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SOIL ORGANIC CARBON DYNAMICS UNDER PERENNIAL ENERGY CROPS

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Sommario

Introduction

Global warming is a term used to describe the gradual temperature rise in the earth probably due to the increase of CO_2 concentration in the atmosphere (Metz *et al.*, 2007) as a consequence of increased use of fossil fuels (Fig. 1.1), land use changes, and soil erosion (Searchinger *et al.*, 2008; Smith, 2008). The main target of Kyoto protocol (1992) and subsequent Doha conference (2012) was to reach an agreement to reduce global greenhouse gas (GHG) emissions. In this context, the European renewable energy directive 2009/28/EC (E.C., 2009) provides a legislative framework for reducing GHG emissions by 20%, while achieving a 20% share of energy from renewable sources by 2020. However, the growing prices of energy and continuous increment in the of demand alternative energy sources to reduce GHG emission (Hillier *et al.*, 2009; Frische *et al.*, 2010) has caused the utilization of food crops such as corn, sugar cane, soybeans, canola and oil palm for bio-fuels production (Pimentel and Patzek, 2005; Fargione *et al.*, 2008) leading to food security issues (Krasuska *et al.*, 2010; Tilman *et al.*, 2011) and land competition problems (Searchinger *et al.*, 2008; Frische *et al.*, 2010). Land competition may cause direct and indirect land use change (LUC and i-LUC), i.e. conversion of undisturbed ecosystems as savannah, grassland, peatlands into agricultural land for food production and / or biofuels, with consequent carbon debts due to high CO_2 emissions from soil as a result of SOM mineralization (Fargione *et al.*, 2008). This mineralization may cause carbon losses up to 60% in temperate areas and 75% in tropical soils (Lal, 2004a). The global historic C loss from soil, because of LUC and soil degradation, was estimated to be circa 50 Pg (Smith, 2008). It should be noted, however, that soil can be transformed from carbon source to carbon sink using correct land management and agronomic practices (Lal and Kimble, 1997; Lal, 2009), as well as converting arable lands into perennial crop lands (Fargione *et al.*, 2008; Powlson *et al.*, 2011) or by restoring forest (Righelato and Spracklen, 2007). In fact, soil contains two times organic C more than the atmosphere and 2.3 times more than the biota (Lal and Kimble, 1997; Schlesinger, 1997), so it can represent a large container of organic carbon (Fig.1.2). It was estimated that by increasing 1 Mg C ha^{-1} in cropland soils, it could compensate between 5 and 15% of global emissions of fossil fuels (Lal, 2004a). The positive aspects of increasing soil organic carbon (SOC) are numerous, such as soil stabilization (Six *et al.*, 2000c; Conant *et al.*, 2004), soil aggregates formation (Bronick and Lal, 2005b; Jastrow *et al.*, 2007), increase of fertility (Lal, 2004a) and soil water capacity (Lal and Kimble, 1997). Ecosystems that can have high potential for carbon sequestration are croplands (Zan *et al.*, 2001; Powlson *et al.*, 2011), grazing / range land (Lal, 2004b) and degraded / desertified soils (Lemus and Lal, 2005; Hillier *et al.*, 2009). On the other hand the conversion of complex ecosystems such as forests, grassland, peatlands into arable lands can cause biodiversity losses (Frische *et al.*, 2010).

Soil C sequestration

The “Soil C sequestration” describe the increases of soil organic carbon (SOC) subtracting C from atmosphere through land management. SOC is the dynamic part of the carbon cycle that includes the carbon contained in the atmosphere, in soil, in water, and in large part of the biosphere, that is both above and belowground biomass (Lal, 2004a). SOC stabilization occurs via biochemical processes that can be both biotic and abiotic. These series of mechanisms are known as humification that transforms organic matter into more stable and resistant forms to decomposition processes (Jastrow *et al.*, 2007). The SOC residence time (τ) in the soil is determined by the ability of the soil to accumulate C (Luo *et al.*, 2003) and the parameters that are involved in C accumulation are water content (Young and Ritz, 2000), pH (Jastrow *et al.*, 2007), nutrient availability (Six *et al.*, 2002), clay content (Six *et al.*, 2000a; Bronick and Lal, 2005b), geological factors (Baldock and Skjemstad, 2000; Six *et al.*, 2000b) and vegetation type (Lal and Kimble, 1997; Anderson-Teixeira *et al.*, 2009). SOC sequestration favors soil stabilization true formation of soil aggregates (Six *et al.*, 2000a) that reduce soil leaching, increases water retention (Lal, 2008b) and fertility (Lal and Kimble, 1997; Lal, 2004a). There are several agronomic techniques to stabilize or increase SOM. For example, in soybean soil amendment application favors SOM accumulation and root proliferation (Hati *et al.*, 2006), while organic fertilization and manure application are the most common practices for maintaining high SOM level (Smith, 2004). Even techniques such as minimum-tillage or no-tillage limit soil disturbance and thus SOM mineralization, favoring crop residues humification (Six *et al.*, 2000a; Mrabet *et al.*, 2001) and soil biodiversity increment (Lupwayi *et al.*, 2001; Bronick and Lal, 2005a). Furthermore, in undisturbed cultivation systems, such as no-tillage practice, fungal hyphae growth is favored that leads to the formation of bridges between soils and litter (Beare *et al.*, 1992) and favor fungal and bacteria proliferation (Haynes and Beare, 1997). Fungi, thanks to their cell wall characteristics, which consist of melanin and chitin, are relatively resistant to degradation (Guggenberger *et al.*, 1999) and their residues contribute to SOM formation (West *et al.*, 1987). On the contrary, the contribution to SOM formation by bacterial components is limited because of more vulnerable membranes (West *et al.*, 1987; Suberkropp and Weyers, 1996). Biochemical and physicochemical process are involved in SOM decomposition, polymerization and turnover (Jastrow *et al.*, 2007). Decomposition involves mainly the cellulose and lignin components of the biomass. Cellulose is easily degradable by bacteria and fungi, while lignin is more complex and is degraded predominantely by fungi (Conesa *et al.*, 2002). Polymerization is generally spontaneous and takes place between soil particles and remaining molecules after degradation processes and is influenced by water content, pH and by catalysts, such as enzymes produced by fungi (Jastrow *et al.*, 2007). Moreover, soil is a complex system where the biological community interacts with soil physical and chemical properties and many times determines its characteristics (Jastrow et al., 2007). The potential for soil to sequester C is linked with regional climate, soil properties and land management (West and Six, 2007) and it is limited (Powlson *et al.*, 2011)

Effects of perennial crops on SOC

Perennial grasses used for energy end use are characterized by high yield potential, deep root systems, low fertilization input and conservative agricultural practices (Lewandowski *et al.*, 2003). SOC accumulation can be favored by the cultivation of perennial crops (Paustian *et al.*, 1997; Lemus and Lal, 2005; Anderson-Teixeira *et al.*, 2009; Powlson *et al.*, 2011), including dedicated energy crops such as switchgrass and Miscanthus (Lemus and Lal, 2005; Clifton-Brown *et al.*, 2007; Liebig *et al.*, 2008). The large belowground biomass production of perennial energy crops and the reduced agricultural practices needed to cultivate them result in low SOM mineralization (Bronick and Lal, 2005b; Lemus and Lal, 2005). Therefore perennial energy crops have potential to store C in the soil and reduce GHG emissions (Zan *et al.*, 2001; Clifton-Brown *et al.*, 2004; Lee *et al.*, 2007). For example, it was demonstrated that by converting cropland to perennial energy crops, the SOC stock can increases from 1 to 1.2 Mg ha^{-1} y^{-1} in switchgrass (Frank *et al.*, 2004; Monti *et al.*, 2012) and from 0.6 to 1.2 Mg ha^{-1} y^{-1} in Miscanthus (Kahle *et al.*, 2001; Clifton-Brown *et al.*, 2007). On the other hand, by converting a grassland to a perennial energy crop, the SOC tends to remain constant (Anderson-Teixeira *et al.*, 2009) because the initial SOC content of grassland is high (Conant *et al.*, 2001). Moreover, converting degraded agricultural soils into perennial energy croplands can result in better soil conditions and quality since the accumulated SOC improve the soil structure (Six *et al.*, 2000a), fertility (Lal, 2004a), reduce soil leaching (Lal and Kimble, 1997). In general, when converting a cropland into a perennial grass, the accumulation of SOC is faster in the first years of introduced new culture, then this process becomes slower over time up to zero change (West and Six, 2007), when a new equilibrium in the system is reached (West and Six, 2007; Smith *et al.*, 2008b). In temperate areas, for example it was indicated that the new equilibrium can be reached after 100 years of land use change (Paustian *et al.*, 1997; Smith *et al.*, 2008b), but in tropical soils equilibrium could be reached faster (Smith *et al.*, 2008a).

Cultivating perennial energy crops can favor SOC due to large and deep root system (Ma *et al.*, 2000a; Monti and Zatta, 2009) that is the main C vehicle into the soil (Kuzyakov, 2002; Nguyen, 2003). However, the large part of C returns to atmosphere through soil respiration, which is the second largest source of CO_2 emission of the eco-system (Raich and Schlesinger, 1992; Kuzyakov, 2006). These two factors, belowground biomass and soil respiration, are closely connected and their appropriate determination and quantification is important to understand the carbon cycle and the real potential of perennial energy crops to input large quantities of C into the soils.

Belowground biomass

The root system is the main water and nutrient absorption organ of a plant (Passioura, 2002). It has been studied mainly to understand complex mechanisms involved in its functioning (Passioura, 1996; King *et al.*, 2003; Dardanelli *et al.*, 2004), like plant adaptability to different environmental conditions (Jackson *et al.*, 1996) or water stress (Robertson and Fukai, 1994; Chaves *et al.*, 2002; Zegada-Lizarazu *et al.*, 2012). Belowground biomass is the main vehicle for storing organic carbon into the soils (Kuzyakov, 2002; Nguyen, 2003). Its development in depth affect the SOC distribution and accumulation within the soil profile (Frank *et al.*, 2004; Lemus and Lal, 2005; Monti and Zatta, 2009). In fact, the belowground biomass developed in deep layers is less subjected to mineralization (Lemus and Lal, 2005), therefore has a greater permanence in the subsoil (Ma *et al.*, 2000c, a).

Through the photosynthetic process atmospheric CO_2 is transformed into carbohydrates (Paul and Foyer, 2001) that are transferred to different plant tissues and used in several metabolic processes (Paul and Pellny, 2003). It was estimated that 50% of the photosynthetic C-derived was exported from shoots to belowground biomass (Vanderwerf *et al.*, 1987; Nguyen, 2003). In a review Nguyen (2003) reported that part of this C is lost by the roots (17%), part by the rhizosphere respiration (12%), and part is lost as soil residues (5%). Plant age plays a primary role in this complex mechanism, since older roots release less C than younger roots (Swinnen *et al.*, 1994). Besides that, a portion of the C fixed by photosynthesis (approximately 30%) can be allocated to mycorrhizae in symbiosis with plant roots (Nehls and Hampp, 2000).

More C is released into the soil through rhizodeposition, i.e. the process by which living roots release organic carbon into their surroundings. The release process occurs through different mechanisms such as i) *Sloughing-off of root border cells* produced during the growth of the root cap (Sievers and Hensel, 1991). In this process, the root apical meristems, that are arranged in layers, sloughs off cells in order to reduce soil resistance to root growth (Bengough and McKenzie, 1997), regulate rhizosphere microbial populations, promote gene expression in symbiotic microorganisms (Hawes *et al.*, 2000), and protect the meristemic apex against heavy metals (Morel *et al.*, 1986; Kozhevnikova *et al.*, 2007). ii) *production of root exudates* (Bretharte and Silk, 1994; Jones, 1999) that are constituted by starch, complex sugars, organic acids, alcohols, proteins, hormones and enzymes (Hodge *et al.*, 1996; Grayston, 2000). In general the microbial component of the soil (Nguyen and Henry, 2002) and root system (Vinolas *et al.*, 2001) assimilate these exudates in order to favor nutrient mobility and up take. Exudates serve also to balance the pH around root and to facilitate root exploration (Bretharte and Silk, 1994; Jones, 1999). Besides that, it is indicated that the production of exudates is enhanced under stress conditions (i.e., nutritional deficiency or toxicity and proliferation of pathogens (Nguyen, 2003). iii) *mucilage secretions* (Abeysekera and Mccully, 1993), that are produced within the Golgi apparatus and are mainly constituted of polymerized sugars (fucose, galactose, glucose, arabinose, etc.) and proteins (Bacic *et al.*, 1987).

Mucilages favour soil aggregates formation (Habib *et al.*, 1990; Morel *et al.*, 1991) and protection against heavy metals (Morel *et al.*, 1986). Its permanence into the soil is limited because of its low molecular weight (Kuzyakov, 2006), for example in corn, 45% of mucilage are degraded by microorganisms in about 2 weeks (Mary *et al.*, 1993; Nicolardot *et al.*, 2001). Moreover, a mucilage film around the roots play an important role in drought resistance (Watt *et al.*, 1993). So, each of these mechanisms play a fundamental role in the root exploration of the soil profile, root cap protection, nutrients availability (N, P, K), and reduced ion toxicity (Morel *et al.*, 1986). In addition, all of these mechanisms are sources of C, essential for the metabolic processes of soil microorganisms (Kuzyakov, 2006).

Soil respiration

Soil respiration is one of the largest flux in most ecosystems (Raich and Schlesinger, 1992) and can account for 60-90% of the total ecosystem respiration (Longdoz *et al.*, 2000). Soil CO_2 flux negatively influence SOC deposition (Paustian *et al.*, 2000) and small changes in soil CO_2 flux could have large impacts on global CO_2 emissions (Schlesinger and Andrews, 2000). Soil temperature is the main factor that influence soil CO_2 flux (Lou *et al.*, 2004; Lee *et al.*, 2007). Soil moisture is another factor that has a significant effect on soil respiration (Hollinger *et al.*, 2004) but independent from that of soil temperature (Frank *et al.*, 2002; Lee *et al.*, 2007).

Kuzyakov (2006) suggest five sources of soil CO_2 efflux (Fig. 1.3), i.e. i) root respiration, ii) rhizo-microbial respiration of rhizodeposits from living roots (root exudates, mucilages, etc.), iii) microbial decomposition of dead plant residues, iv) SOM microbial decomposition and v) priming effect, i.e. faster SOM decomposition and carbon turnover because of the accumulation of large amounts of fresh organic matter into the soil (Kuzyakov, 2002). These five categories can, however, be grouped into two main soil respiration (R_s) sources:

- Autotrophic respiration (R_a) is the soil CO_2 flux derived from the root system, i.e. derives from the photo-assimilates used in the synthesis of new plant tissues and maintenance of living tissues (Luyssaert *et al.*, 2007).

- Heterotrophic respiration (R_h) is the soil CO_2 flux derived from the microorganisms activity such as decomposition of rhizodeposits from living roots, plant residues and SOM.

The boundaries among these two CO_2 sources are, however, not clear. Indeed a clear separation between root respiration and rhizodeposits degradation (root exudates, mucilages, etc.) may not be possible, or even realistic because of impossibility to divide it with conventional techniques described in the paragraph below (Bond-Lamberty *et al.*, 2004). Also it is difficult to distinguish between CO_2 flux from degradation of fresh plant residues and degradation of SOM (Kuzyakov, 2006). Adding to that, CO_2 flux from roots can derive from the symbiosis between roots and ecto and endomycorrhiza fungi, among others (Jones *et al.*, 2004).

In order to have correct net ecosystem exchange values accurate measurements of R_s and R_a are needed (Bond-Lamberty *et al.*, 2004; Smith *et al.*, 2010). Among the different methods developed for determining R_s partitioning, the "*root exclusion technique*" consists on soil CO_2 flux measurements in unplanted (R_h) and planted soils (R_s). This technique can give crude estimations of R_s since soil conditions (i.e. soil water regime and temperature) with and without plants differ considerably (Ross *et al.*, 2001). "*Shading or clipping*" of aboveground biomass is considered a less invasive method. The method is based on inhibiting photosynthesis and thus no new photo-assimilates are made available to the roots system. The advantage of this method is that in the short term water content and nutrient turnover are the same in treated and untreated plots. The disadvantages of this method are that residues of organic compounds previously fixed can be used by the root system (Kuzyakov, 2006), and that root exudation is increased after defoliation/shading (Fu and Cheng, 2004). The "*regression technique"* (Kucera and Kirkham, 1971) is another method to estimate R_s. This method assumes a linear relationship between root biomass and the amount of CO_2 respired by the roots and the rhizosphere microorganisms; the amount of CO_2 derived from SOM decomposition corresponds to the intercept of the regression line between root biomass (independent variable) and total CO_2 emitted from the soil (dependent variable). This technique is simple and has been used by several authors (Brook *et al.*, 1983; Hanson *et al.*, 2000) with similar results, i.e. R_h varies between 40 to 60% of the total S_r. *"Continuous and pulse labeling of plant in* $^{14}CO_2$ *or* $^{13}CO_2$ *atmosphere".* The isotopic technique consists of subjecting the plants for a prolonged period of time to an enriched atmosphere with heavy C isotopes (^{13}C and ^{14}C). The enrichment period usually lasts form early stages of development (first leaves) until the end of the experiment. This technique allows the identification of the proportion of CO_2 derived from plant roots, as indicated by the abundance of ^{13}C or ^{14}C atoms, and the proportion of CO_2 derived from SOM respiration, which is indicated by the abundance of light C atoms (Whipps, 1987). The *"^{13}C natural abundance"* technique is based on the natural abundance of heavy C atoms (^{13}C) and its discrimination during CO_2 assimilation by the photosynthetic apparatus of C_3 plants compared to C_4 plants. Briefly, Rubisco Enzyme (C_3 plants) leads to ^{13}C depletion compared with air CO_2 (around -19 ‰), as a consequence $\delta^{13}C$ concentration in C_3 plant tissues is about -27 ‰. While, phosophoenol pyruvate carboxylase (PEP) (C_4 plants) discriminates less ^{13}C therefore the typical $\delta^{13}C$ value in C_4 plant tissues is around -13 ‰. So in the case of fields are cultivated with C_3 or C_4 plants only, the $\delta^{13}C$ values of the SOM will be close to those of C_3 or C_4 plants (-27 and -13‰, respectively; (Cheng, 1996). This methodology can be easily used in the field because atmospheric isolation is not necessary and new technologies such as NIRS (Horner *et al.*, 2004) or FT-IR spectroscopy (Griffis *et al.*, 2005) can be used, and they permit a continuous and long term measurements of isotopic flux in ecosystem.

Carbon budget – Net Ecosystem Exchange (NEE)

Biomass crops play a fundamental role in reducing CO_2 emissions (Lal and Kimble, 1997; Fargione *et al.*, 2008; Anderson-Teixeira *et al.*, 2009). Moreover, soils, where plant biomass finds its foundation, are the second largest sink of organic carbon (Fig. 1.2) therefore the magnitude of their contribution to reduce GHG emission is of fundamental importance. As shown in Figure 1.4, through the photosynthetic processes atmospheric C is transformed into carbohydrates and amino acids for tissue formation and the maintenance of biochemical processes (Amthor, 2000; Paul and Foyer, 2001). The fixed C is again released into the atmosphere through plant and soil respiration (Raich and Schlesinger, 1992; King *et al.*, 2006). Comparing different biomes (from desert to tropical forest) the global balance between net primary productivity (50-60 Pg C/yr), i.e. the sum of above- belowground and autotrophic respiration, and ecosystem respiration (50-75 Pg C/yr) was considered virtually nil (Houghton and Woodwell, 1989). On the contrary, the close relationship between these two components (Raich and Schlesinger, 1992) showed that an increase of organic matter in the soil corresponds to an increase in soil respiration (Hogberg and Ekblad, 1996).

