon on a field scale. Using a net present value model and a financial balance approach it could be shown that the patchiness can significantly reduce gross margin that can render *Miscanthus* production economically unfeasible.

In conclusion, the analyses show significant carbon sequestration in young commercial *Miscanthus* sites. However, as the majority of that *Miscanthus*-derived carbon is still in a labile state, the *Miscanthus* should be grown on a longer time-scale to ensure benefits. Additionally it was shown, that the introduction of *Miscanthus* to grasslands does not lead to a significant loss of already existing soil organic carbon, and that one time ploughing events associated with *Miscanthus* introduction do not lead to a significant disturbance of soil aggregation. Finally it was shown that crop patchiness on a field-scale has a significant impact on crop yield and the formation of *Miscanthus*-derived carbon stocks.

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You will be missed.

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List of acronyms

GHG:	Greenhouse gas
LULUCF:	Land-use, land-use change, & forestry
SOC:	Soil organic carbon
SOC_t:	Total soil organic carbon
SOC_i:	Initial soil organic carbon (C_3-derived)
SOC_{Mis}:	*Miscanthus*-derived soil organic carbon
S+A:	Sand and stable aggregate
S+C:	Silt and clay
POM:	Particulate organic matter
DOC:	Dissolved organic carbon
rSOC:	Resistant soil organic carbon

Chapter 1

General Introduction

1.1 Climate change mitigation

International ambitions to reduce greenhouse gas (GHG) emissions, identified as the main driver of anthropogenic climate change, led to the adoption of the Kyoto Protocol (United Nations, 1998) by the United Nations Framework Convention on Climate Change (UFCCC). The Protocol describes targets, methods, and a timeframe for the reduction of global GHG emissions. The so-called annex I countries agreed on reducing the global emissions of the major GHGs (standardised by their global warming potential, measured in CO_2 equivalent) by 5 % compared to baseline emission levels (1990).

Ireland committed itself to limit the increase in GHG emissions to a maximum increase of 13 % above 1990 levels (EPA, 2011). To achieve these targets the Department of the Environment, Heritage and Local Government has released the National Strategy on Climate Change (Department of the Environment, 2000; Department of the Environment, 2007) setting out measures to reduce GHG emissions for all relevant sectors. The agricultural sector is the second largest source of GHGs in Ireland (EPA, 2011), contributing about 28 % of the overall anthropogenic GHG emissions in 2009. This makes Ireland an unusual case which primarily can be attributed to the fact that on average the livestock sectors account for over 80 percent of the Irish agricultural output value. The aim is to reduce the agricultural emissions by 2.2 Mt CO_2 equivalents compared to the `business as usual' projected level of 18.7 Mt CO_2 equivalents by the end of the commitment period 2008-2012 (Behan & McQuinn, 2002; Department of the Environment, 2007).

Carbon sequestration due to land-use, land-use change, and forestry (LULUCF; IPCC, 2000) have been recognised in the Kyoto Protocol as a mean of crediting reductions. Eligible LULUCF activities, as agreed on the 7th Conference of Parties (Marrakesh, 2001), are afforestation, reforestation, and deforestation (Article 3.3, Kyoto Protocol), as well as forest management, crop management, grassland management, and revegetation (Article 3.4, Kyoto Protocol). In the agricultural context one focus of research and policy has been on two major strategies, (1) to either reduce direct emissions of CO_2 from soils by conserving existing soil organic carbon (SOC) pools, and by utilising soil carbon sequestration, and (2) to mitigate carbon emissions from fossil fuels by using biomass for energy production.

The strategies are strongly interlinked, as the use of bioenergy can have positive and negative effects on SOC pools in both, direct and indirect processes. Recent research has shown the complexity of the interactions between biomass production and SOC dynamics (e.g. Anderson-Teixeira *et al.*, 2009) and it was shown that GHG mitigation policies which do not take these interactions into account can potentially lead to significant underestimates of GHG emissions (e.g. Hill *et al.*, 2006; Fargione *et al.*, 2008; Searchinger *et al.*, 2008).

1.2 Soil carbon dynamics and land-use change

Generally stable ecosystems that do not undergo permanent, large-scale changes show a steady state carbon balance where uptake and emissions are in equilibrium, however ecosystems with low SOC stocks, either naturally occurring or due to anthropogenic practices, can be managed to foster carbon sequestration. In general, soil carbon sequestration is the long-term incorporation of atmospheric CO_2 into the soil in the form of stable organic compounds. The rate of soil carbon sequestration is depending on (1) the input of photosynthetically derived organic matter, and (2) the rate of removal of organic carbon, through emission into the atmosphere, leaching and runoff of dissolved organic carbon, as well as erosion (Jastrow *et al.*, 2007). Soil carbon sequestration occurs when the above processes are in a disequilibrium where the input of carbon is large than the output. The input of organic matter is depending on the primary production of above and below-ground biomass, as well as on the rate of incorporation of dead above-ground biomass into the soil. Biomass production regulated by climatic factors as well as nutrient availability, the incorporation of organic matter into soil is heavily depending on the soil fauna. Processes that remove carbon from the soil depend on the decomposition rate of soil organic matter. The decomposition rate of organic matter is depending on environmental factors, such as soil pH, soil moisture content, and soil temperature, but also on the composition of the organic material, especially the C:N ratio. Increasing soil carbon stocks has positive impacts on soil quality and fertility; also it has the potential to mitigate CO_2 emissions from agricultural soils and can provide a possible sink for atmospheric carbon. An overview of the pathways in soil carbon sequestration can be found in Figure 1.

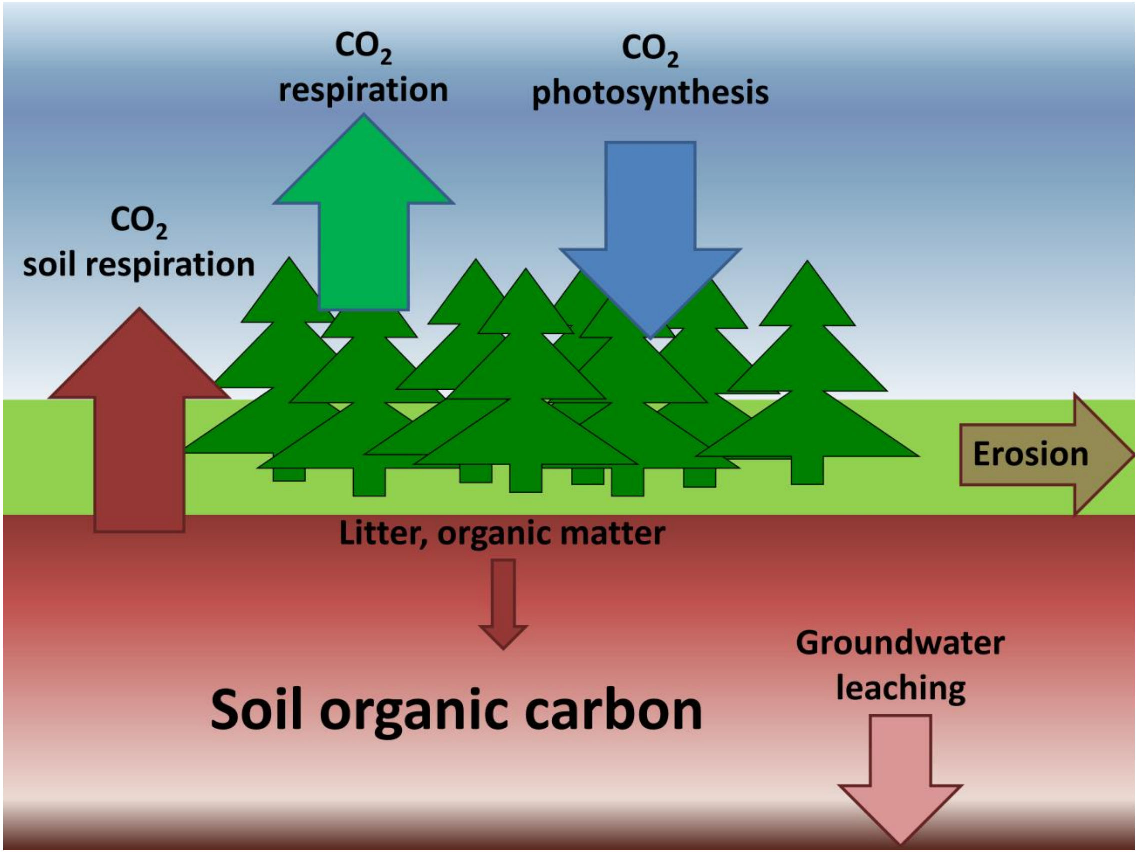

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Figure 1: Diagram of pathways involved in soil organic carbon dynamics

The effects of land-use change on carbon stocks are generally well studied and have been recognised as an important part of the global carbon cycle (Schimel, 1995b). On-going soil disturbance such as ploughing has led to significant emissions of CO_2 into the atmosphere (Roberts & Chan, 1990; Houghton, 1995; Smith, 2008). In the decade from 1990 to 2000 global emissions due to LULUCF have been estimated to be between 0.5 to 2.7 Pg C yr^{-1} (IPCC, 2007). On a long-term scale, land-use change has been estimated to be a major global carbon source, adding about 124 Pg of carbon to the atmosphere, with the majority being lost due to conversion of forestry to agriculture as well as changes from grasslands to croplands (Houghton, 1999; Smith, 2008). Soil disturbance leads to an increased aeration of the soil, as well as a break up of soil aggregates protecting soil organic matter leading to increased mineralisation rates (Roberts & Chan, 1990). Lal (2004) showed that land-use change led to a depletion of up to two thirds of the original SOC contents in agricultural soils equivalent to a loss of 30 to 40 Mg C ha^{-1}. Current losses are difficult to estimate, however Smith (2004a) estimated the croplands in Europe alone lose up to 300 Tg C yr^{-1}.

To increase SOC stocks a number of management techniques can be utilised: (1) afforestation, (2) land-use change to permanent pasture or perennial crops, and (3) reducing tillage. Management systems that favour soil carbon sequestration generally increase the input of organic carbons into soils, either as plant litter or root material, they reduce soil disturbance, and preserve or increase soil quality, structurally as well as biologically (Post & Kwon, 2000). Calculating the global carbon mitigation potential is difficult, as apart from assessing the full potential of measures increasing soil carbon sequestration, restraints regarding land-use change and available materials as well as socio-economic variables need to be taken into consideration (Smith, 2004a). However, recent research has estimated that over the past decades soils have stored between 1 and 2 Pg yr^{-1} in the northern hemisphere (Tans *et al.*, 1990; Ciais *et al.*, 1995; Fan *et al.*, 1998). The carbon sequestration potential in agricultural soils for the EU-15 countries has been estimates to be 16 to 19 Tg C yr^{-1} (Freibauer *et al.*, 2004). In terms of long-term benefits it should be recognised that carbon sequestration is not a continuous sink, SOC stocks will eventually reach an equilibrium state, from where on no further sequestration will occur (Smith, 2004b). The timeframe for this is highly variable: in temperate regions it takes about 100 years to reach a new equilibrium after land-use change; however this process may be much quicker in tropical soils (e.g. Six *et al.*, 2002b; Freibauer *et al.*, 2004). As a compromise the IPCC is suggesting a period of 20 years for SOC to reach an equilibrium state on a global scale (IPCC, 2000).

1.3 Bioenergy crops

The use of biomass for energy production is one of the main strategies in mitigating GHG emissions and achieving independence from fossil fuels. The aim of the European Union (EU) is that by 2020, 20 % of the energy consumed will be produced using renewable sources (European Commission, 2008), with a significant amount being achieved using bioenergy. Until now the major source of biomass in Europe and America have been starch and oil containing crops, such as maize, sugarcane, and rapeseed (Sims *et al.*, 2006) which are used to produce liquid fuels such as bioethanol and biodiesel. International and national policies are supporting the use of bioenergy. In Ireland the introduction of bioenergy has been heavily subsidised by the government, with the recent bioenergy scheme investing 1.6 M € into the planting of *Miscanthus* and willow (Department of Agriculture, 2010).

Within the scientific community the proposal to introduce bioenergy crops is subject to controversial discussion. Recent research suggests that under certain conditions bioenergy production can lead to increasing food prices due to direct and indirect competition. Also, the conversion of native, semi-natural, or generally more diverse ecosystems to large monocultures can lead to a loss of biodiversity (Cook *et al.*, 1991; Koh, 2007; Koh & Wilcove, 2008). Furthermore it has been shown that, looking at the whole production life-cycle, GHG savings can be much lower than initially assumed. Major factors which need to be taken into account are carbon emissions related to machinery for planting, harvesting, transport, and processing (Hill *et al.*, 2006), as well as carbon emissions related to land-use change. Conversion of forest and grassland ecosystems to bioenergy crops have been shown to lead to significant emissions of carbon from vegetation and soils that have under certain conditions been estimated to take up to centuries to offset using bioenergy (Fargione *et al.*, 2008; Searchinger *et al.*, 2008). It is suggested that key benefits of bioenergy use are dependent on management practices, including but not limited to (1) the use of biomass feedstock with low life-cycle emissions such as perennial crops, crop residues, sustainably harvested wood, and (2) avoiding the clearing native ecosystems (Tilman *et al.*, 2009).

The use of perennial, lingo-cellulosic bioenergy crops has been a particular focus in recent research. These so-called second-generation bioenergy crops generally have low fertiliser and pesticide inputs, and due to their perennial nature relatively low establishment costs. Furthermore perennial crops have a high carbon sequestration potential due to high biomass production, deep rooting systems, and the reduction of disturbance as fields are taken out of tillage for the life-cycle of the crop (Kahle *et al.*, 2001; Freibauer *et al.*, 2004). The incorporation of plant litter by the soil fauna is a major source of SOC, and the perennial nature of second generation bioenergy crops not only allows for senescence leading to higher litter input, but it has also been shown that the reduced disturbance has a positive impact on the soil fauna hence enhancing litter incorporation further (Chan, 2001; van Eekeren *et al.*, 2008; Ernst *et al.*, 2009). In Ireland a special focus has been on the perennial grass *Miscanthus x giganteus* (Greef et Deu ex Hodkinson; Greef & Deuter, 1993; Hodkinson & Renvoize, 2001). This rhizomatous C_4-plant, originating from south-east Asia is remarkably adaptable to temperate climates producing up to 25 Mg ha^{-1} yr^{-1} in Europe (Lewandowski *et al.*, 2000); potentially offering higher yields and better economic feasibility than woody bioenergy feedstock such as short rotation coppice (SRC) willow (Styles *et al.*, 2008). Furthermore *Miscanthus* cultivation has been reported to be feasible when grown on marginal lands, defined as agricultural lands with poor conditions, or recently abandoned cropland (Qin *et al.*, 2011), reducing possible food competition as well as possible carbon emissions due to land-use change (Clifton-Brown *et al.*, 2007; Heaton *et al.*, 2008; Qin *et al.*, 2011).

Ireland is located at the northern limit of the range in which *Miscanthus* can be grown economically. Depending on the location, modelled peak yields range between 16 and 26 Mg ha^{-1} dry matter (DM) (Clifton-Brown *et al.*, 2000). However, the harvest yield is estimated to be about 30 % lower due to senescence and harvest losses (Clifton-Brown *et al.*, 2004).

Miscanthus has a high carbon sequestration potential due to its physiological features as well as specific management practices. As a deep rooting crop *Miscanthus* distributes carbon deeper within the soil profile than annual crops or grasses (Neukírchen *et al.*, 1999). Furthermore, it translocates a large proportion of the aboveground carbon into the belowground section during winter senescence to enhance spring growth Kuzyakov & Domanski, 2000). Generally the crop is harvested in spring time, allowing full winter senescence in order to reduce the crops moisture content to a minimum, although the senescence leads to increased litter fall (Beuch, 1999; Clifton-Brown *et al.*, 2007). Furthermore, due to the reduced disturbance the stability of SOC is increased, as aeration is reduced and the formation of stable aggregates is supported, reducing mineralisation rates and therefore benefitting soil carbon sequestration (Beuch, 1999; Balesdent *et al.*, 2000; Six *et al.*, 2000a). Also, the high input of plant material combined with low N inputs from fertiliser lead to a high C:N ratio further inhibiting mineralisation (Schneckenberger & Kuzyakov, 2007).

A number of field experiments have confirmed high carbon sequestration rates under *Miscanthus*, showing high potential to increase SOC stocks under former arable lands and the potential of increasing carbon stock under former permanent pasture. Hansen *et al.* (2004) reported *Miscanthus*-derived carbon sequestration rates of 0.78 and 1.13 Mg ha^{-1} yr^{-1} for two coarse loamy soils, at one location with 9 years of *Miscanthus* cultivation, soils showed no significant differences in SOC stocks compared to grassland reference sites, but a second site with 16 years of *Miscanthus* cultivation showed higher total SOC stocks. Rowe *et al.* (2009) compared four *Miscanthus* sites to adjacent reference sites and found two of the *Miscanthus* site to have significantly higher carbon stocks than the grasslands reference sites, while two sites showed no significant differences. Comparing two different sites Schneckenberger & Kuzyakov (2007) found higher annual *Miscanthus*-derived carbon rates under loamy soils compared to sandy soils (0.23 and 0.11 g C kg^{-1} soil; area based values were not available). Also they found lower total SOC contents under *Miscanthus* compared to grassland. Model estimates showed potential carbon sequestration rates inputs between 0.6 Mg ha^{-1} yr^{-1} (Freibauer *et al.*, 2004) and 0.93 Mg ha^{-1} yr^{-1} (Matthews & Grogan, 2001). In Ireland annual rates of *Miscanthus*-derived carbon have been reported to be between 0.59 Mg ha^{-1} yr^{-1} (Clifton-Brown *et al.*, 2007) and 3.2 Mg ha^{-1} yr^{-1} (Dondini *et al.*, 2009b). All field measurements have so far been carried out on experimental plots. The authors are not aware of any publication reporting on soil carbon sequestration under commercial *Miscanthus* plantations.

Other major greenhouse gases associated with agriculture are nitrous oxide (N_2O) and methane (CH_4). Nitrous oxide is generally associated with fertiliser input (Mosier *et al.*, 1991). As *Miscanthus* has low fertiliser requirements N_2O emissions are generally considered to be low. However, a recent study (Davis *et al.*, 2010) has shown evidence for increased nitrogen fixation in *Miscanthus* fields however exact rates are not yet known. While wetland soils can act as major sources for methane emissions, normal agricultural soils generally act as a CH_4 sink (Don *et al.*, 2011). However on a number of bioenergy crop sites survey by Don *et al.* (2011), methane uptakes were relatively small with values between 2 and 17 kg $CO_{2\ equiv}$ ha^{-1} yr^{-1}.

1.4 Factors influencing soil carbon sequestration and spatial variability

Soil carbon sequestration under *Miscanthus* is mainly driven by the input of fresh soil organic matter and the turn-over rates. Both drivers are influenced by a number of conditions and processes. The importance of these factors varies on different spatial scales.

(1) The main driver on the global scale is climate. As decomposition is temperature dependent, higher temperatures lead to higher decomposition rates and vice versa (e.g. Raich & Potter, 1995; Reichstein *et al.*, 2003). Vleeshouwers & Verhagen (2002) showed that an increase in temperature of 1°C leads to a decrease in SOC stocks of 0.05 Mg C ha^{-1} yr^{-1}. Furthermore the reaction is water limited, and higher precipitation leads to higher decomposition rates (Schlentner & Vancleve, 1985; Davidson *et al.*, 2000). However, areas with frequently occurring anaerobic conditions due to high precipitation and water logging such as wetlands and peatlands will show much lower decompositions rates due to inhibited microbial activity. Climate also influences biomass production and therefore soil organic matter input. Generally *Miscanthus* is more productive in Mediterranean climates due to higher global radiation levels (Clifton-Brown

et al., 2000) and a longer period of minimum temperatures above 10°C (Clifton-Brown & Jones, 1997).

(2) Soil carbon sequestration can also show a high variation even in areas of low to no climatic variation. The main drivers on this regional scale are soil properties and management practice. The main soil properties are soil texture, pH value, and the initial SOC content (Rowe *et al.*, 2009). Generally higher sand content is linked to lower SOC levels (Brogan, 1966; Zhang & McGrath, 2004). Fine soil material (silt and clay) increase physical protection as they offer a larger surface for adsorption of organic material, the potential to enclose organic matter, and are more likely to form stable aggregates (Tisdall & Oades, 1982; Elliott & Coleman, 1988; Oades, 1989). The soil pH controls microbial activity, and therefore the turn-over rates. Higher acidity generally inhibits activity and therefore reduces mineralisation leading to higher accumulation of SOC (Motavalli *et al.*, 1995; Kemmitt *et al.*, 2006). The capacity of soils to accumulate carbon is limited and the initial SOC content is therefore a limiting factor for soil carbon sequestration. The more depleted a carbon pool is, the more SOC can be sequestered (e.g. Grogan & Matthews, 2002). Furthermore soil properties influence the crop performance, therefore having an indirect influence on the biomass input. A number of management practices have been reported to influence soil carbon sequestration. In a *Miscanthus* plantation the main management based drivers for soil carbon sequestration are the harvest practice, possible fertiliser application, and the former land-use. The timing of the harvest directly influences organic matter input. Spring harvest allows winter senescence, significantly increasing litterfall compared to autumn harvest. Also more efficient harvest techniques reduce litter fall during the process, therefore reducing the input of organic matter. Fertiliser application can both enhance and reduce SOC stocks. Organic fertiliser acts as an additional input of carbon, therefore increasing carbon stock, and mineral fertiliser can lower the C:N ratio and therefore increase mineralisation rates. While *Miscanthus* is generally a low input crop, with low to no fertiliser application recommended (Caslin *et al.*, 2010), some farmers may still add both mineral or organic fertiliser to increase the crops performance.

(3) On a field scale, a number of factors can influence soil carbon sequestration. Local differences in soil properties can influence mineralisation rates, as well as the crop performance leading to differences in crop density. Furthermore, a number of studies have reported large open patches in *Miscanthus* fields (Semere & Slater, 2007; Bellamy *et al.*, 2009; Sage *et al.*, 2010), although, so far, the research has been limited to impacts on biodiversity. There have been no publications on the cause of the patchiness as well as its impact on the economic performance and soil carbon sequestration. Lower crop densities and open patches lead to reduced input of organic material and can therefore have a significant impact on soil carbon sequestration.

(4) Soil carbon sequestration is also dependent on processes on the micro-scale. Stability of new carbon input depends on its association with soil particles (e.g. Six *et al.*, 2004). Most of the carbon enters the soil in readily available form and is therefore relatively quickly decomposed by microorganisms (Christensen, 2001). A portion of that carbon is however aggregated and adsorbed to mineral surfaces which makes it less vulnerable to decomposers and significantly reduces the mineralisation rates and therefore the turnover time (Six *et al.*, 2004; Lehmann *et al.*, 2007). To understand the full carbon sequestration potential it is therefore important to have knowledge about the portions of carbon entering the different pools. Soil aggregation is subject to management practices and land-use change, aggregates are sensitive to soil disturbance caused by ploughing leading to a reduction in the stability of associated SOC (Baldock & Skjemstad, 2000).

Acknowledging processes on different spatial scales is also important for refining models for crop yield and soil carbon dynamics. The influence of physiological parameters, climatic conditions, soil properties on crop yield and soil carbon dynamics is well understood (Monteith, 1977; Clifton-Brown *et al.*, 2000; Hastings *et al.*, 2009). However, regional and local processes that are not fully understood can lead to inaccuracies in model prediction that can transfer to large errors when upscaling to regional, national or global scale predictions (Cantarello *et al.*, 2011). Furthermore, the accuracy of decomposition models, and therefore soil organic carbon dynamics is dependent on knowledge of the association of organic carbon with different soil fractions. The RothC model for example divides soil organic carbon into different pools depending on their decomposability (Parton *et al.*, 1987). It has also been shown by Zimmermann *et al.* (2007) and Dondini *et al.* (2009a) that using certain fractionation techniques allows for the identification of soil fractions that are representative the conceptual carbon pools in RothC and therefore allow for further improvement of the model. This emphasises the importance understanding processes on the regional, field, and micro-scale to further improve models.

1.5 Modelling crop yield and economic feasibility

To predict possible *Miscanthus* yields on a large scale a number of models have been developed. In the present study MISCANFOR is used to predict dry matter yields for the surveyed sites as comparable direct measurements were not available. The model is based on an earlier production model, MISCANMOD (Clifton-Brown *et al.*, 2004), which predicts the potential non water-limited yields based on physiological parameters. Yield estimates are based on daily climate data using three components: (1) the radiation interception efficiency of the canopy, which is calculated using a leaf area index estimate based on thermal time, (2) the radiation use efficiency of the intercepted radiation, and (3) an estimate of the end of growing season, either based on flowering time or when the mean daily temperature falls below 10 °C. Hastings *et al.* (2009) modified MISCANMOD to improve process descriptions for evapo-transpiration, soil moisture content, photosynthetically active radiation (PAR), the plants physiological time clock, water stress, possible shoot and rhizome mortality, nutrient translocation to the rhizome, and above ground dry matter moisture content.

As a perennial crop with relatively high establishment costs *Miscanthus* poses a financial risk to potential growers, therefore it is important to estimate the gross margin over the whole life-cycle of the crop. Assessing the economic viability of *Miscanthus* requires knowledge of all involved financial inputs including establishment costs, fertiliser costs, harvest and storage costs, as well as the costs required to take the crop out of production at the end of its life-cycle. Using a financial balance approach the costs and incomes for every year used to calculate the annual gross margin which is then subtracted from the initial establishment costs, taking the interest rates for all debt as well as the inflation into account. The approach allows for identifying the amortisation period and the overall financial gains of the crops life-cycle. While the approach is relatively simple it does not apply a discount rate for the long term investment that *Miscanthus* represents. Using a net present value (NPV) model a set discount rate can be applied to any future incomes, which allows putting future cash flows into relation with the initial investment. As the model output is standardised over the whole life-cycle of the crop the results can be annualised to represent the annual gross margin for the farmer taking an annual discount into account (Styles *et al.*, 2008).

