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**The potential of *Miscanthus* to
sequester carbon in soil:
a study of carbon dynamics in soil
aggregates**

PhD thesis

2010

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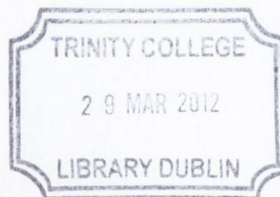
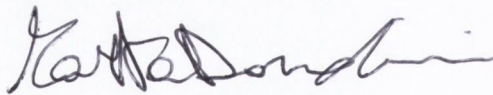
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Summary

The growing of bioenergy crops has been widely suggested as a key strategy in mitigating anthropogenic CO₂ emissions. However, the full mitigation potential of these crops cannot be assessed without taking into account their effect on soil carbon (C) dynamics. Here I analyzed the potential of *Miscanthus*, a bioenergy crop, to sequester C in the soil. I studied the effect of land use change from *Miscanthus* to arable and grassland on the total SOC content, as well as on the C associated with the soil fractions. Finally, in order to predict the potential of *Miscanthus* to sequester C in the soil, I compared direct measurements of soil organic carbon fractions to soil C model predictions made by RothC and a cohort model.

The soil organic matter (SOM) was studied using two different soil physical fractionation methods combined with the application of stable C isotopic methodologies. The combination of these two techniques allows to trace the *Miscanthus*-derived C in various physically protected soil fractions.

Integrated through the whole soil profile, the total amount of soil organic carbon (SOC) was higher under *Miscanthus* than under an arable crop and this difference was largely due to the input of new C. The C stock of the macroaggregates (M) under *Miscanthus* was significantly higher than those in the arable land. Additionally, the C content of the micro-within macroaggregates (mM) were higher in the *Miscanthus* soil as compared with the arable soil. Analysis of the intra-microaggregates particulate organic matter (POM) suggested that the increase C storage in mM under *Miscanthus* was caused by a decrease in

disturbance of M. Thus, the difference in C content between the two land use systems is largely caused by soil C storage in physically protected SOM fractions.

Futhermore this research shows that the conversion from grassland to a one-year-old *Miscanthus* plantation leads to accumulate *Miscanthus*-derived C in the intra-macroaggregate fractions. In the upper 30 cm soil depth, land use has a significant effect on the total SOC content, as well as on the C associated with the soil fractions. In particular, 14 years of *Miscanthus* plantation increased C sequestration in stable and protected soil fractions compared to the other land use systems (e. g. arable land and grassland).

Finally, the results from the comparison of the direct measurements of SOC fractions to model predictions show that when *Miscanthus* is grown on land previously under arable agriculture, the SOC will increase as *Miscanthus* organic material is shown to have a slow decomposition rate. In addition I demonstrated that for measured organic carbon, fractions of different lability are similar to the carbon pools used in the RothC model. The model predictions from RothC and *Miscanthus* yields from MISCANFOR show that, in Ireland, changing the land use from arable to *Miscanthus* plantations has the potential to store between 2 to 3 Mg C ha⁻¹ y⁻¹ depending on the crop yield and the initial soil organic carbon level.

I conclude that when *Miscanthus* is planted on former arable land, the resulting increase in soil C storage contributes considerably to its CO₂ mitigation potential.

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A nonno

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Chapter 1

General Introduction

1.1. Kyoto Protocol

Climate change and global warming have been the subject of increased debate in an agricultural policy context in recent years. In 1997, the United Nations Framework Convention on Climate Change (UNFCCC) adopted the Kyoto Protocol (see UNFCCC (1997) for details). The Protocol outlines targets and timetables for the reduction of human induced sources of global warming. It was agreed that by 2012, global emissions of greenhouse gases (GHGs), expressed in carbon dioxide (CO₂) equivalents, should be five percent less than emission levels recorded in 1990. Ireland's commitment under the protocol is to limit its emissions of greenhouse gases to not more than 13 percent above 1990 levels. In order to achieve this target, the Irish Department of the Environment, Heritage and Local Government has published the National Strategy on Climate Change (2007), which proposes measures for the mitigation of greenhouse gas emissions across different sectors of the economy. The strategy emphasises that the contribution of agriculture towards reaching the target should come from both a reduction in emissions and an increase in sequestration.

Ireland is unusual because more than one third of its human induced greenhouse gas emissions originate in agriculture. In 1998, it was estimated that agriculture, at 35 percent, was the single largest producer of GHGs (Department of the Environment, Heritage and Local Government, 2007). This is primarily due to the structure of Irish agriculture, where the livestock sectors typically account for over 80 percent of agricultural output value. The objective is to achieve a reduction in agricultural emissions of 2.2 million tonnes (Mt) CO₂ equivalents by the end of the commitment period 2008-2012 (Department of the Environment,

Heritage and Local Government, 2007) from a 'business as usual' projected level of 18.7 Mt CO₂ equivalents (Behan *et al.*, 2002).

The Kyoto Protocol establishes the possibility for crediting greenhouse gas emission reductions coming from forestry and agriculture activities (named LULUCF; Watson *et al.*, 2000). Under the Marrakesh accords, eligible LULUCF activities include afforestation, reforestation, and deforestation (under Article 3.3 of Kyoto protocol), forest management, crop management, grassland management and revegetation (under Article 3.4 of Kyoto protocol). Three broad types of LULUCF projects have been identified: avoiding emissions via conservation of existing carbon (C) stocks, increasing C storage by sequestration and substituting C for fossil fuel and energy intensive products.

Specific challenges related to C sequestration include: complex responses of soil C stocks to LULUCF activities, the need to monitor small incremental changes in soil C content relative to large C pools, long-term periods to accrue the full C benefits, high local variability of soil C content and relatively costly soil C measurement procedures (García-Oliva *et al.*, 2004).

Despite the importance of the soil C mitigation potential, only in the recent years projects on soil C management explicitly directed to the mitigation of greenhouse gases emissions have been proposed (Sampson *et al.*, 2000; Follett 2010).

1.2 Land use change and carbon sequestration

Soil C sequestration means increasing soil organic and inorganic stocks through recommended management practices and proper land use. In fact, the impact of

land-use change and management on soil organic carbon (SOC) stocks has implications for soil quality improvement and sustainability, as well as a role in the mitigation of C emission from agricultural land. The commonly recommended land-use changes that lead to SOC sequestration are afforestation and the replacement of arable land with pasture or biomass crops. The SOC sequestration is caused by those management systems that add high amounts of biomass to the soil, cause minimal soil disturbance, conserve and improve soil structure, enhance activity and species diversity of soil fauna and strengthen mechanisms of elemental cycling (Saurerbeck, 2001; West *et al.*, 2002). Maintaining or enhancing carbon storage requires consistent input of carbon, for example from crop residues, compost, cattle slurry or sewage sludge in cultivated soils. However, in cultivated soils where crop residues are incorporated in large quantities, SOM content varies slowly. In the same way, the removal of crop residues does not necessarily induce a rapid decrease of SOM content. It has been shown that the incorporation of fresh organic matter such as green manure or straw in a soil may intensify SOM mineralization. The stimulation of SOM mineralization, named the 'priming effect' by Bingeman *et al.* (1953) has been clearly observed at the rhizosphere scale. The mechanisms leading to the priming effect remain poorly understood (Kuzyakov *et al.*, 2000). It is commonly believed that the low quality of SOM limits the amount of available energy for soil microorganisms, and in turn the rate of SOM mineralization. Thus, the priming effect is often supposed to result from an increase in overall microbial activity due to the higher availability of energy and nutrients released from fresh organic matter (Fontaine *et al.*, 2003).

When natural vegetation is converted to cultivated crops, a rapid decline in soil organic matter (SOM) has been observed (Post & Kwon, 2000). It has been estimated that already within the first few decades of cultivation, soils lose typically 20-30% of the C pool, partly through erosion, but mostly through oxidation of organic matter into CO₂, thus contributing to the CO₂ emissions to the atmosphere (Schlesinger, 1995). Agricultural practices also affect soil structure and, after few years of conversion from grasslands to pastures or cultivated lands, soils experience a substantial reduction in aggregate stability (Elliott, 1996). The breakdown of stable macroaggregates could cause the decomposition of previously physically-protected organic matter through greater exposure to soil organisms and improved aeration (Beare *et al.*, 1994). The magnitude of this mechanism is a function of the soil texture; in coarse-soils, for example, the soil stability mainly depends on roots and fungal hyphae, which are disrupted and rapidly decomposed upon soil disturbance (Tisdall & Oades, 1982). On the other hand, higher clay contents promote organic-mineral complexes which may allow smaller aggregates to persist, protecting the physically stabilized SOM from decomposing following disturbance (Parfitt *et al.*, 1997).

The implications of many factors affecting C sequestration after a change in land use (e.g. the amount of C input into the soil, the distribution of C among the soil profile and the grade of decomposition of the organic matter) may explain the high variability of the results found among different studies and, at the same time, recall the need to assess SOM dynamics in all its aspects, especially in order to predict with a good degree of accuracy the time of C persistence in the soil.

1.3 Bioenergy crops

Several studies on fast-growing perennial bioenergy crops, established on cultivated land, have demonstrated their ability to improve soil quality by increasing C sequestration due to their perennially, high biomass production and deep root systems (Bransby *et al.*, 1998; Ma *et al.*, 2000). The SOC is added to the soil mainly by decomposition and decay of plant material on the surface and by root growth and senescence below the surface. The deep rooting system in bioenergy crops allows for direct movement of C into the soil and makes it less available for removal by harvest. Hence, the C incorporated into the biomass and root system has a high potential for being incorporated into the SOC pool. Climate also affects the aboveground biomass productivity and CO₂ mitigation, but most perennial bioenergy crops can survive extreme climatic conditions due to large nutrient reserves in their root system (Ingram & Fernandes, 2001; Lemus & Lal, 2005). In fact, the seasonal trends in the biomass and nutrient content of shoot and rhizome show that, in general, from mid-summer onwards the proportion of the total nutrients in the shoot declined as the proportion in the rhizome increased. This suggests that efficient internal recycling of nutrients occurred by translocation of nutrients (Beale & Long, 1997). Moreover, adequate water is required by the growing plant to develop and maintain green leaf area throughout the season and to ensure that photosynthesis is not limited by water stress. Herbaceous perennials forming an annual crop of stems have a growth cycle that maximize water content during the growing season, while minimize water content in the harvested biomass. The shoots of these bioenergy crops die annually and

dry-down in the winter, when the available solar radiation is small and decreased water content will be of least importance (Long 1994).

Bioenergy crops can be used as a good option to sequester atmospheric CO₂ by increasing biomass productivity which can be incorporated into existing energy alternatives to improve energy use efficiency. In fact, bioenergy crops are defined as any plant material used to produce biofuel, but those grown specifically for the purpose are characterized by the capacity to produce large volume of biomass, have high energy potential and are adapted to marginal soils (Lemus & Lal, 2005).

First-generation biofuels can be produced from food crops containing starch/sugar/oil, where the conversion technologies and markets are well established, and where it has been possible to quickly take advantage of the commercial opportunity provided by the increase in petroleum prices (Hettinga *et al.*, 2009; Van den Wall Bake *et al.*, 2008). Particularly, where these technologies are applied to annual temperate-climate food crops (cereals, sugar beet, oil seeds), the overall process has relatively low energy output compared to energy inputs (Wu *et al.*, 2008; Farrell *et al.*, 2006). A major attraction of second-generation feedstocks is their abundance, low cost, and high ratio of energy output to input. They are composed mainly of lignocellulosic materials that make up the fibrous and woody structural components of plants. These materials are available as primary (in the field) or secondary (processing) residues from agriculture and forestry; as well as tertiary waste (from urban/industrial activity). Primary sources also include herbaceous (grasses) and woody species grown as crops. The herbaceous and woody species being targeted for development as lignocellulosic

crops are robust perennial species that can resprout from rootstocks after harvest. Woody crops in particular have the potential to provide ecosystem services and therefore to complement rather than compete with conventional agriculture. There is considerable current investment in developing second-generation conversion technologies and biomass sources (Bartle & Abadi, 2009). Of the second-generation bioenergy crops, *Miscanthus x giganteus* (Gref and Deu.) has generated a lot of interest for several different reasons.

Miscanthus is a perennial rhizomatous grass native to East Asian tropic and subtropic region and with a considerable biomass production potential, even under cool temperate climatic condition (Lewandowski *et al.*, 2000). In Europe, *Miscanthus* is used as bioenergy crop with high aboveground biomass-yield potential (Beuch, 2000). Research over the past two decades suggests *Miscanthus* as a potentially important bioenergy crop (Jørgensen *et al.*, 2000). In fact, previous researches have focused on management and economics in relation to productivity and combustion ability. It can be cultivated for 15 up to 25 years without replanting and is harvested yearly, often in the following spring to reduce ash contents. Particularly interesting is that the replacement of conventional cropping systems by this bioenergy crop, may introduce an increase in SOC (Hansen *et al.*, 2004) due to the following characteristics: (1) perennial plants such as *Miscanthus* displace high portions of the assimilated C belowground as a C reservoir for growth in spring (Kuzyakov & Domanski, 2000), (2) *Miscanthus* has a very deep and well-developed root system (Miridokawa *et al.*, 1975; Neukirchen *et al.*, 1999), (3) the absence of soil tillage means less aeration, lower plant residues-decomposition rates, and better C stabilization for longer periods,

(4) a high input of aboveground harvest residues, because harvesting in late winter or early spring leads to an accumulation of stubbles and leaves on the soil surface as pre-harvest losses (Beuch, 1999) and (5) slower decomposition of plant residues (stubbles, leaves and roots) because the absence or reduction of nitrogen (N) fertilization leads to a larger C : N ratio (Schneckenberger & Kuzyakov, 2007). These features of *Miscanthus*' physiology and cultivation lead us to expect that this bioenergy crop has a potential to increase C stores in the soil (Fisher *et al.*, 1994) at least when established on arable land (Foereid *et al.*, 2004).

1.4 Soil organic matter

In soil, plant residues, microbial and animal biomass are bound to mineral constituents to form what is defined as soil organic matter (SOM). The constituents of SOM can be divided into non-humic substances, which are discrete identifiable compounds such as sugars, amino acids and lipids, and humic substances, which are complex largely unidentifiable organic compounds. As organic compounds, both humic and non-humic substances contain C, oxygen (O) and hydrogen (H) and can also contain nitrogen (N), phosphorus (P) and sulfur (S).

The total amount and partitioning of organic matter in the soil is influenced by soil properties and by the quantity of annual inputs of plant and animal residues to the ecosystem. In a given soil ecosystem, the rate of decomposition and accumulation of SOM is determined by such soil properties as texture, pH, temperature, moisture, aeration, clay mineralogy and soil biological activities. A complication is that soil organic matter in turn influences or modifies

many of these same soil properties (Bot & Benites, 2005). An increase in the organic matter content has a lot of advantages for the soil such as: 1) increase in cation exchange capacity (CEC) which in turn increases the ability to attract and retain nutrients, 2) improvement of soil structure (more stable soil aggregates), 3) better water holding capacity, 4) enhancement of growing conditions for soil fauna and flora and 5) construction of a nutrient buffer (Hassink et al., 1993).

Organic matter releases nutrients in a plant-available form upon decomposition. Decomposition of organic matter is largely a biochemical process that occurs naturally; three parallel processes go on during decomposition: (1) degradation of plant and animal remains by cellulases and other microbial enzymes, (2) the increase in the biomass of microorganisms which comprises polysaccharides and proteins and (3) the accumulation or liberation of end products. In the decomposition process, different products are released: CO₂, energy, water, plant nutrients and re-synthesized organic C compounds. The speed of this process is determined by three major factors: soil organisms, the physical environment and the quality of the organic matter (Brussaard, 1994).

Soil biota are the numerically most abundant organisms in terrestrial ecosystems and are the primary decomposers of organic matter in soil. By breaking down C structures and rebuilding new ones or storing the C into their own biomass, the micro-organisms play an important role in nutrient cycling processes. As they break down the organic matter, any excess nutrients (N, P and S) are released into the soil in forms that plants can use. The material produced by micro-organisms is less decomposable than the original plant and animal material, but it can be used by a large number of organisms (Van Veen & Kuikman, 1990).

The SOM is often split up in distinct compounds (or pools), with residence times ranging between a few months for the most labile, to over thousand years for the most recalcitrant and complex pools. This compartmentalization is strictly correlated to the different physical and chemical properties of the SOM, as well as to its microbial degradability (Paul & Clark, 1996). Traditionally, soil aggregation has been linked with either total C (Matson *et al.*, 1997) or organic C levels (Dalal & Mayer, 1986a, 1986b). More recently, techniques have developed to fractionate C on the basis of lability (ease of oxidation), recognizing that these sub-pools of C may have greater effect on soil physical stability and be more sensitive indicators than total C values of C dynamics in agricultural systems (Blair & Crocker, 2000). In their conceptual model, Six *et al.* (2002) distinguish the SOM that is protected either physically or biochemically against decomposition from that which is unprotected. They identified four measurable pools: (i) an unprotected C pool, (ii) a biochemically protected C pool, (iii) a silt and clay-protected C pool, and (iv) a microaggregate-protected C pool. The unprotected SOM pool consists of both the light fraction and the particulate organic matter (POM) fraction, although they are conceptually considered to be identical pools by Six *et al.* (2002). The origin of both light and POM fractions is mainly plant residues, but they may also contain microbial debris. These fractions are highly labile organic matter pools and Six *et al.* (2000) have shown that variation in the light fraction pool is the best indicator of management-induced changes in SOM.

Protected SOM is stabilized by physical, chemical and/or biochemical factors (Six *et al.*, 2002). First, chemical protection occurs through specific bonds of SOM with colloids or clays, and often involves highly stable organic

compounds. Second, biochemical stabilization is caused by the chemical-complexing processes between substrates such as lignins and polyphenols and soil particles, forming recalcitrant SOM compounds. Finally, physical protection occurs through encapsulation of SOM fragments by clay particles or soil aggregates, thereby forming physical barriers between microbes and enzymes and their substrates (Marinissen & Hilenaar, 1997; Jones & Donnelly, 2004).

Even if the heterogeneity of soil structure and nature is well recognized, only in the last decades, the importance to assess soil dynamics in terms of aggregate distribution and stability has been revealed (DeGryze *et al.*, 2004). In fact, previous researches showed that considering and analyzing SOM dynamics in its different pool distribution is one of the best approaches to detect and comprehend the processes involved in soil C, as well as its composition, properties and turnover time.

1.5 SOM separation methods

A wide range of separation methods have been developed to identify the various SOM constituents. Depending on the procedure of separation, distinction can be made between chemical, physical or biological SOM fraction.

Chemical fractionation methods include hydrolyzing labile C with acid (Paul *et al.*, 2001), digesting it with permanganate (Weil *et al.*, 2003), or extracting it with hot water (Gregorich *et al.*, 2003). Each method assumes that the same properties that make SOM degradable by microbial enzymes make it less resistant to chemical attack or more soluble in hot water (McLauchlan, 2004). In chemical fractionations, the use of several solutions (i.e., water or sulphuric acid)

or methods, like gel permeation chromatography or ultrafiltration, allow the extraction of soluble SOM compounds as water soluble organic matter (WSOM), dissolved organic matter (DOC), carbohydrates, sugars, lignin and humic or fulvic acids. However, the separation of SOM in such compounds, does not always separate fraction with different turnover rate (Balesdent, 1996), while this can be often achieved through physical separation techniques, which are also less destructive than chemical extractions. Physical fractionation can help to provide information concerning the architecture of SOM, determining the extent to which residues have been biologically processed and the degree of physical occlusion or organo-mineral complexation (Ellert & Gregorich, 1995).

Conglomerations of primary particles with organic matter, called macroaggregates (0.25-2 mm), are separated by sieving or flotation. Macroaggregates are formed by microaggregates, bound with fresh plant or animals residues (i.e., plant polysaccharides, fungal hyphae or roots) and with silt and clay (Tisdall & Oades, 1982). In this hierarchical division, it is assumed that most of the turnover of SOM is in the light or large fractions, while heavier or smaller compounds are complexed with clay minerals to form very stable and protected C pools (Tisdall & Oades, 1982). The formation of big aggregates promotes the encapsulation of organic matter into the smaller compounds, thus inducing C stabilization in the long term (Six *et al.*, 2000).

Density fractionation is based on the observation that during the humification process, SOM becomes associated with mineral portions of the soil and, thus, more humified particles are thought to be associated with the mineral fraction. Additionally, it must be considered that SOM density increases by itself

during decomposition. The light fraction (LF) is considered to be labile whereas the heavy fraction is assumed to be stabilized onto surfaces of clay particles, making it more resistant to microbial degradation. Sieving soil into different size classes separates small aggregates or particles from larger particles (Kemper & Chepil, 1965; Six *et al.*, 1998), which contain SOC that is partially physically protected from microbial degradation, although not chemically recalcitrant. Dispersion and sieving of the sand-sized organic matter can be used to isolate the particulate organic matter (POM) fraction that is considered labile (Cambardella & Elliott, 1992). Within the separation agents used to achieve this fractionation, sodium iodide and sodium polytungstate are the most common, usually in a range of density from 1.6 to 2.2 g cm⁻³ (Sollins *et al.*, 1984; Cambardella & Elliot, 1992).

