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The impact of land-use change for lignocellulosic biomass crop production on soil organic carbon stocks in Britain

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Declaration

I declare that this thesis has been composed by myself and has not been submitted for any other degree or professional qualification. The work described is my own unless otherwise stated.

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Signature:

Date:

Lay summary

Globally, soils form a major carbon store containing three times more than the atmosphere. This carbon storage is not permanent but sensitive to human influence; depending on how humans manage the land, soil can be either a source or a sink for atmospheric carbon. With increasing concentrations of atmospheric carbon dioxide, mainly due to fossil fuel combustion, research on mitigation measures has increased. This includes identifying land-uses to increase carbon storage in the soil. Planting 'second generation bioenergy' crops that have a high portion of lignocellulosic biomass is one option, as it is expected to increase carbon accumulation in soil instead of the atmosphere and can be used to generate energy instead of fossil fuels. Lignocellulosic biomass refers to the fibrous, woody and generally inedible portion of a plant. The two most widely grown lignocellulosic biomass crops in Britain are *Miscanthus* and short rotation coppice (SRC) willow.

However, we are still uncertain about how changing land-use to lignocellulosic biomass crops affects soil carbon stocks. To address this, soil samples were collected from 93 biomass crop plantations in England and Wales to assess the soil carbon stocks after planting *Miscanthus* and SRC willow on arable and grassland. The results indicate that planting SRC willow on arable land can increase soil carbon stocks, while planting on grassland and planting *Miscanthus* on arable or grassland had no clear effect on soil carbon stocks. Carbon stocks were affected by many other factors such as climate and soil type. This knowledge may be useful for estimating more region-specific changes in soil carbon stocks.

Abstract

The contribution of energy from biomass sources is projected to increase in Britain to assist in meeting renewable energy targets and reducing anthropogenic CO_2 emissions. With increasing concerns over the sustainability of food crop-based biofuels, purpose-grown lignocellulosic biomass crops such as *Miscanthus* and short rotation coppice (SRC) willow have been promoted as more sustainable feedstocks for the production of heat and electricity as well as for the future production of liquid biofuels. With the introduction of the Energy Crops Scheme, land-use change (LUC) for lignocellulosic biomass crop production has become increasingly common in Britain in recent decades. However, there is limited understanding of the impact this has on soil organic carbon (SOC) stocks and limited predictability concerning the overall trajectory, magnitude and rate of SOC changes under a range of different conditions.

Using a chronosequence of 93 biomass crop plantations in England and Wales, mainly of 1 to 14 years age, empirical models were developed to determine the short term trajectory of SOC stocks following LUC from arable and grassland to SRC willow and *Miscanthus* production. SOC stocks were calculated for each site using a fixed sampling depth of 30 cm and estimated changes were inferred by comparing with typical pre-change SOC stocks. These results indicate that only LUC from arable crops to SRC willow demonstrated an overall increase in SOC stocks, by an estimated 15.3 ± 2.2 t C ha^{-1} (± 95% confidence intervals) after 14 years and 68.8 ± 49.4 t C ha^{-1} after 22 years. LUC from arable crops to *Miscanthus* and from both arable crops and grassland to SRC willow and *Miscanthus* demonstrated no overall

net effect on SOC stocks. Soil texture and climate data were measured for each site and multivariable models were created to assess the influence of different environmental conditions on SOC trajectory. In most cases the addition of these explanatory variables improved the model fit, and the models provide some preliminary estimates of more region-specific changes in SOC following LUC.

Since LUC to biomass crops often causes a loss of SOC, at least in the short term, the potential for pyrogenic carbon (PyC) to ameliorate this effect was investigated. Studies indicate that PyC can interact with and stabilise native SOC, a process termed negative priming, although the potential for PyC to reduce LUC-induced losses of SOC by negative priming has not yet been assessed. Although negative priming has been observed in many studies, most of these are long term incubation experiments which do not account for the impact of environmental weathering of PyC on interactions with native SOC. Here the aim was to assess the impact of environmentally weathered PyC on native SOC mineralisation at different points in LUC from arable crops to SRC willow. Soil was sampled to a 5 cm depth from multiple recently established SRC willow plantations approximately 2 years after amendment with PyC. Cumulative CO_2 flux was measured weekly from incubated soil and soil-surface CO_2 flux was also measured in the field. The results demonstrate a PyC-induced increase in CO_2 flux for the surface 5 cm of soil. However, no net effect on soil-surface CO_2 flux was observed in the field. Although the mechanisms for these contrasting effects remain unclear, they do not suggest that PyC can reduce LUC-induced SOC losses through negative priming.

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Dedicated to my grandmother Margaret Burns,

for her wisdom and inspiration.

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Chapter 1 Introduction

1.1 Soil organic carbon

Globally, soils represent the largest carbon (C) reservoir in terrestrial ecosystems and contain 2500 gigatonnes (Gt) C, which is 3.1 times more than the atmosphere (Oelkers and Cole, 2008) and 4.5 times more than all living biomass (Lal, 2004a). Most of this C is organic (1550 Gt), while the rest is inorganic (950 Gt) and stored either in elemental form or in carbonates (Lal, 2004a). Although the organic compounds that are present in the soil include many living organisms such as bacteria, algae, fungi, fauna and plant roots, the term soil organic matter (SOM) often excludes the soil-living population and is generally defined as the decomposing residues of these organisms and all of the organic products derived from their activity, such as faeces, synthesised products, root exudates and humic substances (Soil Science Society of America, 1984). Soil organic carbon (SOC) refers to the carbon content of SOM, which is approximately 58% by mass (Nelson and Sommers, 1996).

Most SOC inputs are plant-derived and enter the soil from above-ground sources, such as woody tissues, leaf litter and crop residues, or below-ground sources, such as dead roots, mycorrhizal hyphae and root exudates. Much of this organic material is labile and decomposes rapidly, with an estimated average turnover time of 32 years (Raich and Schlesinger, 1992; Schlesinger, 1995). However, a small fraction can resist decomposition and remain in the soil for up to hundreds or thousands of years (Campbell *et al*., 1967; Martel and Paul, 1974). This variation in turnover time largely relates to the selective preservation of certain organic compounds that are more resistant to degradation due to their chemical composition (Schlesinger, 1990; Dean and Gorham, 1998). Von Lutzow *et al*. (2006) distinguish between the 'primary recalcitrance' of original plant material and the 'secondary recalcitrance' of microbial products, humic polymers and black carbon (BC). In addition to substrate composition, the rate of decomposition also depends on environmental conditions such as soil moisture, temperature, pH and aeration (Davidson *et al*., 2006). Stabilisation of SOM by various mechanisms also affects the rate of turnover (Six *et al*., 2002). SOM may become physically protected by occlusion within aggregates or small pores as: (i) soil aggregates form physical barriers between microbes and substrate; (ii) SOM may become positioned in pores that are too small for bacteria or fungi to penetrate, and; (iii) aggregates may become partially anaerobic due to the slow diffusion of O_2 through the small intra-aggregate pores (Marinissen and Hillenaar, 1997; Six *et al*., 2002). Chemical protection also occurs through interactions with mineral surfaces (Kleber *et al*., 2005; Von Lutzow *et al*., 2006) or other organic compounds (Sollins *et al*., 1996; Kelleher and Simpson, 2006; Von Lutzow *et al*., 2006; Bachmann *et al*., 2008).

The overall C content of a soil can be determined as the net balance of all C fluxes over time. Most undisturbed soils exist in a state of dynamic equilibrium, as C inputs are continuously matched by outputs at a steady rate (Schlesinger, 1990). However, disturbances induced by human activities, such as land-use change (LUC), can have a major impact on the balance between soil C inputs and outputs (Guo and Gifford, 2002). Changes in vegetation can cause quantitative changes to C inputs to the soil, through the amount of net primary production and/or exogenous organic matter amendments, as well as qualitative changes by altering the chemical composition of plant litter (Lugo and Brown, 1993; Guo and Gifford, 2002). The latter influences SOC outputs, as chemical composition affects decomposition rates (Schlesinger, 1990; Fan and Liang, 2015), in addition to management practices that increase erosion and cause physical disturbance of the soil, e.g. tillage, grazing and irrigation (Beare *et al*., 1994; Biederbeck *et al*., 1994; Bremer *et al*., 1994). LUC disturbs the soil C balance and often results in a net increase or decrease in SOC stocks when a new equilibrium is reached. Subject to LUC, the soil C pool may form either a net sink or source of C to the atmosphere (Guo and Gifford, 2002; Poeplau and Don, 2013).

1.2 Impact of land-use change on soil organic carbon stocks

1.2.1 Global trends in land-use change

With an increasing global population that is expected to exceed 9 billion by 2050, the demand for food and resources is continuously increasing (UN, 2014). Growing demands for food, animal feed, forestry and energy have been reflected in land-use changes across the globe (Spiertz and Ewert, 2009). It has been widely documented that disturbance to terrestrial ecosystems by changes in land-use and management practices has significantly affected soil C stocks and the overall dynamics of C cycling regionally and globally (Schlesinger, 1985; Mann, 1986; Post and Mann, 1990; Davidson and Ackerman, 1993; Poeplau and Don, 2013). At present, LUC contributes 0.9 ± 0.5 Gt C yr^{-1} to the atmosphere, which is approximately 18% of all anthropogenic greenhouse-gas (GHG) emissions (IPCC, 2013). Between 1850 and 1998, 136 ± 55 Gt C was released into the atmosphere as a result of LUC of which almost 90% was from deforestation (IPCC, 2000). Forest ecosystems are major C stores and cover an estimated 30% of the earth's land surface, however, global forest area is estimated to have decreased by 20% since 1850 (Houghton, 1999). The main drivers of deforestation include the demand for fuel, wood, paper products, cattle ranching and agriculture (Boahene, 1998). Deforestation is currently estimated to contribute 6-17% of total global anthropogenic CO_2 emissions (Van der Werf *et al*., 2009).

The total historic loss of soil C resulting from peatland destruction is only a fraction of that related to deforestation, but has increased dramatically in recent decades (Hooijer *et al*., 2010). Although peatlands are significant C stores containing 15-35% of the global terrestrial soil C reservoir (Post *et al*., 1982; Gorham, 1991), the capacity of these ecosystems to accumulate C has been disrupted by their exploitation for forestry, agriculture and biofuel plantations (Hooijer *et al*., 2010). It is estimated that 65 million hectares (M ha) of global peatlands are currently degraded, causing a net release of 0.8 Gt C yr^{-1} to the atmosphere, half of which originates from Southeast Asia alone (Parish *et al*., 2008). Peat oxidation in Southeast Asia, which is occurring mainly for the establishment of *Acacia* pulp wood and palm oil plantations, is estimated to contribute the equivalent of 1.3–3.1% of current global CO_2 emissions from the combustion of fossil fuels (Hooijer *et al*., 2010).

Palm oil plantations currently cover over 16 M ha of land distributed throughout many tropical countries and yield over 53 M t yr^{-1} of edible oil (FAO, 2015). Palm oil accounts for 34% of global vegetable oil consumption and is also used as a source of biodiesel (FAO, 2015). It has been estimated that LUC from forest to palm oil plantations on peat soils causes a net release of over 1300 tonnes of CO_2 equivalent per hectare (CO_2 Eq ha^{-1}) during the first 25-year cycle (Germer and Sauerborn, 2008). This is much greater than C emissions resulting from the conversion of forests on mineral soils which causes an estimated net release of approximately 650 t CO_2 Eq ha^{-1} over a 25-year cycle (Germer and Sauerborn, 2008). Despite the emission savings derived from the substitution of fossil fuels, it is estimated that the conversion of lowland tropical and peatland rainforests to palm biodiesel causes emissions of around 610 t ha^{-1} and 3000 t ha^{-1} respectively after 50 years, producing a C debt that would take 86 and 420 years to repay (Fargione *et al*., 2008). In contrast, the conversion of tropical grasslands to palm oil production may form a C sink of up to 135 t ha^{-1} over a 25-year cycle (Germer and Sauerborn, 2008).

In addition to palm oil plantations in Southeast Asia, the production of biofuels has increased globally, largely driven by rising crude oil prices, concerns over energy security and the impacts of climate change (Berndes *et al*., 2003; Sims *et al*., 2006; Demirbas, 2008; Solomon, 2010). Presently, the leading commercial biofuel industries are food crop-based biofuels, also known as ‘first generation biofuels’, which utilise plant sugar or starch to produce ethanol or oilseed to produce biodiesel. Global ethanol production in 2012 was an estimated 83.1 billion litres, with the USA and Brazil the two largest producers accounting for a combined 87% of total production (Figure 1.1). While biodiesel production remains comparatively low at 22.5 billion litres in 2012, the industry is growing rapidly with the USA, Argentina and the EU as the largest producers (Figure 1.2). Ethanol is mostly manufactured from sugarcane in tropical regions and cereals in temperate regions, such as corn in USA and China and wheat in the EU (Langeveld *et al*., 2014). The main feedstocks for producing biodiesel are soybean (USA, Brazil and Argentina), rapeseed (EU) and

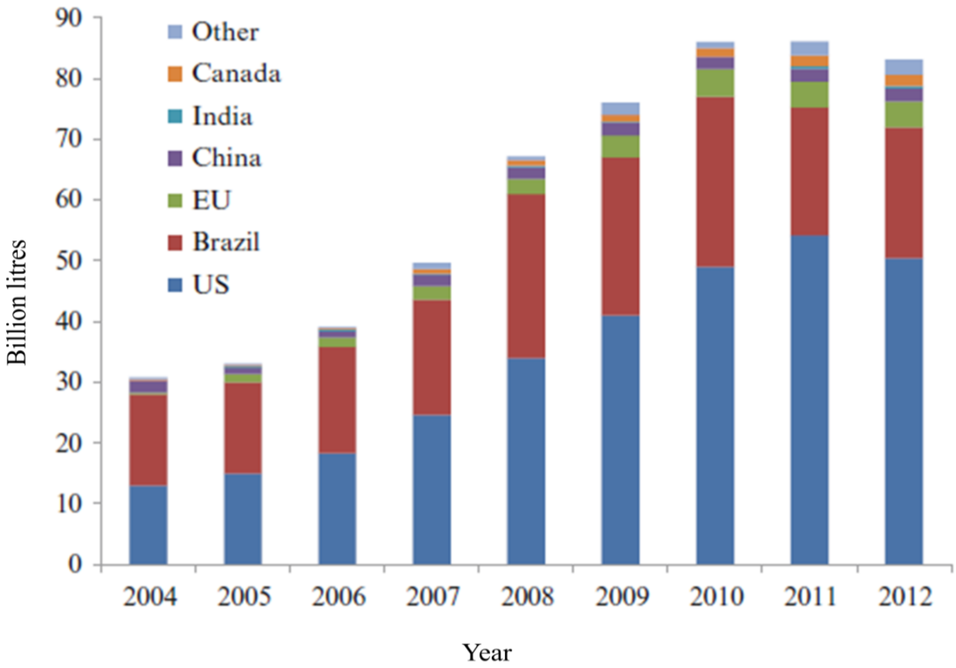

Figure 1.1 Global ethanol production (Timilsina and Shrestha, 2014).

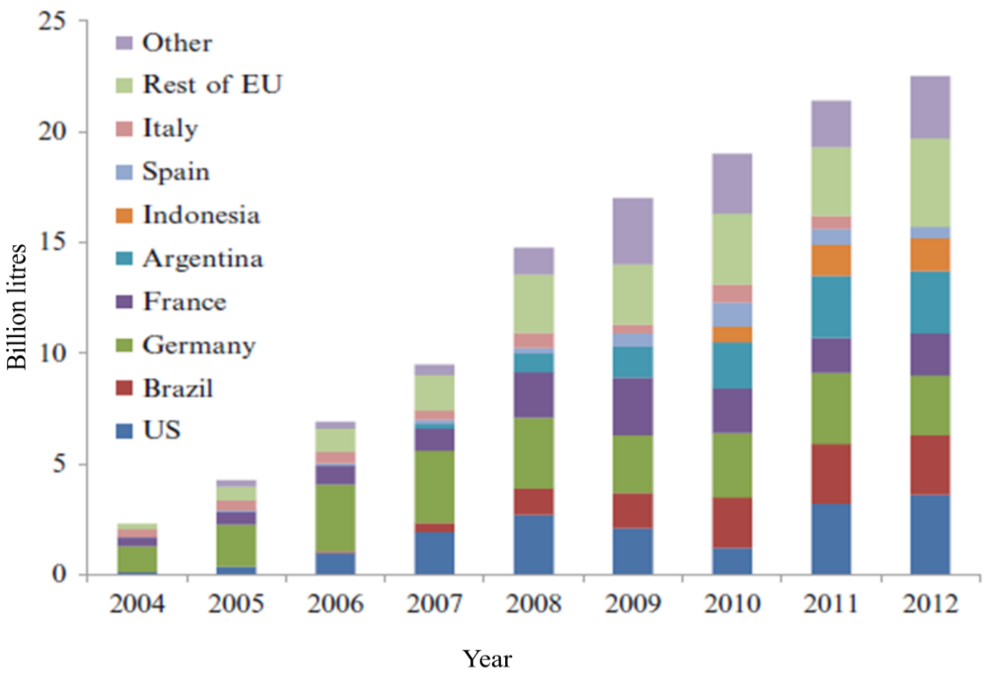

Figure 1.2 Global biodiesel production (Timilsina and Shrestha, 2014).

oil palm (Indonesia and Malaysia) (Langeveld *et al*., 2014; Timilsina and Shrestha, 2014).

Although biofuels have been promoted as a low- or zero-C fuel source, C savings are highly variable and largely dependent on a variety of factors such as the type of feedstock, production processes, yield levels, conversion efficiency and changes in land-use (Sims *et al*., 2006). An expansion of food crop-based biofuel production has the potential to cause substantial LUC both directly and indirectly as unintended LUC may also occur elsewhere to maintain the food supply (Searchinger, 2010). Studies indicate that the impact of LUC has the potential to negate any C savings from fossil fuel substitution and may even result in a net increase in CO_2 emissions due to significant loss of C from biomass and soil (Fargione *et al*., 2008; Searchinger *et al*., 2008; Plevin *et al*., 2010). The impact of biofuel production on the global C balance can be quantified by calculating GHG payback times (GPBT), which are defined as the amount of time required for biofuel GHG offsets to repay the debt created from initial losses in ecosystem stocks caused by LUC (Elshout *et al*., 2015).

A recent study used simulation models to calculate global GPBTs for the replacement of natural vegetation with five biofuel systems: production of ethanol from corn grain, sugarcane sucrose and winter wheat grain for the replacement of fossil petroleum, and the production of biodiesel from rapeseed and soybean oil for the replacement of fossil diesel (Elshout *et al*., 2015). Overall GPBTs ranged between 1 and 162 years and the location of crop cultivation was identified as the most important driving factor with the longest GPBTs occurring in the tropics (Figure 1.3). Farm management was also important as farming with higher inputs of fertiliser and irrigation reduced GPBTs by 45–79% compared to low-input farming

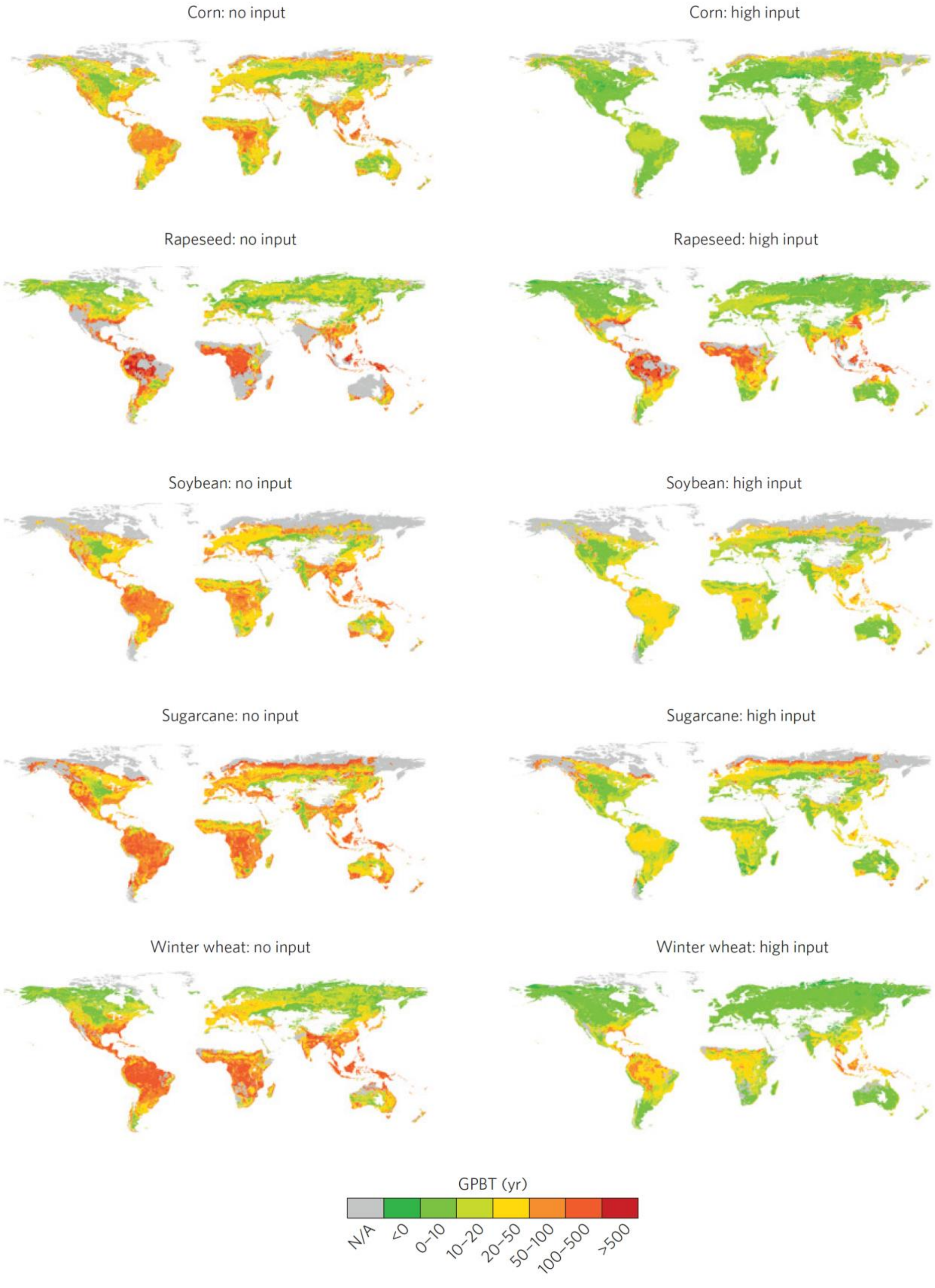

Figure 1.3 Global maps of GPBTs for five biofuel systems under no-input and high-input farm management. White areas were deemed unsuitable for agricultural land use (e.g. deserts, ice cover) and grey areas were excluded because modelled crop yields were below the threshold (Elshout *et al*., 2015).

(Figure 1.3). Although GHG emissions were higher from fertiliser and machinery for high- compared to low-input farming, these were offset by higher crop yields (Elshout *et al*., 2015). Ethanol production from sugarcane in tropical regions under no-input farming had the longest GPBT, with a global median of 60 years (95% range of 8 to 209 years), while corn and winter wheat in temperate regions under high-input farming had the shortest GPBTs, with a global median of 6 (95% range of 0 to 29 years) and 8 (95% range of 0 to 57 years) years respectively (Elshout *et al*., 2015).

Elshout *et al*. (2015) focused on the conversion of natural vegetation, however, this may not reflect recent or future trends in LUC. Other studies indicate that greater GHG savings are likely to result from the conversion of other land-uses. For example, although the conversion of natural vegetation to sugarcane in Brazil has occurred previously, between 2000 and 2009 it was estimated that, in areas that account for 90% of production, approximately 70% was from pasture and 25% from grain crops (Mello *et al*., 2014). A recent study calculated GPBTs for the conversion of natural vegetation, pasture and cropland to sugarcane production in Brazil using 75 pairwise comparisons derived from 135 study sites. The authors estimate a 16% increase in SOC stocks for the conversion of cropland to sugarcane and a 5–6 year GPBT for the conversion of pasture, with a soil C debt of 3 years (Mello *et al*., 2014). This was much shorter than a 17 year GPBT for the conversion of natural vegetation to sugarcane, with a soil C debt of 8 years (Mello *et al*., 2014).

However, these GPBTs do not include potential GHG emissions from biomass and soil related to indirect LUC, which may occur as global biofuel production increases pressure on land supply and food production. A study using economic and terrestrial biogeochemistry models estimated that indirect LUC is likely to be responsible for twice as much GHG emissions as direct LUC over the 21st century (Melillo *et al*., 2009). Using a global agricultural model, Searchinger *et al*. (2008) estimated that corn-based ethanol would incur a GPBT of 167 years, largely as a result of indirect LUC as farmers convert forest and grassland to replace cropland diverted to biofuels (Searchinger *et al*., 2008). This estimate assumed an increase in yield per hectare of 11.5% between 2007 and 2016, and model estimates for GHG emissions were even greater when lower yield increases were assumed (Searchinger *et al*., 2008). Similarly, Yang and Suh (2015) predicted that expansion of biofuels in the USA is likely to bring less-fertile 'Conservation Reserve Program' (CRP) land into production and that, without yield increases, corn ethanol would be unlikely to provide any C benefits for >100 years after LUC. When productivity improvements were simulated GPBTs of <20 years were predicted only when more productive CRP land was converted to corn production, but results were sensitive to crop yields and a more typical scenario produced a GBPT range of 19 to 43 years (Yang and Suh, 2015).

With increasing concerns over the sustainability of food crop-based biofuels (Crutzen *et al*., 2007; Searchinger *et al*., 2008; Whitaker *et al*., 2010), efforts are being made to develop 'advanced biofuels' from non-food biomass sources. Evidence indicates that purpose-grown biomass crops that are produced for a high yield of lignocellulosic biomass, also known as 'second generation biofuels', may provide a more sustainable feedstock for the production of liquid biofuels (Whitaker *et al*., 2010). Lignocellulosic biomass refers to the fibrous, woody and generally inedible portion of a plant that consists of a mixture of lignin, cellulose and hemicellulose. However, second generation technologies are not yet commercially viable on a global scale and will require further investment in research as well as policy support mechanisms to overcome key technical and economic challenges to commercialisation (Sims *et al.*, 2010).

1.2.2 UK land-use change

1.2.2.1 Recent trends in land-use change

Changing land-use in the UK in recent decades has been characterised by a decrease in agricultural land area and an increase in forest cover (Rounsevell and Reay, 2009). Recent surveys indicate that 17.2 M ha of land in the UK is currently used for agriculture, which is 71% of the total land area (DEFRA, 2015). Although this figure has been largely stable in recent years, agricultural land area has declined by 13% since 1961 when there was 19.8 M ha of agricultural land (FAO, 2015). Driven by a range of socio-economic factors, arable and permanent grassland both declined by 13% during this time (FAO, 2015). As agricultural land area has decreased, UK forest cover has increased from just 5% of land area at the start of the 20th Century to 13% of total land area by 2014 (Forestry Commission, 2014). Of this forested area, 51% is conifer woodland and 49% is broadleaved forests (Forestry Commission, 2014). The establishment of conifer plantations, such as the native Scot's pine and imported Sitka spruce, forms the largest single LUC in upland UK over the past century and occurred at rates of up to 40,000 ha yr^{-1} in the early 1970s (Farmer and Nisbet, 2004; Stott and Mount, 2004).

Modelling studies attempting to assess the effects of historic afforestation on C stocks in the UK indicate that this expansion in forest area formed a net C sink

(Dewar and Cannell, 1992; Cannell *et al*., 1993; Cannell and Dewar, 1995). Net removal of CO_2 by British plantation forests into trees, litter and wood products is estimated to have increased from zero in 1920 reaching 2.5 M t C yr^{-1} in 1990 (Cannell and Dewar, 1995). This model assumed a C sink of zero for plantation forests in 1925, since there is little evidence of afforestation prior to this date (Malcolm, 1991; Cannell and Dewar, 1995). Using the same C accounting model, another study estimated that net C removal by forests in Northern Ireland increased over the same period to 0.18–0.23 M t C yr^{-1} in 1990 (Cannell *et al*., 1996). However, as these forests reached maturity, exceeding the age for maximum net CO_2 removal, and afforestation rates decreased, forestry activities in the UK became a net source of CO_2 between 1990 and 1995 (Webb *et al*., 2013). With renewed afforestation, forestry is estimated to have formed a net C sink since 1996 (Webb *et al*., 2013). It has been estimated that British forests remove 4 M t C yr^{-1} from the atmosphere (Read *et al*., 2009) and contain 150 M t in biomass and 664 M t in the soil (Read *et al*., 2009; Vanguelova *et al*., 2013).

However, these estimates are based on modelling approaches and there remain relatively few studies in which SOC has been measured directly. Although there are a number of national soil-monitoring networks in the UK which measure and estimate SOC stocks (Bradley *et al*., 2005; Emmett *et al*., 2008; Lilly *et al*., 2011), none of these are comprehensive in their coverage of forest soils (Vanguelova *et al*., 2013). Therefore, large uncertainties remain concerning the magnitude and direction of SOC change following afforestation (Reynolds, 2007). Studies using non-UK datasets indicate that planting broadleaf trees may have little effect on SOC stocks and that conifer planting, especially in high rainfall areas may even cause a decline in

SOC stocks (Guo and Gifford, 2002). It is suggested that losses in SOC caused by drainage and disturbance during site preparation and planting may offset C from litter inputs for over 20 years following the planting of both deciduous and coniferous forests (DEFRA, 2009). However, long-term trends have suggested that the initial increase in CO_2 production is eventually offset by increased C sequestration following renewed afforestation (Hargreaves *et al*., 2003).

It has been suggested that a future decline in agricultural land area may occur in the UK as less land may be required to maintain food production, due to the combined effects of climate and technological improvements on agricultural productivity (Rounsevell and Reay, 2009). Although socio-economic factors are expected to have a more predominant effect in shaping future land-use policy, an increasing interest in climate change mitigation and the need to meet associated international obligations are also likely to have a significant impact (Pacala and Socolow, 2004). With increasing awareness of the influence of human activities on SOC stocks, research efforts are being made to identify land-uses that can increase SOC storage and utilise the C sink capacity offered by global agricultural and degraded soils (Smith *et al*., 2000; Lal, 2004b; Ostle *et al*., 2009).

1.2.2.2 Lignocellulosic biomass crops

Under the Kyoto Protocol the EU has committed to reduce its overall GHG emissions by 20% compared to 1990 levels by 2020 and by 80% compared to 1990 levels by 2050 (UNFCCC, 1997). To achieve its share of emission reductions, the UK government introduced the Climate Change Act (2008) which established a legally binding target for a reduction of GHG emissions by 34% compared to 1990 levels by 2020 (DECC, 2008). Bioenergy has been identified as one option that can help to reduce emissions as part of a wider strategy to decarbonise the energy sector (DECC, DEFRA, DfT, 2012). Under the EU Renewable Energy Directive (2009/28/EC), the UK government is committed to producing 15% of all energy and 10% v/v of liquid transport fuels from renewable sources by 2020. Although the contribution from biomass-derived energy remains low, accounting for just 3% of electricity and 1% of total heating in the UK, this is projected to increase to an estimated 5–11% and 6% respectively by 2020 (DECC, DEFRA, DfT, 2012).

With the introduction of the Energy Crops Scheme in 2000, LUC for lignocellulosic biomass crop production has become increasingly common in the UK in recent decades (DEFRA, 2013). Although lignocellulosic feedstocks are not yet commercially viable for biofuel production, these crops are currently being grown for heat and co-fired electricity generation (Travaini *et al*., 2014). *Miscanthus* x *giganteus*, a rhizomatous perennial grass, and short-rotation coppice *Salix* spp. (SRC willow) are the most prevalent purpose-grown lignocellulosic biomass crops in the UK and currently cover estimated areas of 8000 ha and 3000 ha respectively (DEFRA, 2013). A future increase is also projected with 0.93–3.63 M ha of land being identified as 'available' for the production of lignocellulosic biomass crops (DECC, DEFRA, DfT, 2012). A large number of life-cycle assessment (LCA) studies have been used to assess the environmental impacts of heat and/or electricity production from *Miscanthus* and SRC willow. LCA is a 'cradle-to-grave' systems analysis tool that has been widely used to compare the energy requirements, GHG balance and other environmental impacts of bioenergy production chains with the fossil energy chains they replace. A recent review of 26 studies published between

1990 and 2009 on the energy and GHG balance of SRC woody crops estimated between 90 and 99% GHG emission reductions from the production of heat and/or electricity from SRC compared to conventional coal (Djomo *et al*., 2011). Reported values for the intensity of GHG emissions ranged from 0.7 to 10 g CO_2 equivalent per megajoule of energy (g CO_2 Eq MJ^{-1}) for biomass of SRC willow compared to 96.8 g CO_2 Eq MJ^{-1} from coal (Djomo *et al*., 2011).

Other studies have also calculated comparable GHG emission savings for the replacement of fossil fuels with *Miscanthus* for the production of heat and/or electricity in temperate Europe (Styles and Jones, 2007; Styles and Jones, 2008; Hillier *et al*., 2009; Tonini *et al*., 2012). For example, Styles and Jones (2007) calculated GHG emission reductions of almost 90% when 30% of peat and 10% of coal electricity generation was substituted with co-fired *Miscanthus* and SRC willow in Ireland. GHG savings were greatest for the replacement of dairy agricultural systems and peat fuel chains and lowest for the replacement of sheep agriculture and coal fuel chains with 35,209 and 23,251 kg CO_2 equivalent per hectare per year (kg CO_2 Eq ha^{-1} yr^{-1}) respectively for *Miscanthus* and 29,019 and 18,028 kg CO_2 Eq ha^{-1} yr^{-1} for SRC willow (Styles and Jones, 2007). Another LCA study estimated even greater GHG emission reductions for the production of heat and electricity in Denmark using *Miscanthus* and SRC willow planted on arable land compared to a reference fossil energy chain (Tonini *et al*., 2012). In this instance SRC willow provided the largest GHG savings of approximately 82,000 kg CO_2 Eq ha^{-1} yr^{-1} compared to approximately 45,000 kg CO_2 Eq ha^{-1} yr^{-1} for *Miscanthus* (Tonini *et al*., 2012). Although these studies generally indicate the potential for significant GHG savings there are large variations between studies, which are often difficult to compare due to a lack of transparency and/or common methodology (Rowe *et al*., 2011). There is not yet a well-defined procedure to account for SOC changes in LCA studies and as a result this is often ignored or included in a coarse manner owing to limited experimental data relating to the effects of LUC to biomass crops on SOC stocks (Don *et al*., 2012; Goglio *et al*., 2015).

Previous studies aiming to assess the effects of LUC for biomass crop production mainly utilise a single-site paired plot approach, which consists of comparing adjacent fields that share similar characteristics and are both pre- and post-LUC. Such studies have typically inferred an increase in SOC following LUC from arable land to *Miscanthus* (Kahle *et al*., 2002; Hansen *et al*., 2004; Dondini *et al*., 2009). For example, Dondini *et al*. (2009) reported significantly higher SOC content in the 0-30 cm soil profile of a 14-year old *Miscanthus* plantation than under an adjacent arable crop, with 131 and 106 t C ha^{-1} respectively. Assuming both fields began with the same SOC content, soil under the *Miscanthus* plantation gained 25 t C ha^{-1}, equivalent to 25% of SOC under arable crops. These results are similar to those of Hansen *et al*. (2004), who also reported significantly higher SOC content under a 16-year old *Miscanthus* plantation compared to an adjacent arable crop.