1.6 Stable carbon isotope signature in C_3 and C_4 plants

Tracking organic matter from different sources within the elemental cycles, taking different ecological and spatial scales into account is a difficult process. In recent years, the analysis of stable isotopes has been shown to be a reliable, and relatively cost efficient tool to understand the fate of organic matter within and between ecosystems (Balabane & Balesdent, 1992; Balesdent & Balabane, 1992; Flessa *et al.*, 2000; Garten & Wullschleger, 2000; Foereid *et al.*, 2004; Pelz *et al.*, 2005). Measuring stable carbon isotopes is an important tool to identify sources of soil organic matter. The stable carbon isotope ^{13}C has a natural abundance of 1.11 % however a number of physical and chemical processes can lead to differences in the ratio of ^{13}C and ^{12}C in organic material due to discrimination, these differences can be tracked throughout the carbon cycle and help to identify possible sources of organic compounds. The stable carbon isotope signature is described using the δ notation. It is defined as the ratio of the $^{13}C/^{12}C$ of the given sample and the $^{13}C/^{12}C$ of a reference material. The reference for the $\delta^{13}C$ is the Pee Dee Belemnite (South Carolina, United States) with a $^{13}C/^{12}C$ ratio of 0.10112372. The $\delta^{13}C$ value is calculated using Equation 1 and given in the unit per mill [‰]

$$(1) \qquad \delta^{13}C = ((R_{sample-}R_{Reference})/R_{Reference}) * 1000$$

with R_{sample} being the $^{13}C/^{12}C$ ratio of the sample and $R_{Reference}$ being the $^{13}C/^{12}C$ ratio of the Pee Dee Belemnite.

To identify sources of SOC using ^{13}C three methods are currently applied: (1) pulse labelling, (2) continuous labelling, and (3) natural abundance (Kuzyakov & Schneckenberger, 2003). The first two methods use ^{13}C enriched CO_2 to label specific plants. Organic compounds derived from the labelled plants can then be tracked through the carbon cycle. The third method comprised natural discrimination of the stable carbon isotopes during the formation of organic compounds. During the process of photosynthesis plants generally discriminate against the heavier carbon isotope ^{13}C (Farquhar *et al.*, 1989), leading to a depletion of ^{13}C levels in plant organic material and therefore a lower $\delta^{13}C$ value compared to the atmosphere. The depletion is based on the fact that the heavier ^{13}C forms slightly more stable chemical bonds, furthermore it diffuses more slowly, therefore entering stomata at a lower rate (O'Leary, 1988). The level of discrimination depends on the photosynthetic pathway. In plants with a C_3 photosynthetic pathway two major forms of isotopic fractionation occur. The difference from the $\delta^{13}C$ of atmospheric CO_2 ($\Delta\delta$) due stomatal diffusion is about 4.4 ‰. Fractionation due to carboxylation shows a $\Delta\delta$ of about 28 ‰. With a $\delta^{13}C$ of ca. 8 ‰ for atmospheric CO_2, plant material would show a $\delta^{13}C$ of -12 and -37 ‰ if stomatal diffusion or carboxylation would be the limiting factor or isotope fractionation, respectively. The median $\delta^{13}C$ measured in C_3 plants is about 27 ‰, showing that both processes influence the isotope fractionation with a stronger influence from the carboxylation (O'Leary, 1988). C_4 plant material shows a significantly higher $\delta^{13}C$ value. While C_4 plants use a different enzyme, phosphoenolpyruvate (PEP) carboxylase, to catalyse photosynthesis, which has a $\Delta\delta$ of about -6 ‰ leading to $\delta^{13}C$ of about -2 ‰ for plant material (Farquhar, 1983), measurements show a $\delta^{13}C$ of -14 ‰ (O'Leary, 1988). These results show that carboxylation is not the limiting factor, but diffusion. However assuming that diffusion is the only factor causing fractionation plant material would have a $\delta^{13}C$ of -12 ‰. The additional depletion of ^{13}C is explained by the physiology of the C_4 pathway. The products of the PEP carboxylase (usually malate) are transported into the bundle sheath cell, where they are decarboxylised to CO_2 and pyruvate, the CO_2 is then refixed by RuBisCO. Farquhar (1983) argues that the further discrimination in ^{13}C is caused by a slow leak of CO_2 from the bundle sheath cells. Due to the preference of RuBisCO for the lighter ^{12}C isotope the leaking CO_2 would be enriched in ^{13}C leading to a further reduction of the $\delta^{13}C$ value of the plant material.

As plant material from C_3 and C_4 plants show significantly different $\delta^{13}C$ values, the analysis of the stable isotope signature can be used to trace the source of SOC of a particular C_4 or C_3 plant if it is introduced to an area of no former history with the respective plant, providing a powerful tool in determining the source of SOC under maize, switchgrass, or *Miscanthus* (Balesdent *et al.*, 1990; Garten & Wullschleger, 2000; Hansen *et al.*, 2004).

1.7 Aims and outline of the thesis

This work is part of the multidisciplinary SIMBIOSYS project (Sectoral IMpacts on BIOdiversity and ecoSYStem services, http:\\www.simbiosys.ie). The aim of the project was to analyse the impacts of human actions on biodiversity and ecosystem services in different sectors of human activity. The sectors studied were bioenergy production, wind energy, road construction, and aquaculture. As part of the project Chapters 2 to 4 aim to assess the ecosystem service soil carbon sequestration in a dedicated bioenergy crop, additionally chapter 3 aims to assess fuel production.

As shown above, not all of the different processes influencing soil carbon sequestration on different spatial scales, are fully understood. While large scale models provide a good overview of expected *Miscanthus* yields and soil carbon sequestration rates, smaller scale processes may lead to substantial differences in yields realised by producers and soil carbon sequestration rates compared to the models. The aim of this work is to analyse processes influencing soil carbon sequestration, as well as crop yield while down-scaling from a regional to a micro scale. Based on the literature summarised in this section the major hypotheses are, that (1) *Miscanthus* cultivation will lead to a significant amount of *Miscanthus*-derived carbon which can be measured using the ^{13}C natural abundance method. (2) The introduction of *Miscanthus* to a grassland site will lead to a significant reduction in the SOC stocks due to soil disturbance when breaking up the grassland and planting the rhizomes. (3) Soil properties will have a significant influence on the soil carbon sequestration. (4) Crop patchiness will lead to a significant reduction in both yield and *Miscanthus*-derived carbon stocks on a field scale, and (5) soils under former grasslands show a higher quantity of stable aggregates and therefore more carbon will enter a long-term pool, compared to a former arable land.

To test the above mentioned hypotheses Chapters 2 to 4 focus on commercial farms cultivating *Miscanthus* in south-east Ireland. The surveyed sites were a subset of the field sites selected for the SIMBIOSYS project. All sites were either planted on grassland (permanent pasture, set aside, or silage) or arable land. As the national bioenergy scheme subsidising the planting of *Miscanthus* in Ireland was introduced in 2006, there is no commercial plantation prior to that year. Therefore, all sites can be considered to be in the establishment phase.

To assess the impact of land-use change to *Miscanthus* on soil aggregates and different carbon pools a soil fractionation was carried out (Zimmermann et al., 2007). Chapter 4 also comprises aerial imagery of a subset of the field sites to assess the patchiness, furthermore two models are used (1) the MISCANFOR model to assess the potential *Miscanthus* yields for the specific sites (Hastings et al., 2009), and (2), based on the modelled yields, a net present value (NPV) model as well as a financial balance approach to assess the economic impacts of crop patchiness on the biomass yield (Styles et al., 2008; Styles & Jones, 2008). Chapter 5 will synthesise the results of the previous sections and put them in the context of the different spatial scales in which soil carbon sequestration and the influencing factor were observed.

The following points describe the main objectives.

- To quantify soil carbon sequestration and possible soil organic carbon losses linked to the planting process under *Miscanthus x giganteus* regional scale with an emphasis on the influence of the former land-use and soil properties. The analysis was carried out on commercial farms to provide insight into possible differences between estimates based on experimental plots and commercial farming.

- To analyse the SOC associated with different soil aggregates, with a special emphasis on how the former land-use influences different carbon pools under *Miscanthus*.
- To estimate the influence of crop patchiness on soil carbon sequestration and crop yield on a field scale, providing information on the environmental and economic impact of open patches in *Miscanthus* fields.

Chapter 2

Soil carbon sequestration during the establishment-phase of *Miscanthus x giganteus:* a regional scale study

Based on: Zimmermann, J, Dauber, J, and MB Jones (2012): Soil carbon sequestration during the establishment-phase of *Miscanthus x giganteus*: a regional scale study. *Global Change Biology Bioenergy*, Vol. 4, Issue 4, pp. 453-461.

2.1 Abstract

The use of biomass for energy production is considered a promising way to reduce net carbon emissions and mitigate climate change. However, land-use change to bioenergy crops can result in carbon emissions from soil and vegetation in amounts that could take decades to compensate. Perennial grasses such as *Miscanthus* offer a possible solution to this problem as measurements on experimental plots planted with *Miscanthus* have shown significant carbon sequestration in the soil. It can, however, be expected that sequestration potentials in commercial use might differ from those measured in experimental plots due to different farming practices and soil characteristics. For this study, *Miscanthus* plantations on 16 farms in SE Ireland as well as on-farm controls representing the former land-use (grassland and tillage) have been examined. The *Miscanthus* plantations were 2 to 3 years old. Soil organic carbon (SOC) content and a number of soil properties were measured and the amount of *Miscanthus*-derived carbon was determined using the ^{13}C natural abundance method. On both former tillage fields and grasslands, although there were no significant differences in SOC contents between *Miscanthus* and control sites, it was shown that 2 to 3 years after *Miscanthus* establishment, 1.82 ± 1.69 and 2.17 ± 1.73 Mg ha^{-1} of the SOC under former-tilled and former grassland respectively were *Miscanthus*-derived. Mixed-effects models were used to link the total SOC concentrations and *Miscanthus*-derived carbon to the land-use parameters as well as to soil properties. It was shown that on control sites, pH had an effect on total SOC. In the case of *Miscanthus*-derived carbon, the initial SOC content, pH, former land-use and crop age had significant effects.

2.2 Introduction

The production of biofuels, particularly in North America and Europe, has recently increased significantly (Sims *et al.*, 2006). The main drivers of this increase are changes in national and international legislation to reduce greenhouse gas (GHG) emissions and independence from fossil fuels The increase in production of bioenergy crops is accompanied by a rising number of concerns questioning the benefits of biofuels in terms of environmental sustainability and GHG reductions (e.g. De Oliveira *et al.*, 2005; Hill *et al.*, 2006). Tilman *et al.* (2009) conclude that biofuels require a sophisticated approach in terms of feedstock and cultivation as well as management, as uncontrolled clearing of natural ecosystems and the replacement of food crops can lead to loss of biodiversity as well as increasing food prices.

Particularly important aspects of the recent debate have been the effects of land-use change on GHG emissions, and the so-called ‘carbon debt’. The term describes the direct and indirect carbon emissions due to loss of above and belowground biomass as well as soil disturbance, which first have to be balanced before any GHG benefit can be derived from the use of biofuels (Searchinger *et al.*, 2008). Recent studies (Fargione *et al.*, 2008; Gibbs *et al.*, 2008; Searchinger *et al.*, 2008) estimated the ‘payback period’ to be up to centuries depending on the type of land-use change and the biofuel system. However, Fargione *et al.* (2008) showed the potential of so-called second generation bioenergy crops to reduce the payback period to zero, if cultivated on abandoned croplands. The term second generation bioenergy crops usually describes lignocellulosic feedstock, e.g. perennial grasses (e.g. switchgrass or *Miscanthus*) or woody species (e.g. short rotation coppice such as willow) (Somerville, 2007; Yuan *et al.*, 2008). Perennial crops in particular increase the carbon sequestration potential in soils due to both physiological and management features. Perennial crops translocate large proportions of carbon to the root system or rhizomes as a reserve for spring growth (Kuzyakov & Domanski, 2000). They are mainly harvested in spring allowing senescence and accumulation of plant litter (Beuch, 1999; Clifton-Brown *et al.*, 2007). Also, in comparison with arable lands, the minimization of soil disturbance reduces mineralization rates of soil organic matter (Beuch, 1999). Soil disturbance, e.g. due to ploughing processes, is reported to reduce physical protection of soil organic matter, and therefore increase rates of mineralization and loss of soil organic carbon (SOC) (Roberts & Chan, 1990). Evidence for increased mineralization due to soil disturbance is particularly seen in SOC losses linked to the conversion of grasslands to crop-lands (Poeplau *et al.*, 2011).

In Ireland, the planting of *Miscanthus* (*Miscanthus* × giganteus, Greef and Deu.), a perennial, rhizomatous, C_4 grass originating from SE Asia, has been subsidized by the government (Department of Agriculture, 2010). Although recent studies on experimental plots have confirmed the ability of *Miscanthus* to sequester carbon (Clifton-Brown *et al.*, 2007; Dondini *et al.*, 2009b), it might be anticipated that sequestration potentials in commercial use would substantially differ from those measured in experimental plots due to a wider range of soils and climate conditions (Rowe *et al.*, 2009) as well as differences in farming practices, e.g. fertilizer application and harvesting practice.

In Ireland, both grassland and arable land are being converted to *Miscanthus*. Due to regular disturbance, tilled land is generally associated with lower carbon stocks than grassland (Smith, 2004a; Soussana *et al.*, 2004; Smith, 2008); therefore, the additional disturbance due to *Miscanthus* establishment is not expected to lead to an additional soil carbon loss. Furthermore, the introduction of perennial grasses has been reported as a viable option to facilitate soil carbon sequestration in croplands (Freibauer *et al.*, 2004).

However, conversion of grassland to *Miscanthus* is accompanied by a considerable soil disturbance as a result of ploughing (Caslin *et al.*, 2010). Consequently, while grassland is reported to have a significant carbon sequestration potential, disturbance can lead to a rapid reversal of previously sequestered carbon (Conant, 2010). A loss of SOC following any disturbance will require a certain time to regenerate, therefore adding to the carbon debt. The conversion of permanent grassland to *Miscanthus* in Ireland is expected to be particularly significant as more than 90% of the agricultural land is dedicated to permanent grasslands (Donnelly *et al.*, 2011). As the soil carbon stocks under grassland are dependent on management (Conant *et al.*, 2001; Jones & Donnelly, 2004; Chan *et al.*, 2011), the carbon debt resulting from conversion of grassland to *Miscanthus* is likely to alter with farming practice.

The aim of this work was to assess the impact on soil carbon stocks of converting grasslands and tilled lands to the perennial bioenergy crop *Miscanthus*. In particular, we measured (1) the changes in total SOC stocks, comparing *Miscanthus* fields that were planted either on former grasslands or on former arable sites with adjacent control sites to assess possible direct impacts of *Miscanthus* establishment on soil carbon; (2) the amount of carbon sequestered by *Miscanthus* using the ^{13}C natural abundance; and (3) the impacts of the former land-use as well as soil particle size distribution and pH on carbon stock changes due to both conversion and sequestration, as both have been reported to have a potential effect on SOC dynamics (Brogan, 1966; Motavalli *et al.*, 1995).

2.3 Materials and methods

2.3.1 Field site selection

Data were collected from 16 farms in south east Ireland planted with *Miscanthus* × giganteus. Eight of the plantations were established on grassland and eight on tilled land. The locations of the field sites are shown in Figure 2. The climate conditions were similar at all sites with a mean annual temperature of about 9.3 °C and mean annual precipitation of about 830 mm. Criteria for the field site selection were absence of recent application of organic fertilizers, an elevation below 120 m a.s.l., a minimum field size of 2 ha and the availability of an on-farm control site. The control site had to be an adjacent field representing the former land-use of the *Miscanthus* field to ensure comparability between the soils of the two fields. The first commercial *Miscanthus* fields were planted in 2006; therefore, only fields planted in 2006 or 2007 were selected. *Miscanthus* is planted in the form of rhizomes; prior to planting, the fields are treated with round-up (Monsanto, Creve Coeur, Missouri, United States) and ploughed. It was also important for the analysis, that no sites had previously been used for cultivating a C_4-crop (i.e. maize). Table 1 lists the properties of the sampled farms summarised for 0 to 30 cm depth. Soils from four forms of cultivation were sampled, *Miscanthus* planted on former-tilled land (MT), tillage control (CT), *Miscanthus* planted on former grassland (MG) and grassland control (CG). *Miscanthus* fields and control fields were sampled as matched pairs with one pair per farm, securing independence of the samples with respect to individual farming practises.

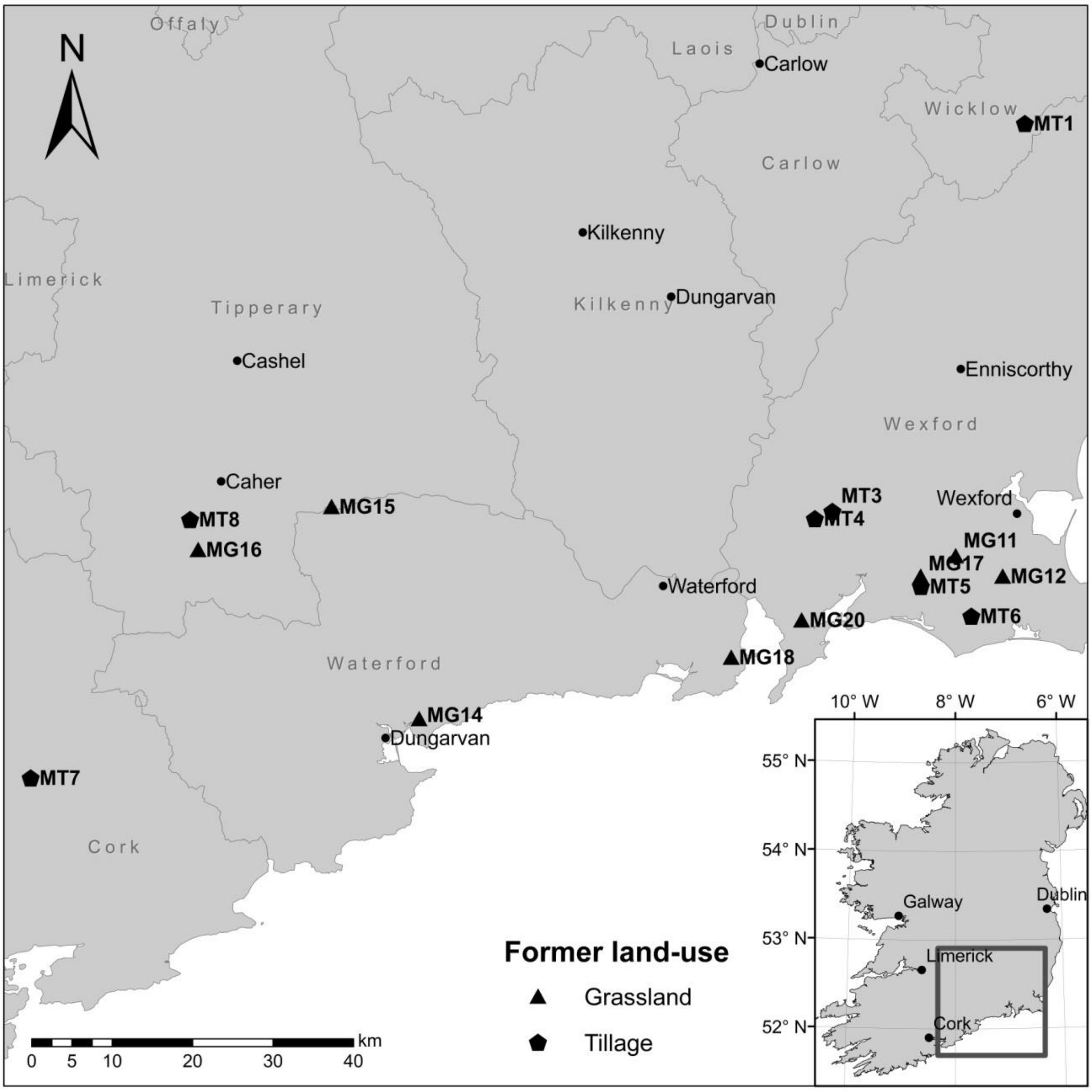

Figure 2: Locations of the field sites and the former land-use of the *Miscanthus* fields.

Table 1: Parameters of the *Miscanthus* sites. Elevation was measured using one GPS measurement. Soil organic carbon and *Miscanthus*-derived carbon are summed over 30 cm soil depth and averaged over the subplots, all other values are averaged over 30 cm sample depth and the subplots.

Site ID	Former land-use	*Miscanthus* planted in	Control land-use	Elevation [m a.s.l.]	Clay [%]	Silt [%]	Sand [%]	pH	Bulk density [g cm^{-3}]	$\delta^{13}C$ [‰]		Total SOC [Mg ha^{-1}]		SOC_{Mis} [Mg ha^{-1}]
										Miscanthus	Control	*Miscanthus*	Control	
MT1	tilled land	2006	recently ploughed	110	3.6	20.7	75.7	6.16	0.99	-27.85	-28.23	75.12	95.66	1.84
MT3	tilled land	2006	barley	73	4.6	21.9	73.5	5.98	1.03	-29.17	-29.21	74.54	81.94	4.63
MT4	tilled land	2006	barley	35	4.7	24.7	70.6	6.89	0.98	-27.41	-28.12	78.93	69.27	3.43
MT5	tilled land	2006	recently ploughed	38	12.2	34.8	53.0	6.39	1.04	-27.94	-28.55	74.73	69.06	2.67
MT5a	tilled land	2006	recently ploughed	38	11.6	29.9	58.6	6.44	0.91	-27.79	-28.27	58.90	45.37	1.68
MT6	tilled land	2006	recently ploughed	13	11.5	31.0	57.5	6.29	1.17	-27.40	-27.81	51.26	41.70	1.33
MT7	tilled land	2007	barley	109	6.7	26.2	67.2	6.62	1.11	-28.67	-28.46	59.76	44.36	-0.51
MT8	tilled land	2007	barley	73	4.0	18.2	77.8	5.95	1.12	-27.72	-27.76	42.91	36.68	0.13
MG11	grassland	2007	pasture	90	7.1	29.7	63.2	6.37	1.01	-28.89	-29.14	83.10	81.79	0.99
MG12	grassland	2006	pasture	22	6.8	25.8	67.3	6.02	1.24	-27.95	-28.82	67.90	72.12	3.43
MG14	grassland	2007	pasture	8	4.1	18.5	77.4	5.32	0.96	-28.31	-28.94	74.07	73.87	2.72
MG15	grassland	2007	pasture	24	3.6	14.9	81.5	5.60	1.10	-27.56	-28.22	84.92	81.50	3.50
MG16	grassland	2007	pasture	74	4.7	17.0	78.3	6.17	1.09	-28.20	-28.42	61.40	58.94	0.59
MG17	grassland	NA	pasture	33	8.1	27.3	64.7	5.62	1.08	-29.59	-29.98	107.77	116.16	1.84
MG18	grassland	2006	silage	56	4.8	19.8	75.5	5.68	1.02	-27.97	-28.49	67.39	77.51	2.74
MG20	grassland	2006	set-aside	32	9.9	27.1	63.1	6.78	0.83	-28.30	-28.67	90.25	83.11	2.08

2.3.2 Soil sampling and sample preparation

For the soil sampling, a nested study design was used. On each *Miscanthus* and control field, three subplots were sampled, using a Pürckhauer type single gauge auger (Ø 18 mm, 100 cm length). To account for small-scale variations, seven samples were taken in each subplot and mixed prior to soil analysis. Samples were taken to a depth of 30 cm and then divided into three layers (0 - 10, 10 - 20 and 20 - 30 cm). In addition, one undisturbed soil sample (Ø 5.6 cm) to the depth of 30 cm was taken on each subplot for bulk density measurement; again the core was subdivided into 10 cm steps prior to analysis. The litter horizon, generally consisting of leaves and stem parts and varying in thickness up to 3 cm, was removed before the sampling.

The soil samples were sieved using a 2 mm meshed sieve. Approximately 5 g of the fresh soil was used for gravimetric water content measurement. The remaining soil was air dried. For the soil carbon analysis, subsamples of ca. 20 g were taken and roots and biomass larger than 2 mm were removed. The samples were then powdered using a ball mill and samples of ca. 30 mg were weighed into silver capsules. Any carbonate carbon was removed using acid fumigation (Harris *et al.*, 2001).

The pH_{aq} was measured using 5 g air-dried soil suspended in distilled water. Clay, silt and sand content were determined from the air-dried soil using the hydrometer method (Gee & Bauder, 1986). For the method 50 g of soil are dispersed in 1L measuring cylinder filled with water. The dispersed sediment increases the water density. According to Stoke's law, the settlement time of the dispersed soil particles is directly related to the particle size, therefore, by measuring the water density at two distinct times (208 sec and 5 hours after dispersing the soil) it is possible to calculate the amount of silt and silt and clay particles dispersed, as the different sized soil particles settle in different timeframes. As the values are calculated in %, sand content can easily be calculated, once silt and clay proportions are known. The Soil bulk density was measured for the 0 - 10, 10 - 20, and the 20 - 30 cm layers. An undisturbed core of known volume was oven-dried, passed through a 2 mm sieve and weighed. The weight was then divided by the volume. The value was corrected for stone content by subtracting stone mass and volume from soil mass and volume prior to the calculation.

2.3.3 Analyses of carbon

Miscanthus-derived carbon (SOC_{Mis}) was measured using the ^{13}C natural abundance method. Photosynthesis leads to a depletion of ^{13}C in plant biomass compared with the atmosphere, but the degree of depletion varies with the photosynthesis pathway. Due to the differences in the photosynthesis pathway, C_4 plants show distinctly higher ^{13}C than C_3 plants (Smith & Epstein, 1971). In an environment with no previous C_4 history, the ^{13}C abundance provides a signal to estimate the SOC, which is derived from C_4-plants such as maize or *Miscanthus* (Balesdent *et al.*, 1990; Foereid *et al.*, 2004). The SOC content and the $^{13}C/^{12}C$ ratio were analysed by the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). The ^{13}C abundance is expressed in $\delta^{13}C$ according to the equation:

$$(2) \qquad \delta^{13}C = ((R_{sample-}R_{Reference})/R_{Reference}) * 1000$$

with R_{sample} being the isotope ratio $^{13}C/^{12}C$ of the sample, and $R_{Reference}$ being the $^{13}C/^{12}C$ ratio of the international PDB carbon standard (PeeDee formation belemite).