Biological separation empirically separates labile SOC from recalcitrant SOC by allowing microbes to mineralize SOC under controlled temperature and moisture conditions and in the absence of new organic inputs (McLauchlan, 2004). This method assumes that microbes will mineralize the most labile C first, with recalcitrant C being mineralized later. The technique involves measuring CO₂ produced by mineralization of SOC during the course of a laboratory incubation of soil (Alvarez & Alvarez, 2000; Pastor *et al.*, 1993). Although some aggregate structure is destroyed during sieving that occurs before the incubation, some aggregates survive this process and are intact during the incubation. Thus, some of the labile C that was protected by aggregate structure in the field becomes available for microbial mineralization during the incubation (Kristensen *et al.*, 2003). Additionally, a direct measure of the pool size of living soil organisms,

microbial biomass, can be quantified with either chloroform fumigation incubation or chloroform fumigation extraction (Beck *et al.*, 1997; Paul *et al.*, 1999). Other frequent biomass determinations are based on the microscopic counting of fungi and bacteria, or by the measurements and determination of cellular compounds, nucleic acids, chitin or glucosamines.

1.6 Stable isotopes and soil carbon sequestration

In recent years a lot of emphasis has been placed on better understanding the C cycle across different ecological scales, ranging from patch, stand, and landscape to global scales. In particular, stable analyses of C ($\delta^{13}\text{C}$) had been applied to help meet the challenge of closing gaps in our understanding of soil C turnover and belowground processes (Balesden & Mariotti, 1996; Boutton, 1996; Amundson *et al.*, 1998; Allen *et al.*, 2000; Jackson *et al.*, 2000).

^{13}C represents approximately 1.11 atom% of the earth's C (Craig, 1957), but biological material varies around this average value as a result of isotopic discrimination during biological, physical and chemical processes (Blair *et al.*, 1985; Galimov, 1985). Stable isotope abundances are expressed using the δ notation in per mil (‰), as the deviation of the isotopic ratio of the sample from that of an internationally accepted standard, where:

$$\delta^{13}\text{C} = [(R_{\text{SAMPLE}} - R_{\text{STANDARD}}) / R_{\text{STANDARD}}] \times 1000$$

where $R_{\text{SAMPLE}} = {}^{13}\text{C}/{}^{12}\text{C}$ of the sample and $R_{\text{STANDARD}} = {}^{13}\text{C}/{}^{12}\text{C}$ of the PDB standard ($R_{\text{PDB}} = 0.0122372$).

The utility of ^{13}C isotopic tracers for SOM studies derives from the fact that all plants discriminate against ^{13}C during photosynthesis but to different degrees depending on their photosynthetic pathway (Farquhar *et al.*, 1989; Bernoux *et al.*, 1998). Plants are “depleted” of ^{13}C relative to the atmosphere and this depletion is caused by enzymatic and physical processes that discriminate against ^{13}C in favour of ^{12}C . Discrimination varies among plants using different photosynthetic pathways: the Calvin cycle (C_3), Hatch-Slack cycle (C_4) and Crassulacean acid metabolism (CAM) (O’Leary 1981, 1988).

The C_3 pathway begins with the diffusion of CO_2 from the atmosphere into the air-filled spaces within the leaf. This diffusion occurs through the still air occupying stomatal pores. Such diffusion has an apparent fractionation ($\Delta\delta$) of $\sim 4.4\text{‰}$ due to the slower motion of the heavier ^{13}C -containing CO_2 molecules. Within the leaf, the carboxylating enzyme ribulose biphosphate carboxylase / oxygenase (rubisco) discriminates further against the ^{13}C , with a $\Delta\delta$ of about 29‰ . If the atmospheric diffusion were the sole limitation for CO_2 uptake, then we would expect to see only the fractionation of 4.4‰ . This 4.4‰ would be subtracted from the $\delta^{13}\text{C}$ value for CO_2 in the atmosphere, which is about -8‰ , yielding a $\delta^{13}\text{C}$ of -12‰ . At the opposite extreme, if enzyme activity were the sole limitation for CO_2 uptake, then only the rubisco fractionation would be observed. These conditions would yield a predicted leaf $\delta^{13}\text{C}$ value of about -37‰ . In fact, $\delta^{13}\text{C}$ value for C_3 plants lie between these extremes, with a median of about -27‰ . Variation about this median depends on the balance between diffusive supply and enzymatic demand of CO_2 .

Isotopic composition of C_4 plants differs substantially from that of C_3 plants. The initial step in C_4 photosynthesis is the same: the diffusion of CO_2 from the atmosphere into the leaf via stomata. However, C_4 photosynthesis is catalyzed by a different enzyme, phosphoenolpyruvate (PEP) carboxylase, which has a different discrimination, approximately -6‰, for the fixation of CO_2 (Farquar 1983). If this enzymatic fractionation were fully and exclusively expressed relative to the atmospheric CO_2 , it would yield tissue values around -2‰. Diffusion-limited uptake would be the same as that for C_3 plants, namely -12‰. One may expect C_4 plants to lie between these extremes, analogous to C_3 plants. In fact, measured $\delta^{13}C$ value for C_4 plants lie below this range, clustering around -14‰. These surprisingly negative values result from the unique physiology of C_4 photosynthesis. The C_4 compounds produced by PEP carboxylase are transported into the bundle sheath, which is the cylinder of vascular tissue enclosed in the center of the leaf. Inside the bundle sheath, the C_4 compounds are catabolized to C_3 compounds, releasing CO_2 , which accumulates to high concentrations. The released CO_2 is then refixed by rubisco, the same enzyme used by C_3 photosynthesis. The negative $\delta^{13}C$ values are caused by a slow leak of enriched CO_2 from the bundle sheath. The leaking CO_2 pool is enriched in ^{13}C by the preference of rubisco for the light isotope. As the enriched CO_2 leaks out, it depletes the $\delta^{13}C$ of the CO_2 left behind (Ehleringer & Pearcy, 1983; Berry 1989). Support for this mechanism comes from evidence that C_4 plants with the most developed bundle sheaths tend to exhibit the most negative $\delta^{13}C$ (Hattersley 1982; Henderson *et al.* 1992; Sandquist & Ehleringer 1995).

In conclusion, the isotopic composition of SOM closely resembles the isotopic composition of the vegetation from which it was derived because of the fractionation during C fixation (Peterson & Fry, 1987; Nadelhoffer & Fry, 1988). When one type of vegetation is replaced with another, $\delta^{13}\text{C}$ values can be used to identify SOM derived from residues from the original vegetation and from the new vegetation (Cerri *et al.*, 1985; Balesdent *et al.*, 1987; Bernoux, 1998).

1.7 Modelling soil organic carbon

The dynamic changes of SOC have a strong effect on atmospheric composition and the rate of climate changes. Where direct measurements are not available, GHG fluxes are often estimated using numerical soil / ecosystem models. Models are not substitutes for direct measurements, but they do allow integration of the various factors controlling decomposition processes and SOM dynamics. Most importantly, they can be used to predict changes in SOM under the different management and climatic conditions that may occur in the future (Jones and Donnelly, 2004). The applications of simulation models range across scales from field or plot level (Jenkinson *et al.*, 1987) to regional (Parton *et al.*, 1987) and global scale (King *et al.*, 1996; Cao & Woodward, 1998). Many soil C models have been relatively successful in simulating C dynamics at the field and regional scale, but they generally need some site-specific calibration to provide reliable predictions (Paustian *et al.*, 1997).

Most of the models can describe the turnover rate of SOC as a sum of multiple and parallel compartment, and each compartment has its own turnover rate. Those compartment models require the size and turnover rate for each

compartment, which is difficult to obtain from field studies (Yang *et al.*, 2003). In fact, classical descriptions of SOM have normally combined chemical extractions with the identification of specific chemical compounds, but this approach has contributed little to a functional understanding of soil processes (Collins *et al.*, 2000). As an alternative, researchers studying ecosystem functioning have tended to adopt a model where organic C is located in more-or-less discrete “pools” in the soil. There is at present little agreement on the precise definition of most of these pools and they can mean different things to different researchers (Jenkinson *et al.*, 1992; Smith *et al.*, 2002). To some they mean different fractions of plant residues at different stages of decomposition and associated with different soil particle size fractions (Six *et al.*, 2002a). To others they are interpreted as chemical fractions containing specified chemical structures or functional groups (Christensen, 1996; Post & Kwon, 2000). Nevertheless, several models have been described for the turnover of organic C in soil under field conditions over the years-to-centuries time scale. These models assume that the SOM may be treated as a compartmental system which is made up of a finite number of macroscopic subsystems, called compartments or pools, each of which is homogeneous and well mixed, and the compartments interact by exchanging material (Atkins, 1969). There may be inputs from the environment into one or more of the compartments and there may be outputs from one or more compartments into the environment. The transfer of material from one compartment to another is usually assumed to occur by first-order processes, i.e., the rate of transfer of material from compartment j to any other compartment k is proportional to the current amount of material in the compartment j , with the constant of proportionality being a first

order rate transfer coefficient. This results in a linear system of model equations. These compartmental models are mechanistic since they arise from making assumptions about the kinetics involved (e.g. first-order kinetics), writing down differential equations that represent these assumptions and solving these equations to obtain a model with parameters that can be physically interpreted. A mechanistic model often also has a level of empiricism. For example, the rate coefficients of the resulting differential equation systems may vary empirically with abiotic conditions such as soil moisture content and soil temperature. These empirical relationships could be any arbitrary functions which may only have the desired shape in order to fit the available data points. Parameters in such empirical models often do not have any theoretical and physical interpretation, and any connections between this family of curves and the underlying physical processes are often tenuous (Parshotam, 1996). To date, the Century model (Parton *et al.*, 1988) and the Rothamsted soil-C turnover model (Jenkinson & Rayner, 1977; Jenkinson, 1990) have undergone the most field testing and validation. These models and other soil-C turnover models are increasingly being used in climate-change and land-use studies.

Both the RothC and CENTURY models are classified as ecosystem-level models by Paustian *et al.* (1997) to distinguish them from another group, classified as macro-scale models. A macro-scale model such as the Terrestrial Ecosystem Model (Melillo *et al.*, 1993) assesses soil C change at very large spatial resolutions, typically thousands of square kilometers. Compared with the ecosystem models they have a simple structure (fewer plant, litter and soil components) and employ more general rate-controlling factors. More recent

developments by Falloon *et al.* (1998) have used RothC on a scale closer to the macro-scale models to estimate C sequestration at the regional level using the simulation model linked to spatially explicit data. By linking geographical information systems that contain detailed information on soils, land use and climate to dynamic simulation models such as RothC for the turnover of organic C, it is possible to estimate the impacts of land use and climatic changes on C stocks in soil at the macro-scale (Jenkinson *et al.*, 1991).

1.8 Objectives and outlines of this thesis

Based on further literature research which is presented in Chapter 2 through 5, the main objectives of this dissertation are:

- To measure the total C sequestered in a *Miscanthus* soil and its distribution in different soil aggregates.
- To quantify the amount of C₄- and C₃- derived C under a 14-years-old *Miscanthus* plantation, established on previous arable land, and under a 1-year-old *Miscanthus* plantation, previously under grassland.
- To compare SOC stocks between different land-use systems and quantify the relative distribution of SOC in different aggregate size fractions.
- To compare field measurements in Ireland to soil model predictions in order to estimate the long-term potential of *Miscanthus* to sequester C in the soil.

In particular, with this dissertation I will study the following hypothesis:

H1: The land use change from arable to *Miscanthus* cultivation increases C storage in physically protected SOM fractions and, consequently, the soil C stocks under *Miscanthus* contribute considerably to its CO₂ mitigation potential.

H2: Land use has a significant effect on the total SOC content, as well as on the C associated with the soil fractions. In particular, 14 years of *Miscanthus* plantation increase C sequestration in stable and protected soil fractions compared to other land use systems.

H3: Measured soil fractions can be related to RothC pools.

Chapters 2 through 4 focus on experimental sites located at the Teagasc Research Center, Oak Park, Carlow, Ireland. In particular, Chapter 2 reports on SOM dynamics through four soil depths after 14 years of land use change from arable land to *Miscanthus* plantation. In order to understand the cause of the difference in soil C content between these two land use systems, I also analyze the C content of various soil fractions combining soil physical fractionation technique with the ¹³C isotopic analysis.

Chapter 3 has the aim of quantifying the amount and distribution of SOC under different land use systems. In this experiment I use the same method as the previous research to analyze the C content of various soil fractions under a 1 year old *Miscanthus* plantation, established on grassland. Hence, I compare soil C stocks and the relative distribution of SOC in the aggregate size fractions between different land use systems.

Chapter 4 focuses on the comparison between direct measurements of SOC fractions and model predictions made by RothC and a cohort model. The targets of this experiment are to predict C sequestration resulting from changing the land use from arable to *Miscanthus* plantations and to demonstrate that measured organic C fractions of different lability are similar to the C pools used in RothC. To achieve this last objective, I applied a soil fractionation technique that is different to that used for the previous experiments.

Chapter 5 presents the general discussion, in which I synthesize findings of all previous chapters. I will also discuss the use and limitations of soil physical fractionation techniques in order to study soil C dynamics.

Chapter 2

Carbon sequestration under *Miscanthus*: a study of ^{13}C distribution in soil aggregates¹

Based on: Dondini M, Van Groenigen KJ, Del Galdo I, Jones MB (2009) Carbon sequestration under *Miscanthus*: a study of ^{13}C distribution in soil aggregates. *Global Change Biology-Bioenergy*, **1**, 321-330.

2.1 Abstract

The growing of bioenergy crops has been widely suggested as a key strategy in mitigating anthropogenic CO₂ emissions. However, the full mitigation potential of these crops cannot be assessed without taking into account their effect on soil C dynamics. Therefore, we analyzed the C dynamics through four soil depths under a 14 year old *Miscanthus* plantation, established on former arable land. An adjacent arable field was used as a reference site. Combining SOM fractionation with ¹³C natural abundance analyses, we were able to trace the fate of *Miscanthus*-derived C in various physically protected soil fractions. Integrated through the whole soil profile, the total amount of SOC was higher under *Miscanthus* than under arable crop, this difference was largely due to the input of new C. The C stock of the macroaggregates (M) under *Miscanthus* was significantly higher than those in the arable land. Additionally, the C content of the micro-within macroaggregates (mM) were higher in the *Miscanthus* soil as compared with the arable soil. Analysis of the intra-microaggregates particulate organic matter (POM) suggested that the increase C storage in mM under *Miscanthus* was caused by a decrease in disturbance of M. Thus, the difference in C content between the two land use systems is largely caused by soil C storage in physically protected SOM fractions. We conclude that when *Miscanthus* is planted on former arable land, the resulting increase in soil C storage contributes considerably to its CO₂ mitigation potential.

2.2 Introduction

In recent years, climate change impacts and GHG emissions from different forms of land use have emerged as key factors shaping agricultural policies world wide. Whereas agriculture is one of the largest sources of anthropogenic GHG emissions, it also provides several possibilities for GHG mitigation (IPCC 2001). Specifically, the conversion of surplus agricultural land to bioenergy crops provides great potential for CO₂ mitigation across Europe (Smith *et al.* 2000). Biomass energy is close to 'carbon neutral', that is to say, it produces energy while only releasing C to the atmosphere that has been captured during the growing cycle of the plant, rather than emitting C that has been locked away from the atmosphere in fossil reserves for millions of years. Bioenergy crops can take many forms and can be converted to a number of different products. Many crop species are multipurpose in that they can be used to produce more than one type of energy product (Sims *et al.*, 2006).

In recent years, *Miscanthus* (*Miscanthus x giganteus* Greef and Deu.) has received much attention as a potential bioenergy crop (Styles *et al.*, 2007). This perennial rhizomatous grass, which is native to East Asian tropical and subtropical regions, has a considerable biomass production potential even under temperate climatic conditions (Lewandowski *et al.*, 2000). Clifton-Brown *et al.* (2004) estimated peak annual yields across Europe ranging from 13 t ha⁻¹ in Finland and Sweden to 25.8 t ha⁻¹ in Belgium. By both displacing C released through burning fossil fuels and by soil C sequestration, growing *Miscanthus* for

electricity production could mitigate 9% of the total European C emissions in 1990 (Clifton-Brown *et al.*, 2004).

Previous research on *Miscanthus* primarily focused on management and economics in relation to establishment and productivity, harvest and storage, and combustion feasibility (Jørgensen *et al.*, 2000). Even though soil C represents the largest C pool in most agro-ecosystems (IPCC, 2001), research on SOC dynamics under *Miscanthus* plantation remains scarce (Hansen *et al.*, 2004). In particular, little is known about C dynamics below the top soil layer. Assessing the effect of agricultural practices on C stocks in top soil alone may lead to erroneous conclusions regarding soil C storage potential (Baker *et al.*, 2007). To determine the CO₂ mitigation potential of *Miscanthus*, we therefore need to measure its effect on soil C dynamics through the whole soil profile.

Several features of *Miscanthus*' physiology and the agricultural practices associated with its cultivation suggest a large potential for soil C sequestration. Firstly, perennial plants such as *Miscanthus* allocate high proportions of the assimilated C belowground as a C reservoir for growth in spring (Kuzyakov & Domanski, 2000). Secondly, when *Miscanthus* is planted on former arable land, the absence of soil tillage results in less aeration, lower decomposition rates and increased soil C stabilization (Beuch, 1999; Clifton-Brown *et al.*, 2007). Thirdly, as *Miscanthus* systems are typically harvested after aboveground biomass has senesced, stems and leaves accumulate on the soil surface as pre-harvest losses, causing high soil C input rates of aboveground biomass (Beuch, 1999). Finally, *Miscanthus* systems typically receive little or no N fertilizer, leading to increased

soil C : N ratios and a slower decomposition of plant residue (Schneckenberger & Kuzyakov, 2007).

To accurately determine the potential for soil C sequestration following land use changes, we need to know the fate of new soil C input. Most organic matter enters the soil as readily recognizable plant litter and is mineralized within months (Christensen, 2001). A small portion, however, may be stabilized through interactions with mineral surfaces for periods up to thousands of years (Six *et al.*, 2004; Lehmann *et al.*, 2007). In many soils, such as Mollisols and Alfisols, strong feedbacks exist between SOM stabilization and aggregate turnover (Jastrow & Miller, 1998; Six *et al.*, 2004). In these soils, the deposition and transformation of SOM is a dominant aggregate stabilizing mechanism. Soil aggregate structure is usually hierarchical (Tisdall & Oades, 1982; Oades & Waters, 1991) with primary particles and silt-sized aggregates (<50 μm diameter) bound together to form microaggregates (50-250 μm diameter). These primary and secondary structures, in turn, are bound into macroaggregates (>250 μm diameter).

Current evidence suggests that microaggregates are formed inside macroaggregates, and that factors increasing macroaggregate turnover decrease the formation and stabilization of microaggregates (Angers *et al.*, 1997; Gale *et al.*, 2000; Six *et al.*, 2000, 2004). However, microaggregates, and smaller aggregated units, are generally more stable and less susceptible to disturbance than macroaggregates (Tisdall & Oades, 1982; Dexter, 1988; McCarthy *et al.*, 2008). Soil C storage following land use change has previously been attributed to changing C contents of microaggregates within macroaggregates (Six *et al.*,

2004). For these reasons, soil physical fractionation forms a useful tool to evaluate changes in soil C and SOM dynamics.

Isotopic labelling of C allows the tracing of newly sequestered C into SOM pools (Balesdent *et al.*, 1987). Using a simple isotopic dilution model, differences in ^{13}C -signature between C_3 and C_4 plants have been used to trace newly sequestered C (e.g. Jastrow *et al.*, 1996; Collins *et al.*, 1999). This is particularly the case when C_4 plantation are grown on soils which have retained a predominantly C_3 signal, associated with a C_3 vegetation history. The combination of SOM fractionation techniques with ^{13}C natural abundance analyses offers an elegant approach to investigate small shifts on soil C stores that would be significant in the long term, but that might not be detected by conventional methodologies in the short term (Del Galdo *et al.*, 2003).

In this study, we report on soil C sequestration beneath a 14 years old Irish *Miscanthus* plantation established on former arable land. Measurements of the $^{13}\text{C}/^{12}\text{C}$ ratios in the soil down to 60 cm depths in *Miscanthus* plots and in nearby references plots with a C_3 plant history allowed us to estimate the fraction of SOC derived from *Miscanthus* as well as changes in overall SOC storage.

2.3 Materials and methods

2.3.1 Study site and soil sampling

The experimental site was located at the Teagasc Research Centre, Oak Park, Carlow, Ireland (52°51' N 6°54' W, 50 m a.s.l.). The soil at this site has a loamy

sand texture, with a pH of 6.9. Mean annual precipitation and annual temperature are 830 mm and 9.3 °C, respectively. At the experimental site, we established three sampling plots (15x30 m) in a *Miscanthus* plantation and three plots on adjacent arable land. The *Miscanthus* field (30x120 m) was established in 1994. Although a detailed historical record of past land use is not available, the entire experimental site had been under cultivation for at least 15 years before the *Miscanthus* plantation was established, and has been cropped exclusively with C₃ crops. Aboveground standing biomass of the *Miscanthus* was harvested annually in March – April.

In order to collect undisturbed soil cores, two different soil sampling procedures were used. In June 2007, four cores (Ø 10 cm) were taken down to 60 cm in each plot and were divided in four soil layers (0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm). These samples were used for bulk soil analyses. In addition, four cores (Ø 5.6 cm) per plot were collected and divided into 0-15 and 15-30 cm soil layers. These samples were used in the soil fractionation procedure. All soil samples were combined by depth increment. Soil samples were air-dried and soil density and gravel content were measured by conventional methodologies. The four cores were sieved to pass through an 8 mm sieve by gently breaking apart the soil. Aboveground biomass production in the *Miscanthus* plots was determined by harvesting 2x2 m quadrats in each plot. The mean peak dry matter yield (November 2007) was 16 t ha⁻¹ and the mean harvest dry matter yield (April 2008) was 13 t ha⁻¹. Plant, litter and root material were collected from the *Miscanthus* plots three times during the growing season of 2007 for isotopic analyses. All plant samples were combined per plot and were air-dried.