This increase in SOC is mainly attributable to increased C input and reduced soil disturbance. Selected for trials in Europe due to its high productivity (Lewandowski *et al*., 2000; Clifton-Brown *et al*., 2007), *Miscanthus* is a fast growing C_4 grass which has been reported to achieve 37% higher photosynthetic uptake than native C_3 plants in the UK (Beale and Long, 1995). Significant soil C inputs result from stems and leaves accumulating on the soil surface as *Miscanthus* is harvested after senescence (Christian *et al*., 2006). In addition to high aboveground productivity, *Miscanthus* allocates large proportions of assimilated C belowground as a C reservoir for growth in spring, also contributing large amounts of C to the SOC pool (Kuzyakov and Domanski, 2000). As inputs of plant-derived C increase, decomposition also decreases in the absence of tillage. This has been confirmed using physical fractionation techniques. For example, Dondini *et al*. (2009) observed an increase of SOC content in physically protected fractions under *Miscanthus* compared to an adjacent arable field.

Modelling studies have predicted an increase in SOC following LUC from arable to SRC willow production (Lemus and Lal, 2005; Amichev *et al*., 2012). However, field studies in temperate Europe are scarce and produce inconsistent trends. For example, Jug *et al*. (1999) reported that SOC increased by 20% after 10 years under one SRC willow plantation established on arable land in Germany but no increase was observed at two other sites, as increases in the 0-10 cm layer were balanced by a decrease in the 10-30 cm layer. Other studies also indicate that most SOC accumulation occurs in the top layer (Makeschin, 1994; Zan *et al*., 2001) and this has mainly been attributed to the accumulation of leaf, woody and root litter production, as well as reduced soil disturbance and increased transfer of assimilates into the external mycelium of mycorrhizal fungi (Makeschin, 1994; Zan *et al*., 2001; Grogan and Matthews, 2002; Baum *et al*., 2009; Amichev *et al*., 2012).

Paired plot studies typically infer no significant change in SOC following LUC from grassland to *Miscanthus* in temperate Europe (Hansen *et al*., 2004; Clifton-Brown *et al*., 2007; Schneckenberger and Kuzyakov, 2007). For example, Schneckenberger and Kuzyakov (2007) reported no significant differences between SOC contents under a 9-year old and a 12-year old *Miscanthus* plantation and reference grassland

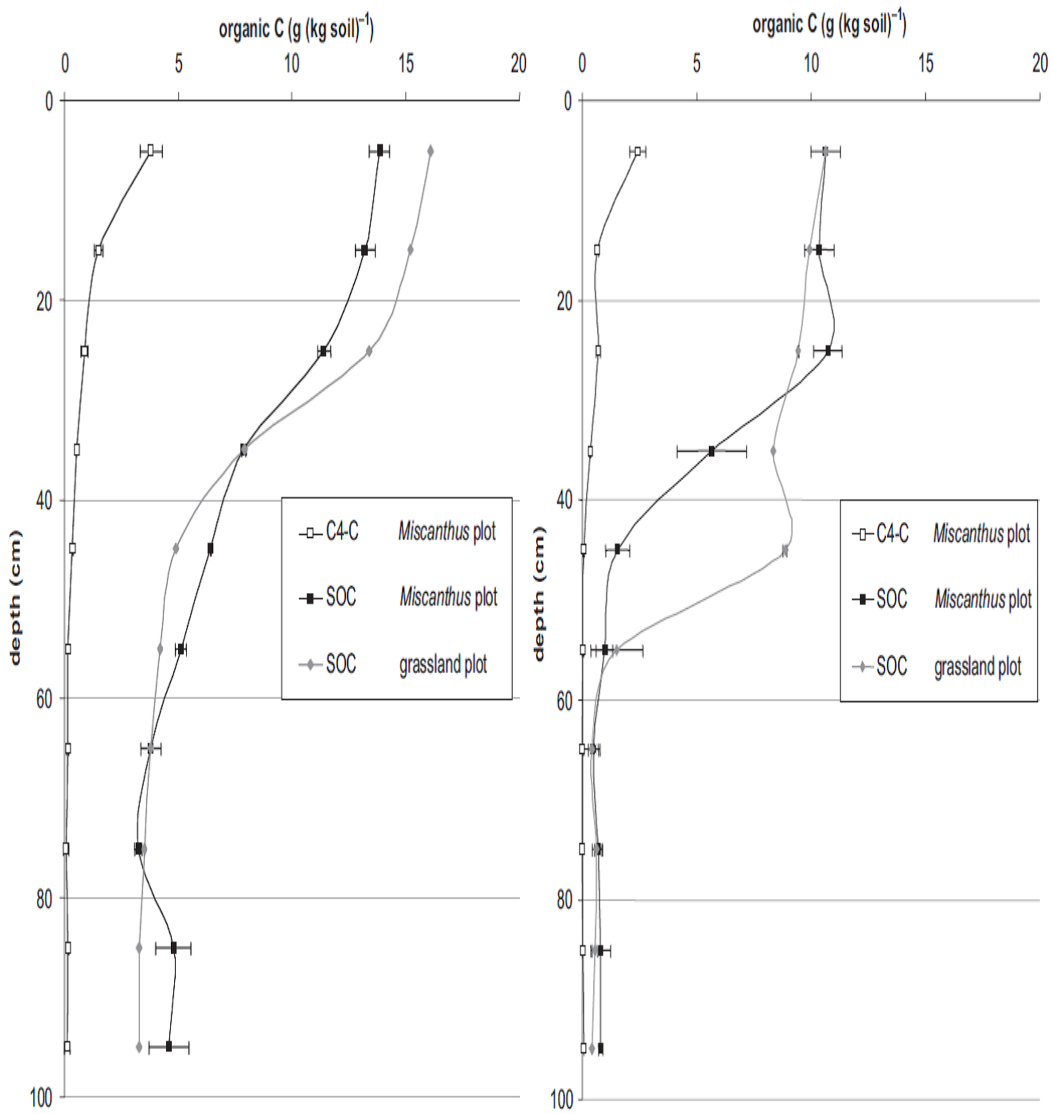

Figure 1.4 Comparison of SOC in a *Miscanthus* plantation and a reference grassland site for a loamy soil (left) and sandy soil (right). No significant differences in SOC were observed to a 1 m depth between: (i) the 9-year old *Miscanthus* and grassland for a loamy soil (11.2 and 12.1 kg C m^{-2} respectively) and; (ii) the 12-year old *Miscanthus* and grassland for a sandy soil (6.4 and 7.0 kg C m^{-2} respectively). ^{13}C natural abundance ratios demonstrated that in both soils there was a significant contribution of *Miscanthus* (C_4)-derived C to a 1 m depth. There was significantly greater *Miscanthus*-derived C in the top 10 cm of the loamy soil than the sandy soil (3.0 and 2.4 g C_4-C kg $soil^{-1}$ respectively) but with no significant differences beyond this depth (Schneckenberger and Kuzyakov, 2007).

sites (Figure 1.4). Another study also reported similar SOC concentrations for a 9-year old *Miscanthus* and a reference grassland, but with moderately higher SOC under a 16-year old *Miscanthus* plantation (Hansen *et al*., 2004). However, studies indicate that a loss of SOC may occur following LUC from grassland to SRC willow (Makeschin, 1994; Jug *et al*., 1999). For example, Jug *et al*. (1999) reported that SOC decreased by 15% after 7 years under one SRC plantation established on grassland. These results demonstrate that LUC for lignocellulosic biomass crop production has the potential to both increase and decrease SOC stocks. To limit the impact on food production, establishing biomass crops on more marginal land has been encouraged (Campbell *et al*., 2008). However, most studies assessing impacts on SOC stocks have been carried out mainly using single site comparisons and on experimental sites rather than commercial plantations. Therefore, the impact of such a planting strategy on crop yields and SOC stocks remains unclear. Since areas with the highest predicted yields for lignocellulosic biomass crops co-locate with higher grade food producing areas (Lovett *et al*., 2009), yields on more marginal land may be lower. Therefore, a large number of study sites representing LUC under a broader range of conditions are required to ascertain a more general understanding of LUC on SOC stocks.

1.3 Potential role for pyrogenic carbon in land-use change scenarios

Monitoring and management of SOC stocks are essential for accurate C accounting and for climate change mitigation. In addition to preserving natural C stores such as forests and peatlands, it has recently been suggested that LUCs that are likely to result in an accumulation of SOC could be utilised for C abatement purposes (Smith *et al.*, 2000). However, there are currently many difficulties associated with predicting the effects of implementing such strategies. For many LUCs there remains insufficient data relating to the trajectory, magnitude and rate of SOC changes under a range of different conditions. This is a prerequisite for predicting the potential short and long term benefits and for identifying suitable land to inform policy implementation. Furthermore, even with improved predictability, the land that is available may not produce the intended benefits. Additionally, with increasing demand for food and other commodities, there are many scenarios where future LUC is more likely to be driven by socio-economic rather than environmental factors. Therefore, in many cases a loss of SOC may be difficult to avoid and in these circumstances, pyrogenic C (PyC), another biomass-derived 'eco-engineering' option, could be used to augment SOC stocks and ameliorate some of the deleterious effects of LUC.

Pyrogenic carbon (PyC) purposefully produced for use as a soil amendment is also frequently termed 'biochar'. This is the porous, carbonaceous solid residue of thermochemical decomposition of biomass at elevated temperatures in a low oxygen environment. This form of PyC has mainly been proposed as a strategy for long term C sequestration (Pessenda *et al.*, 2001; Masiello, 2004; Krull *et al.*, 2006; Preston and Schmidt, 2006) that is simultaneously capable of improving soil quality (Joseph *et al.*, 2010; Montanarella and Lugato, 2013; Woolf *et al.*, 2010). Although the stable aromatic C core of PyC accounts for the largest proportion of GHG emission reductions potentially available from its use (Roberts *et al.*, 2010), there may be additional direct and indirect emission reductions. Studies indicate that PyC has the potential to both interact with and stabilise native SOC (Singh and Cowie, 2014). Therefore, PyC application during land-use transition may have the potential to not only offset any LUC-induced SOC losses with C sequestered in the stable aromatic portion of PyC, but possibly reduce such losses through stabilisation of native SOC.

Alteration of the turnover rates of native SOC by the addition of PyC is often referred to as ‘priming’, with increased or decreased turnover rates being defined as positive or negative priming respectively. Although uncertainty exists regarding the mechanisms responsible and the conditions required for these effects, priming appears to be the result of a complex interaction of both biotic and abiotic processes (Zimmerman *et al*., 2011). Many studies report a short term PyC-induced increase in soil CO_2 emissions following fresh PyC additions (Hamer *et al*., 2004; Wardle *et al*., 2008; Zimmerman *et al*., 2011). In some cases this can be attributed to the mineralisation of labile C compounds present in the PyC (Smith *et al*., 2010; Zimmerman, 2010; Cross and Sohi, 2011). However, there is evidence to suggest that mineralisation of this labile C also causes accelerated decomposition of native SOC by stimulating microbial activity (Hamer *et al*., 2004; Luo *et al*., 2011; Farrell *et al*., 2013; Luo *et al*., 2013). This could occur following the alleviation of constraints on microbial activity and C mineralisation, such as nutrient or water availability or soil pH (Luo *et al*., 2011; Zimmerman *et al*., 2011).

Negative priming has been observed in the short and longer term in both laboratory incubation and field studies, indicating that PyC can reduce native SOC mineralisation. Possible mechanisms include surface sorption of native SOC (Zimmerman *et al*., 2011), inhibition of microbial activity by PyC-associated volatile organic compounds (VOCs) (Spokas *et al*., 2010; Spokas *et al*., 2011), a temporary shift to mineralisation of labile C present in the PyC or enhanced physical protection of SOC by PyC-induced organo-mineral interactions (Keith *et al*., 2011; Zimmerman *et al*., 2011; Singh and Cowie, 2014). Observations of large quantities of non-black C (nBC) in the *terra preta* soils (Glaser *et al*., 2001; Liang *et al*., 2010) may provide further evidence for PyC-induced stabilisation of native SOC stocks. However, this increase may also relate to higher net primary productivity and C inputs due to increased soil fertility (Downie *et al*., 2011). Although priming effects vary between studies, most evidence indicates that any PyC-induced increase in CO_2 production is likely to be a short term effect, with a negligible impact on SOC stocks in the longer term (Woolf and Lehmann, 2012). However, the long term direction and duration of any priming effects remain unclear, with few studies investigating the impact of environmental weathering of PyC on interactions with native SOC (Spokas, 2013).

1.4 Thesis structure

This thesis is structured around four journal paper-style chapters. The primary aim of this thesis was to assess the impact of LUC from conventional agriculture to lignocellulosic biomass crop production on SOC stocks on a landscape scale in Britain to provide an improved understanding of the short term effects and how this LUC may be better managed in the future. The first of the four journal paper-style chapters presents results that aim to address this knowledge gap. Subsequent chapters present results of experiments and additional data analyses that aim to further improve our understanding of SOC stocks. Since soil bulk density is required to convert SOC from a mass to an area basis and, therefore, to assess the impact of LUC on SOC stocks, statistical models were required to estimate this characteristic.

Statistical models were developed for the purpose of providing accurate estimates of soil bulk density of lignocellulosic biomass cropland soils in Britain. The performance of these models was compared with published models in the literature to select the best-fit model and the results of this comparison have been presented in Chapter 3. Since the dataset presented in Chapter 2 demonstrated short-term LUC-induced losses of SOC stocks, an experiment was carried out to investigate the potential for PyC to reduce these losses by stabilising native SOC. The results of this experiment are presented in Chapter 4. To improve our understanding of the impacts of direct PyC amendments to soil and optimise its function as a C sequestration tool capable of augmenting SOC stocks, a deeper insight into the abundance of pre-existing black carbon (BC) and its importance in the global C cycle is essential. Further analysis of the dataset presented in Chapter 4 was carried out to yield a statistical model that could be used for rapid estimation of soil BC content to help address gaps in our inventories of global PyC stocks and the PyC cycle. The results of this experiment have been presented in Chapter 5.

1.5 Thesis objectives and overview

The results presented in this thesis aim to address an important knowledge gap concerning the effects of LUC for lignocellulosic biomass crop production on SOC stocks in Britain. To provide an improved understanding of SOC stocks, 6 principle objectives were set to:

1. Determine the general trajectory of SOC stocks for the initial 13 to 22 years following the establishment of commercial lignocellulosic biomass crops;

Miscanthus x *giganteus* and SRC willow, on former arable and grassland in Britain.

2. Investigate the influence of soil texture (clay, silt and sand) and climate (mean annual precipitation and mean annual temperature) as controlling factors on SOC changes following LUC to lignocellulosic biomass crops.
3. Using biomass crop-specific soil data, develop pedotransfer functions for estimating bulk density of lignocellulosic biomass cropland soils in Britain and compare their performance with that of published pedotransfer functions derived from other land-uses.
4. Assess whether environmentally weathered PyC reduces LUC-induced losses of SOC through negative priming.
5. Assess the sensitivity of priming effects to LUC-induced changes in soil physicochemical properties, such as SOC content, pH and soil bulk density, to elucidate any potential consequences for the timing of PyC application to land in transition.
6. Develop and evaluate the performance of statistical models for the estimation of soil BC content using soil C and N data obtained from SRC willow plantations in Britain.

This thesis is composed of four journal paper-style chapters reporting original research findings, with each chapter containing an introduction, methods, results, discussion and conclusion section. Chapter 2 addresses Objectives 1 and 2, Chapter 3 addresses Objective 3, Chapter 4 addresses Objectives 4 and 5 and Chapter 5 addresses Objective 6 as outlined below.

Chapter 2: Empirical models were developed to determine the general trajectory of SOC stocks following LUC from conventional agriculture to lignocellulosic biomass crops in Britain. Soil samples were taken from 93 commercial plantations of different ages and this chronosequence dataset was used to derive general carbon response functions to provide a general measure of change across multiple sites in Britain (Objective 1). Soil texture and climate data for each site were used to create multivariable models to assess the influence of different environmental conditions on SOC changes following LUC (Objective 2).

Chapter 3: Since soil bulk density is required to calculate SOC stocks, pedotransfer functions were evaluated for the purpose of predicting soil bulk density of lignocellulosic biomass crops in Britain. Using bulk density measurements of the 0-15 cm soil profile and other soil properties from a large number of biomass crop plantations, regression models were developed and validated, and their performance for predicting bulk density compared with a range of published models (Objective 3). This also helped to select a pedotransfer function for estimating bulk density of the 15–30 cm soil profile, which was used to calculate SOC stocks on an area basis for the 0–30 cm soil (Objective 1).

Chapter 4: In this chapter the impact of environmentally weathered PyC on native SOC mineralisation was assessed for land in transition to lignocellulosic biomass crop production. To do this, soil was collected and incubated from multiple recently established SRC willow plantations approximately 2 years after amendment with PyC. Cumulative CO_2 flux measurements from incubated soil as well as field flux measurements were used to test the hypothesis that environmentally weathered PyC can reduce native SOC mineralisation by negative priming (Objective 4). By sampling sites of different ages this study also aims to assess the sensitivity of priming effects to changes in soil properties following LUC to help elucidate any potential consequences for the timing of PyC application (Objective 5).

Chapter 5: To help address gaps in our inventories of global PyC stocks and provide an improved understanding of the PyC cycle, statistical models were developed and validated using more easily obtainable and available data for the estimation of soil BC content (Objective 6). Soil was collected from control and PyC-amended experimental plots representing the soil background BC content and elevated levels of BC following purposeful amendment with PyC. Using soil C and N data of SRC willow plantations with varying OC status and with relatively low and high concentrations of BC, statistical models were developed using regression analysis and an artificial neural network approach and their predictive capabilities evaluated and compared.

Chapter 6: This chapter addresses the overall aim of the thesis by presenting a general discussion of the impacts of LUC to lignocellulosic biomass crop production on SOC stocks, as well as the potential for an improved future management of LUC and implication for policy makers.

Chapter 7: This chapter concludes with a summary of the major findings of the above chapters and recommendations for further research.

Chapter 2 The impact of land-use change from conventional agriculture to lignocellulosic biomass crop production on soil organic carbon stocks in Britain

Project aim, scope and experimental design were conceived by the candidate's supervisors with assistance from the Carbo-BioCrop consortium members. The candidate carried out site visits for soil sampling, sample preparation, laboratory analysis, data analysis and writing of the chapter. Rebecca Rowe selected study sites and provided support with field work planning, logistics and with soil sampling. Mike Duvall assisted with laboratory analysis. This chapter forms the basis for a manuscript published in *Biomass and Bioenergy* as:

McClean GJ, Rowe RL, Heal KV, Cross A, Bending GD, Sohi SP (2015) An empirical model approach for assessing soil organic carbon stock changes following biomass crop establishment in Britain. Biomass and Bioenergy, 83, 141–151.

DOI: 10.1016/j.biombioe.2015.09.005.

Type of paper: Original research article

2.1 Introduction

Globally, soils represent the most important long term organic carbon store in terrestrial ecosystems, containing 4.5 times as much carbon (C) as all living biomass (Lal, 2004a) and 3.1 times as much as the atmosphere (Oelkers and Cole, 2008). Soil organic carbon (SOC) storage results from a dynamic equilibrium between C continuously entering the soil through organic matter inputs and leaving through decomposition and mineralisation, dissolved organic carbon leaching and erosion. Land-use change (LUC) from natural to agro-ecosystems has a major impact on this balance and is the second largest source of anthropogenic greenhouse gas (GHG) emissions after fossil fuel combustion (IPCC, 2013). This sensitivity to human impact is recognised in the Kyoto Protocol and signatory states are obliged to report SOC stock changes resulting from LUC in their annual GHG inventories. Consequently, efforts are being made to identify land-uses that increase SOC storage and utilise the C sink capacity offered by global agricultural and degraded soils (Smith *et al*., 2000; Lal, 2004b).

Bioenergy has been identified as one such option which may have the potential to offset anthropogenic CO_2 emissions through soil C sequestration as well as fossil fuel substitution (Smith *et al*., 2000; Hillier *et al*., 2009). Consequently, LUC from conventional agriculture to purpose-grown lignocellulosic biomass crop production has become increasingly common in Europe in recent decades (Don *et al*., 2012). Purpose-grown biomass crops have been promoted as a source of lignocellulosic feedstock for the production of heat and electricity as well as for the future production of liquid biofuels (Gomez *et al*., 2008). It has been suggested that lignocellulosic biomass crops are a more sustainable resource than using food crop-based biofuels (Crutzen *et al*., 2007; Searchinger *et al*., 2008; Whitaker *et al*., 2010). Studies indicate that lignocellulosic biomass crops require fewer inputs and can grow on marginal land (Hastings *et al*., 2009; Hillier *et al*., 2009; Tilman *et al*., 2009) but concerns remain over competing land-use where purpose-grown biomass crops will replace food production.

Miscanthus x *giganteus* and short-rotation coppice *Salix* spp. (SRC willow) are the most prevalent lignocellulosic biomass crops in the UK and currently cover estimated areas of 8000 ha and 3000 ha respectively (DEFRA, 2013). However, this may increase, with 0.93–3.63 M ha of land being identified as available for lignocellulosic biomass crop production in the UK (DECC, DEFRA, DfT, 2012). Although life-cycle assessment (LCA) studies indicate that *Miscanthus* and SRC willow have significant potential for GHG mitigation through fossil fuel substitution (Styles and Jones, 2007; Styles and Jones, 2008; Hillier *et al*., 2009; Djomo *et al*., 2011; Tonini *et al*., 2012), a lack of experimental data relating to the effects of LUC on SOC stocks remains a barrier to their promotion (Don *et al*., 2012; Goglio *et al*., 2015).

The effects of LUC on SOC stocks are difficult to assess and long term monitoring of SOC stocks through repeated assessment of soil inventories is time-consuming and complex, often showing insignificant changes in SOC or inconsistent temporal and spatial trends (Fahey *et al*., 2005; Johnson *et al*., 2007; Hopkins *et al*., 2009; Kiser *et al*., 2009; Goglio *et al*., 2015). The potential to measure changes in SOC over time is limited with detectability dependent on the number of samples taken as well as the rate of change (Smith, 2004; Schrumpf *et al*., 2011). Attempts have been made to develop simple and cost-effective practical indicators of SOC stock changes that would avoid repeated sampling (Sohi *et al*., 2010; Culman *et al*., 2012). However, such measurements have not been widely tested and require validation for a range of soil and land-use types. Due to the many problems associated with long term measurements, methods have been developed that use a space-for-time substitution to infer changes over time by sampling sites of different ages (Pickett, 1989).

Previous studies aiming to assess the effects of LUC for biomass crop production mainly utilise a single-site paired plot approach, which consists of comparing adjacent fields that share similar characteristics and are both pre- and post-LUC. Such studies often infer short term gains in SOC following LUC from arable crops to *Miscanthus* in temperate Europe (Hansen *et al*., 2004; Kahle *et al*., 2007; Dondini *et al*., 2009) while trends are inconsistent for LUC from arable crops to SRC willow with observations of increases or no changes in SOC stocks (Jug *et al*., 1999). Studies typically infer no significant change in SOC following the conversion of grassland to *Miscanthus* (Hansen *et al*., 2004; Clifton-Brown *et al*., 2007; Schneckenberger and Kuzyakov, 2007) and a loss of SOC following LUC from grassland to SRC willow (Makeschin, 1994; Jug *et al*., 1999). Variation in the trajectory and magnitude of change between studies reflects the sensitivity of SOC to many factors such as biomass crop type, previous land-use, climate and soil texture (Keoleian and Volk, 2005). As a result, SOC changes inferred from these inter-site comparisons are often related to site-specific conditions and do not provide for a general measure of change. Therefore, a large number of study sites representing LUC under a range of conditions would be required to ascertain a more general understanding of LUC. However, this paired plot approach is constrained by the availability of suitable reference sites and it is often difficult to identify and sample a large number of sites in this way.

The chronosequence method offers an alternative approach to the single-site comparison to assess temporal trends where multiple sites of different ages can be assumed to follow the same general trajectory (Walker *et al*., 2010). The carbon response function (CRF) concept was developed as a simple statistical tool to estimate the average annual change in SOC following LUC (West *et al*., 2004; Vesterdal *et al*., 2011). It has been used to analyse long term chronosequence datasets to estimate the effects of major LUCs in temperate Europe (Stevens and Van Wesemael, 2008; Poeplau *et al*., 2011). With this approach, regression models are fitted to the dataset and using maximum likelihood the best-fit model, or 'general carbon response functions' (CRF_{gen}), is identified to provide an overall measure of change across multiple sites (Vesterdal *et al*., 2011). To investigate the influence of environmental parameters on SOC change rate and to improve the model fit, these variables are used in a more complex model designated 'specific carbon response functions' (CRF_{spec}) for the purpose of more region-specific estimates (Poeplau *et al*., 2011; Vesterdal *et al*., 2011). These empirical models are more transparent and less complex than process-based simulation models although they require large datasets to provide reliable estimates of temporal trends in SOC following LUC. The main objectives of this study are: (i) to develop an empirical model to assess short term trajectory in SOC stocks following LUC for lignocellulosic biomass crop production in Britain and; (ii) assess the effects of environmental parameters on SOC changes. The main purpose is to assist in targeting future research efforts and to provide preliminary evidence in a format suitable for policy makers.

2.2 Materials and methods

2.2.1 Site selection

Letters were sent to commercial growers of SRC willow and *Miscanthus* x *gigeanteus* plantations with questionnaires designed to assess the suitability of land, and seek permission, for inclusion in this research project. A list of 150 commercial

SRC willow and 121 *Miscanthus* plantations was compiled, from which 45 SRC willow and 48 *Miscanthus* plantations in England and Wales were selected for soil sampling. To limit variance arising from atypical site factors the following were excluded from the list: (i) sites with anomalously high SOC content (>8% SOC) or wetland soil; (ii) crops established on reclaimed land, and; (iii) land where organic fertiliser (sewage sludge or manure) had been applied in the past five years. To reduce bias, only one field was sampled on a given farm, even if another plantation of a different stand age was present. Sites were selected to obtain as far as possible a broad and even range of stand age and an equal representation of SRC willow and *Miscanthus* plantations that had been established on arable and permanent grassland. All of the plantations were between 1 and 14 years old at the time of sampling, with the exception of one 22-year old SRC willow crop. This was owing to the relatively recent emergence of these crops as a biomass resource in Europe. The number of plantations that had been established on grassland was limited. Therefore, all available sites established on permanent grassland were sampled and these were supplemented by sites established on set-aside fields that had been under grassland management for at least five years prior.

Many of the sites from each biomass crop type were generally located in the same geographical area (Figure 2.1) with broadly similar climatic characteristics and soil texture (Table 2.1). The distribution of sites was affected by historic planting efforts, with a concentration of SRC willow in the north-east and *Miscanthus* in the south-west of England (Figure 2.1). Site climate was characterized using mean annual precipitation (MAP) and mean annual temperature (MAT), based on 1981–2010 observations, obtained for the Met Office weather station nearest to each study site.

Table 2.1 Summary of site characteristics for each LUC. Clay and SOC are weighted averages for the 0–30 cm soil profile using BD values for the 0–15 and 15–30 cm increments.

	Arable to SRC willow (0–14 yrs)	Arable to SRC willow (0–22 yrs)	Arable to *Miscanthus*	Grass to SRC willow	Grass to *Miscanthus*
n	29	30	37	15	11
Clay (%)					
Mean	17.60	18.27	19.88	15.91	22.54
Standard deviation	4.76	5.97	5.18	4.87	5.04
Median	16.86	16.98	19.09	15.74	23.41
Range	21.34	27.77	19.89	20.94	20.36
IQR (inter quartile range)	15.00 to 19.78	15.45 to 20.80	15.78 to 24.76	13.21 to 16.47	20.40 to 24.69
MAP (mm)					
Mean	657.9	657.9	837.9	717.3	899.4
Standard deviation	73.1	71.8	174.1	123.9	146.6
Median	620.2	635.7	751.1	659.7	918.2
Range	253.2	253.2	523.1	496.4	372.9
IQR	614.6 to 659.7	614.6 to 659.7	659.7 to 1017.4	656.6 to 496.4	708.5 to 372.9
MAT (°C)					

	Arable to SRC willow (0–14 yrs)	Arable to SRC willow (0–22 yrs)	Arable to *Miscanthus*	Grass to SRC willow	Grass to *Miscanthus*
Mean	10.0	10.1	10.1	10.1	10.6
Standard deviation	0.6	0.6	0.7	0.9	0.4
Median	10.0	10.0	10.1	10.5	10.8
Range	2.1	2.1	2.3	2.4	1.3
IQR	9.9 to 10.6	9.9 to 10.8	9.9 to 10.8	9.2 to 10.8	10.1 to 1.3
SOC (%)					
Mean	2.15	2.26	2.28	2.20	2.82
Standard deviation	1.18	1.29	0.74	1.24	0.99
Median	1.84	1.86	2.11	1.65	2.53
Range	4.29	4.55	3.20	4.20	2.71
IQR	1.39 to 2.51	1.42 to 2.55	1.77 to 2.71	1.33 to 2.96	2.05 to 2.71

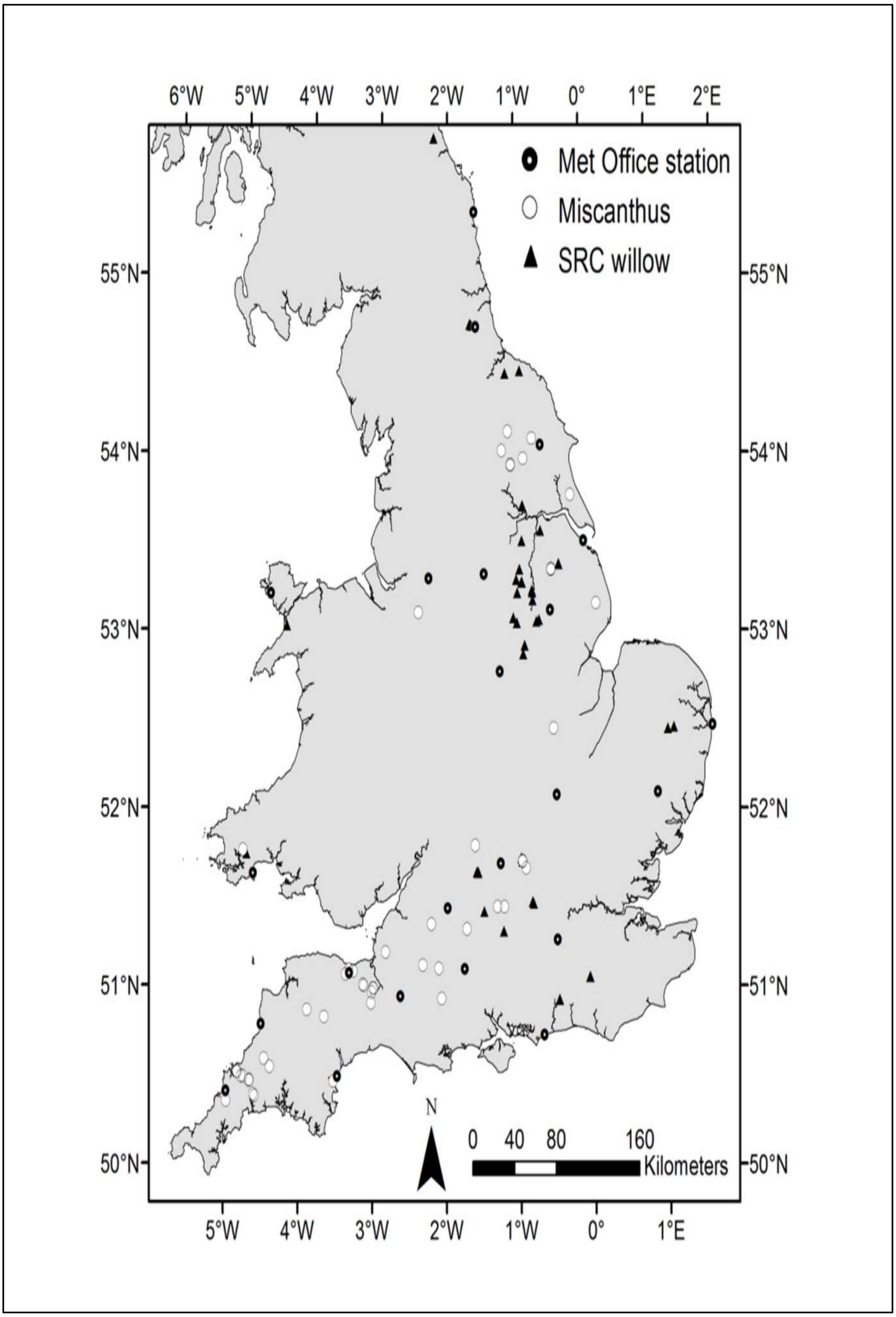

Figure 2.1 Locations of study sites and nearest Met Office weather stations.

Soil at 70% of the sites was classified as ‘medium’ textured (15–30% clay), while 26% of the sites were ‘light’ (<15% clay) and 4% as ‘heavy’ textured (>30% clay). Since soil texture is a major controlling factor of SOC content (Feller and Beare, 1997), information on soil texture was sought in the questionnaires and an effort was made to avoid soils with high clay contents and to select sites of broadly similar texture to ensure a similar site trajectory of SOC. However, a more accurate classification of soil texture based on percentage clay, silt and sand abundance could only be determined *post hoc*.

2.2.2 Soil sampling

Soil sampling at the 93 study sites was undertaken between March and November 2011. Each field was divided into a grid with 100 intersections using a scale appropriate to field size, of which 25 were selected for sampling using a random number generator. Soil cores of 30 mm diam. were taken using a bi-partite gouge auger and absorbing hammer (Van Walt, Haslemere, UK) to 30 cm depth and divided into two layers: 0–15 and 15–30 cm. Where sampling was prevented by the presence of roots or large stones, a full sample was taken from within 10 cm of the grid intersection. Partial samples were discarded to prevent a depth bias. Samples were combined for each of the two layers and stored at 4°C for a maximum of 2 weeks before processing for analysis to reduce the impact of storage on microbial activity (Zelles *et al*., 1991). Field work campaigns were therefore limited to a maximum of 2 weeks and, on return to the laboratory, samples were frozen until all field work was complete. Three additional cores of 50 mm diam. were taken to 15 cm depth from randomly selected intersections, using a specialised ring corer kit to measure soil bulk density (BD) (Van Walt, Haslemere, UK).

2.2.3 Soil analysis

After thawing, the composite soil samples were sieved manually using a 5.6 mm sieve and homogenised using the cone and quarter method (Raab *et al.*, 1990). This procedure involved piling the sample into a cone, which was flattened and divided into four quarters, with two opposite quarters removed before re-piling the remaining sample into a cone and repeating the process until the desired sample size was obtained. A representative sub-sample was then collected and air-dried at room temperature for 7 days, before being crushed with a pestle and mortar, sieved (<2 mm) and milled to a fine powder using a MM200 ball mill (Retsch GmbH, Haan, Germany). 20 mg of sample was analysed for total C and N by dry combustion using a TruMac elemental analyser (Leco, St. Joseph, MI, USA).