The calculation of the *Miscanthus* derived fraction of the SOC is based on the isotope mass balance. This requires knowledge of the $\delta^{13}C$ values of (1) the SOC after the *Miscanthus* cultivation ($\delta^{13}C_{new}$), (2) the SOC before the *Miscanthus* cultivation ($\delta^{13}C_{old}$), and (3) the *Miscanthus* plant material ($\delta^{13}C_{Mis}$). With x being the fraction of SOC_{Mis}, the isotope mass balance is written as:

$$(3) \qquad \delta^{13}C_{new} = x\delta^{13}C_{Mis} + (1-x)\delta^{13}C_{old}$$

To calculate the SOC_{Mis} fraction, the equation can be rewritten as:

(4) $x = (\delta^{13}C_{new} - \delta^{13}C_{old})/(\delta^{13}C_{Mis} - \delta^{13}C_{old})$

As the $\delta^{13}C$ of the SOC before the *Miscanthus* introduction is not known, the $\delta^{13}C$ of the corresponding depths of the control sites is used instead. Therefore, the reference sites must not have any C_4 history as this would bias the results. The $\delta^{13}C$ of the *Miscanthus* plant represents an average of shoot, root and rhizome material (value taken from M. Dondini, personal communication). Carbon contents are expressed in Mg ha^{-1} for the soil depths of 0 - 10, 10 - 20 and 20 - 30 cm using the measured soil bulk densities. To verify if the selected sites are representative of carbon stocks on Irish permanent grasslands, the SOC data were compared with a SOC survey conducted on permanent grasslands in southeast Ireland by Zhang & McGrath (2004)

In the subsequent analysis, the term initial SOC (SOC_i) was introduced as an estimate for SOC contents directly after the conversion to *Miscanthus*. SOC_i was calculated by subtracting the SOC_{Mis} from the total SOC stock in the *Miscanthus* sites.

2.3.4 Statistical analysis

The dataset was tested for normality. As clay, sand and silt content were not normally distributed, a log_{10}-transformation was performed before further statistical analysis. Due to the nature of the isotope mass balance, negative SOC_{Mis} values result from higher $\delta^{13}C$ values in the control site compared with the corresponding *Miscanthus* site. Negative SOC_{Mis} values can therefore indicate a C_4-history or a local source (e.g. cow dung) of high $\delta^{13}C$. As the analysis is based on the assumption that the control site represents the $\delta^{13}C$ value prior to *Miscanthus* planting, with *Miscanthus* being the only source of higher ^{13}C carbon, a higher $\delta^{13}C$ value in the control site renders a matched pair unfeasible for the analysis. As SOC_{Mis} values can be close to zero, inaccuracy in measurement can also lead to negative values. Therefore, to avoid positive bias, only negative outliers were removed. Data points outside the 1.5 interquartile-range were considered outliers (Tukey, 1977).

To analyse the significance of differences in SOC and SOC_i contents between (former) land-use (LU_f), treatment (T), and sample depth (D), one-way analyses of variance (one-way ANOVA) were calculated using the total SOC and SOC_i, respectively, as a response variable and the site parameters as explanatory variables. The term 'treatment' is used for the generalized current land-use, distinguishing between control and *Miscanthus* sites. The dataset was split and different one-way ANOVAs were calculated with (1) (former) land-use as explanatory variable for each sample depth and treatment, (2) treatment as explanatory variable for each sample depth and (former) land-use, and (3) depth as explanatory variable for each treatment and (former) land-use. As the *Miscanthus* and control sites were sampled as matched pairs, a nested ANOVA was conducted when testing for differences in treatment, adding the factor farm to the error structure. In the case of sample depth, again a nested ANOVA was conducted with the factor subplot as included in the error structure to account for the nesting structure.

To analyse the effects of former land-use on SOC_{Mis} again different ANOVAs were used for each sample depth. As in the case of SOC and SOC_i, the factor sample depth was non-independent and a nested ANOVA with the factor subplot added to the error structure had to be conducted to analyse differences within the soil profile. All ANOVAs were calculated using the R-software Version 2.12.1 (R Development Core Team, 2010).

Due to the nested design of the experiment, the soil properties show within-farm correlation. To account for that, linear mixed-effects models were used to analyse the effects of soil properties on SOC dynamics. SOC_i and total SOC stocks, for *Miscanthus* and control, respectively, were used as a single response variable. As fixed effects, former land-use (LU_f), treatment (T), soil pH and the soil particle size distribution including all interaction terms were used. Both pH and particle size distribution have been reported to have significant effects on SOC (Brogan, 1966; McGrath & Zhang, 2003). With the use of the combined response variable, conclusions on changes in SOC directly after *Miscanthus* planting can be drawn by using treatment as an explanatory variable. The variables Farm (F) and Field (FLD) were included as random effects to account for the nested design of the experiment. As the particle size distribution parameters sand, silt and clay content are not independent, a different model has been calculated for each single parameter. The factor 'sample depth' violates the assumption of independence; therefore, the data were pooled over depth. Initially, a model using the fixed effects and all interaction terms was generated. To optimize the model structure, the significance of the model terms was tested and non-significant terms were dropped stepwise (P-value > 0.05).

To explain variations in soil carbon sequestration, the SOC_{Mis} content was used as response variable. The former land-use and soil properties (pH, soil particle size distribution, SOC_i) including their possible interactions, as well as crop age and an interaction term of crop age and former land-use, were used as fixed effects. The interaction term was introduced to take account of different annual sequestration rates under the two former land-uses. In addition to pH and particle size distribution, SOC_i has been identified as source variability for soil carbon sequestration rates (Grogan & Matthews, 2002; Chan *et al.*, 2010). The factor Farm was used as random effect to account for the nesting structure. Again, different models were calculated for sand, silt, and clay content. All mixed-effects models were calculated with the R-software Version 2.12.1 (R Development Core Team, 2010) using the nlme package (Pinheiro *et al.*, 2010).

2.4 Results

2.4.1 Survey of the field sites

The survey of the *Miscanthus* fields showed variations in the crop density and height. Even though we ruled out *Miscanthus* sites with organic fertilizer application, on one site, a recent spread of manure could be observed. However, retrieving information on the kind and amount of fertilizer spread as well as on crop yield was not possible.

On most farms, *Miscanthus* showed patchy growth with stem density varying from under 1 up to 20 m^{-2} or higher. Patches of low density showed up to 100% of grass and weed cover.

2.4.2 Soil organic carbon stocks

A summary of the SOC stocks is given in Table 2. Compared with tillage control, *Miscanthus* planted on tillage shows a trend of higher SOC contents throughout the soil profile with the difference being significant in the upper 10 cm, while SOC_i shows no significant differences between *Miscanthus* planted on tillage and the corresponding control. *Miscanthus* planted on grassland shows significantly lower SOC and SOC_i contents in the upper 10 cm and a trend towards higher contents from 20 to 30 cm compared with the grassland control. Pooled over soil depth, no significant difference can be observed. Comparing the two control types, grassland shows higher SOC values than tillage throughout the soil profile, with significant differences from 0 to 20 cm sampling depth. Under former tillage, 2.97% of the SOC was *Miscanthus*-derived, while under former grassland the amount was 2.42%. A summary of the SOC_{Mis} stocks is given in Table 3. Under former tillage, 2.97% of the SOC was *Miscanthus*-derived, while under former grassland, 2.42% of the SOC was *Miscanthus*-derived. A summary of the SOC_{Mis} stocks is given in Table 3. Within the upper 10 cm of the soil profile, former grassland shows significantly higher SOC_{Mis} contents than former tillage. From 10 to 30 cm sample depth, the differences in C_4derived SOC contents were not significant. Figure 3 shows the SOC and SOC_{Mis} contents throughout the soil profile. The tillage control sites show no significant differences between the sample depths. The grassland control shows a significant decline in SOC contents with sampling depth. *Miscanthus* sites planted on tillage show a significant difference between 10 - 20 and 20 - 30 cm. *Miscanthus* sites planted on grassland show no significant differences throughout the soil profile.

Table 2: Total and initial soil organic carbon (SOC) stocks in the tillage control (CT), grassland control (CG), *Miscanthus* planted on tillage (MT) and *Miscanthus* planted on grassland (MG).

Depth [cm]	Total SOC stocks [Mg ha^{-1}] CT		MT		CG		MG	
0 - 10	19.02	±*8.69*	22.14	±*5.91*	31.24	±7.16	26.60	±*6.40*
10 - 20	22.66	±*8.87*	23.50	±*5.22*	28.21	±5.82	28.50	±*6.60*
20 - 30	18.19	±*6.53*	19.08	±*5.20*	20.81	±6.59	24.24	±*9.18*
Total	59.87	±*20.50*	64.72	±*13.68*	80.26	±17.36	79.34	±*17.00*
Depth [cm]	**Initial SOC stocks [Mg ha^{-1}]**		**MT**				**MG**	
0 - 10			21.22	±5.54			25.51	±6.68
10 - 20			22.83	±4.79			28.40	±6.54
20 - 30			18.74	±4.92			25.21	±9.43
Total			62.80	±12.52			79.12	±18.33

Table 3: *Miscanthus*-derived carbon (SOC_{Mis}) stocks in *Miscanthus* planted on tillage (MT) and *Miscanthus* planted on grassland (MG)

Depth [cm]	SOC_{Mis} [Mg ha^{-1}] MT		MG	
10	0.90	±*0.69*	1.59	±*0.83*
20	0.62	±*0.65*	0.57	±*0.58*
30	0.30	±*0.46*	0.01	±*0.84*
Total	1.82	±*1.69*	2.17	±*1.73*

2.4.3 Influence of soil properties on soil carbon dynamics

The final model explaining the changes in SOC within 0 to 30 cm soil depth contains the terms treatment, pH and their interaction (see Table 4). None of the soil particle size distribution parameters had a significant effect. Figure 4 shows that on control sites pH has a negative effect on SOC stocks, whereas on *Miscanthus* sites, planted on grassland as well as on tilled fields, no effect can be seen. The final model explaining SOC_{Mis} reports significant effects of soil pH, SOC_i, crop age, and former land-use (see Table 4).

Table 4: Model parameters of the final mixed-effect models explaining effects on changes in soil organic carbon and on *Miscanthus*-derived carbon.

Model	Variable	Value	SE	Df	*p*
Changes in soil organic carbon					
	(Intercept)	146.54	21.55	55	< 0.001
	Treatment	-71.22	30.77	15	0.035
	pH	-12.72	3.47	55	< 0.001
	Treatment * pH	11.69	5.03	55	0.024
Miscanthus-derived carbon					
	(Intercept)	-13.10	3.11	27	< 0.001
	age	2.28	0.49	13	< 0.001
	former land-use	-1.45	0.58	13	0.026
	SOC_i	0.08	0.03	27	0.013
	pH	1.33	0.47	27	0.009

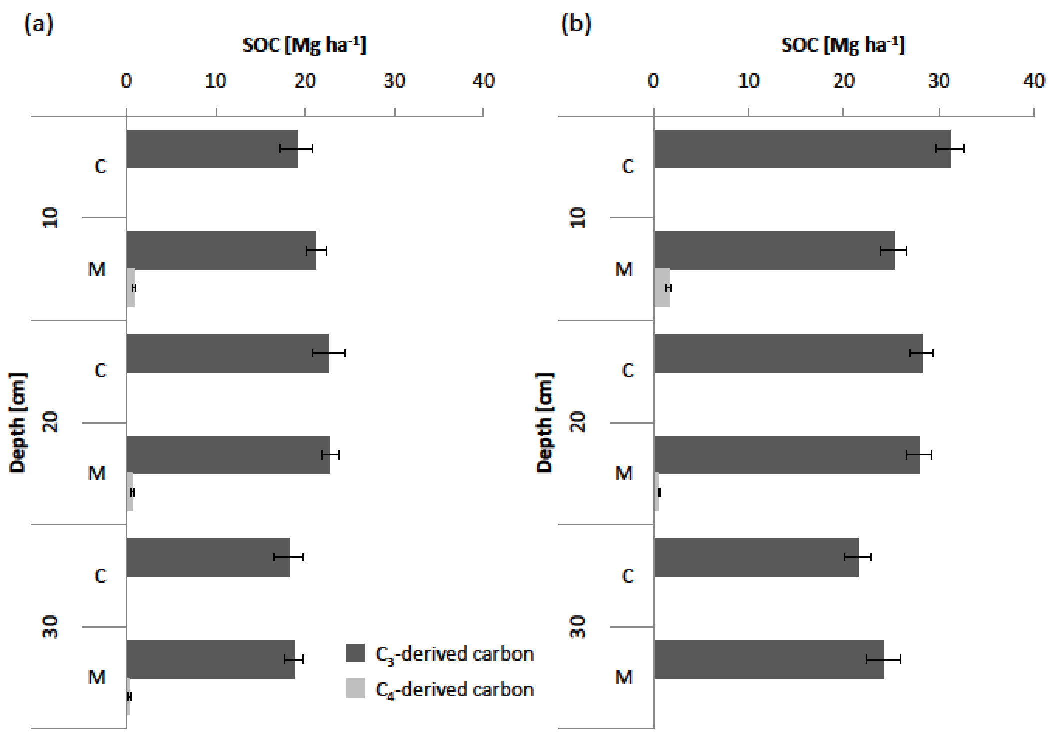

Figure 3: Soil organic carbon (SOC) contents vs. depth under the control (C) and *Miscanthus* (M) sites for (a) tillage and (b) grassland. The error bars indicate standard error.

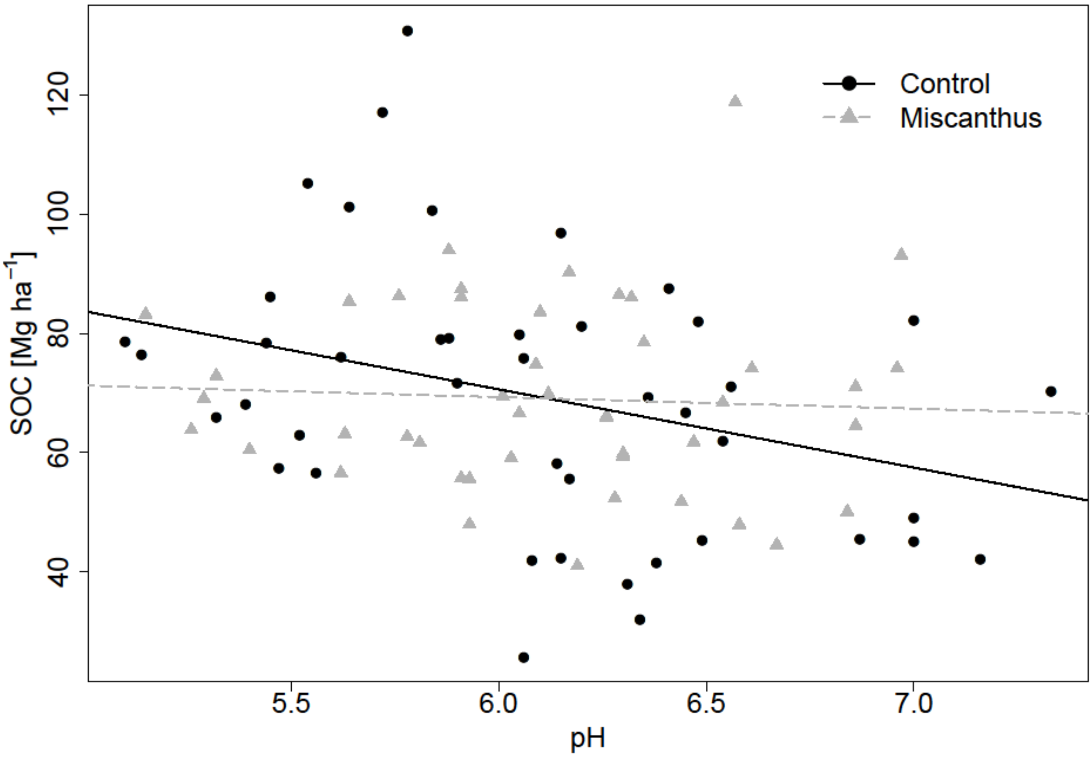

Figure 4: Total soil organic carbon (SOC) vs. pH. Points show the measured values. Lines show the results of the mixed-effects model.

2.5 Discussion

The measured SOC concentrations in grasslands are well within range of the data reported in Zhang & McGrath (2004) for south east Ireland. As expected, grassland shows significantly higher total SOC contents than tilled sites; however, tilled sites show a high variability. Pooled over 0 - 30 cm, the data show no significant changes in total SOC 2 to 3 years after the introduction of *Miscanthus* to both former grasslands and former tillage fields. SOC_i, as an estimator for SOC stock directly after the *Miscanthus* introduction, shows no significant differences between the controls and the *Miscanthus* fields. Assuming that the control sites represent the same carbon levels as the *Miscanthus* sites before transformation to the bioenergy crop, this leads to the following possible explanations: (1) there is no major loss of SOC due to the soil disturbance caused by the introduction of *Miscanthus* either on grasslands or on former-tilled lands and (2) in addition to the sequestration of SOC_{Mis}, an amount of C_3-plant derived carbon is sequestered, which cannot be detected using the ^{13}C natural abundance method as it only allows to distinguish SOC fractions from sources with different $\delta^{13}C$ values.

Depending on the former land-use, both explanations have to be considered. Lands under annual tillage have low baseline SOC contents and disturbance does not lead to additional losses. Furthermore, although not significant, the trend of higher SOC_i stocks in the upper 10 cm of the *Miscanthus* fields compared with the corresponding controls could indicate sequestration of C_3-plant derived carbon.

Due to the disturbance when establishing *Miscanthus* on grassland, a substantial loss of soil carbon was expected (Roberts & Chan, 1990; Reeder *et al.*, 1998); however, no significantly lower SOC_i stocks under *Miscanthus* compared with the grassland control were observed. Recent work on *Miscanthus* in Ireland suggests that carbon losses due to *Miscanthus* planting are lower than previously assumed (Donnelly *et al.*, 2011). The main effect was a shift of carbon from the upper 10 cm into to lower soil layers, indicating a redistribution of SOC into deeper soil layers. In addition, the incorporation of above-ground biomass due to ploughing can partly compensate for losses of SOC in the soil profile.

The results of the ^{13}C natural abundance method show a significant soil carbon sequestration by *Miscanthus* on both former arable and former grasslands. The annual carbon sequestration (0.62 ± 0.59 Mg ha^{-1} on former tillage and 0.90 ± 0.53 Mg ha^{-1} on former grasslands) corresponds well with rates reported by Matthews & Grogan (2001) (0.93 Mg ha^{-1}), Freibauer *et al.* (2004) (0.6 Mg ha^{-1}) and Smith (2004a) (0.62 Mg ha^{-1}). Clifton-Brown *et al.* (2007) reported an annual sequestration of 0.59 Mg ha^{-1} for a site in Ireland.

The upper 10 cm of former grasslands shows significantly higher SOC_{Mis} contents than former-tilled sites. This indicates a higher rate of litter incorporation under former grasslands. An explanation is a possible higher activity of the soil fauna. Among others, Don *et al.* (2009) showed the importance of earthworms in incorporation of litter biomass into soil. As long-term conventional tillage has generally a negative impact on earthworm population (e.g. Chan, 2001; van Eekeren *et al.*, 2008), and recovery is reported to take several years (van Eekeren *et al.*, 2008), it is suggested that *Miscanthus* sites established on tilled fields might show lower earthworm abundance than former grasslands. However, this hypothesis could not be tested in this study.

As expected, the conversion of tilled lands to the perennial crop *Miscanthus* leads to no direct carbon debt. Furthermore, 2 to 3 years after conversion, the *Miscanthus* fields show a trend towards higher carbon contents originating from *Miscanthus*, confirming the high potential of *Miscanthus* to improve soil carbon stocks when planted on tilled lands (Smith, 2004a; Rowe *et al.*, 2009).

The conversion of grassland to *Miscanthus* leads to a significant loss of SOC in the upper 10 cm of the soil profile. However, the data indicates a relocation of SOC within the soil column rather than emission. Altogether, assuming a constant annual carbon sequestration of 0.90 ± 0.53 Mg C ha^{-1}, the soils can regain pre-*Miscanthus* carbon levels in ca. 4 to 5 years after conversion just by *Miscanthus*. It is important to note that even though in this study the introduction of a bioenergy crop led to no significant loss of SOC, it did not account for losses in aboveground and root biomass. It is therefore possible that carbon emissions to the atmosphere due to *Miscanthus* cultivation on grasslands are underestimated.

An effect of soil particle size distribution on both total SOC and soil carbon sequestration has been reported in the literature (e.g. Brogan, 1966; Kahle *et al.*, 2001); however, in this study, neither could be seen. On control sites, a negative effect of pH on SOC was found. Former studies report that the relationship between pH and SOC is very complex with different possible interactions (Motavalli *et al.*, 1995; Kemmitt *et al.*, 2006). However, a low soil pH does inhibit microbial activity and therefore slows down degradation of soil organic matter (Kemmitt *et al.*, 2006). Also, recent input of low pH organic fertilizer might lead to a peak in SOC values connected to low pH. This is supported by the fact that under *Miscanthus*, the effect of pH on total SOC is much weaker.

The differences in SOC_{Mis} are explained by different variables. The model shows that the ^{13}C natural abundance method is able to pick up annual soil carbon sequestration as age has a positive effect on SOC_{Mis}. The positive effect of pH on carbon sequestration seen in the model is in contradiction with the negative effect on total SOC in the control sites. This shows the complexity of the interaction between pH and SOC in general. The explanation of that effect requires a more specific study of these interactions.

The positive relationship between SOC_i and SOC_{Mis} contradicts the findings of Grogan & Matthews (2002) who argued that the rate of carbon sequestration declines with the initial carbon pool size. However, an explanation of the relationship between the different SOC pools requires a detailed understanding of the local carbon dynamics as well as farming practices. For instance, unaccounted application of organic fertilizer will potentially increase the SOC_i contents and at the same time stimulate growth of *Miscanthus* and therefore increase the aboveground and belowground biomass (Smith & Slater, 2010), increasing the soil carbon sequestration.

The study shows significant soil carbon sequestration under *Miscanthus*, even after only 2 years from plantation. It can be seen that the loss of SOC due to soil disturbance caused by the introduction of *Miscanthus* does not necessarily contribute to the carbon debt, as in this study, no significant loss could be seen. Also, this study showed that on average, *Miscanthus* has the potential to regenerate SOC stocks to pre-*Miscanthus* levels within 4 to 5 years.

However, compared with the data reported in the literature, a large variability can be seen in soil carbon sequestration. Significant effects of pH and the SOC_i as well as the former land-use on soil carbon sequestration were observed. This implies that the net carbon balance of *Miscanthus* can change even on a regional scale, showing the importance of local management on soil carbon dynamics associated with the introduction of *Miscanthus*. Even though the assessment of the general suitability of *Miscanthus* for carbon mitigation was not the scope of this study, the information is valuable for the development of models and life-cycle analysis for *Miscanthus* cultivation as well as for underlining the importance of planning and management of bioenergy crops on a local basis.

Chapter 3

Assessing long-term stability of newly sequestered carbon under *Miscanthus x giganteus* during the establishment phase

Based on: Zimmermann, J, Dondini, M, and MB Jones (submitted): Assessing the direct impacts of the establishment of Miscanthus x giganteus on soil organic carbon in Ireland.

Abstract

In recent years the use of biomass for energy production has become an increasingly important measure for mitigating global change. While national and EU legislators advocate the further development of the bioenergy sector, the scientific debate has been inconclusive. There is particular concern that land-use change to bioenergy production can lead to increased CO_2 emissions. These emissions result from the loss of vegetation and the soil disturbance when ploughing natural vegetation and pastures as a preparation for planting bioenergy crops. The use of *Miscanthus x giganteus* as a bioenergy feedstock offers a possible solution, as it shows a high soil carbon sequestration potential across Europe. Furthermore, as shown in the previous Chapter, no significant differences in soil organic carbon (SOC) stocks between *Miscanthus* fields and adjacent control sites could be measured. However, it may be possible that initial ploughing may lead to a disruption of existing aggregates and therefore to on-going losses of soil organic carbon to the atmosphere. The aim of the present study was to analyse impacts of land-use change to *Miscanthus* on different soil fractions as well as the total SOC, as well as *Miscanthus*-derived SOC stocks.

Four young commercial *Miscanthus* sites, as well as adjacent sites representing the former land-use, in SE Ireland were analysed for changes in total SOC and newly sequestered *Miscanthus*-derived C.

The fraction with which the SOC is associated significantly influences its decomposability and turn-over time. Using the ^{13}C natural abundance method, we found that newly sequestered C is mainly found as particulate organic matter (79.7 %) and therefore is in a labile state with short turn-over times. No significant differences were found in the distribution of the different soil fractions, and SOC between the *Miscanthus* and the control sites, and it was shown that the share of fractions on the bulk soil, as well as the total SOC associated with these fractions in young *Miscanthus* sites is mainly depending on the previous land-use.

3.1 Introduction

In the previous chapter it was shown that annual soil carbon sequestration rates of young *Miscanthus* crops is similar to those reported in the literature for older crops. Also, no significant differences in total SOC stocks between *Miscanthus* sites and adjacent control sites could be observed, indicating no major SOC loss due to *Miscanthus* planting. However, to understand the fate of newly sequestered carbon as well as to fully understand the impacts of *Miscanthus* planting it is important to measure the stability of the *Miscanthus*-derived carbon under these young *Miscanthus* sites.

The use of *Miscanthus x giganteus* (Greef et Deu ex Hodkinson et Renvoize) (Greef & Deuter, 1993; Hodkinson & Renvoize, 2001) as feedstock for bioenergy production has been a focus in recent research (Lewandowski *et al.*, 2000; Foereid *et al.*, 2004; Clifton-Brown *et al.*, 2007; Heaton *et al.*, 2008; Styles *et al.*, 2008). This perennial, rhizomatous C_4 grass, originating from south-east Asia has shown a remarkable adaptability to temperate climates achieving high biomass yields in Europe and North America (Clifton-Brown *et al.*, 2004; Heaton *et al.*, 2008; Hastings *et al.*, 2009). Generally the introduction of perennial crops as bioenergy feedstock is considered a viable alternative to overcome some of the negative aspects of annual crops such as maize, soybean, or oil seed rape (Tilman *et al.*, 2009). The major concerns associated with annual crops are (1) a possible increase in food prices due to competition with food crops (Koh & Ghazoul, 2008), (2) negative impacts on biodiversity and associated ecosystem services, such as pollination and biocontrol, due to high intensity farming, therefore extensive use of fertiliser and pesticides, as well as regular disturbance, and the destruction of (semi-) natural habitats (e.g. Cook *et al.*, 1991; Koh, 2007; Landis *et al.*, 2008), and (3) the loss of soil organic carbon due to ongoing soil disturbance in annual cropping systems (e.g. Roberts & Chan, 1990; Paustian *et al.*, 2000b; Smith, 2008). These losses, as well as the loss of above-ground vegetation, are depending on both the introduced bioenergy crop as well as the ecosystem that is replaced. Fargione *et al.* (2008) estimated that, depending on these factors, the losses can be up to 3452 Mg C ha^{-1}. These carbon emissions can potentially outweigh carbon benefits due to bioenergy use for up to four centuries (Fargione *et al.*, 2008; Gibbs *et al.*, 2008; Searchinger *et al.*, 2008).