In order to understand if soil can be considered a C container greater understanding of the C cycle has become important for mitigation GHG emissions, food and energy security and biodiversity (Smith *et al.*, 2012). There are a number of papers in the last decades that give carbon balance budgets at local to continental levels. Monitoring C cycle is important for net ecosystem exchange (NEE), or net ecosystem production (NEP), computation, that is the difference between CO_2 entering an ecosystem and all the CO_2 leaving the ecosystem during growing period (Smith *et al.*, 2010). In order to calculate the carbon balance of an eco-system it is necessary to quantify the gross primary production (GPP) and heterotrophic respiration (R_h). GPP represents the gross uptake of CO_2 that is used in photosynthesis (Ciais *et al.*, 2010) and it is the sum of net primary production (NPP), i.e. sum of above and below ground biomass, and autotrophic respiration (R_a):

$\text{GPP} = \text{NPP} + R_a$

However part of the assimilated carbon was used by living roots to produce organic compounds like exudates, secretions, and sloughed-off root cells and part was used for the roots turnover, that are used by soil microorganisms activities which contributes to R_h. Thus in eco-systems net ecosystem Exchange (NEE) is given by:

$\text{NEE} = \text{GPP} - R_h$

Effect of Nitrogen fertilization on NEE

Crop response to fertilization depends on species, climatic conditions, rainfall, harvest time and soil mineralization (Vogel *et al.*, 2002). Nitrogen fertilization plays an important role in production cost, energy consumption, and environment management (McLaughlin and Kszos, 2005; Nelson *et al.*, 2006). In agriculture 50% of energy inputs derive from nitrogen fertilization (Barbanti *et al.*, 2006) so it's accurate management result in optimized production systems. On the other hand, excessive N fertilization create problems of leaching (Dinnes *et al.*, 2002), NH_3 (Bouwman *et al.*, 2002a), N_2O (Mosier *et al.*, 1996; Bouwman *et al.*, 2002b) and NO_x (Lee *et al.*, 1997; Olivier *et al.*, 1998) emissions.

Nitrogen fertilization stimulates root development (Lemus and Lal, 2005) which in turn results in SOC accumulation (Schuman *et al.*, 2002). For example such relationships were noted in semiarid soils (Rasmussen and Rohde, 1988), annual crops (Grant *et al.*, 2001) and rangeland (Schuman *et al.*, 2002). In any case, the positive environmental effects of increasing SOC through larger root systems is counterbalanced by CO_2 costs associated with the N fertilizer production (Gregorich *et al.*, 1996). The low agricultural inputs like N fertilization of perennial energy crops such as switchgrass and Miscanthus are considered a source of short-term CO_2 emissions mitigation

(Dohleman *et al.*, 2012). Indeed, it is reported that perennial energy crops do not respond significantly to elevated N fertilization levels at both above- and belowground biomass levels (Ma *et al.*, 2001; Clifton-Brown *et al.*, 2007; Lee *et al.*, 2007). The range of optimal N fertilization in these species varies from 70 to 100 kg ha^{-1} (McLaughlin and Walsh, 1998; Cadeaux and Ng, 2012), 2012) with acceptable production using 50 kg N ha^{-1} (McLaughlin and Kszos, 2005; Cadeaux and Ng, 2012). Another aspect to take in consideration in a well managed fertilization program is the harvest time. In general, harvests carried out at the end of the growing season or after first cold allow most of nutrient to move back to the rhizomes (Dohleman *et al.*, 2012). Such nutrient reserves allow the crops a rapid re-growth in the next spring (Vogel *et al.*, 2002; Heaton *et al.*, 2009). Therefore, the need of supplemental fertilization is reduced (Lewandowski *et al.*, 2003). In addition, the harvest frequency has a significant effect on nutrient reserves. For example a double harvest system in switchgrass (the first during full flowering and second one in autumn) remove twice N than a single cut system at the end of growing season (McLaughlin and Kszos, 2005). Besides that, optimizing N application allows at the same time good productivity and environmental and economic benefits (Lemus *et al.*, 2008), such as reduced of N_2O emission (up to 40%; Monti *et al.* (2012)).

Effect of ashes recycling on NEE

The resulting ashes from the combustion processes of biomass can be a source of plant nutrients such as Ca, K, and P (Weber *et al.*, 1985; Perucci *et al.*, 2006; Moilanen *et al.*, 2012). But at the same time the addition of ash to the soil can influence soil pH (Silfverberg and Huikari, 1989; Perucci *et al.*, 2008), microbial activity (Perkiomaki and Fritze, 2002), field capacity (Chang *et al.*, 1977), soil structure and soil salinity (Clapham and Zibilske, 1992). Studies concerning the influence of ash in the soil properties were mainly carried out in North Europe (Moilanen *et al.*, 2002; Saarsalmi *et al.*, 2012). For example ash addition in peatlands reduce soil acidity and increase soil CO_2 flux, probably because of enhanced soil microbiological activity (Weber *et al.*, 1985; Moilanen *et al.*, 2002). Some studies were also conducted in alkaline soils, which are typical of Mediterranean areas, and in contrast with the results obtained in acid soils, ash addition caused the reduction of microbial biomass and enzymatic activity (Perucci *et al.*, 2006; Perucci *et al.*, 2008).

Cultivating perennial energy crops on cropland and / or marginal lands can contribute to the reduction of GHG emission through C storage into the soil (Anderson Teixeira et al., 2009; Fargione et al., 2008; Powlson et al., 2011). In this thesis two cases of land use change were studied:

1) *from cropland to switchgrass in high productive area (IT)* where (i) evaluating evaluated the interactive effects of ash amendments and nitrogen fertilization on the above and belowground biomass production of switchgrass in Po valley (Italy), (ii) soil C content after land use change from cropland to switchgrass and (iii) estimation of Net Ecosystem Exchange (NEE).

2) *from marginal grassland to Miscanthus in Wales* evaluating the (i) soil organic carbon (SOC) stock variation by converting grasslands to different Miscanthus genotypes; ii) the relationship between carbon accumulation rate and root biomass; iii) estimation of the fate of soil carbon over the life cycle of a *Miscanthus* crop.

FIGURE INTRODUCTION

Fig. 1.1 Global GHG emissions (Boden *et al.*, 2012)

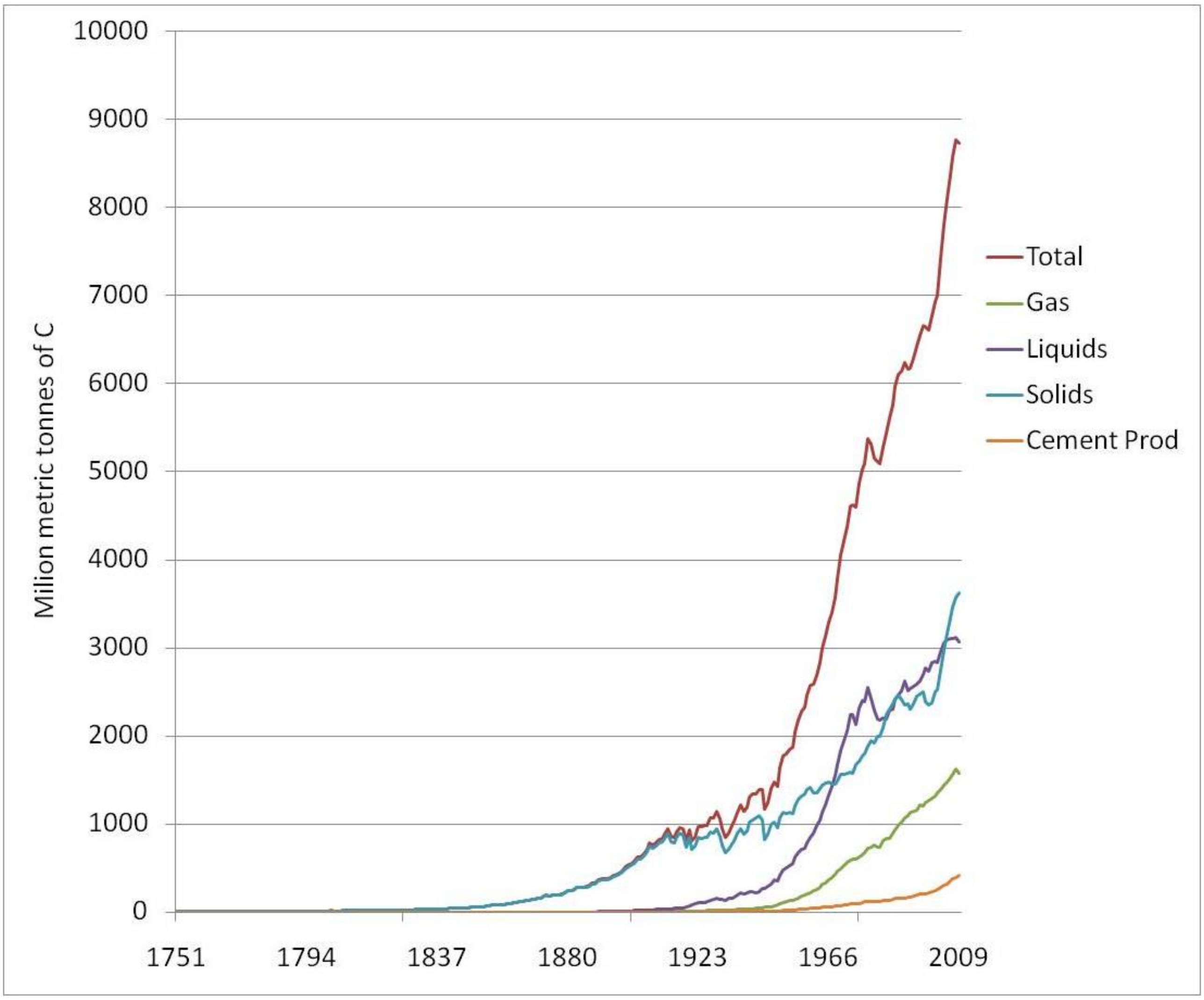


Figure 1.2. Role of soil in C cycling (adapted from Lal & Kimble, 1997)

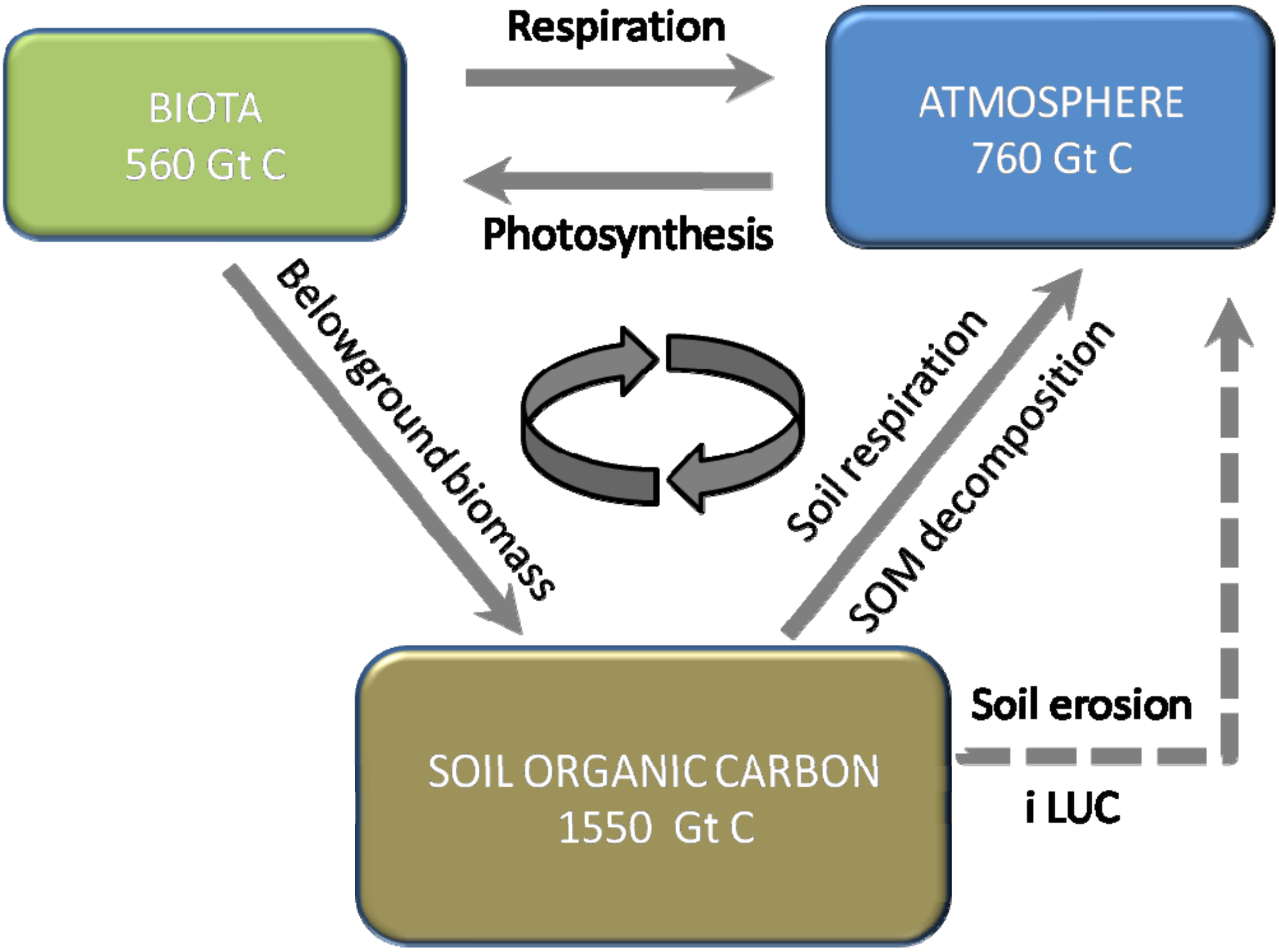


Figure 1.3. Source of CO_2 efflux from soil (adapted from Kuzyakov, 2006).

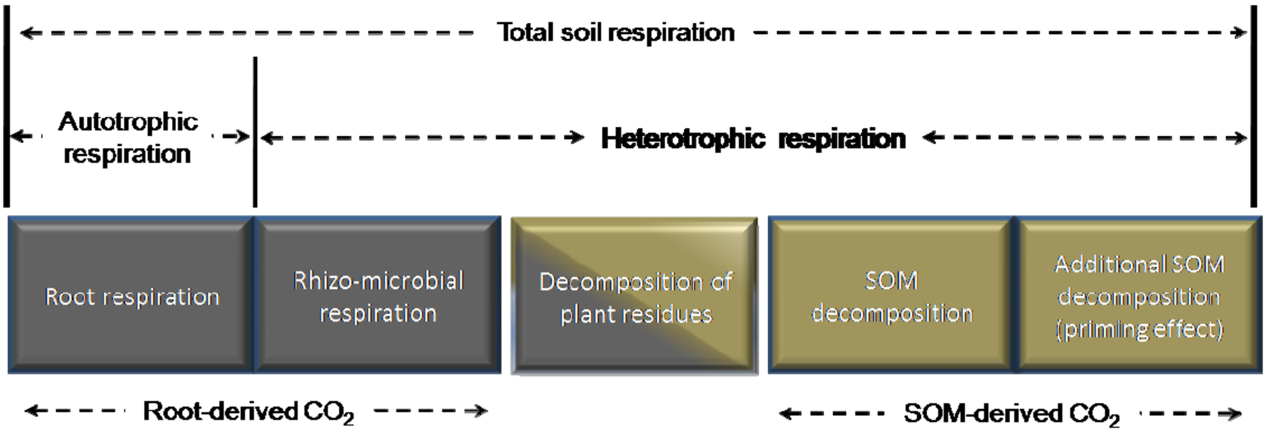
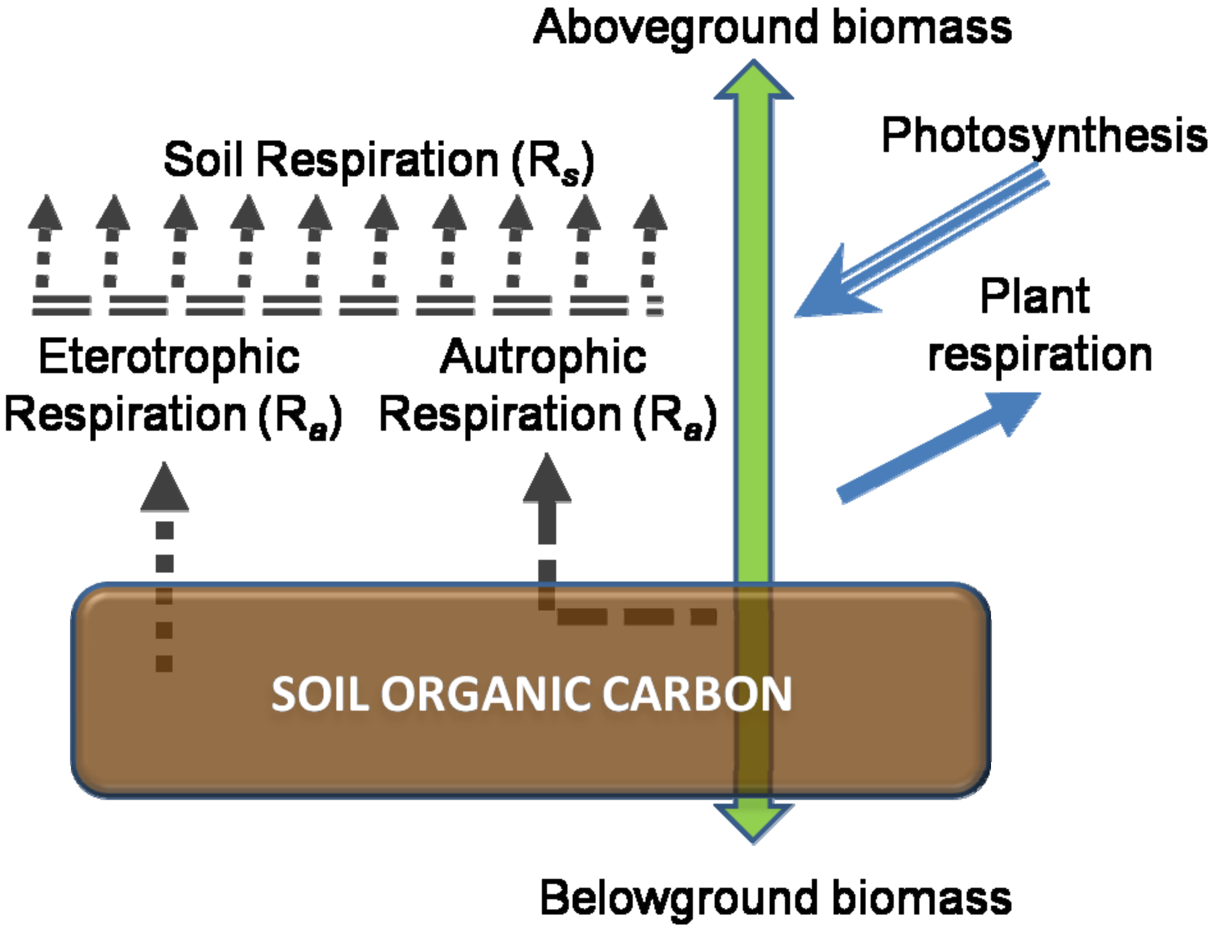


Figure 1.4. Simplified scheme of atmospheric-biota-soil inter-relations on carbon fluxes.



EXPERIMENT 1:

Effect of biomass-ash amendments and nitrogen fertilization on above- and belowground biomass production of switchgrass (Panicum virgatum L.)