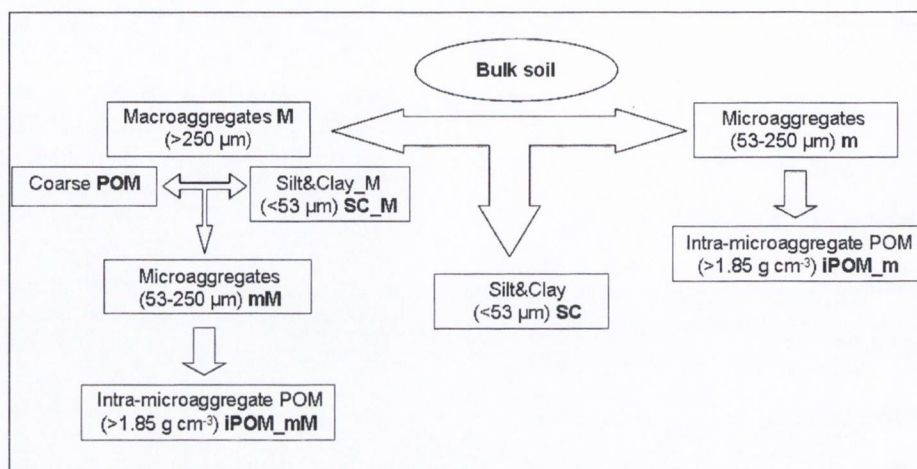


Figure 1: Fractionation scheme adopted in this study, modified after Six et al. (1998, 2002). M = macroaggregates (>250 μm size aggregates); m = microaggregates (53–250 μm size aggregates); SC = silt&clay 53 μm size class fraction; coarse POM = >250 μm size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates; iPOM_mM = intra-aggregate particulate organic matter within mM; iPOM_m = intra-aggregate particulate organic matter within m.

2.3.2 Soil fractionation

All samples from the 0-15 cm and 15-30 cm soil layers were fractionated by size and density (Fig. 1). Briefly, two sieves (250 and 53 μm mesh size) were used to separate macroaggregates (>250 μm ; M), microaggregates (53-250 μm ; m) and the silt & clay fraction (<53 μm ; SC). A subsample was submerged for 5 min in room-temperature-deionized water, on top of a 250 μm sieve. Aggregate separation was achieved by manually moving the sieve up and down 3 cm with 50 repetitions during a period of 2 min. After the 2 min cycle, the M fraction was gently backwashed off the sieve into an aluminum pan. Water plus soil that went through the sieve was poured onto a 53 μm sieve and the sieving procedure was repeated. Material <53 μm was left to settle for 24 hours in plastic bottles, the supernatant was poured off and the SC fraction were washed into another aluminum pan. All the fractions were oven dried at 50 $^{\circ}\text{C}$.

In the second step, the M fraction was separated into coarse particulate organic matter (>250 μm ; coarse POM), microaggregates (53-250 μm ; mM) and silt and clay (<53 μm ; SC_M) by using the methodology described in Six *et al.* (2000). Subsamples of fraction M were immersed in deionized water on top of a 250 μm mesh screen and gently shaken with 50 glass beads ($\varnothing$ 5.4 mm) for 10 min. The coarse POM was retained on the 250 μm screen, while a continuous and steady water flow ensured that the mM were flushed onto a 53 μm sieve. Once the M fraction was broken up entirely, the material on the 53 μm sieve was wet-sieved. The fraction retained on the 53 μm sieve (i.e. water-stable mM) and the fraction that passed through the 53 μm sieve (i.e. SC_M) were collected and dried at 50 °C.

Density fractionation was carried out by following the method described in Six *et al.* (1998). Subsamples (5 g) of the oven-dried m and mM fractions were suspended in 35 mL of a 1.85 g cm⁻³ of sodium polytungstate (SPT) solution and slowly shaken by hand. Material remaining on the cap and sides of the centrifuge tube was washed into suspension with 10 mL of the SPT solution. After 20 min of vacuum (138 kPa), the sample was centrifuged (1250 g) at 20 °C for 60 min. The floating material was aspirated onto a 20 mm nylon filter, thereby removing all undecomposed litter and root fragments from the soil samples. The heavy fraction was rinsed twice with 50 mL of deionized water and dispersed by shaking it with 30 mL of 0.5% sodium hexametaphosphate for 18 h. The dispersed heavy fraction was passed through a 53 μm sieve and the material remaining on the sieve, i.e. the intra-aggregate particulate organic matter (iPOM), was dried (50 °C) and weighed.

2.3.3 Total C and ^{13}C analysis

The C contents and $\delta^{13}\text{C}$ signature were measured for all soil and plant samples. Sample preparation prior to isotopic analyses was similar for all samples. Three grams of each sample was ball milled and sub-samples of 30 mg were weighed into Ag capsules. Soil carbonates were removed using HCl fumigation as described by Harris *et al.* (2001). Total C and $\delta^{13}\text{C}$ were determined at the UC Davis Stable Isotope Facility using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter.

2.3.4 Data analysis

By convention, the ^{13}C abundance in a sample is expressed in delta-units ($\delta^{13}\text{C}\text{‰}$) according to the following equation:

$$\delta^{13}\text{C}\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where R_{sample} is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the international Pee Dee formation belemnite carbonate standard (PDB). The fraction of the soil C pool that is C_4 -derived (f_{M}) is calculated by using the isotope mass balance equation (Balesdent *et al.*, 1987):

$$f_{\text{M}} = (\delta_2 - \delta_0)/(\delta_1 - \delta_0)$$

where δ_2 is the $\delta^{13}\text{C}$ of the soil under *Miscanthus*, δ_0 is the $\delta^{13}\text{C}$ of the soil before introduction of *Miscanthus* and δ_1 is the $\delta^{13}\text{C}$ of the *Miscanthus* plant material entering the soil. As δ_0 was not known, the value from relevant depths of the reference field was used instead. The δ_1 represents an average of $\delta^{13}\text{C}$ values determined for litter, rhizomes and roots from *Miscanthus*. Carbon concentrations were expressed on an aggregate basis while the C contents of SOM fractions were calculated on an area basis, correcting for soil depth and bulk density.

We estimated the retention coefficient of *Miscanthus*-derived C at our site as the ratio of the annual quantity of C retained in the soil over the total annual input of *Miscanthus* C derived from above- and below- ground biomass (Hansen *et al.*, 2004). In our calculations, we assumed that the yield data of 2007 were representative for the last 14 years. This approach assumes a full grown crop after 1 year, which overestimates the actual soil C input rate in the first years after the establishment of the *Miscanthus* field. On the other hand, the approach also assumes that yield did not decline over time, which underestimates the actual rate of soil C input (Clifton-Brown *et al.*, 2007).

2.3.5 Statistical analysis

. The results of the experiment were tested for normal distribution using the Shapiro-Wilk Test and the Sig. value was > 0.05 (i.e., the data have a normal distribution) The results of the experiment were then analysed as a split-plot design, with land use as the main plot treatment. An ANOVA was conducted for each soil depth using the SPSS 16.0 software package, with blocks as random effects and fractions as a fixed effect. For the soil profile data an ANOVA was

conducted with blocks as random effects and depth as fixed effect. The separation of means was tested using Tukey's honestly significant difference with a significance level of $P < 0.05$.

2.4 Results

2.4.1 Soil profile

Integrated through the whole soil profile, the total SOC content under *Miscanthus* is significantly ($P < 0.01$) higher than under arable crops (131.3 vs. 105.8 Mg C ha⁻¹). Within soil layers, significant differences between the two land use systems were found at the 0-15 and 15-30 cm depth (Fig. 2b). Top soil under both land use systems contained significantly more C than lower soil layers.

The distribution of soil C with depth differed between land use systems. Under *Miscanthus*, 82.1% of the total C stock was found in the upper 30 cm. In the arable soil, on the other hand, the upper 30 cm contained only 61.9% of the total C stock. Soil C content in both the 0-15 cm and the 15-30 cm layers were significantly higher under *Miscanthus* (58.0 and 49.7 Mg C ha⁻¹) than in the arable soil (42.1 and 34.7 Mg C ha⁻¹). At lower soil depths, C contents did not differ between land uses.

The input of C₄-derived C increased $\delta^{13}\text{C}$ values at all depths under *Miscanthus* relative to the arable soil (Fig 2a). In the 0-15 cm soil layer, 42.6% of the total C pool in the 0-15 cm layer was *Miscanthus* derived. This value declined to 36.6% in the 15-30 cm layer, to 9.3% in the 30-45 cm and to 8.8% in the 45-60

cm layers (Fig. 2b). Integrated through the whole soil profile, 45.1 Mg C ha⁻¹ under *Miscanthus* was C₄-derived.

After 14 years of *Miscanthus* plantation, the estimated total biomass recycled to the soil was 170 Mg DM ha⁻¹, which is equivalent to a C input to the soil of 77 Mg C ha⁻¹ (Table 1). This estimate resulted in a retention coefficient of 0.5.

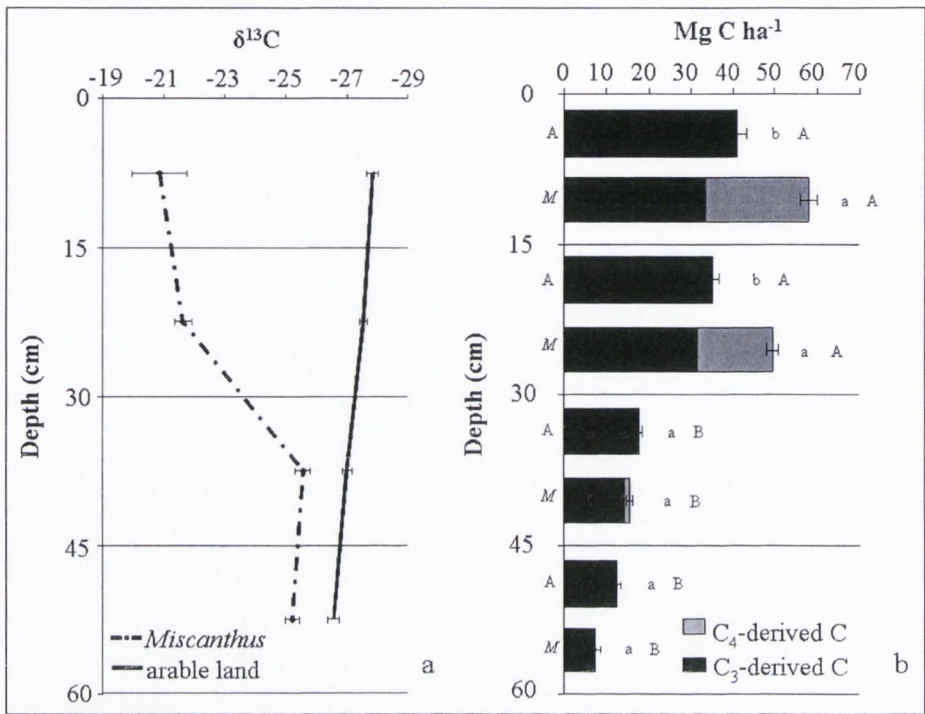


Figure 2: a) $\delta^{13}\text{C}$ values and b) C content in the bulk soil, at four soil depths, for the arable land (A) and the *Miscanthus* (M) systems. Within each depth, values followed by a different lowercase letter are significantly different ($P < 0.05$) between land use systems (A and M). Within each land use system, values followed by different capitals are significantly different ($P < 0.05$) between depths. For *Miscanthus* soils, the C₃- and C₄-derived C are indicated.

Table 1: Peak yield, estimated C balance and coefficient of retention for the *Miscanthus* plantation. DM = dry matter

Peak yield (Mg DM ha ⁻¹ yr ⁻¹) ^a	16.0
Recycled above ground biomass (Mg DM ha ⁻¹ yr ⁻¹) ^b	6.9
Recycled below ground biomass (Mg DM ha ⁻¹ yr ⁻¹) ^c	6.5
Sum of biomass recycled to soil (Mg DM ha ⁻¹ yr ⁻¹)	13.4
Total soil input of carbon (Mg C ha ⁻¹ yr ⁻¹) ^d	6.0
Carbon derived from <i>Miscanthus</i> (Mg C ha ⁻¹ yr ⁻¹) ^e	3.2
Coefficient of retention ^f	0.5

^a Data based on mean peak yields in 2007.

^b Data based on difference between peak yields and harvested biomass in 2007, we assumed a 30% harvest loss of above ground biomass (Clifton-Brown *et al.*, 2007)

^c Below ground biomass estimated as proportion of the above ground biomass, allowing a turnover time of 4 years (Hansen *et al.*, 2004). Ratio above/below ground biomass from Clifton-Brown *et al.* (2007).

^d 45% of the total biomass recycled to the soil.

^e Data from Fig. 2.

^f Ratio of the quantity of C retained in soil to the total input of *Miscanthus* C.

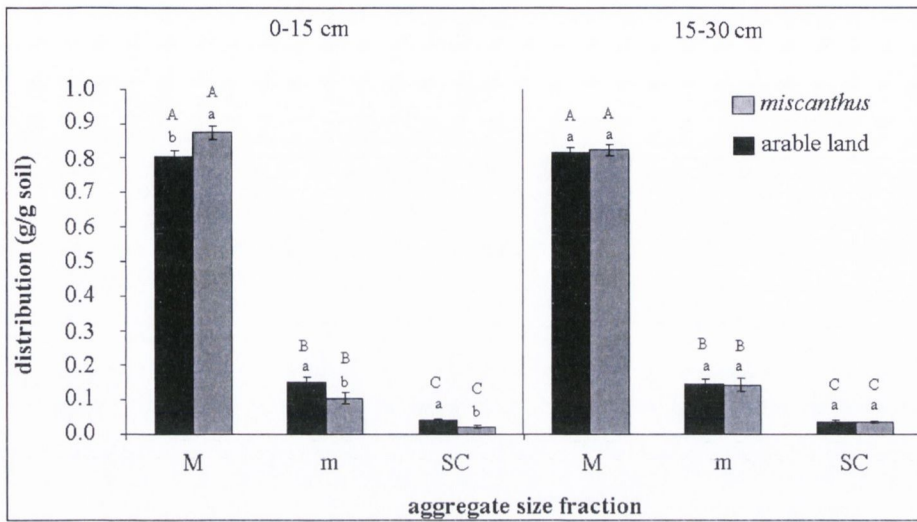


Figure 3: Weight distribution among aggregate size classes, at two soil depths, for the arable land and the *Miscanthus* systems. Within each depth, values followed by a different lowercase letter are significantly different ($P < 0.05$) within aggregate size and among land use systems. Within depth and land use system, values followed by a different capital are significantly different ($P < 0.05$) between soil fractions. M = macroaggregates; m = microaggregates; SC = silt&clay.

2.4.2 Aggregate size fractions

Aggregate size distributions followed the same pattern across both land use systems and with soil depths, with the highest percentage of soil being present as M, followed by m and SC (Fig. 3). In the 0-15 cm layer, *Miscanthus* soil has a higher percentage of M compared to the arable land, while the arable soil contained a higher proportion of m and SC fractions. Here, the difference in the weight distribution of the aggregate size classes between land uses was consistent with their respective C content. Macroaggregates under *Miscanthus* contained $57.4 \text{ Mg C ha}^{-1}$ in the 0-15 cm layer, and $45.4 \text{ Mg C ha}^{-1}$ in the 15-30 cm layer. These values are significantly higher than the C content of the same fraction in the arable soil.

Both in the 0-15 cm and 15-30 cm soil layers, the $\delta^{13}\text{C}$ values of all aggregate size fractions were significantly higher under *Miscanthus* than in the arable soil (Table

2). In the 0-15cm and 15-30 cm soil layers under *Miscanthus*, about 92.9 % and 80.7% of all C₄-derived C was located in the macroaggregates, respectively. In the macroaggregates, the average amount of C₃-derived C under *Miscanthus* was significantly higher than in the arable soil. In the microaggregates on the other hand, the average amount of C₃-derived C under *Miscanthus* was significantly lower (Fig. 4) than in the arable soil.

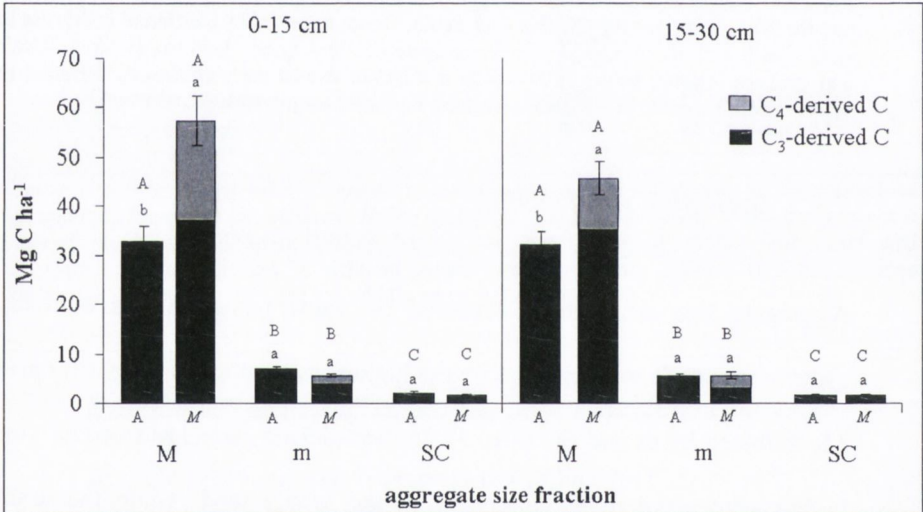


Figure 4: C content in the aggregates size classes, at two soil depths, for the arable land (A) and the *Miscanthus* (M) systems. For *Miscanthus* soils, the C₃- and C₄-derived C are indicated. See Fig. 3 for the explanation of the symbols. M = macroaggregates; m = microaggregates; SC = silt&clay.

Table 2: $\delta^{13}\text{C}$ values for soil aggregate fractions.

$\delta^{13}\text{C}$				
Depth (cm)	Land use	Soil aggregates		
		M	m	SC
0-15	<i>Miscanthus</i>	-21.45	-23.24	-26.20
		(1.19)	(1.14)	(0.55)
	Arable land	-26.43*	-27.50*	-27.11
		(0.57)	(0.03)	(0.04)
15-30	<i>Miscanthus</i>	-23.80	-22.50	-25.20
		(1.10)	(1.89)	(0.94)
	Arable land	-27.12*	-26.92*	-27.01*
		(0.06)	(0.20)	(0.08)

Mean values are shown (n = 3) with standard error in parentheses. Statistically significant differences are given after two-way ANOVA. Within each depth, values followed by a star symbol (*) are significantly different within aggregate size and among land use systems. M = macroaggregates; m = microaggregates; SC = silt&clay.

2.4.3 Intra-macroaggregate organic matter

Under *Miscanthus*, the mM fraction contained 42.8 Mg C ha⁻¹ and 32.2 Mg C ha⁻¹ at 0-15 cm and 15-30 cm depths, respectively (Fig. 5). These values are significantly higher than C contents found for the same fraction at the arable site (22.4 Mg C ha⁻¹ and 19.3 Mg C ha⁻¹ at 0-15 cm and 15-30 cm depth, respectively). As such, around 50% of the difference in C content between the two land use systems can be ascribed to soil C storage in the mM fraction. The C content of coarse POM and SC_M was also significantly higher under *Miscanthus* than in the arable soil, but these differences had a relatively small effect on the total C budget. Of all the C₄-derived C in the M fraction under *Miscanthus*, 62.1% (0-15 cm soil layer) and 61.4% (15-30 cm soil layer) was found in the mM fraction. The average amount of C₃-derived C in the mM fraction under

Miscanthus was higher than in the arable soil; however, this difference was not statistically significant (Fig. 5).

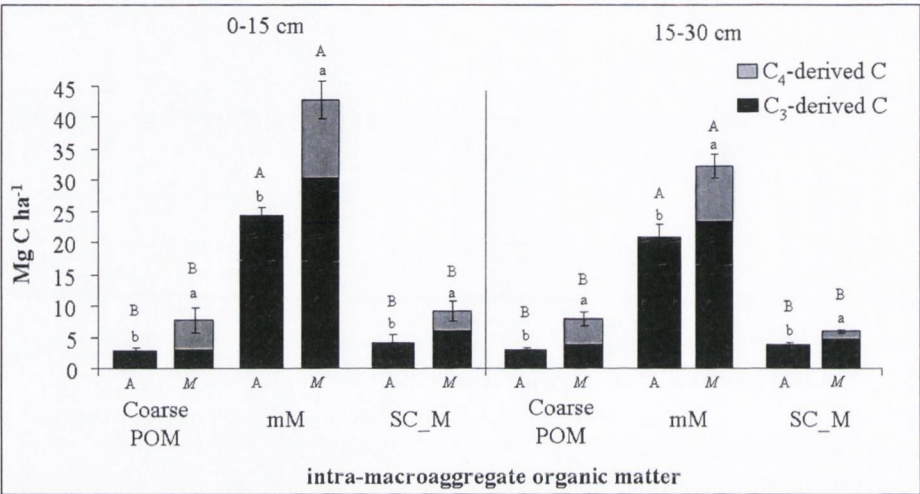


Figure 5: C content in the intra-macroaggregate organic matter, at two soil depths, for the arable land (A) and the *Miscanthus* (M) systems. See Fig. 3 for the explanation of the symbols. For *Miscanthus* soils, the C₃- and C₄-derived C are indicated. Coarse POM = >250 μ m size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates.