As a dedicated perennial bioenergy crop, *Miscanthus* does not necessarily compete with food production, also it has the potential to be grown on marginal lands therefore not competing for high quality agricultural land (Qin *et al.*, 2011), furthermore it requires low inputs of fertiliser and pesticides (Caslin *et al.*, 2010), reducing its impact on biodiversity, potentially even offering habitat for some species (Semere & Slater, 2007; Rowe *et al.*, 2009; Dauber *et al.*, 2010). Additionally, *Miscanthus* shows a high soil carbon sequestration potential compared to annual crops or grassland systems (Hansen *et al.*, 2004). Generally the cultivation of perennial crops decreases soil disturbance as the field is taken out of tillage. Soil disturbance has been identified to be a major driver of soil organic carbon loss (Paustian *et al.*, 2000a), due to increased aeration and a reduction in the physical protection of soil organic matter leading to increased decomposition rates (Oades, 1984; Roberts & Chan, 1990). In particular the land-use change from forest or grassland to arable has been shown to lead to substantial losses in the soil organic carbon stocks (Houghton *et al.*, 1999; Chen *et al.*, 2005; Poeplau *et al.*, 2011). The crop is usually harvested in spring time to allow winter senescence to reduce plant moisture content. Leaving the crop standing over winter increases litter fall, therefore leading to the accumulation of biomass (Beuch, 1999). Additionally, as a rhizomatous crop it allocates a large proportion of the above ground carbon into the roots and rhizomes during winter senescence further increasing soil organic carbon stocks (Kuzyakov & Domanski, 2000).

A number of studies have confirmed the soil carbon sequestration potential in experimental plots throughout Europe (Hansen *et al.*, 2004; Clifton-Brown *et al.*, 2007; Schneckenberger & Kuzyakov, 2007; Dondini *et al.*, 2009b). Annual carbon sequestration rates reported in the literature ranged from 0.7 to 3.2 Mg C ha^{-1}. However differences in sampling techniques make comparisons difficult. In Ireland the planting of *Miscanthus* has recently been subsidised by the government (Department of Agriculture, 2010), leading to an increased abundance of the crop in the farming landscape allowing for on-farm research. In the previous chapter soil carbon sequestration rates during the establishment phase of commercial *Miscanthus* plantations in south-east Ireland were measured showing comparable rates to those reported in earlier field trials. However, information on the fate of this newly sequestered carbon was not available.

The stability of soil organic matter (SOM) and SOC towards decomposition is depending on three factors, (1) chemical processes, especially through bonds of SOM with colloids and clays, leading to highly stable organic compounds, (2) biochemical processes leading to chemical-complex formation between organic compounds and soil particles, and (3) physical protection reducing the accessibility of organic carbon for decomposers (Jones & Donnelly, 2004). Unprotected, or labile, organic carbon is easily accessible for the soil fauna while organic carbon that is coated in soil particles (e.g. silt or clay) or is incorporated into stable aggregates is generally less accessible and therefore more resistant to decomposition (Six *et al.*, 2000b; Six *et al.*, 2002b). Generally, stable aggregates also reduce aeration, leading to a further reduction in oxidation of organic carbon (Roberts & Chan, 1990).

The majority of organic carbon enters the soil as particulate organic matter in the form of dead plant material. As this unprotected stage is more accessible to decomposers it generally shows short turn-over times (Six *et al.*, 2002b). Stabilisation of soil organic carbon occurs through a number of processes. Initially unstable aggregates are formed by biological, chemical and physical processes, such as interaction with bacteria and fungi, the aggregation of soil organic matter around growing roots, forming of chemical bonds, and the coating of organic matter with silt or clay particles. Long-term protection is achieved due to further stabilisation of these newly formed aggregates such as ageing, exposure to dry-wet cycles, and biological processes such as root-growth (Six *et al.*, 2002b).

Freshly sequestered particulate organic matter is also highly susceptible to land-use change (Six *et al.*, 2000a), therefore to assess the sustainability of soil carbon sequestration by *Miscanthus* it is crucial to know about the time-frame in which stabilisation processes occur. Furthermore, it is not known how ploughing prior to *Miscanthus* planting (Caslin *et al.*, 2010) affects the stability of pre-existing C_3 carbon stocks. While the study in Chapter 2 showed no significant reduction in C_3 carbon stocks after

Miscanthus planting a disruption of stable aggregates due to ploughing may lead to an on-going loss of soil organic carbon.

To analyse the amount of carbon associated with different stage of protection a number of separation methods, including chemical (Gregorich *et al.*, 2003; Weil *et al.*, 2003) as well as physical methods and density separation (Tisdall & Oades, 1982; Cambardella & Elliott, 1992; Zimmermann *et al.*, 2007) have been developed. While chemical methods provide strong insights into the composition of SOM and SOC, it does not always reflect the different turn-over times. Physical fractionation methods have been shown to achieve better results (Ellert *et al.*, 1995; Balesdent *et al.*, 1996).

The aim of the present study was (1) to quantify the proportion of freshly derived as well as old C associated with the labile and stable fractions, and (2) to assess the impact of land-use change on the overall proportion of these fractions in commercial fields planted with *Miscanthus*. As all fields were planted in 2006/2007 the sites proved valuable for studying early stages of *Miscanthus*-derived soil carbon sequestration. Using the approach described by Zimmermann *et al.* (2007) soil samples from the sites were fractionated using a combination of chemical, physical, and density-separation methods. The methodology was selected as it is more cost and time efficient than other physical fractionation methods (Reeder *et al.*, 1998; Six *et al.*, 2002a), also the fractions are well representative of the conceptual carbon pools described in the RothC model (Coleman & Jenkinson, 1996b). The comparability allows for the data to be used in further parameterising and testing of the model. These advantages have led to a widespread use of the fractionation method in *Miscanthus* and other research (Dondini *et al.*, 2009a; Xu *et al.*, 2011; Poeplau & Don, 2013). The method separates soils into two labile (dissolved organic matter, DOC, and particulate organic matter, POM), two physically protected (sand and stable aggregates, S+A, and organic carbon protected by silt and clay particles, S+C) and an inert fraction, resistant to chemical oxidation (resistant soil organic carbon, rSOC). The separation of the physically protected fractions enables an understanding of the possible impacts of soil disturbance on soil organic carbon pools. Large aggregates, while more stable than particulate organic matter, are more susceptible to tillage processes than the smaller fraction protected by silt and clay, or the resistant fraction (Six *et al.*, 2002b). It is hypothesised that (1) a large portion of fresh *Miscanthus*-derived carbon will be found in the POM fraction, (2) ploughing of a grassland before *Miscanthus* plantation will lead to a reduction of carbon in the S+A fraction, (3) a long-term arable site will have lower total soil organic carbon stocks than grassland sites due to long term losses because of soil disturbance, and (4) that the difference between grassland and tillage will be most apparent in the S+A fraction.

3.2 Materials & Methods

3.2.1 Field site selection

Soil samples were collected in May/June 2010 on a four of commercial farms growing *Miscanthus* located in south east Ireland. All *Miscanthus* fields were planted in the years 2006/2007, and have been harvested annually from the second year after establishment during spring. Two former land-use categories were sampled, tilled land and grassland. The locations of the farms are shown in Figure 5. The selection criteria were a maximum elevation of 120 m a.s.l., a minimum field size of 2 ha, and the availability of an on-farm control site. The control site was an adjacent field representing the former land-use of the *Miscanthus*. For the analyses it was important that both the *Miscanthus* and the control sites had not recently been used to cultivate a C_4 crop (i.e. maize). An overview of the soil properties in the field sites is given in Table 5.

Figure 5: Location of the field sites and former land-use of *Miscanthus* fields.

3.2.2 Soil sampling

Four different land-use types were sampled, *Miscanthus* planted on grassland (MG), grassland control (CG), *Miscanthus* planted on former tilled land (MT) and tillage control (CT). The experimental design was nested with each pair of *Miscanthus* and control site being nested in farm. Per field 16 cores were taken using a gauge auger (Ø 5.6 cm) down to 30 cm depth. The cores were then subdivided into three 10 cm sections and finally pooled over the site. The litter horizon, consisting mostly of leaf and shoot material of varying thickness up to 3 cm was removed prior to sampling. An additional four cores was taken in each field to determine soil bulk density. The samples were separated into the three depth increments prior to measurement. Soil bulk density for each depth increment was measured by determining the weight of a known volume of oven dried soil (105 ºC), after stones (> 2 mm) have been removed. The core weight and volume was corrected for stone content and the bulk density was then calculated by dividing the corrected soil weight by the corrected volume.

Table 5: Parameters of the sampled *Miscanthus* sites, elevation was measured using one GPS measurement. Particle size distribution, bulk density, and pH values are averaged over 30 cm sample depth and the subplots.

Site ID	Former land-use	Miscanthus planted in	Elevation (m a.s.l.)	Treatment	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Bulk density (g cm^{-3})
MT6	Tilled land	2006	13	Control	10	10.0	29.4	60.6	0.77 ±0.03
					20	10.2	32.4	57.4	0.98 ±0.03
					30	16.0	29.8	54.2	1.25 ±0.03
				Miscanthus	10	11.4	31.4	57.2	1.19 ±0.01
					20	10.0	30.8	59.2	1.17 ±0.07
					30	11.6	32.0	56.4	1.39 ±0.07
MT8	Tilled land	2007	73	Control	10	4.0	16.4	79.6	1.00 ±0.09
					20	3.8	16.8	79.4	1.11 ±0.13
					30	6.0	18.6	75.4	1.24 ±0.11
				Miscanthus	10	2.0	18.6	79.4	1.14 ±0.05
					20	4.4	16.4	79.2	1.15 ±0.05
					30	4.0	22.4	73.6	1.17 ±0.10
MG11	Grassland	2007	90	Control	10	5.4	22.4	72.2	1.01 ±0.32
					20	5.4	26.8	67.8	0.64 ±0.40
					30	7.4	31.4	61.2	1.23 ±0.46
				Miscanthus	10	5.4	33.6	61.0	1.02 ±0.05
					20	7.6	30.4	62.0	1.00 ±0.05
					30	11.4	33.8	54.8	1.43 ±0.09
MG14	Grassland	2007	8	Control	10	3.8	13.8	82.4	1.04 ±0.06
					20	2.2	16.8	81.0	0.85 ±0.04
					30	6.2	22.4	71.4	1.14 ±0.00
				Miscanthus	10	4.4	19.4	76.2	0.84 ±0.01
					20	3.8	18.6	77.6	1.00 ±0.04
					30	4.2	20.2	75.6	1.17 ±0.09

3.2.3 Sample preparation and soil fractionation

The soil was air-dried and approximately 90 g was passed through a 2 mm mesh-sized sieve. For each category, the samples were pooled over the subplots. The samples were then fractionated using physical and chemical methods according to Figure 6. Thirty grams of the sample was added to 161 ml of deionized water and dispersed using a calibrated ultra-sonic probe (VC 750, Sonics & Materials Inc, Newtown, USA) at 22 J ml 1. The suspension was then washed through a 63 µm aperture size sieve. The suspension <63 µm was centrifuged at 1000 g to separate the clay and silt fraction (S+C) and the dissolved organic carbon (DOC). The S+C fraction was dried at 40 °C and weighed. A known volume of the remaining suspension was passed through a 0.45 µm aperture filter which was then dried at 40 °C and weighed to account for any S+C left in the suspension. The filtrate was frozen and stored for DOC measurement. The fraction >63 µm, containing the sand fraction and stable aggregates (S+A) as well as the particulate organic matter (POM), was dried at 40 °C and weighed. To separate the S+A from the POM fraction a density fractionation was applied. The >63 µm fraction was transferred to a centrifuge tube and dispersed in approximately 30 ml of sodium polytungstate (SPT) (Sometu, Berlin) set to a density of 1.8 g cm^{-3}, leaving the light fraction (POM) floating on top and the heavy fraction (S+A) settled at the bottom of the centrifuge tube. The dispersion was then centrifuged for 15 min at 1000 g and left settling overnight. After that the sample was carefully placed in a freezer in an upright position. Once the sample was frozen the POM could easily be separated from the S+A fraction by melting it using deionized water. The POM fraction was collected in a 25 µm aperture size nylon bag, cleared of all remaining SPT using deionized water, dried at 40 °C, and weighed. To remove any remaining SPT from the S+A fraction a subsample was placed on a 0.45 µm filter and rinsed with deionized water. The weight of the S+A fraction could be determined using the mass balance as the weight of the POM fraction and the POM and S+A fraction was known. Sodium hypochlorite (NaOCl) oxidation was used to extract a chemically resistant fraction (rSOC) from the fraction <63 µm (S+C). Following a modified method after Kaiser & Guggenberger (2003) 500 g of the S+C fraction were oxidized for 18 hours at 25 °C with 25 ml of 6 % NaOCl solution, adjusted to pH 8 using HCl. The sample was then centrifuged at 1000 g for 15 minutes, decanted and washed with deionised water, then centrifuged again. For each sample the oxidization was repeated twice.

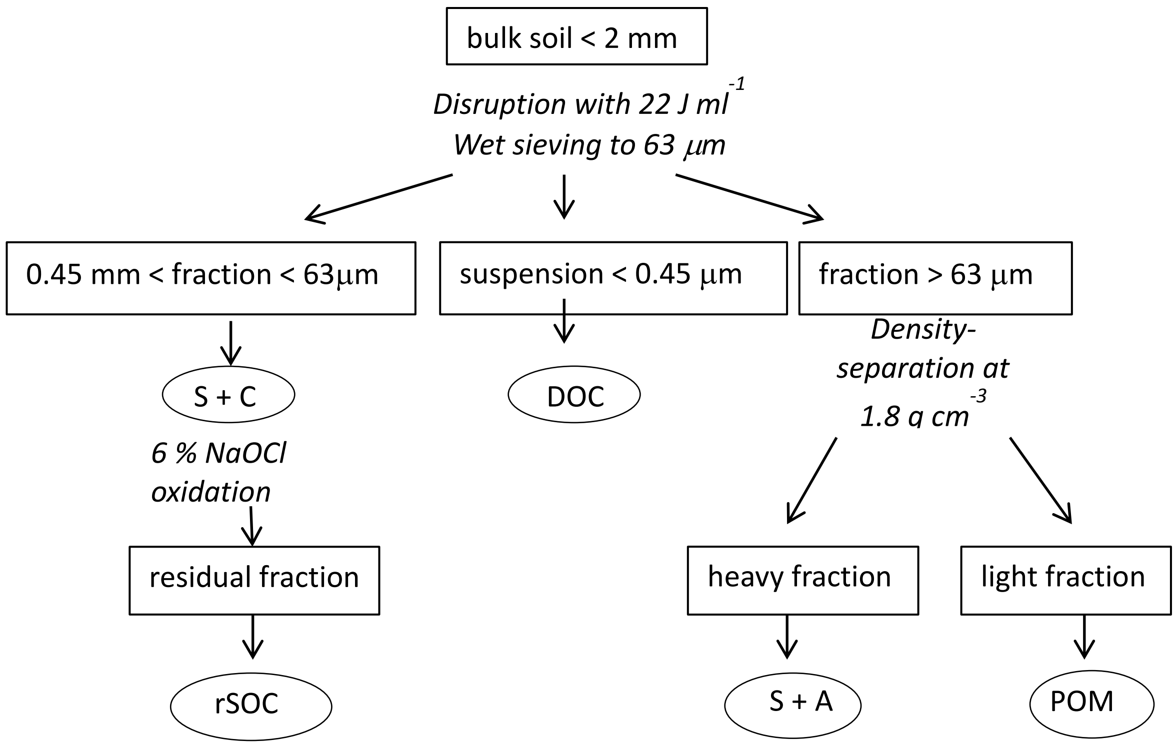

Figure 6: Diagram of the fractionation procedure; S+C = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, S+A = sand and stable aggregates, and POM = particulate organic matter (Zimmermann *et al.*, 2007).

3.2.4 Total SOC and Miscanthus-derived C analysis

Total soil organic carbon (SOC_t) and $\delta^{13}C$ values were determined for each fraction. Each sample of the solid fraction was ground and an appropriate amount was weighed into Ag capsules (1 mg for the POM fraction, 30 mg for all other fractions). The samples were then fumigated with HCl to remove all carbonate carbon following the method of Harris *et al.* (2001). All SOC_t and $\delta^{13}C$ values were measured by the UC Davis Stable Isotope Facility, California, USA, using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon

Ltd, Cheshire, UK). Total dissolved carbon and $\delta^{13}C$ in the liquid samples was measured using a O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX), again interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments).

The amount of *Miscanthus*-derived carbon (SOC_{Mis}) was determined using the ^{13}C natural abundance method. Generally photosynthesis leads to a discrimination against the heavier ^{13}C isotope in the plant organic matter compared to atmospheric CO_2. The degree of the discrimination is dependent on the photosynthetic pathway with organic matter in C_4-plants shows distinctly higher ^{13}C abundance than in C_3-plants. In an environment with only one source of C_4-derived soil organic carbon (e.g. *Miscanthus*) the isotopic signal can be used to quantify the amount of carbon derived by that given source (Balabane & Balesdent, 1992; Balesdent & Balabane, 1992) using the isotope mass balance.

The ^{13}C abundance is expressed as $\delta^{13}C$, relative to the international PDB carbon standard (PeeDee formation belemite) according to the equation

$$(5) \qquad \delta^{13}C = ((R_{sample-}R_{Reference})/R_{Reference}) * 1000$$

where R_{Sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{Standard}$ the $^{13}C/^{12}C$ ratio of the PDB carbon standard.

Having knowledge about (i) $\delta^{13}C$ of SOC before *Miscanthus* plantation ($\delta^{13}C_{old}$), (ii) $\delta^{13}C$ of SOC after *Miscanthus* plantation ($\delta^{13}C_{new}$), and (iii) $\delta^{13}C$ of *Miscanthus* plant material ($\delta^{13}C_{Mis}$) the stable isotope mass balance can be used to calculate the fraction of *Miscanthus*-derived carbon. With x being the fraction of $\delta^{13}C_{Mis}$ the isotope mass balance is written as

$$(6) \qquad \delta^{13}C_{new} = x\delta^{13}C_{Mis} + (1-x)\delta^{13}C_{old}$$

To then calculate the *Miscanthus*-derived fraction the equations is solved for x as following

$$(7) \qquad x = (\delta^{13}C_{new} - \delta^{13}C_{old})/(\delta^{13}C_{Mis} - \delta^{13}C_{old})$$

Because the $\delta^{13}C_{old}$ value of the *Miscanthus* plots is not known, it is assumed that the control sites represent the $\delta^{13}C$ value of the *Miscanthus* sites prior to *Miscanthus* planting at the corresponding depths. The method requires that no other source of C_4 derived carbon is, or was present at the surveyed sites. The $\delta^{13}C$ of the *Miscanthus* plant represents an average of shoot, root and rhizome material (value taken from M. Dondini, personal communication). All carbon contents are measured from the depths 0 - 10 cm, 10 - 20 cm, and 20 - 30 cm. Using the measured bulk density the measured carbon contents given in g C kg^{-1} soil were converted into carbon stocks (Mg C ha^{-1}).

3.2.5 Statistical analysis

To analyse the significance of differences between groups, linear mixed effects models were applied. This was necessary to account for the nested structure of the experimental design. Three response variables were tested: (1) share of soil fraction on bulk soil (SF), (2) total soil organic carbon (SOC_t), and (3) *Miscanthus*-derived carbon (SOC_{Mis}). Former land-use (LU_f, grassland vs. tillage), treatment (T, *Miscanthus* vs. control), and sample depth (D) were used as explanatory variables, farm (F) was used as random effect. An initial model using all explanatory variables, as well as all possible interactions was created, then, in a stepwise approach all non-significant terms ($p > 0.05$) were removed. The final models are shown in Table 6. To test differences between different levels with an explanatory variable a general linear hypothesis function in combination with a Tukey post-hoc test was used. All statistical analysis was carried out using the R software (R Development Core Team, 2010), including the packages NLME (Pinheiro *et al.*, 2010), and MultComp (Hothorn *et al.*, 2008).

Table 6: Summary of the linear mixed-effects models. SF = Soil fraction, LU_f = former land-use, D = sample depth, and Dens = crop density (open patch vs. high crop density). Significance levels: * p < 0.05 ** p < 0.01.

Response	Fixed effect	dF	dF density	F-value	p-value
Share of soil fraction					
	(Intercept)	1	60	0.36	0.55
	SF	2	60	118.01	< 0.01
	LU_f	1	2	0.09	0.79
	SF:LU_f	2	60	10.49	< 0.01
Total soil organic carbon					
	(Intercept)	2	60	38.81	< 0.01
	SF	2	60	20.95	< 0.01
	LU_f	1	2	12.40	0.07
	D	2	60	15.76	< 0.01
	SF:LU_f	2	60	17.33	< 0.01
	LU_f:D	2	60	6.45	< 0.01
Miscanthus-*derived carbon*					
	(Intercept)	1	46	5.51	0.02
	SF	4	46	9.42	< 0.01
	D	2	46	12.09	<0.01

3.3 Results

3.3.1 Impact of land-use on the distribution of soil fractions

The distribution of the different fractions shows no significant differences between *Miscanthus* and the control sites, for both former tilled and former grassland sites and the respective controls. Also the distribution of the soil fractions does not change significantly with increasing soil depth. Patterns in the distribution of soil fractions vary strongly between farms, however, the MG and CG sites showed significantly higher proportion of the S+A fraction (59.9 % ±8.8 SE and 61.0 % ±3.7 SE) and a significantly lower proportion of the S+C (+rSOC) fraction (38.4 % ±3.61 SE and 37.8 % ±3.5 SE), compared to the MT and CT sites (47.3 % ±10.7 SE and 48.9% ±12.2 SE for the S+A fraction, and 51.3 % ±10.3 SE and 50.1 % ±12.0 SE for the S+C fraction) (all values averaged over depth). The contribution of the solid fractions to the bulk soil is shown in Figure 7; the full datasets for all sites are shown in the supplementary materials. The best fit model is shown in

Table 6. As the rSOC fraction is not physically separated from the S+C fraction, these two fractions are not separated when considering their contribution to the bulk soil.

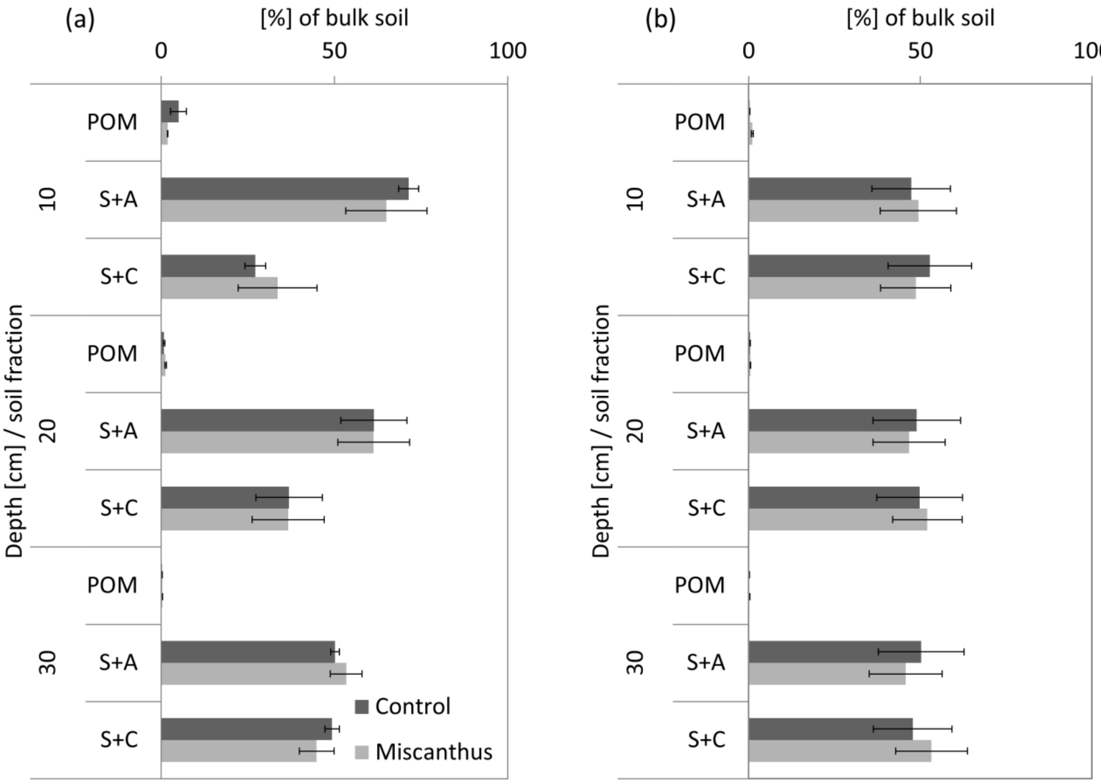

Figure 7: Distribution of solid fractions within the bulk soil in *Miscanthus* and control sites for (a) (former) grassland, and (b) (former) tilled land. The error bars show the standard error.

3.3.2 Distribution of total SOC stocks within soil fractions

Both grassland control sites as well as *Miscanthus* planted on grassland (CG and MG) contained significantly higher SOCt stocks than tillage control sites and *Miscanthus* planted on tillage (CT and MT) sites (106.6 Mg ha^{-1} ±7.3 SE and 97.0 Mg ha^{-1} ±16.3 SE vs. 55.7 Mg ha^{-1} ±3.1 SE and 46.1 Mg ha^{-1} ±0.3 SE). Again, no significant differences between the *Miscanthus* and the control sites could be found for both former land-uses. As seen in Figure 8, significant differences in the distribution of C among the different fractions could be seen between the two former land-use categories. A significantly higher share of the total SOC levels was found in the S+A fraction in MG and CG sites compared to the MT and CT sites (45.4 % ±13.2 SE and 47.1 % ±5.2 SE vs. 14.4 % ±3.2 SE and 13.0 % ±3.3 SE, for *Miscanthus* and control respectively), while the share of the S+C fractions on SOCt showed a reverse pattern (31.8 % ±10.6 SE and 27.6 % ±0.7 SE vs. 59.7 % ±1.0 SE and 62.8 % ±2.1 SE, for MG and CG, and MT and CT respectively). The other fractions contained relatively similar shares of the total SOC stocks in all four sites with no clear patterns regarding the influence of (former) land-use and *Miscanthus* cultivation. The POM fraction contained 10.4 % ±1.8 SE and 16.0 % ±4.6 SE of the total C stock for MG and CG respectively. The rSOC fraction contained 10.5 % ±1.0 SE and 7.5 % ±10.5 SE of the SOC_t¬ stocks under MG and CG respectively. The share of C in the POM fraction for MT and CT was 10.5 % ± 0.6 SE and 8.0 % ± 0.2 SE and that of the rSOC fraction 13.4 % ± 1.2 SE and 14.5 % ± 1.0 SE for MT and CT, respectively. In all sample categories the share of DOC was under 3 %.