Inorganic C content was measured using an automated acidification module and coulometric titration (CM 5012 and CM 5130, UIC, Joliet, Illinois). 50–100 mg of each sample was loaded into a reaction tube and acidified using 8 ml of 2 *M* perchloric acid ($HClO_4$). During this procedure carbonates are released as CO_2 into a carrier gas stream that had been purged of CO_2 to a coulometer cell. As CO_2 enters the cell it is quantitatively absorbed by ethanolamine (C_2H_7NO) to form a titratable acid which causes a colourimetric pH indicator to fade in colour. This fade is detected by a photodetector as a percentage of transmittance change and a titration current is triggered to electrochemically produce a base proportional to the transmittance change. The weight of CO_2 is then calculated from the quantity of electricity required to titrate the acid using Faraday's laws of electrolysis (Hirmas *et al.*, 2012). For all samples, SOC content was determined by subtracting the inorganic C from the total C content.

Abundance ratios of clay- (<2 µm), silt- (2–63 µm) and sand-sized (63–2000 µm) primary particles were determined for the soil mineral fraction using a laser diffractometer (Beckmann Coulter LS230, High Wycombe, UK). Samples containing inorganic C >0.01% by weight were treated to remove carbonates prior to analysis. For this, 20 g of each sample was acidified with 20 ml of 1 *M* sodium acetate (NaOAc), adjusted to pH 5 with glacial acetic acid (CH_3COOH). Acidified samples were maintained at 70°C overnight in a water bath and then centrifuged at 2500 rpm and the supernatant discarded. After carbonate removal, 10 g of all samples were treated for the removal of organic matter with 20 ml of 30% w/w hydrogen peroxide (H_2O_2) and the suspension maintained at pH 5 with 0.1 *M* NaOAc buffer. The mixture was left at room temperature for 1 hour and then heated to 70°C for 24 hours using a water bath. Each residue was then rinsed three times with deionised water and oven dried overnight at 80°C (Lavkulich and Wiens, 1970; Dumat *et al*., 1997).

Oxidised, carbonate-free residues were dispersed by treating overnight with 25 ml of 4% w/v sodium hexametaphosphate $(NaPO_3)_6$ before being placed in an ultrasonic bath for 10 minutes and sieved (<1 mm) prior to analysis with the laser diffractometer. The sample pretreatment methods that were used here for organic matter removal and soil particle dispersion were first tested for their effectiveness at achieving soil aggregate separation to help ensure the accuracy and reliability of soil particle size distribution measurements (see Appendix 1). The >1 mm residue was isolated by vacuum filtration then oven-dried at 80°C and weighed. The volume of the >1 mm fraction was estimated using an assumed grain density of 2.65 g cm^{-3} (Freeze and Cherry, 1979) and particle size distribution calculated for the <2 mm sample. This procedure was used to prevent particles >1 mm from damaging the lens of the laser diffractometer.

Samples collected for BD measurements were returned to the laboratory, oven dried at 105°C for 48 hours and sieved (<2 mm) using a mechanical roller mill to separate coarse fragments from fine earth. Collected samples were weighed to calculate BD of the fine earth (BD_{fe}) [Equation (2.1)], correcting for the volume of coarse fragments with an assumed density of 2.65 g cm^{-3} [Equation (2.2)] (Cools and De Vos, 2010).

$$BD_{fe}\ (g\ cm^{-3}) = \frac{(soil\ with\ gravel\ (g) - coarse\ fragments(g))}{volume\ of\ corer(cm^{-3}) - \left(\frac{coarse\ fragments(g)}{2.65}\right)} \quad (2.1)$$

$$BD_{corrected}\ (g\ cm^{-3}) = BD_{fe} \times diameter\ of\ corer\ (cm) \times 10 \times \left(1 - \left(\frac{coarse\ fragments(g)}{(2.65 \times volume\ of\ corer(cm^{-3}))}\right)\right) \quad (2.2)$$

2.2.4 Data analysis

2.2.4.1 Pedotransfer function to estimate soil bulk density

To calculate SOC density on an area basis, it was first necessary to obtain BD values for the 15–30 cm layer since these were not measured due to limited time and resources. To do this, a pedotransfer function (PTF) was developed to derive estimates of 15–30 cm soil BD using regression analysis, with the 0–15 cm BD measurements as the dependent variable and the following measured soil or site properties as independent variables: % SOC, % total nitrogen, % clay, % silt, % sand and time since conversion (years). PTF development is discussed in more detail in Chapter 3. Briefly, regression models were developed using a training dataset with

70% of the BD data and validated with the remaining 30% of the dataset. A wide range of published PTFs were also validated using the biomass crop soil data. Results indicate that separating the data according to crop type did not improve model performance. Therefore, the same PTF was used for SRC willow and *Miscanthus*. Two linear equations provided the best predictive models and 12 of the published models performed equally as well. These models include various nonlinear functions and multiple variables including interaction terms. Since the goodness of fit is comparable for each of these models, the simplest model was selected which was a linear model with SOC as the only independent variable [Equation (2.3)]. The PTF was also used to estimate BD for the 0–15 cm soil layer for three of the sites with missing data.

$$BD(g\ cm^{-3}) = 1.49 - (0.09 \times SOC) \quad (2.3)$$

where SOC is soil organic carbon (%).

2.2.4.2 Carbon response functions

CRFs were developed for each LUC based on the approach developed in a number of recent studies (West *et al*., 2004; Stevens and Van Wesemael, 2008; Poeplau *et al*., 2011; Vesterdal *et al*., 2011). Since each biomass plantation did not have a reference arable or grassland site to serve as a baseline measurement, the CRF approach was modified to model SOC density (t ha^{-1}) in absolute values, rather than relative changes from a baseline (% ΔSOC), over time. SOC density (t ha^{-1}) was calculated using the fixed depth approach to 30 cm depth [Equation (2.4)] using results from the 0–15 and 15–30 cm soil samples:

$$SOC\ density\ (t\ ha^{-1}) = \sum_{i=1}^{n} C\ concentration\ (\%\ mass)_i \times BD\ (g\ cm^{-3})_i \times depth\ (cm)_i \quad (2.4)$$

Regressions fitted to the SOC density values included linear, quadratic, cubic, power and exponential functions. Statistical models were selected to most adequately describe the trend in SOC stocks over time (± Wald 95% confidence intervals) for each LUC using the corrected Akaike information criterion (AICc) [Equation (2.5)] and designated general carbon response functions (CRF_{gen}). Due to the limited number of observations for each LUC, AICc rather than AIC was used as a model selection criterion since this includes a correction factor which is recommended for smaller datasets, i.e. where *n/k* <40 (Burnham and Anderson, 2002). Although AICc provides a criterion for selecting the most likely *true* model from a set of candidate models, it does not provide an absolute measure of performance (Burnham and Anderson, 2002). For this purpose, predictive capacity was evaluated using a set of statistical criteria. Overall model robustness was evaluated using the model efficiency index (EF) [Equation (2.6)]. Root mean square prediction error (RMSPE) [Equation (2.7)] was used to measure the overall prediction error. Estimated SOC stock changes were inferred for each LUC by comparing against typical pre-change SOC stocks. It was not appropriate to extrapolate pre-change SOC stocks from the free-intercept models since these could not be expected to account for any initial changes following LUC. Therefore, typical pre-change SOC stocks were approximated using the National Soils Inventory (NSI) (DEFRA, 2009) with mean values of 77 and 96 t C ha^{-1} to a fixed depth of 30 cm for mineral soils under arable and grassland management respectively. The standard error bars for these mean SOC values were not included in the literature.

$$AICc = \left(n\ln\left(\frac{SSE}{n}\right) + 2(k)\right) + \left(\frac{2k\ (k+1)}{n-k-1}\right) \quad (2.5)$$

$$EF = \frac{\left(\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2 - \sum_{i=1}^{n}\left[\left(P_i - O_i\right)^2\right]\right)}{\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2} \quad (2.6)$$

$$\text{RMSPE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(P_i - O_i\right)^2} \quad (2.7)$$

where n is the total number of observations, SSE is the sum of squared errors of prediction and k is the number of parameters plus 1, P_i are the predicted values, O_i the observed values and $\bar{O}$ the mean of the observed data.

Specific CRFs (CRF_{spec}) were also created to assess the influence of other explanatory variables on changing SOC stocks (Table 2.2). Clay, silt and sand density (t ha^{-1}) was used instead of relative abundances (%) since these provided a better fit and enable greater predictive accuracy. Exponential and power functions were selected for CRF_{gen} [Equations (2.8–2.9)] which were enhanced for CRF_{spec} by entering explanatory variables in a hierarchical manner as direct effects on model coefficients to increase EF and decrease RMSPE [Equations (2.10–2.11)]. The order of the variables (x_1, x_2…) indicates their degree of influence with x_1 having the greatest effect. Explanatory variables were added individually (with age in the model but no other X variables) and associated coefficients used to indicate either a positive or negative effect on each response function (Poeplau *et al*., 2011). Since sampling season is a categorical variable with spring, summer and autumn assigned values of 1, 2 and 3 respectively, effects on the rate of change are from changing season in the order of spring to autumn.

Table 2.2 Explanatory variables used to develop CRF_{spec}.

Variable	Units / categories	Method / description	Direct or indirect measurement
Clay density	$t\ ha^{-1}$	Laser diffraction	Direct
Silt density	$t\ ha^{-1}$	Laser diffraction	Direct
Sand density	$t\ ha^{-1}$	Laser diffraction	Direct
Mean annual precipitation	mm	Interpolated data based on 1981-2010 observations	Indirect
Mean annual temperature	°C	Interpolated data based on 1981-2010 observations	Indirect
Season	spring / summer / autumn	Season during which sampling occurred.	Direct

Exponential CRF_{gen}: $SOC\ density\ (t\ ha^{-1}) = a + be^{ct}$ (2.8)

Power CRF_{gen}: $SOC\ density\ (t\ ha^{-1}) = at^{b}$ (2.9)

Exponential CRF_{spec}: $SOC\ density\ (t\ ha^{-1}) = a + (b_0 +\ b_1x_1 + \cdots\ b_ix_i)e^{ct}$

(2.10)

Power CRF_{spec}: $SOC\ density\ (t\ ha^{-1}) = (a_0 + a_1x_1 + \cdots a_ix_i)t^{b}$ (2.11)

where *t* is time after LUC (years), *a*, *b*, and *c* are constants and *x* denotes the explanatory variable. All regression analysis, curve fitting and checking of residuals for normal distribution using the Shapiro Wilk test were carried out using Genstat 16 (VSN International, Hemel Hempstead, UK). *SSE* values were obtained from Genstat 16 (VSN International, Hemel Hempstead, UK) and AICc calculated using the method of Motulsky and Christopoulos (2004).

2.3 Results

2.3.1 Arable to SRC willow

Two CRFs were established to describe changes in SOC stocks following conversion of arable land to SRC willow: (i) for the initial 14-year period and (ii) including the 22-year old site. Dual analysis was carried out to enable comparison of all LUCs over a similar time frame and since this 22-year site was not identified as an outlier using the Grubb's test. In both cases, exponential and power functions provided the lowest AICc scores, with a difference of less than two between them (ΔAICc $\leq$2), indicating these are the best predictive models for estimating SOC stocks (Table 2.3). Although both models are considered equally likely to be the *true* model, since EF was slightly higher for the exponential model this was selected for the CRF_{gen}. The CRF_{gen} estimated an increase in SOC stocks from years 2-14 of 42.2 ± 19.1 t ha^{-1}, with SOC accumulating at a rate of 3.5 ± 1.6 t ha yr^{-1} (Figure 2.2a). Compared to the NSI average of 77 t ha^{-1} for arable land, there was an increase of 15.3 ± 2.2 t ha^{-1} occurring at a rate of 1.1 ± 0.2 t ha yr^{-1}. The CRF_{gen} also estimated an increase in SOC stocks from years 2-22 of 78.5 ± 51.0, with an accumulation rate of 3.9 ± 2.6 t ha yr^{-1} (Figure 2.2b). There was an increase of 68.8 ± 49.4 t ha^{-1} from the NSI average, with SOC accumulating at a rate of 3.1 ± 2.2 t ha yr^{-1}.

EF was improved for both the 14-year and 22-year CRFs (from 0.08 to 0.51 and from 0.17 to 0.57 respectively) with the addition of explanatory variables (Table 2.4). Sampling season, clay density and MAT all had an effect on temporal trends in SOC. In both cases a predicted positive effect on the response function occurred from spring to autumn (Table 2.5). For the 0–14 year period, the 3 SRC willow plantations that were sampled in autumn had the highest mean SOC stock (± standard deviation) of 119 ± 43.4 t ha^{-1}, compared to a mean of 94.8 ± 33 t ha^{-1} for the 9 plantations sampled in summer and a mean of 65.7 ± 28 t ha^{-1} for the 17 plantations sampled in spring (Figure 2.3). The same overall trend was observed for the 0–22 year period and the 22-year old SRC willow plantation was sampled in summer, giving a mean SOC stock of 100.6 ± 36.1 t ha^{-1} for the 10 plantations that were sampled in summer (Figure 2.3). A negative effect of clay density on the response function indicates lower SOC accumulation for more clayey soils (Table 2.5). A positive effect of MAT indicates greater SOC accumulation in warmer regions (Table 2.5).

Table 2.3 Model evaluation for each LUC.

LUC	Function	EF	AICc
Arable to SRC willow (after 14 years)	Cubic	0.09	215.9
	Quadratic	0.09	213.1
	Linear	0.06	211.3
	Power	0.07	206.1
	Exponential	0.08	205.8
Arable to SRC willow (after 22 years)	Cubic	0.20	222.1
	Quadratic	0.17	220.4
	Linear	0.17	217.8
	Power	0.16	213.2
	Exponential	0.17	212.9
Arable to *Miscanthus* (after 13 years)	Cubic	0.09	222.3
	Quadratic	0.04	221.7
	Linear	0.04	219.1
	Exponential	0.04	214.5
	Power	0.06	214.0
Grass to SRC willow (after 14 years)	Cubic	0.13	123.9
	Quadratic	0.08	120.1
	Linear	0.07	116.4
	Power	0.05	111.0
	Exponential	0.08	110.5
Grass to *Miscanthus* (after 13 years)	Cubic	0.36	81.5
	Quadratic	0.06	78.4
	Linear	0.06	73.2
	Power	0.07	66.1
	Exponential	0.11	65.6

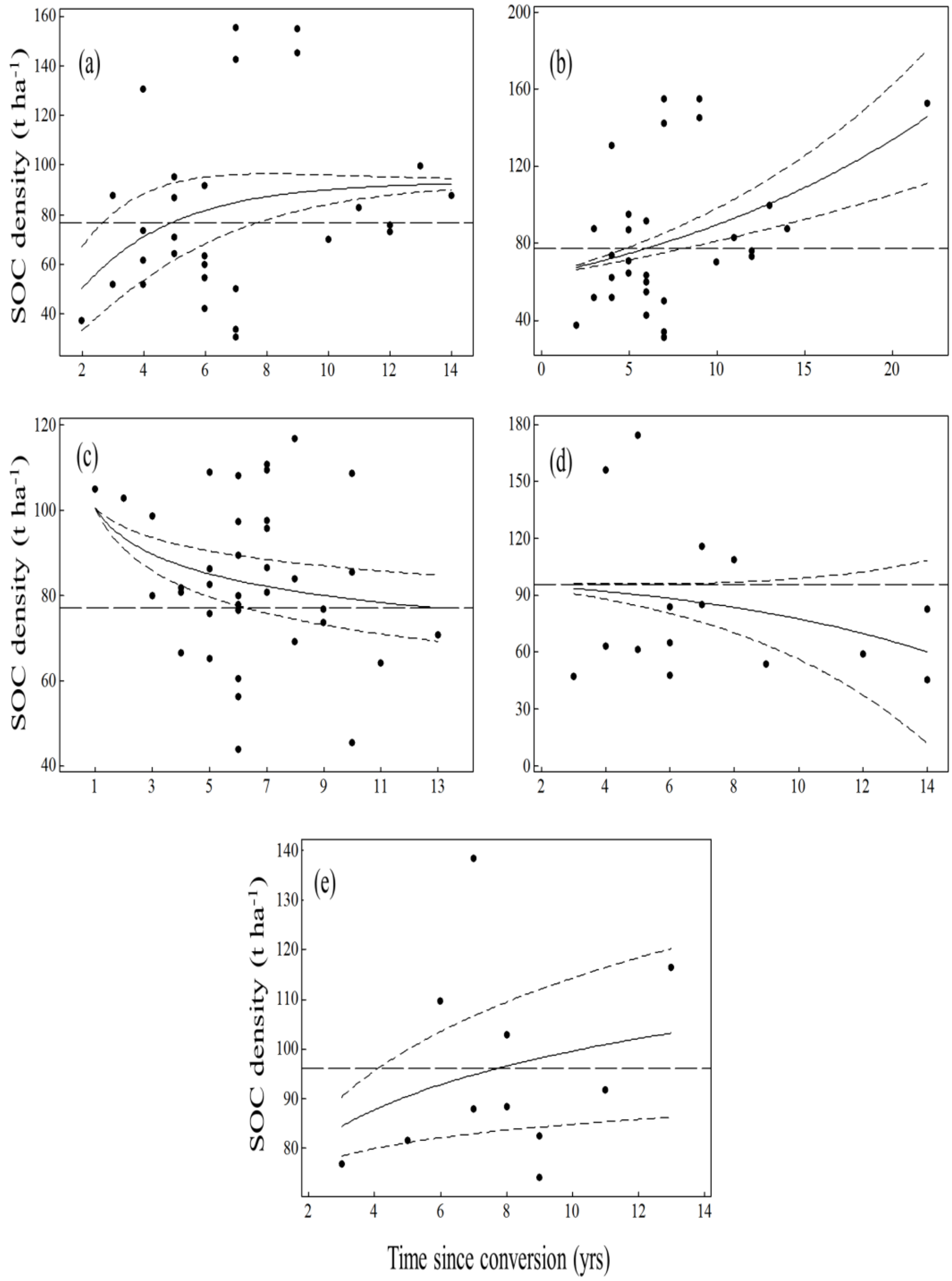

Figure 2.2 Changing SOC stocks (t C ha^{-1} ± 95% confidence intervals) after LUC: (a) arable to SRC willow 0–14 yrs; (b) arable to SRC willow 0–22 yrs; (c) arable to *Miscanthus*; (d) grass to SRC willow; (e) grass to *Miscanthus*. Dashed horizontal line represents NSI average values: 77 t C ha^{-1} for arable and 96 t C ha^{-1} for grassland.

Table 2.4 Model performance of CRFs for each LUC.

LUC	Function	Model	Equation	EF	RMSPE (SOC t ha^{-1})
Arable to SRC willow (after 14 years)	Exponential	General	93.12 – 83.37 x exp(0.72 x age)	0.08	33.5
		Specific	-13.64 + (-7.38 + 20.12 x season – 0.04 x clay density + 7.38 x MAT) x exp(0.05 x age)	0.51	24.4
Arable to SRC willow (after 22 years)	Exponential	General	24.05 + 39.04 x exp(0.05 x age)	0.17	33.5
		Specific	-43.50 + (17.87 + 21.71 x season – 0.05 x clay density + 7.97 x MAT) x exp(0.03 x age)	0.57	24.1
Arable to *Miscanthus* (after 13 years)	Power	General	100.46 x $age^{-0.10}$	0.06	17.3
		Specific	No variables entered or removed		
Grass to SRC willow (after 14 years)	Exponential	General	105.44 – 8.39 x exp(0.12 x age)	0.08	36.8
		Specific	92.73 + (1.08 x 10^{-3} – 1.56 x sand density – 1.07 x 10^{-3} x season + 2.39 x MAP) x exp(1.15 x age)	0.17	35.0
Grass to *Miscanthus* (after 13 years)	Power	General	72.39 x $age^{0.14}$	0.07	18.0
		Specific	(193.83 – 0.02 x sand density – 0.02 x silt density – 0.05 x MAP – 2.32 x MAT) x $age^{0.07}$	0.47	13.6

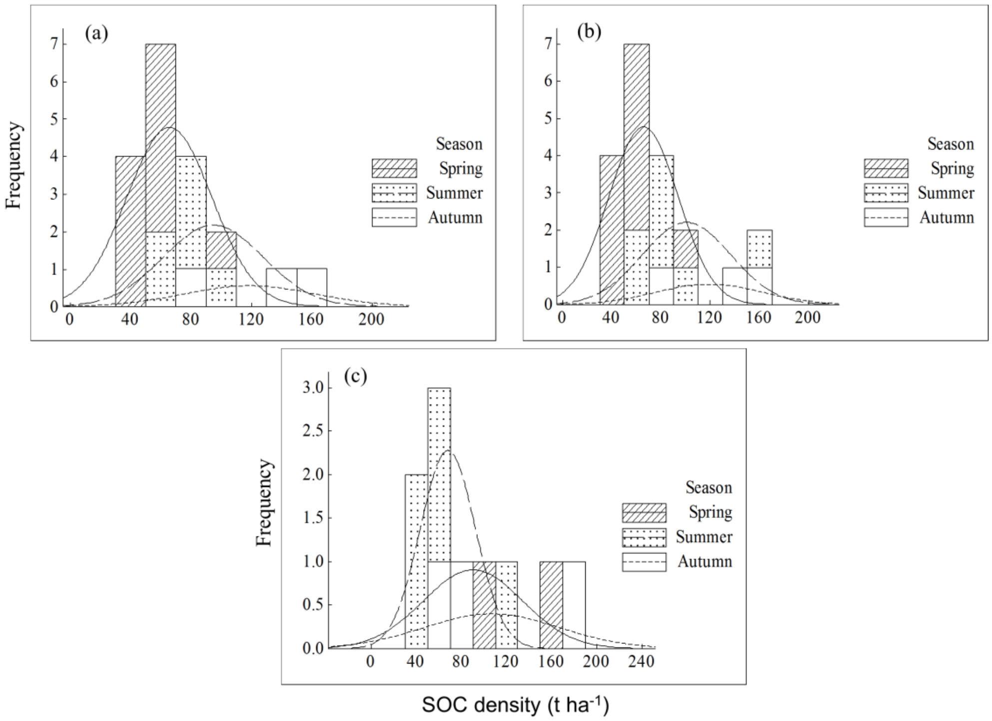

Figure 2.3 Histograms showing frequency of SOC density (t C ha^{-1}) for SRC willow by sampling season: (a) arable to SRC willow 0–14 yrs; (b) arable to SRC willow 0–22 yrs; (c) grass to SRC willow.

Table 2.5 Explanatory variables used to develop CRF_{specs}. + indicates a positive and – a negative effect on the response function. Blank cells indicate variables were not included in the CRF for the respective LUC.

LUC	Explanatory variable					
	Clay density	Silt density	Sand density	MAP	MAT	Season
Arable to SRC willow (0-14 yrs)	–				+	+
Arable to SRC willow (0-22 yrs)	–				+	+
Arable to *Miscanthus*						
Grass to SRC willow			–	–		–
Grass to *Miscanthus*		–	–	–	–	

2.3.2 Arable to *Miscanthus*

Again, exponential and power functions provided the lowest AICc scores for LUC from arable to *Miscanthus* (ΔAICc $\leq$2), indicating these are the best predictive models for estimating SOC stocks (Table 2.3). Since EF was slightly higher for the power model this was selected for the CRF_{gen}. The CRF_{gen} estimated a decline in SOC stocks from years 1–13 of -23.5 ± 7.8 t ha^{-1} occurring at a rate of -2.0 ± 0.7 t ha yr^{-1} (Figure 2.2c). However, there was no difference between estimated SOC stocks after 13 years and the NSI average. No additional variables improved the model fit.

2.3.3 Grass to SRC willow

Again, exponential and power functions provided the lowest AICc scores for LUC from grass to SRC willow (ΔAICc $\leq$2), indicating these are the best predictive models for estimating SOC stocks (Table 2.3). Since EF was slightly higher for the exponential model this was selected for the CRF_{gen}. From years 3–14 the CRF_{gen} follows a slight downward trend but with no demonstrable overall change in SOC, with a model estimate of -33.5 ± 51.0 t ha^{-1} (Figure 2.2d). Similarly there was no difference between the estimated SOC stocks after 14 years and the NSI average. There were fewer study sites for SRC willow established on grass than on arable land which may contribute to the broader 95% confidence intervals. EF was improved from 0.08 to 0.17 by the addition of the explanatory variables sand density, sampling season and MAP (Table 2.4). Negative effects of sand density and MAP indicate greater SOC losses may occur in sandier and wetter soils (Table 2.5). A negative effect of season indicates decreasing SOC stocks from sites sampled in the order of spring to autumn (Table 2.5). Although the 3 SRC willow plantations that were sampled in autumn had the highest mean SOC stock of 106.8 ± 59.9 t ha^{-1}, SOC stocks still appear to decrease from spring to autumn as the 5 plantations sampled in spring had a higher mean SOC stock than the 7 plantations sampled in summer with 90.2 ± 44 and 67.9 ± 24.5 t ha^{-1} respectively (Figure 2.3).

2.3.4 Grass to *Miscanthus*

Again, exponential and power functions provided the lowest AICc scores for LUC from grass to *Miscanthus* (ΔAICc ≤2), indicating these are the best predictive models for estimating SOC stocks (Table 2.3). Since EF was slightly higher for the exponential model this was selected for the CRF_{gen}. From years 3-13 the CRF_{gen} follows a slight upward trend but with no demonstrable overall change in SOC, with a model estimate of 19.0 ± 23.0 t ha^{-1} (Figure 2.2e). There was also no difference between estimated SOC stocks after 13 years and the NSI average. Again the limited number of sites is reflected in the broad 95% confidence intervals. EF was improved from 0.07 to 0.47 with the addition of the explanatory variables sand density, silt density, MAP and MAT (Table 2.4). Negative effects of sand and silt density, MAP and MAT indicate potential SOC losses or less accumulation in lighter textured soils and/or in warmer and wetter regions (Table 2.5).

2.4 Discussion

LUC from arable crops to SRC willow production was estimated to increase SOC stocks by 15.3 ± 2.2 t ha^{-1} after 14 years and by 68.8 ± 49.4 t ha^{-1} after 22 years. Both CRFs project an exponential curve that begins below the NSI average at year 2 and follows an upward trajectory (Figures 2.2a-b). This indicates an initial decline in SOC stocks after LUC followed by a period of recovery. An initial loss could be expected to result from the disruption of aggregates caused by soil disturbance, leading to the accelerated decomposition of SOC that has lost physical protection (Guo and Gifford, 2002). After this loss, SOC is predicted to accumulate to a level above the NSI average. An expected increase in SOC has previously been attributed to reduced tillage, increased C inputs from leaf, woody and root litter production and by increased transfer of assimilates into the external mycelium of mycorrhizal fungi (Verwijst and Makeschin, 1996; Bowman and Turnbull, 1997; Ek, 1997; Grogan and Matthews, 2002). The 14-year CRF predicts a decline in the rate of accumulation and possibly reaching a new equilibrium (Figure 2.2a). However, the 22-year CRF projects a continued increase, but with a large uncertainty reflected by the broad 95% confidence intervals (Figure 2.2b). These results demonstrate a short term increase in SOC stocks but it is unclear from the parameterised model whether further increases should be expected beyond this initial period, or when a new equilibrium may be reached.

In contrast, LUC from arable crops to *Miscanthus* production did not lead to a demonstrable overall increase in SOC stocks, with no difference between estimated SOC stocks after 13 years and the NSI average (Figure 2.2c). SOC stocks measured for sites 1–2 years old are considerably higher than the NSI average and this produces a negative exponent used to predict a loss over time. Rather than these sites having atypically large pre-change SOC stocks, it is possible that this disparity relates to the influence of changing establishment practices on SOC over time. Low C input arable soils have previously been identified as having a large C storage potential (Smith *et al*., 2000) and paired plot studies have inferred a significant increase in SOC for LUC from arable crops to *Miscanthus* (Hansen *et al*., 2004;

Dondini *et al*., 2009). However, these site-specific effects are not reflected by the CRF_{gen}. Instead, the difference in SOC stocks under *Miscanthus* and SRC willow established on arable land could be due to poor crop yields for *Miscanthus* that have previously been reported and attributed to poor management practices for a newly emerging crop (Lewandowski *et al*., 2000). Although crop yield data could not be obtained for these commercial plantations, patchy *Miscanthus* crop establishment was observed at some sites. It is also possible that the performance of *Miscanthus* in trials using experimental sites does not adequately reflect that of commercial planting which, due to economic factors, may be more likely to occur on lower grade land. Since the resolution of agricultural land classification maps in Britain is not suitable for the assessment of single fields, we were unable to verify the quality of land for our study sites. However, research from another study using focus groups of farmers (Sherrington *et al*., 2008), as well as communication with the growers within this study, both suggests a tendency to select the least productive agricultural land for biomass crop establishment.

No demonstrable changes in SOC stocks are predicted following LUC from grassland to either SRC willow or *Miscanthus* (Figures 2.2d-e). LUC to SRC willow follows a slight downward trend and LUC to *Miscanthus* follows a slight upward trend but in both cases with broad 95% confidence intervals. Both CRFs begin just below the NSI averages, suggesting an initial decline in SOC stocks following LUC. Fewer study sites were available for biomass crops established on grassland which may contribute to the large uncertainty reflected by the broad 95% confidence intervals. These results may support single site paired plot studies that reported no significant differences in SOC between *Miscanthus* and adjacent grassland sites

(Hansen *et al*., 2004; Clifton-Brown *et al*., 2007; Schneckenberger and Kuzyakov, 2007). Fewer studies have compared SOC stocks for grassland and SRC willow but significant losses have been reported (Makeschin, 1994; Jug *et al*., 1999). The CRF developed in the present study indicates that significant losses may occur in some soils but, due to the large uncertainty, does not provide evidence for an overall loss following LUC in Britain. It may be expected that any short term losses incurred by LUC from grassland to *Miscanthus* or SRC willow would have been detected in the present study since other studies of LUCs that act as C sources in temperate Europe have shown that SOC reached a new equilibrium in a similar time frame (Poeplau *et al*., 2011).

EFs of the CRF_{gen} were low with a range of 0.06-0.17 (Table 2.4) and there was a large degree of uncertainty surrounding the estimated changes in SOC stocks. Other explanatory variables were used to enhance the model fit, with soil texture, climate and sampling season all having an effect on measured SOC stocks. Sampling season improved the model fit for LUC to SRC willow. There was a positive effect on the response function after LUC from arable land, with increasing SOC stocks from sites sampled in the order of spring to autumn. Conversely, there was a negative effect after LUC from grassland since SOC is higher at sites sampled in spring than in summer. However, in both cases SOC stocks were highest at the sites sampled in autumn (Figure 2.3). This may relate to fine root growth, which begins in spring and continues until early autumn (Rytter and Hansson, 1996), or increased litter inputs and decomposition during the course of the year. Although care was taken to remove root material passing through the 2-mm sieve, some fine roots may have remained, which may also have influenced the results.

Clay density improved the model fit for LUC from arable to SRC willow with a negative effect indicating lower SOC accumulation for more clayey soils. This may reflect a slower rate of change, which would be consistent with trends reported in other studies investigating long term changes in SOC stocks (Poeplau *et al*., 2011) as well as studies that have assessed changes in specific SOC fractions following LUC (Sohi *et al*., 2010). In any case, this negative effect on temporal trends indicates that the projected increase in SOC over time is not simply an effect of collinearity between clay and stand age. Sand and silt density improved the model fit for LUC from grassland to SRC willow and *Miscanthus* with both variables having a negative effect on the response function. There was no demonstrable overall change in SOC stocks for either LUC, although sandier soils may be more susceptible to SOC losses. These effects of soil texture can be explained by the higher proportion of mineral and aggregate bound SOC in clayey soils which is more resistant to decomposition than the particulate SOC that is more abundant in sandy soils (Six *et al*., 2002). If SOC is assumed to follow a ‘slow in, fast out’ trend then it may be ‘slower in’ for clayey soils which have a greater C storage capacity in the long term.

Climatic factors improved EF with potentially greater SOC losses and/or less accumulation in warmer and wetter regions following the conversion of grassland. There is evidence that greater SOC accumulation may have occurred in warmer regions following the establishment of SRC willow on arable land. This could indicate that SOC losses may be accentuated or are more likely to occur in warmer and wetter regions where conditions favour microbial activity. Where SOC accumulation occurs the C inputs may have a greater effect on the SOC balance than decomposition, with larger inputs in warmer regions due to higher net primary production (Thornley and Cannell, 1997; Post and Kwon, 2000; Poeplau *et al*., 2011).

This study utilised a large chronosequence dataset to capture a large number of sites from across England and Wales, in order to assess the general trajectory of short term SOC stocks following biomass crop establishment. The 95% confidence intervals are broad owing to additional effects of other superimposed explanatory variables on SOC and the limited time frame under investigation. A paired sites approach would have allowed for site-based relative changes in stocks (% ΔSOC) to have been modelled rather than absolute values. However, it would also have compromised the number of sites that could be sampled since suitable reference sites may not have been available in many cases and also considering the fixed resources that were available. While providing a useful baseline for change, the paired sites approach cannot eliminate all background variation since it is rare that two fields will share the exact same site history and also owing to intra-site variability in soil properties. Furthermore, the same factors that influence initial SOC are also liable to affect ΔSOC. In this study an attempt was made to incorporate these explanatory variables in the definition of CRFs so their influence on temporal trends may be captured.

2.5 Conclusion

The results presented here indicate that commercial planting of SRC willow on arable land caused a net increase in SOC stocks, while planting on grassland had no demonstrable net effect on SOC stocks. For *Miscanthus*, there was no demonstrable net effect on SOC stocks following commercial planting on arable or grassland. Fewer study sites were available for biomass crops established on grassland which may contribute to the large uncertainty reflected by the broad 95% confidence intervals. Further research would be required to reduce this uncertainty and determine the likely effects of LUC from grassland on the overall GHG mitigation potential of lignocellulosic biomass crops. The data presented here suggests that C sequestration benefits of lignocellulosic biomass crops may previously have been over-emphasised, at least for *Miscanthus*, and that crop performance in a commercial setting may not reflect that of experimental field trials. It is likely that increases in SOC can occur for both SRC willow and *Miscanthus* under certain conditions and the effects of environmental parameters on SOC trajectory require further investigation. Since SOC stock changes generally follow a 'slow in, fast out' trend, further increases may occur outside of the time-frame of this study. For more reliable longer term predictions, process-based models can be used in conjunction with the experimental data presented here.

Chapter 3 Estimating bulk density of lignocellulosic biomass cropland soils in Britain using pedotransfer functions

The aim and experimental design for this chapter were conceived by the candidate with advice from supervisors. The candidate carried out site visits for soil sampling, sample preparation, laboratory analysis, data analysis and writing of the chapter. Rebecca Rowe provided support with field work planning, logistics and with soil sampling. Mike Duvall assisted with laboratory analysis. This chapter forms the basis for a manuscript intended for submission to *Soil Use and Management*.