Abstract

Switchgrass is one of the most promising perennial energy crops. It could significantly contribute to limiting GHG emissions through replacing fossil fuels whilst also sequestering a considerable amount of carbon into the soil. Nonetheless, the production of CHP from biomass generates a large amounts of ash which has to be opportunely allocated or profitably recycled, e.g. as nutrient amendments (Perucci *et al.*, 2006). Therefore, the objective of this study was to (i) evaluate the interactive effects of ash amendments and nitrogen fertilization on the above and belowground biomass production of switchgrass in Po valley (Italy), (ii) determine the soil C content after land use change from cropland to switchgrass and (iii) estimation of Net Ecosystem Exchange (NEE). Nitrogen fertilization did not influence crop productivity in the first two years while after second year a rate of 100 kg ha^{-1} y^{-1} of N seems to be the more adequate fertilization. Ash addition did not have an effect on above and belowground biomass, instead it reduced soil CO_2 flux, but the effect gradually decreased with the age of plantation. Belowground biomass productivity increased from 8.5 (T_0) to 22.5 (T_5) Mg ha^{-1}. Higher increments were noticed in upper soil layers. SOC increased in the whole soil profile analysed (0-60 cm) but significant increments were found only in deep layers (30-60 cm) probably because the "priming effect" increased microbial activity and consequently reduced SOC in shallow layers (0-30 cm). The Net Ecosystem Exchange (NEE) increased with the application of ash to the soil but its effect gradually decreased with the time. On average NEE was

9.5 Mg ha^{-1} y^{-1}. High switchgrass yield could be obtained with "low input", instead large amount of belowground biomass did not promote SOC accumulation but stimulated a "priming effect". Switchgrass was confirmed to be environmental friendly thanks to positive NEE.

Keywords: switchgrass, bioenergy, SOC, land use change, ash, soil CO_2 flux, priming effect

Introduction

The increasing emissions of greenhouse gases (GHG's) into atmosphere has encouraged interest in the role of soil and plant biomass on reducing atmospheric GHG concentrations (Cole *et al.*, 1997; Paustian *et al.*, 1997; Watson *et al.*, 2000). Soils contain about 2.7 times the carbon present in the atmosphere (Schlesinger, 1997) and biomass cultivation may favour soil carbon storage which varies between 1 and 2 x 10^6 Mg C yr^{-1} (Cannell, 2003). For example, it was estimated that one-meter soil depth may contain from 30 to 800 Mg ha^{-1} of soil organic carbon (SOC) (Lal, 2004b). Carbon sequestration is an important component of the life cycle for the production of bioenergy crops (Adler *et al.*, 2007) and may be a key aspect in determining GHG reduction potential compared to fossil fuels (Anderson-Teixeira *et al.*, 2009). Land use change from cropland to grassland leads to substantial storage of SOC (Lemus and Lal, 2005; Fargione *et al.*, 2008; Anderson-Teixeira *et al.*, 2009; Powlson *et al.*, 2011), but also raises ethical issues if bioenergy crops are cultivated on conventional land used for food or feed production (Field *et al.*, 2007). In addition to that, by using croplands for bioenergy end use may cause displacement of food production in peatlands, grassland and forest causing a carbon debt because of SOM mineralization due to disturbance of ecosystem (Fargione *et al.*, 2008). So cultivation of perennial grasses in marginal or degraded lands would be advisable because of their adaptability to poor soil conditions and environmental benefits (Campbell *et al.*, 2008). Moreover, the SOC increase entails a number of agronomic advantages such as aggregation of clay particles (Six *et al.*, 2002), water drainage (Lal and Kimble, 1997) and increasing the residual fertility (Lal, 2004a). For example, the increase of one ton of SOC can increase wheat (27-40 kg ha^{-1}) and maize (3-10 kg ha^{-1}) production (Lal, 2004a). Perennial crops such as switchgrass are considered one of the most promising crops for SOC storage and reduction of GHG emissions (McLaughlin and Kszos, 2005; Sanderson, 2008; Anderson-Teixeira *et al.*, 2009). Due to its well-developed root system, that can reach 3 m in depth and 30 Mg ha^{-1} of belowground biomass (Ma *et al.*, 2000b), switchgrass can stock large quantities of SOC in deep soil layers (Ma *et al.*, 2000a; Sommer *et al.*, 2000; Frank *et al.*, 2004; Lemus and Lal, 2005). Despite the fact that high belowground biomass input into the soil is not necessarily linked to increment of soil C concentration because of the so-called "priming effect" (i.e. increment of soil microbiological activity due to higher fresh organic matter supply (Kuzyakov, 2002). Monti *et al.* (2012) reported that converting cropland to switchgrass generally increase soil C stocks at a rate of 1 to 1.2 Mg ha^{-1} y^{-1}. Usually the "priming effect" occurs in the vicinity of living roots because of the presence of exuded organic substances like mucilage and sloughing-off the root cap cells (Kuzyakov, 2002) that are easily degraded by soil microorganisms (Nguyen, 2003). This increase of microbiological activity may change the intensity of soil organic matter (SOM) decomposition (Paterson *et al.*, 2003) that causes increment of soil CO_2 flux (Kuzyakov, 2006) and fast substitution of SOC (Kuzyakov, 2002). Soil respiration is one of the larger C flux in most ecosystems (Kuzyakov, 2006) and can account for 60-90% of total ecosystem respiration (Longdoz *et al.*, 2000). Soil respiration negatively influences SOC deposition (Paustian *et al.*, 2000) and small changes on soil CO_2 flux using crop management (such as no- minimum tillage) or perennial crops could have large impacts on global CO_2 emissions (Paustian *et al.*, 2000; Schlesinger and Andrews, 2000). In fact, belowground biomass and soil respiration are the two main factors that are involved in the SOC dynamics and that can influence the real potential to stock SOC (Schlesinger and Andrews, 2000).

Nitrogen fertilization can stimulate root development (Ma *et al.*, 2001; Lemus and Lal, 2005) and plays an important role on cost, energy consumption, and environment management (McLaughlin and Kszos, 2005; Stehfest and Bouwman, 2006) and can cause leaching (Cambardella *et al.*, 1999; Dinnes *et al.*, 2002), NO_x and N_2O emissions (Lee *et al.*, 1997; Bouwman *et al.*, 2002a). Several studies have been carried out to determine the appropriate nitrogen fertilization requirement of switchgrass (Ma *et al.*, 2000b; Muir *et al.*, 2001; Vogel *et al.*, 2002; Lee *et al.*, 2007; Sanderson, 2008). The annual nitrogen requirement of switchgrass was estimated between 70 to 100 kg ha^{-1} (McLaughlin and Walsh, 1998) but 50 kg ha^{-1} could be considered adequate (McLaughlin and Kszos, 2005). In this studies many different environmental conditions were taken in to account from southern to northern U.S.A. but information on Mediterranean areas are limited. Ashes that are generated by production of CHP by biomass can be considered as a way to recycle nutrients from elements such as Ca, K, and P (Demeyer *et al.*, 2001; Perucci *et al.*, 2006; Moilanen *et al.*, 2012). Ashes can influence soil pH (Saarsalmi *et al.*, 2012), microbial activity (Perkiomaki and Fritze, 2002; Perucci *et al.*, 2008), field moisture capacity (Adriano and Weber, 2001) and soil salinity (Clapham and Zibilske, 1992). Studies concerning the influence of residues from combustion were mainly carried out in Northern Europe in forests (Moilanen *et al.*, 2012), where the addition of ashes to peatlands reduces soil acidity and favours soil microbiological activity, which in turn increases of soil CO_2 flux (Weber *et al.*, 1985; Moilanen *et al.*, 2002), while in alkaline soils, such as Poggio Renatico, enzymatic activity and microbiological C-mass was reduced (Perucci *et al.*, 2006).

The objective of this study (i) was to evaluate the interactive effects of ash amendments and nitrogen fertilization on the biomass production of above and belowground switchgrass in the Po valley (Italy), (ii) to determine the soil C content after land use change from cropland to switchgrass and (iii) estimation of Net Ecosystem Exchange (NEE).

Materials and Methods

Field setting

The experiment was carried out at Poggio Renatico, Ferrara, Italy (5 m a.s.l, 44°37' N, 11°45' E). According to the USDA classification the soil was loam (30% sand, 51% silt, 19% clay), pH (in H_2O) was 8.0 and organic matter content of 1.57% (Walkley and Black, 1934). Typically, the area is characterized by cold winters and hot summers, while the annual rainfall is about 646 mm (average of 30 years). The maximum daily temperature is 30.4 °C in July and in average daily temperature during the growing season (April-October) is 18.8°C.

Before switchgrass establishment the site was under annual crop rotation (wheat-maize-wheat-sugar beet). Seedbed preparation was: ploughing at 0.3 m soil depth, mechanical weeding, rotating-harrowing and vibrocultivating. Switchgrass (cv. Alamo) was sowed on May 8th, 2007 with an experimental sowing machine (Vignoli) at a rate of 6 kg ha^{-1} using pure live seeds (PLS). The row distance was set at 20 cm and seeds were sown 2-3 cm depth in the soil. Plots size were 151 m^2 (16.0 x 9.5 m) replicated four times.

Fertilization rate

Nitrogen fertilization was applied at rates of 0, 50 and 100 kg of N ha^{-1} (N_0, N_1 and N_2 respectively) and were tested in combination with two ash levels (0 and 500 kg ha^{-1}). The ash level was obtained by multiplying switchgrass ash content (5%) by the potential harvested biomass (10 Mg ha^{-1}). Nitrogen fertilization was done by hand with Carbamide ($CO(NH_2)_2$) at the same time as ash addition after rhizome emergence.

Aboveground and belowground biomass collection and determination

The aboveground biomass production was measured by weighing 7.5 m^2 of biomass per plot cut at the end of the growing season. Sub samples (~200 g) were taken and dried at 105°C in order to calculate the dry matter content. Another sub-sample (~100 g) from each plot was taken and dried at 60°C for laboratory analysis.

Root biomass was determined by collecting one soil core per plot at the end of each growing season from 2008 until 2011. Root samples were taken within the row and midway between two adjacent plants at 0.2 m intervals to a depth of 1.2 m with an auger (74 mm Ø, 1.2 m height). Samples were temporarily stored at -18 °C before root separation. In order to separate roots from soil, samples were first kept in a solution of oxalic acid (2%) for 2 h, and then washed in a hydraulic sieving-centrifugation device. Once cleaned, roots were hand-recovered from the water using a 0.5 mm mesh sieve (Vamerali et al., 2003). Root dry matter (RDM; Mg ha^{-1}) was determined by drying the root samples at 105°C for 24 h.

RDM was used to determine the shape of root apparatus through the asymptotic function proposed by Gerwitz and Page (1974) then modified by Gale and Grigal (1987) and corroborated by a number of studies (Stone and Kalisz, 1991; Jackson *et al.*, 1996; Monti and Zatta, 2009):

$$Y = 1 - \beta^{d}$$

Where Y is the cumulative root fraction from the soil surface to depth d in centimetres (120 cm in this study); β is a dimensionless parameter describing the shape of the vertical root distribution within the considered soil layer. Briefly, higher β values correspond to a greater proportion of roots at depth, while lower β values imply a greater proportion of roots near the soil surface.

Soil moisture measurements

In the period March - September 2011 the soil moisture content over multiple depth intervals (0.1 m until 1.6 m depth) was monitored every month using a portable soil moisture probe (Diviner 2000; Sentek Sensor Tech., Stepney, Australia). Diviner 2000 utilises the frequency domain reflectometry (FDR) as sensor technology. To convert scaled frequency readings (SF) into volumetric water content (θ_v), the following equation was used which was specifically calibrated for this instrument in a soil with 1.39 dry bulk density (Groves and Rose, 2004):

$$SF = 0.3531\,\theta_v{}^{0.2621}$$

Access tubes for the diviner probe were installed in the centre of each plot. Tubes were placed into soil holes having 5 mm greater diameters than the PVC tubes. Along with the tubes a soil water mixture was inserted into the hole thus to improve the contact between pipe and soil.

Soil respiration measurements

Following full rhizome emergence one PVC collar (100 mm Ø, 50 mm height) per plot was placed in the interrow at about 5 cm deep in the soil. CO_2 soil fluxes (R_s), i.e., the combined autotrophic (R_a) and heterotrophic (R_h) ecosystem respiration, were measured at approximately monthly intervals by an infrared gas analyzer (EGM-4; SRC1/EGM4, PP-System) equipped with a soil chamber and soil temperature probes. Flux data were collected between 10:00 and 13:00 h in each measuring day. R_a is CO_2 flux derived by roots respiration, while R_h is CO_2 flux derived by soil microorganisms and soil microfauna (Kuzyakov, 2006).

The regression technique (Kucera and Kirkham, 1971) was used in order to separate R_a and R_h. Briefly, it is assumed the linear relationship between root biomass and the amount of CO_2 respired by roots and rhizosphere microorganisms; the amount of CO_2 derived from SOM decomposition corresponds to the intercept of the regression line between root biomass (independent variable) and total CO_2 evolved from the soil (dependent variable).

Soil collection and determination of soil organic carbon (SOC)

To determine soil organic carbon (SOC) before switchgrass establishment (T_0) soil cores were taken randomly across the field at two depths (0-30 and 30-60 cm) in April 2007. Two and four years after crop establishment another set of soil cores were taken at the same depths as T_0, but the first layer was subdivided into three soil layers (0-5, 5-15, 15-30 cm). In both cases samples were taken at the end of March before rhizomes started re-growing. Soil and roots were separated by hand and then air dried until constant weight before milling. Soil organic carbon concentration (%) and stable carbon isotope ratio ($^{13}C/^{12}C$) was determined by an isotope ratio mass spectrometer (ANCA-MS system, ltd) in 120/150 mg soil samples. Before analysis the inorganic soil C was removed by acidification (Bundy and Bremen, 1972).

The soil bulk density was calculated on the sieved dried samples (Ellert *et al.*, 2001) taken at two depths (0-20 and 20-40 cm) because it was assumed that bulk density does not change below 20 cm (Gifford and Roderick, 2003).

The spatial coordinates method (Zan *et al.*, 2001) was used for carbon mass (Mc, Mg ha^{-1}) per unit volume calculation that was obtained by multiplying soil bulk density (BD, Mg m^{-3}), horizon thickness (T, m) and C concentration (C_{cont}, kg Mg^{-1}) as given by Ellert *et al.* (2001):

$$M_c = BD * C_{cont} * T * 10000 \text{ m}^2 \text{ ha}^{-1}$$

The switchgrass contribution to soil carbon sequestration (F) was calculated using the following equation (Balesdent, 1987):

$$F = \frac{(\delta_n - \delta_0)}{(\delta_r - \delta_0)}$$

where δ_0 and δ_n are soil organic C isotope abundance before planting of switchgrass and after *n* years of cultivation, respectively; δ_r is the carbon isotope abundance of switchgrass roots.

Carbon budget – Net Ecosystem Echange (NEE)

There are number of papers in the last decades that give carbon balance budgets at the site to continental scales. Usually the net ecosystem exchange (NEE), or net ecosystem production (NEP), of CO_2 is the difference between CO_2 entering an ecosystem and all the CO_2 leaving the ecosystem during growing period (Smith et al., 2010). In order to obtain the carbon balance it is necessary to quantify the gross primary production (GPP). GPP represents the gross uptake of CO_2 that is used in photosynthesis (Ciais *et al.*, 2010) and is the sum of net primary production (NPP), i.e. sum of above and below ground biomass, and autotrophic respiration (R_a):

$$\text{GPP} = \text{NPP} + R_a$$

However part of carbon was used by living roots to organic compounds like exudates, secretions and sloughed-off root cells and part for the roots turnover, that were used by soil microorganisms that contributes to R_h. As well as crop residues any old roots are subjected to decomposition by microorganisms during growing seasons. Thus in cropland Net Ecosystem Exchange (NEE) is given by:

$$\text{NEE} = \text{GPP} - R_s$$

Statistical analysis

A completely randomized block design with four replicates was adopted with plots of 151 m^2 (16.0 x 9.5 m). Root data from the four replicates were analyzed by repeated measures analyses of variance, using depth as the repeated factor. Statistical analyses were performed using CoStat v6.204 (Monteray, USA). Following significant ANOVA results, means were separated using Tukey's LSD test at ($P \leq 0.05$). The statistical significance of the regression coefficients was tested by analyzing the variance of the regression (ANOVA) through splitting the sum of square into regression and residual components (F-statistic for $P \leq 0.05$).

Results

Climatic conditions

The average precipitation changed significantly during the five experimental growing seasons (from March to September). Compared to the long-term average (376 mm), precipitation was 37% and 42 % lower in 2007 and 2011, respectively. In 2008 and 2010 precipitation was 23% and 49% higher than the long-term average. Even though the 2008 and 2010 growing seasons were rainy, there was no significant decrease in ambient temperatures compared to long-term average, but were lower compared to 2009 and 2011 (Table 2.2). Average soil temperatures increased from 2008 to 2011, reaching a maximum of 23.8°C in the last experimental year.

Above and belowground biomass

Addition of ash did not influenced aboveground biomass and no interaction was found between ash and nitrogen fertilization. Figure 2.1 shows the effects of different nitrogen fertilization levels on the aboveground dry biomass (ADB) production of switchgrass in a five-year-old stand. In all treatments ADB increased continuously from 2007 until 2009. Significant differences between N_2 (23.3 Mg ha^{-1}) and N_1 and N_0 (17.3 and 15.3 Mg ha^{-1}, respectively) were found only in 2009. It is also important to note that maximum productivity was reached in 2009 (Fig. 2.1). Afterwards, ADB decreased significantly; in 2010 ADB was 46.2% and 20.2% lower in N_0 and N_2 than in 2009. In N_0

and N_1 small decrements in yield were detected in 2011 as compared to 2010, while yields in N_2 remained stable.

Root dry matter (RDM) increased from 8.5 Mg ha^{-1} in 2008 up to 22.5 Mg ha^{-1} in 2011. No significant differences, however, were found both among nitrogen fertilization and ash treatments. Instead interaction was found between depths and years (Fig. 2.2) since RDM increase significantly from 2008 till 2011 in the top layers (0-60 cm), while no significant differences were found in deeper layers.

The model proposed by Gale and Grigal (1987) was used to describe the vertical root distribution. Higher β value, i.e. indicating that more roots are concentrated in deeper layers, was obtained in 2008 (β= 0.980) when 50% of RDW was concentrated in the upper 40 cm soil depth (Fig. 2.2). In the following years the proportion of RDW in upper layer slowly increase, reaching a maximum of 60% in 2011 (β= 0.974). Anyway belowground biomass increased from 2.1 to 4.5 Mg ha^{-1} in 40-120 cm soil profile.

Soil C and N content

Table 2.3 shows that after 5 years of switchgrass cultivation (T_5) the soil C content (%) increased significantly (+23.4%) in deep layers (30-60 cm deep), while in the upper layers only a small increase was detected (+14.3%). Soil organic carbon (SOC) increased with time from 25.7 in T_0 to 30.0 Mg ha^{-1} in upper layers (0-30 cm deep); but the differences were not significant. On the other hand in deeper layers SOC increased significantly from 16.2 in T_0 to 21.2 Mg ha^{-1} in T_5. As for the C isotope ratio, it changed significantly only in the upper layers; 9.8% of such change was derived from the switchgrass implantation. Small increments of N content (%) were detected in both layers, but the differences between T_0 and T_5 were not significant.

Because there was no significant differences between nitrogen fertilization and ash addition, as well as no interactions among them were found, only the differences between soil depths were analyzed.