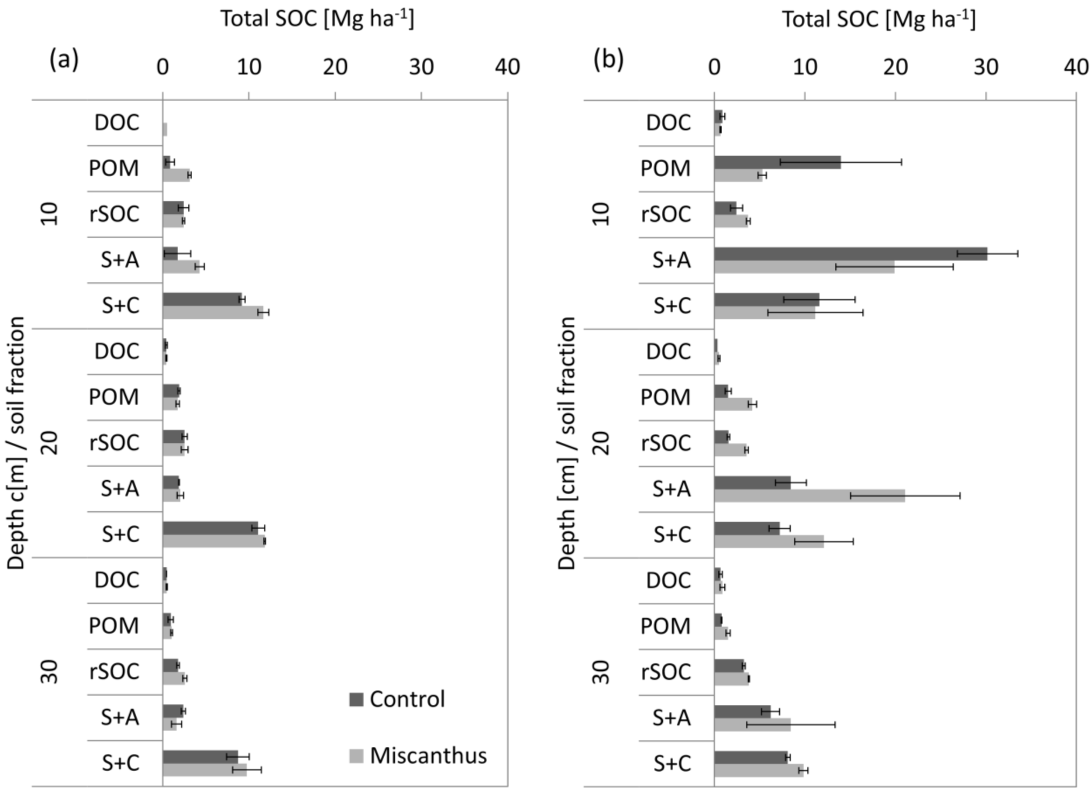

Figure 8: Total soil organic carbon within the soil fractions in *Miscanthus* and control sites, for (a) (former) grassland, and (b) (former) tilled land. The error bars show the standard error.

3.3.3 Distribution of *Miscanthus*-derived carbon stocks within soil fractions

Miscanthus-derived C stocks are 2.2 Mg ha^{-1} ±2.4 SE and 4.5 Mg ha^{-1} ±2.2 SE, for former grasslands and former tilled lands, respectively. In both land-uses the majority of *Miscanthus*-derived C is found in the top 10 cm of the soil profile. As seen in Figure 9, the majority of *Miscanthus*-derived C is found in the POM fraction (76.9% ±3.2 SE), which shows significantly higher SOC_{Mis} values than the other fractions. The S+A, S+C, and rSOC fractions show increase SOC_{Mis} stocks in the top 10 cm (0.6 Mg ha-1 ±0.2 SE, 0.5 Mg ha^{-1} ±0.2 SE, and 0.1 Mg ha^{-1} ±0.06 SE, respectively). The SOC_{Mis} values in these fractions vary strongly between farms and no influence of former land-use can be recognised. SOC_{Mis} in the DOC fraction was not significantly different form zero. In the lower sampling depths only POM showed values significantly different from zero. The δ^{13}C values as well as the SOC_{Mis} stocks can be found in the supplementary materials.

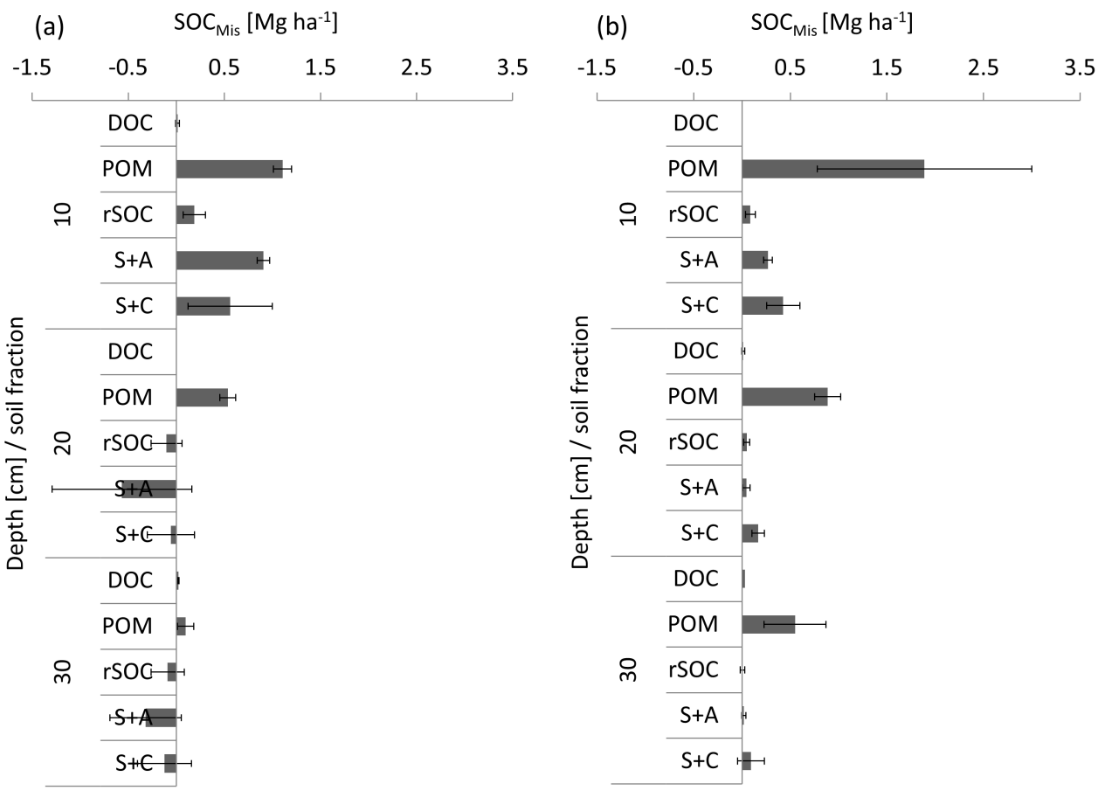

Figure 9: *Miscanthus*-derived carbon within the soil fractions in open patches and high crop density patches, for (a) (former) grassland, and (b) (former) tilled land. The error bars show the standard error.

3.4 Discussion

The commercial sites recently established in Ireland provided insight into the C dynamics linked to the land-use change from tillage and grassland to a perennial bioenergy crop and into the fate of newly derived. Applying the fractionation to the surveyed sites enabled us to further understand the SOC dynamics under *Miscanthus* plantations in the establishment phase.

The results show that the initial ploughing carried out before *Miscanthus* planting (Caslin et al., 2010) has no significant impact on the proportions of the soil fractions described by Zimmermann et al. (2007) as well as on the amount of C associated with these fractions. The lack of change in SOCt values are in accordance with recent studies, showing that *Miscanthus* plantation does not lead to a significant SOC loss (Zatta et al., 2012; Zimmermann et al., 2012). Furthermore, as land-use change to *Miscanthus* leads to no significant changes in the proportion of stable fractions, it can be assumed that no further destabilisation of C_3 (or old) C occurred. This can also be seen in Dondini et al. (2009a), who reported similar C_3 carbon stocks in a 14-year old *Miscanthus* sites and an adjacent arable control site. These results show that the land-use change to bioenergy crops does not necessarily lead to a significant C debt due to losses of SOC. Moreover, it suggests that changing a cropping system from an annual to a perennial regime does not affect the formation of stable aggregates within the first three to four years, indicating slower aggregate formation speed as reported by Jastrow (1996), who found a significant increase in macroaggregates in the first 4 years of a tall grass prairie restoration chronosequence. However, it has to be mentioned that initial aggregate formation processes cannot be observed, as the methodology is not able to detect newly formed unstable macroaggregates, because the treatment with the ultrasonic probe destroys this initial stage of aggregate formation (Six et al., 2002). The results show, that in the early stage of *Miscanthus* plantation the influence of the previous land-use regime on the distribution of the soil fractions, as well as on the total SOC is more important than the processes linked to land-use change.

As expected, at the early stage of *Miscanthus* cultivation, the majority of *Miscanthus* derived C was found in the POM fraction. The majority of *Miscanthus*-derived C is found in the top 10 cm of the soil column and all four sites show stabilization of newly sequestered carbon as SOC_{Mis} can be found in all stable fractions, including rSOC. However, since all four sites showed different patterns no influence of the former land-use on that process could be identified. In the deeper soil increments, *Miscanthus* derived carbon was not significantly different from zeros with partly negative values. These negative values are likely to be caused by inaccuracies when measuring the $\delta^{13}C$ values. As the sites are still in an early stage, SOC_{Mis} levels in lower depths are likely below the detection limit of the ^{13}C natural abundance method, especially when separating the soil into the different fractions. A comparison of the *Miscanthus*-derived C stocks found in the present study with values measured by Dondini et al. (2009a) on a 14 year old *Miscanthus* site in Co. Carlow, Ireland showed significantly lower SOC_{Mis} values in the present study for all fractions except for the POM fraction, where similar values are found in both studies (2.5 Mg ha^{-1} in the present study and 2.62 Mg ha^{-1} in Dondini et al. (2009b)). As the sites show similar climatic conditions as well as similar soil properties, these similar SOC_{Mis} values in the POM fraction may be attributed to an equilibrium of C associated with the POM fraction. Due to high input rates of litter under *Miscanthus* (Beuch, 1999) it can be assumed that the *Miscanthus*-derived C stocks in the POM fraction build up quickly after planting the crop. These results suggest that for the POM fraction, the equilibrium between input of fresh plant material and output due to decomposition and association with stable aggregates can be reached in a short time after planting. However it would require continuous long-term measurements to confirm this hypothesis.

While the direct impacts of land-use change on soils under young *Miscanthus* fields where small, the study provided interesting insights on the distribution of soil fractions under the two different land-uses grassland and tillage. As expected, SOC_t stocks found under grassland, as well as *Miscanthus* planted on grassland are larger than under (former) tillage sites. Generally tillage sites are depleted of SOC due to long-term disturbance (Paustian et al., 2000). This is also confirmed by an earlier study on the same sites as well as a number of additional sites in the region (Zimmermann et al., 2012).

Grassland sites also generally showed a higher share of the S+A fraction, compared to the S+C fraction, which can be attributed to the lack of disturbance due to ploughing. However, other than expected, the tillage sites showed no clear pattern that would indicate aggregate disruption due regular ploughing, such as reported by Six et al. (2000b), who found a reduction in macroaggregates in a number of long-term agricultural sites in the United States following similar agricultural activity.

The absolute difference in SOC_t stocks between the S+A fractions in the (former) grassland samples compared to the (former) tillage samples are much larger than the share of the respective fraction in the bulk soil. As the S+A fraction also contains the sand fraction, this may be due to a shift in the sand/stable ration within the aggregate towards more sand. However Six et al. (2000b) report a strong depletion of SOC in microaggregates under long-term conventional tillage compared to native vegetation and no-till agriculture.

While Poeplau and Don (2013) show similar results in a number of studies across Europe, the low SOCt values in the S+A fraction of tillage sites contradict measurements obtained on an arable site in Co. Carlow, Ireland, conducted by Dondini et al. (2009a) using the same methodology. While the total SOC in the combined S+A and S+C fractions reported by Dondini et al. (2009a) is similar to the C stocks found in the present study (30.9 Mg C ha^{-1} and 34.95 Mg C ha^{-1}, respectively), Dondini et al. (2009a) reported them to be equally distributed among these fractions. The site used by Dondini et al. (2009a) were in close proximity to the sites sampled in this experiment, and show similar climatic conditions, furthermore reported soil properties (pH and soil texture) are also similar. A possible reason for these differences is the historical land-use, as the site used by (Dondini et al., 2009a) was an experimental site in the Teagasc, Oak Park research facility, while the sites used in this research are commercial sites. The Oak Park research facility is situated on an old estate that was only made accessible to agriculture in 1960 by the Irish Land Commission. The *Miscanthus* site itself was only converted from forest to arable land 20 years before the *Miscanthus* crop was planted.

The amount of C found in the rSOC fraction is similar in all treatments as well as to the values reported by Dondini et al. (2009a), showing that this fraction is highly resistant to any form of disturbance. This fraction represents old long-term stabilized C which is highly resistant to decomposition (Eusterhues et al., 2003).

In conclusion the study shows, that up to four years after planting of *Miscanthus*, the majority of newly sequestered C is found in the relatively labile particulate organic matter. Our results therefore suggest that to achieve long-term C benefits from *Miscanthus*, cultivation needs to be maintained. As particulate organic matter is highly sensitive to land-use change any benefits in terms of long-term soil C storage will likely be negated when taking a *Miscanthus* plantation out of production before any soil C stabilization occurred. However, our study also shows that the conversion from grassland or tillage to *Miscanthus* neither significantly disturbs stable aggregates nor does it lead to a significant reduction in associated C stocks. This may imply that, once C has entered more stable stages, it is resistant to single disturbances such has the planting of *Miscanthus*. Continuous disturbance, such as long term arable farming, however shows to have a significant effect on C associated with stable aggregates. Land-use change from *Miscanthus* back to arable lands could therefore potentially reverse any soil C benefits due to *Miscanthus* cultivation.

In order to fully quantify the effects of planting *Miscanthus* for a full crop cycle of 20 or more years long-term studies of C stabilization under *Miscanthus* are required. Furthermore, studies of the breakdown of stabilized *Miscanthus*-derived C at the end of the crops life-cycle will be necessary to optimize C benefits in possible crop rotations with *Miscanthus* and arable crops.

Chapter 4

Assessing the impact of within crop heterogeneity (‘patchiness’) in young *Miscanthus x giganteus* fields on crop yield and soil carbon sequestration

Based on: Zimmermann, J, Styles, D, Hastings, A, Dauber, J, and MB Jones (in press): Assessing the impact of within crop heterogeneity ('patchiness') in young *Miscanthus x giganteus* fields on economic feasibility and soil carbon sequestration. *Global Change Biology Bioenergy*.

4.1 Abstract

In Ireland *Miscanthus x giganteus* has the potential to become a major feedstock for bioenergy production However, under current climate conditions Ireland is situated on the margin of the geographic range where *Miscanthus* production is economically feasible, it is therefore important to optimise the yield as well as other ecosystem services such as carbon sequestration offered by the crop.

A survey of commercial *Miscanthus* fields showed a large number of open patches. These patches can potentially influence the crop yield and the soil carbon sequestration. Especially the reduction in yield may have a significant negative impact on the economic viability of the crop. The aim of this research is to assess patchiness on a field-scale and to analyse the impacts on crop yield and soil carbon sequestration.

Analysis of remote sensing images showed an average of 372.5 patches per hectare, covering an average of 13.7 % of the field area. Using net present value models and a financial balance approach it could be shown that patchiness has a significant impact on amortisation time for initial investments and might reduce gross margins by more than 50%. Total and *Miscanthus*-derived soil organic carbon was measured in open patches and adjacent plots of high crop density showing significantly lower *Miscanthus*-derived carbon stocks in open patches compared to high crop density patches (0.47 Mg C ha^{-1} ±0.42 SD and 0.91 Mg C ha^{-1} ±0.55 SD). Using GIS modelling it could be shown that on a field scale *Miscanthus*-derived carbon stocks were reduced by 7.38 % ±7.25 compared to a theoretical non-patchy field. However total soil organic carbon stocks were not significantly different between open patches and high crop density plots as the *Miscanthus* sites were only three to four years old, indicating no impact on the overall carbon sequestration on a field scale. Therefore long term experiments are necessary to further assess possible impacts on soil carbon sequestration.

4.2 Introduction

The survey conducted in Chapter 2 showed, that soil carbon sequestration rates on commercial *Miscanthus* plantations is similar to rates reported in earlier publications. However, a large number of open patches was observed on all surveyed sites. The aim of this chapter is to analyse the impacts of crop patchiness on biomass yield and soil carbon sequestration.

In recent years the use of biomass for energy production, particularly in Europe and North America, has increased significantly (Sims *et al.*, 2006). The main drivers of this development are the possible reduction of greenhouse gas (GHG) emissions and independence from fossil fuels. While national and international legislation is promoting the use of bioenergy by setting mandatory renewable energy targets or subsidising biofuel production (e.g. European Parliament & Council, 2009; Department of Agriculture, 2010) the costs and benefits of producing bioenergy generated a controversy within the scientific community. Major concerns are the impact on biodiversity and the efficiency of carbon saving (e.g. Dauber *et al.*, 2010; Anderson-Teixeira *et al.*, 2011; Don *et al.*, 2011; Jorgensen, 2011).

The use of *Miscanthus x giganteus* (Greef et Deu ex Hodkinson et Renvoize) (Greef & Deuter, 1993; Hodkinson & Renvoize, 2001) as bioenergy crop has been a focus research in the last decade (e.g. Lewandowski *et al.*, 2000; Clifton-Brown *et al.*, 2007; Styles *et al.*, 2008). This perennial, rhizomatous C_4 grass, originating from Southeast Asia is highly adaptable to most of European climates with estimated yields between 13 and 25.8 Mg ha^{-1} (Clifton-Brown *et al.*, 2004). In Ireland the introduction of *Miscanthus* has been subsidised by the government for the last few years with the most recent bioenergy scheme having come into operation in August 2012 (Department of Agriculture, 2010). In the Irish context *Miscanthus* has been estimated to have both economic and environmental benefits with gross margins of 326 to 383 € ha^{-1} (Styles *et al.*, 2008), therefore a viable alternative to conventional crops, and a carbon mitigation potential of 4.0 to 5.3 Mg C ha^{-1} yr^{-1} which includes soil carbon sequestration as well as amount of fossil fuel substitutes by potential bioenergy use (Clifton-Brown *et al.*, 2007). However, the estimates of the gross margin are particularly dependent on market dynamics and the total biomass yield.

Miscanthus has been shown to sequester significant amounts of carbon into the soil (e.g. Clifton-Brown *et al.*, 2007; Dondini *et al.*, 2009b), furthermore it has been shown in Chapters 2 and 3 that the introduction of *Miscanthus* to arable or grassland does not lead to a significant reduction in soil organic carbon. In order to optimise carbon benefits from *Miscanthus* it is important to understand all factors influencing soil carbon sequestration.

The survey of commercial *Miscanthus* fields conducted in Chapter 2 showed a significant amount of open patches in all visited sites. Also, studies conducted in the UK have reported patchiness in *Miscanthus* fields (Semere & Slater, 2007; Bellamy *et al.*, 2009; Sage *et al.*, 2010). These earlier studies focussed on the impact of patchiness on biodiversity, however it can be expected that the patchiness has a significant impact on the biomass yield, which especially in the Irish context can compromise the economical performance of *Miscanthus*. Economic studies show relatively low sensitivity of the economic viability of *Miscanthus* production to a reduction in the expected yields (Styles & Jones, 2008; Styles *et al.*, 2008), however, as Ireland is situated on the margin of economically viable *Miscanthus* production (Clifton-Brown *et al.*, 2004; Stampfl *et al.*, 2007) site specific yield losses due to gaps in the crop cover, which are not covered by economic models might render the *Miscanthus* production not economically feasible. Furthermore, due to its high establishment costs, *Miscanthus* represents a considerable financial risk to producers and the financial returns especially in the first years of production are important to amortise initial debt and therefore for the perception of *Miscanthus* by farmers (Styles *et al.*, 2008). Furthermore, it can be expected that in open patches sequestration of *Miscanthus*-derived soil organic carbon is significantly lower than in areas of normal or high crop density, as the main sources of soil organic carbon are plant litter and root material (e.g. Schneckenberger & Kuzyakov, 2007).

While soil carbon sequestration currently has no direct impact on the economic feasibility of *Miscanthus* it is still an important ecosystem service. Land-use change related carbon dynamics are an important part of the national greenhouse gas inventory report (NIR) as defined in the Kyoto protocol (United Nations, 1998) and the loss of soil organic carbon due to land-use change has been identified as a major factor in increasing atmospheric CO_2 levels (Smith *et al.*, 2008). Within the 1990s soils have emitted about 1.6 ±0.8 Pg C yr^{-1} of carbon to the atmosphere due to land-use change (Schimel *et al.*, 2001; IPCC, 2007). Historical carbon losses due to cultivation and disturbance have been estimated to be between 40 and 90 Pg carbon globally (Schimel, 1995a; Houghton, 1999; Houghton *et al.*, 1999; Lal, 1999). The support of soil carbon sequestration through clean development mechanisms (CDM) under the Kyoto Protocol is currently focussed on afforestation and reforestation, however the importance of soil carbon sequestration in agriculture in relation to land-use, land-use change, and forestry (LULUCF) is well recognised (IPCC, 2000; 2006). It is likely that in future soil carbon sequestration in agriculture will become a part of the NIR, and that carbon credits will be allocated to this ecosystem service.

The aim of this work is to assess the patchiness in commercial *Miscanthus* fields and analyse the impacts on the crop yield and soil carbon sequestration using an integrated field-measurement, and remote sensing approach. The study comprised three major steps. (1) Field measurements of soil carbon sequestration in open patches and high crop density plots in *Miscanthus* fields, (2) assessment of the patch properties in selected fields using remote sensing, (3) assessment of the impact of patchiness on soil carbon sequestration and crop yield on a field scale. It is hypothesised that the patchiness will significantly reduce the crop yield and soil carbon sequestration on a field-scale, and that the yield reduction will significantly increase the amortisation time, as well as lower the gross margin for *Miscanthus* producers.

4.3 Materials and Methods

4.3.1 Field sites

The soil sample collection was conducted in May/June 2010. Figure 10 shows the locations and the field codes of the sites. Further information is shown in Table 7. All *Miscanthus* fields were planted in 2006 or 2007, so that the *Miscanthus* plantations were at the end of the establishment phase (Karp & Shield, 2008) at the time the experiment was conducted. The selection criteria were an elevation of maximum 120 m a.s.l., a minimum field size of 2 ha, and the availability of an adjacent on-farm control site. The control site was a field representing the former land-use, grassland or tilled land, of the *Miscanthus* field. For the analyses it was important that both the *Miscanthus* and the control sites had not recently been used to cultivate a C_4 crop (i.e. maize). The planting of the *Miscanthus* crop has been carried out by an external contractor; therefore the farmers were not able to provide information on planting techniques used. However, as all farms were supplied by the same contractor it can be assumed that no differences in planting technique were apparent.

Table 7: Parameters of the sampled *Miscanthus* sites, elevation was measured using one GPS measurement. Particle size distribution, bulk density, and pH values are averaged over 30 cm sample depth and the subplots.

Site ID	Former land-use	*Miscanthus* planted in	Elevation [m a.s.l.]	Clay [%]	Silt [%]	Sand [%]	pH	Bulk density [g cm^{-3}]
MT3	tilled land	2006	73	4.6	21.9	73.5	5.98	1.03
MT5	tilled land	2006	38	12.2	34.8	53.0	6.39	1.04
MT6	tilled land	2006	13	11.5	31.0	57.5	6.29	1.17
MG11	grassland	2007	90	7.1	29.7	63.2	6.37	1.01
MG18	grassland	2006	56	4.8	19.8	75.5	5.68	1.02
MG20	grassland	2006	32	9.9	27.1	63.1	6.78	0.83

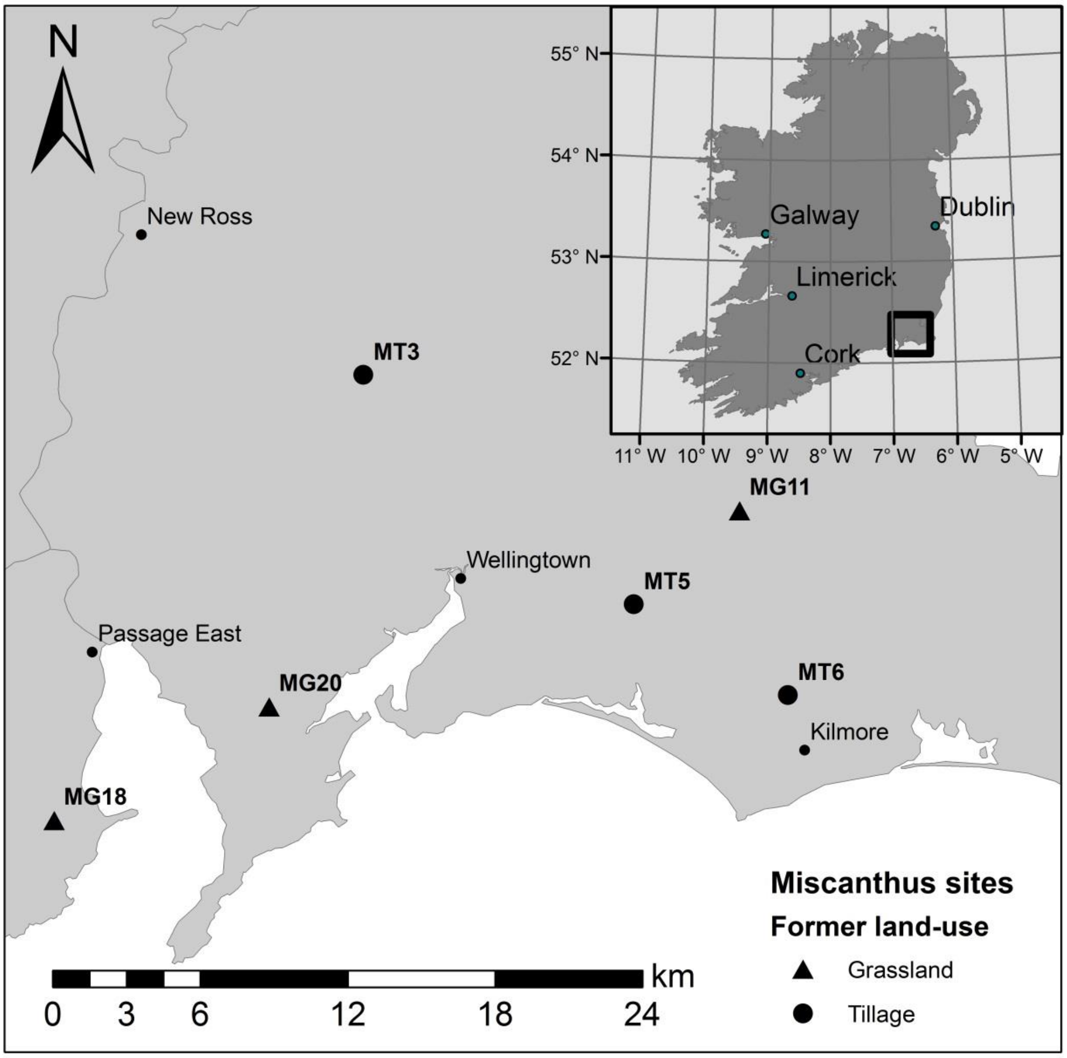

Figure 10: Location of fieldd sites and former land-use of *Miscanthus* fields.