3.1 Introduction

To understand the role of the soil organic carbon (SOC) pool in the global carbon (C) cycle and the sensitivity of this pool to disturbances such as land-use change, accurate measurements and monitoring of SOC stocks are essential. To derive inventories of SOC stocks, it is necessary to convert SOC concentrations recorded in percentages of dry mass to mass of SOC per unit area [Equation (3.1)] and this requires measuring the bulk density (BD) of the soil. In addition to its use in converting SOC from a mass basis to an area basis, BD is a critical characteristic of soil which plays an important physical and biological role due to the direct effect it has on properties such as aeration (Mouazen *et al*., 2003), moisture availability and hydraulic conductivity (Dam *et al.*, 2005), as well as the indirect effect it has on root growth (Dexter, 2004) and crop yield (Reichert *et al*., 2009). Soil BD is often a required input parameter for models predicting soil processes (Heuscher *et al*., 2005) and is essential for assessing the potential contribution of SOC accumulation to climate change mitigation. However, measuring BD is labour intensive and time-consuming and it is often difficult to extract and trim volumetric soil samples if there are gravel, stones or other materials present in the soil profile. Consequently, BD measurements are frequently missing from soil survey databases (Sequeira *et al*., 2014). This paucity of data led to the development of various pedotransfer functions (PTFs), which are physical-mathematical models used to estimate soil properties that are often difficult or time-consuming to directly measure, such as BD, using more easily obtainable and available data.

$$SOC\ density\ (t\ ha^{-1}) = \sum_{i=1}^{n} C\ concentration\ (\%\ mass)_i \times BD\ (g\ cm^{-3})_i \times depth\ (cm)_i \quad (3.1)$$

An important observation for PTF development was that BD tends to decrease as soil organic matter (SOM) content increases (Curtis and Post, 1964) and many predictive functions are based on this relationship. Although it was initially suggested that the relationship between SOM and BD may be universal (Jeffrey, 1970), other studies demonstrated that this varied depending on factors such as soil depth (Harrison and Bocock, 1981; Huntington *et al*., 1989; Tranter *et al*., 2007; Perie and Ouimet, 2008), taxonomy (Alexander, 1980; Salifu *et al*., 1999), land-use and vegetation (Alexander, 1980; Celik, 2005; Gebrelibanos and Assen, 2013). Studies have also investigated the role of other variables in PTFs, primarily through multiple regression analysis, and observed that a range of soil properties relate to BD, such as soil texture, pH and exchangeable cations (Adams, 1973; Rawls, 1983; Heuscher *et al*., 2005; De Vos *et al*., 2005), in addition to site characteristics, geology and horizon designation

(Harrison and Bocock, 1981; Leonaviciute, 2000; Calhoun *et al*., 2001; Jalabert *et al*., 2010).

A wide range of PTFs have been developed in recent decades, with many using relatively small datasets which derive from a single land-use such as forest soils (Honeysett and Ratkowsky, 1989; Tamminen and Starr, 1994; De Vos *et al*., 2005; Jalabert *et al*., 2010). However, many of these PTFs may have limited potential for predicting soil BD in environments other than those from which they were calibrated. For example, De Vos *et al*. (2005) observed a large variation in performance when PTFs were applied to datasets derived from contrasting environments. Indeed, other studies demonstrated improved predictive accuracy from the partitioning of data according to soil taxonomic groups (Manrique and Jones, 1991; Heuscher *et al*., 2005), land-use and vegetation (Celik, 2005; Gebrelibanos and Assen, 2013).

As a result, recent studies have attempted to develop PTFs for different soil functional types using larger datasets (Hollis *et al*., 2012; Sequeira *et al*., 2014). Hollis *et al*. (2012) stratified a large dataset of BD measurements from England and Wales into broad functional groupings with the aim of developing separate PTFs that are applicable across all soil types within Europe. In recent decades, LUC from conventional agriculture to lignocellulosic biomass crops, such as short rotation coppice (SRC) willow and *Miscanthus* x *giganteus*, has become increasingly common in Europe (Don *et al*., 2012). However, the performance of established PTFs within this context has not yet been evaluated.

The main aims of this study were:

1. To develop and validate regression models for predicting soil BD using soil properties measured for biomass crops in Britain.
2. To evaluate the performance of a wide range of PTFs for estimating BD of lignocellulosic biomass cropland soils in Britain and compare the performance of published PTFs with models developed using biomass crop-specific soil data.

3.2 Materials and methods

3.2.1 Model development

The dataset for this study consists of soil BD measurements that were taken from 90 biomass crop plantations in Britain between March and November 2011. Site selection, soil sampling and analytical methods are outlined in more detail in Chapter 2.2. Briefly, BD of the 0-15 cm soil layer was measured using a 50 mm diam. specialised ring corer kit (Van Walt, Haslemere, UK) at 44 SRC willow and 46 *Miscanthus* x *giganteus* plantations. Each BD value represents the mean of triplicate measurements taken from random locations within the selected field. Other soil parameters measured at each site were: % SOC, total nitrogen (TN), clay (<2 μm), silt (2-63 μm) and sand (63-2000 μm) content.

Model development and validation was conducted using the holdout validation method (Aggarwal, 2015) to enable a comparison of PTFs developed using biomass crop-specific soil data and published PTFs that also used this method (Hollis *et al*., 2012). The data was divided into SRC willow and *Miscanthus* and subdivided randomly into two sets: (i) a training dataset with 70% of the data for model development and; (ii) a validation dataset with 30% of the data. Another training and validation dataset was also created for all biomass crop soil data combined. Descriptive statistics for each set of data are listed in Tables 3.1–3.3. Forward stepwise linear regression was carried out on each set of training data to identify the best-fit linear relationship between BD and the independent variables: % SOC, % TN, % clay, % silt, % sand, time since conversion (years) and various two-way interaction terms between these independent variables. Where an interaction term provides the best-fit model, two exploratory models were proposed and evaluated, one including interaction effects and one with main effects only. Collinearity diagnostics were performed and if two variables were collinear then the least significant covariant was removed. Interaction terms were not created for collinear variables. Curve fitting was also carried out using polynomial and other mathematical functions that have previously been reported to provide best-fit models for estimating soil BD. Corrected Akaike information criterion (AICc) [Equation (3.2)] was used to select the best-fit exploratory models which were then validated. The model with the lowest AICc score was selected and multiple lowest scoring models were selected where the difference in AICc scores was less than or equal to 2 (ΔAICc $\leq$2) (Burnham and Anderson, 2002).

$$AICc = \left(n\ln\left(\frac{SSE}{n}\right) + 2(k)\right) + \left(\frac{2k\ (k+1)}{n-k-1}\right) \quad (3.2)$$

where n is the total number of observations, SSE is the sum of squared errors of prediction and k is the number of parameters plus 1.

All regression analysis, curve fitting and checking of residuals for normal distribution using the Shapiro Wilk test were carried out using Genstat 16 (VSN International, Hemel Hempstead, UK). SSE values were obtained from Genstat 16

Table 3.1 Descriptive statistics of measured parameters for SRC willow training (T) and validation (V) datasets.

	Bulk density (g cm^3)		Time since conversion (years)		Organic carbon (%)		Total nitrogen (%)		Clay content (%)		Silt content (%)		Sand content (%)	
	T	V	T	V	T	V	T	V	T	V	T	V	T	V
No. of observations	31	13	31	13	31	13	31	13	31	13	31	13	31	13
Minimum	0.84	0.81	1	2	0.83	0.89	0.06	0.07	8.12	8.94	22.31	44.48	3.99	0.34
Maximum	1.64	1.60	13	22	5.80	6.51	0.66	0.73	27.33	36.32	75.13	75.91	66.68	44.76
Range	0.80	0.79	12	20	4.97	5.62	0.60	0.65	19.21	27.38	52.82	31.43	62.68	44.41
First quartile	1.17	1.14	4	3	1.61	1.34	0.13	0.14	13.95	13.43	47.59	47.52	18.71	7.04
Median	1.28	1.30	5	5	1.99	2.10	0.19	0.17	16.61	16.53	58.41	55.57	25.17	22.22
Third quartile	1.40	1.46	8	10	3.06	4.38	0.26	0.28	18.62	24.73	62.51	65.83	37.56	39.08
Mean	1.27	1.28	6	7	2.45	2.75	0.22	0.23	16.35	18.99	55.81	57.38	27.84	23.63
Standard error	0.03	0.07	1	2	0.22	0.53	0.02	0.05	0.73	2.23	2.13	2.97	2.58	4.55
Standard deviation	0.18	0.24	3	6	1.25	1.91	0.13	0.18	4.07	8.04	11.89	10.70	14.37	16.41

Table 3.2 Descriptive statistics of measured parameters for *Miscanthus* training (T) and validation (V) datasets.

	Bulk density (g cm^3)		Time since conversion (years)		Organic carbon (%)		Total nitrogen (%)		Clay content (%)		Silt content (%)		Sand content (%)	
	T	V	T	V	T	V	T	V	T	V	T	V	T	V
No. of observations	32	14	32	14	32	14	32	14	32	14	32	14	32	14
Minimum	0.80	0.80	1	3	1.25	1.23	0.13	0.12	8.99	13.95	25.39	40.15	6.30	6.63
Maximum	1.74	1.38	10	12	4.52	4.90	0.47	0.45	29.68	28.80	70.96	65.53	65.62	45.90
Range	0.94	0.58	9	9	3.27	3.67	0.35	0.33	20.69	14.85	45.57	25.38	59.32	39.27
First quartile	1.11	0.99	4	4.75	1.94	1.89	0.21	0.19	16.10	16.34	49.79	55.93	14.66	14.47
Median	1.20	1.26	6	5	2.43	2.36	0.26	0.24	20.75	19.80	59.00	60.56	18.16	17.95
Third quartile	1.37	1.31	7	9	3.01	2.99	0.33	0.29	25.26	22.41	62.14	64.75	33.06	25.61
Mean	1.25	1.17	6	6.29	2.59	2.51	0.28	0.25	20.30	19.95	55.23	58.24	24.47	21.81
Standard error	0.04	0.05	0	0.80	0.15	0.26	0.02	0.02	1.00	1.15	1.90	2.23	2.53	3.06
Standard deviation	0.20	0.19	2	3.00	0.84	0.96	0.09	0.09	5.67	4.29	10.72	8.34	14.30	11.45

Table 3.3 Descriptive statistics of measured parameters for all biomass crops training (T) and validation (V) datasets.

	Bulk density (g cm^3)		Time since conversion (years)		Organic carbon (%)		Total nitrogen (%)		Clay content (%)		Silt content (%)		Sand content (%)	
	T	V	T	V	T	V	T	V	T	V	T	V	T	V
No. of observations	63	27	63	27	63	27	63	27	63	27	63	27	63	27
Minimum	0.80	0.81	1	1	0.83	0.93	0.06	0.06	8.12	11.49	22.31	35.77	0.34	5.97
Maximum	1.74	1.53	21	12	6.51	5.40	0.73	0.50	36.32	30.36	75.91	68.62	66.68	52.73
Range	0.94	0.72	20	11	5.68	4.46	0.67	0.44	28.20	18.87	53.60	32.85	66.33	46.76
First quartile	1.12	1.13	4	3	1.71	1.88	0.15	0.18	14.16	16.30	48.25	54.61	15.53	17.28
Median	1.28	1.24	6	5	2.26	2.44	0.23	0.26	18.23	18.03	57.11	59.94	22.60	20.28
Third quartile	1.38	1.38	8	6	3.01	3.12	0.28	0.33	22.57	21.96	63.03	63.10	35.60	27.49
Mean	1.25	1.24	7	5	2.53	2.60	0.24	0.27	18.40	19.40	55.65	57.50	25.95	23.10
Standard error	0.03	0.03	0	0	0.16	0.20	0.02	0.02	0.73	0.98	1.47	1.55	1.94	2.09
Standard deviation	0.21	0.17	4	2	1.24	1.06	0.13	0.10	5.80	5.10	11.66	8.08	15.39	10.85

(VSN International, Hemel Hempstead, UK) and AICc calculated using the method of Motulsky and Christopoulos (2004).

3.2.2 Model validation

The best-fit exploratory models for individual land-use categories and for all data combined were validated and AICc was used as a model selection criteria. Given the relatively small sample size AICc rather than AIC was used. This includes a correction factor which is recommended for smaller datasets, i.e. where *n/k* <40 (Burnham and Anderson, 2002). The best-fit model for SRC willow was then validated for *Miscanthus* and the best-fit *Miscanthus* model validated for SRC willow. Although AICc provides a criterion for selecting the most likely *true* model from a set of candidate models, it does not provide an absolute measure of performance (Burnham and Anderson, 2002). To assess the latter, predictive capacity was evaluated against a set of statistical criteria. Overall goodness of fit was assessed using the model efficiency index (EF) (Green and Stephenson, 1986; Loague and Green, 1991) [Equation (3.3)]. Prediction accuracy was assessed using root mean square prediction error (RMSPE) [Equation (3.4)], which is a measure of overall prediction error and mean prediction error (MPE) [Equation (3.5)], which indicates systematic under- or over-estimation (De Vos *et al*., 2005; Jalabert *et al*., 2010).

$$EF = \frac{\left(\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2 - \sum_{i=1}^{n}\left[\left(P_i - O_i\right)^2\right]\right)}{\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2} \quad (3.3)$$

$$RMSPE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(P_i - O_i\right)^2} \quad (3.4)$$

$$MPE = \frac{1}{n}\sum_{i=1}^{n}\left(P_i - O_i\right) \quad (3.5)$$

where n is the total number of observations, P_i are the predicted values, O_i the observed values and $\bar{O}$ the mean of the observed data.

A list of published PTFs was compiled of which 24 models were validated for each land-use category and all of the data combined. Other published models were identified but could not be validated as they required parameters which had not been measured in this study. Models using SOM were validated by applying a conversion factor of 1.72 to SOC, which assumes that SOM contains 58% organic carbon (Nelson and Sommers, 1996). All regression analysis and checking of residuals for normal distribution using the Shapiro Wilk test were carried out using Genstat 16 (VSN International, Hemel Hempstead, UK).

3.3 Results

3.3.1 Models for individual land-use categories

SOC provided the best-fit linear model with BD for the SRC willow training dataset (Table 3.4). SOC was also selected as the best-fit independent variable for all nonlinear exploratory models except for an exponential function with an interaction effect of TN and clay. When interaction effects were removed, SOC provided the model of best-fit. The linear, quadratic, square root and two exponential models provided the lowest AICc scores and were selected as the best-fit exploratory models (Table 3.4). When these were validated, all of the models except for the exponential model with an interaction term produced a similar score (ΔAICc $\leq$2) (Table 3.5). All of these models have an EF of 0.35–0.37 for both training and validation and, therefore, explain a similar amount of variation in the BD data. The models also have

Table 3.4 Evaluation of exploratory models for soil bulk density for SRC willow and *Miscanthus*.

Land-use	Function	Equation	EF	MPE	RMSPE	AICc
SRC willow	Linear	$1.49 – (0.09 \times SOC)$	0.37	0.00	0.14	-118.5
	Quadratic	$1.46 – (0.06 \times SOC) – ((4.0 \times 10^{-2}) \times SOC^2)$	0.37	0.00	0.14	-117.2
	Cubic	$1.43 – (0.02 \times SOC) – (0.02 \times SOC^2) + ((0.2 \times 10^{-2}) \times SOC^3)$	0.37	0.00	0.14	-115.7
	Exponential	$1.42 \times e^{-0.03 \times (TN \times clay)}$	0.37	0.00	0.14	-118.6
		$1.52 \times e^{-0.07 \times (SOC)}$	0.36	0.00	0.15	-118.3
	Power	$1.45 \times SOC^{-0.17}$	0.31	0.00	0.15	-115.7
	Natural logarithm	$1.44 – (0.22 \times (Ln(SOC)))$	0.32	0.00	0.15	-116.3
	Square root	$1.72 – (0.30 \times (\sqrt{(SOC)}))$	0.35	0.00	0.15	-117.8
Miscanthus	Linear	$1.61 – ((2.45 \times 10^{-3}) \times (SOC \times silt))$	0.58	0.00	0.13	-129.2
		$1.70 – (0.17 \times (SOC))$	0.41	0.00	0.16	-123.9
	Quadratic	$1.67 – ((3.34 \times 10^{-3}) \times (SOC \times silt)) – ((0.3 \times 10^{-5}) \times (SOC \times silt)^2)$	0.59	0.00	0.13	-128.3
		$2.05 – (0.44 \times (SOC) + (0.05 \times (SOC)^2)$	0.54	0.00	0.14	-124.9
	Cubic	$1.69 – ((3.82 \times 10^{-3}) \times (SOC \times silt)) + ((0.6 \times 10^{-5}) \times (SOC \times silt)^2) – ((6.21 \times 10^{-8}) \times (SOC \times silt)^3)$	0.59	0.00	0.13	-126.8
		$2.04 – (0.43 \times (SOC) + (0.04 \times (SOC)^2) + ((5.1 \times 10^{-4}) \times (SOC)^3)$	0.54	0.00	0.14	-123.4
	Exponential	$1.83 \times e^{-0.15 \times (SOC)}$	0.53	0.00	0.14	-125.1
	Power	$1.74 \times (SOC^{-0.37})$	0.54	0.00	0.14	-125.6
	Natural logarithm	$1.67 – (0.47 \times (Ln(SOC)))$	0.54	0.00	0.14	-122.9
	Square root	$1.72 – (0.30 \times (\sqrt{(SOC)}))$	0.53	0.00	0.14	-122.3

Table 3.5 Validation of selected best-fit exploratory models for soil bulk density for SRC willow and *Miscanthus*.

Land-use	Function	Equation	EF	MPE	RMSPE	AICc
SRC willow	Linear	$1.49 - (0.09 \times SOC)$	0.37	0.00	0.19	-41.8
	Quadratic	$1.46 - (0.06 \times SOC) - ((4.0 \times 10^{-2}) \times SOC^2)$	0.37	0.01	0.19	-39.9
	Exponential	$1.42 \times e^{-0.03 \times (TN \times clay)}$	0.25	-0.02	0.20	-39.5
		$1.52 \times e^{-0.07 \times (SOC)}$	0.36	0.00	0.19	-41.7
	Square root	$1.72 - (0.30 \times (\sqrt{(SOC)}))$	0.35	0.00	0.19	-41.5
	*Miscant*hus best-fit PTF	$1.61 - ((2.45 \times 10^{-3}) \times (SOC \times silt))$	0.21	-0.04	0.21	-38.9
Miscanthus	Linear	$1.61 - ((2.45 \times 10^{-3}) \times (SOC \times silt))$	0.26	0.00	0.16	-50.3
	Quadratic	$1.67 - ((3.34 \times 10^{-3}) \times (SOC \times silt)) - ((0.3 \times 10^{-5}) \times (SOC \times silt)^2)$	0.04	-0.06	0.18	-44.7
	Willow best-fit PTF	$1.49 - (0.09 \times SOC)$	0.47	0.00	0.13	-55.0

similar prediction accuracy as MPE was very low both in training and validation and RMSPE for each model was 0.19 g cm^{-3} for the validation dataset.

For the *Miscanthus* training dataset, an interaction effect of SOC and silt provided the best-fit linear, quadratic and cubic models (Table 3.4). SOC provided the best-fit model when interaction effects were removed and for all other nonlinear exploratory models. The linear and quadratic models with interaction effects provided the lowest AICc scores and were selected as the best-fit exploratory models (Table 3.4). When these were validated, the linear model with an interaction effect represents the best predictive model (Table 3.5). For the two training datasets, exploratory models had a higher EF for the *Miscanthus* training dataset than for SRC willow. However, when the *Miscanthus* exploratory models were validated they produced a lower EF than the SRC willow exploratory models. The linear model developed from the SRC willow dataset was also validated for the *Miscanthus* dataset and the linear model developed from the *Miscanthus* dataset was also validated for the SRC willow dataset. The *Miscanthus* model did not perform as well as the exploratory models for the SRC willow validation dataset. However, the SRC willow model performed better for the *Miscanthus* validation dataset than any of the exploratory models and even had a higher EF for the *Miscanthus* than the SRC willow validation dataset (Table 3.5). This indicates that the SRC willow linear model provides the best predictive model for both the SRC willow and *Miscanthus* datasets.

AICc scores indicate that 7 of the 24 published models are considered more likely to be the *true* model for the SRC willow validation dataset, while 9 of the published models can be considered equally likely and 8 are less likely to be the *true* model (Table 3.6). However, EF, MPE and RMSPE indices demonstrate that the predictive

Table 3.6 Performance of published models for predicting soil BD for the SRC willow validation dataset. Models are ordered hierarchically with the best-fit (lowest AICc score) at the top. All models above the single horizontal line are considered more likely to be the *true* model than the best-fit exploratory model developed in this study (ΔAICc >2). Models below the single horizontal line are considered equally likely (ΔAICc ≤2) and models below the double horizontal line are considered less likely to be the *true* model than the best-fit exploratory model (ΔAICc >2). Units are % except for the following symbols: † = mg g^{-1}, * = ‰, ‡ = g kg^{-1} and $^{+}$ = g g^{-1}.

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
Manrique & Jones 1991a	1.51 – (0.11 x (SOC))	Mostly USA	0.36	-46.2	-0.01	0.19
Callesen *et al.*, 2003a	1.69 – (0.10 x (√ (SOC†)))	Sweden, Finland, Norway, Denmark	0.35	-46.0	0.00	0.19
Alexander 1980b	1.72 – (0.29 x ($SOC^{0.5}$))	USA	0.35	-46.0	-0.00	0.19
Alexander 1980a	1.66 – (0.31 x ($SOC^{0.5}$))	USA	0.35	-46.0	0.00	0.19
Manrique & Jones 1991b	1.66 – (0.32 x ($SOC^{0.5}$))	Mostly USA	0.35	-46.0	0.00	0.19
Hollis *et al.*, 2012e	1.49 – (0.33 x (LN(SOC)))	Britain / Europe	0.33	-45.4	-0.02	0.19
Callesen *et al.*, 2003b	1.83 – (0.13 x (√(SOC†)))	Sweden, Finland, Norway, Denmark	0.32	-45.3	-0.01	0.19
Adams 1973	100 / (SOM / 0.22 + (100 – SOM) / 1.27)	Britain	0.34	-43.7	0.01	0.19
Benites *et al.*, 2007	1.57 – (0.5 x 10^{-3} x ($clay^{*}$) – 0.01 x (SOC^{*}))	Brazil	0.33	-43.6	-0.02	0.19
Bernoux *et al.*, 1998	1.40 – (0.04 x (SOC) – 4.7 x 10^{-3} x (clay))	Brazil	0.30	-43.0	0.02	0.20

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
Hollis *et al.*, 2012a	$1.59 – (0.47 \times e^{0.06 \times (SOC)}) – (0.08 \times LN(7.5))$	Britain / Europe	0.25	-42.0	0.00	0.20
Hollis *et al.*, 2012b	$0.81 + (0.82 \times e^{-0.28 \times (SOC)}) + (1.41 \times 10^{-3} \times (sand)) – (1.03 \times 10^{-3} \times (clay))$	Britain / Europe	0.32	-41.2	0.01	0.19 0.193357
Huntington *et al.*, 1989	$e^{(0.26 – 0.15 \times LN(SOC) – 0.10 \times (LN(SOC^2)))}$	USA	0.18	-40.7	0.05	0.21
Prevost 2004b	$0.16 \times 1.56 / (1.56 \times (SOM^{+}) + 0.16 \times (1-(SOM^{+})))$	Canada	0.18	-40.7	0.03	0.21
Hollis *et al.*, 2012d	$0.70 + (0.75 \times e^{-0.23 \times (SOC)}) + (8.69 \times 10^{-4} \times (sand)) – (5.16 \times 10^{-4} \times (clay))$	Britain / Europe	0.26	-40.1	0.03	0.20
Howard *et al.*, 1995	$1.3 – (0.28 \times LN((SOC‡)/10))$	Britain	0.04	-40.4	0.03	0.23
Hollis *et al.*, 2012c	$1.13 – (0.11 \times LN(SOC)) + (0.06 \times LN(7.5)) + (2.25 \times 10^{-3} \times (sand))$	Britain / Europe	0.16	-38.3	0.04	0.21
Prevost 2004a	$e^{-1.81 – 0.89 \times LN(SOM+) – 0.09 \times (LN(SOM+)^2)}$	Canada	0.00	-38.0	0.06	0.23
Tomasella & Hodnett 1998	$1.58 – 0.05 \times (SOC) – 0.01 \times (silt) – 0.01 \times (clay)$	Brazil	0.04	-36.4	0.03	0.23
Tremblay *et al.*, 2002	$0.12 \times (1.4 / (1.4 \times (SOM^{+}) + 0.12 \times (1 – (SOM^{+}))))$	Canada	-0.11	-36.5	0.06	0.25
Perie & Ouimet 2008b	$0.11 \times (1.77 / (1.77 \times (SOM^{+}) + 0.11 \times (1 – (SOM^{+}))))$	Canada	-0.22	-35.2	0.07	0.26
Federer, 1983	$0.11 \times (1.45 / (1.45 \times (SOM^{+}) + 0.11 \times (1 – (SOM^{+}))))$	USA	-0.22	-35.2	0.07	0.26
Perie & Ouimet 2008a	$1.98 + (4.11 \times (SOM^{+}) – 1.23 \times LN(SOM^{+}) – 0.10 \times (LN(SOM^{+})^{2}))$	Canada	-0.15	-33.9	0.06	0.25
Kaur *et al.*, 2002	$e^{0.31 – 0.19 \times (SOC) + 0.02 \times (clay) – 4.76 \times 10^{-4} \times (clay)^2) – 4.32 \times 10^{-3} \times (silt)}$	India	-0.84	-24.9	0.09	0.32

Table 3.7 Performance of published models for predicting soil BD for the *Miscanthus* validation dataset. Models are ordered hierarchically with the best-fit (lowest AICc score) at the top. Models above the double horizontal line are considered equally likely (ΔAICc $\leq$2) and models below the double horizontal line are considered less likely to be the *true* model than the best-fit exploratory model (ΔAICc >2). Units are % except for the following symbols: † = mg g^{-1}, * = ‰, ‡ = g kg^{-1} and $^{+}$ = g g^{-1}.

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
Manrique & Jones 1991a	1.51 – (0.11 x (SOC))	Mostly USA	0.47	-55.0	0.00	0.13
Callesen *et al*., 2003b	1.83 – (0.13 x (√(SOC†)))	Sweden, Finland, Norway, Denmark	0.45	-54.6	0.00	0.13
Alexander 1980b	1.72 – (0.29 x (SOC$^{0.5}$))	USA	0.44	-54.2	0.00	0.14
Benites *et al*., 2007	1.57 – (0.5 x 10^{-3} x (clay*) – 0.01 x (SOC*))	Brazil	0.51	-54.1	0.00	0.13
Callesen *et al*., 2003a	1.69 – (0.10 x (√ (SOC†)))	Sweden, Finland, Norway, Denmark	0.45	-53.9	0.00	0.13
Manrique & Jones 1991b	1.66 – (0.32 x (SOC$^{0.5}$))	Mostly USA	0.42	-53.7	0.01	0.14
Alexander 1980a	1.66 – (0.31 x (SOC$^{0.5}$))	USA	0.42	-53.7	0.00	0.14
Hollis *et al*., 2012e	1.49 – (0.33 x (LN(SOC)))	Britain / Europe	0.41	-53.4	-0.03	0.14
Prevost 2004b	0.16 x 1.56 / (1.56 x (SOM^{+}) + 0.16 x (1-(SOM^{+})))	Canada	0.45	-52.6	0.00	0.13
Prevost 2004a	$e^{-1.81 – 0.89 \text{ x LN(SOM+)} – 0.09 \text{ x (LN(SOM+)^2)}}$	Canada	0.30	-52.6	0.07	0.15
Tremblay *et al*., 2002	0.12 x (1.4 / (1.4 x (SOM^{+}) + 0.12 x (1 – (SOM^{+}))))	Canada	0.41	-51.6	0.01	0.14
Hollis *et al*.,	0.81 + (0.82 x $e^{-0.28 \text{ x (SOC)}}$) + (1.41 x 10^{-3} x	Britain / Europe	0.49	-51.5	0.00	0.13

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
2012b	(sand)) – (1.03 x 10^{-3} x (clay))					
Howard *et al.*, 1995	1.3 – (0.28 x LN((SOC‡)/10))	Britain	0.31	-51.4	0.02	0.15
Adams 1973	100 / (SOM / 0.22 + (100 – SOM) / 1.27)	Britain	0.40	-51.3	-0.01	0.14
Federer, 1983	0.11 x (1.45 / (1.45 x (SOM^{+}) + 0.11 x (1 – (SOM^{+}))))	USA	0.38	-50.9	0.01	0.14
Bernoux *et al.*, 1998	1.40 – (0.04 x (SOC) – 4.7 x 10^{-3} x (clay))	Brazil	0.37	-50.8	0.01	0.14
Perie & Ouimet 2008b	0.11 x (1.77 / (1.77 x (SOM^{+}) + 0.11 x (1 – (SOM^{+}))))	Canada	0.36	-50.6	0.01	0.14
Hollis *et al.*, 2012d	0.70 + (0.75 x $e^{-0.23 \text{ x (SOC)}}$) + (8.69 x 10^{-4} x (sand)) – (5.16 x 10^{-4} x (clay))	Britain / Europe	0.41	-49.5	0.01	0.14
Huntington *et al.*, 1989	$e^{(0.26 – 0.15 \text{ x LN(SOC)} – 0.10 \text{ x (LN(SOC\^{}2)))}}$	USA	0.30	-49.2	0.02	0.15
Perie & Ouimet 2008a	1.98 + (4.11 x (SOM^{+}) – 1.23 x LN(SOM^{+}) – 0.10 x (LN(SOM^{+})^{2}))	Canada	0.37	-48.6	0.01	0.14
Hollis *et al.*, 2012a	1.59 – (0.47 x $e^{0.06 \text{ x (SOC)}}$) – (0.08 x LN(7.5))	Britain / Europe	0.20	-47.3	0.00	0.16
Hollis *et al.*, 2012c	1.13 – (0.11 x LN(SOC)) + (0.06 x LN(7.5)) + (2.25 x 10^{-3} x (sand))	Britain / Europe	0.31	-47.2	-0.02	0.15
Tomasella & Hodnett 1998	1.58 – 0.05 x (SOC) – 0.01 x (silt) – 0.01 x (clay)	Brazil	0.27	-46.6	0.01	0.15
Kaur *et al.*, 2002	$e^{0.31 – 0.19 \text{ x (SOC)} + 0.02 \text{ x (clay)} – 4.76 \text{ x } 10\text{\^{}-4 x (clay)\^{}2) – 4.32 x } 10\text{\^{}-3 x (silt)}}$	India	0.32	-45.1	0.01	0.15

accuracy was not improved for any of these models but is comparable to the best-fit exploratory model. For the *Miscanthus* validation dataset, AICc scores indicate that none of the published models can be considered more likely to be the *true* model than the best-fit exploratory model, while 8 can be considered equally likely and 16 are less likely to be the *true* model (Table 3.7).

3.3.2 Models for all biomass crop soil data

For all biomass crop soil data combined, an interaction effect between TN and silt provided the best-fit linear, quadratic and cubic models (Table 3.8). SOC provided the best-fit model when interaction effects were removed and for all other nonlinear exploratory models. The linear, quadratic and cubic models with interaction effects provided the lowest AICc scores and were selected as the best-fit exploratory models (Table 3.8). In addition to these three exploratory models, the SRC willow linear model was also validated for the combined data. The exploratory linear and the SRC willow linear models provided the lowest AICc scores and represent the best predictive models (Table 3.9). The two linear models also provided the highest EF of 0.11. Although the EF values were much lower for the validation than the training dataset, the randomly selected data has quite a high spread as indicated by the best-fit model for the validation dataset, which had a low EF of 0.15 and a comparable AICc score to the models being tested (Table 3.9, Figure 3.1). Both the exploratory linear and the SRC willow linear model had an MPE of 0.00 and an RMSPE of 0.16 (Table 3.9). AICc scores indicate that none of the published models are considered more likely to be the *true* model than either the best-fit exploratory model or the SRC willow linear model, while 12 can be considered equally likely and 12 are less likely to be the *true* model (Table 3.10). EF was higher for six of the published models and

Table 3.8 Evaluation of exploratory models for soil BD for all of the biomass crop data combined.

Function	Equation	EF	Mean BD ($g\ cm^{-3}$)	MPE	RMSPE	AICc
Linear	$1.51 - (0.02 \times (TN \times silt))$	0.49	1.25	0.00	0.15	-236.6
	$1.55 - (0.12 \times (SOC))$	0.37	1.22	0.03	0.17	-232.7
Quadratic	$1.51 - (0.02 \times (TN \times silt)) - ((2.3 \times 10^{-5}) \times (TN \times silt)^2)$	0.49	1.25	0.00	0.15	-235.5
	$1.65 - (0.19 \times (SOC)) + (0.01 \times (SOC)^2)$	0.47	1.25	0.00	0.15	-232.8
Cubic	$1.37 + (0.02 \times (TN \times silt)) - ((2.14 \times 10^{-3}) \times (TN \times silt)^2) + ((3.6 \times 10^{-5}) \times (TN \times silt)^3)$	0.51	1.25	0.00	0.15	-237.1
	$1.50 - (0.02 \times (SOC)) - (0.05 \times (SOC)^2) + (0.01 \times (SOC)^3)$	0.47	1.25	0.00	0.15	-232.3
Exponential	$1.61 \times e^{-0.10 \times (SOC)}$	0.46	1.25	0.00	0.16	-233.6
Power	$1.52 \times SOC^{-0.24}$	0.46	1.25	0.00	0.16	-228.9
Natural logarithm	$1.51 - (0.31 \times (Ln(SOC)))$	0.44	1.25	0.00	0.16	-228.5
Square root	$1.87 - (0.40 \times (\sqrt{(SOC)}))$	0.46	1.25	0.00	0.16	-230.8

Table 3.9 Validation of selected best-fit exploratory models for all of the biomass crop data combined.

Function	Equation	EF	Mean BD (g cm^{-3})	MPE	RMSPE	AICc
Linear	1.51 – (0.02 x (TN x silt))	0.11	1.25	0.00	0.16	-97.7
Quadratic	1.51 – (0.02 x (TN x silt)) – ((2.3 x 10^{-5}) x (TN x silt)^2)	0.09	1.26	0.00	0.16	-95.6
Cubic	1.37 + (0.02 x (TN x silt)) – ((2.14 x 10^{-3}) x (TN x silt) ^{2}) + ((3.6 x 10^{-5}) x (TN x silt)^3)	0.10	1.27	0.00	0.16	-94.3
Best-fit model for SRC willow	1.49 – (0.09 x (SOC)	0.11	1.25	0.00	0.16	-97.7
Best-fit model for validation dataset	1.40 – (0.06 x (SOC))	0.15	1.24	0.00	0.16	-98.8

Table 3.10 Performance of published models for predicting soil BD for the validation dataset of all of the biomass crop data combined. Models are ordered hierarchically with the best-fit (lowest AICc score) at the top. Models above the double horizontal line are considered equally likely (ΔAICc ≤2) and models below the double horizontal line are considered less likely to be the *true* model than the best-fit exploratory model (ΔAICc >2). Units are % except for the following symbols: † = mg g^{-1}, * = ‰, ‡ = g kg^{-1} and + = g g^{-1}.