Table 2.4 shows the soil carbon and nitrogen concentration, and switchgrass carbon derived at a three depths (0-5; 5-15 and 15-30 cm deep) after 5 growing seasons (T_5). It was found that at 0-5 cm deep the C (+18.9% in average) and N (+12.0% in average) concentrations were higher than in the other two depths. The switchgrass C derived was also higher in the upper layer (23.9%) than in the other two deeper layers (2.8% in average).

Soil CO_2 flux

The seasonal pattern of soil CO_2 flux was similar in all years and it followed that of soil temperature (Fig. 2.3). Fluxes increased rapidly from April to the end of July/August where a peak reached, this was coincident with the highest soil temperatures. The highest peak was reached in 2011 at the end of July (31.2 g m^2 d^{-1}) when soil temperature was 28.1°C, while in July 2008 was detected the lowest value (20.9 g m^2 d^{-1}) when soil temperature was 21.5°C. After the summer peaks, the soil CO_2 fluxes decreased rapidly up to October. From November to March, when usually the temperatures were below zero and there is snow, the fluxes were not detectable. The addition of ash to the soil significantly reduced soil CO_2 fluxes during the different summer periods examined. The reductions were 30%, 29%, and 16% in 2009, 2010, and 2011, respectively.

The relationship between soil CO_2 fluxes and root biomass is presented in Figure 2.4. It was found that the soil CO_2 fluxes were positively and significantly correlated with belowground biomass in 2008, 2009 and 2010 only. Applying regression analysis technique to plots fertilized with and without ash it was found that the heterotrophic respiration (R_h) was 61 and 63% respectively (Fig. 2.4). The best fit was obtained without ash (r = 0.72) compared to that with ash (r = 0.63).

Carbon budget – Net Ecosystem Exchange (NEE)

From 2008 to 2010 the gross primary production (GPP) increased faster in plots without ash addition (from 13.2 to 22.0 Mg C ha^{-1}) than in plots treated with ash (from 12.0 to 21.3 Mg C ha^{-1}).

The Net Ecosystem Exchange (NEE) also increased with time (Fig. 2.5), but it was significantly higher in the ash treated plots than in the plots without ash. The difference between ash and without ash treatments was higher during the first three years of growth (on average +13.5%), while this difference disappeared during the last experimental season (on average 12.8 Mg ha^{-1} y^{-1}). After five years of switchgrass cultivation the NEE was 40 and 36 Mg C ha^{-1} for plot treated with and without ashes.

Discussion

Above and belowground production

Switchgrass is considered one of the main biomass crops that maintain high production levels at different environmental conditions (Lemus *et al.*, 2002; Sanderson, 2008; Follett *et al.*, 2012; Monti *et al.*, 2012). Numerous studies have been carried out on its response to nitrogen fertilization (Ma *et al.*, 2000b; Muir *et al.*, 2001; Lee *et al.*, 2007) and on its ability to import C to the soil (Ma *et al.*, 2000a; Zan *et al.*, 2001; Liebig *et al.*, 2008). As well, switchgrass is considered a "low input" perennial grass (McLaughlin and Kszos, 2005). This is especially true after establishment, as the crop does not need high fertilization inputs and tillage practices (Monti *et al.*, 2012). At the second year of the experiment switchgrass reached a biomass production of 10 Mg ha^{-1} without significant differences among nitrogen treatments and ash addition, indicating that no fertilization or low fertilization rates (such as N_1) could be enough for the successful stand establishment. These results confirm the findings of previous studies where it was assumed that the nitrogen has been added via atmospheric deposition and soil N-mineralization (Dohleman *et al.*, 2012). The peak of productivity was achieved in the fourth year using 100 kg ha^{-1} of N (N_2 = 23.3 Mg ha^{-1}; Fig. 2.1). This exceptional production is probably due to the combination of nitrogen fertilization, optimal temperatures, and well distributed rainfall that permitted the nitrogen uptake (NO_3-N) during the more important growing stages (Vogel *et al.*, 2002; Guretzky *et al.*, 2010). As well, in 2011 switchgrass confirmed its resistance to water stress conditions (Barney *et al.*, 2009; Monti *et al.*, 2012) because it showed excellent yields with modest rainfall (- 40% less than average of last 30 years) if supported by good nitrogen fertilizers (8.2, 14.4 and 18.6 Mg ha^{-1} for N_0, N_1 and N_2 respectively). Its capacity to resist water stress is related to its rapid belowground biomass development allowing switchgrass to exploit deep water reserves (Ma *et al.*, 2000b; Monti and Zatta, 2009). As a shown in figure 2.6 the crop was able to take up water down to 1.6 m depth throughout the growing season in a critical year like 2011.

In contrast to the aboveground biomass, the nitrogen fertilization treatments did not affect the root system development (Fig. 2.2). These results are in agreement with those of Ma *et al.* (2000b) but in contrast with those of Heggenstaller *et al.* (2009). The reason for the different results could be explained by interaction among soil nutrients availability (Ma *et al.*, 2000b), genotypes (Sanderson, 2008) and crop management (Ma *et al.*, 2001). The analysis of the root system by β value parameter (Fig. 2.2), obtained from the model proposed by Gale and Grigal (1987) that describes the development in depth of the root system, it was found that the increment of belowground biomass (from 2008 to 2011) was concentrated mainly in shallow layers (in the first 40 cm). In fact, it was detected a relative and progressive decreases of the β value, indicating that the bulk of the roots was concentrated in surface layers. Anyway, belowground biomass increased from 2.1 (T_0) to 4.5 (T_5) Mg ha^{-1} in 40-120 cm soil profile (+71%), confirming the crop's potential to stock carbon in deep soil layers (Ma *et al.*, 2000a; Liebig *et al.*, 2005; Liebig *et al.*, 2008; Monti and Zatta, 2009). However, ashes, which can be considered a way to recycle nutrients from elements such as Ca, K, and P (Perucci *et al.*, 2006; Moilanen *et al.*, 2012), can influence soil chemical components such as pH (Silfverberg and Huikari, 1989), field capacity (Chang *et al.*, 1977) and soil salinity (Clapham and Zibilske, 1992) but did not have an effect on productivity both in above and belowground biomass.

Soil C and N content

Switchgrass potential to input carbon to the soil has been demonstrated by several authors (Zan *et al.*, 2001; Frank *et al.*, 2004; Sanderson, 2008). In the present study we also found that after five years of switchgrass cultivation SOC increased. The soil carbon concentration increased along the whole profile analyzed but this was only significant in the deep soil layers. In a recent review of switchgrass it was reported that SOC can increase between 1 and 1.2 Mg ha^{-1} y^{-1} when converting a cropland into a switchgrass plantation (Monti *et al.*, 2012). In the present study somewhat higher values were found. After five years of growth the SOC stored was 9.3 Mg ha^{-1} meaning an average of 1.9 Mg ha^{-1} y^{-1}. The low initial SOC, as a consequence of intensive crop cultivation in the preceding decades (Lal and Kimble, 1997), may be the reason for relatively high and fast accumulation of stored SOC under switchgrass (Powlson *et al.*, 2011). After 5 years of switchgrass the SOC increment was 14.3% and 23.4% in the 0-30 cm and 30-60 cm layers, respectively. The lower increment in SOC in the top layers could be related to several factors. For example, the upper layers are more susceptible to mineralization processes because of exposure to seasonal fluctuations in precipitation, soil temperature, and microbial activity (Gupta and Rao, 1994; Lemus and Lal, 2005). The "priming effect", which indicates that a large root biomass can trigger faster metabolic processes by soil microorganisms thus accelerating soil organic matter decomposition and C turnover (Kuzyakov, 2002, 2006), probably played an important role in the carbon dynamics in upper layers. In fact, despite the general increase on SOC, there was a strong relation (R^2=0.93) between root biomass and C concentration (Fig. 2.7), suggesting an increase of microbial activity in the presence of a greater amount of root biomass (Kuzyakov, 2002; Kuzyakov and Larionova, 2005). In the vicinity of roots there are large quantities of exuded organic substances that are utilized in short time by microorganisms that forced intensive C turnover (Kuzyakov, 2002; Nguyen, 2003; Kuzyakov, 2006).This hypothesis, was also demonstrated in Miscanthus (Zatta *et al.*, 2013), and further corroborated by the 'high C-derived from the top 5 cm of soil (table 2.4) where quantity of more active fine-roots is high (Gregory, 2006). The C-derived in the deep layers did not change significantly, because this carbon is more strongly stabilized than in top-layers (Grigal and Berguson, 1998; Powlson *et al.*, 2011). Even though some authors indicated that nitrogen fertilization can increase SOC in the soil because of the enhanced root biomass production (Powlson *et al.*, 2011), we did not find any correlation of such type, as well as no increments on soil N concentration were found, probably because of the capacity of switchgrass to use soil nutrient resources after SOM mineralization.

Soil CO_2 flux

Soil temperature is the main factor influencing soil CO_2 flux (Lou *et al.*, 2004), and in switchgrass the correlation between the two factors was observed by some authors (Frank, 2002; Lee *et al.*, 2007). Our results also show the close relationship throughout the growing seasons between CO_2 flux and soil temperature, in fact peaks of CO_2 fluxes were recorded during the summer (end of July; Fig. 2.3), when plants were on full flowering stage and soil temperature is higher. In the present study the addition of ash to the soil caused a soil CO_2 flux decrease during the warmer months in all of the growing seasons evaluated. This result is in contrast with studies carried out on peatlands in northern Europe in which it was found an increase in soil respiration after the ash addition (Moilanen *et al.*, 2012). This phenomenon has been justified as the addition of ashes reduce soil acidity and in turn increase soil microbiological activity (Weber *et al.*, 1985; Moilanen *et al.*, 2012). On the other hand in alkaline soils, which are typical of Mediterranean areas, the addition of ashes caused the reduction in microbial biomass-C and enzyme activity (Perucci *et al.*, 2006; Perucci *et al.*, 2008). In the present study, soil are sub-alkaline (pH = 8) and consequently the reduction in soil CO_2 flux could be attributed to a reduction in microbiological activity. This hypothesis could be partly confirmed by Figure 2.7, where the correlation between soil C concentration and root biomass disappear in presence of ash ($R^2 = 0.13$) while in plots without ash amendments the correlation is highly significant ($R^2 = 0.93$). So it could be argued that the priming effect in alkaline is reduced through the inhibitory effect that ashes have on microbial activity.

The contribution of autotrophic respiration (R_a) to soil fluxes (R_s), has been reported to vary from 10% to as much as 90% for both forest and non-forest ecosystems (Hanson *et al.*, 2000; Xu *et al.*, 2001). Part of this variability may be due to differences in ecosystems, species, or developmental stages (Hanson *et al.*, 2000; Bond-Lamberty *et al.*, 2004). In the present study the regression technique was used, among the several available, in order to separate autotrophic (R_a) and heterotrophic (R_h) ecosystem respiration (Kucera and Kirkham, 1971) and it was found that heterotrophic respiration (R_h) was 61 and 63% for plots with and without ash respectively (Fig. 2.4). Our results, that are in agreement with those reported on grassland by other authors (Kucera and Kirkham, 1971; Brook *et al.*, 1983; Hanson *et al.*, 2000), indicate that R_h are the main components of soil respiration and that ashes reduced both components of soil respiration. The absence of correlation between soil CO_2 flux and belowground biomass in the last year is probably due by large, older roots, which respire and exude much less C than fine, young roots (Kuzyakov, 2006).

Carbon budget – Net Ecosystem Exchange (NEE)

Factors to be taken into account for NEE calculation are aboveground, belowground biomass, and soil respiration which must be divided into autotrophic (R_a) and heterotrophic respiration (R_h) (Smith *et al.*, 2010). Therefore, the summation of these factors makes the NEE computation highly uncertain (Lauenroth *et al.*, 2006). Despite that some studies indicated that switchgrass C savings can vary from 7.6 to 15 Mg ha^{-1} y^{-1} (Frank *et al.*, 2004; Lee *et al.*, 2007; Liebig *et al.*, 2008). In the present study it was estimated that during the first three years switchgrass NEE was 7.8 and 9.0 Mg ha^{-1} y^{-1} in plots with and without ash, respectively, while in 2011 these differences disappeared. The different results could be related to the different soil CO_2 fluxes caused by the addition of ash as indicated before. Moreover, soil respiration negatively affects soil C sequestration (Paustian *et al.*, 2000) and is responsible for 10% of CO_2 emissions into the atmosphere (Lee *et al.*, 2007) so its reduction can have considerable impacts on global emissions (Schlesinger and Andrews, 2000). Therefore, the addition of ashes to alkaline soils, as in the present case could have significant effects on reduction of CO_2 emissions. It was calculated that the addition of ashes could allow 3.6 Mg C ha^{-1} saving after 5 years of switchgrass cultivation. Then the total NEE in plots with ash, adding the computed increment in SOC, was 49.3 Mg ha^{-1} in contrast to the 45.3 Mg ha^{-1} produced in plots without ashes.

Conclusion

No N fertilization is needed during the first two years after sowing because of utilization of residual soil fertility by switchgrass. Belowground biomass increased from 8.5 to 22.5 Mg ha^{-1} and developed in depth allowing large quantities of carbon to be stocked in the soil after 5 years (9.3 Mg ha^{-1}), especially in the deep layers, while in upper layer carbon stock was limited because of the priming effect. Ashes, the residues after combustion of biomass, can be used as soil nutrient amendments because it was found that ashes did not have any impact either on above and belowground biomass. Instead, ashes had inhibitory effect on soil respiration, which permit to save 4 Mg ha^{-1} of C after 5 years of growing switchgrass.

TABLES

Table 2.1. Chemical characteristics of ash used for fertilization

Element	concentration	unit
Phosphorus (P)	7,00	mg/kg
Potassium (K)	1120,00	mg/kg
Arsenic	<0,10	mg/kg
Cadmium (Cd)	<0,10	mg/kg
Chromium (Cr)	<0,10	mg/kg
Copper (Cu)	<0,10	mg/kg
Mercury (Hg)	<0,10	mg/kg
Manganese (Mn)	<0,10	mg/kg
Nickel (Ni)	<0,10	mg/kg
Lead (Pb)	<0,10	mg/kg
Sodium (Na)	97,72	mg/kg
Aluminum (Al)	0,00	mg/kg
Calcium (Ca)	10,00	mg/kg
Iron (Fe)	0,00	mg/kg
Magnesium (Mg)	1,00	mg/kg
Silicon (Si)	158,00	mg/kg
Titanium (Ti)	0,00	mg/kg
Carbon (C)	14,01	%
Sulfur (S)	0,02	%
Chlorine (Cl)	0,30	%

Table 2.2. Total precipitation, mean air temperature and soil temperature during growing season (March-September) and in long-term average (1961-1990)

Year	Precipitation (mm)	Air Temp (°C)	Soil Temp (°C)
2007	238	18.8	
2008	461	18.2	18.8
2009	335	19.3	20.8
2010	560	18.0	20.4
2011	216	19.4	23.8
Long-term average (30 years)	376	18.0	

Table 2.3. Soil carbon (C) and nitrogen (N) content (%) on two depths before switchgrass cultivation (T_0) after 5 years (T_5). (n.s. = not significant , *and **, statistically significant differences for P≤0.05 and P≤0.01 respectively, with Tukey's test).

Depth	Parameter	T_0	T_5	P≤0.05	C.V. (%)
0-30 cm	N (%)	0.08	0.1	n.s.	6.45
	C (%)	0.72	0.84	n.s.	7.51
	C (Mg ha^{-1})	25.75	30.06	n.s.	7.26
	δC	-25.2	-24.04	*	0.58
30-60 cm	N (%)	0.06	0.08	n.s.	10.65
	C (%)	0.45	0.59	*	3.35
	C (Mg ha^{-1})	16.23	21.18	*	3.35
	δC	-25.38	-25.28	n.s.	0.35

Table 2.4. Soil carbon (C%) and nitrogen (N%) concentration, and switchgrass carbon derived at a three depths after 5 years. Different letters show statistically different means (Tukey's LSD test, P≤0.05).

Soil depth	C (%)		N (%)		Switchgrass C-derived (%)	
0-5	0.963	a	0.108	a	23.9	a
5-15	0.784	b	0.098	b	3.4	b
15-30	0.779	b	0.093	b	2.1	b

FIGURE EXPERIMENT 1

Fig. 2.1. Aboveground biomass (Mg ha^{-1}) of switchgrass at three nitrogen fertilization levels (0, 50 and 100 kg ha^{-1} of N, namely N_0, N_1 and N_2 respectively) from 2008 to 2011. Different letters indicate statistically different means (Tukey's $LSD_{P\leq0.05}$=2.5).

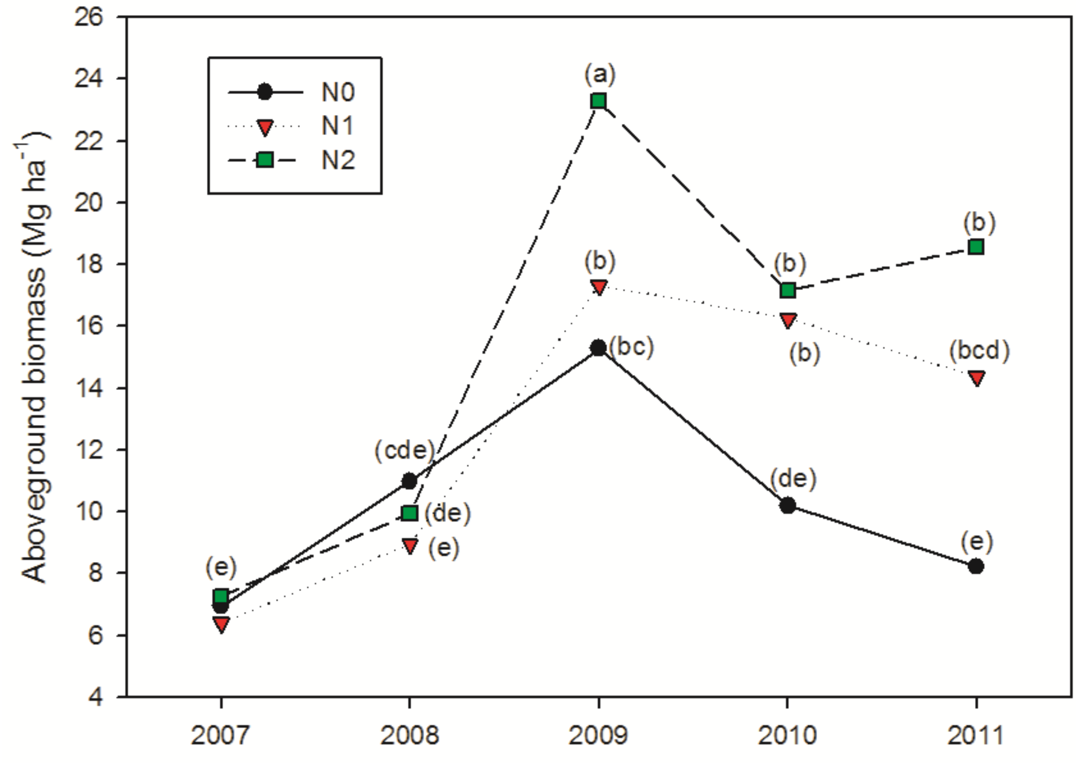


Fig. 2.2. Belowground biomass (Mg ha^{-1}) of switchgrass at six soil depths (0-20; 20-40; 40-60; 60-80; 80-100; 100-120 cm), at the end of growing season at four years (from 2008 to 2011). Bars indicate the least significant difference (Tukey's LSD$_{P\leq0.05}$=0.81). The inset graph shows belowground biomass over soil depth following the β model proposed by (Gale and Grigal, 1987) that describe the shape of the cumulative root distribution over depth.