2.4.4 Intra-microaggregates POM

Under *Miscanthus*, iPOM_mM contained 20.8 Mg C ha⁻¹ at 0-15 cm depth, 8.6 Mg of which was C₄-derived. At the 15-30 cm depth iPOM_mM contained 18.2 Mg C ha⁻¹, 3.1 Mg of which was C₄-derived. In the arable soil, iPOM_mM contained 3.4 Mg C ha⁻¹ at both 0-15 and 15-30 cm soil depth, significantly less than under *Miscanthus*. In the iPOM_mM, the amount of C₃-derived C under *Miscanthus* was significantly higher compared to the arable soil. The iPOM_mM fraction accounted for 30% of all the C₃-derived C in the M fraction under *Miscanthus*, but for only 10% of the C stock in the M fraction in the arable land.

Under *Miscanthus*, the ratio of iPOM_mM to coarse POM was 3.8 and 2.1 for the 0-15 cm and 15-30 cm soil layer, respectively. In the arable soil this ratio was 1.4 and 1.3 for the 0-15 cm and 15-30 cm soil layer, which is significantly lower than under *Miscanthus* (Fig. 6).

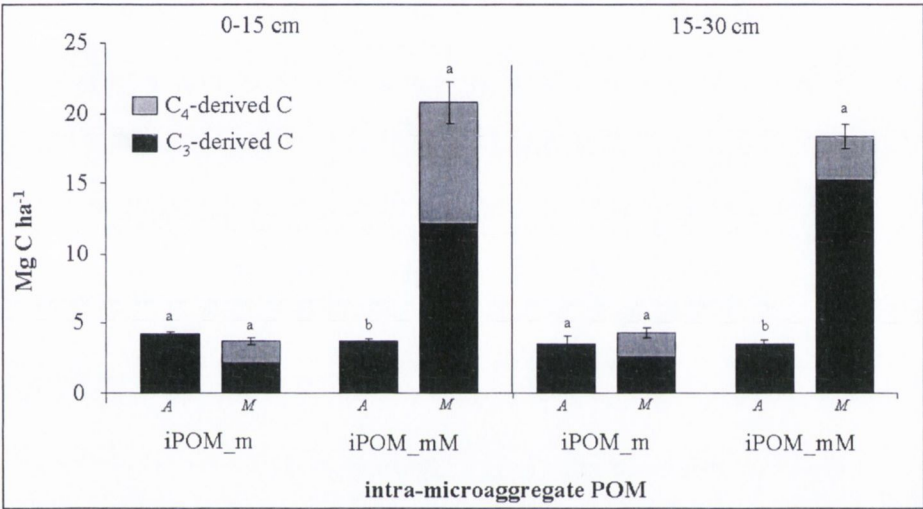


Figure 6: C content in the intra-microaggregate POM, at two soil depths, for the arable land (A) and the *Miscanthus* (M) systems. Values followed by a different lowercase letter are significantly different within aggregate size and among land use. For *Miscanthus* soils, the C₃- and C₄-derived C are indicated. iPOM_m = intra-aggregate particulate organic matter within m.

2.5 Discussion

Assuming that both fields started at the same SOC level, the *Miscanthus* system gained 25.4 Mg C ha⁻¹ through the whole soil profile, or 25.0% of the total soil C stock under the arable site. Over a period of 14 years, these results partially corroborate a study by Hansen *et al.* (2004) on soil C sequestration under two *Miscanthus* plantations at Hornum, Denmark. They found a significant increase in SOC stocks under a 16 years old *Miscanthus* plantation compared to an arable reference site. However, in the same study, no differences were found in soil C

contents between a 9 years old *Miscanthus* plantation and a grassland reference site. Schneckenberger and Kuzyakov (2007) also found no significant differences in SOC stocks between a 12 years old *Miscanthus* plantation and a grassland reference site. A study conducted in Ireland by Clifton-Brown *et al.* (2007) shows a higher SOC under *Miscanthus* than in an adjacent pasture; however, this difference was not statistically significant. Kahle *et al.* (2001) reported that out of four *Miscanthus* plantations, only two showed a significant increase in soil C relative to grassland reference sites. Together, these results suggest that the potential of soil C storage under *Miscanthus* largely depends on the land use system it is replacing. Apparently arable lands provide the largest potential as a soil C sink. Indeed, arable soils have previously been identified as having large C storage potentials, as they are often depleted in soil C (Smith *et al.*, 2000).

In the arable soil, the $\delta^{13}\text{C}$ values of SOM increased with depth. Similar results were found in several other studies (*e.g.*, Veldkamp, 1994; Gregorich *et al.*, 1995; Schneckenberger *et al.*, 2007). In addition to ^{13}C discrimination during decomposition (Ågren *et al.*, 1996), the decrease in $\delta^{13}\text{C}$ of atmospheric CO_2 during the last century contributes to decrease of $\delta^{13}\text{C}$ values in the upper soil horizon (Gregorich *et al.*, 1995).

The SOM under *Miscanthus* was more enriched in ^{13}C compared to the arable site at all depths, indicating the presence of *Miscanthus*-derived C throughout the profile. The *Miscanthus*-derived C was largely concentrated in the top 30 cm. These results correspond well with Schneckenberger & Kuzyakov (2007), who found that under a 12 years *Miscanthus* plantation, about 85% of total C_4 -derived C found in the soil profile was concentrated in the upper 30 cm.

Near the soil surface, soil C input is mostly determined by turnover of large roots and by harvest losses. However, C derived from *Miscanthus* was found also at lower soil depths. Here the C accumulation was most likely caused by fine root turnover and DOC leaching or by the burrowing activity of earthworms (Hansen *et al.*, 2004).

Integrated through the whole soil profile, 45.1 Mg C ha⁻¹ under *Miscanthus* was C₄-derived, which is equivalent to a sequestration rate of 3.2 Mg C ha⁻¹ yr⁻¹. Although our experimental site was two years younger than the site of Hansen *et al.* (2004), our site contains 2.5 times more *Miscanthus*-derived C. The contrast between the two studies is also reflected in the retention coefficient; Hansen *et al.* (2004) reported a value of 0.29 for a 16 years old *Miscanthus* plantation, i.e. far lower than at our site (Table 1). The difference between the two studies may be explained by the relatively low soil C contents at our arable site, indicating that *Miscanthus* was planted on soil that was depleted in C. This was not the case in Hansen *et al.* (2004), where the arable reference site had soil C contents close to the *Miscanthus* site. As such, the potential to harbour and protect new soil C input was relatively high at our site. However, differences in soil mineralogy and climate might also have contributed to differences in the soil C storage potential between the two sites (Kirschbaum, 1994; Silver *et al.*, 2000).

Our results further show that microaggregates within macroaggregates act as a primary site for long-term soil C sequestration under *Miscanthus*. Comparing no-tilled vs. tilled agricultural systems, Six *et al.* (1999) concluded that no tillage practices increase C sequestration in this very same SOM fraction. Moreover, Gulde *et al.* (2008) concluded from a long term agricultural experiment that

increased soil C input through manure application caused soil C sequestration in the mM and iPOM_mM fractions. These results suggest that soil C sequestration in agro ecosystems largely follows a fixed pattern, regardless of the management practices that were applied to increase soil C stocks.

Most of the C_4 -derived C under *Miscanthus* was found in the mM and iPOM_mM (Figs. 5 and 6). Under *Miscanthus*, these fractions also showed an increase of C_3 -derived C relative to the arable soil. These results can be explained by assuming that at the start of the field experiment, the soils of the future *Miscanthus* plantation and the reference site were not fully in equilibrium with their use as arable land. Continued soil disturbance through ploughing would then further decrease soil C_3 stocks in the reference site. Under *Miscanthus*, the lack of disturbance would cause stabilization of C_3 in the mM fraction.

This scenario is in agreement with our finding that in both the 0-15 cm and the 15-30 cm soil layers, the ratio of iPOM_mM to coarse POM was higher under *Miscanthus* compared to the arable site. As described by Six *et al.* (1999), an increase in this ratio indicates a decrease in M turnover. Six *et al.* (2002) suggested that this response is a driving force behind soil C storage in no-till systems. Unfortunately, we have no data on soil C input at the arable site, so that we cannot separate the individual contributions of soil tillage effects and soil C input effects on soil C storage under *Miscanthus*. However, a recent meta-analysis shows that on average, 20 years of no-tillage increases soil C stocks by 16% in the top 30 cm of soils in temperate moist climates (Ogle *et al.* 2005). Since we found a 40% increase in soil C stocks in the top 30 cm after only 14 years, our results

suggests that increased soil C input rates under *Miscanthus* contributed substantially to the measured increase in soil C stocks.

In summary, our study shows that the land use change from arable land to the energy crop *Miscanthus* increased C sequestration in stable and protected soil fractions. Our results support the soil fractionation scheme presented by Six *et al.* (2002) and underline the role of microaggregates in the long term SOC sequestration. Our results are particularly promising in view of the commitment to the Kyoto protocol, since they show that the conversion of surplus agricultural land to bioenergy crops provides great potential for CO₂ mitigation. Although beyond the scope of this study, the data obtained here could be used to feed into a complete life cycle analysis for the C mitigation benefits of growing *Miscanthus* as an energy crop (Styles *et al.*, 2007).

Chapter 3

Soil organic carbon stocks in soil aggregates under different land use systems

3.1 Abstract

During the past two centuries, land-use practices such as deforestation and tillage have resulted in a net loss of soil C to the atmosphere. Recent concerns about rising CO₂ concentrations in the atmosphere have focused attention on the possibility of sequestering C back into the soil system. This may be achieved by means of afforesting and other land-use conversions. Despite decades of research on changes on soil C stocks after land use changes, there are few studies with a direct focus on estimating the SOC stocks after land use change from pasture or cropland to bioenergy crop plantation. This study was conducted with the aim of quantifying the amount and distribution of SOC under different land use systems in Carlow, Ireland. The specific objectives were to: (i) quantify the amount of C₄- and C₃-derived C under a 1-year-old *Miscanthus* plantation previously under grassland, (ii) to estimate SOC stocks in different land use systems (i.e., *Miscanthus*, arable land and grassland), and to (iii) quantify the relative distribution of SOC in different aggregate size fractions. Our results show that the conversion from grassland to *Miscanthus* plantation leads to accumulate C₄-derived C in the intra-macroaggregate fractions, underlining the role of microaggregates in the SOC sequestration processes. In the upper 30 cm soil depth, land use has a significant effect on the total SOC content, as well as on the C associated with the soil fractions. In particular, 14 years of *Miscanthus* plantation increased C sequestration in stable and protected soil fractions compared to the other land use systems.

3.2 Introduction

Following the promising results on soil C sequestration under *Miscanthus* in Carlow, Ireland, in this Chapter I will compare the C dynamics in different soil aggregates under different land use systems (*Miscanthus*, grassland and arable systems).

During the past two centuries, global land-use practices such as deforestation and tillage have resulted in a net loss of soil C to the atmosphere. Recent concerns about rising CO₂ concentrations in the atmosphere have focused attention on the possibility of sequestering C back into the soil system. This may be achieved by means of afforestation and other land-use conversions (DeGryze *et al.*, 2004). It has been noted that these C sinks represent only a partial solution given that the capacity of soils to store C is finite (Hassink, 1996; Six *et al.*, 2002b) and that increases in SOM can be reversed if proper management is not maintained (Paustian *et al.*, 2000).

A number of factors and processes influence the C pools and fluxes, the most significant being soil erosion, deforestation, land use and land use changes (Shrestha *et al.*, 2004). In particular, the latter can cause a change in land cover and associated changes in C stocks (Bolin & Sukumar, 2000). The change from one ecosystem to another could occur naturally or be the result of human activity, such as for food or timber production. Each soil has a C-carrying capacity, i.e. an equilibrium C content depending on the nature of the vegetation, precipitation and temperature (Gupta & Rao, 1994). The equilibrium C stock is the result of a balance of inflows and outflows to the pool (Fearnide & Barbosa, 1998). The equilibrium between C inflows and outflows in soil is disturbed by land use

change until a new equilibrium is eventually reached in the new ecosystem. During this process soil may act either as a C source or as a C sink according to the ratio between inflows and outflows (Guo & Gifford, 2002). Some studies have reviewed the effects of certain land use changes on soil C stocks, such as deforestation for pasture (Neil & Davidson, 2000) and from cultivation and native vegetation to grasslands (Conant *et al.*, 2001). A meta-analysis of the world literature on changes in soil C stocks following land use changes reported that: (1) after natural forests are cleared for pastures, soil C stocks do not generally decline, (2) when established pastures switch to forest, soil C stocks decline under pine plantations but are unaffected by either broadleaf tree plantation or naturally regenerated secondary forest, (3) when native forest is cleared for cropland, soil C stocks are halved in the top soil but not affected at depth, (4) when cropland reverts to forest, there is recovery in C stocks, and (5) when cropland is placed under pasture, soil C stocks increase to depths below 100 cm but the fractional increase decreases with depth (Guo & Gifford, 2002). Despite decades of research on soil C stocks after land use changes, there are few study with direct focus on estimating the SOC stocks after land use change from pasture or cropland to bioenergy crop plantation.

An important aspect in C sequestration studies is to determine the contribution of “new” organic carbon to SOC. Natural differences in the abundance of ^{13}C between C_4 and C_3 plants are frequently used to distinguish between old SOC and new plant-derived C (Kristiansen *et al.*, 2005). The discrimination against ^{13}C is higher at C_3 photosynthesis, making the $\delta^{13}\text{C}$ values of C_3 plants smaller (ca. -27‰) than those of C_4 plants (ca. -12‰) (Ehleringer &

Cerling, 2002). Therefore, by growing a C_4 plant on soils with former C_3 vegetation, the amount of C_4 plant-derived C can be estimated on the basis of the changing $\delta^{13}C$ values of SOC. Nearly all investigations using ^{13}C natural abundance have been conducted with maize (e.g. Ludwig *et al.*, 2003). Focusing solely on one of the various C_4 plants cultivated in temperate climates may yield biased information on C dynamics in soils. Nevertheless, a limited number of investigations with other C_4 crops are available.

In general, mechanistic C sequestration studies can be divided into two groups: (1) studies that correlate total SOM accumulation with ecosystem driving variables (e.g. soil texture, soil structure, annual rainfall, temperature), and (2) studies that compare organic matter fractions among treatments. Fractionation of organic matter implies the separation of the total organic matter into different parts that are thought to be functionally homogeneous with respect to physicochemical properties and turnover rate. The separation can be carried out by physical or chemical means: e.g. sieving, flotation, dispersion (Six *et al.*, 2002a). In the global change context, soil C sequestration through aggregation is an important aspect of better soil management. The SOC in microaggregates is believed to be protected from degradation and hence contribute to C sequestration (Bajracharya *et al.* 1998). Better understanding of the effects of soil management on the distribution and forms of SOC in different soil aggregate size fractions could contribute to an improved understanding and modelling of C dynamics and sequestration.

This study was conducted with the aim of quantifying the amount and distribution of SOC under different land use systems in Carlow, Ireland. The

specific objectives were to: (i) quantify the amount of C₄- and C₃- derived C under a 1-year-old *Miscanthus* plantation previously under grassland, (ii) to compare SOC stocks between two *Miscanthus* systems (1- and 14-years-old plantations), a grassland and an arable land and to (iii) quantify the relative distribution of SOC in different aggregate size fractions.

3.3 Materials and methods

3.3.1 Site description and soil parameters

The site (co-ordinates 52°51'N, 6°54'W) was located at the Teagasc, Oak Park Research Centre in Carlow, Ireland. Prior to 1974, the area had been temperate mixed forest; in 1974 the plot was converted to arable land, cultivating a rotation of winter and spring barley. In 1990 part of the plot was converted to grassland and in 1994 part of the remaining arable land was planted with *Miscanthus* which was harvested each year. In addition, in 2007 a further part of the grassland was planted with *Miscanthus*. To summarize, at the time of the soil sampling in 2008, there were four fields: an area of arable land planted to barley, a 14-years-old *Miscanthus* plantation (*Miscanthus*-14), a grassland and a 1-year-old *Miscanthus* plantation (*Miscanthus*-1).

The soil at the site is described as loamy sand; the mean annual precipitation and annual temperature at the Oak Park Research Centre for the period 1982-2002 were 830 mm and 9.3 °C (climate records supplied by Met Éireann, Irish Meteorological Service), respectively.

3.3.2 Soil sampling and fractionation procedure

At the experimental site, we established three sampling plots (15x30 m) in the *Miscanthus*-1 plantation, grassland, *Miscanthus*-14 and at the arable site.

In 2009, four cores (Ø 5.6 cm) per plot were collected at the *Miscanthus*-1 and grassland sites and divided into 0-15 and 15-30 cm soil layers. All soil samples were combined by depth increment, air-dried and fractionated by size. Briefly, all samples were sieved to pass through an 8 mm sieve by gently breaking apart the soil. Two sieves (250 and 53 µm mesh size) were used to separate macroaggregates (>250 µm; M), microaggregates (53–250 µm; m) and the silt & clay fraction (<53 µm; SC). A subsample was submerged for 5 min in room-temperature-deionized water, on top of a 250 µm sieve. Aggregate separation was achieved by manually moving the sieve up and down 3 cm with 50 repetitions during a period of 2 min. After the 2 min cycle, the M fraction was gently backwashed off the sieve into an aluminum pan. Water plus soil that went through the sieve was poured onto a 53 µm sieve and the sieving procedure was repeated. Material <53 µm was left to settle for 24 hours in plastic bottles, the supernatant was poured off, and the SC fraction were washed into another aluminum pan. All the fractions were oven dried at 50 °C.

The M fraction was further separated into coarse particulate organic matter (>250 µm; CP), microaggregates (53–250 µm; mM) and silt and clay (<53 µm; SC_M) by using the methodology described in Six *et al.* (2000). Subsamples of fraction M were immersed in deionized water on top of a 250 µm mesh screen and gently shaken with 50 glass beads (Ø 5.4 mm) for 10 min. The coarse POM was retained on the 250 µm screen, while a continuous and steady water flow

ensured that the mM were flushed onto a 53 μm sieve. Once the M fraction was broken up entirely, the material on the 53 μm sieve was wet-sieved. The fraction retained on the 53 μm sieve (i.e. mM) and the fraction that passed through the 53 μm sieve (i.e. SC_M) were collected and dried at 50 $^{\circ}\text{C}$ (Fig. 1).

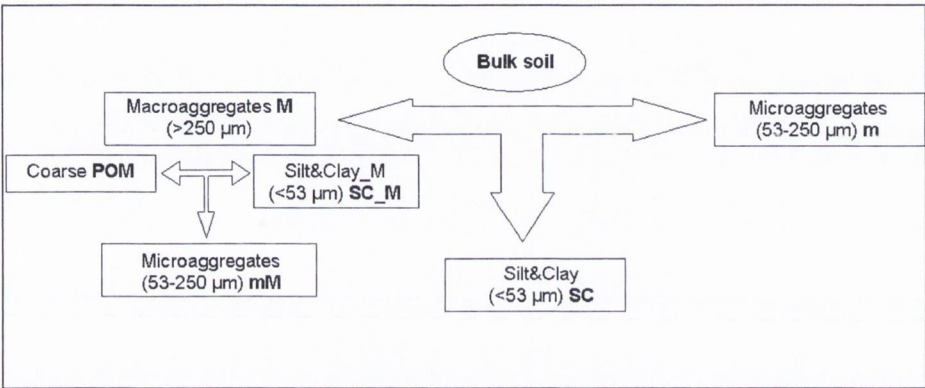


Figure 1: Fractionation scheme adopted in this study, modified after Six *et al.* (1998, 2002). M = macroaggregates (>250 μm size aggregates); m = microaggregates (53–250 μm size aggregates); SC = silt&clay 53 μm size class fraction; coarse POM = >250 μm size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates.

3.3.3 Total C and ^{13}C analysis

The C contents and $\delta^{13}\text{C}$ signature were measured for all soil samples. Three grams of each sample was ball milled and sub-samples of 30 mg were weighed into Ag capsules. Soil carbonates were removed using HCl fumigation as described by Harris *et al.* (2001). Total C and $\delta^{13}\text{C}$ of solid fraction were measured using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter.

3.3.4 Data analysis

By convention, the ^{13}C abundance in a sample is expressed in delta-units ($\delta^{13}\text{C}\%$) according to the following equation:

$$\delta^{13}\text{C}\% = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where R_{sample} is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the international Pee Dee formation belemnite carbonate standard (PDB).

The fraction of the soil C pool that is C_4 -derived (f_M) is calculated by using the isotope mass balance equation (Balesdent *et al.*, 1987):

$$f_M = (\delta_2 - \delta_0)/(\delta_1 - \delta_0)$$

where δ_2 is the $\delta^{13}\text{C}$ of the soil under *Miscanthus*-1, δ_0 is the $\delta^{13}\text{C}$ of the soil before introduction of *Miscanthus*-1 and δ_1 is the $\delta^{13}\text{C}$ *Miscanthus*-1 plant material entering the soil. As δ_0 was not known, the value from relevant depths of the reference field (grassland) was used instead. The δ_1 represents an average of $\delta^{13}\text{C}$ values determined for litter, rhizomes and roots from *Miscanthus*-1. C contents of SOM fractions were calculated on an area basis, correcting for soil depth and bulk density.

3.3.5 Statistical analysis

The results of the experiment were tested for normal distribution using the Shapiro-Wilk Test and the Sig. value was > 0.05 (i.e., the data have a normal distribution). The results of the experiment were analysed as a split-plot design, with land use as the main plot treatment. An ANOVA was conducted for each soil depth using the SPSS 16.0 software package, with blocks as random effects and fractions as a fixed effect. Differences between means were tested with the Tukey procedure for multiple comparison with a significance level of $P < 0.05$.