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
Manrique & Jones 1991b	1.66 – (0.32 x ($SOC^{0.5}$))	Mostly USA	0.15	-99.0	0.00	0.16
Alexander 1980a	1.66 – (0.31 x ($SOC^{0.5}$))	USA	0.15	-99.0	0.00	0.16
Callesen *et al*., 2003a	1.69 – (0.10 x (√ (SOC†)))	Sweden, Finland, Norway, Denmark	0.15	-98.8	0.00	0.16
Alexander 1980b	1.72 – (0.29 x ($SOC^{0.5}$))	USA	0.14	-98.5	0.00	0.16
Hollis *et al*., 2012e	1.49 – (0.33 x (LN(SOC)))	Britain / Europe	0.12	-98.0	0.00	0.16
Benites *et al*., 2007	1.57 – (0.5 x 10^{-3} x (clay*) – 0.01 x (SOC*))	Brazil	0.14	-97.1	0.00	0.16
Manrique & Jones 1991a	1.51 – (0.11 x (SOC))	Mostly USA	0.09	-97.1	0.00	0.16
Callesen *et al*., 2003b	1.83 – (0.13 x (√(SOC†)))	Sweden, Finland, Norway, Denmark	0.08	-96.9	0.00	0.16
Hollis *et al*., 2012c	1.13 – (0.11 x LN(SOC)) + (0.06 x LN(7.5)) + (2.25 x 10^{-3} x (sand))	Britain / Europe	0.18	-96.7	0.00	0.15
Adams 1973	100 / (SOM / 0.22 + (100 – SOM) / 1.27)	Britain	0.12	-96.6	0.00	0.16
Huntington *et al*., 1989	$e^{(0.26 - 0.15 \times LN(SOC) - 0.10 \times (LN(SOC^2)))}$	USA	0.11	-96.3	0.00	0.16
Hollis *et al*., 2012a	1.59 – (0.47 x $e^{0.06 \times (SOC)}$) – (0.08 x LN(7.5))	Britain / Europe	0.11	-96.2	0.00	0.16

Published PTF	Equation	Country	EF	AICc	MPE	RMSPE
Bernoux *et al*., 1998	$1.40 – (0.04 \times (SOC) – 4.7 \times 10^{-3} \times (clay))$	Brazil	0.08	-95.5	0.00	0.16
Howard *et al*., 1995	$1.3 – (0.28 \times LN((SOC‡)/10))$	Britain	0.03	-95.5	0.00	0.17
Hollis *et al*., 2012b	$0.81 + (0.82 \times e^{-0.28 \times (SOC)}) + (1.41 \times 10^{-3} \times (sand)) – (1.03 \times 10^{-3} \times (clay))$	Britain / Europe	0.13	-95.1	0.00	0.16
Hollis *et al*., 2012d	$0.70 + (0.75 \times e^{-0.23 \times (SOC)}) + (8.69 \times 10^{-4} \times (sand)) – (5.16 \times 10^{-4} \times (clay))$	Britain / Europe	0.12	-94.8	0.00	0.16
Prevost 2004b	$0.16 \times 1.56 / (1.56 \times (SOM^{+}) + 0.16 \times (1-(SOM^{+})))$	Canada	-0.03	-92.4	0.00	0.17
Tomasella & Hodnett 1998	$1.58 – 0.05 \times (SOC) – 0.01 \times (silt) – 0.01 \times (clay)$	Brazil	0.03	-92.4	0.00	0.17
Prevost 2004a	$e^{-1.81 – 0.89 \times LN(SOM+) – 0.09 \times (LN(SOM+)^2)}$	Canada	-0.22	-87.6	0.00	0.19
Tremblay *et al*., 2002	$0.12 \times (1.4 / (1.4 \times (SOM^{+}) + 0.12 \times (1 – (SOM^{+}))))$	Canada	-0.32	-85.6	0.00	0.19
Federer, 1983	$0.11 \times (1.45 / (1.45 \times (SOM^{+}) + 0.11 \times (1 – (SOM^{+}))))$	USA	-0.44	-83.2	0.00	0.20
Perie & Ouimet 2008b	$0.11 \times (1.77 / (1.77 \times (SOM^{+}) + 0.11 \times (1 – (SOM^{+}))))$	Canada	-0.44	-83.2	0.00	0.20
Kaur *et al*., 2002	$e^{0.31 – 0.19 \times (SOC) + 0.02 \times (clay) – 4.76 \times 10^{-4} \times (clay)^2) – 4.32 \times 10^{-3} \times (silt)}$	India	-0.82	-73.7	0.00	0.23
Perie & Ouimet 2008a	$1.98 + (4.11 \times (SOM^{+}) – 1.23 \times LN(SOM^{+}) – 0.10 \times (LN(SOM^{+})^{2}))$	Canada	-23.21	-5.46	0.00	0.83

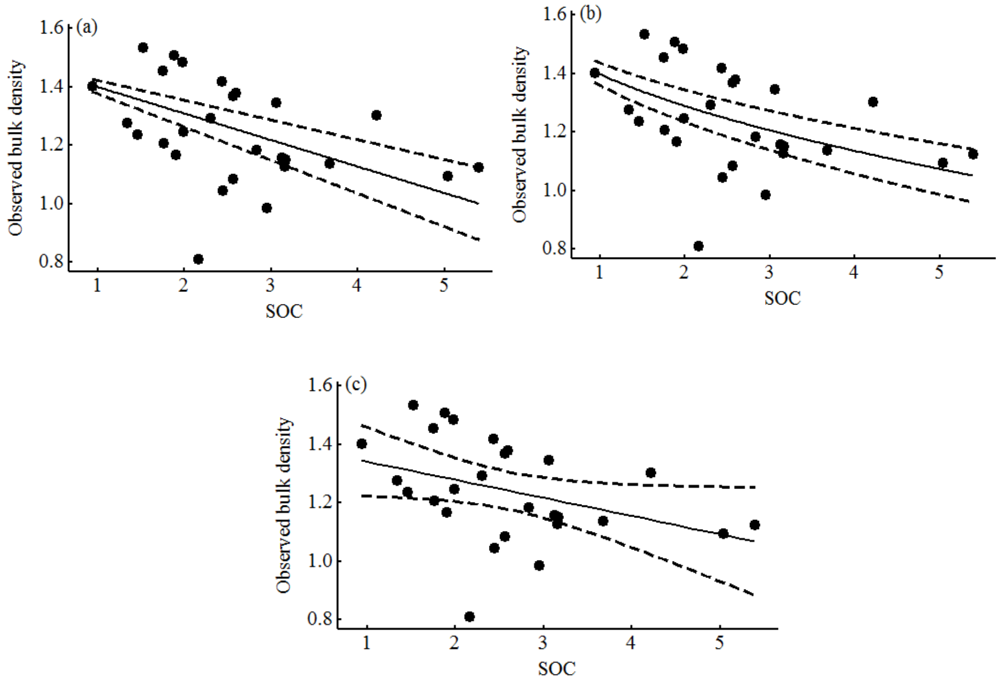

Figure 3.1 Performance of selected PTFs for validation dataset of all of the biomass crop data combined: (a) best-fit exploratory model; (b) best-fit published model (Manrique and Jones, 1991b); (c) best-fit model for validation dataset. Bulk density (g cm^{-3}) is the dependent variable and SOC (%) is the independent variable in each model. Dashed lines represent 95% confidence intervals.

three had an EF of 0.15, which was equal to the best-fit model for the validation dataset. MPE and RMSPE were the same as the exploratory models, with values of 0.00 and 0.16 respectively.

3.4 Discussion

Evaluation of model performance for the individual land-use categories indicates that separating the data according to crop type does not improve model performance. Of the models developed in this study, the same equation provided the best predictive model for SRC willow and *Miscanthus* data as well as for all of the data combined. Furthermore, the same published models performed consistently well for SRC willow and *Miscanthus* data separately as well as for all of the data combined. For SRC willow, 7 of the published PTFs represent a better predictive model than the best-fit exploratory model. These same 7 published PTFs also performed equally as well as the best-fit exploratory models for the *Miscanthus* data and for all of the data combined. Since the best predictive models are the same for both SRC willow and *Miscanthus*, this indicates that considering these perennial biomass crops separately is unlikely to improve model performance for estimating soil BD.

For all of the data combined, two linear equations provided the best predictive models for estimating soil BD. None of the published models evaluated in this study provided a better predictive model than the models developed using biomass crop-specific soil data. However, 12 of the published models performed equally as well. These published models included linear as well as nonlinear functions such as natural logarithm, square root, power and exponential. This demonstrates that a linear model with one independent variable performed equally as well or better than a range of models developed using multiple linear regression and curve fitting. Since various published nonlinear equations performed equally as well as the best-fit developed linear equations, it might have been expected that the nonlinear equations developed here would also have performed equally as well since: (i) they were developed using biomass-crop specific soil data and; (ii) they were developed using a more robust method. Previously multiple linear regression analysis has been used to develop and select nonlinear functions (e.g. Manrique and Jones, 1991; Hollis *et al*., 2012). Therefore, it was expected that curve-fitting would provide a more robust means of fitting parameter values. In the present study, Genstat 16 software was used to fit nonlinear regression models by maximum likelihood (Ross, 1990). However, neither the use of biomass crop-specific soil data nor curve-fitting produced better-fitting nonlinear models.

The independent variables that were selected as the most significant for predicting BD were SOC and an interaction effect of TN and silt. Other studies also report a significant effect of SOM and soil texture on BD. SOC and TN are highly correlated ($r = 0.90$, $p < 0.001$ and $r = 0.96$, $p < 0.001$ for SRC willow and *Miscanthus* respectively) and both are strongly negatively correlated with BD as SOM promotes good soil structure and aggregation of organic compounds with clay and fine silt particles through physicochemical interactions (Tisdall and Oades, 1982; Chaney and Swift, 1984; Chenu *et al*., 1994; Schlecht-Pietsch *et al*., 1994; Caravaca *et al*., 2001). The selection of TN over SOC as the most significant covariant during stepwise linear regression has been observed previously (Benites *et al*., 2007). This has been explained by the inaccuracies associated with dichromate oxidation as a method for soil carbon determination (Benites *et al*., 2007). However, this was not the case in the present study since dry combustion elemental analysis was used to determine SOC. It has also been suggested that the selection of TN over SOC may relate to the possible stabilisation effect of nitrogen on SOM, which can occur depending on the plant species composition and associated lignin inputs (Dijkstra *et al*., 2004).

Although clay, silt and sand have all been included in published models, the selection of silt rather than sand or clay as the most significant covariant is contrary to many other studies which observed that the content of clay and sand sized particles are more strongly related to BD (Bernoux *et al*., 1998; Benites *et al*., 2007; Hollis *et al*., 2012). Other published models that also included silt performed relatively poorly in this study and models that included clay and sand performed well, including models that were developed for application to European soils (Hollis *et al*., 2012). Since the study sites were purposefully selected to avoid soils with a high clay content and to obtain soils with broadly similar texture, this has produced a bias toward medium-textured soils, with 70% classified as 'medium' textured (15–30% clay), 26% as 'light' (<15% clay) and 4% as 'heavy' textured (>30% clay). Therefore, the selection of silt as the more significant covariant in a statistical model derived using a homogenous sampling strategy may relate to the predominance of silty soils rather than the functional importance of silt particles in the soil. It is also possible that the selection of silt over clay is simply an artefact of collinearity associated with compositional data, since, when silt was removed from the model, clay was also a significant covariant. Another possible explanation relates to the potential underestimation of the clay sized fraction and overestimation of the silt sized fraction that can result from using laser diffraction as a particle size analysis method (Di Stefano *et al*., 2010). However, issues over method of particle size determination are not of major concern since a linear model with SOC as the only independent variable explained an equal amount of variance in the data.

Although the same variables were selected, the poor model performance observed in the present study is contrary to the results of many other studies. When the new and published models were validated for all of the data combined, EF was much lower than for the training dataset or for the validation datasets for SRC willow and *Miscanthus*. The EF for the best-fit model for the validation dataset was only 0.15. This indicates that the poor model performance relates to the variability in the validation dataset which cannot be accounted for by any of the measured independent variables using either the developed or published models. Previous studies have suggested that model efficiency may be improved by the stratification of measured data based on soil taxonomy (Manrique and Jones, 1991; Heuscher *et al*., 2005) and soil horizons (Harrison and Bocock, 1981; Leonaviciute, 2000), or incorporation of additional independent variables such as soil physiographic and morphological properties (Calhoun *et al*., 2001). This information is not available for this study but it is possible that model efficiency could be improved by the inclusion of such data.

Hollis *et al*. (2012) considered a range of structural factors as determinants of soil BD and evaluated 18 conceptual groupings using a large dataset derived from England and Wales. The authors concluded that separation into only five broad groupings was justified: (i) volcanic soils; (ii) cultivated topsoils; (iii) compact subsoils; (iv) all other mineral horizons and; (v) all organic horizons. The five regression models explained 40–60% of the variation in each grouping. A similar fit was observed when these models were used to predict soil BD for the individual SRC willow and *Miscanthus* validation datasets in this study. However, the low EF observed in the validation dataset for the combined data suggests that, even for datasets derived from a soil environment similar to that of the development data, other factors are influencing BD and require further investigation.

By only using one training and validation dataset, a high variance in model evaluation is considered one of the main disadvantages of using the holdout validation method. Cross validation is a more robust model validation method which uses multiple training and validation datasets to reduce this variance. Although this may have improved the development of exploratory models, since the aim of this study was to evaluate the performance of models that were developed using holdout validation and draw a comparison with models developed using biomass crop-specific soil data, for this purpose the same model development method was used in this study. Similarly, other approaches for model development may have provided a better fit than the models developed here using regression analysis. Although most studies have tended to use regression analysis to develop PTFs, several non-parametric methods have been proposed as other means of model development, such as artificial neural networks, k-nearest neighbour, random forest algorithm and boosted regression trees (McBratney *et al*., 2002). However, these methods have not been widely used for estimation of soil BD and most of those studies that have used these methods for the purpose of BD estimation have not directly compared the results to regression analysis. For example, Sequeira *et al*. (2014) used the random forest algorithm while Ghehi *et al*. (2012) used the k-nearest neighbour and boosted regression trees and Jalabert *et al*. (2010) also used boosted regression trees to estimate soil BD but none of these studies compared the results of these methods with those of regression analyses on the same datasets. One study that did compare the performance of MLR with artificial neural networks and regression trees for estimating BD using a dataset consisting of 1896 soils reported a better fit for the MLR model, with a r^2 of 0.49 compared to r^2 values of 0.47 and 0.43 for the artificial neural network and regression tree respectively (Tranter *et al*., 2007). Further research is required to investigate the potential of non-parametric methods to estimate soil BD.

Since LUC may affect soil BD, time since conversion was also considered as an independent variable but was not found to be significant for either land-use category, either as a main or interaction effect. Although changes in soil BD with LUC may follow trends in SOC, other effects on soil physical structure may occur. For example, changes in soil water content can cause swelling and shrinking and susceptibility to compaction which would also affect soil BD (Haines, 1923; Berndt and Coughlan, 1976; Hillel, 1998). Such effects are likely to relate to specific soil or climate conditions. Therefore, rather than simply observing a monotonic effect of time since conversion on BD, more soil and climate data may be required to detect these effects. It is possible that changing soil properties with LUC can influence the applicability of PTFs and this may also help to explain the observed poor model performance for developed and published models.

3.5 Conclusion

Partitioning of data according to land-use did not improve model performance for estimating BD of lignocellulosic biomass cropland soils in Britain. The predictive accuracy of PTFs was not improved either by considering SRC willow separately to *Miscanthus* or by considering lignocellulosic biomass crops separately to other land-uses. A simple linear equation with SOC as the only independent variable performed equally as well or better than a range of models developed using multiple linear regression analysis and curve-fitting. However, all of the regression models that were developed in this study as well as published models performed poorly when validated for all of the biomass crop soil data, with the highest EF of 0.15. Various non-parametric methods of model development, such as artificial neural networks, k-nearest neighbour, random forest algorithm and boosted regression tree analysis, as well as other methods of model validation, such as cross validation, may produce better models for estimation of soil BD. However, these have not been widely used for this purpose and will require further research. It is possible there may have been an effect of land-use change on the performance of PTFs since BD may change with the establishment of a new crop. However, this could not be assessed using the soil properties that were measured for this study.

Chapter 4 Using incubation experiments to assess the priming potential of environmentally weathered pyrogenic carbon during land-use transition to biomass crop production

The aim and experimental design of this chapter were conceived by the candidate with advice from supervisors as well as Andrew Cross and Niall McNamara. The candidate carried out site visits for PyC amendment and soil sampling, sample preparation, laboratory analysis, data analysis and writing of the chapter. Sarah McCormack assisted with fieldwork planning and field flux measurements. Andrew Cross also helped with setting up and carrying out incubation experiments. Will Meredith provided training and technical assistance for hydrogen pyrolysis. Elemental and proximate analysis as well as pH and nutrient contents of the PyC was carried out by Maria Borlinghaus. The stability and toolkit assays were conducted by Andrew Cross. This chapter forms the basis for a manuscript accepted for publication in *Global Change Biology Bioenergy* as:

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4.1 Introduction

Land-use change (LUC) from conventional agriculture to lignocellulosic biomass crop production has recently received considerable attention as a prospective carbon (C) abatement strategy (Smith *et al*., 2000; Don *et al*., 2012). Life cycle assessment (LCA) studies indicate that substitution of fossil fuels for bioenergy has significant greenhouse gas (GHG) mitigation potential (Smith *et al*., 2000; Styles and Jones, 2007; Styles and Jones, 2008; Hillier *et al*., 2009; Whitaker *et al*., 2010; Djomo *et al.* 2011; Tonini *et al*., 2012). However, the effects of LUC to biomass crops on soil organic carbon (SOC) stocks remain uncertain (Don *et al*., 2012; Goglio *et al.* 2015). Results from paired-plot studies are highly variable and the trajectory of SOC relates to many factors such as biomass crop type, previous land-use, climate and soil texture (Keoleian and Volk, 2005). Any alteration in SOC stocks will have a subsequent impact on the overall C abatement potential of biomass crops.

It has been suggested that the long term C abatement potential of biomass crops could be enhanced if combined with pyrogenic C (PyC) production and use as a soil amendment (Case *et al*., 2014). This PyC, also frequently termed *biochar*, has been proposed mainly as a strategy for long term C sequestration (Pessenda *et al*., 2001; Masiello, 2004; Krull *et al*., 2006; Preston and Schmidt, 2006) that is simultaneously capable of improving soil quality (Joseph *et al*., 2010; Montanarella and Lugato, 2013; Woolf *et al*., 2010). However, some aspects of PyC function in soil remain poorly understood. For example, concerns persist over the impact of PyC on native SOC mineralisation (Wardle *et al*., 2008; Kuzyakov *et al*., 2009; Luo *et al*., 2011; Keith *et al*., 2011). Alteration of the turnover rates of native SOC after the addition of any substrate is often referred to as ‘priming’, with increased and decreased rates referred to as positive and negative priming respectively. There have been observations of both positive and negative priming following PyC application (Kuzyakov *et al*., 2009; Spokas and Reicosky, 2009; Liang *et al*., 2010; Jones *et al*., 2011; Keith *et al*., 2011; Zimmerman *et al*., 2011). Effects are therefore likely to vary according to the nature and composition of the PyC used and the receiving soil type (Shneour, 1966; Spokas and Reicosky, 2009; Atkinson *et al*., 2010; Lehmann *et al*., 2011).

Although priming effects vary between studies, most evidence indicates that any PyC-induced increase in CO_2 production is likely to be short lived, with a negligible impact on SOC stocks in the longer term (Woolf and Lehmann, 2012). Studies have demonstrated that as positive priming decreases over time, negative priming often occurs and this has been used to further substantiate the environmental benefits of PyC production and soil incorporation strategies (Singh and Cowie, 2014). Based on such assertions, it is possible that PyC application to recently established biomass crops could not only offset any LUC-induced SOC losses with C sequestered in the stable aromatic portion of PyC, but possibly reduce such losses through negative priming as well. However, this potential has not yet been directly investigated even though a number of studies have emerged which have assessed priming effects in the context of recently established biomass crops (Prayogo *et al*., 2013; Case *et al*., 2014; Ventura *et al*., 2015).

In one study, PyC application to a 5-year old *Miscanthus* x *giganteus* plantation was reported to decrease CO_2 flux in the field by 33% over 2 years and by 53% in a 120-day incubation experiment (Case *et al*., 2014). Net CO_2 flux was up to 20% lower in a 90 day incubation experiment using soil from a 14-year old SRC willow plantation mixed with PyC (Prayogo *et al*., 2013). Negative priming was also observed in the field following PyC amendment to a 1-year old SRC willow plantation in the UK and a 2-year old SRC poplar plantation in Italy (Ventura *et al*., 2015). However, in both cases these emission reductions were offset by decomposition of the PyC, with no net effect on CO_2 flux at the Italian site and an increase in CO_2 flux at the UK site (Ventura *et al*., 2015). While these results demonstrate considerable potential for negative priming in soils of biomass crops, these studies report only single-site observations for each biomass crop type. Due to remaining uncertainty over the mechanisms involved and the conditions required for different priming effects to occur, results are likely to vary for different PyC-soil combinations. The long term direction of any priming effects is also unclear, since few studies have investigated the impact of environmental weathering of PyC on interactions with native SOC (Spokas, 2013). Although environmental weathering can influence the sorption (Hale *et al*., 2011; Martin *et al*., 2012) and cation exchange capacity (Steiner *et al*., 2007) of PyC as well as surface group chemistry (Cheng *et al*., 2006, 2008a; Joseph *et al*., 2010), studies assessing the impact of environmental weathering on priming effects are relatively few (Spokas, 2013). Furthermore, the establishment of biomass crops on former agricultural land can be expected to alter soil biological and physicochemical properties over time (McCormack *et al*., 2013), which could affect the response of soil to PyC independent of changes in PyC itself. The aim of this study was therefore to assess the impact of environmentally weathered PyC on native SOC mineralisation at different points in LUC.

The focus of this study is on LUC from arable crops to SRC willow production. Other studies (Jug *et al*., 1999; Lemus and Lal, 2005; Amichev *et al*., 2012) and the data presented in Chapter 2 indicate that this transition may have considerable SOC accumulation potential, but with short term losses owing to initial soil disturbance. Previous studies have reported negative priming both in the laboratory (Prayogo *et al*., 2013) and in the field (Ventura *et al*., 2015) following PyC amendment to soil of single-site SRC willow plantations in the UK. In the present study, the effects of environmentally weathered PyC at various stages of LUC are considered. Using laboratory incubations and field flux measurements from SRC willow plantations of different age, this experiment aims to: (i) test the hypothesis that environmentally weathered PyC can reduce native SOC mineralisation by negative priming (ii) assess the sensitivity of priming effects to changes in soil properties following LUC and elucidate any potential consequences for the timing of PyC application.

4.2 Materials and methods

4.2.1 Site selection

The eight field sites selected for this study are all SRC willow plantations established on former arable land and were chosen from the 93-site chronosequence described in Chapter 2 to provide plantations with a range of different ages. Where multiple plantations in the 93-site chronosequence had the same age, sites were selected with a range of SOC contents and where possible to reflect the trend of increasing SOC content with stand age expected following this LUC in Britain (further discussion in Chapter 2). The plantations that were sampled range in age from 3 to 22 years (Table 4.1). Two of the sites that were originally identified for sampling, a 2-year and a

Table 4.1 Soil and climate characteristics for each study site. Sites are ordered by sampling date.

	Location	Age (yrs)	Field area (ha^{-1})	% SOC (*n* = 3)	% TN (*n* = 3)	BD (g cm^{-3}) (*n* = 3)	Soil pH (*n* = 3)	% clay (*n* = 1)	% silt (*n* = 1)	% sand (*n* = 1)	MAP (mm)	MAT (°C)	Met Office weather station
1	Oxfordshire	22	0.37	7.46 ± 0.48	0.79 ± 0.04	0.78 ± 0.09	6.58 ± 0.22	36.32	55.57	8.11	659.7	10.75	Oxford
2	Oxfordshire	9	3.72	6.90 ± 0.71	0.72 ± 0.06	0.81 ± 0.03	7.25 ± 0.15	27.33	55.23	17.44	659.7	10.75	Oxford
3	Oxfordshire	9	0.76	9.02 ± 0.83	0.66 ± 0.05	1.12 ± 0.07	7.06 ± 0.08	16.38	56.13	27.49	659.7	10.75	Oxford
4	Berkshire	3	10	3.03 ± 0.18	0.26 ± 0.03	1.38 ± 0.20	5.94 ± 0.80	15.16	53.51	31.32	659.7	10.75	Wisley
5	Berkshire	4	10	2.45 ± 0.36	0.22 ± 0.03	1.49 ± 0.14	5.82 ± 0.35	12.20	47.59	40.21	659.7	10.75	Wisley
6	Norfolk	11	11.07	2.39 ± 0.26	0.16 ± 0.03	1.60 ± 0.05	5.98 ± 0.47	18.23	55.49	26.28	613.7	9.90	Wattisham
7	North Yorkshire	5	2.64	2.69 ± 0.33	0.19 ± 0.04	1.24 ± 0.25	5.08 ± 0.16	14.91	59.14	25.95	651.1	9.15	Durham
8	Durham	4	7.05	4.57 ± 0.53	0.27 ± 0.02	1.10 ± 0.09	5.4 ± 0.16	18.62	60.45	20.93	651.1	9.15	Durham

7-year old plantation, were inaccessible at the time of sampling due to flooding. Therefore two other sites within relatively close proximity, a 4-year and a 9-year old plantation were sampled instead. Whilst sampling two 4-year and 9-year old plantations may obscure the upward trend in SOC over time, this arose due to the practical constraints noted. The number of study sites (n = 8) was determined based on available resources, namely incubation storage space and equipment. Descriptive statistics for soil and climate parameters of selected study sites are listed in Table 4.2.

4.2.2 Pyrogenic carbon characterisation

One type of PyC was used in this study, which was produced by slow pyrolysis of *Miscanthus* straw (Pyreg GmbH, Dörth, Germany). Lignocellulosic biomass was selected as feedstock for pyrolysis. This was in anticipation of a scenario where a portion of the biomass harvested would be pyrolysed and used to augment SOC stocks following the establishment of a new biomass crop plantation. Since *Miscanthus* uses the C_4 photosynthetic pathway thus creating the potential for future stable isotope studies, this was selected over SRC willow as the choice of feedstock. Although the target in-kiln processing temperature was 550°C, heat was recycled to the kiln due to the gas flow triggered by *Miscanthus* straw and there was a final production temperature of approximately 800°C. Therefore, the PyC used here is likely to be more stable than most PyC produced for soil application in other studies.

Particle size distribution of the PyC was measured using progressive dry sieving and was as follows: 18% was <0.5 mm, 20% was 0.5–1.0 mm, 35.8% was 1–2 mm and 26.2% was 2–5.6 mm. PyC was characterised by elemental and proximate analysis and the University of Edinburgh stable C (Cross and Sohi, 2013) and labile C (Cross and Sohi, 2011) toolkit assays (Table 4.3). Prior to elemental and proximate analysis, samples were milled to a fine powder using a MM200 ball mill (Retsch GmbH, Haan, Germany) and dried overnight at 105°C. Elemental analysis of PyC was used to determine the total elemental C, H, N and O, which are expressed as a percentage of the dry weight of each sample. Proximate analysis was carried out using thermal gravimetric analysis (TGA / DSC 1, Mettler-Toledo, Leicester, UK) to determine the proportion of free, locked and total volatile matter (FVM, LVM and TVM) and ash content. Samples were first heated to 105°C for 10 min under a N_2 atmosphere to determine the moisture content. Temperature was subsequently increased at 25°C min^{-1} to 900°C and held for 10 min so that volatile matter could be determined gravimetrically after dehydration. Finally air was introduced and the sample combusted at 900°C for 15 min to determine the ash content. The Edinburgh stable C and labile C assays were developed at the UK Biochar Research Centre, University of Edinburgh. For further details on these procedures, see Cross and Sohi (2013) and Cross and Sohi (2011) respectively. Briefly, for the stable C assay, a sample containing 0.100 g C was milled to a fine powder and oxidised with 7 ml of 5% hydrogen peroxide (H_2O_2) (w/v), first at room temperature and then at 80°C (to dryness) over 48 hours. Stable C is expressed as the percentage of initial sample C that remains after treatment, calculated from the gravimetric mass loss and C content before and after oxidation (Cross and Sohi, 2013).

Table 4.2 Descriptive statistics for soil and climate parameters of selected study sites.

	% SOC	% TN	BD (g cm^{-3})	pH	% clay	% silt	% sand	MAP (mm)	MAT (°C)
Mean	4.71	0.38	1.19	6.01	19.46	55.98	24.56	650	10.10
Standard deviation	2.40	0.24	0.29	0.76	7.18	3.91	8.73	14.09	0.75
Median	3.90	0.28	1.18	5.95	18.23	55.57	25.95	656.60	10.75
Range	7.35	0.70	0.98	2.44	24.12	12.86	32.10	46.00	1.60
Interquartile range	2.65 to 7.04	0.22 to 0.65	1.00 to 1.49	5.33 to 6.66	15.10 to 18.62	54.80 to 59.47	20.93 to 28.45	651.10 to 659.70	9.15 to 10.75

Table 4.3 PyC characteristics: % stable and labile C (*n* = 4), total elemental C, hydrogen (H), nitrogen (N), and oxygen (O) and molar oxygen to carbon (O/C), hydrogen to carbon (H/C) and carbon to nitrogen (C/N) ratios (*n* = 1), free, locked and total volatile matter (FVM, LVM and TVM), ash content, black carbon (BC_{hypy}) (*n* = 1) and pH (*n* = 6). Percentages are expressed on a dry weight basis.

(wt %)														
Stable C	Labile C	C	H	N	O	O/C	H/C	C/N	FVM	LVM	TVM	Ash	BC_{hypy} (BC/SOC %)	pH
95.28 ± 0.06	0.11 ± 0.01	77.74	0.97	0.36	4.52	0.04	0.15	253.35	4.01	2.46	6.47	16.41	99.1	9.97 ± 0.10

For the labile C assay, 2 g of PyC was mixed with 19 g of sterilised size-graded quartz sand, inoculated with a solution of soil microbes and micronutrient solution, adjusted to 65% water holding capacity (WHC) and then incubated in flasks containing suspended vials containing soda lime (Fisher Scientific, Loughborough, UK) at 30°C for 2 weeks. Cumulative CO_2 flux was then measured gravimetrically using the alkali trap method, where the amount of CO_2 evolved is proportional to the increase in soda lime mass, and the labile C is expressed as a percentage of the total C content of each sample (Cross and Sohi, 2011). PyC pH was measured using a ratio of 1.0 g of PyC in 20 ml of deionised water and shaking for 1.5 hours before measuring pH to ensure sufficient equilibration between solution and PyC surfaces (Rajkovich *et al*., 2011).

4.2.3 Pyrogenic carbon field application and soil sampling

PyC amendment was carried out between July and November 2011. A grid of 100 intersections was overlain on each study site using a scale appropriate to the field size and further divided into 3 areas of approximately equal size. Within each of the 3 areas, a pair of 2 x 2 metre plots was established at an intersection selected using a random number generator. For each pair, one plot had PyC applied manually to the surface at an application rate of 16 t ha^{-1} and incorporated into the soil surface using a spading fork. The aim was to incorporate PyC to 15 cm depth. However, based on visual assessments this was reduced to approximately 5–10 cm for sites with hard and dry soil. The forking treatment was then also applied to the alternate control plot, located at a 5 m distance from the PyC amended plots. In the context of current literature, the PyC application rate used here (approx. 0.5% by soil mass) is a mid-range experimental rate. At site 8 only, three additional pairs of plots were established just 2 weeks before sampling to compare the effects of weathered and fresh PyC.

In May 2013, 18–22 months after PyC amendment, cores (Ø 30 mm) were taken using an absorbing hammer and bi-partite gouge auger (Van Walt, Haslemere, UK). The length of time between PyC amendment and soil sampling varied between sites since PyC amendment was carried out during visits to the 93-site chronosequence over a period of 9 months. Sampling was to 5 cm depth from the central 1 m^2 of each plot using a ‘W formation’. Ten soil cores were collected from each plot to obtain sufficient material for the laboratory incubations and soil analysis. At Site 3 only two pairs of plots could be sampled due to partial flooding of the field. Samples were combined by plot and stored at 4°C for less than 30 days prior to the incubation experiment. This was to reduce the impact of storage on microbial activity (Zelles *et al*., 1991). An additional core of 50 mm diam. was taken to 5 cm depth from each plot using a specialised ring corer kit to measure soil bulk density (BD) (Van Walt, Haslemere, UK).

4.2.4 Laboratory incubations and carbon dioxide flux measurements

Prior to incubation, soil samples were sieved (<4 mm), with care taken to remove fine roots and stones, and adjusted to 60% WHC, which is considered optimal for soil microbial respiration (Howard and Howard, 1993). To determine the maximum WHC a method similar to Ohlinger (1995) was used. For each sample, triplicates of 20 g of field moist soil were weighed into cellulose filters (Whatman No.1, Sigma-Aldrich, Gillingham, UK; 11 µm retention), which were placed inside plastic funnels with the bottoms sealed. These were saturated in deionised water for 1 hour while covered with plastic film (Parafilm, USA) and placed in a closed plastic box to limit evaporation. After 1 hour the stoppers were removed and samples were left to drain for 3 hours. Samples were then weighed into foil cups, dried at 105°C for 24 hours and then cooled in a desiccator and re-weighed to determine the gravimetric moisture content (GMC) [Equation (4.1)]. The maximum WHC (WHC_{max}) under laboratory conditions was assessed for each sample [Equation (4.2)]. The moisture addition / reduction required to adjust 10 g (dry weight equivalent) of field moist soil to 60% WHC was determined. WHC_{max} was also calculated for the samples from the amended plots to determine the effects of PyC on WHC. Prior to incubation, samples from amended plots were adjusted to the % GMC equivalent to 60% WHC of the control soil. The purpose of using equalised GMC was to remove indirect WHC-related effects of PyC amendment and instead focus on direct priming effects. Since PyC may alter both the distribution of a fixed amount of water within different soil pores as well as the bulk soil water filled pore space (WFPS), using equalised GMC may assist in ascertaining the importance of these effects with respect to priming.

$$GMC\ (\%) = \frac{(field\ moist\ soil\ (g) - oven\ dried\ soil(g))}{oven\ dried\ soil(g)} \times 100 \quad (4.1)$$

$$WHC_{max}(\%) = \frac{(saturated\ soil\ (g) - oven\ dried\ soil(g))}{oven\ dried\ soil(g)} \times 100 \quad (4.2)$$

Incubations were carried out in triplicates of 10 g (dry weight equivalent) of each sample, weighed into 250 ml conical flasks, at 30°C for 10 weeks. Conical flasks were sealed with a rubber stopper to minimise moisture loss. Cumulative CO_2 flux was assessed gravimetrically using the soda lime adsorption method. 1.0–1.5 g of self-indicating, non-hygroscopic soda lime granules were used in each flask (1.0–2.5

mm size; Fisher Scientific, Loughborough, UK). These were weighed into 1.7 ml glass vials, dried at 105°C for 24 hours and cooled in a desiccator before re-weighing and incubation. The vials were suspended from the rubber stopper used to seal each flask. A blank flask containing a soda lime vial but no soil was used for every five flasks, to correct for CO_2 gained during preparation of the vials, from the flask headspace at closure and on re-drying of the soda lime prior to re-weighing. Each vial was weighed and replaced weekly to prevent saturation of the soda lime. Mineralised C was determined gravimetrically as the quantity of CO_2 is proportional to the increase in soda lime mass as the CO_2 reacts with sodium and calcium hydroxides to form carbonates [Equation (4.3)] (Edwards, 1982; Grogan, 1998). The first week was considered as a 'pre-incubation' period during which respiration rate stabilised following sieving and moisture adjustment (Fierer and Schimel, 2003).

$$Mineralised\ C\ (mg\ CO_2 - C) = \left(1.69 \times (mass\ gain\ of\ soda\ lime - mass\ gain\ of\ blank\ flask) \times \frac{12}{44}\right) \times 1000 \quad (4.3)$$

where 1.69 is a conversion factor used to correct for water formed during chemical adsorption (Grogan, 1998) and 12/44 is the ratio of the molar mass of C to molecular weight of CO_2.