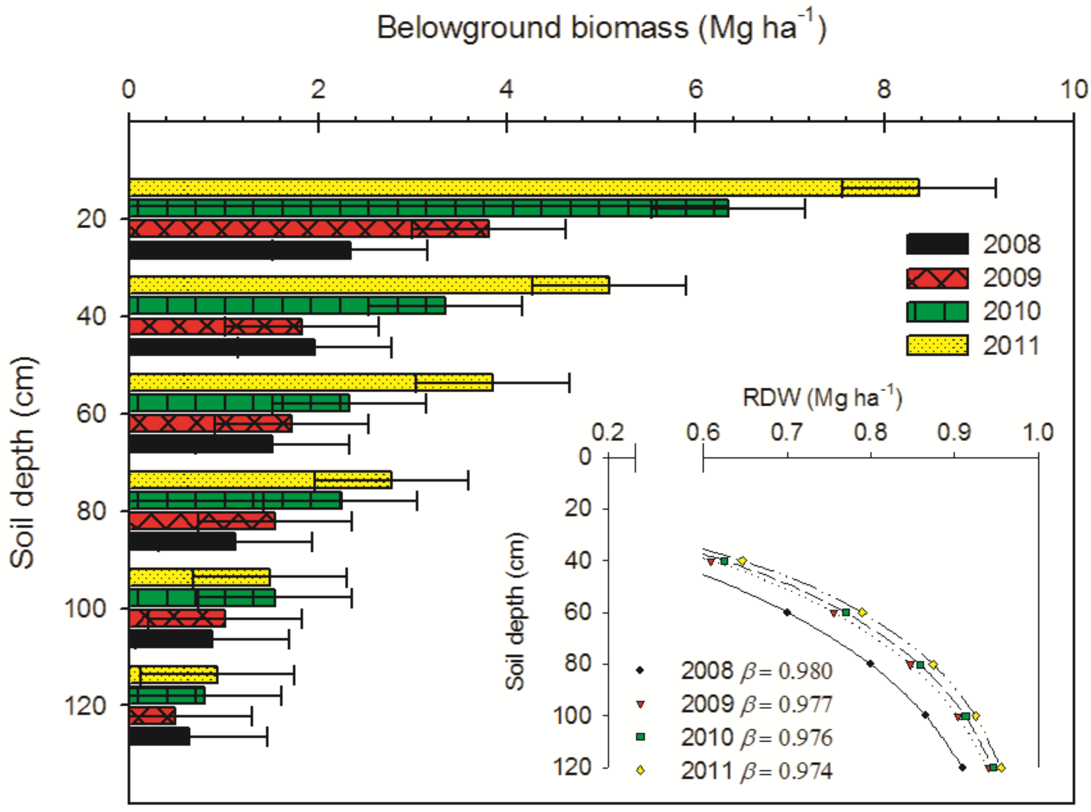


Fig. 2.3. Soil flux (g CO_2 m^2 d^{-1}) and temperature (°C) over growing season from 2008 to 2011 (* and **, statistically significant differences for P≤0.05 and P≤0.01, respectively with Tukey's test).

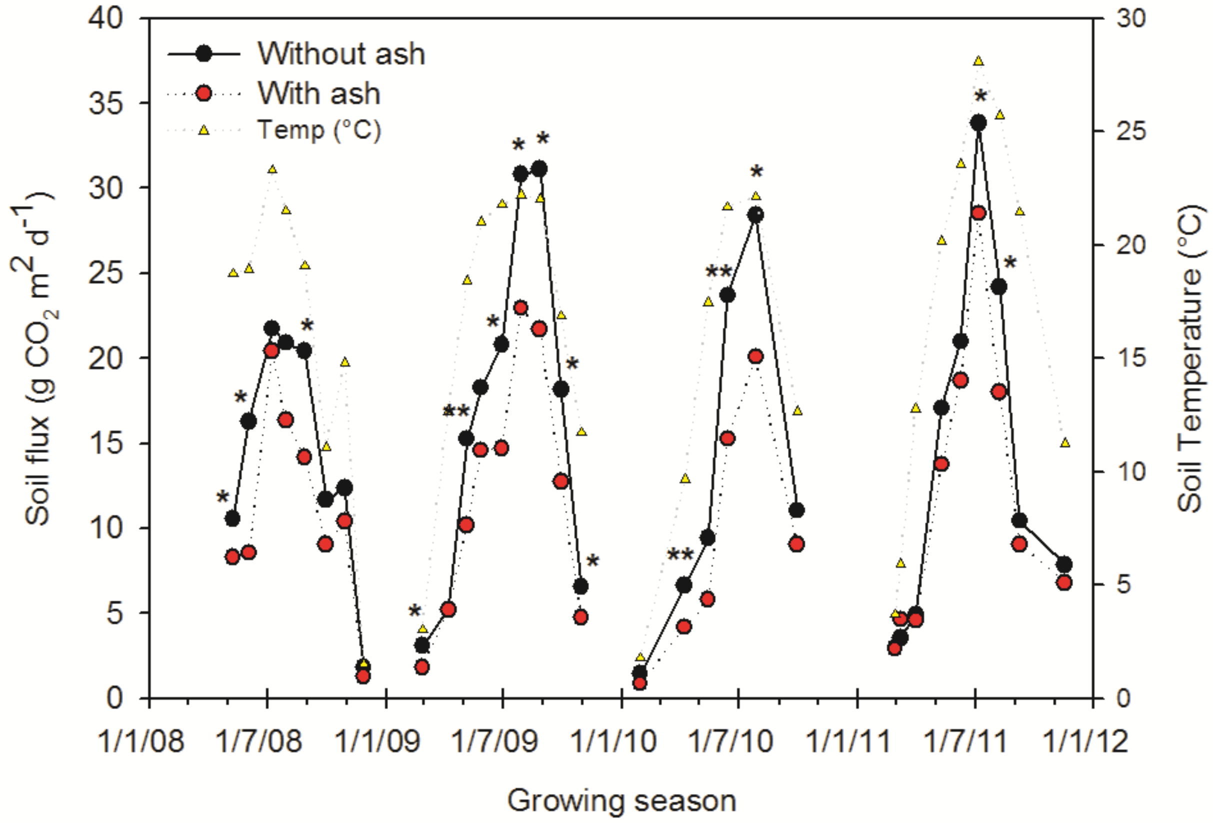


Fig. 2.4. Relationship between Soil flux (g CO_2 m^2 d^{-1}) and belowground biomass (mg cm^{-3}). Symbols "+" indicate if ash was applied to the soil, while symbol "-" indicate that ash was not applied. (** and ***, statistically significant differences for P≤0.01 and P≤0.001 respectively with Pearson's test).

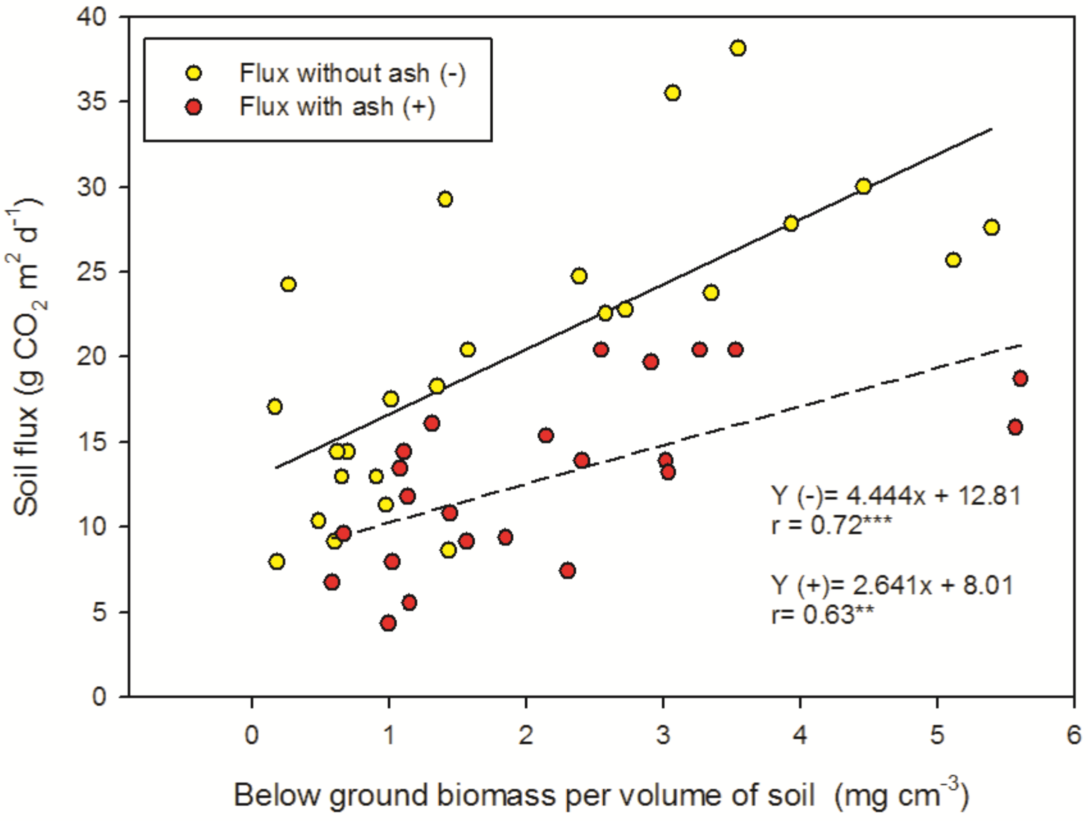


Fig. 2.5. Gross Primary Production (GPP) and Net Ecosystem Exchange (NEE) in term of net carbon saved (Mg ha^{-1}) from 2008 to 2011. Symbols "+" indicate if ash was applied to the soil, while symbol "-" indicate that ash was not applied.

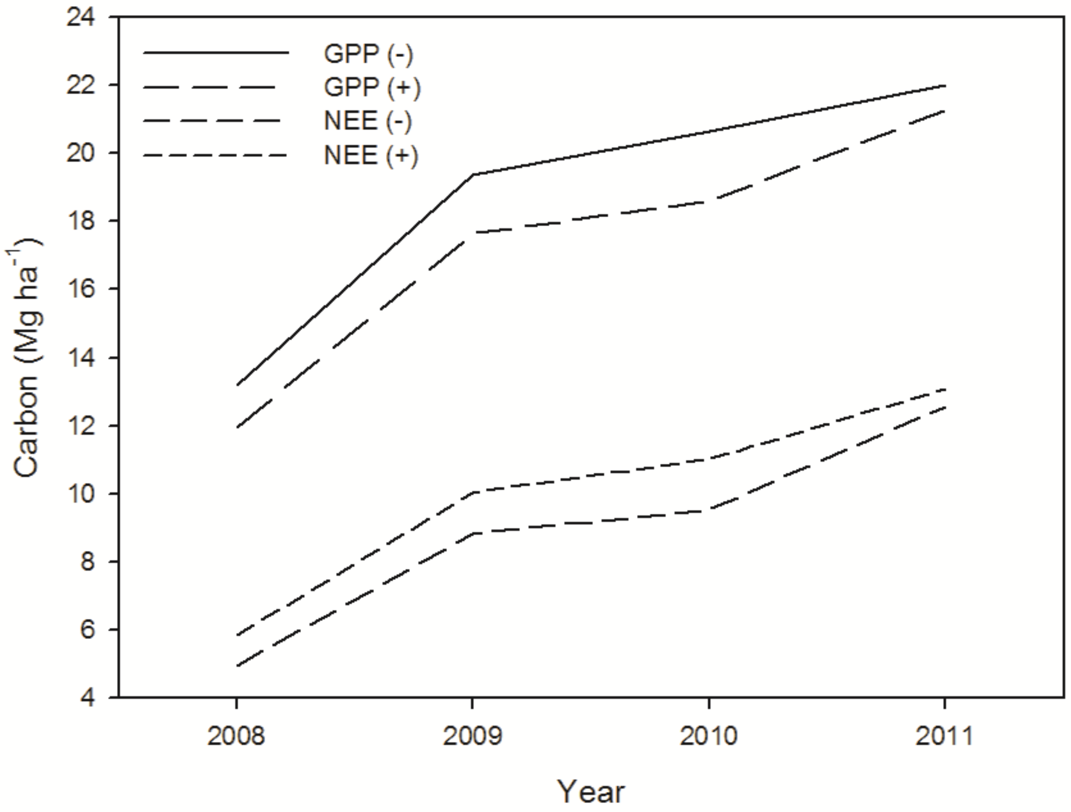


Figure 2.6. Soil moisture pattern along soil profile in 2011

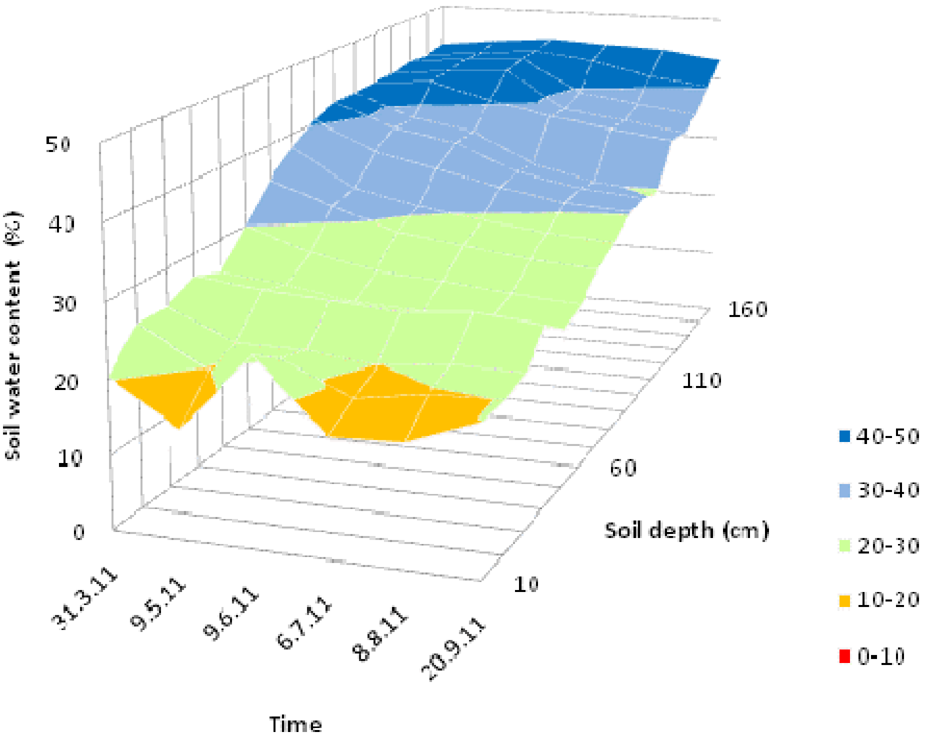
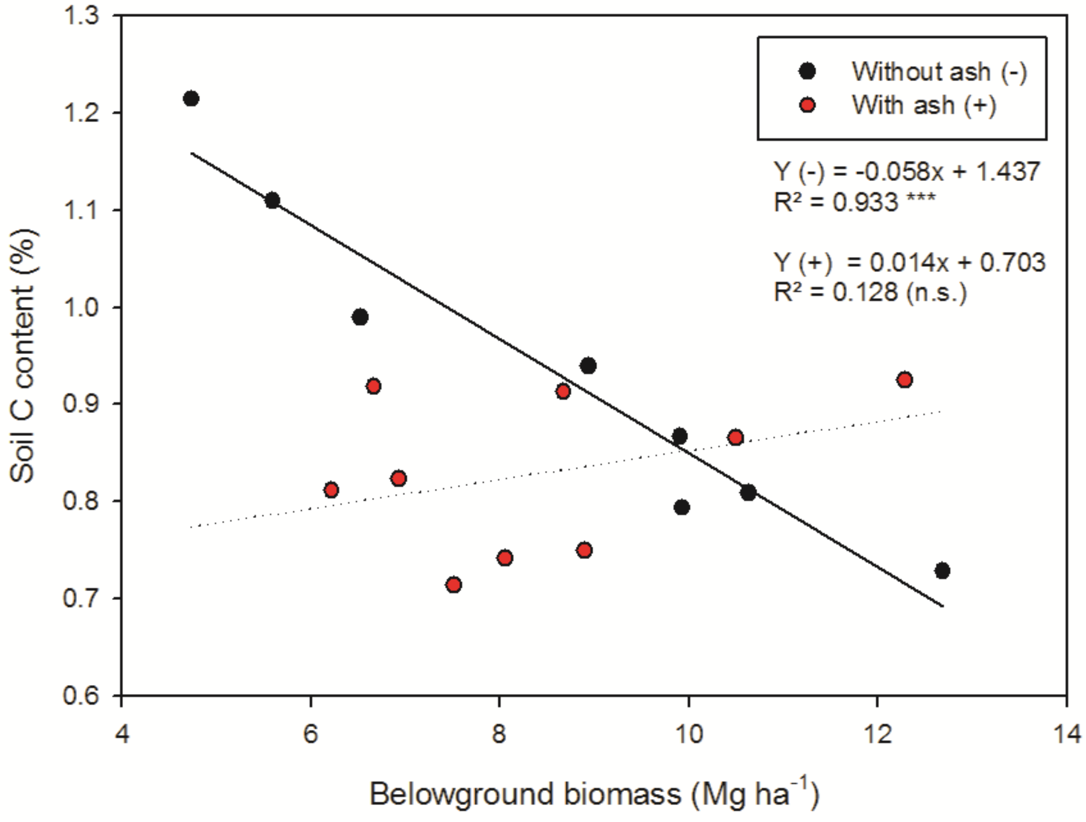


Fig. 2.7. Soil carbon concentration (%) in relation to belowground biomass (Mg ha^{-1}). Symbols "+" indicate if ash was applied to the soil, while symbol "-" indicate that ash was not applied. (n.s. = not significant, *** statistically significant differences for P≤0.001, with Pearson's test).



EXPERIMENT 2:

Land use change from grassland to *Miscanthus*: effects on soil carbon content and estimated mitigation benefit over six years

Abstract

Quantification of the GHG benefits of displacing coal with biomass depends on several factors including the impact of land use change on soil carbon stocks. To reduce competition for land between food and energy crops, the latter should be grown on lower grade land less suitable for the main arable crops. Semi-improved grasslands represent a major land resource for energy crops. In such grasslands, where soil organic carbon (SOC) levels can be high, there have been concerns that the carbon mitigation benefits of bioenergy from *Miscanthus* could be offset by losses in SOC associated with land use change. At a site in Wales (UK), we quantified the relatively short-term impacts (6 years) of four novel *Miscanthus* hybrids and *M. x giganteus* on SOC in improved grassland. After 6 years, using stable carbon isotope ratios ($^{13}C/^{12}C$), the average contribution of Miscanthus to total SOC content in the upper 15 cm was 14.0 ± 2.8% and 9.9 ± 2.0% in the under lying 15-30 cm layer and positively correlated to belowground biomass of different hybrids. Although substantial quantities of new carbon inputs from Miscanthus were detected there was no significant change in total SOC content; however, initial SOC decreased more in the presence of higher belowground biomass. We ascribed this apparently contradictory result to the rhizosphere priming effect triggered by easily available C sources. We interpret our observations to mean that the new labile C from *Miscanthus* has replaced the labile C from the grassland and, therefore, planting *Miscanthus* causes an insignificant change in soil organic carbon. The overall C mitigation benefit is therefore not decreased by depletion of soil C and is due to substitution of fossil fuel by the aboveground biomass, in this instance 73 to 108 Mg C ha^{-1} for the lowest and highest yielding hybrids respectively.