4.3.2 Soil sampling and sample preparation

Soil from four treatments, i.e. high crop-density *Miscanthus*, open patch, for the two former land-use categories, grassland and tillage, respectively, as well as from the respective on farm control sites was collected. The open patch and high crop-density plots were sampled as matched pairs. A matched pair was defined as two adjacent subplots nested within each farm. Within each category four randomly distributed subplots were sampled using a soil auger (Ø 5.6 cm). Five soil samples up to 30 cm soil depth were taken in each subplot situated at least 1m from the edge of the subplot. The soil samples were divided into three depths 0 to 10 cm, 10 to

20 cm, and 20 to 30 cm. Four of the samples were then pooled for each depth to account for small scale variation. The fifth sample was used for bulk density determination. Soil bulk density was measured by weighing a known volume of oven dried soil (105 ºC), afterwards stones (> 2 mm) were removed, weighed and there volume determined by measuring the water extrusion after transferring the stones into a measuring column. The core weight and volume was corrected for stone content and the bulk density was then calculated by dividing the corrected soil weight by the corrected volume.

The collected soil was air-dried and passed through a 2 mm mesh-size sieve and residual biomass larger than 2 mm was removed manually. The soil was then ground using a ball mill and approximately 30 mg were transferred into silver capsules. Any carbonate carbon was removed using the acid fumigation method (Harris et al., 2001). Additionally, soil pH was measured from 3 g soil suspended in 12 ml distilled water using a Jenway 4330 pH meter.

4.3.3 Carbon measurements

Miscanthus-derived carbon (SOC_{Mis}) was determined using the ^{13}C natural abundance method. While photosynthesis generally leads to lower ^{13}C values in plant organic matter compared to atmospheric CO_2, the degree of depletion is dependent on the photosynthetic pathway. Organic matter in C_4-plants shows distinctly higher ^{13}C abundance than in C_3-plants. In an environment with only one source of C_4-derived soil organic carbon (e.g. *Miscanthus*) the isotopic signal can be used to quantify the amount of carbon derived by that given source (Balesdent & Balabane, 1992).

The ^{13}C abundance is expressed relative to the international PDB carbon standard (PeeDee formation belemite) according to the equation

$$(8) \qquad \delta^{13}C = ((R_{sample-}R_{Reference})/R_{Reference}) * 1000$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{Standard}$ the $^{13}C/^{12}C$ ratio of the PDB carbon standard.

Using the stable isotope mass balance the fraction of *Miscanthus*-derived carbon can be calculated, given knowledge about (i) $\delta^{13}C$ of SOC before *Miscanthus* plantation $\delta^{13}C_{old}$), (ii) $\delta^{13}C$ of SOC after *Miscanthus* plantation $\delta^{13}C_{new}$), and (iii) $\delta^{13}C$ of *Miscanthus* plant material $\delta^{13}C_{Mis}$). With x being the fraction of $\delta^{13}C_{Mis}$ the isotope mass balance is written as

$$(9) \quad \delta^{13}C_{new} = x\delta^{13}C_{Mis} + (1-x)\delta^{13}C_{old}$$

To then calculate the *Miscanthus*-derived fraction the equations is solved for x as following

$$(10) \quad x = (\delta^{13}C_{new} - \delta^{13}C_{old})/(\delta^{13}C_{Mis} - \delta^{13}C_{old})$$

The $\delta^{13}C_{old}$ value is not known, however, it is safe to assume that the control sites represent the $\delta^{13}C$ value of the *Miscanthus* sites prior to *Miscanthus* plantation at the corresponding depths. It is important that neither the *Miscanthus* site nor the control site has any C_4 history has this might bias the results. The $\delta^{13}C$ of the *Miscanthus* plant represents an average of shoot, root and rhizome material (value taken from M. Dondini, personal communication). All $\delta^{13}C$ values as well as total SOC values were measured by the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). All carbon contents are measured from the depths 0 - 10 cm, 10 - 20 cm, and 20 - 30 cm. Using the measured bulk density the measured carbon contents given in g C kg^{-1} soil were converted into area based carbon stocks (Mg C ha^{-1}).

4.3.4 Statistical analysis

All datasets showed a normal distribution and no transformations were applied. Due to the nature of the isotope mass balance, negative SOC_{Mis} values result from higher $\delta^{13}C$ values in the control site compared with the corresponding *Miscanthus* site. Negative SOC_{Mis} values can therefore indicate a C_4-history or a local source (e.g. cow dung) of high $\delta^{13}C$. As the analysis is based on the assumption that the control site represents the $\delta^{13}C$

value prior to *Miscanthus* planting, with *Miscanthus* being the only source of higher ^{13}C carbon, a higher $\delta^{13}C$ value in the control site renders a matched pair unfeasible for the analysis. As SOC_{Mis} values can be close to zero, inaccuracy in measurement can also lead to negative values. Therefore, to avoid positive bias, only negative outliers were removed. Data points outside the 1.5 interquartile-range were considered outliers.

The statistical analysis was carried out using linear mixed effects models to account for the nested structure of the experimental design (crop density nested in farm). *Miscanthus*-derived carbon stocks (SOC_{Mis}) as well as total SOC stocks (SOC_t) were used as response variable. As this study is focussing on the field-scale, the soil organic carbon stocks were summed over the 30 cm sampling depth for the statistical analysis. Former land-use (LU_f; grassland vs. tillage) and crop density (Dens; open patch vs. high crop density plot) were used as response variables. To account for possible interactions between the response variables an initial model was run, taking all possible interactions into account. In a stepwise selection process all non-significant terms were removed ($p > 0.05$). To account for the nesting structure of the experimental design the term Farm (F) was introduced as random effect. The analysis was carried using the NLME package in the R-project software (Pinheiro *et al.*, 2010; R Development Core Team, 2010).

4.3.5 Assessment of patchiness

High resolution aerial imagery (Bing maps, Microsoft) was acquired for all field sites. To be suitable for the analysis the imagery required a sufficient resolution to enable patch identification (< 1 m^2). Furthermore, the images must have been recorded when the crop canopy was fully developed (ideally between August and October) since patches cannot be recognised directly after harvest, and are difficult to identify in earlier growth stages or after winter senescence. To assess the number and size of patches in *Miscanthus* fields a geographic information system (GIS)-based analysis of remote sensing imagery was used. Patches were identified using a combination of spatial analysis and manual digitising. Smaller patches are generally shaded by surrounding *Miscanthus* and can therefore be identified as dark areas. The dark areas were identified and converted into polygons. In a second step the polygons were compared with the aerial images and errors were corrected manually (typically large patches that were not shaded.) The finished polygons were then used to analyse the patch number, average patch size, and the overall loss of cropped area due to patchiness. All spatial operations were conducted using ArcGIS 10. Ground-truthing was carried out using hand-held GPS units (Garmin GPS 72). Large patches could be confirmed in the field, however small patches were difficult to identify due to the high crop height and density.

4.3.6 Effect of patchiness on yield

The effect of patchiness on yield was estimated by calculating the loss of total yield in each field due to the reduction in effectively cropped area as a result of patchiness. It was assumed that the yield in the open patches is zero. To assess the economic impact for farmers, two model approaches were used, (1) a net present value model (NPV), and (2) a financial balance approach. Discounted annualised net present values represented the difference between discounted costs and discounted income over the 21 year plantation lifetime, divided by 21 years. The discount rate is applied to discount future cash flows to its present value, so that clash flows at different times can become comparable. The approach was based on an updated version of the NPV model used in Styles *et al.* (2008).

The NPVs are calculated by listing all costs and incomes created for the farmer by *Miscanthus* in each year. The present value of all future cash flows is determined by applying the discount rate to all future costs and incomes and both values following equation (11),

$$(11) \quad PV = \frac{R_t}{(1+i)^t}$$

where PV is the discounted present value, R_t is the cash flow at time t, and i is the discount rate. To calculate the net present value all discounted present values are summed over life-cycle of the crop, leading to equation (12).

$$(12) \quad NPV(i,n) = \sum_{t=0}^{n} \frac{R_t}{(1+i)^t}$$

where *NPV* is the net present value of the overall cash flows (costs and incomes), and *n* is the length of the crop life-cycle in years. The NPV is then annualised by dividing the total discounted costs and incomes by the total crop life cycle in years.

The financial balance approach allowed us to determine how many years after establishment plantations break even under different yield and patchiness scenarios, by simply adding the annual net income to the debt initially created by the producer to establish the crop. The model parameters are shown in Table 8 and apply for both approaches. The NVP approach was employed with an annual discount rate of 5%, for the financial balance approach a 5 % annual interest rate was applied for all remaining debt. The models have been calculated for three peak yield levels representing dry matter harvested off takes (net yield): 10.5 Mg ha^{-1} yr^{-1}, 12 Mg ha^{-1} yr^{-1}, and 13.5 Mg ha^{-1} yr^{-1}, these yields represent the possible range in Ireland (Clifton-Brown *et al.*, 2000; Stampfl *et al.*, 2007). As the estimated yield directly impacts fertiliser inputs and financial returns it was assumed that the impact of patchiness is depending on the expected baseline yield of a theoretical non-patchy field. Furthermore, it was assumed that the peak yield occur from years 3 to 17 after establishment of the crop. Years one and two were set at 30 % and 60 % of the peak yield. For the years 18 to 21 an annual 10 % decline in peak yield was assumed. The models were run for 5 levels of patchiness for each of the three yield types, a baseline of 0 %, as well as 10 %, 20 %, 30 %, and 40 %.

To calculate the impact of crop patchiness on the surveyed sites the total yield was estimated using the MISCANFOR model (Hastings *et al.*, 2009). The model is a semi-mechanistic production model, based on MISCANMOD (Clifton-Brown *et al.*, 2004), an empirical growth model that estimates aboveground biomass yields based on (1) the relationship between leaf canopy light interception and thermal time based on air temperature, and (2) the radiation intercepted and above ground biomass. MISCANFOR further developed MISCANMOD to include genotype-specific process descriptions for the plant growth phase, photo-period sensitivity, temperature dependant radiation-use efficiency, drought and frost kill predictions, nutrient repartition to the rhizome, and moisture content at harvest. The model was run for the year 2009 using soil data from the Harmonized World Soil Database (FAO, 2009), and CRU 2.1 0.25 degree climate data for the period 1970-2002 (Climatic Research Unit, University of East Anglia). The modelled data was used as a baseline representing a non-patchy field. The reduction in crop yield due to patchiness was then calculated by reducing the effectively cropped area by the sum of the area of all patches in the respective fields.

Table 8: List of the financial parameters for the NPV and financial balance model. Fertiliser costs were calculated for a nutrient take-off by a 13.5 Mg ha 1 (dry matter) harvest and scaled down to fit the alternative yield scenarios (not taking patchiness into account). Harvest and storage were also based on 13 Mg ha $^{-1}$ (dry matter) harvest, for alternative scenarios costs were scaled down, also patchiness was taken into account. Removal costs were incurred at the end of year 21.

Parameter	Value	Source
Establishment		
Establishment costs	€ 2595 ha^{-1}	Caslin (2009)
Establishment grant	€ 1295 ha^{-1}	
Total	**€ 1300 ha^{-1}**	
Fertiliser application Costs		DEFRA (2001) (amount) CSO (2012) (Costs)
220 kg 8:5:18 N:P:K	€ 444 t^{-1}	
255 kg CAN	€ 333 t^{-1}	
140 kg Muriate of potash	€ 462 t^{-1}	
Total for 88:11:95 N:P:K	**€ 248 ha^{-1}**	
Spreading	**€ 15 ha^{-1}**	O'Donovan & O'Mahony (2012)
Harvest and storage	**€ 270 ha^{-1} yr^{-1}**	Caslin (2009)
Removal cost	**€ 200**	

4.3.7 *Effect of patchiness on soil carbon sequestration*

To measure the effect of patchiness on soil carbon sequestration the SOC_{Mis} values measured in high crop density *Miscanthus* and open patches were interpolated onto two respective 0.5 m rasters using kriging. The open patch SOC_{Mis} value raster was then clipped using polygons that represented the patchiness for the according field as derived from the aerial images, creating raster files representing SOC_{Mis} values for the modelled patches of each field. This raster was then merged with the high crop density SOC_{Mis} value raster using the mosaic function creating a full coverage for a field of modelled patchiness. The average SOC_{Mis} values were then calculated for each raster in each field as well as the high crop density SOC_{Mis} raster file representing a field with no patches. All spatial operations were conducted using ArcGIS 10 and all raster operations were carried out using the spatial analyst toolbox.

4.4 Results

4.4.1 Remote sensing study

Analysis of the aerial imagery showed that open patches can be classified into three groups: (1) small randomly distributed patches (see Figure 11a to f); (2) linear features with either a number of small patches aligned along a line, or large stretches of open patches (especially visible on Figure 11a to c), and (3) as large open areas with few *Miscanthus* shoots growing (Figure. 11a,f, both in the south-eastern corner of the field.) The results of the GIS-based remote sensing analysis are summarised in Table 9. Standardised to patches per hectare, all sites show similar patch numbers (in average 372.54 ±31.96 SD). The average patch size and total area of open patches per field was 3.67 m^2 ±1.24 SD and 0.50 ha ±0.26 SD, respectively. Considering the patch size distribution, it can be shown that about half of the total open patch area (47.64 % ±22.31 SD) is contributed by patches larger than 5 m2. However the number of large patches is significantly lower than the number of small patches (195.33 ±91.45 SD vs. 1207.50 ±813.87 SD). The loss of cropped area due to open patches calculated using the remote sensing approach is shown in Table 12. The average loss of cropped area is 13.69 % ±4.71 SD. Field MG11 showed the highest, and MG18 the lowest reduction in cropped area.

Table 9: Summary of the patchiness estimated using remote sensing.

Farm	Field size	Number of patches		Average patch size	Total patch area
	[ha]	total	Per ha	[m^2]	[ha]
MG11	2.450	873	356.34	7.93	0.69
MG18	1.061	389	366.78	2.18	0.09
MG20	3.562	1455	408.53	4.72	0.69
MT3	3.691	1298	351.64	5.55	0.72
MT5	3.631	1491	410.63	3.28	0.49
MT6	8.269	3051	368.97	5.78	0.85

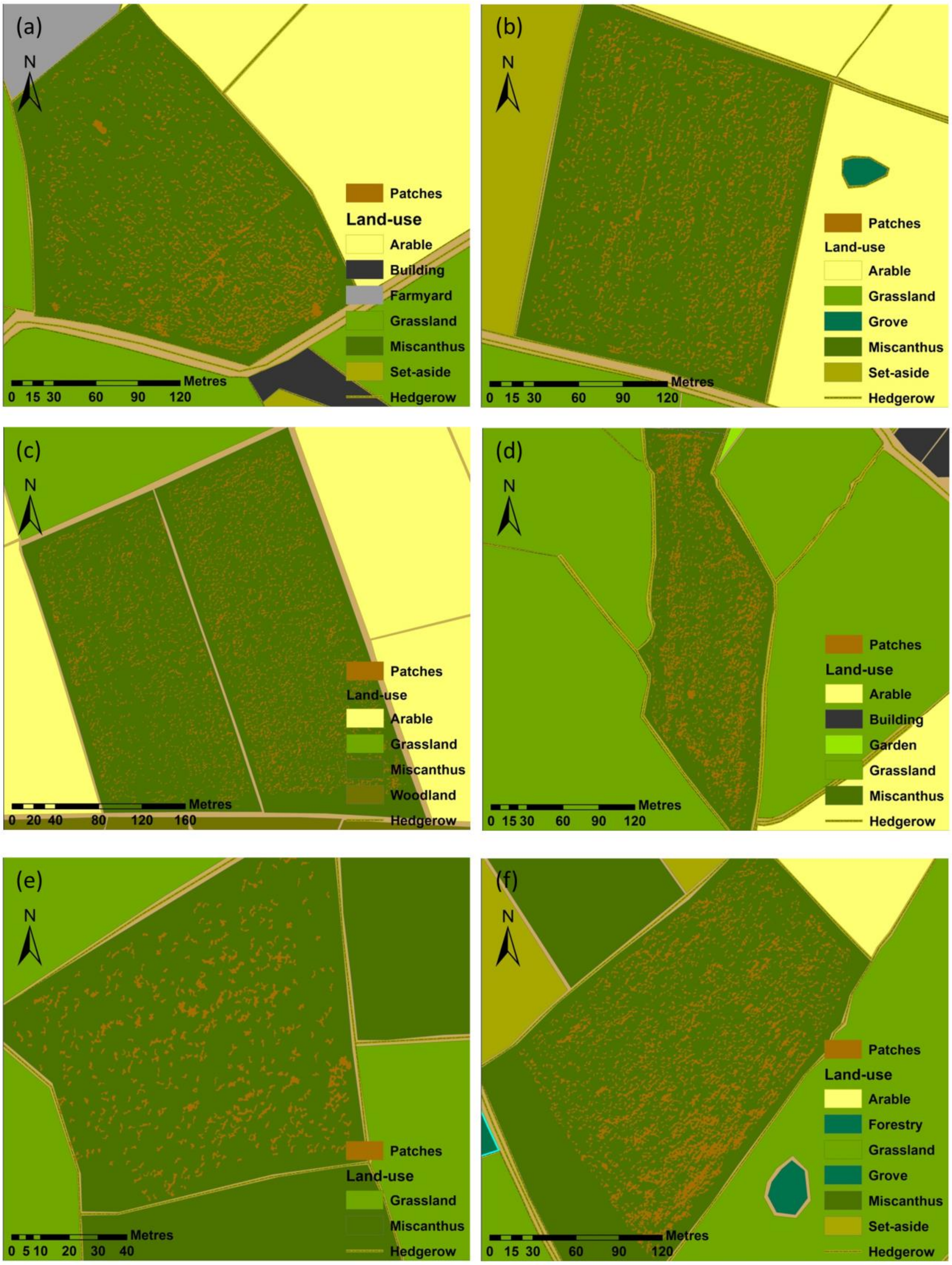

Figure 11: Map of the patches in the Miscanthus field on sites (a) MT3, (b) MT5, (c) MT6, (d) MG11, (e) MG18, and (f) MG20

4.4.2 Impact of patchiness on yield and economic feasibility

As seen in Figure 12, average gross margins are reduced by more than two thirds at patchiness levels of 40% and 30%, respectively. For the high yield estimates (13.5 Mg ha^{-1} yr^{-1}), discounted gross margins almost halve, from 265 € ha^{-1} yr^{-1} to 170 € ha^{-1} yr^{-1}, as patchiness increases from 0% to 20%. Similar proportionate declines occur for the high and low yield levels.

The results of the financial balance approach are shown in Figure 13. Changes in patchiness up to 20 % lead to a payback period between 4 and 7 years for all modelled baseline yields. When looking 30 % and 40 % patchiness, establishment costs are paid back within 9 and 11 years, depending on the baseline yields, independent of the patchiness. Generally the time to pay back initial costs increases with lower assumed yields. The estimated yields of the surveyed *Miscanthus* sites are summarised in Table 3. According to the NPV model, two sites show a reduction in the gross margin of 50% due to patchiness (MG11 and MG20).

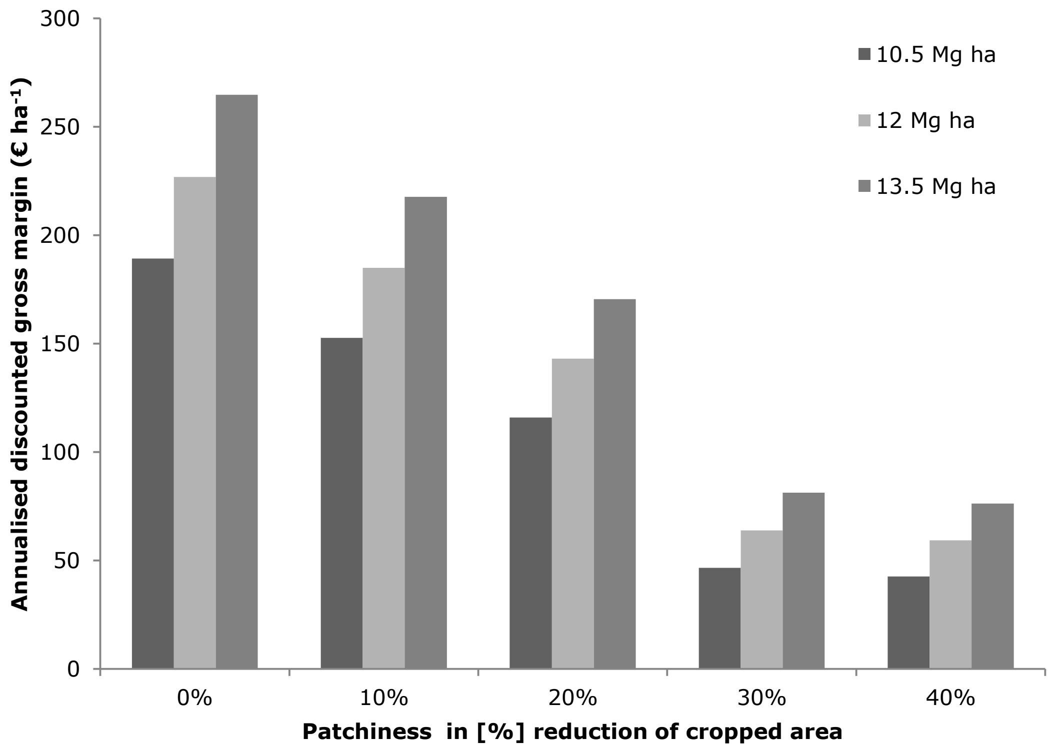

Figure 12: Annualised discounted gross margins under different yield and patchiness scenarios.

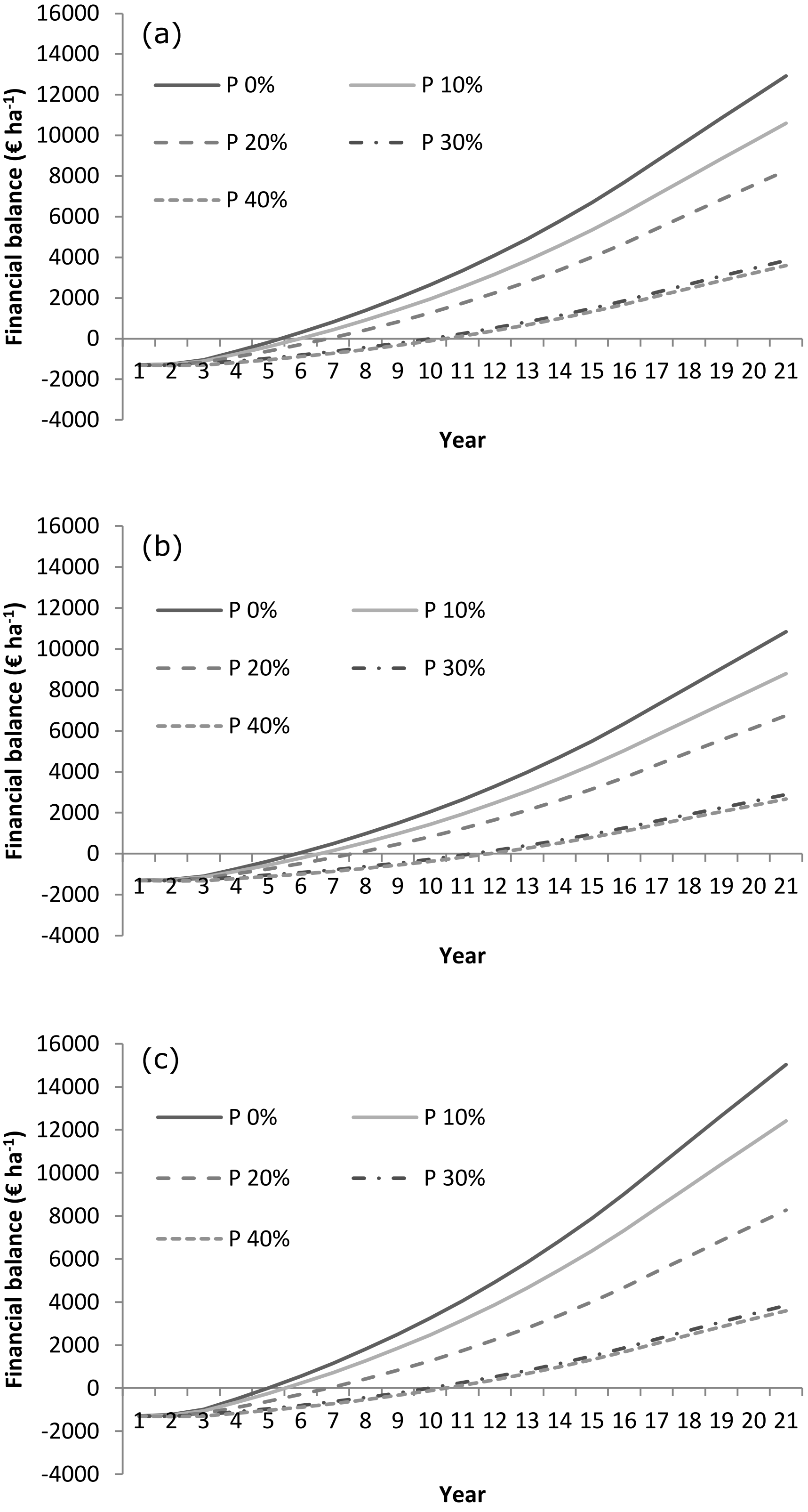

Figure 13: Evolution of financial balance over plantation lifetime for (a) 10.5 Mg ha^{-1} yr^{-1}, (b) 12 Mg ha^{-1} yr^{-1}, and (c) 13.5 Mg ha^{-1} yr^{-1} peak yield

4.4.3 Total soil organic carbon and Miscanthus-derived carbon

The average total soil organic carbon stocks (SOC_t) and the *Miscanthus*-derived carbon stocks (SOC_{Mis}) in either high or low cop density are summarised in Table 10. The final models describing the influence of the parameters former land-use (LU_f) and crop density (Dens) on SOC_t, and SOC_{Mis} are shown in Table 11. The parameter 'Dens' did not show any significant influence on SOC_t and was therefore removed in the model selection process, however *Miscanthus* fields planted on grassland show significantly higher SOC_t values than fields planted on former tilled lands.
The model best explaining SOC_{Mis} shows an influence of the factor 'Dens', with significantly higher SOC_{Mis} values under high crop density plots compared to open patches. The factor LU_f had no significant influence and was therefore removed during the model selection process.