3.4 Results

3.4.1 C_3 - and C_4 - derived C in soil fractions under *Miscanthus*-1

The soil at the site has pH between 6.3 and 7, soil bulk density from 0.9 to 1.3 g cm⁻³, soil C:N ratio from 7.1 to 12.9 and microbial biomass C from 755 to 967 $\mu\text{g soil}^{-1}$ (Tab. 1).

In the 0-15 cm and 15-30 cm soil layers, little evidence of C_4 -derived C was found after one year of *Miscanthus* plantation. However, about 84.1 % and 90.1 % of all C_4 -derived C was located in the macroaggregates, at 0-15 and 15-30 cm respectively. In the M fraction, the average amount of C_3 -derived C under *Miscanthus*-1 was significantly lower than in the grassland soil. In the microaggregates on the other hand, the average amount of C_3 -derived C under *Miscanthus*-1 was higher than in the grassland soil, but this difference was not

statistically significant. In the SC fraction the average amount of C₃-derived C under *Miscanthus*-1 was higher than in the grassland soil (Fig. 2).

In the 0-15 cm soil layer, 59.5% of the derived C in the M fraction under *Miscanthus*-1 was found in the mM fraction. In the 15-30 cm soil layer, just 31.2% of all the C₄- derived C in the M fraction under *Miscanthus*-1 was found in the mM fraction, whereas 60.4% of all the C₄- derived C was found in the CP fraction (Fig. 3).

Table 1: soil characteristics, at two soil depths, for the *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems. MB-C = microbial biomass C.

depth	Land use	clay %	silt %	sand %	pH	bulk density (g cm ⁻³)	C/N	MB-C (µg g soil ⁻¹)
0-15 cm	<i>Miscanthus</i> -14	5	6	89	6.8	0.9	12.9	967
	Arable land	4	7	89	6.3	1.1	10.8	801
	<i>Miscanthus</i> -1	4	12	84	6.8	1.3	8.5	-
	Grassland	4	12	84	6.8	1.3	10.7	1080
15-30 cm	<i>Miscanthus</i> -14	6	6	88	6.9	1.0	13.4	755
	Arable land	4	8	88	6.4	1.2	10.9	768
	<i>Miscanthus</i> -1	4	12	84	6.9	1.3	7.1	-
	Grassland	4	12	84	7.0	1.3	10.0	808

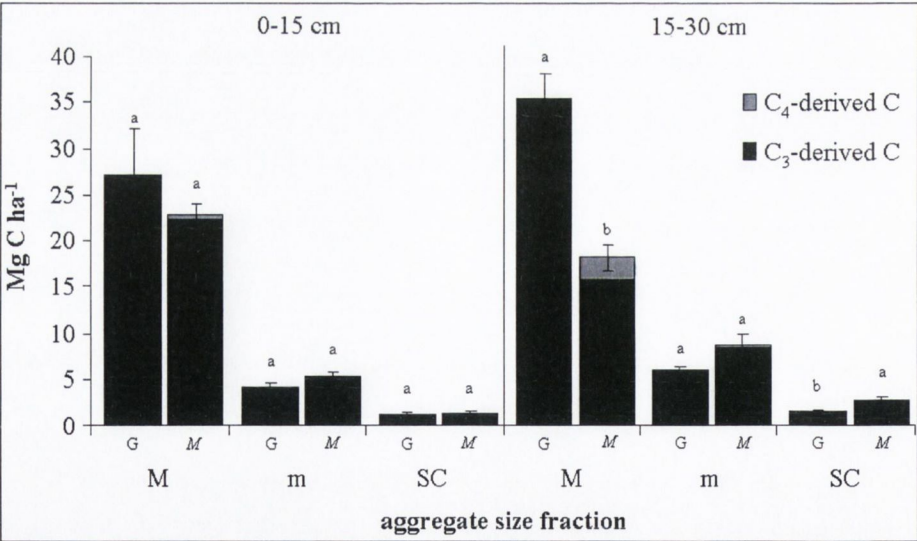


Figure 2: C content in the aggregates size classes, at two soil depths, for the grassland (G) and the *Miscanthus*-1 (M) systems. For *Miscanthus*-1 soils, the C₃- and C₄-derived C are indicated. Within each depth, values followed by a different lowercase letter are significantly different (P<0.05) within aggregate size and among land use systems. M = macroaggregates; m = microaggregates; SC = silt&clay.

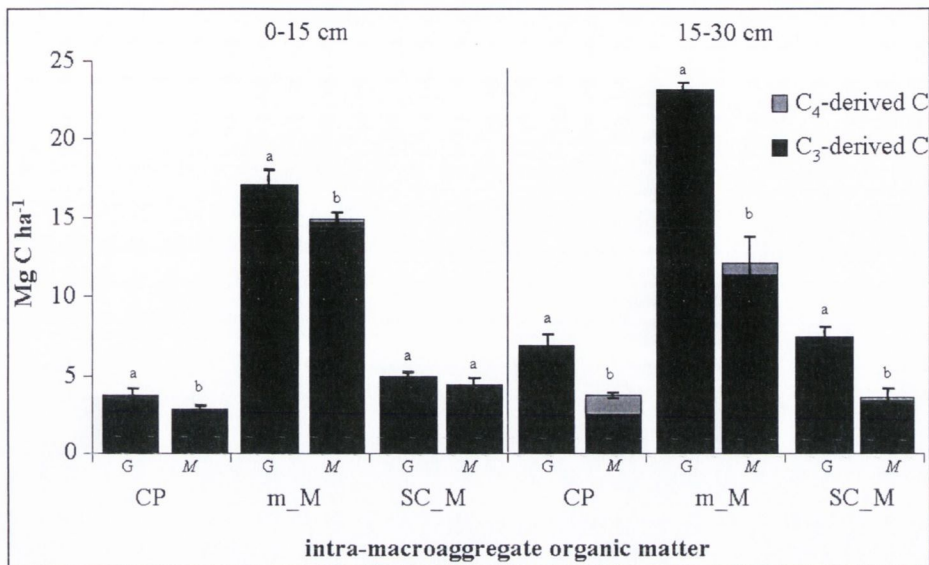


Figure 3: C content in the intra-microaggregate POM, at two soil depths, for the grassland (G) and the *Miscanthus*-1 (M) systems. For *Miscanthus*-1 soils, the C₃- and C₄-derived C are indicated. Coarse POM = >250 µm size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates.

3.4.2 SOC in bulk soil and aggregate fractions

In the 0-15 cm soil layer, the total organic C of the soil among land use systems decreased in the order *Miscanthus*-14 > arable > grassland > *Miscanthus*-1. Under *Miscanthus*-14, the total soil C content was significantly ($P<0.05$) higher than the soil C contents found under the other three land use systems (Fig. 4). In the 15-30 cm soil layer, the total soil C content decreased in the order *Miscanthus*-14 > grassland > arable > *Miscanthus*-1. At this same soil depth, the total C content under *Miscanthus*-1 was significantly ($P<0.05$) lower than the soil C contents found under the other three land use systems (Fig. 5).

At both soil depths, C content of the soil aggregates generally decreased in the following order: macroaggregates > microaggregates > silt & clay associated

C (Figs. 4 and 5). Among systems, differences generally decreased with depth. The largest differences in C among systems were found in the M fraction (Tab. 2).

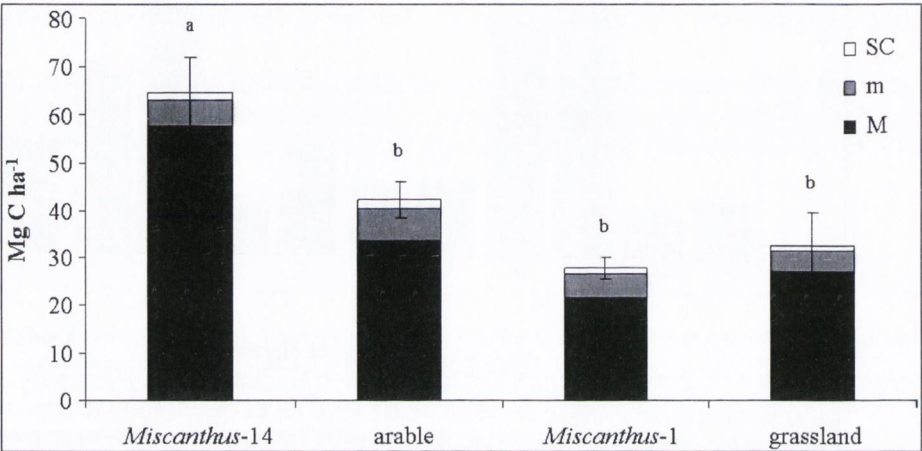


Figure 4: C content in bulk soil and aggregates size classes, at 0-15 cm soil depth, for the *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems. For C contents in bulk soil, values followed by a different lowercase letter are significantly different ($P < 0.05$) among land use systems. M = macroaggregates; m = microaggregates; SC = silt&clay.

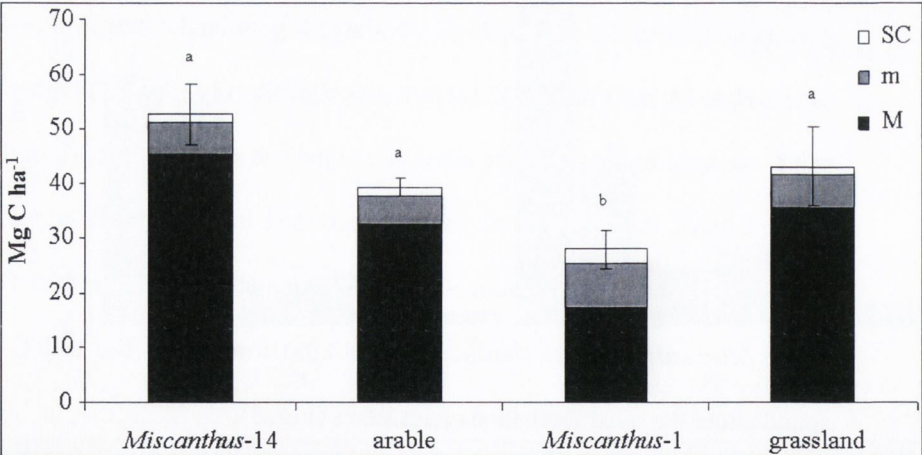


Figure 5: C content in bulk soil and aggregates size classes, at 15-30 cm soil depth, for the *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems. For C contents in bulk soil, values followed by a different lowercase letter are significantly different ($P < 0.05$) among land use systems. M = macroaggregates; m = microaggregates; SC = silt&clay.

3.4.3 C content of intra-macroaggregates organic matter

Under *Miscanthus*-14, the mM fraction contained 42.8 Mg C ha⁻¹ and 32.2 Mg C ha⁻¹ at 0-15 cm and 15-30 cm depths, respectively (Figs. 6 and 7). These values are significantly ($P < 0.05$) higher than C contents found for the same fraction at the arable, *Miscanthus*-1 and grassland sites (Tab. 3). As such, the difference in C content between *Miscanthus*-14 and the other three land use systems can be ascribed to soil C storage in the mM fraction.

At both depths, the C content of CP fraction was significantly higher under *Miscanthus*-14 than in the arable and *Miscanthus*-1 soils, whereas only at the 0-15 cm soil depth, the C content of SC_M was significantly higher under *Miscanthus*-14 than in the other three soils. At 15-30 cm soil depth, the C content of both CP and SC_M were significantly lower under *Miscanthus*-1 than in the grassland site. However, among land use systems, differences in C content of CP and SC_M had a relatively small effect on the total C budget.

Table 2: Multiple comparison of C contents in the aggregate size fractions between *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems, at two soil depths (0-15 and 15-30 cm).

depth (cm)	Land use	M				m				SC			
		<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland	<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland	<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland
0-15	<i>Miscanthus</i> -14	-	*	***	**	-	n.s.	n.s.	n.s.	-	n.s.	n.s.	n.s.
	arable		-	n.s.	n.s.		-	n.s.	**		-	n.s.	**
	<i>Miscanthus</i> -1			-	n.s.			-	n.s.			-	n.s.
	grassland				-				-				-
15-30	<i>Miscanthus</i> -14	-	n.s.	***	n.s.	-	n.s.	n.s.	n.s.	-	n.s.	n.s.	n.s.
	arable		-	n.s.	n.s.		-	n.s.	n.s.		-	**	n.s.
	<i>Miscanthus</i> -1			-	**			-	n.s.		**	-	**
	grassland				-				-				-

*, **, *** Significant at $p < 0.0001$, $p < 0.001$, $p < 0.05$ respectively.

Table 3: Multiple comparison of C contents in the intra-macroaggregate organic matter between *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems, at two soil depths (0-15 and 15-30 cm).

depth (cm)	Land use	CP				m_M				SC_M			
		<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland	<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland	<i>Miscanthus</i> -14	arable	<i>Miscanthus</i> -1	grassland
0-15	<i>Miscanthus</i> -14	-	**	**	n.s.	-	***	***	***	-	***	***	***
	arable		-	n.s.	n.s.		-	**	n.s.		-	n.s.	n.s.
	<i>Miscanthus</i> -1			-	n.s.			-	n.s.			-	n.s.
	grassland				-				-				-
15-30	<i>Miscanthus</i> -14	-	**	*	n.s.	-	**	***	**	-	n.s.	n.s.	n.s.
	arable		-	n.s.	n.s.		-	**	**		-	n.s.	**
	<i>Miscanthus</i> -1			-	**			-	**			-	**
	grassland				-				-				-

*, **, *** Significant at $p < 0.0001$, $p < 0.001$, $p < 0.05$ respectively.

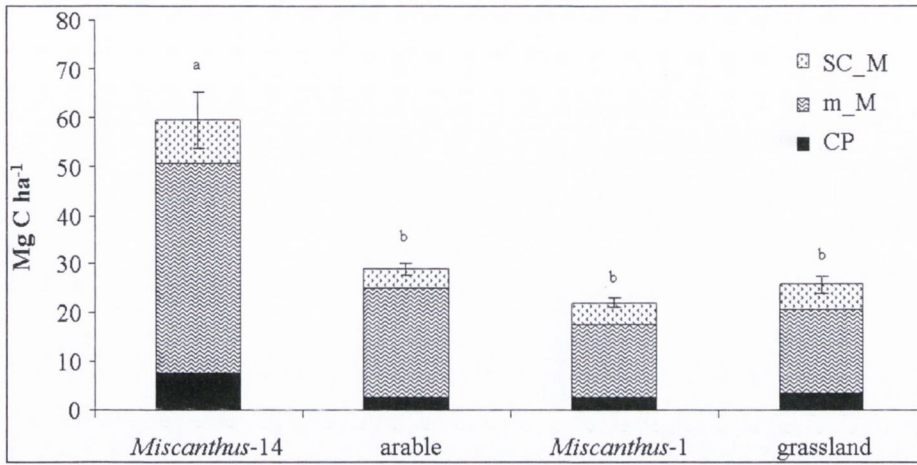


Figure 6: C content in the intra-macroaggregate organic matter at 0-15 cm soil depth, for the *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems. For C content in macroaggregates, values followed by a different lowercase letter are significantly different ($P < 0.05$) among land use systems. Coarse POM = $>250 \mu\text{m}$ size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates.

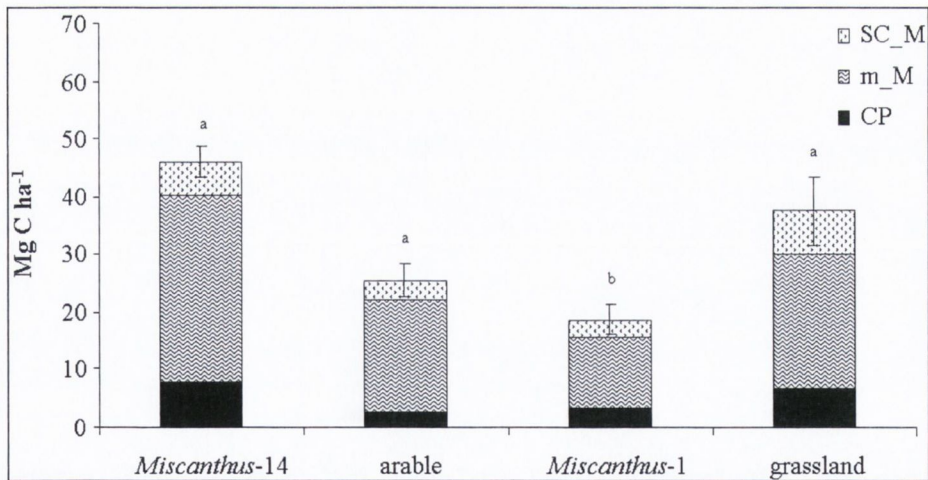


Figure 7: C content in the intra-macroaggregate organic matter at 15-30 cm soil depth, for the *Miscanthus*-14, arable, *Miscanthus*-1 and grassland systems. For C content in macroaggregates, values followed by a different lowercase letter are significantly different ($P < 0.05$) among land use systems. Coarse POM = $>250 \mu\text{m}$ size particulate organic matter; mM = microaggregates within macroaggregates; SC_M = silt&clay within macroaggregates.

3.5 Discussion

As expected, after one year of conversion from grassland to *Miscanthus* plantation, the soil C content under *Miscanthus*-1 decreased compared to the grassland soil. Interestingly, C content in the m and SC fractions under *Miscanthus*-1 was higher than in the grassland soil (Figs. 4 and 5). This difference in C content may be attributed to a transfer of C₃-derived C from M to these fractions and it represents a true stabilization process of old organic C (Six *et al.* 2002). Although the *Miscanthus* plantation was established just one year before the soil sampling, C₄-derived C was found in all the intra-macroaggregates fractions, particularly at 15-30 cm soil depth (Fig. 3). The increased C input in the *Miscanthus*-1 soil and possibly the shift in quality of the litter may have directly promoted C flows into mM and C stabilization in the macroaggregates fractions (Six *et al.* 2002). The presence of C₄-derived C in all the intra-macroaggregates fractions, and in particular in the mM fraction, supports the study by Dondini *et al.* (2009) on soil C sequestration under an established *Miscanthus* plantation. In fact, in this study, most of the C₄-derived C under *Miscanthus* was found in these same fractions, showing that the land use change from arable land to the energy crop *Miscanthus* rapidly increased C sequestration in stable and protected soil fractions.

At our experimental site in Carlow, Ireland, 117.2 Mg ha⁻¹ of C were found in the soil (0-30 cm soil depth) after fourteen years of *Miscanthus* plantation. For the same soil depth, the total C contents in the arable soil (81.3 Mg C ha⁻¹), grassland (75.6 Mg C ha⁻¹) and *Miscanthus*-1 (55.9 Mg C ha⁻¹) were lower than under *Miscanthus*-14. The potential for soil C sequestration under the

established *Miscanthus* field, planted on former arable land, has been reported by Dondini *et al.*, (2009). In our study we also found a higher total soil C content under *Miscanthus*-14 than in the grassland plots. In contrast to this, we found a decrease in total C content after one year of *Miscanthus* plantation on grassland soil. Possible reason could be the relatively low quantities of above- and below-ground biomass residues due to the early stage of the *Miscanthus*-1 plantation. A study by Hansen *et al.* (2004) on soil C sequestration under two *Miscanthus* plantations at Hornum, Denmark, reported a significant increase in SOC stocks under a 16 years old *Miscanthus* plantation compared to an arable reference site. However, in the same study, no differences were found in soil C contents between a 9 years old *Miscanthus* plantation and a five-year-old grassland reference site. A study by Schneckenberger & Kuzyakov (2007) compared the total C stocks of a loamy and a sandy soil with similar cultivation periods of *Miscanthus* in Germany. They found that in the upper 30 cm of the loamy soil, the SOC content under *Miscanthus* was lower than under a grassland reference site. However, at the upper 30 cm of the sandy soil, more SOC was found under *Miscanthus* than in the grassland soil. A study conducted by Kahle *et al.* (2001) reported on the influences of *Miscanthus* cropping on SOM at four sites in Germany. The C concentration in the *Miscanthus* soils were compared with associated grassland areas cropped with *Lolium* ssp. Results from this study showed that, for the sandy substrates, the *Miscanthus* plots were characterized by a significant increase in C concentration compared to the grassland sites. Together, these results suggest that the potential of soil C storage under *Miscanthus* largely depends on the land use system it is replacing as well as on the maturity of the *Miscanthus* plantation (e.g.

higher below- and above-ground biomass) and the soil mineralogy. At our site, total soil C stocks didn't significantly change after conversion from arable crop to grassland. However, at 15-30 cm soil layer, we found an increase in C content of the grassland compared to the arable soil. Post & Know (2000) indicated that there are many factors and processes that determine the direction of change in SOC when vegetation and soil management practices are changed. In particular, when soils are converted from crop to pasture, the processes that may be important for increasing SOC storage include: placing organic matter deeper in the soil either directly by increasing belowground inputs or indirectly by enhancing surface mixing by microorganisms and enhancing physical protection through either intra-aggregate or organomineral complexes.