Keywords: bioenergy, stable carbon isotope, grassland, roots, *Miscanthus*, priming effect, SOC

Introduction

The European renewable energy directive 2009/28/EC (E.C., 2009) provides a legislative framework for reducing GHG emissions by 20%, while achieving a 20% share of energy from renewable sources by 2020. Energy crops, particularly perennial grasses, can contribute to both targets by replacing fossil fuel energy sources, as well as increasing soil organic carbon (SOC) sequestration, i.e. the long-term storage of carbon in soil. It has been estimated that in the next 50-100 years, a more sustainable land use could allow to mitigate 5 to 14% of global carbon emissions by SOC sequestration (Smith *et al.*, 2000; Lal, 2003; Paustian *et al.*, 2004; Smith *et al.*, 2007). Clifton-Brown *et al.,* (2004) estimated, with a simple model, that about 12 Mt C y^{-1} could be sequestered in EU-15 by growing *Miscanthus* on 10% of agricultural land, while Smith *et al.,* (2008b) indicated that SOC may account for up to 89% of the global potential mitigation for agriculture. Land conversion involving energy crops from surplus cropland resulted in 63% of the potential SOC sequestration in Europe (Smith *et al.*, 2000). It should be recognized; however, that SOC sequestration may increase only until an environmental equilibrium is reached or could even show a transient decrease followed by a complete recovery (West and Six, 2007). Converting grassland to *Miscanthus*, for example, was predicted to cause an initial SOC loss followed by a considerable carbon accumulation rate (Anderson-Teixeira *et al.*, 2009; Donnelly *et al.*, 2011). In a recent review on switchgrass, another dedicated perennial energy crop, Monti *et al*., (2012) reported that converting cropland to switchgrass generally increase soil C stock at a rate of 1 to 1.2 Mg ha^{-1}

y^{-1}. Moreover, SOC levels will change with soil tillage, climate, soil type and agricultural management (Lal, 2003). In an extended review, Smith *et al.,* (2008b) reported that -0.25 to 1.30 Mg C ha^{-1} y^{-1} could be mitigated by adopting sustainable cropping practices, and 1.07 to 1.46 Mg C ha^{-1} y^{-1} by converting cropland to native vegetation.

To avoid conflict with food production, energy crops need to be planted on lower grade land unsuitable for arable crops such as wheat (Fargione *et al.*, 2008). Land abandonment may lead to negative effects on biodiversity, causing wild fires and decreased soil fertility (Peco *et al.*, 2012); keeping energy crops out of arable lands may reduce or avoid indirect land use change issues (Lemus and Lal, 2005; Field *et al.*, 2007; Fargione *et al.*, 2008; Frische *et al.*, 2010). It was estimated that in England and Wales, there are 870,000 hectares of marginal and 'idle' lands which could be used for bioenergy crop production, excluding areas of high biodiversity value (Haughton *et al.*, 2009; Turley *et al.*, 2010). However, if the development of energy crops is not properly regulated with regard to land allocation and use of the most suitable crop species, then the environmental and social benefits of biofuels may be substantially diminished. This could include possible conflicts between food and energy production and the consequent social and ethical issues that may arise (Field *et al.*, 2007; Rathmann *et al.*, 2010; Haberl *et al.*, 2011b).

Inappropriate choice of land types and crop types may even increase GHG emissions from soils such that the environmental benefits of growing bioenergy crops are negated (Fargione *et al.*, 2008; Hillier *et al.*, 2009; Frische *et al.*, 2010; Powlson *et al.*, 2011). Therefore, extending knowledge and understanding through quantification of soil carbon stock change under energy crops on different soil types such as poor quality arable or grasslands is crucial for the successful development of these crops and is of strategic value to policy makers.

Belowground biomass is the primary vehicle for soil carbon storage (Kuzyakov, 2002; Nguyen, 2003; Kell, 2011); therefore, perennial grasses are expected to increase soil carbon, mineralisation processes being slower under minimal soil tillage and deeper root system (Lal and Kimble, 1997; Ma *et al.*, 2000b; Monti and Zatta, 2009). Nonetheless, it is still questionable whether high root biomass corresponds to a proportionally high SOC accumulation. Some studies found that a large root biomass can trigger faster metabolic processes by soil microorganisms thus accelerating soil organic matter decomposition and C turnover, namely 'priming effect' (Kuzyakov, 2002). Ultimately, a precise relationship between root biomass and SOC is not easy to establish as soil organic matter decomposition depends on several interacting factors including weather conditions, soil characteristics, soil moisture content, oxygen concentration, microbial population, and anthropologic factors such as soil tillage. For these reasons both losses and gains in SOC were observed in perennial energy grasses such as switchgrass (Frank *et al.*, 2004; Monti *et al.*, 2012) and *Miscanthus* (Hansen *et al.*, 2004; Clifton-Brown *et al.*, 2007). Soil carbon sequestration under pasture management and in converting land use from pasture to forest was investigated in a number of studies (Gifford *et al.*, 1992; Conant *et al.*, 2001; Guo and Gifford, 2002; Paul *et al.*, 2002; Cowie *et al.*, 2006). SOC changes in converting arable land to *Miscanthus* energy crop tend to increase SOC to level similar to perennial grassland (Kahle *et al.*, 1999; Hansen *et al.*, 2004; Dondini *et al.*, 2009a; Dondini *et al.*, 2009b; Zimmermann J *et al.*, 2011; Felten and Emmerling, 2012), whilst changes from pasture to a Miscanthus energy crop does has a small but ambiguous effect on SOC (Foereid *et al.*, 2004; Hansen *et al.*, 2004; Schneckenberger and Kuzyakov, 2007; Blagodatskaya *et al.*, 2011; Zimmermann J *et al.*, 2011). Based upon documented measurements of SOC changes, Hasting *et al.*, (2009) developed a simple model based upon the initial soil carbon before land conversion to *Miscanthus* and its annual harvested yield. Zenone *et al.* (2011) demonstrated using eddy covariance flux measurements that the process of converting grassland to soya crops, using herbicide to kill perennial grass and first tillage resulted in an extra respiration emission of between 1 to 4 Mg C ha^{-1} in the year of conversion. *Miscanthus* is one of the most promising candidate crops for energy-biomass across Europe (Lewandowski *et al.*, 2003; Tuck *et al.*, 2006; Stampfl *et al.*, 2007; Hastings *et al.*, 2009; Zegada-Lizarazu *et al.*, 2010).

In the present study we undertake to understand the fate of *Miscanthus* carbon input into former C3 grassland soil. We compared SOC stocks before and after a six-year cultivation of *Miscanthus* genotypes planted on former grassland. To understand root biomass to SOC relationships belowground biomass was quantified orthogonally: vertically, at two different soil depths, and horizontally at three different positions from the centre of the plant. By analyzing the ratio of stable carbon isotopes (O'Leary, 1988; Farquhar *et al.*, 1989) we estimated to what extent the priming effects counteracted the higher root biomass and finally we estimate the fate of soil carbon over the life cycle of a *Miscanthus* crop.

Materials and Methods

Experimental field site and trial set up

The field experiment was conducted near Aberystwyth in Wales, UK (52°26'N, 4°01'W, 34 m elevation). The soil is classified as a dystric cambisol and a dystric gleysol depending on spatial variation in drainage (FAO, 1988) with a stone fraction (particles >2mm) of approx. 15% (0-30 cm soil layer). Soil texture was 18% clay, 24% silt and 58% sand. Wilt point and field water capacity were estimated to be 150 and 350 mm, respectively using pedo-transfer functions (Campbell, 1985). This field has been part of the experimental station at Aberystwyth and has been used for trials for more than 30 years. It has been re-sown regularly (~5 years) with new grassland mixtures and used for silage and grazing tests. It has occasionally been used for arable plots of oats when flatter better land has been in short supply. Mature established perennial ryegrass was killed with Glyphosate (3 l ha^{-1}) in September 2004 and inversion tilled and re-sown in October 2004 with a ryegrass cover crop. This was subsequently sprayed with Atrazine (3 l ha^{-1}) on the 5th April 2005, one month before the time zero cores were taken (5 May 2005). The fragile biomass fragments were considered to be part of the soil and could not be separately quantified. The soil carbon stocks we determined at time zero are consistent with those expected of grasslands in this climate (Raich and Schlesinger, 1992; Parton *et al.*, 1995; Smith *et al.*, 2005). Four blocks of five 25 m^2 (6.67 m x 3.75 m) plots were marked out with 3m paths between the blocks. Plots were separated by an equivalent of one planting row.

A tank mix of Atrazine (3 l ha^{-1}) was applied on 5 April 2005 to destroy the grass sward (*Lolium perenne*) in the plot areas. Before planting soil cores were extracted on the 9th May 2005 (more below). On 24th May 2005, four novel *Miscanthus* genotypes (Hy1-4, JCB unpublished results) which had been cloned by *in vitro* tillering were planted as bare root transplants of approximately 2g fresh weight, in a similar manner to trees without inversion tillage using a narrow spade. The control genotype, *Miscanthus x giganteus* Greef et Deu (Greef and Deuter, 1993; Hodkinson and Renvoize, 2001) was planted similarly a few days later from fragments of clean overwintering rhizomes. Plants were planted directly (without inversion tillage) at a density of 2 plants m^{-2}. The carbon input from the propagules at planting was negligible (< 20 g DM m^{-2}). No fertiliser was applied over the six years, because soil analysis of the top 20 cm in November 2004 showed stocks were 6.7 Mg N (total) ha^{-1}, 34 kg P ha^{-1} and 120 kg K ha^{-1}, sufficient to cover the requirement of the crop (Cadoux *et al.*, 2012).

Determining stock changes in soil organic carbon

Soil cores were taken to determine bulk density and soil organic carbon (SOC) on the 6th May 2005, before the *Miscanthus* were planted (T_0), and again after 6 years on the 5th May 2011 (T_6).

At T_0 two plots in each of the randomised blocks were randomly selected for coring. In each plot five cores were taken in pre-determined gridded positions with a 7.62 cm diameter corer with straight internal walls. To avoid compressing the sample (resulting in erroneous bulk densities) the corer was inserted and pulled back out every 5 cm down to a depth of 30 cm. Short 5 cm core samples were collated into one bag to make up 0-15 cm and 15 to 30 cm layers.

The *Miscanthus* hybrids tested here form tussocks making it more challenging at T_6 to take representative cores which can be scaled up to Mg SOC per hectare. To address this we developed a more sophisticated sampling strategy that involved taking multiple cores at different positions with each plot. The coring positions were inter-row (C_i), edge of the plant (C_e) and centre of the plant (C_c) (Fig. 3.1). The tussock mass at C_c and C_e is made up of lignified rhizomes and stem bases which are too tough for hand coring. Based on field measurements, C_c, C_e and C_i accounted for 8.1%, 24.5% and 67.4% of the total field area, respectively. The soil column cylinder auger (Eijkelkamp, Giesbeek, The Netherlands) has been developed to take undisturbed soil samples. This corer has a cutting ring with a diameter of 8.5 cm and a depth of ~2cm. After the ring, the internal diameter of the corer is slightly wider allowing the core to be supported, with minimal core sample compression. This allowed entire cores to be extracted from one insertion.

Ideally, soil bulk density would be constant for comparing C mass over time (Ellert *et al.*, 2001; Kimble *et al.*, 2001); however, it may change considerably with soil moisture, depth and physical properties (Harte, 1984; Ellert *et al.*, 2001). Moreover, due to soil tillage, soil mass may decrease from grassland to arable lands (Ellert and Bettany, 1995). By comparing soil height within the plant (C_c and $C_{e)}$ and outside of the plant (C_i) it was estimated that rhizome growth displaced soil by 1-2 cm. To offset rhizome growth and resulting soil displacement, we sampled 1 and 2 cm deeper cores at C_e and C_c, respectively. At C_i, the cores were taken without adding centimetres assuming that bulk density did not change appreciably as no tillage was made during the six year study (Powlson *et al.*, 2011). Cumulative mass coordinates is preferred to obtain a consistent comparison (Gifford and Roderick, 2003), although other authors have used spatial coordinates (Zan *et al.*, 2001).

In both T_0 and T_6 all samples were air dried until constant weight. In 2011 soil and belowground biomass were separated by hand. The air-dried soil was then passed through a 2-mm sieve to remove stones and any remaining fine roots the latter were added to belowground biomass. Belowground biomass was oven dried at 40°C to constant weight.

The *Miscanthus* contribution to soil carbon sequestration (F) was calculated using the following equation (Balesdent *et al.*, 1987):

$$F = \frac{(\delta_n - \delta_0)}{(\delta_r - \delta_0)}$$

where δ_0 and δ_n are soil organic C isotope abundance before planting of *Miscanthus* and after six years cultivation respectively; δ_r is the carbon isotope abundance of cryo-milled *Miscanthus* roots and rhizomes (three repetitions per hybrid). Soil carbon concentration (%) and stable carbon isotope ratio ($^{13}C/^{12}C$) was determined by an isotope ratio mass spectrometer (ANCA-MS system, ltd) in 250/300 mg soil samples, while the inorganic soil C content was determined by acidification of 3 g soil samples in 30 mL of HCl (1 mol/L) (Van Kessel *et al.*, 2000). Soil organic content (SOC) was calculated from the difference of total and inorganic soil carbon. The bulk density was calculated on the sieved dried soil (Ellert *et al.*, 2001). Carbon mass (M_c, Mg ha^{-1}) per unit volume was then calculated by multiplying soil bulk density (BD, Mg m^{-3}), horizon thickness (T, m) and C concentration (C_{cont}, kg Mg^{-1}) as given by Ellert *et al.* (2001):

$$Mc = BD * C_{cont} * D * 10000\ m^2\ ha^{-1}$$

The contribution of harvested biomass to CO_2 mitigation (C_s) compared with coal was calculated using the following equation:

$$C_s = LHV_m * DM * 0.0\ 33 \ \text{kg C ha}^{-1}$$

where LHV_m is calorific value of *Miscanthus* (17.6 MJ kg^{-1}) (Collura *et al.*, 2006), DM is *miscanthus* dry matter (kg ha^{-1}) and 0.033 kg C MJ is the energy intensity of coal (Hastings *et al.*, 2009).

Data analysis

All data were subjected to the analysis of variance (ANOVA). When ANOVA revealed significant differences ($P \leq 0.05$), the Tukey's LSD test was used to separate means (CoStat v6.204, Monteray, USA). In text means are presented with ± standard deviation unless otherwise specified.

Results

Belowground biomass

The term belowground biomass as used here refers to all roots and rhizomes. As expected, in the 0-15 cm layer, the belowground biomass per volume of soil, or the density of belowground biomass, decreased from soil cores taken from C_c (closest to the plant) to C_i (furthest from the plant), were clearly different at C_c, C_e and C_i (Fig. 3.2A). Hybrids did not generally differ in belowground biomass density; the only exception was at C_c between Hy1 and Hy4 (Fig. 3.2A). *Miscanthus* genotype showed some significant differences even in the deeper layer, however these differences were not as large as in the upper one (Fig. 3.2B).

The soil cores Cc, C_e and C_i represent 8.2, 24.6 and 60.7% of an hectare, respectively. Therefore, given the belowground biomass densities, we calculated the belowground biomass (Fig. 3.2C, D). In the upper layer, belowground biomass still showed the highest values in C_c, while unlike density, C_e and C_i showed a similar biomass values (Fig. 3.2C). By contrast, at the deeper layer, the belowground biomass exhibited a reverse trend to biomass density, thus resulting in a quite similar biomass among C_c, C_e and C_i over the 0-30 cm soil layer (Fig. 3.2D).

Estimating the soil organic carbon content

The development of roots and rhizomes, especially in young plants, can be expected to significantly reduce soil bulk density (BD); therefore, to collect an equivalent soil mass after six years we sampled 1- and 2-cm longer soil cores in correspondence of C_e and C_c, respectively. The results showed that BD significantly decreased after six years (Fig. 3.3 inset). However it was only significant at locations C_c, and C_e (Fig. 3.3). Based on the assumption that average biomass density would not appreciably change between two soil profiles of 17 and 18 cm (i.e. 15-32 and 15-33 cm soil layers) we recalculated the equivalent soil mass considering a soil core of 18 cm. Since this equivalent soil mass was not different from T_0 we added the amount of belowground biomass equivalent to that contained in one cm of soil according to the real average belowground biomass (that measured between 15 and 32 cm of depth) (Fig. 3.3).

Although soil C depletion caused by land use change from grassland to *Miscanthus* was evident in all hybrids with a range between -5 (Hy1) and -10 (Hy2) Mg C ha^{-1} (Fig. 3.4), that decrease was not statistically significant when compared to T_0. Therefore, based on field measurements in which C_c,

C_e and C_i accounted for 8.2, 24.6 and 67.4% of total area, we cannot say that *Miscanthus* decreased SOC after six years (Table 3.1). Significant SOC decreases were only found at C_e and C_c for Hy2 (Fig. 3.4).

By the use of carbon isotope technique we could determine the *Miscanthus*-derived C, i.e. the contribution of *Miscanthus* to total SOC after six years. All the hybrids contributed a similar amount of C, which decreased from C_c to $C_{i,}$ averaging 14% and 9.9% in the upper and deeper layers, respectively (Fig. 3.5). Considering the three cores within each plot, Miscanthus-derived C was higher at C_c (18.3%) than Ce (12.8%) and C_i (10.9%) in the upper layer, and in the deeper layer only the C_i samples were significantly lower (Fig. 3.5). *Miscanthus*-derived C positively correlated with belowground biomass (Fig. 3.6); however, the statistically insignificant change of total SOC (Fig. 3.4) might lead one to expect a triggering effect on soil respiration rates and C turnover by higher root and rhizome deposition or by exudates and organic substances produced by living roots, namely the rhizosphere priming effects (Fig. 3.6). In Figure 3.7, the absolute amount of SOC in the C_c upper layer of soil after 6 years correlates negatively with the below ground biomass providing some evidence for this priming effect as the C_3 C is apparently replaced by the C_4-C faster, but this is not reflected in the overall SOC values.

Discussion.

SOC quantification by coring

For practical reasons soil sampling in row crops is often simplified by only sampling between the rows (Zan *et al.*, 2001; Frank *et al.*, 2004; Monti and Zatta, 2009). To obtain the ‘overarching’ SOC estimates reported in this paper we developed a novel sampling strategy to overcome the technical challenges of representative sampling in a tussock forming plant such as *Miscanthus*.

Our three core method (Fig. 3.1) with proportional representation of plant centre, plant edge and inter-row allows defensible up-scaling to units such as Mg of SOC and below ground biomass per hectare. We developed this method to avoid significant damage to the plots caused by digging out entire quadrates (Clifton-Brown *et al.*, 2007). We intend to make further similar samplings at T_{12} and possibly T_{18} so that we can understand carbon dynamics over the likely useful lifespan of the crop (currently estimated to be up to 20 years).