Table 10: Summary of the average total soil organic carbon (SOC_t) and *Miscanthus*-derived soil organic carbon (SOC_{Mis}) stocks.

SOC_t [Mg ha^{-1}]				
Former land-use	**Crop density**	**Sample depth [cm]** 10	20	30
Tillage	H	20.50 (± *2.57)*	20.42 (± *3.91)*	15.73 (± *3.95)*
	L	19.50 (± *3.47)*	20.35 (± *3.22)*	14.73 (± *5.65)*
Grassland	H	28.87 (± *9.55)*	34.25 (± 11.35)	21.83 (± *6.99)*
	L	27.88 (± *5.37)*	38.12 (± *15.67)*	19.76 (± *10.49)*
SOC_{Mis} [Mg ha^{-1}]				
Former land-use	**Crop density**	**Sample depth [cm]** 10	20	30
Tillage	H	1.37 (± *0.67)*	0.94 (± *0.43)*	0.78 (± *0.36)*
	L	0.91 (± *0.75)*	0.60 (± *0.50)*	0.62 (± *0.45)*
Grassland	H	1.71 (± *0.96)*	0.30 (± *0.65)*	0.37 (± *0.67)*
	L	0.78 (± 0.73)	-0.21 (± *0.63)*	0.13 (± *0.50)*

Table 11: Summary of the mixed effects models used to explain differences in total soil organic carbon stock (SOC_t) and *Miscanthus*-derived carbon stocks (SOC_{Mis}); * p <0.05 and ** p <0.01.

Response variable	Explanatory variables	dF	F-value	p-value	Sig.
Total soil organic carbon (SOC_t)					
	Intercept	1	566.54	< 0.01	**
	fLU	1	14.81	< 0.01	**
Miscanthus-derived carbon (SOC_{Mis})					
	Intercept	1	27.08	< 0.01	**
	Dens	1	14.56	< 0.01	**

Table 12 summarises the reduction of *Miscanthus*-derived carbon in the top 30 cm of the soil column due to patchiness compared with a non-patchy field. The average reduction is 7.38 ±7.34 %. The highest reductions are seen on site MG20 and MG11. Site MG18 shows the lowest reduction. An exception is site MT3 showing an increase in *Miscanthus*-derived carbon with increasing patchiness.

Estimated impacts of patchiness on crop yield, cropped area, and Miscanthus-derived carbon (SOC_{Mis}) stocks.

Table 12: Estimated impacts of patchiness on crop yield, cropped area, and Miscanthus-derived carbon (SOC_{Mis}) stocks.

	Yield [Mg ha^{-1}]		**Reduction [%]**	
Farm	**Baseline**	**With patches**	**Cropped area**	**SOC_{Mis}**
MG11	13.2	8.260	-28.238	-11.234
MG18	11.88	10.073	-7.982	-1.745
MG20	13.2	9.181	-19.278	-21.090
MT3	11.88	8.868	-19.501	0.767
MT5	13.2	10.096	-13.541	-8.374
MT6	13.2	10.938	-10.244	-4.998

4.5 Discussion

The analysis showed a similar abundance of patches on all surveyed farms. The categorisation of the patches described earlier allows for possible explanations for the occurrence of patches; (1) linear patches are likely to be explained by congestions in the rhizome planting machinery, which has been reported by land-owners (personal communication), (2) large patches are often situated in depressions (e.g. MG20), suggesting problems with water-logging, and (3) small randomly distributed patches might occur when single rhizomes are damaged during pre-planting storage, which has been reported by land-owners, and therefore are not able to germinate. Furthermore small-scale variation in the soil properties and poor overwintering might also lead to open patches. The authors are not aware of another study quantifying the patchiness in *Miscanthus* fields therefore a comparison with other data is not possible, however similar patchiness of around 25% is reported in commercial *Miscanthus* plantations in Lincolnshire (personal communication Blankney Estates Ltd).

The estimated loss of yield could have a significant impact on the economic viability of *Miscanthus* plantations. The NPV model showed that depending on the expected yield, patchiness can lead to a major reduction gross margin over the whole crop life-cycle. In particular, systems with already low baseline yields might not be able to achieve positive gross margins. In our analysis two sites show a significant reduction in the gross margin with two sites having the gross margin reduced by about 50% (MG11 and MG20). Higher levels of patchiness such as reported in Lincolnshire, UK (25%, personal communication Blankney Estates Ltd) may even lead to a loss of two thirds of the gross margin for farmers, depending on the baseline yield. The financial balance approach shows that *Miscanthus* plantations typically break even after between four and eleven years, with patchiness being the main reason for longer amortisation times. Increased payback periods are likely to have a significant impact on farmers' acceptance of the crop as a possible alternative to conventional crops, reflecting a typical aversion to commit to long-term financial investments in an uncertain economic climate and fluctuating commodity prices (Styles et al., 2008; Augustenborg et al., 2012). The financial balance model indicates that the economic feasibility of *Miscanthus* is relatively robust to patchiness but does not discount future benefits, and may thus provide an "optimistic" representation of long-term investments such as *Miscanthus*-establishment.

While soil carbon sequestration has at present no direct financial implications for *Miscanthus* producers, it is an important ecosystem service as it is recognised as a major greenhouse gas sink (e.g. Smith et al., 2008), and it is likely that in future carbon credits will be allocated to it. Therefore, maximisation of soil carbon sequestration could become an economically, as well as ecologically, advantageous objective.

Field measurements showed a significant reduction in *Miscanthus*-derived carbon in open patches, compared with directly adjacent high crop density plots. This indicates that processes leading to soil carbon sequestration under *Miscanthus* can be categorised into highly localised and more extensive. Localised contributions to the soil organic carbon pool are most likely root excretions and dead root material, while plant litter is generally more evenly distributed especially during harvest (Beuch, 1999; Kahle et al., 1999). This might also have implications for the stability of the carbon sequestered, which is subject to further research.

It has been shown that on a field-scale patchiness can lead to a considerable reduction in *Miscanthus*-derived carbon stocks, the only exception being site MT3. However as MT3 was the first site to be sampled during the field campaign it is possible, that open patches were not correctly identified during this early stage of annual growth. Total SOC stocks did not differ significantly between open patches and high crop density. At this early stage of crop establishment *Miscanthus*-derived carbon does not represent a large portion of the overall soil organic carbon stocks. As shown in Zimmermann *et al.* (2012), there was no significant difference in soil organic carbon stocks between pre-*Miscanthus* land-use and *Miscanthus* plantation. A number of studies have shown a significant shift in the origin of soil organic carbon under *Miscanthus* crops (Schneckenberger & Kuzyakov, 2007; Dondini *et al.*, 2009a), indicating that the reduction of *Miscanthus*-derived carbon input under open patches might lead to significant differences in total soil organic carbon stocks during the *Miscanthus* life-cycle. However, Schneckenberger & Kuzyakov (2007) also found no significant differences in total soil organic carbon contents between grasslands and a 9 year old *Miscanthus* site. Long-term changes in soil organic carbon stocks might therefore depend on the former land-use. As most patches had a high cover of grasses and other plants, it is therefore possible that losses in *Miscanthus*-derived carbon will be compensated by inputs of C_3-plant derived carbon. To assess the long-term impact of patchiness on soil organic carbon stocks it is necessary to conduct further research on older plantations.

From an economic point of view it is in the best interest of *Miscanthus* producers to maximise the crop yield. Taking measures to minimise patchiness, such as careful soil preparation and planting should be management priorities. The analysis of remote sensing imagery showed that it is possible to reduce patchiness by about 50% through the avoidance of large patches, therefore significantly reducing the gross margin losses to the farmer. Depending on the source of patchiness, it may be possible to replant open patches. However if underlying site specific properties such as water-logging or small-scale variations in soil properties inhibit *Miscanthus* growth it may be assumed the that the area is unsuitable for *Miscanthus* establishment. Replanting small random patches is difficult as they can often not be identified due to the height and density of the *Miscanthus* vegetation. In addition in small patches it is difficult for young infill plants to establish and survive as they are outcompeted for light by the more vigorous established plants (personal comms, Blankney Estates). However it was shown that the contribution of small patches towards overall patchiness is lower than that of large patches.

This study showed the importance of assessing crop patchiness in *Miscanthus* stands at the field scale especially for economic considerations. Analysis of the impact of patchiness on crop yield and *Miscanthus*-derived carbon stocks showed considerable reductions in both parameters. Using net present value models and a financial balance approach, it was shown that measured levels of patchiness can significantly reduce gross margins and can potentially render *Miscanthus* uneconomical for farmers. Especially in Ireland, where crop yields are already relatively low, patchiness can seriously undermine the economic viability of this energy crop. The study also shows a significant reduction in the *Miscanthus*-derived portion of the soil organic carbon stocks under open patches. However long-term studies are required to assess if this will lead to an overall reduction in soil organic carbon stocks under *Miscanthus* as grasses and weeds growing in the patches may show similar soil carbon sequestration rates to *Miscanthus* and therefore compensate reductions in soil carbon sequestration.

In conclusion, patchiness can be significantly reduced through careful site selection and preparation, and by avoiding congestions in the planting machinery. Areas that are prone to water-logging are unsuitable for *Miscanthus* cultivation and should be avoided. Large open patches identified after establishment may be replanted. Randomly occurring small patches are difficult to identify on site, however their proportion of overall patchiness is relatively small and losses in soil carbon sequestration might be compensated by a more abundant non-crop vegetation. Overall, further research on the reasons for and the impacts of crop patchiness in *Miscanthus* stands will be required to fully understand possible challenges and benefits.

Chapter 5

General discussion and perspectives

The SIMBIOSYS project's overall goal was to assess the impact of human actions on biodiversity, and ecosystem services in different sectors of human activity (http://www.simbiosys.ie). As part of the project, the aim of this study was to assess soil organic carbon dynamics under *Miscanthus x giganteus* in realistic farming conditions. Soil carbon sequestration is considered to be a significant sink for atmospheric CO_2 and therefore a viable option for mitigating global change. Because of that it is considered a major regulating ecosystem service (Millennium Ecosystem Assessment, 2005) and is likely to become part of the National Greenhouse Gas Inventory as described in the Kyoto Protocol (United Nations, 1998).

The project focussed on farms growing *Miscanthus* in south east Ireland. As information on farmers growing *Miscanthus* was only available from two rhizome distributors (Quinns of Baltinglass, Co. Wicklow, and JHM Crops, Co. Limerick) the research was limited to farmers that have been provided by either of these companies. Initially 84 farms were contacted and general information acquired. Following a set of criteria 16 sites were selected for the present study. The criteria were absence of recent application of organic fertilizers, an elevation below 120 m a.s.l., a minimum field size of 2 ha and the availability of an on-farm control site. The control site had to be an adjacent field representing the former land-use of the *Miscanthus* field to ensure comparability between the soils of the two fields. As the first commercial *Miscanthus* fields were planted in 2006 only fields planted in the years 2006 or 2007 were selected. As all fieldwork was carried out in the years 2009 and 2010 all sites could be considered in the late establishment phase during the course of this work.

Soil organic carbon stocks under *Miscanthus* fields were measured and compared to control, furthermore potential influencing factors were identified (Chapter 2). Using soil fractionation the stability of newly sequestered carbon as well as the impact of land-use change on previously existing carbon stocks was assessed (Chapter 3). On commercial *Miscanthus* fields a factor significantly influencing soil carbon sequestration, as well as the crop yield is a large number of open patches. The patchiness was assessed using remote sensing analysis and the impact on crop yield and soil carbon sequestration was measured using an integrated field and geographic information system (GIS) approach (Chapter 4).

The study was partly able to offer insights into the main objectives provided in Chapter 1. The applied methodology was able to measure *Miscanthus*-derived carbon stocks and shows the influence of a number of soil properties, however there is still high uncertainty regarding the source of variation between different farms. It could be shown, that while most of the newly sequestered carbon is found in the labile POM fraction there is indication for formation of stable aggregates and therefore stabilisation. However, the age of the *Miscanthus* sites was too young to provide further information regarding long-term stabilisation.

An important finding is, that other than expected converting grassland to *Miscanthus* does not lead to a significant reduction in soil organic carbon stocks and therefore not contribute to a carbon debt. Also, so significant differences in soil fraction could be observed when comparing *Miscanthus* and control sites, indication no disruption of stable aggregates. Finally, it could be shown, that crop patchiness may have a serious impact on the economic viability of growing *Miscanthus*, as the yield loss might lead to significantly reduced gross margins for the farmer.

The following sections will discuss the above mentioned points, as well as the implications of looking at different spatial scales when measuring the ecosystem services, carbon sequestration and biomass production.

5.1 Soil carbon sequestration during the establishment phase of *Miscanthus x giganteus*

The results in Chapters 2 and 3 show, that *Miscanthus*-derived soil organic carbon can be identified from two to four years after the introduction of *Miscanthus* to a site. Annual sequestration rates shown in Chapter 3 were 0.90 Mg ±0.53 ha^{-1} yr^{-1} and 0.62 ±0.59 Mg ha^{-1} yr^{-1} on former grassland and former tilled land, respectively. Generally, the reported *Miscanthus*-derived carbon values are similar to values reported in earlier studies both modelled, (0.93 Mg ha^{-1} yr^{-1}, Matthews & Grogan, 2001, 0.6 Mg ha^{-1} yr^{-1}, Freibauer *et al.*, 2004, and 0.62 Mg ha^{-1} yr^{-1}, Smith, 2004a), as well as measured (0.77 and 1.13 Mg ha^{-1} yr^{-1}, Hansen *et al.*, 2004, and 0.59 Mg ha^{-1} yr^{-1}, Clifton-Brown *et al.*, 2007), showing that commercial plantations have a similar carbon sequestration potential as experimental plots. The results of this study however also showed, that even on a regional scale soil carbon sequestration can vary substantially. Part of the variability was explained using mixed effects models, showing significant negative relationship of pH and a positive relationship of initial soil organic carbon with *Miscanthus*-derived carbon values. However more research is needed to further understand these relationships.

The high variation needs to be taken into consideration in order to maximise soil carbon sequestration when planning biomass production. While the measured values well represent modelled values, the high variation in measurements shows, that if locations with low soil carbon sequestration rates could be identified and therefore avoided average national carbon sequestration rates could be significantly increased, which would be especially important if carbon credits would be allocated to soil carbon sequestration.

The majority of the newly sequestered carbon was found in the top 10 cm of soil. This may either indicate a progression of new *Miscanthus*-derived carbon down the soil column, be a result of different input rates of biomass in the different soil depths, or a combination of both processes.

The stability of soil organic carbon is strongly determined by the soil fraction it is associated with (Six *et al.*, 2000c). Unprotected soil organic carbon such as particulate organic matter is more susceptible to decomposition and shows lower turn-over times (Six *et al.*, 2000a), the formation of aggregates increases physical protection and therefore long-term stability (Tisdall & Oades, 1982; Six *et al.*, 2000a). Measuring *Miscanthus*-derived carbon stocks in the soil fractions derived by the method after Zimmermann *et al.* (2007) allowed some insight into the fate of newly sequestered carbon. As seen in Chapter 3, the majority of the newly sequestered carbon is found in the POM fraction, where *Miscanthus*-derived carbon stocks are significantly higher than zero in all three depth increments, and therefore in a labile state. However, there is some evidence of stabilisation of soil organic carbon in the top 10 cm of the soil column, as both the S+A fraction, as well as the S+C fraction show increased *Miscanthus*-derived carbon values. As the *Miscanthus* fields sampled in this study were still in the establishment-phase there is no direct conclusions regarding long-term stability, however results shown in Dondini *et al.*

(2009a) and Poeplau & Don (2013) show conclusive evidence for long-term stabilisation of soil organic carbon sequestered by *Miscanthus*.

An interesting result was the significant difference in *Miscanthus*-derived carbon stocks between the two former land-uses. While the study could not provide any evidence, it can be hypothesised that earthworm activity is responsible for this. Ernst *et al.* (2009) found that earthworm activity is an important factor in soil carbon sequestration, as arable lands generally show lower earthworm abundance (Chan, 2001; Jouquet *et al.*, 2007) higher carbon sequestration rates in grassland may be explained by a higher activity in former grasslands. To confirm this hypothesis further detailed research is required.

It can be concluded that commercial farms show similar soil carbon sequestration rates than previous experimental plots, and indicators for stabilisation of *Miscanthus*-derived carbon can even be found in these young fields. However, commercial sites showed a much higher variability in carbon sequestration than earlier experimental studies. While part of this variability could be explained by the former land-use, pH, and pre-*Miscanthus* SOC stocks there is still a high level of uncertainty regarding the factors influencing local soil carbon sequestration rates.

The main limiting factor of this study regarding soil carbon sequestration rates and the further fate of *Miscanthus*-derived carbon in the soil is the young age of the commercial *Miscanthus*-plantations. Generally, *Miscanthus*-derived carbon levels are relatively low potentially leading to large errors, also possible inter-annual variation may lead to a strong bias. Furthermore, stabilisation processes, such as the incorporation of SOC in stable aggregates, are relatively slow and are therefore difficult to quantify in young fields. To better quantify soil carbon sequestration it is necessary to continuously monitor total SOC stocks, as well the carbon stocks associated with different soil fractions under *Miscanthus* fields. Also a more detailed analysis of influencing factors, such as soil properties and climate conditions are important in further research of soil carbon sequestration under *Miscanthus*.

5.2 Impacts of land-use change on pre-*Miscanthus* soil organic carbon stocks

While the young age of the *Miscanthus* sites surveyed in this study was a potentially limiting factor when looking at the sequestration of *Miscanthus*-derived carbon, it allowed studying the direct impacts of land-use change to *Miscanthus* on the existing carbon stocks.

The effects of land-use change on existing carbon stocks have been the subject of some controversy in the scientific community (Fargione *et al.*, 2008; Searchinger *et al.*, 2008). It has been assumed, that due to soil disturbance caused by initial ploughing prior to *Miscanthus* planting (Caslin *et al.*, 2010), significant amounts of soil organic carbon will be released into the atmosphere leading to a so-called "carbon debt". This debt would need to be compensated for, before the use of bioenergy will create any carbon benefit.

Chapter 2 of this study showed no significant changes in the overall soil organic carbon stocks between the control and the *Miscanthus* sites. This was expected when *Miscanthus* was planted on arable lands as these are already depleted in soil organic carbon, however when planted on grasslands, higher losses were expected. Looking at the depth profile it could be seen that in the top 10 cm, the *Miscanthus* sites showed significantly lower soil organic carbon values than the control sites, this however could not be found in the deeper layers. A trend for higher soil organic carbon values at 20 to 30 cm depth under *Miscanthus* compared to the grassland indicates a redistribution of carbon due to ploughing, rather than a loss. These results indicate that land-use change to *Miscanthus* does not necessarily lead to a significant soil organic carbon loss, as soil organic carbon stocks are shown to be rather resistant to one-time ploughing events.

It may be possible that disruption of stable aggregates due to the initial ploughing may lead to an on-going loss of soil organic carbon, as it becomes more accessible to decomposers (Six *et al.*, 2000a) which the methodology applied in Chapter 2 would have not been able to pick up. However, Chapter 3 showed that carbon in stable aggregates as well as that protected by silt and clay particles is not susceptible to single ploughing events, as no significant differences in the distribution of soil fractions as well the carbon associated with these fractions could be found between the *Miscanthus* and the control sites. This confirms the results from the initial regional-scale study in Chapter 2.

These results have important implications for bioenergy production. The initial loss of soil organic carbon and the associated reduction in the greenhouse gas mitigation potential have been a strong argument against the use of bioenergy. While this study is limited to one bioenergy system, it indicates that the loss of soil organic carbon does not necessarily contribute to a carbon debt, confirming Tilman *et al.* (2009) who argued that, while bioenergy production can pose a number of environmental risks, it can be, when managed sustainably, highly beneficial. Further research on this subject is highly encouraged, as it is important to confirm the results on a larger scale, but also to look at different bioenergy systems with different trajectories of land-use change.

The analysis of the previous land-use data also indicated that long-term arable land-use had a significant impact on both the distribution of soil fractions, as well as the carbon associated with them. In comparison to grassland sites, long-term arable sites showed a shift from the stable aggregates fraction to the silt and clay fraction, indicating a breakup of the stable aggregates. Furthermore the stable aggregates under arable land were strongly depleted in soil organic carbon, confirming results reported by Six *et al.*, 2002b) on the effect of tillage on stable aggregates. The time-frame of this depletion is somewhat ambiguous. While the two arable sites surveyed in this study showed similar results, the study by Dondini *et al.* (2009a), using the same methodology, reported much higher carbon contents in the stable aggregates in a long-term arable site. However, the experimental site Dondini *et al.* (2009a) used, is situated at Teagasc Oak Park, Co. Carlow, Ireland, a former estate that was only made available to agriculture in 1960 by the Irish Land Commission. It is therefore possible that the breakdown of stable aggregates due to ploughing is a very slow process, and that carbon associated with this fraction is highly resistant to short and mid-term disturbance. In conclusion it is hypothesised, that *Miscanthus*-derived carbon in stable aggregates as well as protected by silt and clay particles is potentially resistant to land-use change up to decades after a *Miscanthus* field is taken out of production. However, more research is required to test this hypothesis.

5.3 Implications of crop patchiness for commercial *Miscanthus* cultivation

Initial surveys of the *Miscanthus* sites showed a large number of open patches in the crop. The patch size varied from one to hundreds of square meters in size. Open patches are likely to result in a significant loss in crop yield, and therefore an economic threat to farmers. While the impact of patchiness on biodiversity has previously been studies (Semere & Slater, 2007; Bellamy *et al.*, 2009; Sage *et al.*, 2010), there has been no attempt to quantify the overall patch area on a field scale. As Ireland is already situated at the margin of the area in which *Miscanthus* can be grown economically viable, any further reduction in yield can have significant impacts on the profitability of *Miscanthus* production.

As shown in Chapter 4, the analysis of satellite imagery proved to be a powerful tool to assess patchiness in *Miscanthus* fields. The use of remote sensing tools allowed us to identify and locate the patches, as well as to measure the patch size. The analysis showed an average number of 372.54 ±31.96 patches ha^{-1}, which accounted for a loss of 13.69 ±4.71% of the cropped area. Applying net present value, and financial balance models it could be shown that the patchiness had a significant impact on the gross margin of *Miscanthus* producers. This has serious implications for biomass production, as farmers may be discouraged to engage into the long-term commitment of *Miscanthus* production due to negative experiences of other producers. It further shows the importance of further research into the subject. While Chapter 2 provided some indication as to the reasons for open patches in the crop cover, more detailed research field studies will be required. Knowledge of the reasons for patchiness is important to (1) avoid patchy *Miscanthus* fields in the first place, and (2) to provide detailed knowledge of where it is possible to fill patches in the crop cover, and where *Miscanthus* production may not be suitable.

The analysis of the size distribution of the patchiness showed that about 50 % of the total patch area was contributed by patches larger than

5 m^2, even though these patches only contributed for 13.92 % of the total patch number. Large patches can be avoided by taking the relief into account and by improving the planting machinery, therefore reducing overall yield loss by half. Small patches are difficult to identify and therefore difficult to replant. While it was not subject of this study, retaining a number of open patches may however have positive impacts on biodiversity and associated ecosystem services. Within-field heterogeneity has been shown to be potentially beneficial for e.g. spiders, carabids, and birds (Benton *et al.*, 2003) and may therefore increase biocontrol, benefitting the Miscanthus crop as well as surrounding fields.

Measurements of soil organic carbon in open patches and high crop density patches showed significantly lower *Miscanthus*-derived carbon stock in the open patches compared to high crop density patches (1.51 ±*0.31* Mg ha^{-1} and 2.78 ±*0.25* Mg ha^{-1}, respectively). Extrapolated to the field scale the losses were on average of 7.38 ±7.34 % of the *Miscanthus*-derived carbon in a hypothetical non-patchy field. While this is a significant loss there were no significant differences in the total soil organic carbon stocks. As shown in Chapter 2 the amount of *Miscanthus*-derived carbon at this early stage of *Miscanthus* plantation, while significantly different from zero, does not lead to a significant difference in the overall total carbon stocks, therefore long-term measurements and more spatially accurate models are required to assess the impact of patchiness on long-term carbon sequestration, especially the impact of possible C_3 vegetation with in the patches is an important factor to consider, as it may balance the reduction in *Miscanthus*-derived carbon.

5.4 Assessing ecosystem services on different spatial scales

In conclusion, using the examples of soil carbon sequestration and crop yield, the present study showed the importance of assessing ecosystem services on different spatial scales. While in average soil carbon sequestration rates under *Miscanthus* were well according with rates predicted in previous studies, the high variation between farms rates varied substantially between relatively close locations. These results emphasise the importance of measuring local factors when assessing land-use change related soil organic carbon dynamics. Including knowledge of local soil properties into the selection process for sites suitable for *Miscanthus* planting may significantly increase the regional or even national soil carbon sequestration potential, as sites with disadvantageous soil properties can be excluded. However, as this study only explained part of the variability in soil carbon sequestration rates, it is crucial to further investigate local factor influencing soil carbon sequestration.

Crop patchiness has been identified to be an important factor influencing both the sequestration of *Miscanthus*-derived carbon and the crop yield on the field scale. Processes that lead to patchiness, such as water-logging and problems with the planting machinery, cannot be predicted by large-scale models and it has been shown that while expected yields are economically viable (Styles *et al.*, 2008) patchiness can significantly reduce gross margins. This further underlines the importance of knowledge of local factors when planting *Miscanthus*, as well as the importance of improving the planting process, especially reducing mortality during storage, and avoiding rhizome jams in the planting machinery.