4.2.5 Carbon dioxide flux measurements in the field

At each site, soil-surface CO_2 flux measurements were made at the 3 pairs of 2 x 2 metre plots, each consisting of a PyC-amended and a control plot, except site 3 where only two pairs of plots could be sampled due to partial flooding of the field. Soil-surface CO_2 flux was measured in the field immediately before soil sampling in

May 2013, using a dynamic closed chamber infra-red gas analyser (IRGA) (EGM-4 PP Systems, Amesbury, Massachusetts, USA). CO_2 flux measurements were taken before soil sampling to reduce the effects of disturbance. At each plot, the respiration chamber was rotated into the soil surface and five measurements were taken in a 'W formation' from the central 1 m^2. Although care was taken to reduce the effects of soil disturbance, due to the presence of leaf litter and understory, it is possible that in circumstances where significant downward pressure was required to ensure an airtight seal this may also have disrupted soil aggregates and/or damaged roots below the surface. Ambient soil temperature (HI993310 Hanna Instruments, Leighton Buzzard, UK) and soil moisture (Moisture Meter HH2, Delta-T Devices, Cambridge, UK) were also recorded at each plot, again using a 'W formation' from the central 1 m^2.

4.2.6 Soil chemical and physical analysis

After sieving (<4 mm) the composite samples for each plot, a sub-sample was taken for C and N analysis. These samples were air-dried at room temperature for 7 days, before being crushed with a pestle and mortar, sieved (<2 mm) and milled to a fine powder using a MM200 ball mill (Retsch GmbH, Haan, Germany). 15–20 mg of the control plot samples and 5–10 mg of sample from PyC amended plots were analysed for total C and N by dry combustion using a NA 2500 Elemental Analyser (Carlo Erba, Milan, Italy). Inorganic C content was measured using an automated acidification module and coulometry (CM 5012 and CM 5130, UIC, Joliet, Illinois). 50–100 mg of each sample was acidified using 8 ml of 2 *M* perchloric acid ($HClO_4$) and, as carbonates were released as CO_2, the acid-evolved gas was measured by coulometric titration. For each sample, SOC content was determined by subtracting the inorganic C from the total C content.

Soil used for GMC determination (see 3.2.4) was subsequently oven dried at 105°C and used for soil pH measurements. 10 g of soil was added to a beaker with 25 ml of deionised water, stirred rigorously and then left for 30 min, stirred again and pH measured using the same equipment as for PyC above. The electrode was held in suspension for 30 seconds before each measurement was taken. Samples collected for BD measurements were returned to the laboratory, oven dried at 105°C for 48 hours and sieved (<2 mm) to separate coarse fragments from fine earth. Collected samples were weighed to calculate BD of the fine earth (BD_{fe}) [Equation (4.4)], correcting for the volume of coarse fragments with an assumed density of 2.65 g cm^{-3} [Equation (4.5)]. Soil WFPS was then calculated using GMC and BD [Equation (4.6)]:

$$BD_{fe}\ (g\ cm^{-3}) = \frac{(soil\ with\ gravel\ (g) - coarse\ fragments(g))}{volume\ of\ corer(cm^{-3}) - \left(\frac{coarse\ fragments(g)}{2.65}\right)} \quad (4.4)$$

$$BD_{corrected}\ (g\ cm^{-3}) = BD_{fe} \times diameter\ of\ corer\ (cm) \times 10 \times \left(1 - \left(\frac{coarse\ fragments(g)}{(2.65 \times volume\ of\ corer(cm^{-3}))}\right)\right) \quad (4.5)$$

$$WFPS\ (\%) = \left(\frac{(GMC(\%) \times BD(g\ cm^{-3}))}{\left(1 - \left(\frac{BD(g\ cm^{-3})}{2.65}\right)\right) \times 100} \times 100\right) \quad (4.6)$$

4.2.7 Black carbon quantification

To enable the comparison of primed CO_2 flux from incubated PyC-amended and control soil samples, CO_2 flux was expressed in relation to the non-black C (nBC) concentration of each sample. Hydrogen pyrolysis (hypy) was used to isolate and quantify black C (BC), with nBC calculated as the difference between BC and SOC (Ascough *et al*., 2009; Meredith *et al*., 2012). By expressing the CO_2 flux in terms of the nBC content, both the background BC at each site and the PyC in the amended plots are excluded from the calculations. The comparison rests on the premise that PyC remaining by the time of the incubation is completely stable.

The BC in the samples collected from each plot was isolated using hypy and quantified by dry combustion elemental analysis. The fresh PyC itself was also tested to assess thermochemical stability. Prior to hypy, samples were air-dried at room temperature for 7 days, before being crushed with a pestle and mortar, sieved (<2 mm) and milled to a fine powder using a MM200 ball mill (Retsch GmbH, Haan, Germany). Samples containing inorganic C >0.01% by weight were pretreated to remove carbonates by acid digestion with 1 *M* hydrochloric acid (HCl) and heating to 80°C for 24 hours. For each sample, 500 mg was loaded with a Mo catalyst 5% by weight using an aqueous / methanol solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$] and placed inside borosilicate glass reactor inserts, which were sealed at each end using quartz wool (Figure 4.1). Inserts were weighed both before and after hypy in order to measure the loss in sample weight. The samples were pyrolysed with resistive heating from 50 to 250°C at 300°C min^{-1}, then from 250 to 550°C at 8°C min^{-1} and finally held at 550°C for 2 minutes under hydrogen pressure of 150 bar. A hydrogen sweep gas flow of 5 L min^{-1}, measured at ambient

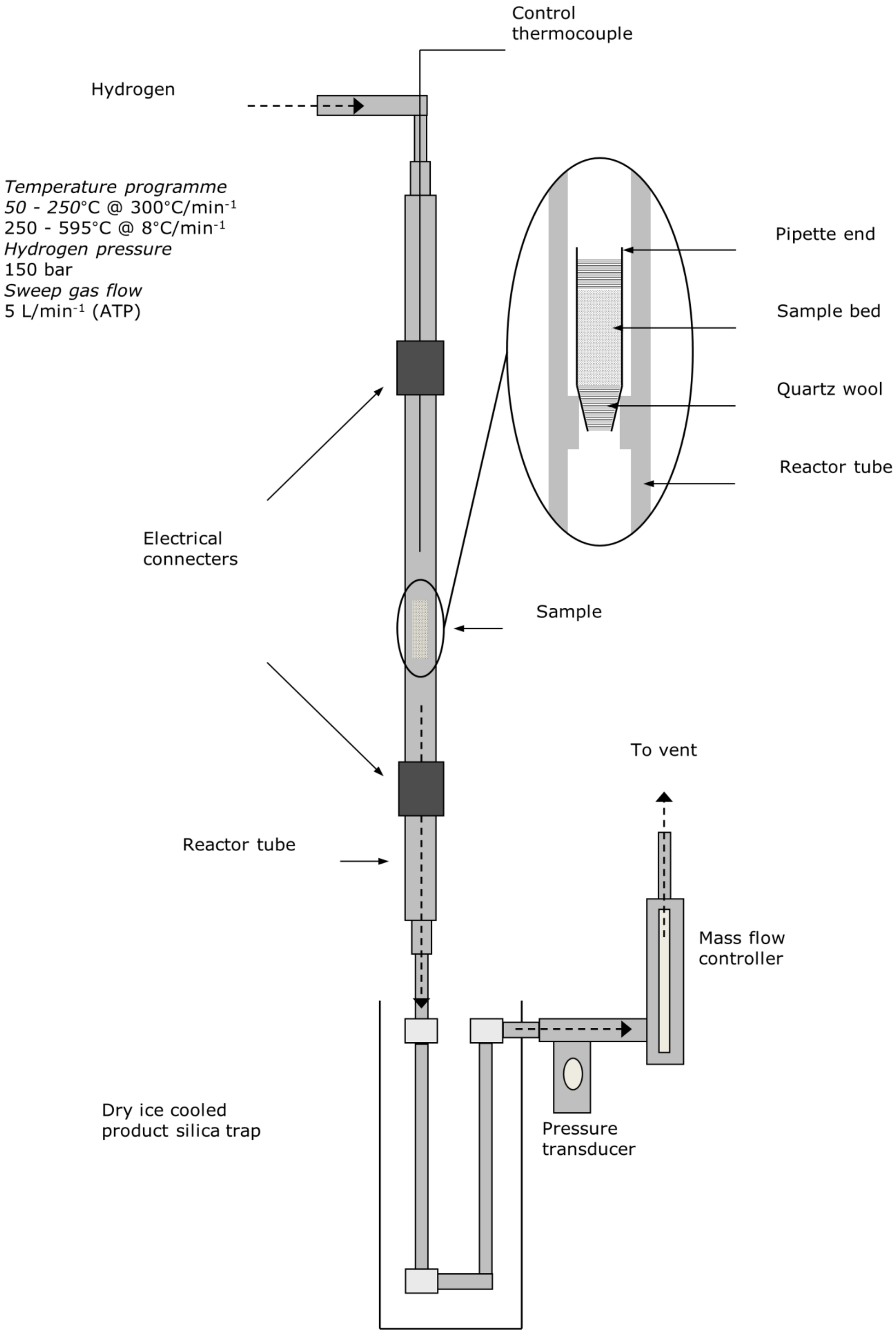

Figure 4.1 Schematic representation of the hydrogen pyrolysis apparatus (Meredith *et al.*, 2012).

temperature and pressure, ensured the nBC products were quickly removed from the reactor and trapped on dry ice cooled silica (Meredith *et al.*, 2004).

The hypy residue for each sample was analysed for total C using a NA 2500 Elemental Analyser (Carlo Erba, Milan, Italy). BC_{hypy} content was quantified by comparing the initial and residual SOC contents [Equation (4.7)]:

$$BC_{hypy}(BC/SOC\,\%) = \frac{Residual\ SOC\ (mg\ in\ hypy\ residue\ incl.spent\ catalyst)}{Initial\ SOC\ (mg\ in\ catalyst\ loaded\ sample)} \times 100 \quad (4.7)$$

4.2.8 Statistical analysis

All statistics were carried out using SPSS 19 (IBM, Armonk, New York, USA). For the incubation experiment, linear mixed-effect models were created using the restricted maximum likelihood (REML) procedure, to assess the significance of PyC amendment and incubation time on cumulative CO_2 flux (mg CO_2-C g^{-1} nBC). Models were first created for each site and then for all paired plots with weathered PyC together. Weekly flux measurements per plot are the arithmetic means for the triplicate flasks. For individual sites, treatment (PyC amended v control) and incubation time (week of incubation) are fixed effects and 'plot pair' is a random effect. Another random effect for 'site' is introduced for the model using all paired plots. Separate models were created for site 8 for plots with weathered and fresh PyC and a model to test the significance of PyC age (weathered v fresh). A linear mixed-effect model was also created to assess the significance of variables affecting soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) for all paired plots with weathered PyC. For this model there are fixed effects for treatment (PyC amended v control), soil temperature (°C) and WFPS (%) and random effects for 'site' and 'plot pair'. Soil-surface flux measurements for each plot are arithmetic means of the 5 measurements taken. Due to the limited number of observations per parameter, models were not used for individual sites. However, it was possible to create a model for site 8 plots with weathered and fresh PyC, testing the significance of PyC age (weathered v fresh).

Another linear mixed-effect model was created to assess the effects of PyC amendment after weathering on soil physicochemical properties across sites. In this case treatment (PyC amended v control) is the fixed effect with random effects for 'site' and 'plot pair'. A general linear model (GLM) was used to test the effects of various site variables on the additional C mineralised from the control soil after normalising for nBC content. This additional C was used for inter-site comparison of priming effects. For the purpose of assessing the influence of site variables on PyC-SOC interactions and possible priming effects, only the incubation flux data was used since field flux comprises root as well as soil respiration. For all models, residuals were checked for normality using the Shapiro Wilk test.

Two sets of correlations were carried out to further explore the relationships and unexplained variance from the GLM. Correlations were carried out of soil and site variables with: (i) specific nBC mineralisation rates (mg CO_2-C g^{-1} nBC); and (ii) ratios of C mineralised in amended and control soil. Pearson correlation coefficients (r) were reported for normally distributed data and Spearman rank coefficients (r_s) for non-normally distributed data. Post-hoc power analysis established the minimum sample size that would be required to obtain a t statistic equal to or larger than a critical value, with $\alpha = 0.05$ and power $(1-\beta) = 0.8$. Student's t-distribution is assumed for both Pearson and Spearman correlation coefficients (Kendall and Stuart, 1979).

4.3 Results

4.3.1 Cumulative carbon dioxide flux under controlled conditions

Over the 10-week incubation period, PyC amendment had a significant impact on soil CO_2 flux. For seven of the eight sites cumulative CO_2 flux (mg CO_2-C g^{-1} nBC) was significantly higher for soil containing weathered PyC (Table 4.4, Figures 4.2–4.3). There was also a significant effect across sites ($p < 0.001$, Table 4.4, Figure 4.3). There was no significant difference in CO_2 flux for site 8 between soil with fresh and weathered PyC over the 10 week period ($p = 0.111$, Table 4.5, Figure 4.3). Weekly CO_2 flux rate significantly decreased over time from both the amended and control soil for all sites ($p < 0.001$, Table 4.4, Figures 4.2–4.3). The mean cumulative flux across sites was 86.9 ± 4.3 mg CO_2-C g^{-1} nBC from the soil with weathered PyC compared to 71.7 ± 3.5 mg CO_2-C g^{-1} nBC from the control, a difference of 21 ± 11%.

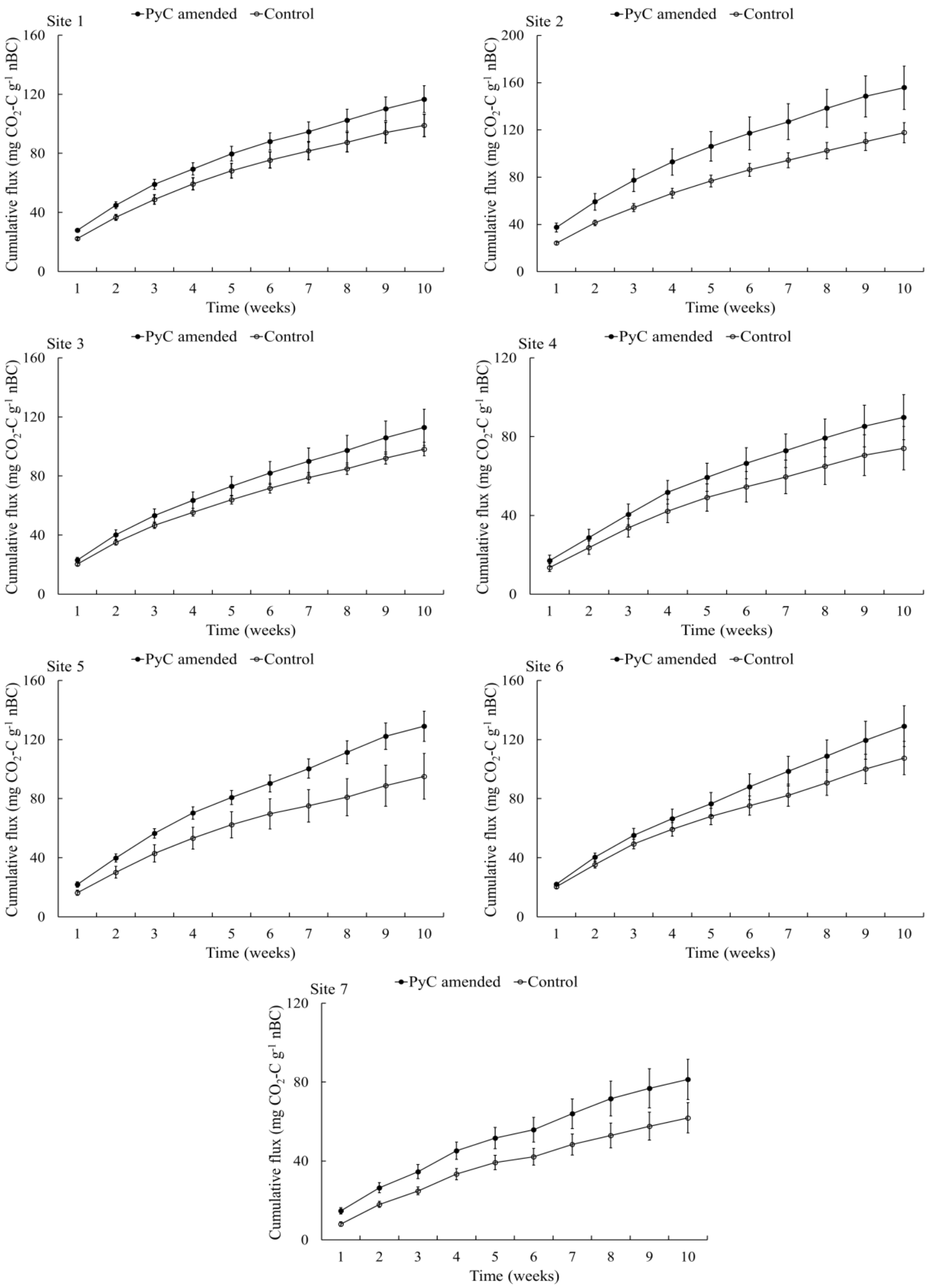

Figure 4.2 Weekly cumulative flux (mg CO_2-C g^{-1} nBC) from incubated soil from sites with weathered PyC and controls. Data points represent the mean of the replicate flasks that were incubated ± standard error (*n* = 9 [with 3 replicate flasks x 3 plots per treatment] except for Site 3 where *n* = 6 [with 3 replicate flasks x 2 plots per treatment, since 1 pair of plots was inaccessible due to flooding]).

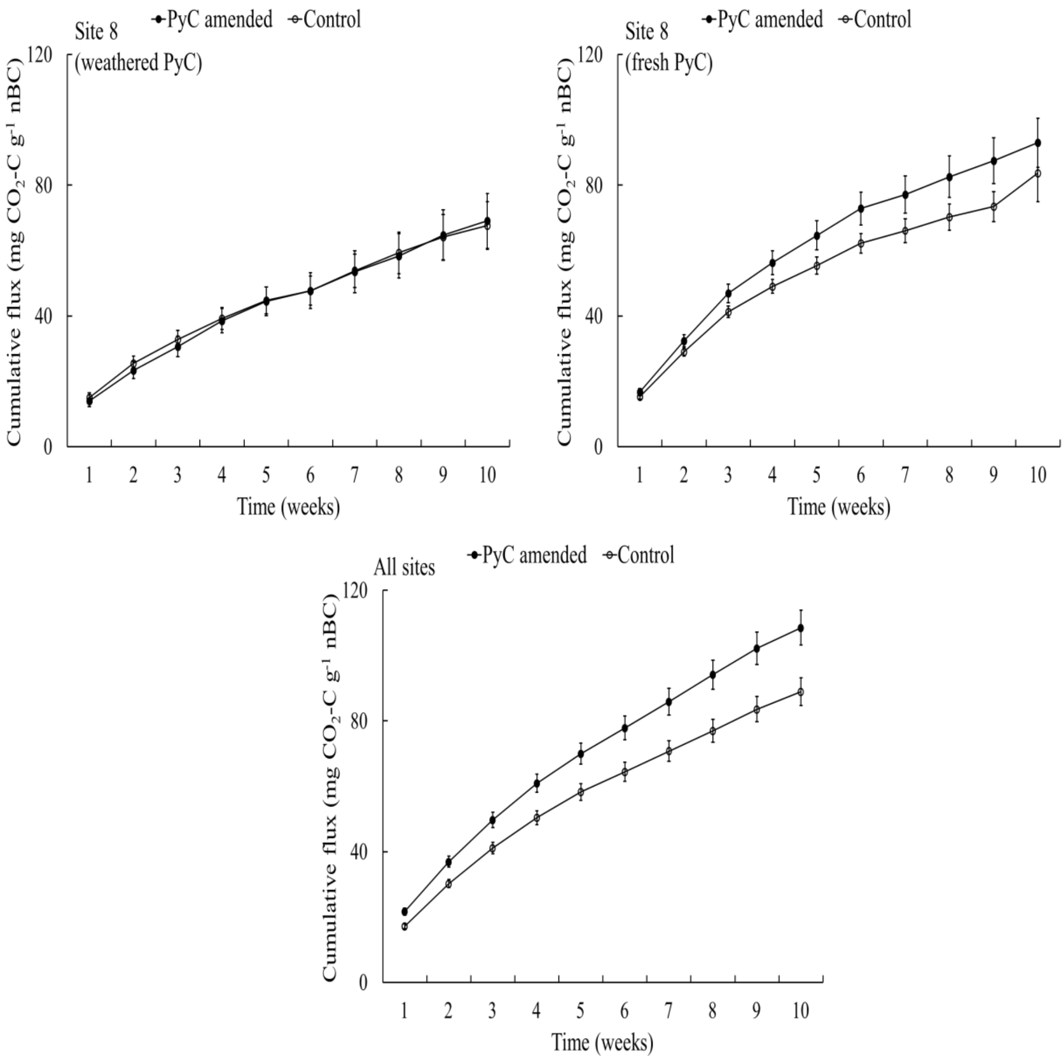

Figure 4.3 Weekly cumulative flux (mg CO_2-C g^{-1} nBC) from incubated soil from site 8 and from all paired plots with weathered PyC and respective controls across all sites. Data points represent the mean of the replicate flasks that were incubated ± standard error (n = 9 for site 8 [with 3 replicate flasks x 3 plots per treatment] and for all sites combined n = 78 [since n = 9 for each site x 8 sites in addition to n = 6 for site 3]).

Table 4.4 Variables affecting weekly cumulative CO_2 flux (mg CO_2-C g^{-1} nBC) from soil with weathered and fresh PyC incubated under controlled conditions for 10 weeks. Results are from linear mixed-effect models with fixed effects for treatment (PyC amended v control) and time (week of incubation) and random effects for site and plot pair (*n* = 27 for each site for each treatment [with 1 mean weekly flux measurement for each plot x 3 plots x 9 weeks, not including the pre-incubation week] except site 3 where *n* = 18 [with 1 mean weekly flux measurement for each plot x 2 plots, since 1 pair of plots was inaccessible due to flooding, x 9 weeks] and for all sites *n* = 207 [since *n* = 27 for each site x 7 sites in addition to *n* = 18 for site 3]).

	Independent variable			
	Treatment		Time	
Dependent variable: CO_2 flux	F-statistic	P value	F-statistic	P value
Site 1	14.799	<0.001	41.094	<0.001
Site 2	13.193	0.001	11.529	<0.001
Site 3	7.861	0.008	18.246	<0.001
Site 4	12.114	0.001	18.438	<0.001
Site 5	26.661	<0.001	12.347	<0.001
Site 6	11.774	0.001	8.431	<0.001
Site 7	14.210	0.001	17.405	<0.001
Site 8: weathered PyC	0.394	0.533	9.620	<0.001
Site 8: fresh PyC	1.654	0.205	13.894	<0.001
All sites: weathered PyC only	47.130	<0.001	88.46	<0.001

Table 4.5 Variables affecting weekly cumulative CO_2 flux (mg CO_2-C g^{-1} nBC) at site 8 from soil with weathered and fresh PyC incubated under controlled conditions for 10 weeks. Results are from linear mixed-effect models with fixed effects for treatment (PyC amended v control), time (week of incubation) and PyC age (weathered v fresh) and a random effect for plot pair (n = 54 for each treatment [with 1 mean weekly flux measurement for each plot x 6 plots x 9 weeks, not including the pre-incubation week]).

	Independent variable					
	Treatment		Time		PyC age	
Dependent variable: CO_2 flux	F-statistic	P value	F-statistic	P value	F-statistic	P value
Site 8: weathered and fresh PyC	1.443	0.233	13.983	<0.001	4.157	0.111

4.3.2 Carbon dioxide flux measured in the field

No significant differences were observed in the field between soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) from the plots with weathered PyC and the control plots (p = 0.191, Table 4.6, Figure 4.4). There were also no significant differences in CO_2 flux at site 8 from the plots with fresh and weathered PyC (p = 0.583, Table 4.7). Soil temperature (°C) and WFPS (%) both had a significant impact on CO_2 flux (p = 0.023 and 0.025 respectively, Table 4.6). The mean CO_2 flux from the soil with weathered PyC was 108.9 ± 6.5 mg CO_2-C m^{-2} h^{-1}, which was not significantly different to the control soil mean of 108.2 ± 6.1 mg CO_2-C m^{-2} h^{-1}. The mean CO_2 flux from all PyC amended soil (both before and after weathering) was 108.9 ± 6.1 mg CO_2-C m^{-2} h^{-1}, which was also not significantly different to the control soil mean of 112.4 ± 6 mg CO_2-C m^{-2} h^{-1}.

Table 4.6 Variables affecting CO_2 flux (mg CO_2-C m^{-2} h^{-1}) measured in the field from plots with weathered PyC. Results are from a mixed-effect model with fixed effects for treatment (PyC amended v control), soil temperature (°C), % WFPS and random effects for site and plot pair (*n* = 23 for each treatment [with 1 mean flux measurement for each plot x 3 plots x 7 sites in addition to 1 mean weekly flux measurement for each plot x 2 plots for site 3, since 1 pair of plots was inaccessible due to flooding]).

	Independent variable					
	Treatment		Soil temperature (°C)		Water filled pore space (%)	
Dependent variable	F-statistic	P value	F-statistic	P value	F-statistic	P value
CO_2 flux (mg CO_2-C m^{-2} h^{-1})	1.812	0.191	5.805	0.023	5.481	0.025

Table 4.7 Variables affecting CO_2 flux (mg CO_2-C m^{-2} h^{-1}) measured in the field at site 8 from plots with weathered and fresh PyC. Results are from a mixed-effect model with fixed effects for treatment (PyC amended v control) and PyC age (weathered v fresh) and a random effect for plot pair (*n* = 12 for each treatment [with 1 mean flux measurement for each plot x 6 plots]).

	Independent variable			
	Treatment		PyC age	
Dependent variable	F-statistic	P value	F-statistic	P value
CO_2 flux (mg CO_2-C m^{-2} h^{-1})	0.526	0.492	0.331	0.583

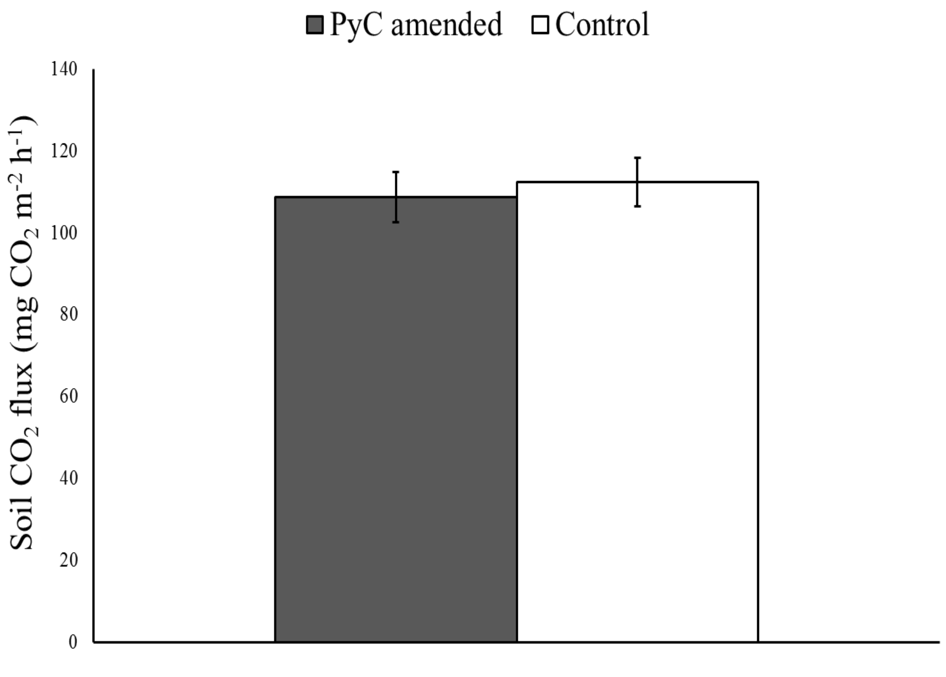

Figure 4.4 Soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) measured in the field from soil with weathered PyC and control soil. Bars represent the mean across all sites ± standard error (*n* = 115 [with 5 replicate measurements from each plot x 3 plots per site x 7 sites in addition to 5 replicate measurements from each plot x 2 plots per treatment for site 3]).

4.3.3 Changes in soil physicochemical properties

PyC amendment significantly altered various soil physicochemical properties (Table 4.8, Figures 4.5–4.6). Soil carbon was affected, with significantly higher % BC and C:N (both $p < 0.001$, Table 4.8) in the PyC amended soil relative to the control, but with significantly lower % nBC ($p < 0.001$, Table 4.8). PyC amendment also significantly increased soil pH, % GMC, % WHC and % WFPS ($p = 0.031$, $p < 0.001$, $p < 0.001$ and $p = 0.024$ respectively, Table 4.8) relative to the control and significantly reduced soil BD ($p < 0.001$, Table 4.8). There was no significant difference in % TN ($p > 0.05$, Table 4.8) between the amended and control soil.

Table 4.8 The effects of PyC amendment after weathering on soil physicochemical properties. Results are from linear mixed-effect models with a fixed effect for treatment (PyC amended v control) and random effects for site and plot pair (*n* = 23 for each treatment [with 1 measurement for each plot x 3 plots per site x 7 sites in addition to 1 measurement for each plot x 2 plots for site 3]).

	Independent variable	
	Treatment	
Dependent variable	F-statistic	P value
% SOC	36.625	<0.001
% BC_{hypy}	50.040	<0.001
% nBC	32.940	<0.001
% TN	2.491	0.129
CN ratio	45.533	<0.001
pH	5.340	0.031
BD (g cm^{-3})	327.381	<0.001
% GMC	30.399	<0.001
% WHC	16.964	<0.001
% WFPS	5.851	0.024

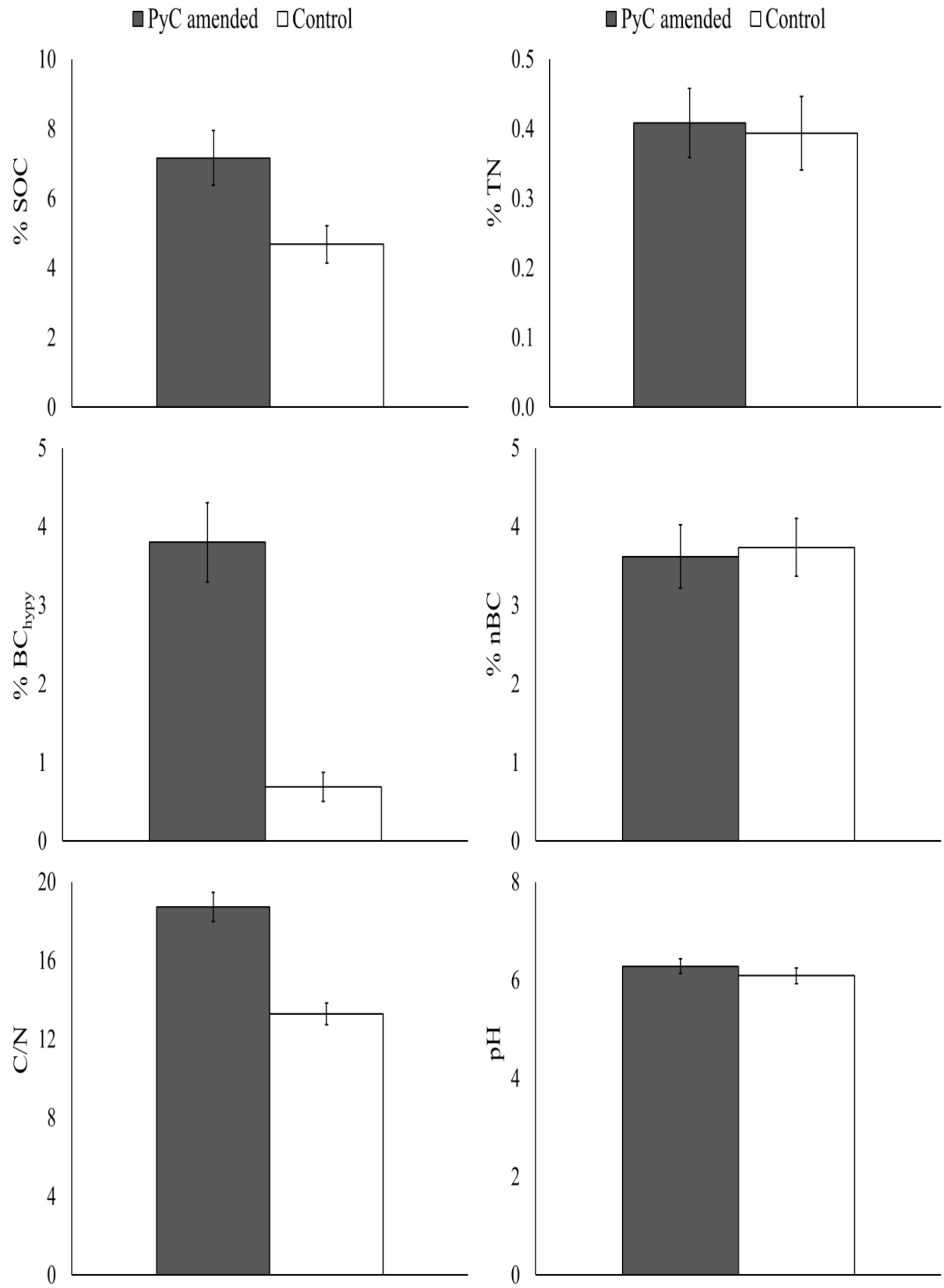

Figure 4.5 The effects of PyC amendment after weathering on soil chemical properties. Bars represent the mean across all sites ± standard error (*n* = 23 [with 1 measurement for each plot x 3 plots per site x 7 sites in addition to 1 measurement for each plot x 2 plots for site 3]).