Evidence for microbial 'priming' effects

A positive correlation between belowground biomass and SOC might be expected (Ma *et al.*, 2000b; Lemus and Lal, 2005; Field *et al.*, 2007; Monti and Zatta, 2009). The *Miscanthus* hybrids in our experiment accumulated significantly different quantities of belowground biomass, but this was not reflected in the total SOC after six years. Curiously the genotype with the highest belowground biomass (Hy2), led to the highest SOC reduction from the values measured at the start of the trial though this was significant only at C_e (Fig. 3.4). The absence of a significant correlation between increase in SOC and belowground biomass might be explained by a triggering effect of belowground biomass on soil metabolism, namely the 'rhizosphere priming effect'. This attempts to explain the faster decomposition of SOC by micro-organisms in response to a higher fresh organic matter supply (Kuzyakov, 2002, 2006). This hypothesis seems to be corroborated by a significant correlation between belowground biomass and *Miscanthus*-derived C in C_c upper layer (Fig. 3.5), suggesting that a priming effect occurred in the direct vicinity of living roots (Kuzyakov, 2002). SOC depletion observed in Fig. 3.6 could be explained by increased priming effect due to high organic matter (Mary *et al.*, 1993; Asmar *et al.*, 1994) causing increased mineralization which has been reported to reach up to 400% (Kuzyakov, 2002). Therefore, soil respiration may correlate with biomass deposition rates, and where belowground biomass accumulated in greater amounts it was also degraded more rapidly. It is unclear why the extent of this priming effect varied with *Miscanthus* genotype; for example Hy4 produced higher root biomass than Hy2, 65.5 and 58.6 mg m^{-3}, respectively but the latter showed a higher contribution (+5%) to SOC. A possible explanation could be that priming effects were driven by variable amounts of more labile organic substances (e.g. polysaccharides, carbohydrates and celluloses) or recalcitrant (e.g. lignin, waxes and suberins) carbon pools deriving from belowground biomass (Nguyen, 2003; Jones and Donnelly, 2004; Fioretto *et al.*, 2005; Kuzyakov and Larionova, 2005; Jastrow *et al.*, 2007; Lal, 2008a). In conclusion, possibly due to priming effects, belowground biomass seems, by itself, not sufficient for predicting SOC dynamics. Further studies are required to understand better the proportion of autrophic and heterotrophic soil respiration underlying our observations in SOC dynamics.

Soil carbon stocks and the saturation point

Another possible explanation of the unexpected association between SOC variation irrespective of genotype and belowground biomass could be the saturation of SOC level, implying that C stock was saturated with respect to C inputs (Freibauer *et al.*, 2004; Stewart *et al.*, 2007; Powlson *et al.*, 2011). The potential for soil to sequester C is linked with regional climate, soil properties and land management (West and Six, 2007) and it is know that grasslands tend to have high SOC content (Guo and Gifford, 2002). This hypothesis seems, however, in contrast with the considerable variation of SOC found in C_i, C_e and C_c, that showed SOC values from 2.2% to 3.3% in the upper layers, and from 0.8% to 2.1% in the deeper layers thus suggesting that C stock in the soil was not saturated. We might expect, in subsequent samplings after longer time periods (e.g. 12, 18 years) that SOC levels would correlate with differences in carbon partitioning of the genotypes. For example, in Denmark soil organic matter remained relatively constant for the first 11 years following establishment with *M. x giganteus* on a grassland site (Foereid *et al.*, 2004).

Impacts of land use change from grassland to Miscanthus on SOC

There is evidence that conversion of some land uses to energy crops, particularly the annuals, may cause significant SOC losses (Lal, 2004b; Fargione *et al.*, 2008; Searchinger *et al.*, 2008). The conversion of natural to agricultural ecosystems, for example, led to a SOC depletion of 60% in temperate regions, and up to 75% in tropical regions (Lal, 2004b). A decrease of SOC was also found when energy crops were planted on forest lands (Murty *et al.*, 2002), peatlands (Page *et al.*, 2002; Inubushi *et al.*, 2003), savanna (Fargione *et al.*, 2008) or former grasslands (Follett, 2001; Tilman *et al.*, 2006). However, St. Clair *et al.*, (2008) included land use change and its associated soil carbon change in a life cycle analysis of energy crops and suggested a neutral effect of planting *Miscanthus* on grassland. Anderson-Teixeira *et al.* (2009) reported that grassland conversion to *Miscanthus* significantly reduced SOC in the first 2-3 years because of ploughing and soil tillage; however, SOC was completely recovered in subsequent years. In contrast, perennial grasses planted on arable lands considerably increased soil carbon reserves (Kort *et al.*, 1998; Field *et al.*, 2007; Lee *et al.*, 2007; Fargione *et al.*, 2008; Anderson-Teixeira *et al.*, 2009; Hillier *et al.*, 2009; Monti and Zatta, 2009). SOC increased up to 18% under a 3 year-old switchgrass stand (Zan *et al.*, 2001), and up to 29% under a 16 year-old *Miscanthus* stand (Hansen *et al.*, 2004) both planted on croplands. In the future, with increasing population and food production requirements the main land resource for energy crops will be lower grade agricultural land often not used for arable crops (Haberl *et al.*, 2011a).

In Wales and England it is estimated that 870,000 hectares of marginal and ‘idle’ lands, excluding areas of high biodiversity value, are potentially available for bioenergy crop production (Turley *et* *al.*, 2010). Data from the present six year study, will reassure policy makers that planting on these semi-permanent grasslands with a range of *Miscanthus* genotypes did not deplete SOC significantly over the 6 years. It is highly unlikely with increasing stand age that SOC levels will deplete relative to T_0, and following the trends from arable land, it is likely there is some scope for SOC increases up to the soil type-environmental equilibrium (Jones and Donnelly, 2004; Powlson *et al.*, 2011). There is undoubtedly some value of this small but significant carbon sequestration sink, which we hope to quantify in years to come.

The immediate carbon benefits of *Miscanthus* cultivation are the substitution of fossil carbon sources when the crop is used to produce energy. *Miscanthus* biomass is a solid fuel, and therefore it is reasonable to use it to substitute coal. Combining accurate yield records from annual harvests made in February (unpublished) and the calorific value of these *Miscanthus* genotypes (Hodgson, unpublished, but it is close to published values of 17.6 MJ kg^{-1} (Collura *et al.*, 2006)) we can calculate the carbon substitution benefit. These figures show for the five genotypes over the six years that the 'coal' carbon substitution ranged from 70 (Hy3) to 103 (Hy1) Mg CO_2 ha^{-1}. Adding in the belowground C content (SOC and roots and rhizomes), the total C saved ranged from 73 (Hy3) to 109 (Hy2) Mg CO_2 ha^{-1}. We conclude the carbon benefit of growing *Miscanthus* as an energy crop on improved grasslands in the UK was largely from fossil fuel substitution. This study was over six years, growing *Miscanthus* for longer periods may slightly increase the role of soil carbon sequestration, but is unlikely to be significant in the overall carbon mitigation benefit when planted on improved grassland in the UK.

Conclusions

We are not aware of previous studies addressing the effects on SOC variation of land use change from grassland to Miscanthus. The present study shows that SOC of a grassland was not affected by a 6-year cultivation of Miscanthus. Moreover, different Miscanthus genotypes showed variable root biomass development, but these differences did not reflect in SOC variations. We conjectured that this was related to a priming effect, that is a faster root biomass degradation by soil microorganisms triggered by a higher amount of available biomass, an hypothesis which seems consistent with the higher contribution of *Miscanthus*-derived carbon to SOC stock, as evidenced through carbon isotope ratios, by genotypes showing the highest root biomass.

TABLES

Table 3.1. Analysis of variance: effects of depth and hybrid, between T_0 and T_6, on measured soil parameters (* and **, statistically significant differences for P≤0.05 and P≤0.01, respectively). BD, bulk density; SOC, soil carbon content; C_{mis}, *Miscanthus* derived C; CV (%), coefficient of variation. Depth x hybrid interaction was never significant.

Soil core position	Soil parameter	Depth	Hybrid	CV
Centre of the plant (C_c)	BD	*	n.s	11.0
	C_{mis}	**	**	2.8
	SOC	**	n.s.	12.4
Edge of the plant (C_e)	BD	n.s.	n.s.	9.4
	C_{mis}	*	**	1.9
	SOC	**	n.s.	11.8
Interrow (C_i)	BD	n.s.	n.s.	8.3
	C_{mis}	n.s.	**	1.3
	SOC	**	n.s.	12.9

FIGURE EXPERIMENT 2

Fig. 3.1. Example of soil core samplings taken in each plot: interrow (C_i), edge of the plant (C_e) and centre of the plant (C_c). Photo 23 May 2012.

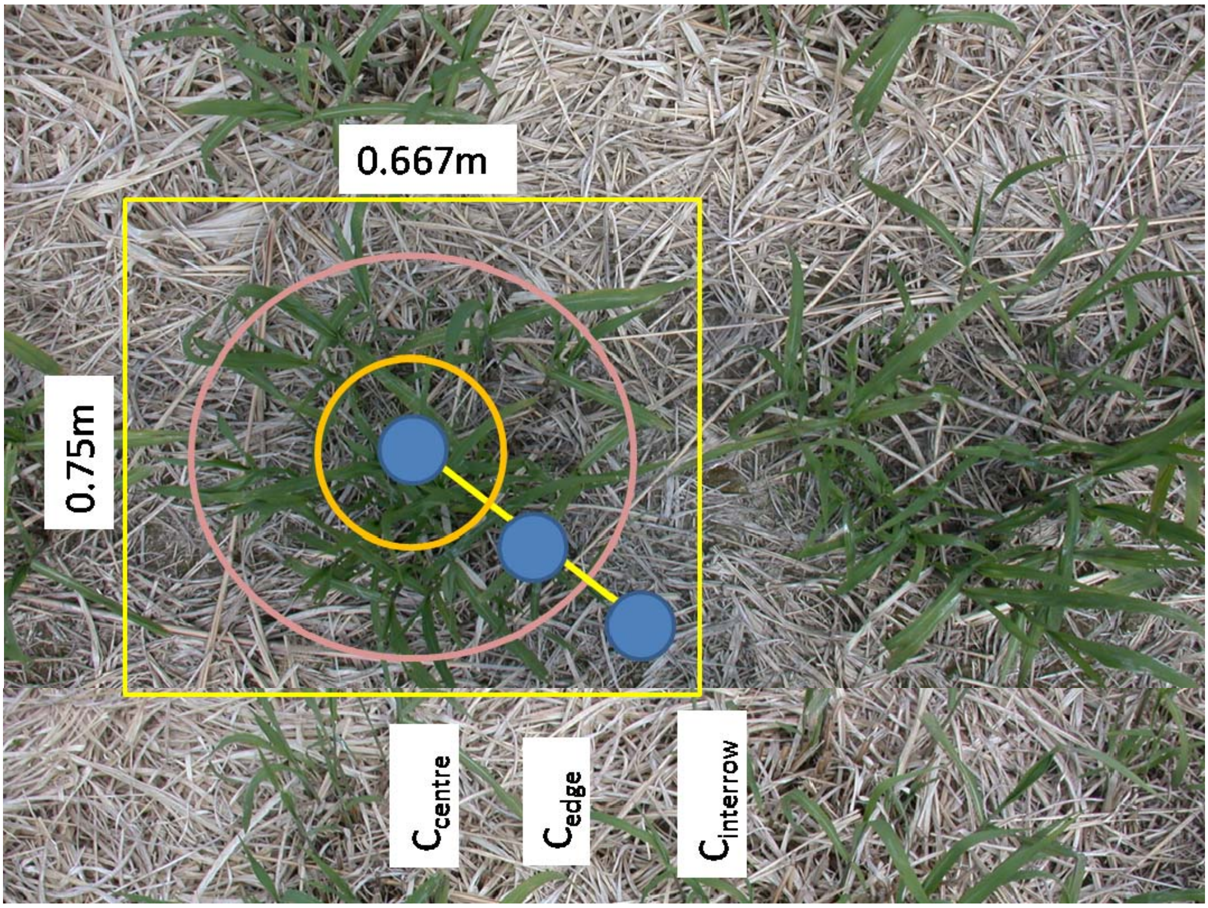


Fig. 3.2. Belowground biomass (roots plus rhizome) of *Miscanthus* hybrids at C_c, C_e and C_i (centre, edge and interrow, respectively, see Fig. 1) at two soil depths: 0-15 (2A) and 15-30 (2B) cm. The belowground biomass per hectare contributed by C_c, C_e and C_i (Fig. C, D) were calculated using the corresponding areas represented by each core position in one hectare (8.2%, 24.6% and 67.2%, in that order). Different lower case letters show statistically different means (Tukey's LSD test, $P \le 0.05$) within a core position. NS = not significant.

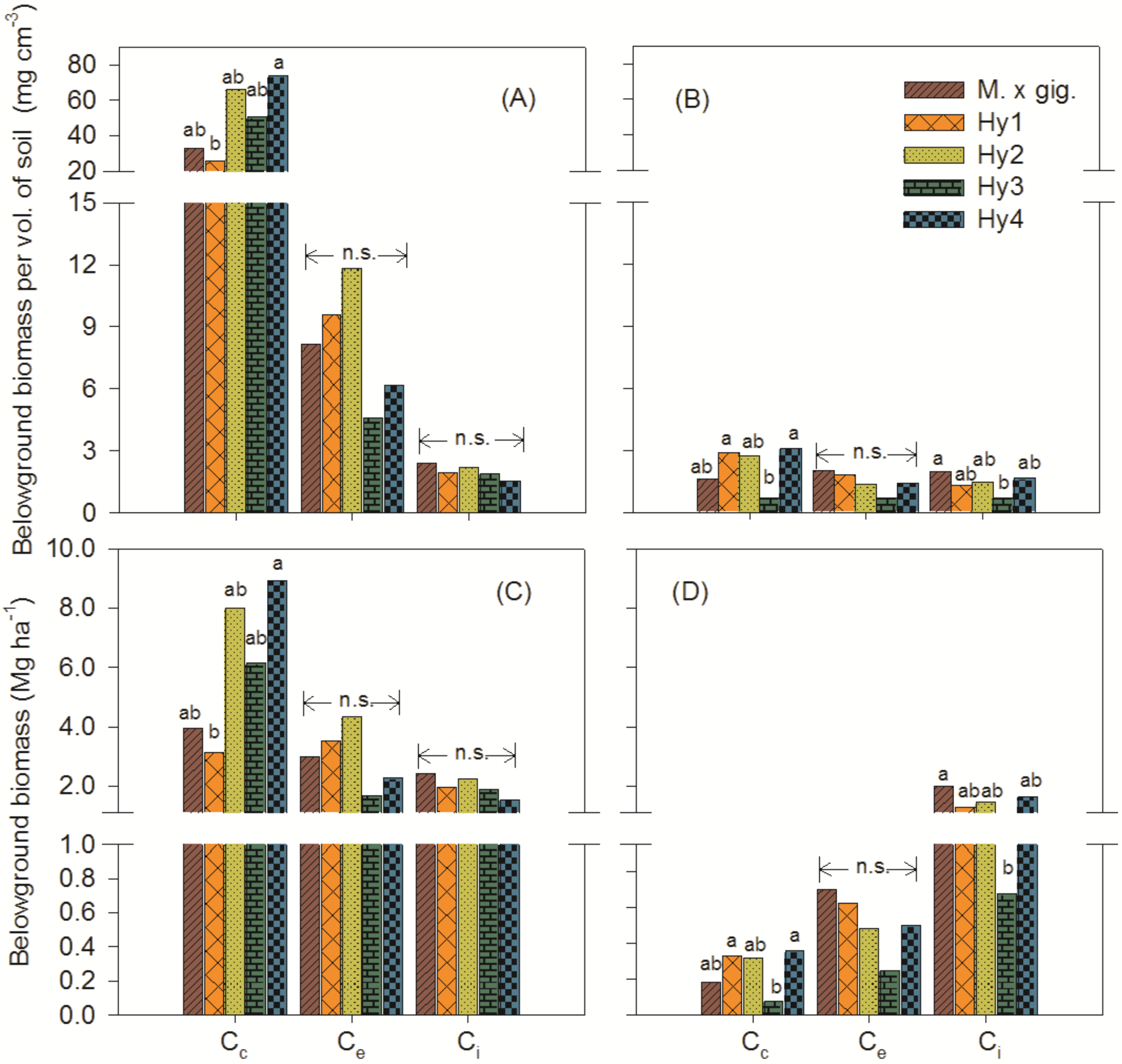


Fig. 3.3. Soil bulk density of the cropland (T_0) and after 6 years of *Miscanthus* (gray graph) at C_c, C_e and C_i (centre and edge of the plant and interrow, respectively) upper layer. The equivalent soil mass (red graph) refers to the real amount of sampled soil as 1 and 2-cm longer cores were taken at C_e and C_c, respectively, to offset the decrease of bulk density due to *Miscanthus* root and rhizome development. The inset graph shows the effect of the belowground biomass development on bulk density. Different letters indicate statistically different means (Tukey's LSD test, P≤0.05).

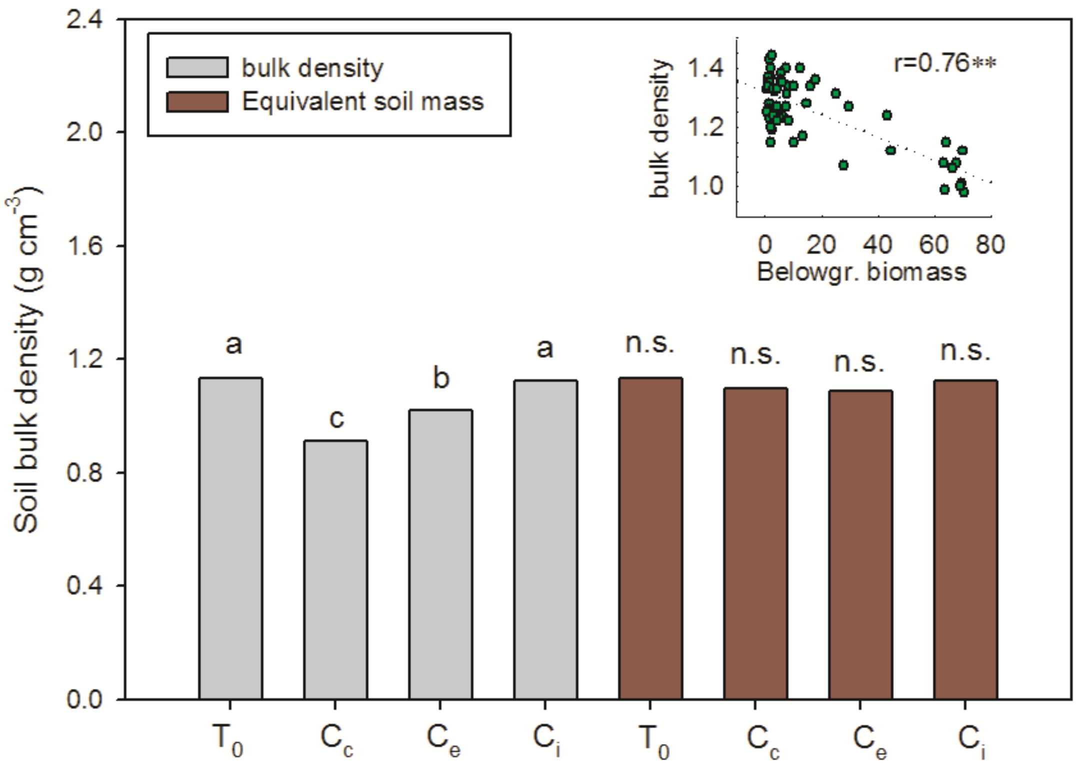


Fig.3.4. Differences between soil organic carbon (SOC, Mg ha^{-1}, 0-30 cm) after six years of *Miscanthus* hybrids (M. x gig. and Hy1 to 4) and SOC of the grassland just before *Miscanthus* plantation in the same profile (T_0). C_c, C_e and C_i indicate the amount of SOC at plant centre, plant edge and interrow, respectively.