At both soil depths, the microaggregates within macroaggregates - associated C, differed significantly between land use systems (Tab. 3). In particular, at 0-30 cm soil depth, microaggregates within macroaggregates - associated C, increased in the order *Miscanthus*-1 < arable < grassland < *Miscanthus*-14 among land use systems. Our results agree with studies by Six *et al.* (2000) and Pulleman *et al.* (2005), who used the same methods as in our study to isolate microaggregates out of stable macroaggregates instead of total soil samples. They showed that microaggregate formation and associated C stabilization increase with decreasing intensity of macroaggregate disturbance, related to either dry-wet cycles (Denef *et al.*, 2001) or tillage intensity (Six *et al.*, 2000). In the short-term, drying and wetting inhibits C-stabilization within macroaggregates due to an enhanced macroaggregate turnover which inhibits the formation of new microaggregates within macroaggregates (Denef *et al.*, 2001).

Greater macroaggregate stability, absence of tillage practices and reduced susceptibility to rapid wetting and drying reduce macroaggregate turnover in no-tillage systems and especially under permanent grassland when compared with conventional-arable land (Pulleman *et al.*, 2005).

In conclusion, our study indicates that, in the upper 30 cm soil depth, land use has a significant effect on the total SOC content, as well as on the C associated with the soil fractions. In particular, the energy crop *Miscanthus*-14 increased C sequestration in stable and protected soil fractions compared to the other land use systems. Furthermore, our research shows that conversion from grassland to *Miscanthus*-1 plantation leads to accumulate C₄-derived C in the intra-macroaggregate fractions, underlining the role of microaggregates in the SOC sequestration processes.

Chapter 4

The potential of *Miscanthus* to sequester carbon in soils: comparing field measurements in Carlow, Ireland to model predictions²

Based on: Dondini M, Astley Hastings A, Saiz G, Jones MB, Smith P (2009) The potential of *Miscanthus* to sequester carbon in soils: comparing field measurements in Carlow, Ireland to model predictions. *Global Change Biology-Bioenergy*, **1**, 413-425.

4.1 Abstract

Growing bioenergy crops such as *Miscanthus* has the potential to mitigate atmospheric CO₂ emissions by the replacement of fossil fuels and by storing C in the soil due to land use change. Here we compare direct measurements of SOC fractions made in Carlow (Ireland) to model predictions made by RothC and a cohort model. Our results show that when *Miscanthus* is grown on land previously under arable agriculture, the SOC will increase as *Miscanthus* organic material is shown to have a slow decomposition rate. In addition we demonstrate that for measured organic C, fractions of different lability are similar to the C pools used in RothC. Using the model predictions from RothC and *Miscanthus* yields from MISCANFOR we predict that in Ireland, changing the land use from arable to *Miscanthus* plantations has the potential to store between 2 to 3 Mg C ha⁻¹ y⁻¹ depending on the crop yield and the initial soil organic C level.

4.2 Introduction

The results of the previous Chapters underlined the ability of *Miscanthus* plantation to sequester C in stable and protected soil fractions, but to better understand the potential of this bioenergy crop to reduce CO₂ emission in the atmosphere we need to quantify its total C mitigation potential.

In recent years, *Miscanthus* (*Miscanthus x giganteus* Greef & Deuter ex Hodkinson & Renvoize) has received much attention as a potential bioenergy crop (Heaton *et al.*, 2004; Clifton-Brown *et al.*, 2007; Styles *et al.*, 2007). This perennial rhizomatous grass (PRG), which is native to East Asian tropical and subtropical regions but is also endemic at high latitudes up to 45° N, has a considerable biomass production potential even under temperate climatic conditions (Lewandowski *et al.*, 2000; Hastings *et al.*, 2009a and b). As well as producing combustible material that substitutes for fossil fuels, PRGs accumulate C in the soil. The increase in stored SOM results from the relatively large quantities of belowground biomass, as well as from the dead above- and belowground biomass that has been incorporated into the soil.

It is important to consider changes in C stored in SOM and standing vegetation when quantifying the impact of growing bioenergy crops on GHG emissions. Global biogenic GHG fluxes are large, and prior to human intervention the 120 Pg of C absorbed from the atmosphere each year by photosynthesis was roughly balanced by soil and plant respiration (IPCC, 2007). This balance has been disturbed by changing land use and increasing fossil fuel combustion. Most ecosystems achieve a stable SOC level in a few centuries with an estimated exponential change time constant of approximately 30-40 years

(Odell *et al.*, 1984). Due to this, each land use change will result in a net addition or depletion of SOC resulting in a net C flux (Smith, 2008). Uncertainty in flux estimation will result in a large error in the estimated net biogenic GHG accumulation in the atmosphere. This error can be reduced by detailed measurements of crop growth, harvested material and the partition of C between grain, shoot and root along with associated SOC changes. These data can be used in soil decomposition models to compare the flux and the end points of the simulation to quantify the error (Hastings *et al.*, 2009).

To accurately determine the potential for SOC sequestration following land use changes, we need to know the fate of new soil C input. Most organic matter enters the soil as readily recognizable plant litter and is mineralized within months (Christensen, 2001). A small portion, however, may be stabilized through interactions with mineral surfaces for periods up to thousands of years (Six *et al.*, 2004; Lehmann *et al.*, 2007).

A crucial factor for the decomposition of organic material is its accessibility to microbes and consequently, plant debris not physically protected is attacked first. Physical protection can be achieved through aggregation and adsorption of SOM on mineral surfaces, which strongly reduces its decomposability. Aggregates are vulnerable to changes in land use and management (Baldock & Skjemstad, 2000) as they can lose physical protection if the soil is disturbed mechanically by tillage.

Soil physical fractionation techniques, such as size and density separations, can be used to obtain a particulate organic matter (POM) fraction that is predominantly plant derived, has a turnover time of 10-50 years (Six *et al.*, 2000)

and is expected to be sensitive to land use and management. Furthermore, POM in silt and clay particles is more resistant to decomposition than that located in the stable aggregates (e.g. micro- and meso-aggregates). Eusterhues *et al.* (2003) found that SOC resistant to oxidation is made up of very refractory and slowly cycling C, which can be extracted as a non-oxidizable SOM fraction from the silt and clay fractions.

Isotopic labelling of C allows the tracing of newly sequestered C into SOM pools (Balesdent *et al.*, 1987). Using a simple isotopic dilution model, differences in ^{13}C -signature between C_3 and C_4 plants have been used to trace newly sequestered C (e.g. Jastrow & Miller, 1998; Collins *et al.*, 2000). This is particularly the case when C_4 plantations are grown on soils which have retained a predominantly C_3 signal, associated with a previous C_3 vegetation history. The combination of SOM fractionation techniques with ^{13}C natural abundance analyses offers an elegant approach to investigate small shifts on soil C stores that would be significant in the long term, but that might not be detected by conventional methodologies in the short term (Del Galdo *et al.*, 2003).

Where direct measurements are not available, biogenic GHG fluxes are often estimated using numerical soil / ecosystem models. There are many types of SOC decomposition models including: 1) single pool first order decomposition rate models, 2) food-web models using nitrogen (N) and C interchanges between soil organisms, 3) cohort models describing decomposition as a continuum and 4) process based multi compartment models such as RothC (Coleman & Jenkinson, 1999) and CENTURY (Parton *et al.*, 1987; Smith *et al.*, 2001). These models

have varying levels of complexity and their utility will depend on the data sets available for their parameterization.

Conceptually, SOM can be divided into a number of ‘pools’ according to their mineralization rate by soil organisms or lability. The RothC (Coleman & Jenkinson, 1999) model, for example, divides soil input organic material into decomposable (DPM) and resistant plant material (RPM), with the ratio depending on origin of the plant material. DPM and RPM decompose at different rates into microbial biomass (BIO) and humus (HUM) releasing CO₂. BIO and HUM then decompose at different rates producing more CO₂, BIO and HUM; the partitioning of the products of the decomposition depending on the soil clay content. In RothC the rates of decomposition are: DPM 0.1 years (y), RPM 3y, BIO 15y and HUM 50y and these are modified by temperature, soil moisture and cover vegetation. The numerical model RothC, based on this concept is a “process-based multi-compartment” model.

Bosatta & Ågren (1985, 1991) proposed a generic theory for the dynamics of C and N in the organic matter of the soil. This generic mathematical model considers the input of litter or organic material at various time intervals as separate cohorts of SOM with a quality distribution that degrades in a similar manner. The microbial community is assumed to be C limited and the quality of the SOM associated with C availability. A similar model can be applied to the N availability and this introduces another set of functions to limit SOM degradation. This mathematical theory, sometimes called a “continuum model” or a “cohort model” (Smith *et al.*, 1998; Smith *et al.*, 2001), is formalized in an analytical model referred to as Q-SOIL, used to model litter decomposition experiments

(Bosatta & Ågren, 1996; Ågren & Knecht, 2001). The “process based multi compartment” and single exponential models can be shown to be special cases of this generic model (Bosatta & Ågren 1991). The rate of decomposition and the partitioning of the SOM residues from each SOM pool at any time depends on the soil clay content, the moisture content, the pH, the climatic conditions and whether SOM level is stable or is changing due to the land use and / or vegetation change. These predictive variables are either implicitly or explicitly incorporated in all models.

Previous research on *Miscanthus* has primarily focused on management and economics in relation to establishment and productivity, harvest and storage, and combustion feasibility (Jørgensen & Schwarz, 2000; Dondini *et al.*, 2009) but *Miscanthus* crop experiments that also report SOC levels are limited (Dondini *et al.*, 2009). Shoji *et al.* (1990) reported on growth and chemical composition of *Miscanthus sinensis* in various locations in Japan. They estimated that the total amount of organic C, which would be dominated by cellulose and lignin, was 99 Mg C ha⁻¹ after 20 years of *Miscanthus* plantation. A study by Hansen *et al.* (2004) on C sequestration through a soil profile (0-100 cm) reported 92 Mg C ha⁻¹ after 9 years and 102 Mg C ha⁻¹ after 16 years of *Miscanthus* cropping in Hornum, Denmark. This study also compared the C stocks of *Miscanthus* plantation to a C₃ pasture, used as a control, which maintained 91-92 Mg C ha⁻¹ over the same period. Studies conducted on SOC changes on *Miscanthus* experimental plots in Germany have shown that the C₄-SOC incorporation observed after 10 years of land use of arable (maize) farming was smaller than that of *Miscanthus* plantation over the same period. The increment rate for SOC

under *Miscanthus* was similar to that observed in C₃ reference site established at the same time on long-term arable land (Kuzyakov *et al.*, 2006; Dorodnikov *et al.*, 2007). Another study on *Miscanthus* experimental plots in Germany reported that the SOC accumulation was higher on loamy than sandy soils for *Miscanthus*. This was explained by slower decomposition of plant residues in loamy soil due to less aeration, as well as by higher protection of SOC by clay particles (Schneckenberger & Kuzyakov, 2007). Kahle *et al.* (2001) published a time series of SOC changes over a 9 year period of *Miscanthus* cropping and reported a significant, year-on-year, increase in SOC from 60 to 80 Mg C ha⁻¹, measured to 1 m soil depth, when established on land that had been a C₃ grass pasture. The control plot, retained as a C₃ grass pasture, exhibited no significant SOC change at 61 ± 4 Mg C ha⁻¹ over the same period. Finally, a study by Clifton-Brown *et al.* (2007) reported that after 15 years of conversion from a C₃ pasture to a *Miscanthus* crop the SOC to a depth of 30 cm was 64.2 ± 7 Mg C ha⁻¹ whilst the unchanged control C₃ pasture had a SOC of 59.7 ± 7 Mg C ha⁻¹.

In this study, we have measured soil C sequestration beneath a 14 year old *Miscanthus* plantation established on former arable land in Ireland. Measurements of SOC fractions, the C location in the soil aggregate size fractions and the C₃ - C₄ origin of the SOC were compared to model predictions made by RothC and a cohort model to understand the decomposition rate of *Miscanthus*-derived organic matter. In addition, this decay rate is used to estimate the potential SOC accumulation of growing this bioenergy crop when it is planted on former arable land under current climatic conditions.

4.3 Materials & Methods

4.3.1 Site description and soil parameters

The site (co-ordinates 52°51'N, 6°54'W) was located at the Teagasc, Oak Park Research Centre in Carlow, Ireland. Prior to 1974, the area had been temperate mixed forest; in 1974 the plot was converted to arable land, cultivating a rotation of winter and spring barley. In 1994 part of the plot was planted with *Miscanthus* which was harvested each year.

The soil at the site is described as a sandy loam with 2-3% clay. The FAO soil data base show the whole site is a Luvisol with a soil water field capacity (FC) of 379 mm, a wilt point of 155 mm and a SOC of 90 Mg ha⁻¹ (Global Soil Task Group 2000). The mean annual precipitation and annual temperature at the Oak Park Research Centre for the period 1982-2002 are 830 mm and 9.3 °C, respectively.

4.3.2 Soil sampling and fractionation procedure

At the experimental site, three sample plots (15x30 m) were established in a *Miscanthus* plantation and three plots of the same size, were established on adjacent arable land. In June 2007 four cores (Ø 5.6 cm) per plot were collected at 0-15 and 15-30 cm soil depth. All soil samples were then combined per plot and depth increment. Soil samples were air-dried and soil bulk density was measured by conventional methodology (Blake & Hartge, 1986).

Soil samples were fractionated by means of physical and chemical procedures, as shown in Figure 1. Thirty grams of the soil (< 2 mm) were added to 150 ml water and dispersed using a calibrated ultrasonic probe-type (VC 750, Sonics & Materials Inc, Newtown, USA) with an output-energy of 22 J ml^{-1} . Application of more energy may disrupt coarse sand-sized SOM (Amelung & Zech, 1999). This dispersed suspension was then wet sieved over a $63 \mu\text{m}$ aperture sieve until the rinsing water was clear. The fraction $> 63 \mu\text{m}$, containing the sand fraction and stable aggregates (S + A) together with POM, was dried at 40°C and weighed. The suspension $< 63 \mu\text{m}$ was filtered through a $0.45 \mu\text{m}$ aperture nylon mesh and the material $> 0.45 \mu\text{m}$ was dried at 40°C and weighed. An aliquot of the filtrate was frozen to measure the amount of dissolved SOC (DOC). POM was separated by stirring the fraction $> 63 \mu\text{m}$ with sodium polytungstate (Sometu, Berlin, Germany) at a density of 1.8 g cm^{-3} . The solution was vortexed until well mixed to enable the organic material to float on the surface. The mixture was centrifuged for 15 min at 1800 rpm and left overnight for further settling. After the sample had separated in the dense medium, it was carefully placed in a freezer in an upright position. When frozen, the sample was removed from the freezer and the surface material (material whose density is $< 1.87 \text{ g cm}^{-3}$) was immediately recovered using deionized water. To remove SPT from the now organic rich sample, the floatant was placed on a filter and rinsed using deionized water. As fine filtration (e.g., 0.45mm) was desired, it helped to add the sample in a number of aliquots with 10mL of deionized water, and then let the deionized water filter through before adding more with assistance from a vacuum pump (Wurster *et al.*, 2010). Both fractions were dried at 40°C and

weighed. A chemically resistant C fraction (rSOC) was extracted from the fraction $< 63 \mu\text{m}$ (s + c) by NaOCl oxidation. Oxidation was done with 6% (60 g L^{-1}) NaOCl at room temperature after a modified method of Kaiser & Guggenberger (2003). One gram of s + c was oxidized for 18 hours at 25 °C with 50 ml of 6 % NaOCl adjusted to pH 8 with concentrated HCl. The oxidation residue was centrifuged at 1000 g for 15 minutes, decanted, washed with deionised water and centrifuged again. This oxidation step was repeated twice.

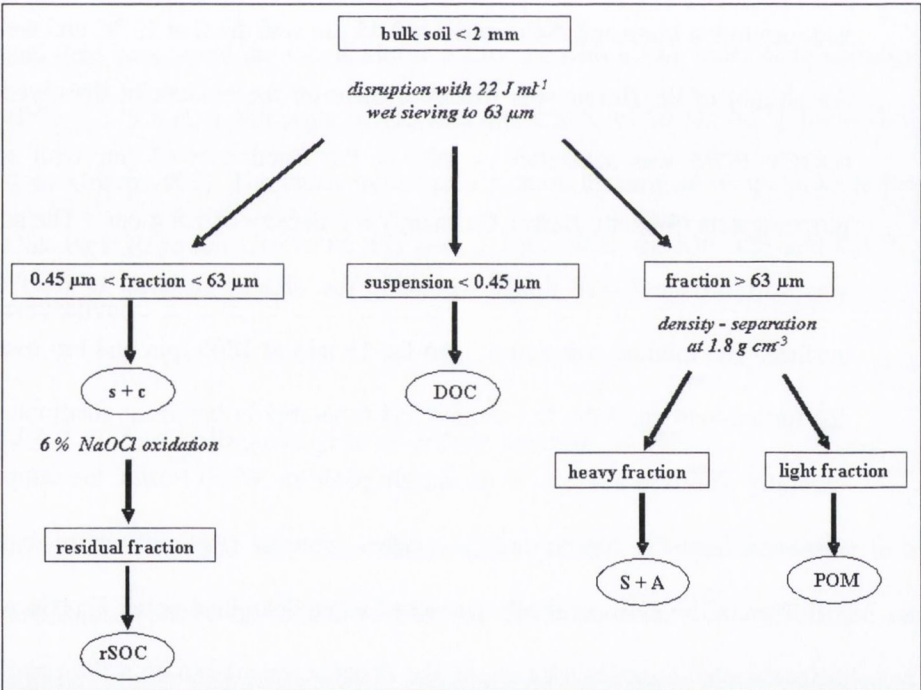


Figure 1: Diagram of the fractionation procedure; s + c = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, S + A = sand and stable aggregates, and POM = particulate organic matter (Zimmermann *et al.*, 2007).

4.3.3 Total C and ^{13}C analysis

The C contents and $\delta^{13}\text{C}$ signature were measured for all soil samples. Three grams of each sample were ball milled and sub-samples of 30 mg were weighed into Ag capsules. Soil carbonates were removed using HCl fumigation as described by Harris *et al.* (2001). Total C and $\delta^{13}\text{C}$ were determined for all samples at the UC Davis Stable Isotope Facility. Total C and $\delta^{13}\text{C}$ of solid fraction were measured using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach, UK) interfaced with a CN sample converter. The liquid samples were analyzed for DOC and ^{13}C using an O.I. Analytical Model 1010 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

4.3.4 Data analysis

By convention, the ^{13}C abundance in a sample is expressed in delta-units ($\delta^{13}\text{C}\text{‰}$) according to the following equation:

$$\delta^{13}\text{C}\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where R_{sample} is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the international Pee Dee formation belemnite carbonate standard (PDB).

The fraction of the soil C pool that is C₄-derived (f_M) is calculated by using the isotope mass balance equation (Balesdent *et al.*, 1987):

$$f_M = (\delta_2 - \delta_0) / (\delta_1 - \delta_0)$$

where δ_2 is the $\delta^{13}\text{C}$ of the soil under *Miscanthus*, δ_0 is the $\delta^{13}\text{C}$ of the soil before introduction of *Miscanthus* and δ_1 is the $\delta^{13}\text{C}$ *Miscanthus* plant material entering the soil. As δ_0 was not known, the value from relevant depths of the reference field was used instead. The δ_1 represents an average of $\delta^{13}\text{C}$ values determined for litter, rhizomes and roots from *Miscanthus*. C contents of SOM fractions were calculated on an area basis, correcting for soil depth and bulk density. The data was analysed to divide the SOC into pools that would be used in the models and to analyse the difference between the arable and *Miscanthus* sites. To quantify fractions, comparable with RothC pools, soil C values from the measured fractions were calculated for the whole soil as sum of the two layers. These C values were then conceptually summarized and split (Fig. 2) according to Zimmermann *et al.* (2007); in particular, we used a splitting ratio for DPM to RPM pools equal to 0.142 and for BIO to HUM pools equal to 0.026.

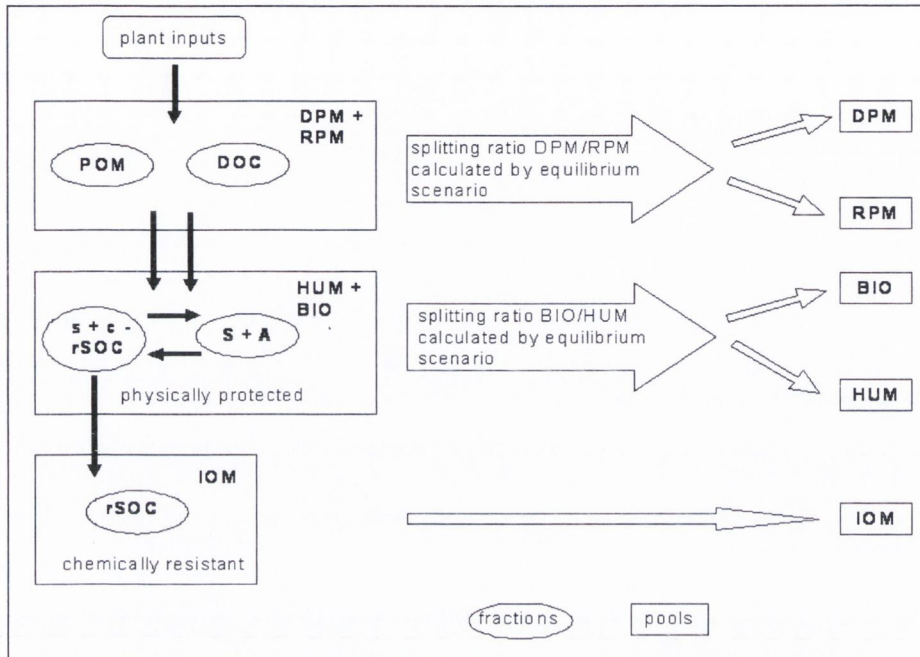


Figure 2: Concept of summarizing and splitting SOC fractions and pools (Zimmermann *et al.*, 2007). s + c = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, S + A = sand and stable aggregates, and POM = particulate organic matter; DPM = decomposable plant material; RPM = resistant plant material; BIO = biomass; HUM = humus; IOM = inert organic matter.