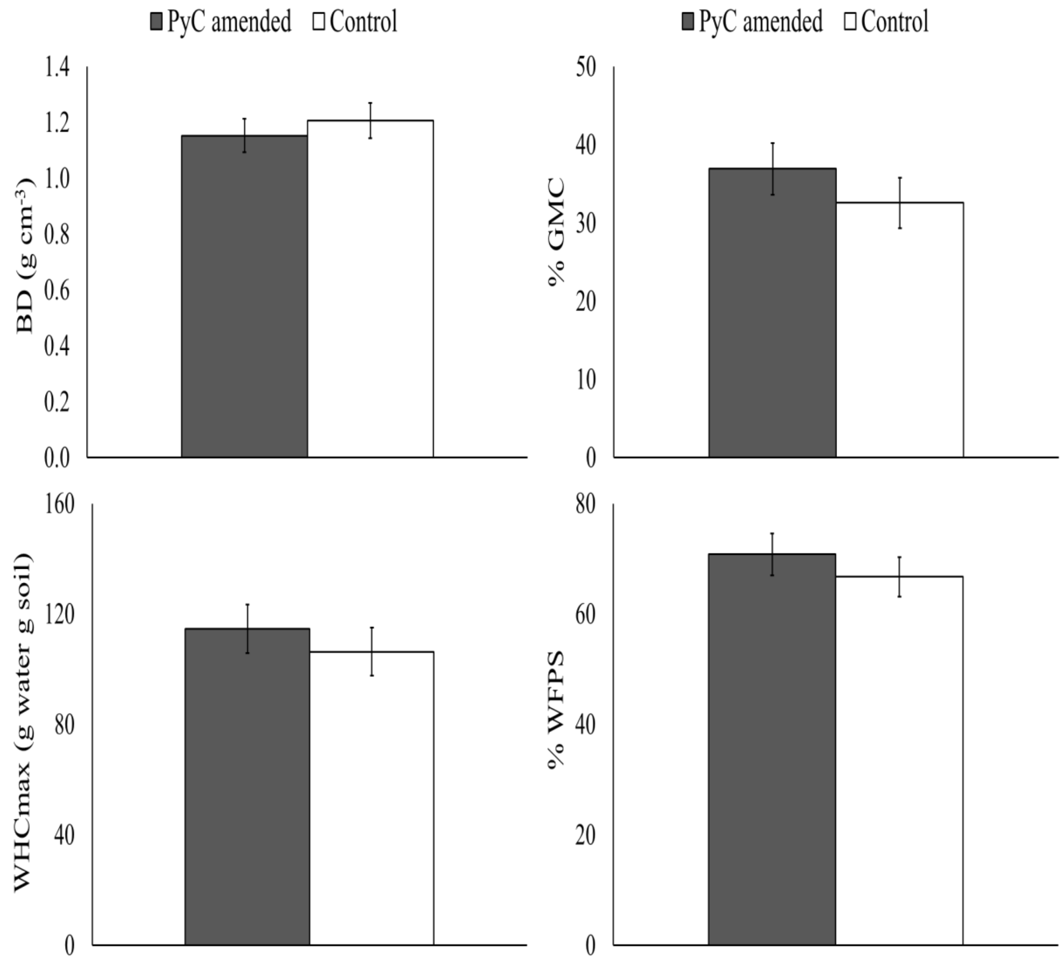

Figure 4.6 The effects of PyC amendment after weathering on soil physical properties. Bars represent the mean across all sites ± standard error (*n* = 23 [with 1 measurement for each plot x 3 plots per site x 7 sites in addition to 1 measurement for each plot x 2 plots for site 3]).

None of the site properties had a significant effect on additional C (mg CO_2-C g^{-1} nBC) (p >0.05, Table 4.9). No statistically significant correlations were observed between site properties and additional C, expressed in absolute or proportional terms (p >0.05, Table 4.10). None of the observed correlation coefficients are indicative of a strong relationship (in all cases coefficients <0.4, Table 4.10) and post-hoc power analysis shows the minimum number of samples required for these relationships to be significant (Table 4.10).

Table 4.9 The effects of various site properties on additional C (mg CO_2-C g^{-1} nBC). Results are from a GLM (n = 23 for each treatment [with 1 measurement for each plot x 3 plots per site x 7 sites in addition to 1 measurement for each plot x 2 plots for site 3]).

	Dependent variable	
	Additional C (mg CO_2-C g^{-1} nBC)	
Independent variable	F-statistic	P value
Stand age	0.005	0.953
Initial SOC	1.789	0.208
% TN	0.943	0.353
Initial pH	1.855	0.201
Δ pH	0.555	0.475
Initial BD (g cm^{-3})	0.756	0.411
Δ BD (g cm^{-3})	0.849	0.379
Δ WFPS (%)	0.091	0.769
% clay	0.107	0.761
MAP (mm)	0.027	0.881
MAT (°C)	0.015	0.911

4.4 Discussion

4.4.1 Effects of PyC on cumulative CO_2 flux under controlled conditions

In the incubation study reported here, field plots were sampled to a 5 cm depth and incubated under constant temperature and moisture. This was intended to isolate the effect of environmentally weathered PyC on C cycling processes, from that of established environmental factors. This surface soil layer was expected to contain a high concentration of PyC and thus clear evidence for any priming effects that might be occurring in the soil. At the end of the 10 week incubation period, cumulative CO_2 flux was significantly higher from soils with weathered PyC than control soils. These results indicate the potential for a sustained positive priming effect for the surface 5 cm of soil. Spokas (2013) also reported an increase in CO_2 production from soil

Table 4.10 Results of correlations between additional C (both absolute and relative amounts) mineralised from soil with weathered PyC and various site properties. Pearson correlation coefficients (r) are displayed for normally distributed data and Spearman rank coefficients (r_s) for non-normal data (n = 26). Post-hoc power analysis assumes Student's t-distribution to determine the minimum sample size required to obtain a t statistic equal to or larger than a critical value with $\alpha = 0.05$ and power $(1-\beta) = 0.8$.

	Additional C (mg CO_2-C g^{-1} nBC)	Required minimum sample size for a significant effect	Additional C (%)	Required minimum sample size for a significant effect
Age of stand	$r_s = 0.151$, $p = 0.492$	170	$r_s = 0.128$, $p = 0.561$	236
SOC conc.	$r_s = -0.050$, $p = 0.819$	1568	$r_s = -0.030$, $p = 0.893$	4359
TN conc.	$r_s = -0.009$, $p = 0.968$	48449	$r_s = -0.017$, $p = 0.939$	13578
pH	$r = 0.259$, $p = 0.234$	58	$r = 0.183$, $p = 0.403$	116
Δ pH (absolute)	$r_s = 0.148$, $p = 0.501$	178	$r_s = 0.168$, $p = 0.445$	137
Δ pH (relative)	$r_s = 0.146$, $p = 0.506$	182	$r_s = 0.172$, $p = 0.433$	131
Initial BD (g cm^{-3})	$r = -0.033$, $p = 0.881$	3602	$r = -0.048$, $p = 0.828$	1702
Δ BD (absolute)	$r_s = 0.004$, $p = 0.985$	245276	$r_s = 0.030$, $p = 0.891$	4359
Δ BD (relative)	$r = 0.375$, $p = 0.078$	28	$r = 0.340$, $p = 0.112$	34
Δ WFPS (absolute)	$r = 0.326$, $p = 0.128$	37	$r = 0.288$, $p = 0.183$	47
Δ WFPS (relative)	$r_s = 0.189$, $p = 0.388$	108	$r_s = 0.190$, $p = 0.386$	107
Clay content	$r_s = -0.070$, $p = 0.752$	799	$r_s = -0.092$, $p = 0.676$	462
Silt content	$r_s = -0.147$, $p = 0.502$	180	$r_s = -0.098$, $p = 0.656$	407
Sand content	$r = 0.047$, $p = 0.833$	1775	$r = 0.053$, $p = 0.809$	1395
MAP	$r_s = -0.016$, $p = 0.942$	15329	$r_s = -0.013$, $p = 0.954$	23220
MAT	$r_s = 0.026$, $p = 0.908$	5804	$r_s = 0.001$, $p = 0.998$	3924429
PyC weathering	$r_s = 0.078$, $p = 0.724$	643	$r_s = 0.053$, $p = 0.809$	1395

incubated with weathered PyC relative to the control soil. This increase was attributed to microbial mineralisation of either the weathered PyC or of labile C compounds sorbed to the surface of the PyC. Without direct source-partitioning using isotopic labelling, PyC mineralisation could not be confirmed, but no alteration was observed in the O:C ratio or a deterioration in the physical appearance of PyC under SEM as a result of weathering (Spokas, 2013). Without source-partitioning, it is not possible to preclude PyC mineralisation in the present study also. However, characterisation of the PyC indicates a high aromaticity and stability, most likely due to the high production temperature (Bruun *et al*., 2011; Cross and Sohi, 2011).

The results of hypy indicate that 99.1% of the PyC is composed of a highly recalcitrant fraction of BC which is highly resistant to environmental degradation over millennia (Ascough *et al*., 2010). Previous studies have shown that hypy reliably isolates a consistent portion of the BC continuum, which consists of poly-aromatic structures with >7 rings and has a H/C atomic ratio of <0.5 (Ascough *et al*., 2010; Meredith *et al*., 2012). Further evidence of the impervious nature of the PyC is provided by the University of Edinburgh stability assay. This uses an accelerated aging technique to simulate PyC degradation in soil (Cross and Sohi, 2013). Results indicate that 95.3% of the C would resist degradation in soil for at least 100 years under temperate environmental conditions. Since the incubation period is equivalent to approx. 2.5 years under UK field conditions (Cheng *et al*., 2008b), the PyC is unlikely to measurably degrade in the timeframe of this incubation.

Several studies using fresh PyC in other land-use contexts have reported a short term increase in CO_2 flux and have attributed this to the mineralisation of a relatively small component of labile C present in the PyC or a co-metabolic effect on the mineralisation of both PyC and native SOC (Hamer and Marschner, 2005; Hamer *et al*., 2004; Luo *et al*., 2011; Major *et al*., 2010; Cross and Sohi, 2011; Troy *et al*., 2013). There is no evidence for such a short term effect in the present study as fresh PyC amendment at site 8 had no effect on CO_2 flux in either the laboratory or the field. This most likely relates to the high production temperature (800°C) of the PyC, which has a labile C content of just 0.11 ± 0.01%.

As Spokas (2013) suggested, it is also possible that the higher CO_2 flux measured here derives from the mineralisation of more labile C that has adsorbed to PyC surfaces. Due to the large surface area and high porosity of PyC, this may provide a favourable habitat for microorganisms, with access to labile substrate and refuge from predators (Neher *et al*., 1999; Bardgett, 2005). However, it has previously been argued that sorption of labile C may inhibit SOC mineralisation as soluble constituents diffuse and sorb into the micropores that are too small for microorganisms to access (Hamer *et al*., 2004; Hilscher *et al*., 2009; Cross and Sohi, 2011; Zimmerman *et al*., 2011). It is likely that the bioavailability of sorbed compounds will therefore vary with the physical properties of PyC. Although surface area and pore size were not measured, this high temperature PyC may have a high sorption affinity for SOC and fine pore size as both are reported to increase with production temperature (Kasozi *et al*., 2010; Warnock *et al*., 2007). Since no reduction in CO_2 flux was observed in this study, it is possible that prior adsorption resulted in the mineralisation of labile C compounds during incubation.

Few incubation studies have investigated priming effects from PyC in soils of perennial biomass crops and only one was identified that used soil from a SRC willow plantation (Prayogo *et al*., 2013). This incubation study using fresh PyC reported no net effect on CO_2 production for a low PyC application rate (0.5% w/w) and negative priming for a high application rate (2% w/w) to soil sampled from 0-30 cm depth (Prayogo *et al*., 2013). A negative priming effect has also been observed following PyC amendment to a *Miscanthus* x *giganteus* plantation (Case *et al*., 2014). In this study, following application of PyC at a rate of 49 t ha^{-1}, CO_2 flux was reduced by 53% in a 120-day incubation using soil collected from the field 10 months after PyC amendment (Case *et al*., 2014). Since a low application was used in the present study and both of these studies reported negative priming at higher application rates, it is possible that this may indicate a threshold effect for priming mechanisms. However, the effects of increasing application rate are inconsistent with other studies reporting no effect for other land-uses (Zhang *et al*., 2012a). Further research is required to assess the effects of different application rates with environmental weathering of PyC for perennial biomass crops.

In the present study a small increase in soil pH was observed following PyC amendment, with a mean pH of 6.09 ± 0.16 for the control soil and a mean of 6.28 ± 0.15 for the PyC amended soil, and a liming effect has previously been identified as a potential cause for positive priming from PyC (Farrell *et al*., 2013). However, both Case *et al*. (2014) and Prayogo *et al*. (2013) also reported an increase in pH following PyC amendment, neither of which were accompanied by positive priming. Since the mean soil pH of the sites in the present study (6.01) was lower than both of these studies (pH >7), an alleviation of an existing pH constraint on C utilisation is more likely to have occurred here, which may at least partially explain the higher CO_2 flux observed for PyC amended soil in the top 5 cm. However, this is unlikely to be the driving mechanism for positive priming for all sites since soils with a range of pH were used in this study and positive priming was not significantly related to pH.

PyC amendment significantly altered other soil physicochemical properties in the present study. These effects may partially explain the increase in CO_2 flux observed in the top 5 cm through the alleviation of constraints on C utilisation. PyC amendment reduced soil BD, hence an increase in porosity and oxygen diffusion may have stimulated microbial activity (Torbert and Wood, 1992; Beylich *et al*., 2010). Since the amended and control soils were adjusted to equalised GMC, the PyC may also have reduced water availability which could further have enhanced aerobic respiration. Another potential cause for the increase in CO_2 flux is through enhanced nutrient availability which can occur due to increased pH, which increases P availability in soil (Cui *et al*., 2011), and greater cation exchange capacity, which allows for the retention of nutrients such as K (Liang *et al*., 2006; Major *et al*., 2010). Further research is required to determine the effects of PyC amendment on soil microbial community structure and, by identifying shifts in functional taxa that are sensitive to certain conditions, this may help to elucidate the mechanism responsible for the observed positive priming. For example, a decrease in the fungal growth/bacterial growth ratio may indicate a liming effect (Bardgett, 2005; Rousk *et al*., 2009; Watzinger *et al*., 2014) which could also reduce the abundance of mites, causing an increase in the microbial grazers they prey on and subsequently increasing microbial respiration (Hagvar and Amundsen, 1981; Kajak, 1995).

4.4.2 Effects of PyC on soil-surface CO_2 flux in the field

Soil-surface CO_2 flux measurements were taken to confirm if the effects observed in the laboratory were demonstrable under field conditions. Despite the increase in CO_2 flux from PyC amended soils incubated under controlled conditions, no significant differences in soil-surface CO_2 flux were observed between amended and control plots in the field. These contrasting results observed between the laboratory and field indicate that at least two mechanisms are occurring under different conditions and/or at different soil depths.

Similar WHC conditions were present in the incubations, where the control and amended soils received equalised GMC (equivalent to 60% WHC) and in the field (all sites were within 50-70% WHC), suggesting similarly optimal conditions for microbial activity in both the laboratory and the field. It was expected that PyC would increase aeration and oxygen diffusion (Torbert and Wood, 1992; Beylich *et al*., 2010) in the field and the laboratory. However, soil cores sampled from the amended plots show an increase in WFPS ($p < 0.024$). It is possible that a reduction in soil aeration in the field reduced aerobic activity, while the opposite occurred in the laboratory. Other studies have reported an increase in methanogenesis following PyC amendment (Knoblauch *et al*., 2011; Zhang *et al*., 2012b) but, although this was not measured in the present study, the rapid field flux rates suggest predominantly aerobic respiration is occurring. Since WFPS was reported to increase with PyC amendment in the present study, it is possible that positive priming may have been caused by the removal of these controls on soil respiration in the laboratory rather than reflecting their *in-situ* effect. However, Case *et al*. (2014) also adjusted soils to equalised GMC prior to incubation and observed a reduction in both BD and WFPS following PyC amendment. In that study, these physical effects did not appear to stimulate microbial activity suggesting that minor variations in moisture contents may not be the cause of the significant increase in CO_2 flux observed in the laboratory in the present study. Further research would be required to assess the effects of PyC on aerobic and anaerobic respiration under changing environmental conditions. In the present study, soil-surface CO_2 flux at each site was only measured on a single day, at which time no PyC-induced priming effects were observed. However, since PyC can alter soil WHC (Case *et al*., 2012) and thermal properties (Genesio *et al*., 2012; Meyer *et al*., 2012), changes in environmental conditions over time will affect PyC-amended soil differently from unamended soil (Case *et al*., 2014). Therefore, continuous soil respiration monitoring is required to assess the dependence of PyC-induced priming effects on changing environmental conditions.

Since soil-surface CO_2 flux also includes root respiration, it is possible that a reduction in root respiration in the PyC amended plots could explain the differences observed between the laboratory and field flux measurements. PyC may impact plant productivity subsequently feeding back into effects on root respiration and it is possible that PyC could reduce root growth or even cause root mortality. PyC-induced changes to physicochemical soil properties and possible interference with plant chemical signals both have the potential to influence plant interspecific competition and root growth, particularly in biomass cropping systems with diverse understorey vegetation (McCormack *et al*., 2013). It has been suggested that PyC absorption of secondary metabolites may lessen the plant's ability to establish mycorrhizal symbioses, which may reduce plant nutrient uptake (Bais *et al*., 2006). Interference with plant defence chemicals may also increase plant susceptibility to disease, which would reduce primary productivity and subsequently root respiration (Bais *et al*., 2006). Further investigation would be required to confirm any such effect on root respiration, which could be achieved by using root exclusion methods that have been developed to enable the estimation of root respiration indirectly by comparing measured CO_2 flux rates with and without the presence of roots (Yiqi and Zhou, 2010). However, Ventura *et al*. (2015) observed a relative increase in CO_2 flux from PyC amended plots in the presence of SRC willow roots, although this was attributed to a root-induced positive priming effect on PyC decomposition rather than altered root respiration rates.

It is also possible that differences observed between the laboratory and field flux measurements may relate to the presence/absence of leaf litter. Prayogo *et al*. (2013) reported increased negative priming following PyC amendment to soil in the presence of litter. Although in their case lesser negative priming was also observed in the absence of litter, it is possible that the nature of PyC-SOC interactions will vary directly with substrate or indirectly through PyC-induced changes to soil physicochemical properties. The effects of PyC may also vary with soil depth. Changes in the distribution of SOC may occur, either directly through PyC-SOC interactions such as adsorption or increased aggregation, or indirectly by altering the physicochemical properties of the soil such as BD and thermal conductivity. For example, it has previously been reported that a reduction in the supply of fresh SOC could prevent the decomposition of SOC in deeper soil layers (Fontaine *et al*., 2007). Therefore, increased stabilisation of labile C in the surface layer may reduce the delivery of labile C to the subsoil which would otherwise activate the mineralisation of slower-cycling C in the deeper soil layers (Fontaine *et al*., 2007). Further research is required to explore these mechanisms and determine how PyC may impact the distribution of labile C, soil microbial structure and SOC mineralisation throughout the soil profile. For example, a decline in *K*-strategists in the deeper soil layers may indicate a reduction in decomposition of more stable SOC and this may be accompanied by an increase in *r*-strategists in the surface layer where a greater portion of labile C may be mineralised (Fontaine *et al*., 2011).

Few studies have investigated the impact of environmental weathering on priming effects of PyC, despite its importance for understanding the environmental benefits of PyC as a C abatement strategy in the longer term (Spokas, 2013). Although the direction and magnitude of priming effects varies between studies in the short term, it has been suggested that any positive priming is likely to be short lived, with negative priming or no effect on SOC decomposition more likely to occur in the longer term (Woolf and Lehmann, 2012; Singh and Cowie, 2014). This potential for PyC to reduce soil CO_2 emissions has been suggested as a means to improve the C abatement potential of lignocellulosic biomass crops (Case *et al*., 2014). Recent studies have observed negative priming with environmental weathering of PyC in soils of biomass crops, both in the laboratory (Prayogo *et al*., 2013) and in the field (Case *et al*., 2014; Ventura *et al*., 2015). The results of the incubation experiment presented here indicate the potential for a positive priming effect for the surface 5 cm of soil from multiple SRC willow plantations, which is in contrast to the single-site observations of previous studies. Although this positive priming appears to be offset in the field, with no net effect on CO_2 flux, these results suggest that the direction of priming effects may also vary in the longer term for different PyC-soil combinations.

4.4.3 Sensitivity of priming effects to changes in soil properties following LUC

Study sites were selected with different stand ages to assess the sensitivity of priming effects to changes in soil properties following LUC. It was expected that certain LUC-induced changes may have an impact on PyC-SOC interactions, however, stand age did not have a significant effect on additional C ($p > 0.05$). For example, soils in minimum till systems such as SRC willow can become compacted over time which affects soil invertebrates by reducing habitable pore space, fungal hyphae and water (Whalley *et al*., 1995). Since PyC reduces BD and alleviates compaction, greater effects on microbial activity may have been expected for older sites. It has also been suggested that these biomass crops can increase soil acidity over time (Jug *et al*., 1999; Makeschin, 1994) due to reduced alkaline inputs and nitrification-induced loss of base cations (Vanmiegroet and Cole, 1985), which also impacts on soil organisms and plant productivity (Bardgett, 2005). Previous studies have observed differential effects of PyC for soils of different pH (Blagodatskaya and Kuzyakov, 2008; Luo *et al*., 2011), however, neither the initial pH of the receiving soil nor observed changes in pH (ΔpH) had an effect on additional C in the present study.

These results indicate that changes in soil properties during LUC from arable to SRC willow may not affect PyC-SOC interactions, which could be predominantly governed by soil factors independent of this. A relationship has previously between observed between the SOC status of a receiving soil and priming effects (Cross and Sohi, 2011) with indications that PyC may stabilise labile C in soils of higher SOC status. It may have been expected that increased C inputs and accumulation of leaf litter with stand age would alter the dynamics of PyC-SOC interactions and exhibit negative priming. However, SOC content had no effect on additional C, indicating that changes in C quantity and quality had no demonstrable influence on priming effects. The range of SOC between sites is similar to that of Cross and Sohi (2011), although there was no clear correlation between stand age and SOC content. Therefore, there may be different consequences of LUC for soil substrates in the sites sampled in this study which could obscure temporal trends. Soil texture might also have been expected to influence priming effects since PyC may provide a favourable habitat for microorganisms, which may be important for soils with low clay content. However, none of the soils used in this study were particularly low in clay and soil texture did not significantly affect additional C.

4.5 Conclusion

Results from the incubation presented here indicate the potential for a sustained positive priming effect for the surface 5 cm of soil that was detectable in soil collected 18–22 months after amendment with PyC. Across all sites the mean cumulative CO_2 flux was 21% higher from soil incubated with weathered PyC than the control soil. Due to the high aromaticity and stability of the PyC used in this study, this increase in CO_2 flux is unlikely to relate to decomposition of the PyC indicating that a positive priming effect has been observed. Positive priming may be partially explained by PyC-induced changes in soil physicochemical properties, such as increased soil pH or reduced water availability, through the alleviation of constraints on C utilisation. However, these effects are unlikely to be the driving mechanism for positive priming for all sites since: (i) soils with a range of pH were used in this study and positive priming was not significantly related to pH and; (ii) other studies have also observed a reduction in water availability following PyC amendment but did not report positive priming. It is therefore suggested that this increase in C mineralisation may relate to prior adsorption and subsequent mineralisation of labile C compounds during incubation.

Despite the increased CO_2 flux observed under controlled conditions, no net effect on CO_2 flux was observed in the field. It is possible that this increase has been offset by a contrasting PyC-induced effect such as a reduction in either root respiration or SOC mineralisation in the deeper soil layers. Increased sorption of labile C in the surface layer may have reduced the delivery of labile C to the subsoil which would otherwise activate the mineralisation of slower-cycling C in the deeper soil layers. Further research is required to assess the impact of PyC on (i) the distribution and mineralisation of SOC throughout the soil profile and; (ii) root respiration. Since previous studies have observed negative priming at higher PyC application rates, further work is also required to investigate the effects of different application rates with environmental weathering of PyC for perennial biomass crops. For the PyC and application rate used in this study, results suggest that PyC does not reduce LUC-induced SOC losses through negative priming. Furthermore, positive priming observed in the laboratory incubation was not sensitive to changes in soil properties that follow LUC from arable crops to SRC willow.

Chapter 5 A statistical model for the estimation of soil black carbon content

The aim and experimental design for this chapter were conceived by the candidate and supervisors. The candidate carried out site visits for PyC amendment and soil sampling, sample preparation, laboratory analysis, data analysis and writing of the chapter. Will Meredith provided training and technical assistance for hydrogen pyrolysis.

5.1 Introduction

Land-use change (LUC) can have a major impact on bulk soil organic carbon (SOC) stocks, and certain LUCs have previously been identified and investigated (see Chapter 2) for their carbon (C) storage potential (Smith *et al*., 2000; Lal, 2004b). However, most SOC stock changes following LUC reflect alterations in the more active SOC pools, at least in the short term. Black carbon (BC) is a component of SOC that displays distinct physical and chemical properties. BC has a high level of recalcitrance and provides a more permanent C store than other forms of C (Schmidt and Noack, 2000). To better understand how direct BC amendment to soil might contribute to C storage, a deeper insight into the abundance and behaviour of pre-existing BC is needed.

The term 'black carbon' encompasses a broad continuum of recalcitrant carbonaceous materials of both pyrogenic and petrogenic origin (Schmidt and Noack, 2000; Schmidt *et al*., 2001; Masiello, 2004; Dickens *et al*., 2004; Preston and Schmidt, 2006). This includes the solid residues derived from the incomplete combustion of fossil fuels or biomass (char), the products formed during the condensation of hot combustion gases (soot) and inert graphitic C from rocks (Novakov, 1984; Goldberg, 1985; Akhter *et al*., 1985; Dickens *et al*., 2004). BC is ubiquitous in the environment where the primary sources are pyrogenic (Goldberg, 1985). Globally, approximately 12–24 megatonnes (Mt) C yr^{-1} is produced as BC from the combustion of fossil fuels (Penner *et al*., 1993) and 50–270 Mt C yr^{-1} from biomass burning (Kuhlbusch and Crutzen, 1995). Although a fraction of BC particles are dispersed globally by atmospheric or fluvial transport, most remain locally in the soil and can accumulate to a relatively high abundance (Masiello, 2004). For example, it has been estimated that BC may account for up to 45% of organic carbon (OC) in frequently burned agricultural soils (Skjemstad *et al*., 2002). Even though conversion rates for biomass C exposed to fire are only 1.4–1.7% (Kuhlbusch and Crutzen, 1995), the production of BC is an important process in the global C cycle. The formation of BC and its subsequent deposition and persistence in soils represents an important sink in the terrestrial C cycle, by enhancing the transfer of biogenic C from the faster-cycling atmosphere-biosphere system into a slower-cycling soil C pool (Schmidt and Noack, 2000). However, the magnitude of this sink is unclear and there are considerable uncertainties associated with quantitative estimates of BC fluxes and stocks (Krull *et al*., 2008).

The recalcitrance of BC is explained primarily by its chemical composition as its structure is dominated by condensed aromatic ring configurations (Tang and Bacon, 1964; Schmidt *et al*., 2001; Simpson and Hatcher, 2004; Preston and Schmidt, 2006). Estimates for the half-life of BC in soil are 5000–7000 yr (Preston and Schmidt, 2006), compared to a mean residence time for bulk SOC of 300 and 2500 yr for the surface and subsoil respectively (Fontaine *et al*., 2007). Based on the proposed recalcitrance of BC and estimated production rates, BC would be expected to comprise 25–125% of the soil and sediment SOC pool (Masiello and Druffel, 1998; Schmidt and Noack, 2000; Forbes *et al*., 2006). However, global estimates of BC are 10–30% of SOC (Cusack *et al*., 2012) and 5–10% of OC in marine sediments (Griffin and Goldberg, 1975; Suman *et al*., 1997; Verardo, 1997; Gustafsson and Gschwend, 1998; Masiello and Druffel, 1998). In addition to understanding its role as a C sink, quantifying the BC content of soil is important for assessing the global response of soil C to climate change (Lehmann *et al*., 2008). An improved understanding of the behaviour of BC in soil would assist in optimising its function as a beneficial soil amendment (Joseph *et al*., 2010). It would also inform its use as a tracer for the Earth's fire history (Bird and Cali, 1998), in conjunction with radiocarbon dating (Ascough *et al*., 2009).

To develop a comprehensive assessment of global soil BC stocks, accurate and comparable analyses of the BC content of soil are essential (Schmidt *et al*., 2001). However, isolating and quantifying BC in soil is challenging due to limited consensus on its definition. BC is still generally defined by operational rather than chemical parameters and the various methodologies that have emerged each identify only a portion of the BC continuum (Schmidt *et al*., 2001; Currie *et al*., 2002; De la Rosa *et al*., 2011). The methods for quantifying BC in the environment can be broadly classified as optical, thermal, and chemical. While atmospheric BC is typically quantified using optical methods, most methods for measuring BC in soil are based on its resistance to thermal ("dry") or chemical ("wet") oxidation. Since

BC is more resistant to oxidation than more labile, non-BC components, the residue following oxidation is often measured as the BC component.

The methodological dependence of BC quantification in soils has been examined in a number of inter-comparison studies which demonstrate the unreliability of BC data obtained using oxidative methods (Horvath, 1993; Ten Brink *et al*., 2004; Watson *et al*., 2005; Hitzenberger *et al*., 2006; Hammes *et al*., 2007; Bae *et al*., 2007; Reisinger *et al*., 2008). For example, Schmidt *et al*. (2001) reported variation over two orders of magnitude (up to a factor of 571) for BC in eight soil samples using six established methods. This variation reflects both inconsistencies in the definition of BC and a variety of positive and negative methodological biases (Nguyen *et al*., 2004; Hammes *et al*., 2007; Meredith *et al*., 2012). Since chemical and thermal oxidation methods all involve a similar process, i.e. heating samples to high temperatures, they are all susceptible to the same positive biases associated with the incomplete removal of non-BC (Knicker *et al*., 2007) and formation of condensation products (Simpson and Hatcher, 2004). Knicker *et al*. (2007) demonstrated that 12% of organic C derived from plant waxes was resistant to chemical oxidation due to hydrophobicity rather than chemical recalcitrance. To mitigate such biases, pretreatment of the sample or additional characterisation of the extraction residue are possible, however, a risk with these modifications is handling and transfer losses which can cause underestimation of the BC fraction (Elmquist *et al*., 2004).

Hydrogen pyrolysis (hypy) has recently gained prominence as a technique of considerable utility for BC isolation and quantification across a range of environmental matrices including soil (Ascough *et al*., 2009; Meredith *et al*., 2012; McBeath *et al*., 2015). This method uses pyrolysis assisted by high hydrogen pressure (150 bar) with a dispersed sulphided molybdenum (Mo) catalyst to reductively remove labile organic matter and isolate the stable polycyclic aromatic carbon (SPAC) pool (McBeath *et al*., 2015). The principle is that thermally labile macromolecular organic matter is reductively liberated by conversion into dichloromethane-soluble oil, leaving behind a highly condensed aromatic carbonaceous material that is chemically recalcitrant (Meredith *et al*., 2012). This residue has a defined chemical structure consisting of peri-condensed clusters of seven or more aromatic rings (coronene) (Meredith *et al*., 2012; McBeath *et al*., 2015). By varying the hypy operating conditions, lignocellulosic, humic and other labile OC is fully removed by 550°C, with hydrogasification of the SPAC pool not commencing until over 575°C (Meredith *et al*., 2012).

While oxidation methods are selective for hydrophobic compounds which may also be chemically resistant (Knicker *et al*., 2007), the range of conditions required using hypy to reliably and reproducibly isolate only the chemically resistant fraction appears constrained, typically ±2°C (1σ) at 550°C (Ascough *et al*., 2009; Meredith *et al*., 2012). Furthermore, the hydrogen atmosphere in hypy stabilises radicals by hydrogen capping before any recombination reactions that would cause char formation. Therefore, the high hydrogen pressure in hypy suppresses stereochemical rearrangement that can bias BC quantification during oxidation through condensation reactions (Meredith *et al*., 2012). Hypy, therefore, has key advantages over other methods of BC quantification, with the potential to provide a uniform and standardised procedure for isolating BC from a range of materials. However, practical challenges have prevented the widespread commercialisation of hypy which remains an expensive and time-consuming technique. Significant research benefits would be offered by developing a low-cost practical indicator of a sample's BC content.

Predictive functions, or pedotransfer functions (PTFs), have been developed for the purpose of estimating many soil properties that are difficult or expensive to measure. PTFs draw on more readily-obtainable measurements or available existing data (see Chapter 3). Although the focus of most PTF research to date has been on soil hydraulic properties, a wide variety of PTFs have also been developed to estimate other soil physical, mechanical, chemical and biological properties (McBratney *et al*., 2002). PTFs have been derived using various mathematical methods, the most common of which are regression analysis and artificial neural networks (ANNs) (McBratney *et al*., 2002; Merdun *et al*., 2006). While regression analysis uses an *a priori* equation to relate input and output data, ANNs establish a mathematical model in a manner considered analogous to that executed by the human brain. This procedure involves constructing a network of processing units or *neurons* which are arranged in groups or layers. An ANN normally consists of three layers: (i) an input layer; (ii) a hidden layer and; (iii) an output layer. The hidden layers extract information from inputs and are used to predict the outputs. The mathematical model of a neural network consists of a set of simple functions that link neurons in these adjacent layers by adaptable communication paths termed *connectors* that are parameterised with numerical values, or *synaptic weights*, indicating the strength of the connection. One of the advantages of this method compared to regression models is that there is no assumed structure of the model (Gershenfeld, 1999), and many studies have reported that ANNs perform better than regression models in predicting various soil properties (Schaap *et al*., 1998; Koekkoek and Booltink, 1999).

Despite the difficulties involved in measuring soil BC content, employing a statistical model for use as a practical indicator of this soil property has not previously been attempted. This is despite the perturbation to soil carbon-to-nitrogen ratio (C/N) that might be anticipated when stable BC enters soil with a minor amount of associated stable N. Therefore, it is expected that soil BC content could be reliably estimated using such easily obtainable and available soil properties measured using elemental analysis.

In the present study statistical models were developed and validated using soil data from short rotation coppice (SRC) willow plantations as a first step towards generating a simple statistical tool for the estimation of soil BC. Although the sites in this study are under similar management, they are at different stages of transition and represent a range of OC contents. Soil was collected from control and PyC-amended experimental plots representing the soil background BC content and elevated levels of BC following purposeful amendment with pyrogenic carbon (PyC). Using soils of varying OC status and with relatively low and high concentrations of BC, the main aim of this study was to develop and evaluate the predictive capabilities and sensitivity of statistical models to estimate soil BC content using soil C and N data.

5.2 Materials and methods

5.2.1 Dataset

The data used in this study concern soil properties of eight SRC willow plantations in England in transition from arable crops. Site selection, PyC amendment, soil sampling and analytical methods have already been described in detail in Chapter 4.2 and experimental methods are briefly outlined here. A grid of 100 intersections was overlaid on each study site using a scale appropriate to the field size and further divided into three areas of approximately equal size. Prior to PyC amendment, soil cores were taken to a 15 cm depth from 25 randomly selected intersections across each field and combined to determine OC status. The sites ranged from 1.9–6.5% OC. Within each of the three areas, a pair of 2 x 2 m plots was established at a randomly selected intersection. For each pair, PyC derived from *Miscanthus* straw that was pyrolysed at 800°C was applied manually to the surface of one plot at an application rate of 16 t ha^{-1} and incorporated to 15 cm depth using a spading fork. The forking treatment was also applied to the corresponding control plot, located at a 5 m distance from the PyC amended plots. Soil cores were then taken 18–22 months after PyC amendment to a 5 cm depth from the central 1 m^2 of each plot using a 'W formation' and combined by plot. At site 8 only, three additional pairs of plots were established just two weeks before sampling. This was to assess the priming impact of freshly applied PyC which is explored further in Chapter 4.

The composite soil sample for each plot was homogenised separately using the cone and quarter method (Raab *et al*., 1990) and analysed for total carbon (TC) and nitrogen (TN) content by dry combustion using a NA 2500 Elemental Analyser

(Carlo Erba, Milan, Italy). Inorganic C was measured using an automated acidification module and coulometry (CM 5012 and CM 5130, UIC, Joliet, Illinois) and OC content was determined by subtracting the inorganic C from the TC. BC was isolated and quantified using hypy. Samples were loaded with a Mo catalyst and pyrolysed with resistive heating to 550°C under high hydrogen pressure to reductively remove the non-BC organic matter and semi-labile PyC (<7 peri-condensed rings) and isolate the SPAC component (Meredith *et al*., 2012; McBeath *et al*., 2015).