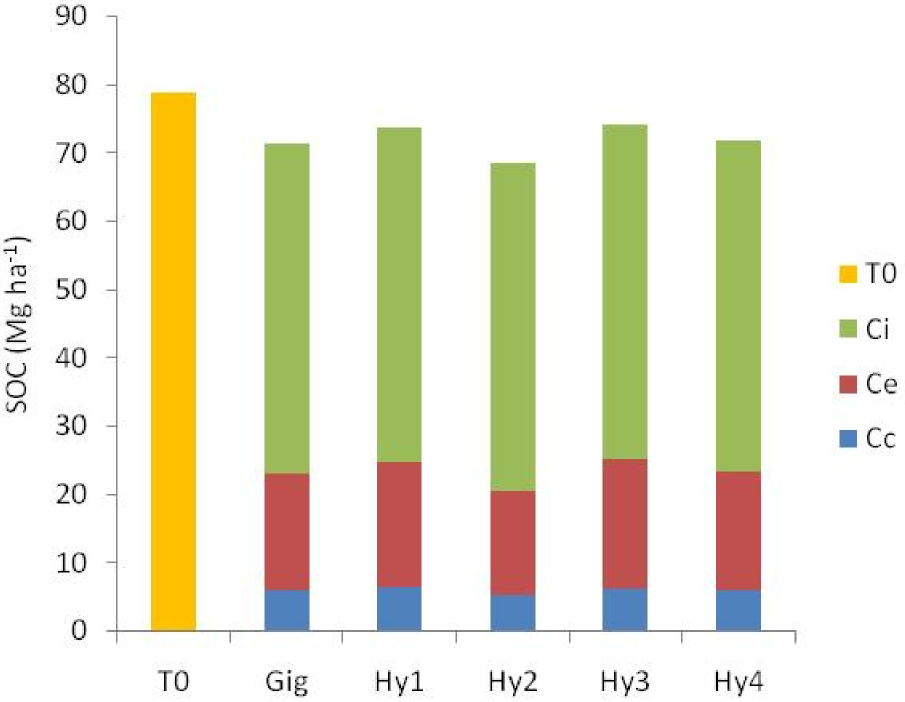


Fig. 3.5 *Miscanthus* derived C (%) in the upper (0 to 15 cm) and deeper layer (15 to 30 cm). C_c, C_e and C_i indicate centre and edge of the plant and interrow, respectively. Different letters indicate statistically different *Miscanthus* C-derived in the two soil layers (Tukey's LSD test, P≤0.05).

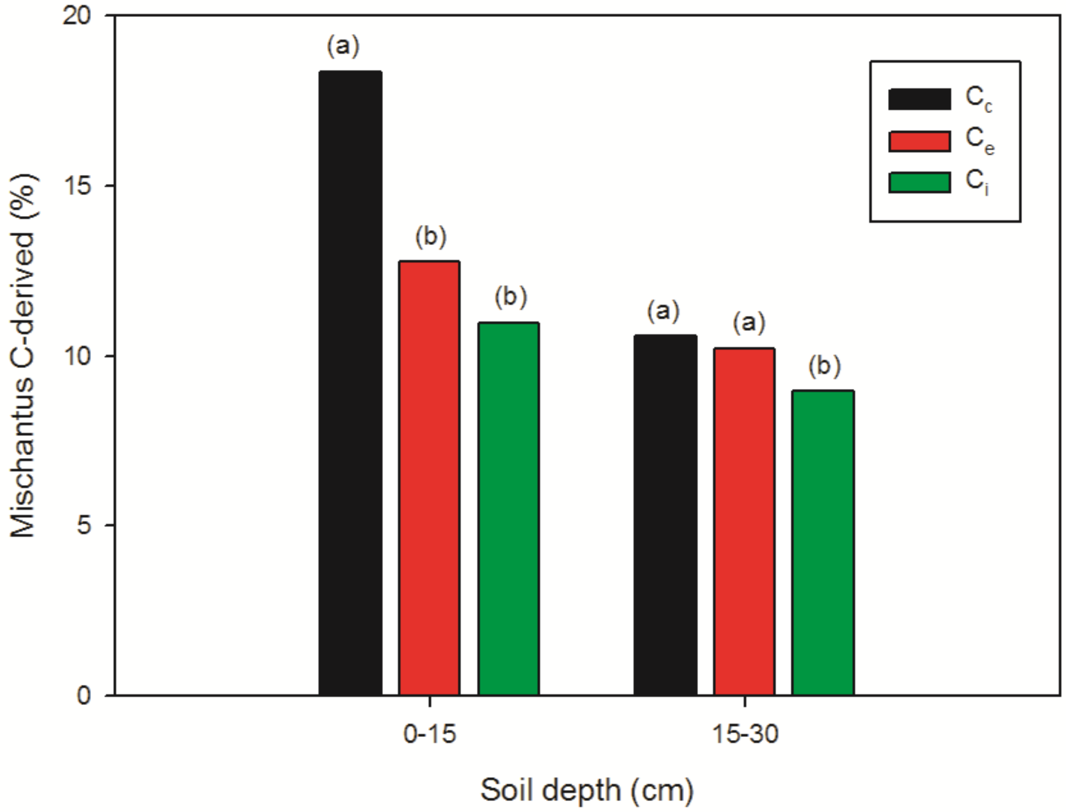


Fig 3.6. Correlation between belowground biomass and *Miscanthus* contribution to total SOC (C_{mis}) in the upper layer (0-15 cm) after six years of five *Miscanthus* genotypes (*M.* x *giganteus*, Hy2 to 4) grown in a former grassland in Aberystwyth, Wales, UK.

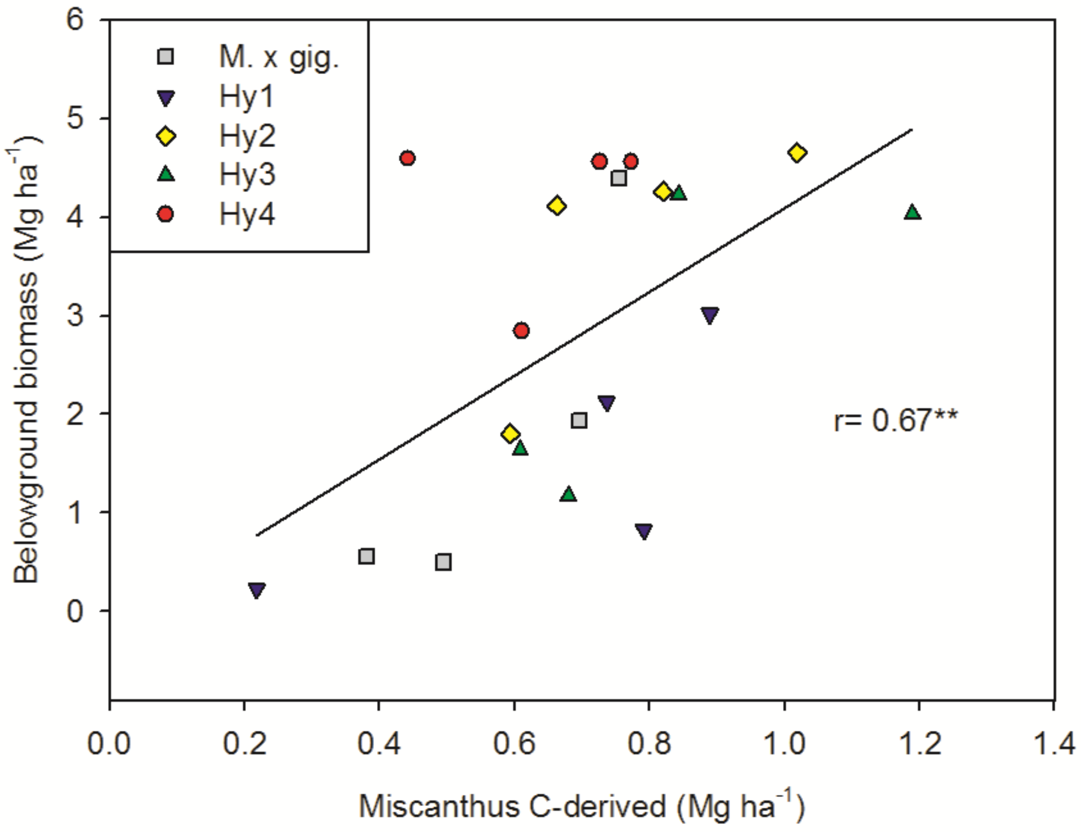
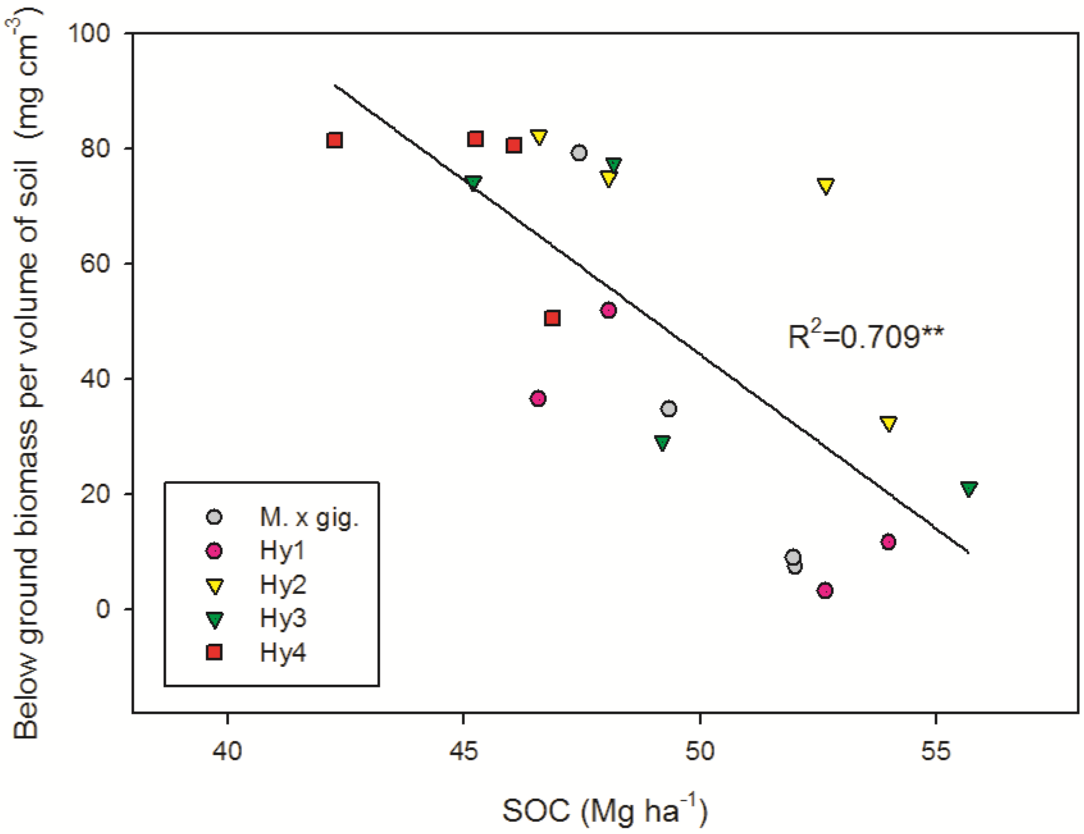


Fig. 3.7. Correlation between belowground biomass intensity (mg cm^{-3}) and SOC (Mg ha^{-1}) in the upper layer (0-15 cm) after six years in the centre of the plant (C_c).



FINAL CONSIDERATIONS

Increasing CO_2 concentration in the atmosphere is mainly due to the increased use of fossil fuels (Fig. 1.1; Boden *et al.* (2012)), but also agriculture (Smith, 2008) and ecosystems disturbances (Houghton and Woodwell, 1989) has significantly contributed. Leaching and land use change that are the main causes of SOM mineralization (Fargione *et al.*, 2008; Lal, 2008b; Smith, 2008) and therefore increased CO_2 emissions. Soil contains twice the C of the atmosphere (fig. 1.2), and historically releases of 40-90 Pg of C into the atmosphere have been estimated (Smith, 2008). Agricultural lands cover about 50% of Earth's surface, being responsible for 10-12% of GHG emissions (Monti *et al.*, 2012). Appropriate agricultural practices may help to reduce CO_2 emissions. Conservative tillage system, such as minimum and no-tillage, for example can contribute to reduce CO_2 emissions thanks to decreased leaching and SOM mineralization (Paustian *et al.*, 2000; Six *et al.*, 2000a). Such reductions favor SOC accumulation which in turn contribute to the stabilization of aggregates and SOM accumulation in deeper soil layers (Lal and Kimble, 1997).

Croplands and marginal lands are considered to be the ecosystems with the greatest potential for SOC accumulation (Powlson *et al.*, 2011). By introducing perennial energy crops in such land, the change in land uses can contribute to the reduction of GHG emission through C storage into the soil and conservative agricultural practices which fit well to the characteristic of these crops (Anderson-Teixeira *et al.*, 2011; Powlson *et al.*, 2011). In this thesis two cases of land use change were studied: 1) from cropland to switchgrass and 2) from marginal grassland to Miscanthus.

In the first study, the change in land use from cropland to switchgrass resulted in 1.9 Mg ha^{-1} y^{-1} of stored C confirming results obtained by other authors under different environmental conditions, such as Canada (Zan *et al.*, 2001), South Dakota (Lee *et al.*, 2007), Nord Dakota (Frank *et al.*, 2004) and Nebraska (Varvel *et al.*, 2008; Follett *et al.*, 2012). A large amount of organic carbon was stored in deep layers as a consequence of the large root biomass developed in those layers (table 2.3). The changes in land use; however, resulted in a greater increment in belowground biomass in the first 40 cm of soil in the soil profile (+68%) than in lower layers (+54%) (fig. 2.2). Significant increments in SOM were found only in deeper layers (table 2.3). So, there was not a direct relationship between belowground biomass accumulation and SOC storage. It is possible; however, that the large belowground biomass developed in shallow layers stimulated soil microbiological activity causing the priming effect (Kuzyakov, 2006). The hypothesis of priming effect could be corroborated by the increased soil CO_2 flux with time (fig. 2.3) and by the soil switchgrass C-derived in the upper layer where more fresh organic is accumulated, higher rhizo-deposition and gas exchange occurs. Switchgrass contribution to C turnover was relevant only in the first 5 cm of soil (20%) and quickly decreased in deeper layers (table 2.4). So, after 5 years of land use change the part of SOM most recalcitrant and stable was not affected by turnover. From the above discussion it is possible to say that SOC increment was mainly derived from labile C. If the field was returned to cropland, 60% of root system would be subject to rapid mineralization because of plowing (~30 cm) so, in the short term, the soil C sequestration may be limited. It should also be said that SOM stabilization can vary from 10 to 100 years (Jones and Donnelly, 2004), so long term monitoring (e.g. 10, 15, 20 years) would be recommended.

Agriculture causes 50% of CH_4 and N_2O global emissions that, after CO_2, are the main factors affecting global temperature rise (Forster and Taylor, 2006). Their emissions can be reduced by decreasing nitrogen fertilization (Metz *et al.*, 2007). In our study at Poggio Renatico, Italy (Experiment 1) besides looking at the effects of land use changes we evaluated the effects of three levels of nitrogen fertilization (0, 50 and 100 kg N ha^{-1}). It was clearly seen that switchgrass did not require nitrogen fertilization during establishment period (first 2 years), while later on 100 kg N ha^{-1} maintained constant productivity over time (fig. 2.1). At the last sampling year, lower fertilization rates (50 kg N ha^{-1}) showed slight decrements in productivity, but there were not statistically significant. At belowground level no significant differences were found between fertilization treatments, neither significant differences were found on soil CO_2 fluxes. These results confirm that switchgrass is a low input crop and that it maintains high levels of productivity with low fertilization rates (between 50 and 100 kg N ha^{-1}). This is especially true if the harvest is done at the end of growing season, i.e. when a larger proportion of the nutrients have been translocated to the roots (Dohleman *et al.*, 2012).

Combustion, which is how most switchgrass is currently used for energy production, produce ashes as a by-product that can be used as soil amendment and / or fertilizer (Perucci *et al.*, 2006). Considering that switchgrass ash content is on average 5% (Monti *et al.*, 2008) and hypothesizing a productivity of 10 Mg ha^{-1} the amount of ash amendments that can be returned to the soil is 0.5 Mg ha^{-1}. In the present study such amount was applied in combination with nitrogen fertilization in order to simulate recycling of mineral elements into the soil. Our results showed that ashes did not influence above- and belowground biomass, and inhibited soil CO_2 flux in the warm-summer months. Reduction in soil respiration is probably due to enhanced soil microbiological activity, which inhibits the heterotrophic soil respiration component (Perucci *et al.*, 2006). Soil respiration is one of the main sources of C in the atmosphere and its reduction due to the addition of ash (1 Mg C ha^{-1} y^{-1}) as soil amendments can have significant effects on the global C cycle (Schlesinger and Andrews, 2000). The quality of ash, however, plays an important role because of the risk of increasing the presence of heavy metals in the soils. Therefore more detailed longer-term studies are definitely needed. Moreover, it was confirmed that switchgrass is an environment friendly crop because the Net Ecosystem Exchange (NEE) obtained from the balance between C input (above-, belowground biomass and autotrophic respiration) and C output (heterotrophic respiration), can reach up to 9 Mg ha^{-1} y^{-1} of stored C.

In conclusion, land use change from cropland to switchgrass using a low input system seems to have positive effects on SOC and NEE. Moreover, the recycling of residual ashes (from the combustion process) as a soil amendment reduced CO_2 emissions with positive effects on final C balance. Recently some ethical issues, such as food security, have emerged from the cultivation of bio-energy crops on croplands (Field *et al.*, 2007). It should be stressed; however, the relevant environmental benefits, such as SOC sequestration, that introducing perennial energy crops have in croplands. Besides that, SOC sequestration favors soil stabilization through the formation of soil aggregates that reduce soil leaching, increase soil water capacity and fertility (Lal, 2004a). In addition there are political aspects that are rarely considered in the evaluation of the introduction of perennial energy crops into croplands. For example the no longer valid set-a-side regulation (Regulation (EEC) 1272/88) that introduced entitlements for removing croplands (10/15% of E.U. croplands) in order to reduce cereals surplus production. It is necessary; however, to evaluate in detail if introducing perennial energy crops in lands previously used for set-a-side really will create food security problems. Last but not the least, consideration must also be given to the problem in developed countries of a diet (rich in meat) that requires large amounts of energy, water and cereals (Haberl *et al.*, 2011a; Haberl *et al.*, 2011b) as well as the amount of food that is wasted which can reach 50% (Fox and Fimeche, 2013).

The second study (experiment 2) was carried out in a Miscanthus plantation established in Wales, UK on marginal land previously cultivated with *Lolium perenne*. In this study five Miscanthus hybrids were compared. Soil cores were taken in three different positions (inter-row, edge of the plant and centre of the plant). Belowground biomass changed among genotypes and decreased progressively from center of the plant to interrow (fig. 3.2). In this experiment belowground biomass was also not correlated with SOC and the genotypes with high belowground biomass caused slight SOC decline. Therefore the priming effect not only accelerated C replacement in the vicinity of the plant (20%) and in upper layer (14%), but also led to a decrease of the most stable SOM component (Kuzyakov, 2006). Unlike experiment 1, a slight decrease after land use change (~10 Mg h^{-1}) was predicted (Anderson-Teixeira *et al.*, 2009) because of high initial SOC content that is typical of grassland (Conant *et al.*, 2001). In C-rich ecosystems such as grassland a further SOC increase creates doubt because they are ecosystems that probably already reached equilibrium.

In conclusion, in both cases there was no correlation between root biomass and SOC accumulation, while a relationship was found between root biomass and C-derived, especially in soil upper layer. The main cause is the priming effect, i.e. large amounts of fresh organic matter released by the root systems did not favor SOC accumulation but stimulated soil microbiological activity. The increase of microbiological activity was highlighted in experiment 1 through the increase in soil respiration, while in experiment 2 was evidenced by C-derived that was much greater in the vicinity of plants, where there was more root biomass than at sample points more distant to the plant. Soils with low SOC content, such as experiment 1, trend to increase SOC level despite the priming effect. SOC increment was higher in deeper layers because they are characterized by lower mineralization. In soils with high SOC content, such as experiement 2, the priming effect caused a partial depletion of soil C stock, especially in the vicinity of the plant (but not significant) and accelerated carbon substitution.

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