4.3.5 Statistical analysis

The results of the direct measurements were analysed as a split-plot design, with land use as the main plot treatment. For the bulk soil data an ANOVA was conducted using the SPSS 16.0 software package, with blocks as random effects and depth as fixed effect. An ANOVA was conducted for each soil depth with blocks as random effects and fractions as a fixed effect. The separation of means was tested using Tukey's honestly significant difference with a significance level of $P < 0.05$.

4.3.6. *Soil organic material input: measurement and model*

Plant, litter and root material were collected from the *Miscanthus* plots three times during the growing season of 2007 for isotopic analyses. All plant samples were combined per plot and were air-dried. The site measurements of the *Miscanthus* yields were made for two years. Aboveground biomass production in the *Miscanthus* plots was determined by harvesting 2x2 m quadrats in each plot. These were compared to predictions made with MISCANFOR model (Hastings *et al.*, 2009a) which was used with meteorological data from the Climate Research Unit of the University of East Anglia (CRU) (Mitchell *et al.*, 2004) and estimated soil properties from the sites with reference to the FAO-IGBP data for the soils of the area to predict the crop yield for the entire 14 years of *Miscanthus* cultivation. The organic C input to the soil was calculated from the peak yield using the relationship proposed by Clifton-Brown *et al.* (2007), which is: 33% of peak yield for surface input of stem and leaves and 10% for root turnover. The annual organic matter input for the arable was estimated from Coleman *et al.*, (1997) as the farm management was similar (1.3 Mg ha⁻¹) but forest phase plant input was adjusted so that the total modelled SOC matched the experimental value minus the inert SOC (rSOC); an input of 5.8 Mg ha⁻¹ achieved this match.

4.3.7 *Modelling soil organic carbon*

RothC was used to calculate the starting point for the SOC at the initialization of the *Miscanthus* crop experiment. To achieve this, RothC was run to equilibrium for a mixed forest with an annual C input of 5.8 Mg ha⁻¹ and a RPM/DPM ratio

of 0.25. This established the soil C equilibrium and partitioning at the start of the land use change to arable cultivation for 20 years, modelled with an annual C input of 1.3 Mg ha^{-1} and a RPM/DPM ratio of 1.44. The soil C content after 20 years of arable cultivation established the soil C status of the soil prior to *Miscanthus* plantation. RothC was then run from this point to calculate three scenarios: 1) the soil C status of the *Miscanthus* plantation after 14 years, with a RPM/DPM ratio of 0.52, 2) a continuation of the arable land use for 14 years and 3) the arable crop management of the site for 14 years with no input to mimic the C_3 soil C change under *Miscanthus*. The meteorological data used to drive the RothC model was the mean of the period 1900-2002. A single exponential model with a time constant of 30 years was used to confirm the change from the forest equilibrium degraded by arable use to the estimated *Miscanthus* equilibrium value of SOC from RothC.

A multi-pool multi-cohort model was developed based on Bosatta & Ågen (1985, 1991) and was used to match the decay of the plant input and the resulting C_4 -derived C accumulation and the decay of the initial C_3 in the soil at the start of *Miscanthus* cropping. The initial SOC was partitioned into three pools (0, 0.44 & 0.46) with decay rates of 0.1, 30 and 500 years respectively. Each annual input cohort of plant organic matter had three pools (0.48, 0.47 & 0.05) with decay rates of 0.1, 30 and 500 years respectively. This model was then used to predict the average soil C change for Ireland on a $5' \times 5'$ grid block in $\text{Mg C ha}^{-1}\text{y}^{-1}$ for the first 20 years of a *Miscanthus* plantation on available arable land. The initial soil C was obtained from the IGBP-FAO soil C map (Global Soil Data Task Group 2000) and the peak yield for *Miscanthus* was obtained from

MISCANFOR (Hastings *et al.*, 2009a and b) using the meteorological data for the period 1982-2002 from the CRU climate data (Mitchell *et al.*, 2004). The results were mapped using ArcGIS™ software.

4.4 Results

4.4.1 Measured soil fractions

In the top 30 cm of soil, the total soil organic C content under *Miscanthus* was significantly higher than under the arable crop (103.7 vs. 72.4 Mg C ha⁻¹; P-value < 0.01). However, in both soil types, the major part of C was found in the s + c and S + A soil fractions. Together, these two fractions contained 30.9 Mg C ha⁻¹ in the arable soil. The rSOC contained 14.3 Mg C ha⁻¹, POM 5 Mg C ha⁻¹ and DOC 2 Mg C ha⁻¹. Under *Miscanthus*, s + c and S + A soil fractions contained a total of 35.8 Mg C ha⁻¹ and rSOC contained 11.9 Mg C ha⁻¹, while POM and DOC contained just 7.9 Mg C ha⁻¹ and 3.7 Mg C ha⁻¹, respectively. The $\delta^{13}\text{C}$ values of all fractions were higher under *Miscanthus* than in the arable soil (Table 1). Under *Miscanthus*, about 55.7 % of all C₄-derived C was found in the s + A fraction. This value declined to 27.8 % in the s + c, to 7.9 % in the DOC and just 6.5 % and 2.1 % in the POM and the rSOC fraction, respectively (Fig. 3).

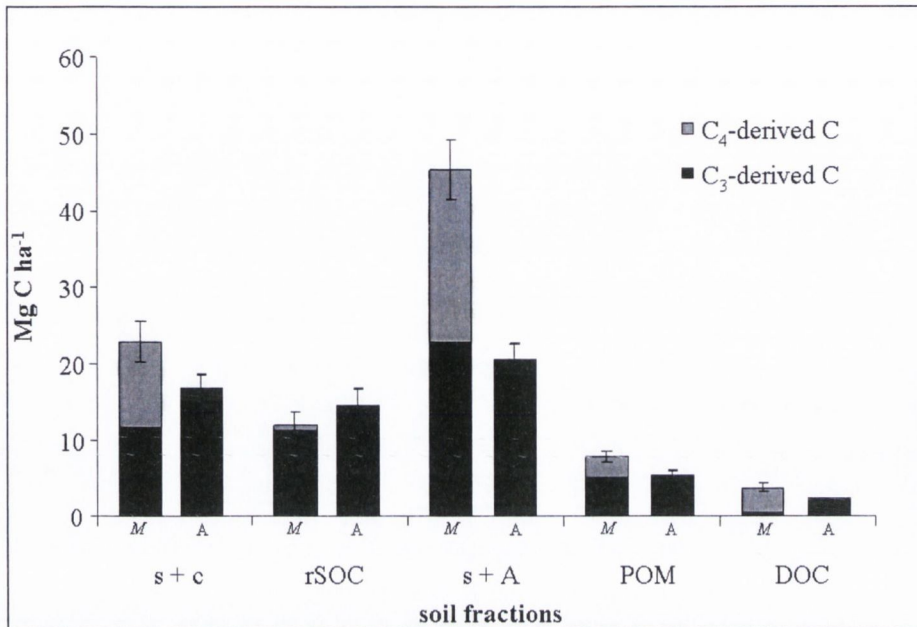


Figure 3: Partitioning of total soil C between different soil fractions under *Miscanthus* plantation (M) and arable crop (A). For *Miscanthus* soils, the C₃- and C₄-derived C are indicated. s + c = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, S + A = sand and stable aggregates, and POM = particulate organic matter.

Table 1: $\delta^{13}\text{C}$ values for soil fractions, at two soil depths, for the *Miscanthus* and the arable systems. Values are means (n = 3) with standard error in parentheses.

Depth (cm)	Land use	Measured soil fraction				
		s + A	s + c	rSOC	POM	DOC
0-15	<i>Miscanthus</i>	-20.48	-24.83	-25.66	-23.07	-25.34
		(0.8)	(0.8)	(0.6)	(0.1)	(0.1)
	Arable	-27.25	-27.32	-26.96	-26.67	-26.35
		(0.01)	(0.09)	(0.05)	(0.2)	(0.2)
15-30	<i>Miscanthus</i>	-19.58	-25.08	-26.00	-21.40	-25.86
		(0.6)	(0.7)	(0.2)	(0.4)	(0.06)
	Arable	-27.58	-27.56	-26.74	-27.33	-26.71
		(0.08)	(0.04)	(0.04)	(0.08)	(0.3)
Source of variation						
Land use		*	*	*	***	*
Depth		n.s.	n.s.	n.s.	n.s.	n.s.
Land use x Depth		n.s.	n.s.	n.s.	*	n.s.

Statistically significant differences are given after two-way ANOVA (n.s. = not significant; *P < 0.05; ***P < 0.001).

4.4.2 Model fit to measured soil fractions

RothC predictions of the initial SOC level of the site before conversion to *Miscanthus* was based upon the equilibrium prediction for a mixed forest using the mean meteorological conditions for the period 1900-2002 and then 34 years of arable wheat-barley intensive farming when the 'arable' SOC measurements were made. Total soil C contents changed in relation to the type of cultivation. Under forest cover the predicted soil C content was $120.5 \text{ Mg C ha}^{-1}$; after the conversion to arable land in 1974, this predicted value declined to $78.7 \text{ Mg C ha}^{-1}$ during a period of 20 years and to $71.3 \text{ Mg C ha}^{-1}$ after 34 years. However, the conversion of the 20 years-old barley plantation to *Miscanthus* predicted an increase of the soil C content up to $103.6 \text{ Mg C ha}^{-1}$ after 14 years of plantation, $58.5 \text{ Mg C ha}^{-1}$ of which are C_3 -derived C. It is interesting to note the good correlation between the total C content estimated by RothC and the directed measurements made in 2008 at the *Miscanthus* and arable sites (Fig. 4).

To make comparisons with modelled pools, SOC contents in the measured fractions were summarized and split as explained above. In the arable soil under equilibrium conditions, the largest part of the modelled SOC appeared in the HUM pools. The IOM pools contained about 20 % and RPM pools about 4.6 % of total SOC. The smallest pools were DPM and BIO, with about 0.6 % and 0.04 % of total SOC, respectively. Moreover, the measured pools were similar to the RothC predictions (Fig. 5). In fact, 69 % of total SOC was found in the measured HUM pools, followed by the 8.4 % in the RPM pools.

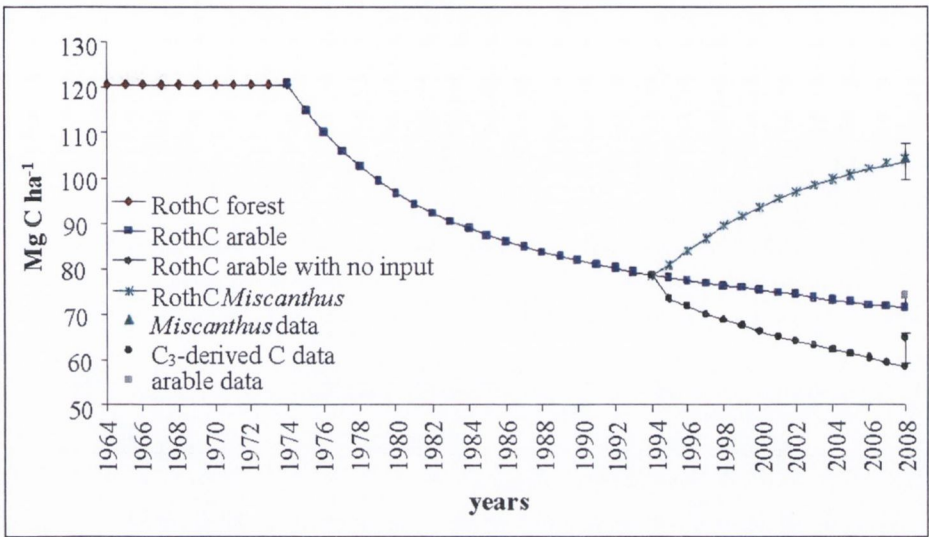


Figure 4: Soil C content of different plantations during a period time of 44 years at Carlow, Ireland.

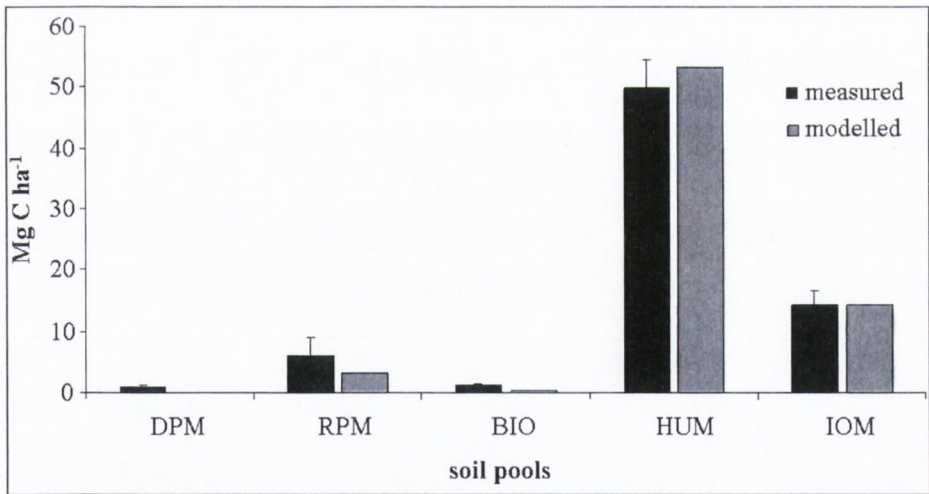


Figure 5: Partitioning of total C of the arable soil into measured fractions and RothC modelled pools. DPM = decomposable plant material; RPM = resistant plant material; BIO = biomass; HUM = humus; IOM = inert organic matter.

The distribution of the C₃-derived C among modelled soil pools followed the same pattern as found for the arable soil. In particular, HUM pools contained 80 % and IOM pools contained about 19 % of total SOC. The smallest pools

were RPM, DPM and BIO. Here the best correlation between modelled and measured pools was found for the HUM pool. Measured RPM, DPM and BIO pools contain together 7 % of total SOC, whereas these same modelled pools contain together just 0.04 % of total SOC (Fig. 6).

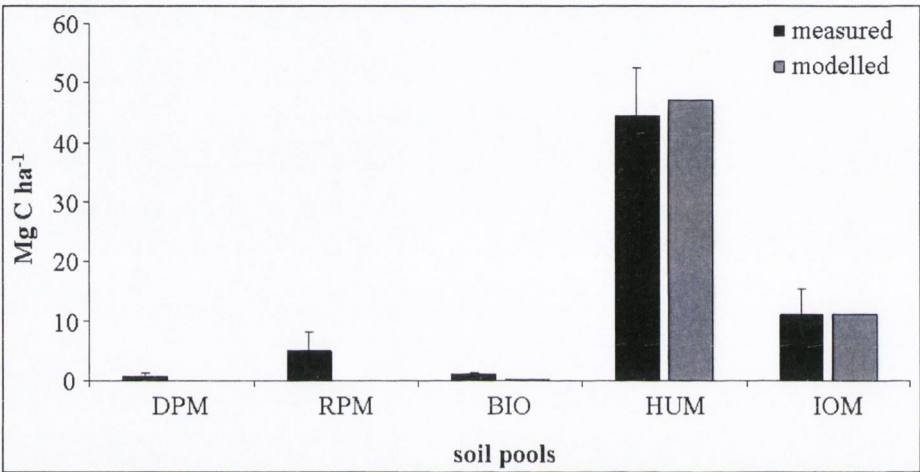


Figure 6: Partitioning of total C₃- C of the *Miscanthus* soil into measured fractions and RothC modelled pools. DPM = decomposable plant material; RPM = resistant plant material; BIO = biomass; HUM = humus; IOM = inert organic matter.

4.4.3 Predicting carbon mitigation in Ireland

The cohort model was run on the same data set using the annual MISCANFOR predictions of peak yield to calculate the plant organic matter input each year and it gave similar results to the RothC predictions for total SOC (Fig. 7).

The predictions of potential annual accumulation of soil C under *Miscanthus* plantations grown on arable land across Ireland for each 5' x 5' grid block, based upon the mean *Miscanthus* dry matter yield calculated by MISCANFOR and using the cohort model to calculate average SOC change per year over the 20 year expected life of the crop for each grid block (Fig. 8)

showed a mean accumulation of $3 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ for the geographical area of Ireland.

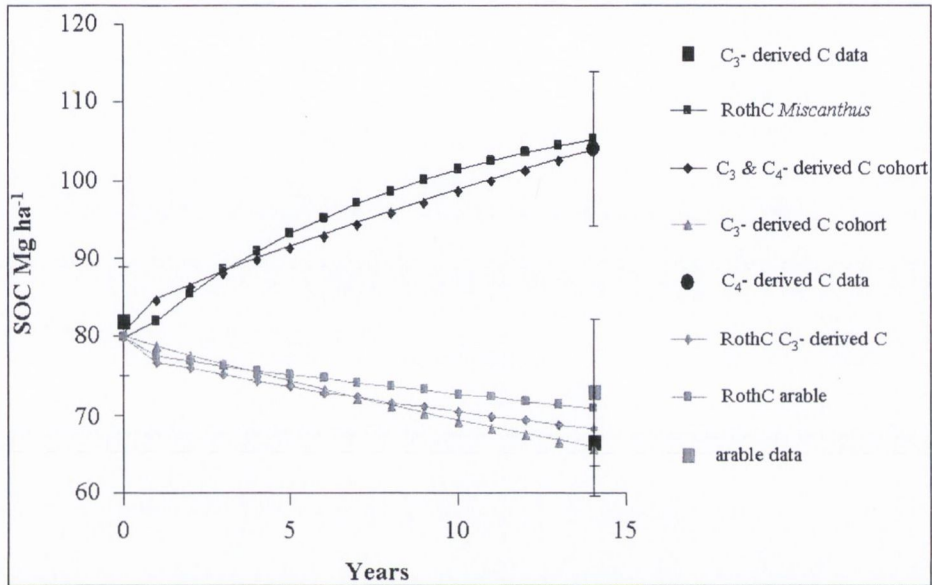


Figure 7: Comparison of soil C contents measured and modelled by two different C models RothC and cohort model over the 14 year site experiment.

4.5 Discussion

Process-based soil C models such as RothC are important tools for projecting likely changes in soil C over long time periods, and for studying historic and future changes in SOC under variable climatic and management conditions (Smith *et al.*, 2005). Typically, these models separate SOC into pools characterized by different turnover times. Zimmerman *et al.* (2007) demonstrated that it is possible to identify SOC fractions experimentally, and these are quantitatively related to SOC pools used in RothC. In our study we applied the same soil fractionation method used by Zimmermann *et al.* (2007) and we found that the RothC individual pools were comparable to the measured pools and

matched to within one SE (Figs. 5 and 6). In addition, in our study, the use of the ^{13}C natural abundances analysis allowed the partitioning of the SOC under *Miscanthus* between C_3 - and C_4 -derived C. As expected, we isolated an old and stable SOC fraction (rSOC), which was almost entirely formed by C derived from the previous vegetation (Fig. 3). Furthermore, we isolated two fractions from the soil samples, which consisted of either stable microaggregates or silt and clay particles (s + c and S + A). Under *Miscanthus*, the C content of these two fractions was about 50% C_4 -derived C and the C_3 -derived C was no different to the C content of the same fractions in the arable soil. Together these results suggest a slow decomposition rate in *Miscanthus* soil, promoting the accumulation of C_4 -derived C and preventing losses of C_3 -derived C.

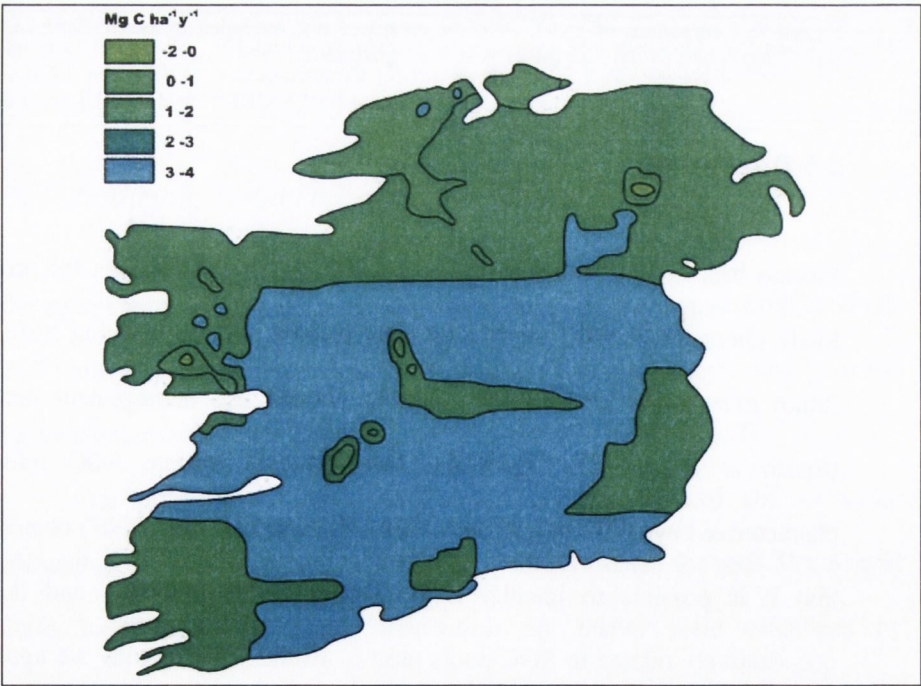


Figure 8: Carbon mitigated by an increase in soil organic carbon from the plant residue input from a *Miscanthus* bioenergy plantation. This value is calculated from input from *Miscanthus* plant debris calculated by the MISCANFOR model and its decomposition along with the initial soil carbon using the cohort model. The mitigation is expressed in $\text{Mg C ha}^{-1} \text{ y}^{-1}$.