While measurements of soil organic carbon stocks in the bulk soil provide information on the status quo it is important to further understand soil organic carbon dynamics in order to assess long-term benefits. Investigating the micro-scale enables additional knowledge on stability and turn-over times of soil organic carbon stocks as it is depending on its association with the different soil fractions. While young *Miscanthus* fields already show measurable stocks of *Miscanthus*-derived carbon, the fractionation showed that a large proportion of that carbon is present as particulate organic matter which is highly labile. This shows that looking at the micro-scale is crucial to assess the sustainability of soil carbon sequestration and that in order to optimise soil carbon sequestration long-term cultivation of *Miscanthus* is required. Unfortunately, due to the young age of the investigated *Miscanthus* plantations, it was not possible to assess the time-frame in which long-term stabilisation occurs. However, Dondini *et al.* (2009a) showed that in a 14 year old *Miscanthus* site about 83.5 % of *Miscanthus*-derived carbon were found in the stable S+A and S+C fractions.

Investigating processes that influence soil organic carbon dynamics on different spatial scales is also crucial for modelling approaches. While field measurements offer a detailed view on spatially and temporally explicit soil organic carbon dynamics, they are limited due to physical and financial restraints. In particular the work on perennial crops such as *Miscanthus* with a crop cycle of more than 20 years requires substantial labour and funding to assess soil organic carbon dynamics throughout the crops life-cycle in particular when taking spatial variability into account.

To assess carbon sequestration and emissions related to land-use change on a larger spatial and temporal scale, explicit models are required. In an agricultural context two basic parameters are required to assess soil carbon dynamics, (1) available organic matter, i.e. growth rate and potential litterfall of the crop that is examined, and (2) decomposition rates, allowing quantifying inputs and outputs of soil organic carbon. Two main types of models can be distinguished, (1) regression, using empirical functions, and (2) mechanistic models based on physiological processes (Spitters, 1990). While general physiological processes determining growth are well established (Monteith, 1977) local processes are often not well understood and can cause a significant bias in model predictions. Many models are therefore combining mechanistic and empirical approaches to increase prediction accuracy. Semi-mechanistic *Miscanthus* growth models include MISCANMOD (Clifton-Brown *et al.*, 2000) and MISCANFOR (Hastings *et al.*, 2009), further improving process descriptions of the former. Coupled with carbon dynamics models such as CENTURY (Parton *et al.*, 1987; Smith *et al.*, 2001) or RothC (Coleman & Jenkinson, 1996a) it is possible to create large-scale estimates and predictions of soil organic carbon dynamics (Matthews & Grogan, 2001; Foereid *et al.*, 2004; Dondini *et al.*, 2009a). However as the present study shows, a number of local factors such as small scale variation in soil properties cannot be picked by recent models, rendering them unsuitable for small scale predictions of soil carbon sequestration and leading to a large bias when predicting larger scale variations. While small scale measuring information on soil properties is labour intensive and expensive, and therefore often unavailable for more spatially explicit models, other information can be utilised to improve the spatial resolution of biomass yield, and soil carbon sequestration models.

Especially topography allows estimating microclimatic variables and certain soil properties, such as possible water logging. Models capable of predicting small scale variation in soil carbon sequestration may be an important tool when optimising large scale soil carbon sequestration rates. Combined with local soil property measurements, based on the model predictions, they can be used to predict land suitability for *Miscanthus* production in regards to soil carbon sequestration, and may even be able to predict areas with a high potential patchiness. Increasing the accuracy of models allows improvement in the analysis of future developments such as the time-frame of soil organic carbon stabilisation as reported in Chapter 3, the up-scaling of soil organic carbon dynamics to national level in order to implement it into the national greenhouse gas inventory (O'Brien, 2007), and the prediction of changes in soil organic carbon stocks due to different scenarios of land-use change (Smith *et al.*, 1997; Fitton *et al.*, 2011).

5.5 General perspectives for *Miscanthus* production

Regarding the soil carbon sequestration potential, as well as the fact that no significant carbon debt is created, Miscanthus offers a viable greenhouse gas mitigation option. While this study focussed on *Miscanthus x giganteus*, the results can be applied to other perennial crops, such as switch grass or short rotation coppice willow or poplar, as the physiological features as well as management practices fostering the mitigation potential apply for those crops as well. Recent studies have shown, that under European conditions *Miscanthus* has a higher biomass production potential than the other crops mentioned (Styles *et al.*, 2008; Smeets *et al.*, 2009) and may therefore be preferable, however, as this study showed, biomass production may be considerably lower due to patchiness. While this study did not focus on reasons for patchiness, it could be shown that improvement of the planting machinery, improvements in rhizome storage to reduce mortality, and a more careful site selection, especially avoiding areas with water-logging, may significantly increase crop growth and therefore biomass production.

5.6 Future perspectives

The presented study suggests a number of opportunities for future research.

- A detailed analysis of soil properties would allow for more insight into local factors influencing soil carbon sequestration, and offer possibilities to better predict soil carbon sequestration rates prior to *Miscanthus* plantation. Factors identified in this study require more detailed investigation, especially the higher soil carbon sequestration rates under former grassland, compared to former tilled land, and the influence of the soil pH value.
- The causes for patchiness require a more detailed study, especially looking at small random patches. A focus should be on the effect of small scale differences in soil properties, microclimatic factors, and rhizome quality.
- A long term study of the sites would offer insights into the dynamic of newly sequestered carbon. Especially the transition from the labile to the stable fractions needs to be examined in more detail.
- A study of the fate of *Miscanthus*-derived carbon after a field is taken out of *Miscanthus* production is crucial to assess long-term benefits of soil carbon sequestration under *Miscanthus*.
- To further calibrate predictive models to take into account local factors and to increase their mechanistic content. Especially the value of high resolution topography data, climatic data, and aerial photography as possible indicators for potential soil carbon sequestration rates and biomass yield need to be tested.

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Appendix

Annex 1: Average soil bulk density [g cm^{-3}] for all farms surveyed

Depth	0 - 10 cm		10 - 20 cm		20 - 30 cm	
Farm	***Miscanthus***	**Control**	***Miscanthus***	**Control**	***Miscanthus***	**Control**
MT1	0.98	0.82	0.97	0.97	1.02	1.16
MT3	0.95	1.02	1.07	1.00	1.08	0.97
MT4	0.95	0.81	1.06	0.80	0.94	0.95
MT5	0.85	0.66	0.94	0.89	0.94	1.07
MT5a	0.99	0.82	1.07	0.95	1.04	1.09
MT6	1.13	0.77	1.20	0.98	1.19	1.25
MT7	0.95	0.71	1.06	1.00	1.33	1.26
MT8	0.95	1.00	1.12	1.11	1.30	1.24
MG11	0.93	1.01	1.17	0.64	0.93	1.23
MG12	1.23	1.22	1.34	0.94	1.15	1.25
MG14	0.80	1.04	1.04	0.85	1.03	1.14
MG15	1.00	0.99	1.20	0.88	1.11	1.18
MG16	0.97	1.05	1.26	0.84	1.05	1.18
MG17	0.78	0.94	1.46	0.70	0.98	0.85
MG18	0.91	0.97	1.04	0.72	1.10	1.23
MG20	0.69	0.88	0.95	0.85	0.86	1.14

Annex 2: Soil particle size distribution for all *Miscanthus* fields planted on tillage as well as tillage control sites. Texture determined using the UK-ADAS texture triangle

	Depth	0 - 10 cm				10 - 20 cm				20 - 30 cm			
Farm	Treatment	Clay [%]	Silt [%]	Sand [%]	Texture	Clay [%]	Silt [%]	Sand [%]	Texture	Clay [%]	Silt [%]	Sand [%]	Texture
MT1	Control	3.6	20.2	76.2	loamy sand	3.4	20.4	76.2	loamy sand	5.4	24.2	70.4	sandy loam
MT1	Miscanthus	3.2	25	71.8	sandy loam	3.2	22.4	74.4	loamy sand	3	11.8	85.2	loamy sand
MT2	Control	3.8	27.6	68.6	sandy loam	4	27.8	68.2	sandy loam	3.2	25	71.8	sandy loam
MT2	Miscanthus	5	23.4	71.6	sandy loam	2.6	27.2	70.2	sandy loam	3.4	28.2	68.4	sandy loam
MT3	Control	3	21	76	loamy sand	5	19	76	loamy sand	4	20.2	75.8	loamy sand
MT3	Miscanthus	4.8	23.6	71.6	sandy loam	4.6	24.4	71	sandy loam	6.2	23	70.8	sandy loam
MT4	Control	4.2	23.4	72.4	sandy loam	3.8	22.8	73.4	sandy loam	4.4	22.4	73.2	sandy loam
MT4	Miscanthus	5.8	26.4	67.8	sandy loam	6	25.8	68.2	sandy loam	3.8	27.6	68.6	sandy loam
MT5a	Control	13.4	28.4	58.2	sandy loam	11.6	24.2	64.2	sandy loam	14.2	35.2	50.6	loam
MT5a	Miscanthus	9.2	28.8	62	sandy loam	9	32.4	58.6	sandy loam	12	30.2	57.8	sandy loam
MT5	Control	13.2	36	50.8	loam	13	37.4	49.6	loam	14.4	35.6	50	loam
MT5	Miscanthus	11	38.8	50.2	loam	8.8	29.2	62	sandy loam	12.6	31.8	55.6	sandy loam
MT6	Control	10	29.4	60.6	sandy loam	10.2	32.4	57.4	sandy loam	16	29.8	54.2	sandy loam
MT6	Miscanthus	11.4	31.4	57.2	sandy loam	10	30.8	59.2	sandy loam	11.6	32	56.4	sandy loam
MT7	Control	4.4	23	72.6	sandy loam	6.2	23	70.8	sandy loam	8	29.4	62.6	sandy loam
MT7	Miscanthus	4.6	26.6	68.8	sandy loam	5.4	26.8	67.8	sandy loam	11.4	28.2	60.4	sandy loam
MT8	Control	4	16.4	79.6	loamy sand	3.8	16.8	79.4	loamy sand	6	18.6	75.4	sandy loam
MT8	Miscanthus	2	18.6	79.4	loamy sand	4.4	16.4	79.2	loamy sand	4	22.4	73.6	sandy loam

Annex 3: Soil particle size distribution for all *Miscanthus* fields planted on grassland as well as grassland control sites. Texture determined using the UK-ADAS texture triangle

	Depth	**0 - 10 cm**				**10 - 20 cm**				**20 - 30 cm**			
		Clay	**Silt**	**Sand**		**Clay**	**Silt**	**Sand**		**Clay**	**Silt**	**Sand**	
Farm	**Treatment**	**[%]**	**[%]**	**[%]**	**Texture**	**[%]**	**[%]**	**[%]**	**Texture**	**[%]**	**[%]**	**[%]**	**Texture**
MG11	Control	5.4	22.4	72.2	sandy loam	5.4	26.8	67.8	sandy loam	7.4	31.4	61.2	sandy loam
MG11	Miscanthus	5.4	33.6	61	sandy loam	7.6	30.4	62	sandy loam	11.4	33.8	54.8	sandy loam
MG12	Control	7.8	20.8	71.4	sandy loam	6.8	26.6	66.6	sandy loam	6	29.4	64.6	sandy loam
MG12	Miscanthus	6.2	25.4	68.4	sandy loam	6	25.6	68.4	sandy loam	8.2	27.2	64.6	sandy loam
MG13	Control	2	6.8	91.2	sand	4	14.8	81.2	loamy sand	1.2	8.8	90	sand
MG13	Miscanthus	2.2	13.8	84	loamy sand	2	9.4	88.6	sand	2.6	13.6	83.8	loamy sand
MG14	Control	3.8	13.8	82.4	loamy sand	2.2	16.8	81	loamy sand	6.2	22.4	71.4	sandy loam
MG14	Miscanthus	4.4	19.4	76.2	loamy sand	3.8	18.6	77.6	loamy sand	4.2	20.2	75.6	loamy sand
MG15	Control	1.4	14	84.6	loamy sand	1.4	14	84.6	loamy sand	4.2	16	79.8	loamy sand
MG15	Miscanthus	3.6	13.4	83	loamy sand	2	14.6	83.4	loamy sand	9	17.6	73.4	loamy sand
MG16	Control	3.8	11	85.2	loamy sand	3.6	15.6	80.8	loamy sand	5.6	19	75.4	loamy sand
MG16	Miscanthus	4	18.4	77.6	loamy sand	5.6	15	79.4	loamy sand	5.6	22.8	71.6	sandy loam
MG17	Control	8	26.2	65.8	sandy loam	6.8	27.4	65.8	sandy loam	12.2	30.6	57.2	sandy loam
MG17	Miscanthus	5.4	26	68.6	sandy loam	7.4	26.8	65.8	sandy loam	8.6	26.6	64.8	sandy loam
MG18	Control	2	17.4	80.6	loamy sand	3.6	13.6	82.8	loamy sand	7.6	27.6	64.8	sandy loam
MG18	Miscanthus	3.8	20	76.2	loamy sand	5.8	17	77.2	loamy sand	5.8	23	71.2	sandy loam
MG20	Control	7	31.2	61.8	sandy loam	7.8	26.2	66	sandy loam	17.6	29	53.4	sandy loam
MG20	Miscanthus	7.4	26.2	66.4	sandy loam	7.2	23.8	69	sandy loam	12.2	26	61.8	sandy loam

Annex 4: $\delta^{13}C$ value (‰) of all farm studied in Chapter 2 for *Miscanthus* and control sites.

Treatment	Miscanthus			Control		
Farm	10 cm	20 cm	30 cm	10 cm	20 cm	30 cm
MT1	-27.83	-27.92	-27.79	-28.39	-28.5	-27.81
MT3	-29.00	-29.22	-29.30	-29.22	-29.19	-29.22
MT4	-27.16	-27.55	-27.50	-28.20	-28.26	-27.90
MT5a	-27.77	-28.03	-28.02	-28.49	-28.57	-28.58
MT5	-27.59	-27.94	-27.84	-28.30	-28.30	-28.22
MT6	-27.06	-27.66	-27.48	-27.83	-27.86	-27.74
MT7	-28.58	-28.76	-28.65	-28.55	-28.60	-28.23
MT8	-27.71	-27.82	-27.63	-27.74	-27.88	-27.67
MG11	-29.29	-29.17	-28.23	-29.88	-29.14	-28.40
MG12	-27.46	-28.21	-28.17	-29.49	-28.67	-28.30
MG14	-28.37	-28.30	-28.24	-29.74	-29.12	-27.97
MG15	-27.75	-27.64	-27.27	-28.89	-28.20	-27.57
MG16	-28.11	-28.35	-28.13	-29.12	-28.45	-27.68
MG17	-29.45	-29.70	-29.63	-30.52	-29.85	-29.56
MG18	-27.71	-28.23	-27.98	-29.60	-28.64	-27.77
MG20	-28.33	-28.39	-28.17	-28.78	-28.86	-28.38

Annex 5: Total soil organic carbon stocks (Mg C ha^{-1}) of all farm studied in Chapter 2 for Miscanthus and control sites

	Miscanthus			Control		
Farm	10	20	30	10	20	30
MT1	26.80	25.75	22.57	31.71	38.48	24.54
MT3	25.16	26.66	22.72	29.75	29.71	24.95
MT4	27.03	29.00	22.90	22.36	25.68	21.23
MT5a	25.64	26.87	22.21	18.13	24.98	25.95
MT5	21.22	21.63	16.05	13.47	15.56	16.34
MT6	17.74	16.86	16.66	12.14	15.15	14.41
MT7	23.48	22.87	13.40	12.77	19.26	12.33
MT8	10.01	15.79	16.15	11.86	12.42	12.39
MG11	35.43	29.43	18.23	33.00	27.19	21.60
MG12	23.72	24.27	19.91	28.53	24.54	21.54
MG14	24.78	28.47	20.82	31.90	25.94	16.03
MG15	30.45	28.32	26.15	30.16	28.16	23.18
MG16	19.31	20.76	21.33	22.21	21.60	15.12
MG17	30.32	37.38	42.33	44.22	40.55	31.39
MG18	21.20	24.2	21.99	30.31	28.16	19.06
MG20	27.03	35.18	28.05	29.25	29.52	24.34

Annex 6: *Miscanthus*-derived carbon (SOC_{Mis}) stocks (Mg C ha^{-1}) and share of SOC_{Mis} on total soil organic carbon (%) of all farm studied in Chapter 2

	SOC_{Mis} stocks [Mg C ha^{-1}]			SOC_{Mis} share on total SOC [%]		
Farm	10	20	30	10	20	30
MT1	0.90	0.88	0.07	3.31	3.34	0.14
MT3	1.97	1.72	0.95	7.83	6.48	4.24
MT4	1.67	1.20	0.56	6.19	4.20	2.41
MT5a	1.05	0.86	0.75	4.15	3.12	3.24
MT5	0.89	0.45	0.34	4.21	2.08	2.18
MT6	0.83	0.20	0.29	4.70	1.24	1.63
MT7	-0.04	-0.19	-0.28	-0.22	-0.92	-2.55
MT8	0.05	0.00	0.04	0.19	0.00	0.25
MG11	0.94	-0.06	0.11	3.20	-0.12	1.06
MG12	2.65	0.63	0.15	11.2	2.69	0.76
MG14	1.81	1.28	-0.37	7.47	4.57	-1.70
MG15	2.00	0.96	0.54	6.51	3.31	1.84
MG16	1.08	0.10	-0.59	5.69	0.54	-2.74
MG17	1.70	0.31	0.03	5.59	0.82	0.01
MG18	2.06	0.59	-0.27	9.74	2.40	-1.27
MG20	0.75	1.00	0.33	2.58	2.64	1.26

Annex 7: Soil organic carbon immediately after introduction of Miscanthus (Mg C ha^{-1}) (estimated as the difference of total SOC and *Miscanthus*-derived SOC) in all farms studies in Chapter 2

Farm	10	20	30
MT1	25.9	24.87	22.51
MT3	23.19	24.95	21.77
MT4	25.36	27.8	22.34
MT5a	24.59	26.01	21.46
MT5	20.33	21.18	15.71
MT6	16.91	16.65	16.37
MT7	23.53	23.06	13.68
MT8	9.95	15.79	16.11
MG11	34.49	29.49	18.13
MG12	21.07	23.64	19.76
MG14	22.97	27.19	21.19
MG15	28.45	27.36	25.61
MG16	18.23	20.66	21.93
MG17	28.62	37.07	42.3
MG18	19.09	23.61	22.26
MG20	26.28	34.18	27.72

Annex 8: $\delta^{13}C$ [‰] values for all samples in the farms surveyed in Chapter 3.

Depth	Farm Fraction	MG11 Control	Miscanthus	MG14 Control	Miscanthus
10 cm	POM	-29.1	-26.0	-29.6	-25.1
	S+A	-30.3	-29.1	-29.7	-29.1
	S+C	-30.1	-28.9	-29.3	-28.9
	rSOC	-30.7	-29.2	-29.9	-29.6
	DOC	-29.6	-28.7	-28.8	-28.9
20 cm	POM	-28.8	-27.1	-28.6	-25.7
	S+A	-29.6	-29.4	-28.5	-29.3
	S+C	-29.2	-28.9	-28.2	-28.8
	rSOC	-29.5	-29.2	-28.2	-29.6
	DOC	NA	-29.1	-29.4	-29.4
30 cm	POM	-28.6	-26.8	-26.8	-26.6
	S+A	-28.5	-28.3	-27.8	-28.7
	S+C	-28.5	-28.2	-27.6	-28.3
	rSOC	-28.5	-28.1	-27.7	-28.8
	DOC	-29.1	-28.2	-29.0	-28.7

Depth	Farm Fraction	MT6 Control	Miscanthus	MT8 Control	Miscanthus
10 cm	POM	NA	-14.0	-28.0	-23.0
	S+A	-27.6	-25.7	-27.8	-27.1
	S+C	-27.7	-26.9	-27.7	-27.3
	rSOC	-27.6	-26.8	-27.7	-27.3
	DOC	NA	-26	NA	-27.2
20 cm	POM	-27.8	-17.2	-27.8	-21.2
	S+A	-27.7	-27.0	-27.5	-27.5
	S+C	-27.7	-27.4	-27.6	-27.4
	rSOC	-27.6	-27.2	-27.5	-27.4
	DOC	-27.4	-26.5	-27.8	-28.0
30 cm	POM	-28.5	-17.5	-27.6	-22.4
	S+A	-27.4	-27.1	-27.3	-27.3
	S+C	-27.5	-27.2	-27.1	-27.2
	rSOC	-27.0	-26.9	-26.9	-27.1
	DOC	-27.9	-26.8	-27.9	NA

Annex 9: Share of the soil fraction on the bulk soil in [mass %] for all farms studies in Chapter 3

	Farm	MG11		MG14	
Depth	Fraction	Control	Miscanthus	Control	Miscanthus
10 cm	POM	7.3	2.0	2.7	1.9
	S+A	68.5	53.2	74.4	76.7
	S+C	30.1	45	24.1	22.2
20 cm	POM	1.1	1.5	0.6	1.1
	S+A	51.8	51.0	71.0	71.6
	S+C	46.5	47.1	27.3	26.3
30 cm	POM	0.4	0.4	0.2	0.5
	S+A	51.4	48.9	48.9	58.0
	S+C	47.3	49.9	51.4	39.9

	Farm	MT6		MT8	
Depth	Fraction	Control	Miscanthus	Control	Miscanthus
10 cm	POM	0.3	1.3	0.3	0.8
	S+A	35.9	38.4	58.8	60.5
	S+C	64.9	58.9	40.7	38.5
20 cm	POM	0.5	0.5	0.4	0.5
	S+A	36.2	36.2	61.8	57.3
	S+C	62.3	62.2	37.3	41.9
30 cm	POM	0.2	0.3	0.2	0.2
	S+A	37.8	35.1	62.8	56.3
	S+C	59.2	63.7	36.4	42.8

Annex 10: Total soil organic carbon stocks [Mg C ha^{-1}] for all farms studied in Chapter 3

	Farm	MG11		MG14	
Depth	Fraction	Control	Miscanthus	Control	Miscanthus
10 cm	DOC	1.2	0.6	0.6	0.7
	POM	20.7	5.7	7.3	4.8
	rSOC	3.1	3.9	1.8	3.5
	S+A	33.5	13.4	26.8	26.4
	S+C	15.5	16.4	7.6	5.9
20 cm	DOC	NA	0.4	0.4	0.6
	POM	1.9	4.7	1.2	3.7
	rSOC	1.7	3.7	1.4	3.3
	S+A	6.7	15.0	10.1	27.1
	S+C	8.4	15.3	6.0	8.9
30 cm	DOC	0.8	0.6	0.5	1.1
	POM	0.8	1.7	0.8	1.3
	rSOC	3.4	3.7	3.1	3.9
	S+A	7.2	3.6	5.2	13.3
	S+C	8.4	10.3	7.9	9.3

	Farm	MT6		MG14	
Depth	Fraction	Control	Miscanthus	Control	Miscanthus
10 cm	DOC	NA	0.4	NA	0.6
	POM	0.7	3.6	1.0	2.6
	rSOC	2.3	3.0	2.6	1.8
	S+A	1.2	2.8	2.2	5.8
	S+C	8.6	12.0	9.8	11.3
20 cm	DOC	0.4	0.5	0.3	0.3
	POM	2.0	1.6	1.7	1.9
	rSOC	2.9	2.8	2.1	2.2
	S+A	1.5	2.0	2.2	2.1
	S+C	11.2	12.6	11.0	11.1
30 cm	DOC	0.4	0.4	0.5	NA
	POM	1.0	1.4	0.8	0.7
	rSOC	2.0	2.7	1.5	2.4
	S+A	1.8	1.8	3.0	1.4

Annex 11: Share of the SOC associated with each fraction on the total SOC of the depth increment [%] for all farms studies in Chapter 3

Depth	Farm Fraction	MG11 Control	Miscanthus	MG14 Control	Miscanthus
10 cm	DOC	1.6	1.5	1.4	1.7
	POM	28	14.3	16.5	11.7
	rSOC	4.2	9.8	4.0	8.5
	S+A	45.3	33.4	60.8	63.8
	S+C	21.0	40.9	17.3	14.3
20 cm	DOC	NA	1.0	1.8	1.4
	POM	10.0	11.9	6.2	8.5
	rSOC	9.2	9.5	7.3	7.7
	S+A	36.0	38.4	53.0	62.1
	S+C	44.8	39.2	31.6	20.3
30 cm	DOC	4.1	3.0	2.9	3.9
	POM	4.0	8.7	4.3	4.4
	rSOC	16.5	18.7	17.7	13.4
	S+A	34.9	17.9	29.9	46.0
	S+C	40.5	51.7	45.2	32.3

Depth	Farm Fraction	MT6 Control	Miscanthus	MT8 Control	Miscanthus
10 cm	DOC	NA	2.0	NA	2.5
	POM	5.4	16.5	6.6	11.8
	rSOC	17.8	13.8	16.3	8.2
	S+A	9.2	12.7	14.4	26.3
	S+C	67.5	55.1	62.7	51.2
20 cm	DOC	2.4	2.7	2.0	1.7
	POM	11.4	8.1	9.8	10.6
	rSOC	16.1	14.6	12.3	12.6
	S+A	8.4	10.2	12.9	11.8
	S+C	61.6	64.4	63.1	63.2
30 cm	DOC	2.4	2.5	3.9	NA
	POM	6.6	7.8	6.4	5.6
	rSOC	12.9	15.6	11.7	18.7
	S+A	11.6	10.5	23.1	10.5

Annex 12: Total C_{Mis} [Mg C ha^{-1}] for each sample on each farm studied in Chapter 3

Depth	Farm / Fraction	MG11	M14	MT6	MT8
10 cm	DOC	0.0	0.0	NA	NA
	POM	1.0	1.2	3.0	0.8
	rSOC	0.3	0.1	0.1	0.0
	S+A	0.8	1.0	0.3	0.2
	S+C	1.0	0.1	0.6	0.3
20 cm	DOC	NA	0.0	0.0	0.0
	POM	0.5	0.6	1.0	0.8
	rSOC	0.1	-0.3	0.1	0.0
	S+A	0.2	-1.3	0.1	0.0
	S+C	0.2	-0.3	0.2	0.1
30 cm	DOC	0.0	0.0	0.0	NA
	POM	0.2	0.0	0.9	0.2
	rSOC	0.1	-0.3	0.0	0.0
	S+A	0.1	-0.7	0.0	0.0
	S+C	0.2	-0.4	0.2	0.0