Assuming that both fields started at the same SOC level, the top 30 cm of the *Miscanthus* system soil gained about 32 Mg C ha⁻¹ over the arable site, with a total C content of about 103 Mg C ha⁻¹. A study conducted in Ireland by Clifton-Brown *et al.* (2007) showed a higher SOC under *Miscanthus* after 16 years than in an adjacent pasture; however, this difference was not statistically significant. Kahle *et al.* (2001) reported that out of four *Miscanthus* plantations, only two showed a significant increase in soil C relative to grassland reference sites. However, studies conducted in Asia, where *Miscanthus* is a native species, reported higher soil C content than in Europe. In fact, a study by Chiang *et al.* (2004) on long established *Miscanthus* ecosystems in the Ta-Ta-Chia area of the Yu-Shan National Park in Taiwan reported a total SOC of 183-226 Mg C ha⁻¹ with Histic podsols being formed having a 30 cm O horizon. The protected area was established in 1949 and before that it was not known to be cultivated and was on the edge of a temperate deciduous C₃ forest, whose boundaries had migrated naturally with time. The SOC levels were assumed to be in equilibrium. They also reported a description of the complete soil profiles and a natural C isotope analysis of the SOC to a depth of 1 m. Levels of SOC are reported in Japanese Andisols where *Miscanthus* had been cultivated for over three centuries for fodder and thatching material, but here, the *Miscanthus* residue was burnt on a periodic basis to avoid colonization by other plants and the burnt material impacted the SOC (Golchin *et al.*, 1997). This Japanese study measured the SOC in the histic O layer and analyzed the humic material in the lower layers of the pedon. The histic layers between 11 and 21 cm have 15-21% of C and the lower levels between 1-2%. The total SOC levels are between 165 and 308 Mg ha⁻¹ for

these Andisols. Together, these results suggest that the potential for soil C storage under *Miscanthus* largely depends on the land use system it is replacing (Dondini *et al.*, 2009). Moreover, differences in soil mineralogy and climate contributed to differences in the soil C storage potential between different experimental sites (Kirschbaum, 1994; Silver *et al.*, 2000).

To match the C₄ accumulation from *Miscanthus* in the RothC model, we have had to decrease the DPM/RPM ratio to a value of 0.52, lower than that for grassland, to account for the recalcitrant nature of the plant organic matter input. This supports other observations reported for other experimental sites that *Miscanthus* accumulated SOM faster than C₃-plantations. In Japan a review of *Miscanthus* ecology and agronomy by Stewart *et al.* (2009) provided anecdotal evidence that the plant organic material from *Miscanthus* decays at a slow rate. They reported that the common practice in centuries old plantations, grown for forage and building material, was to frequently burn vegetation to stop a build up of POM and to reduce the spread of weeds and pioneer plants. Foereid *et al.* (2004) showed that the stability of *Miscanthus*-derived SOM was correlated with the time of *Miscanthus* cultivation. The *Miscanthus*-derived C in the 11-year old *Miscanthus* field had a mean residence time (MRT) not much longer than the fresh residues (1 year), while the MRT of the older field (18-year old) was longer (3.5 years). In contrast, a study by Wynn & Bird (2007) on the natural ¹³C-labelling of C₃ and C₄-SOC collected from across major environmental gradients in Australia, reported that C₄-SOM decomposes twice as fast than C₃ counterparts in mixed C₃-C₄ environments. Together these results suggest that the recalcitrant nature of the SOM under *Miscanthus* plantation is strictly related to the soil type,

the nature of the organic matter input, the plantation management and the climatic conditions at our experimental site.

In this study, the initial conditions for the site SOC were taken to be the values in the RothC simulation after an equilibrium SOC was achieved under mixed forest, assuming that the latter has been established for centuries. The actual history is not known, but the validity of the assumption is strengthened by the similarity between the measured partitioning of the SOC and the modelled predictions (Fig. 4). The partitioning of the SOC at this time was taken as the initial conditions for the RothC simulation of the *Miscanthus* site. C₃-SOC was modelled as a continuation of the arable site with zero input and *Miscanthus* C₄-SOC was modelled using a zero SOC starting point and using the annual input calculated from MISCANFOR simulations for entire period calibrated by site measurements made in 2007/8. The C₃ and the C₄ simulations at each year were added to obtain the total SOC and the total SOC changes over the 14 years showed a good agreement with the experimental measurements (Fig. 7).

Here we estimate that the potential SOC equilibrium level under a long term *Miscanthus* plantation is around 160 Mg C ha⁻¹ and that if planted on 10 % of Irelands arable land (1,215,000 ha in 2005) the total SOC mitigation potential would be 364.5 Mg C y⁻¹, in addition to the 671 Mg C y⁻¹ mitigated by replacing coal by *Miscanthus* as a furnace fuel (Styles *et al.*, 2007).

In summary, our study shows that the land use change from arable land to the energy crop *Miscanthus* increased C sequestration in all soil fractions and, more generally, growing perennial grasses on C-depleted soil (e.g., agricultural land) provides an immediate SOC-sequestration benefit (Anderson-Teixeira *et*

al., 2009). Our results support the soil fractionation scheme presented by Zimmermann *et al.* (2007) in order to extract soil pools that match RothC theoretical pools. Our predictions on total C mitigation under *Miscanthus* suggest that energy crops have the potential to substantially reduce GHG emissions in Ireland (Styles *et al.*, 2007).

Chapter 5

Conclusions and perspectives

Conclusions and perspectives

In this study SOM pool composition, dynamics and responses to land use changes were assessed. The objectives of this dissertation were first to measure the total soil C sequestered under an established *Miscanthus* plantation and its distribution in different soil aggregates (Chapter 3). Second, the amount of C₄- and C₃-derived C under a 1-year-old *Miscanthus* plantation was quantified (Chapter 4). Third, SOC stocks in different land use systems (*Miscanthus* plantations, grassland and arable land) were investigated and the relative distribution of SOC in different aggregate size fractions was investigated (Chapter 4). Finally, in order to estimate the long-term potential of *Miscanthus* to sequester C in the soil, field measurements in Ireland were used to validate soil model predictions (Chapter 5).

In this Chapter, I summarize the general conclusions that arise from the studies previously described and I also review the advantages and disadvantages of the two soil fractionation methods applied in this research.

5.1 Soil carbon sequestration under *Miscanthus*

The results of this dissertation show that 14 years of *Miscanthus* plantation increased soil organic C by 25.0% through the whole soil profile, compared to a reference soil kept under agricultural management.. Although the *Miscanthus*-derived C was largely concentrated in the top 30 cm, the presence of *Miscanthus*-derived C was detected throughout the profile. Near the soil surface, soil C input is mostly determined by turnover of large roots, harvest losses and by

aboveground material, while at lower soil depths the C accumulation was most likely caused by fine root turnover and DOC leaching. Integrated through the whole soil profile, 45.1 Mg C ha⁻¹ under *Miscanthus* was C₄-derived, which is equivalent to an annual C₄-derived C incorporation rate of 3.2 Mg C ha⁻¹ yr⁻¹. Furthermore, the results reported in Chapter 4 show that the conversion from grassland to *Miscanthus* plantation leads to accumulate C₄-derived C. In fact, after one year of *Miscanthus* plantation, in the top 30 cm soil depth, 2.9 Mg C ha⁻¹ was C₄-derived, a value similar to the one measured for the 14 year old *Miscanthus* plantation (3.2 Mg C ha⁻¹ y⁻¹).

In Europe, previous researches on *Miscanthus* have focused on management and economics in relation to productivity and combustion characteristics. Even though soil C represents the largest C pool in most agro-ecosystems, research on SOC sequestration under *Miscanthus* plantation remains scarce. Analyzing the results from the researches on *Miscanthus* conducted in Europe (Table 1), except for few cases, the land use change to *Miscanthus* results in a gain of soil C compared with the reference sites. Moreover, the results reported in these same studies have shown the presence and the resulting accumulation of *Miscanthus*-derived C in the soil. Certainly, the magnitude of these processes differs between studies. At our site in Carlow Ireland, the high C sequestration may be explained by the relatively low soil C content at the time of planting, indicating that *Miscanthus* was planted on soil that was depleted in C; as such, the potential to harbour and protect new soil C input was relatively high at our site. In addition, the study on the SOC under the new established *Miscanthus* plantation supports and strengthens the findings on the annual incorporation of

C₄-derived C under the 14 years *Miscanthus* plantation at our site, demonstrating that in the *Miscanthus* soil the decomposition rate is slow, promoting the accumulation of C₄-derived C.

These results suggest that the potential of soil C storage under *Miscanthus* largely depends on the land use system it is replacing. Moreover, differences in aboveground biomass and age of *Miscanthus* plantations at the research sites might also have contributed to differences in soil C storage (Table 1).

5.2 Soil organic carbon stocks in soil aggregates under different land use systems

The results from Chapter 4 indicate that, in the upper 30 cm soil depth, land use has a significant effect on the total SOC content, as well as on the C associated with the soil fractions. In particular, the energy crop *Miscanthus*-14 increased C sequestration in stable and protected soil fractions compared to the other land use systems (e. g. grassland and arable land).

When considering the conversion from grassland to *Miscanthus* plantation, the effects of C stores and dynamics were much less clear, as expected since only one year had passed after the establishment of the new plantation. However, SOM pools of the *Miscanthus* soil in this system responded similarly, even if only as a trend, to the SOM dynamics of the system investigated in Chapter 3.

The results reported in this dissertation go beyond the pure analysis of the total C, looking also at the C dynamics in different soil aggregates. From this research results that soil aggregation was enhanced by the land use change to

Miscanthus and the stabilization of soil C under this bioenergy crop was induced by the promotion of macroaggregates (M) and their occluded microaggregates (mM). Our results agree with studies by Six et al. (2000) and Denef et al. (2001), who used the same device as in our study to isolate microaggregates out of stable macroaggregates instead of total soil samples. They showed that microaggregate formation and associated C stabilization increase with decreasing intensity of macroaggregate disturbance. Greater macroaggregate stability and the absence of tillage practices reduced macroaggregate turnover in no-tillage systems and especially under permanent grassland when compared with conventional-arable land.

Conversion of cultivated land to *Miscanthus* resulted in increased aggregation and a greater SOC stock. However, the magnitude of the C sinks, the rate of C sequestration, and the period of C sequestration differs substantially between the established and the new *Miscanthus* systems (*Miscanthus*-14 and *Miscanthus*-1). Nevertheless, the microaggregates occluded within the macroaggregates and their capacity to protect C in the longer term was crucial for the SOC sequestration in the *Miscanthus* systems.

Table 1: Harvestable biomass and C sequestration of *Miscanthus x giganteus* plantations in Europe

Site	Soil texture	<i>Miscanthus</i> age (year)	harvested biomass (t DM ha ⁻¹ y ⁻¹)	Reference site	Depth (cm)	SOC <i>Miscanthus</i> (Mg C ha ⁻¹)	SOC reference site (Mg C ha ⁻¹)	C gained under <i>Miscanthus</i> (Mg C ha ⁻¹)	C _d -derived C (Mg C ha ⁻¹)	C _d -derived C incorporation (Mg C ha ⁻¹ y ⁻¹)	Source
Germany											
52°37'14"N, 010°48'53"E	loamy	7	6	grassland	0-25	110	97	14	-	-	Kahle <i>et al.</i> , 2001
49°52'11"N, 009°54'31"E	silty loam	8	7	grassland	0-25	128	126	3	-	-	
49°52'11"N, 009°54'31"E	sandy loam	8	13	grassland	0-25	128	151	-23	-	-	
53°52'43"N, 012°37'04"E	silty loam	9	16	grassland	0-25	87	58	29	-	-	
Germany											
48°43'N,9°13'E	loamy	9	-	grassland	0-30	54	70	-16	8	0.9	Schneckenberger & Kuzyakov, 2007
					0-100	112	121	-9	11	1.2	
52°21'N, 13°19'E	sandy	4	-	grassland	0-30	46	40	6	5	1.3	
					0-100	70	64	6	6	1.5	
Denmark											
56.50°N, 09.26°E	loamy	9	8	grass	0-20	34	40	-6	4	0.5	Hansen <i>et al.</i> , 2004
					20-50	40	39	1	2	0.2	
					50-100	17	12	5	0.2	0.02	
					0-100	91	91	0	7	0.8	
56.50°N, 09.26°E	loamy	16	11	grain	0-20	42	44	-2	13	0.8	
					20-50	44	34	10	4	0.3	
					55-100	20	14	6	1	0.1	
					0-100	106	92	14	18	1.1	
Ireland											
52°39'N, 07°50'W	loam/sandy loam	15	9	pasture grass	0-10	30	28	2	7	0.5	Clifton-Brown <i>et al.</i> , 2007
					10-20	23	24	-1	1	0.1	
					20-30	11	7	4	0.4	0.03	
					0-30	64	60	5	9	0.6	
Ireland											
52°51'N, 6°54'W	sandy loam	14	16	barley	0-15	58	42	16	25	1.8	Dondini <i>et al.</i> , 2009
					15-30	50	35	15	18	1.3	
					30-45	15	18	-3	1	0.1	
					45-60	8	11	-3	1	0.1	
					0-60	131	106	25	45	3.2	

Unfortunately, even though *Miscanthus* plantations have been established all around Ireland, there has been no published research on *Miscanthus* in terms of C sequestration and dynamics in different soil aggregates in this country. Without further researches on these topics, the potential of bioenergy crops to sequester C in the soil in this region remains uncertain. Hence, in order to support and improve the information stated in this dissertation, there is significant scientific merit in establishing work that will specifically determine the soil C dynamics under *Miscanthus* in the Republic of Ireland.

5.3 Comparing field measurements to model predictions

Process-based soil C models such as RothC are important tools for projecting likely changes in soil C over long time periods, and for studying historic and future changes in SOC under variable climatic and management conditions.

The comparison of nine SOC models against long-term datasets described by Smith *et al.* (1997) showed that the models fell into two groups in terms of overall performance. The group containing SOMM, ITE and Verberne performed significantly poorer than the group containing RothC, CANDY, DNDC, CENTURY, DAISY and NCSOIL (see Smith *et al.*, 1997 for models detail). Some of this difference could be ascribed to the level of site-specific calibration used. The other main factor was that all of the models in the poorer performing group were less developed for land-use systems other than that for which they were developed in the first place. In terms of general applicability, RothC model performed well in terms of simulating soil C processes and it would appear to be one of the most suitable model to use as a basis to evaluate carbon sequestration by

short-rotation coppice systems (Smith *et al.*, 1997). An important aspect of this model is that RothC can operate in two modes; if the organic C inputs are known, it can be run in 'forward mode' to calculate how this input will decay in a particular soil under a particular climate, and how this influences SOC. However, generally, these organic C inputs (i.e. from litter decay, root death, root exudation, turnover of microbial biomass, etc.) are not accurately known, and the model can, therefore, be run in 'reverse' mode to calculate what these inputs must be in order to match an observed change in SOC levels. The advantage of RothC is the small number of parameters needed to initialize the model and it has been applied to sites with diverse agricultural management worldwide (Smith *et al.*, 1997).

Typically, soil C models separate SOC into pools characterized by different turnover times. A study conducted by Zimmermann *et al.* (2007) on soil samples from agricultural (arable, temperate grassland, or alpine pasture) sites demonstrated that it is possible to identify SOC fractions experimentally, and these are quantitatively related to SOC pools used in RothC.

In this study I applied the same soil fractionation method used by Zimmermann *et al.* (2007) for the *Misacanthus* and arable soils. I found that the RothC individual pools were comparable to the measured pool. In addition, the use of the ^{13}C natural abundances analysis allowed the partitioning of SOC under *Misacanthus* between C_3 - and C_4 -derived C. As expected, I isolated an old and stable SOC fraction (rSOC), which was almost entirely formed by C derived from the previous vegetation. Furthermore, I isolated two fractions from the soil samples, which consisted of either stable microaggregates or silt and clay particles (s + c and S + A). Partitioning the C contents of all fractions between C_3 - and C_4 -

derived C I could demonstrate that in the *Miscanthus* soil the decomposition rate is slow, promoting the accumulation of C₄-derived C and preventing losses of C₃-derived C.

In this study I estimate that the potential SOC equilibrium level under a long term *Miscanthus* plantation is around 160 Mg C ha⁻¹ and that if planted on 10% of Irelands arable land (1,215,000 ha in 2005) the total SOC mitigation potential would be 364.5 Mg C y⁻¹, in addition to the 671 Mg C y⁻¹ mitigated by replacing coal by *Miscanthus* as a furnace fuel. This prediction suggests that growing perennial grasses on C-depleted soil (e.g., agricultural land) provides an immediate SOC-sequestration benefit and that energy crops have the potential to mitigate C emissions (through fossil C not emitted and SOC sequestered).

Furthermore, the results of this study support previous researches on SOC sequestration; in fact, from the point of view of climate change, it is recognized that in order to achieve acceptable levels of GHGs there is the necessity to obtain a sharp decrease of their content in the next period of 20-30 years (IPCC, 2001). C sequestration is likely to be particularly effective in reducing atmospheric CO₂ levels in the first 20-30 years of its implementation and it should be included in any set of measures aimed to reduce atmospheric CO₂ concentration (Mondini & Sequi, 2008). During this critical period, C sequestration has been shown to be the most cost effective and feasible measure aimed at the reduction of emissions of GHGs (Marland et al., 2001).

5.4 Comparing soil fractionation techniques

Two different fractionation schemes were used to find fractions that are (1) C enriched, thus forming a pool where C is sequestered and (2) quantitatively related to SOC pools used in RothC. A complex and labor-intensive fractionation scheme (Fig. 1 in Chapter 3; from here on called “scheme A”) isolating small but potentially very sensitive C pools is compared with a much simpler fractionation scheme (Fig. 1 Chapter 5; from here on called “scheme B”). Both fractionation schemes distinguish between POM and mineral-associated organic matter.

Fractionation scheme A separates different soil aggregate size classes and the organic matter enclosed within macroaggregates. This fractionation further separates POM according to size: intra-POM (53-250 μm), mostly stabilized within microaggregates, vs. coarse-POM (250-2000 μm) occluded within macroaggregates. Furthermore, this fractionation technique allows distinguishing between the intra-POM occluded in the microaggregates (iPOM_m) and in the micro-within the macroaggregates (iPOM_{mM}). In general, fractionation scheme A results a useful tool to follow the distribution of the organic matter and the C associated with different soil aggregates.

Fractionation scheme B is much simpler and divides POM associated with the soil fraction $<63 \mu\text{m}$ in two fractions according on its density: the total light POM (POM) has a density $<1.85 \text{ g cm}^{-3}$ and the total heavy POM (s + A) has a density $>1.85 \text{ g cm}^{-3}$. Light POM is thought to be younger and more plant derived, while total heavy POM is older and more microbially derived (Zimmermann *et al.*, 2007). This method has some advantages over fractionation scheme A in that it enables more efficient separation and recovery of the sample while requiring

less SPT per sample. Freezing the SPT solution ensures that any material close to the dense media's specific gravity does not remix, which often occurs when using a filtration funnel or centrifuge tube upon minor agitation. It is relatively easy to remove all the surface material using deionized water from a wash bottle so that there is no loss of material; this may be critical when sample material is limited. It is easy to contaminate and difficult to capture all the floatant when pouring or aspirating material from a centrifuge tube (Wurster *et al.*, 2010).

Fractionation schemes A and B are complementary approaches. Fractionation scheme A focuses on mechanistic fractions, but tends to underestimate total POM, due to the many experimental steps. Fractionation scheme B increases the sensitivity of C measurements in comparison with a total C analysis and determines soil fraction quantitatively related to SOC pools used in RothC model.

5.5 Future perspectives

The following research perspectives arose from this thesis:

- The knowledge on the potential of *Miscanthus* to sequester C in the soil would be improved if more studies based on direct measurements were extended to the whole Europe.
- When possible, experiment investigating on soil C sequestration under *Miscanthus* should be carried out not just analyzing the total C content but also using soil fractionation technique combined with isotopic analysis. In fact, it has been well documented and tested that the combination of these

two techniques detects changes in SOC concentrations in the short time and with a good degree of accuracy.

- When investigating the effect of land use change in C sequestration, the belowground biomass under the different land use systems should be measured more intensively. Indeed, trends and differences in belowground biomass can often explain some trends and differences found in SOC concentration and sequestration.
- In this dissertation I demonstrate a good fitting of the SOC observed (measured) and modelled data. It will require further studies on established *Miscanthus* plantations in Ireland to validate the estimates reported in this dissertation on the total SOC mitigation potential of this bioenergy crop. Indeed, this research provides a very useful starting point for comparisons and further studies.

Table of Acronyms

M = macroaggregates.

m = microaggregates.

SC = silt & clay (53 μm size class fraction).

Coarse POM = coarse particulate organic matter.

mM = microaggregates within macroaggregates.

SC_M = silt & clay within macroaggregates.

iPOM_mM = intra-aggregate particulate organic matter within mM.

iPOM_m = intra-aggregate particulate organic matter within m.

s + c = silt and clay.

rSOC = resistant soil organic carbon.

DOC = dissolved organic carbon.

S + A = sand and stable aggregates.

POM = particulate organic matter.

DPM = decomposable plant material.

RPM = resistant plant material.

BIO = biomass.

HUM = humus.

IOM = inert organic matter.

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Dublin, March 2010

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