The dataset consists of 26 pairs of plots since one pair from site 2 was inaccessible due to flooding and site 8 consists of 6 pairs of plots. Each plot pair was treated as a unit and the 26 units were divided randomly into two sets: (i) a training dataset with 70% of the data for model development and; (ii) a validation dataset with 30% of the data. Both the training and validation sets have a balanced number of PyC amended and control plots to prevent confounding, and plot pairs were considered as units to prevent an overoptimistic model fit. The same subsets were used in the derivation and validation of models developed using both regression and ANN methods to enable a reliable comparison between the two model approaches. Descriptive statistics for each subset of data are listed in Table 5.1.

5.2.2 Model development

5.2.2.1 Regression models

Regression models were developed for predicting SPAC using the training dataset with 70% of the data and validated with the remaining 30% of the data. Simple linear regression (SLR) models were developed for each of the following predictor

Table 5.1 Descriptive statistics of measured parameters for training (T) and validation (V) datasets.

	SPAC (%)		TC (%)		OC (%)		TN (%)		C/N	
	T	V	T	V	T	V	T	V	T	V
No. of plot pairs	18	8	18	8	18	8	18	8	18	8
Minimum	0.07	0.11	2.15	2.30	2.15	2.27	0.12	0.19	9.91	10.54
Maximum	8.88	4.22	15.15	11.41	14.29	8.74	0.82	0.70	26.79	24.21
Range	8.81	4.11	13.00	9.11	12.14	6.47	0.70	0.51	16.88	13.67
First quartile	0.29	0.29	3.00	3.32	3.00	3.32	0.20	0.24	13.03	12.73
Median	1.81	1.88	5.10	5.61	5.10	5.61	0.28	0.27	17.22	15.49
Third quartile	3.49	3.42	8.89	7.70	7.78	6.56	0.71	0.52	19.18	20.41
Mean	2.44	1.99	6.36	5.62	6.17	5.28	0.40	0.35	16.58	16.42
Standard deviation	2.56	1.52	3.84	2.63	3.61	2.11	0.25	0.18	4.37	4.45

variables: TC, OC, TN and C/N. Curve fitting was also carried out to assess various nonlinear relationships between the response and each of the predictor variables. The following non-linear regression (NLR) models were considered and fitted to the data: quadratic, cubic, exponential, power, natural logarithm and square root. Stepwise multiple linear regression (MLR) was also carried out using a forward selection procedure with variables with a p-value <5% selected for inclusion. Regression model equations are displayed in Table 5.2. All regression analysis, curve fitting and checking of residuals for normal distribution using the Shapiro Wilk test were carried out using SPSS V.19 (IBM, Armonk, New York, USA).

Table 5.2 Regression models developed in this study.

Model type	Function	Model no.	Equation
SLR	Linear	1a	$-1.17 + (0.57 \times TC)$
		1b	$-1.35 + (0.62 \times OC)$
		1c	$0.33 + (5.26 \times TN)$
		1d	$-2.44 + (0.29 \times (C/N))$
NLR	Quadratic	2a	$0.65 – (0.04 \times TC) + (0.04 \times TC^2)$
		2b	$0.50 – (0.02 \times OC) + (0.04 \times OC^2)$
		2c	$-2.13 + (19.50 \times TN) – (14.63 \times TN^2)$
		2d	$-8.74 + (1.07 \times (C/N)) – (0.02 \times (C/N)^2)$
	Cubic	2e	$-3.85 + (2.36 \times TC) – (0.31 \times TC^2) + (0.01 \times TC^3)$
		2f	$-3.53 + (2.21 \times OC) – (0.29 \times OC^2) + (0.01 \times OC^3)$
		2g	$-3.20 + (28.90 \times TN) – (38.17 \times TN^2) + (17.04 \times TN^3)$
		2h	$5.45 – (1.55 \times (C/N)) + (0.13 \times (C/N)^2) – ((2.81 \times 10^{-3}) \times (C/N)^3)$
	Exponential	2i	$0.58 \times e^{0.18 \times TC}$
		2j	$0.55 \times e^{0.20 \times OC}$
		2k	$1.08 \times e^{1.80 \times TN}$
		2l	$e^{-0.51 + (0.08 \times (C/N))}$
	Power	2m	$0.09 \times (TC^{1.66})$
		2n	$0.09 \times (OC^{1.72})$
		2o	$5.37 \times (TN^{0.82})$
		2p	$-136.84 + (124.37 \times ((C/N)^{0.01}))$
	Natural logarithm	2q	$3.21 + (3.37 \times (Ln(TC)))$
		2r	$3.41 + (3.53 \times (Ln(OC)))$
		2s	$4.93 + (2.27 \times (Ln(TN)))$
		2t	$-11.41 + (4.99 \times (Ln(C/N)))$
	Square root	2u	$-4.54 + (2.89 \times (\sqrt{(TC)}))$
		2v	$-4.90 + (3.07 \times (\sqrt{(OC)}))$
		2w	$-1.86 + (7.11 \times (\sqrt{(TN)}))$
		2x	$-7.48 + (2.46 \times (\sqrt{(C/N)}))$
Stepwise MLR	Linear	3a	$-1.08 + (1.14 \times OC) – (8.72 \times TN)$

5.2.2.2 Artificial neural network models

The type of ANN used in this study was the multilayer perceptron (MLP) ANN which is the most commonly used neural network structure in ecological and soil science (Agyare *et al.*, 2007; Arshad *et al.*, 2013). The MLP ANN uses a feed-forward algorithm where the weighted connections feed activations only in the forward direction from an input layer to the output layer. In this neural network, an input vector of neurons x_l (l = 1,…, N_i) is transmitted through a connection that is multiplied by a weight w_{jl} to give the hidden unit zj (j = 1,…, N_h) as follows:

$$z_j = \sum_{l=1}^{N_i} w_{jl} x_l + w_{j0} \quad (5.1)$$

where N_h is the number of hidden units and N_i is the number of input units. Hidden units consist of the weighted input and a bias w_{j0} which is a constant input of 1 added to the weight. These inputs are passed through an activation function f, commonly a sigmoid or hyperbolic tangent to accommodate the nonlinearity of input-output relationships, to produce:

$$r_j = f\left(\sum_{l=1}^{N_i} w_{jl} x_l + w_{j0}\right) \quad (5.2)$$

These outputs from hidden units then pass through another layer of filters:

$$v_k = \sum_{j=1}^{N_h} u_{kj} r_j + u_{k0} \quad (5.3)$$

and feed into another activation function F to produce output y (k = 1,…,N_0):

$$y_k = F(v_k) \quad (5.4)$$

Three sets of input units were considered here: (i) all of the predictors: TC, OC, TN and C/N; (ii) TC and TN and; (iii) OC and TN. The best ANN architecture for each set of input units was selected by finding the optimal number of hidden neurons through training of various architectures using a trial and error method. Using both sigmoid and hyperbolic activation functions, one and two hidden layers were tested with hidden neurons in each layer varying from one to six. Data was normalised between 0 and 1 for the sigmoid activation function and between -1 and 1 for the hyperbolic tangent activation function. Once the best ANN architecture was trained, it was then validated using the same subset of data as for the regression models. ANN models were developed using SPSS V.19. Architecture and parameter estimates for ANNs are displayed in Figure 5.1 and Table 5.3 respectively.

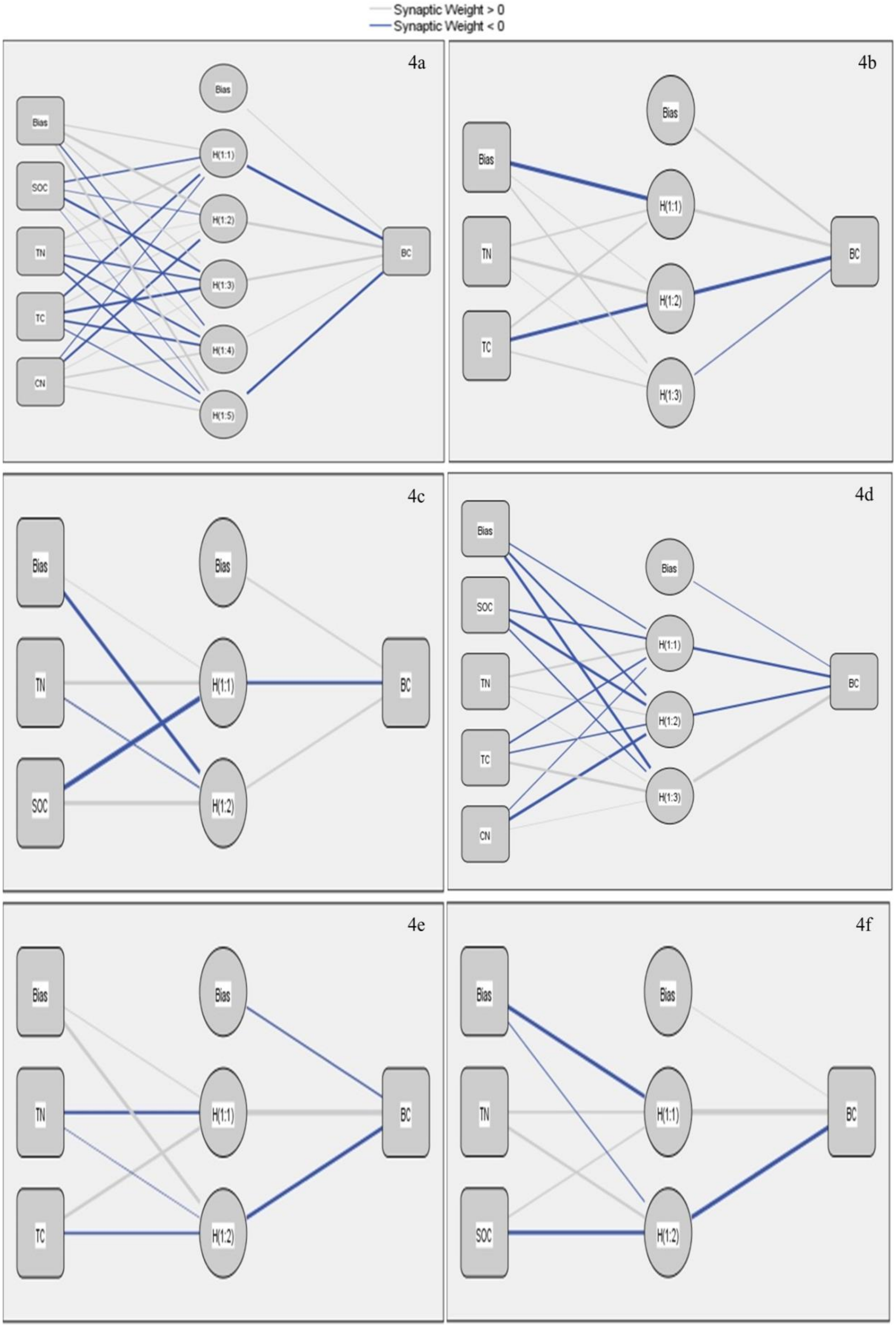

Figure 5.1 Architecture of the ANN models developed in this study.

Table 5.3 Parameter estimates for ANN models developed in this study.

Model	Predictor		Predicted					
			Hidden layer					Output layer
			H(1:1)	H(1:2)	H(1:3)	H(1:4)	H(1:5)	SPAC
4a	Input layer	(Bias)	0.329	1.140	0.327	-0.340	0.868	
		OC	-0.394	-0.077	-0.698	0.072	-0.056	
		TN	0.433	0.001	-0.423	-0.542	-0.429	
		TC	-0.672	0.290	-0.890	-0.553	-0.308	
		C/N	-0.291	-0.732	0.130	0.401	0.345	
	Hidden layer	(Bias)						0.207
		H(1:1)						-2.167
		H(1:2)						0.970
		H(1:3)						0.754
		H(1:4)						0.195
		H(1:5)						-0.990
4b	Input layer	(Bias)	-1.855	0.064	0.608			
		TN	0.462	0.873	0.063			
		TC	0.754	-1.268	0.173			
	Hidden layer	(Bias)						0.859
		H(1:1)						0.880
		H(1:2)						-1.395
		H(1:3)						-0.116
4c	Input layer	(Bias)	0.068	-1.502				
		TN	1.016	-0.266				
		OC	-1.538	1.303				
	Hidden layer	(Bias)						0.710
		H(1:1)						-0.986

Model	Predictor		Predicted					
			Hidden layer					Output layer
			H(1:1)	H(1:2)	H(1:3)	H(1:4)	H(1:5)	SPAC
		H(1:2)						0.874
4d	Input layer	(Bias)	-1.281	-2.035	-3.202			
		OC	-1.635	-2.512	-1.420			
		TN	1.668	0.990	0.703			
		TC	-1.438	-1.393	2.479			
		C/N	-1.017	-3.183	0.535			
	Hidden layer	(Bias)						-0.732
		H(1:1)						-2.184
		H(1:2)						-1.749
		H(1:3)						5.690
4e	Input layer	(Bias)	1.567	5.765				
		TN	-2.832	-1.125				
		TC	4.115	-2.068				
	Hidden layer	(Bias)						-1.603
		H(1:1)						6.427
		H(1:2)						-4.882
4f	Input layer	(Bias)	-4.330	-0.691				
		TN	1.777	3.033				
		OC	0.752	-3.683				
	Hidden layer	(Bias)						0.094
		H(1:1)						5.359
		H(1:2)						-5.270

5.2.3. Model evaluation criteria

The Corrected Akaike information criterion (AICc) [Equation (5.5)] was used for model selection.

$$AICc = \left(n\ln\left(\frac{SSE}{n}\right) + 2(k)\right) + \left(\frac{2k\ (k+1)}{n-k-1}\right) \quad (5.5)$$

where n is the total number of observations, *SSE* is the sum of squared errors of prediction and for regression models k is the number of parameters plus 1 and for ANNs k is the number of weights used:

$$k = (N_i + 1)N_h + (N_h + 1)N_0 \quad (5.6)$$

where 1 is due to bias.

Model performance was evaluated using a set of statistical criteria. Overall goodness of fit was assessed using the model efficiency index (EF) (Green and Stephenson, 1986; Loague and Green, 1991) [Equation (5.7)]. Prediction accuracy was assessed using root mean square prediction error (RMSPE) [Equation (5.8)], which is a measure of overall prediction error, and mean prediction error (MPE) [Equation (5.9)], which indicates systematic under- or over-estimation (De Vos *et al*., 2005; Jalabert *et al*., 2010).

$$EF = \frac{\left(\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2 - \sum_{i=1}^{n}\left[\left(P_i - O_i\right)^2\right]\right)}{\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2} \quad (5.7)$$

$$RMSPE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(P_i - O_i\right)^2} \quad (5.8)$$

$$MPE = \frac{1}{n}\sum_{i=1}^{n}\left(P_i - O_i\right) \quad (5.9)$$

where n is the total number of observations, P_i are the predicted values, O_i the observed values and $\bar{O}$ the mean of the observed data.

5.3 Results and discussion

The stepwise MLR equation with the predictor variables OC and TN provided the lowest AICc score for the validation dataset and was selected as the most likely *true* model (Table 5.4). This equation provided the best-fit to the validation dataset with an EF of 0.92. This has also been illustrated in the plot of the predicted vs the observed values for the most likely *true* model for each model type (SLR, NLR, stepwise MLR and ANN) in which the scatter is noticeably lower for the MLR model (Figure 5.2). As well as providing the best model fit, the stepwise MLR model also had the highest prediction accuracy for the validation dataset with an MPE of -0.01 and a RMSPE of 0.43.

Although various ANN models performed better for the training dataset, with higher EFs and lower AICc scores than the stepwise regression model, none of these models performed as well for the validation dataset according to any of the evaluation criteria (Table 5.4). This demonstrates that the added flexibility and complexity of the ANN models did not improve model performance compared to the MLR approach and instead resulted in over-fitting. Although ANN models have previously been reported to provide a better model fit for the prediction of complex relationships that could not easily be described by regression functions, such as soil hydraulic properties (Schaap *et al*., 1998; Koekkoek and Booltink, 1999), the relationship between soil OC, TN and BC content is more generalised. This study demonstrates that soil BC content can be estimated with a high degree of accuracy using a simple

Table 5.4 Evaluation of model performance for training (T) and validation (V). The best predictive model for each model type (SLR, NLR, stepwise MLR and ANN) has been highlighted.

Model no.	Model name	EF		MPE		RMSPE		AICc	
		T	V	T	V	T	V	T	V
1a	SLR (TC)	0.73	0.34	0.00	0.18	1.32	1.20	21.4	7.6
1b	SLR (OC)	0.75	0.59	0.00	0.05	1.26	0.95	18.2	-0.01
1c	SLR (TN)	0.27	0.00	0.00	0.17	2.16	1.47	56.8	14.1
1d	SLR (C/N)	0.25	0.61	0.00	0.00	2.18	0.92	57.6	-0.8
2a	Quadratic (TC)	0.77	0.25	0.00	0.22	1.21	1.28	16.2	11.3
2b	Quadratic (OC)	0.79	0.52	0.00	0.02	1.15	1.03	12.9	4.3
2c	Quadratic (TN)	0.30	0.15	0.00	0.12	2.12	1.36	56.7	13.3
2d	Quadratic (C/N)	0.29	0.63	0.00	-0.05	2.13	0.89	57.0	-0.15
2e	Cubic (TC)	0.83	0.58	0.00	0.08	1.05	0.96	7.8	4.1
2f	Cubic (OC)	0.83	0.62	0.00	-0.04	1.04	0.90	7.3	2.3
2g	Cubic (TN)	0.30	0.18	0.00	-0.03	2.12	1.34	58.1	14.8
2h	Cubic (C/N)	0.30	0.60	0.00	-0.02	2.11	0.93	57.9	3.3
2i	Exponential (TC)	0.78	0.29	-0.01	0.19	1.17	1.24	12.8	8.8
2j	Exponential (OC)	0.80	0.50	-0.02	-0.03	1.13	1.04	10.2	3.2
2k	Exponential (TN)	0.25	0.04	-0.02	0.14	2.19	1.44	57.8	13.5
2l	Exponential (C/N)	0.19	0.48	-0.06	-0.20	2.27	1.06	60.4	3.6
2m	Power (TC)	0.76	0.26	0.09	0.34	1.24	1.27	16.6	9.4
2n	Power (OC)	0.78	0.52	0.07	0.13	1.17	1.02	12.8	2.3
2o	Power (TN)	0.27	-0.06	-0.03	-0.09	2.16	1.52	56.6	15.1
2p	Power (C/N)	-17.1	0.60	10.27	0.03	10.74	0.93	172.3	-0.6
2q	Natural logarithm (TC)	0.62	0.44	0.00	0.12	1.56	11.12	33.5	8.6
2r	Natural logarithm (OC)	0.64	0.61	0.00	0.07	1.52	0.92	31.6	3.0
2s	Natural logarithm (TN)	0.29	-0.07	0.00	-0.21	2.13	1.53	55.7	19.0

Model no.	Model name	EF		MPE		RMSPE		AICc	
		T	V	T	V	T	V	T	V
2t	Natural logarithm (C/N)	0.27	0.63	0.00	0.01	2.15	0.90	56.5	2.0
2u	Square root (TC)	0.68	0.37	0.00	0.11	1.43	1.17	27.2	10.4
2v	Square root (OC)	0.70	0.60	0.00	0.04	1.38	0.93	24.5	3.2
2w	Square root (TN)	0.28	-0.02	0.00	0.11	2.14	1.49	56.2	18.2
2x	Square root (C/N)	0.26	0.62	0.00	0.01	2.17	0.91	57.0	2.5
3a	Stepwise MLR (OC & TN)	0.94	0.92	0.00	-0.01	0.60	0.43	-34.3	-23.8
4a	ANN with hyperbolic function (OC, TN, TC & C/N)	0.99	0.90	0.00	0.03	0.29	0.48	-78.03	-8.8
4b	ANN with hyperbolic function (TC & TN)	0.97	0.79	-0.04	-0.06	0.40	0.67	-57.9	-3.1
4c	ANN with hyperbolic function (OC & TN)	0.97	0.86	0.00	0.23	0.46	0.55	-48.4	-9.1
4d	ANN with sigmoidal function (OC, TN, TC & C/N)	0.98	0.59	0.01	-0.08	0.34	0.94	-67.7	13.1
4e	ANN with sigmoidal function (TC & TN)	0.99	0.82	0.01	-0.07	0.29	0.63	-81.5	-4.9
4f	ANN with sigmoidal function (OC & TN)	0.98	0.63	0.00	0.36	0.35	0.90	-68.5	6.3

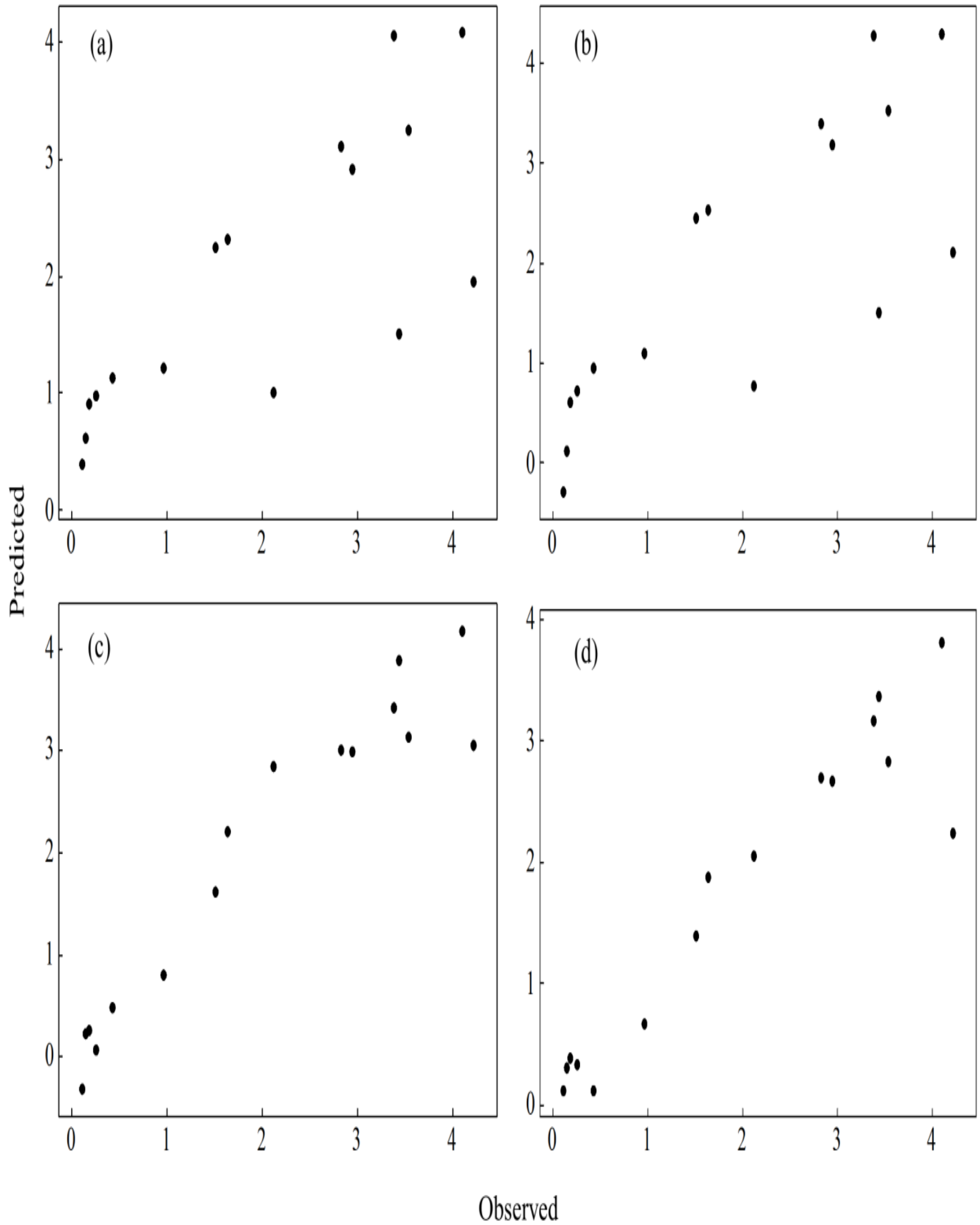

Figure 5.2 Scatterplots of predicted vs observed values for the validation dataset for the most likely *true* model for each model type (SLR, NLR, stepwise MLR and ANN): (a) model 1d SLR (C/N); (b) model 2p power (C/N); (c) model 3a stepwise MLR (OC & TN) and; (d) model 4a ANN with hyperbolic function (OC, TN, TC & C/N).

MLR equation with only two predictor variables, OC and TN. As expected, since PyC amendment increases the OC but not the TN of the soil, OC has a positive and TN a negative model coefficient. Although OC and TN were collinear for the unamended soils, this was not the case for the entire dataset (VIF <5) and these model coefficients can be reliably interpreted.

Although C/N performed better than any of the other predictor variables individually for most of the linear and nonlinear functions, none of these equations performed as well as the stepwise MLR equation with OC and TN. For the dataset used in this study, BC content can be reliably and accurately predicted irrespective of the OC status of the soil. It should be noted, however, that organic or inorganic fertilisers had not been applied to any of the study sites in the five years prior to PyC amendment. This model may not provide as reliable an indicator of BC content in organic soils or in circumstances where other organic or synthetic amendments are present to impact soil OC or TN content. In these cases, additional input parameters may be required for model calibration. However, for mineral soils without fertiliser application, soil C and N data can provide a useful practical indicator for soil BC content.

The MLR model was then used to predict soil BC content of the 0-15 cm samples taken from across each site (n = 25) prior to any PyC amendment and these BC values were compared with those that were measured for the 0-5 cm control soil samples to investigate potential cross contamination of PyC between the amended and control plots. Figure 5.3 demonstrates a reasonably close similarity between the predicted site and measured control plot values. However, negative BC values were predicted for five of the sites and in all cases the predicted values were lower than the measured values. The prediction of negative values might have been expected where the BC content may be close to zero due to the negative bias associated with the model. Furthermore, since it is reasonable to assume BC content may decrease with soil depth, negative estimates may arise from using the model to extrapolate to values of the covariates that lie outside the values used to train the model. Despite the good model fit and high prediction accuracy in model training and validation for the 0-5 cm soil data, the prediction of negative values for the 0-15 cm soil data remains potentially problematic for the derivation of BC stocks from C and N data derived from other soil depths.

Although the control and amended plots were separated by a 5 m distance, dispersion of PyC and cross contamination may have resulted from high winds and flooding which are known to have affected several sites in the time period between PyC amendment and soil sampling. Sites 3 and 8 had the highest BC contents of the control soil samples with 2.1–3.1% and 1.3–1.9% respectively, although none of the control BC values were identified as outliers according to Grubb's test. Although these values are high relative to the other sites it is unlikely to result from contamination with PyC since the model predicts relatively high BC values for the 0-15 cm soil samples that were collected prior to PyC amendment (Figure 5.3).

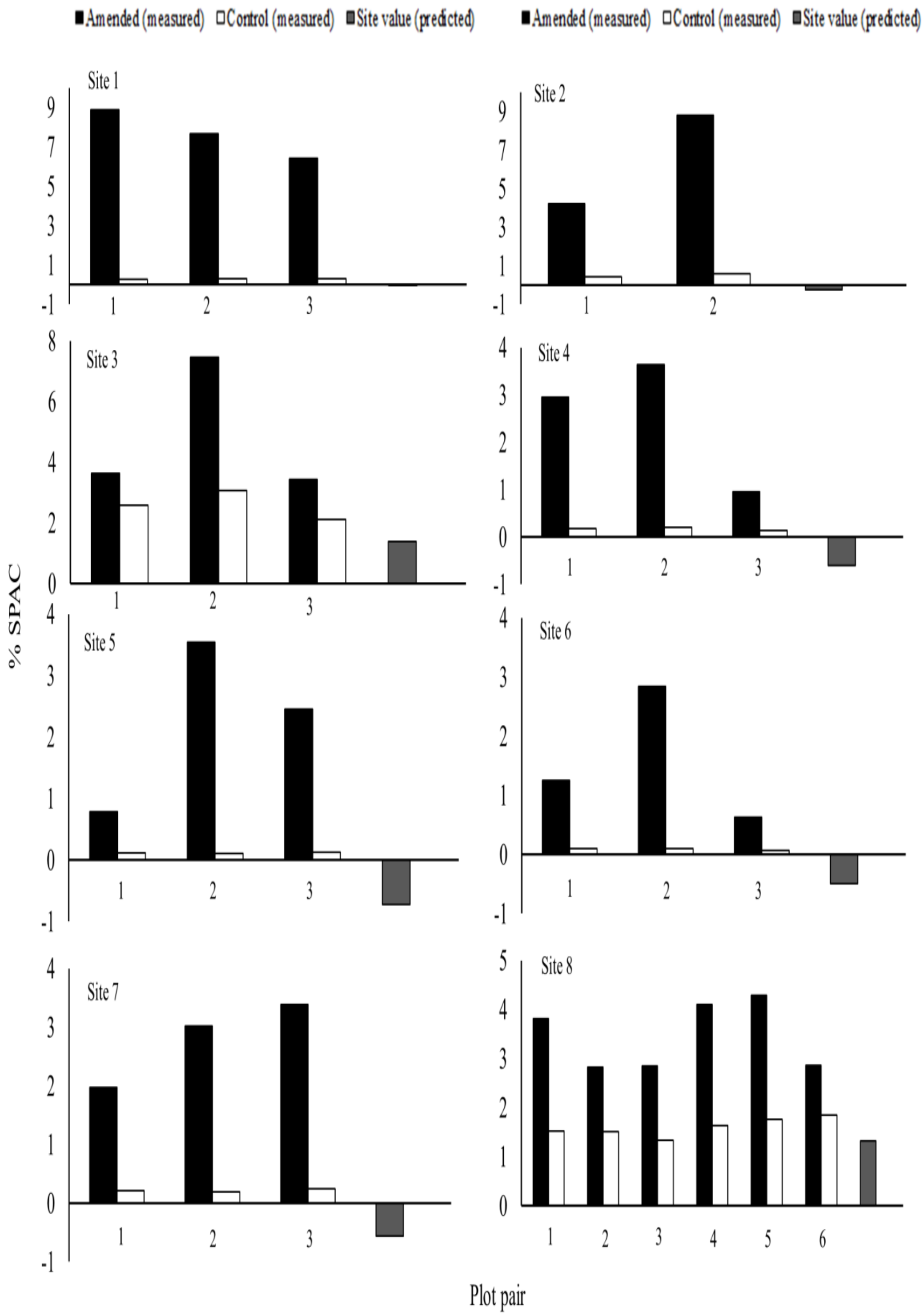

Figure 5.3 Comparison by site of the measured SPAC (%) for both the amended and control plots and those predicted for the 0-15 cm soil sample taken from across each site. Note different y-axis scales.

The presence of PyC in the control soils might have been expected to increase the soil C/N but neither site 3 or 8 had unusually high C/N and for site 3 the control C/N is even lower than for the 0–15 cm soil sample (Figure 5.4). It should be noted, however, that for two of the plot pairs from site 3 the C/N for the amended plots were lower than the 0–15 cm sample (Figure 5.4) and the OC content of the amended plots for the same plot pairs are not much greater than the control soil samples (Figure 5.5). This may indicate PyC dispersion from the amended plots but it is unlikely that cross contamination of control plots has occurred and the relatively high BC content for the control soil samples at these sites may relate to a nearby industrial source.

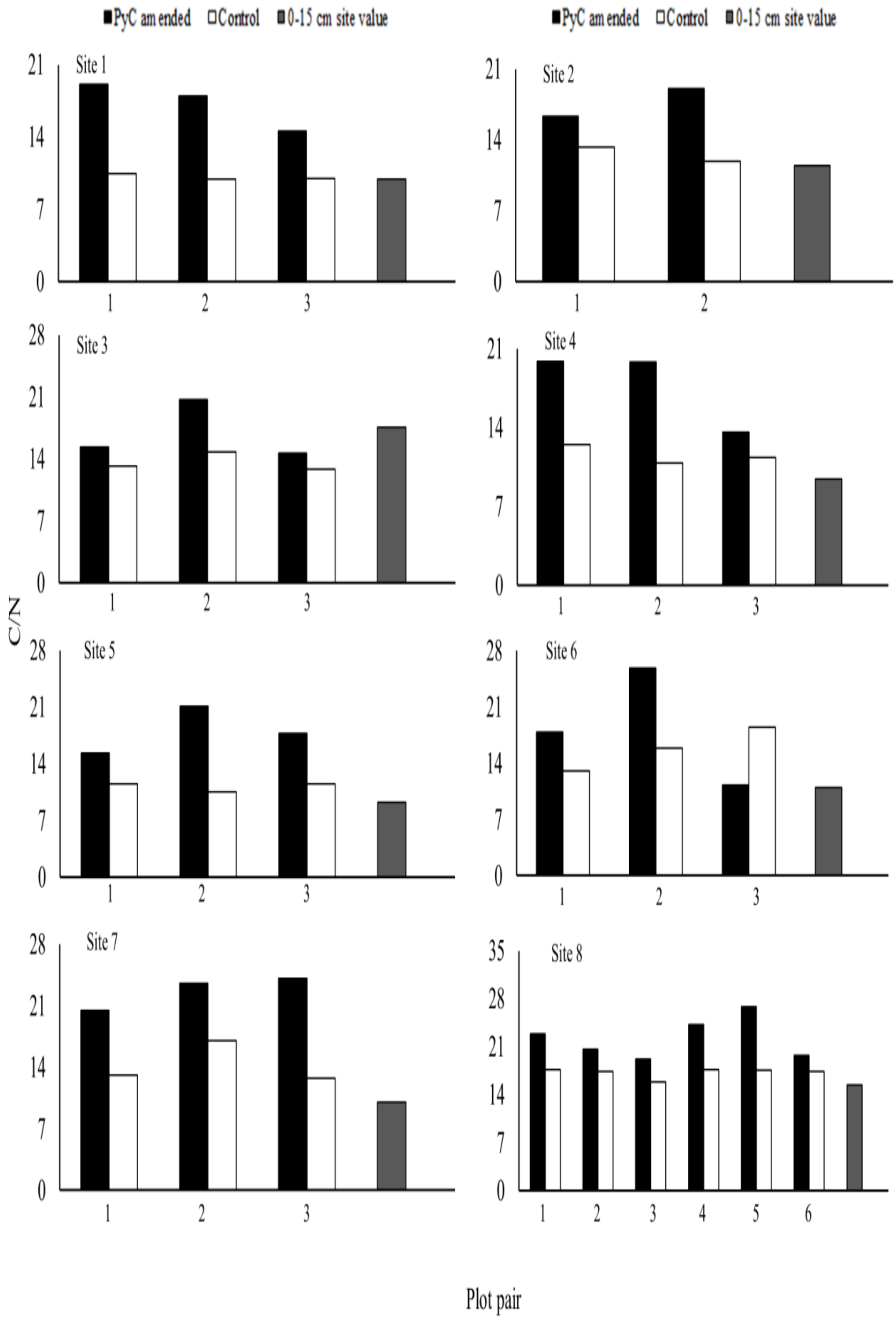

Figure 5.4 Comparison by site of the measured C/N for the amended and control plots and for the 0-15 cm soil sample taken from across each site. Note different y-axis scales.

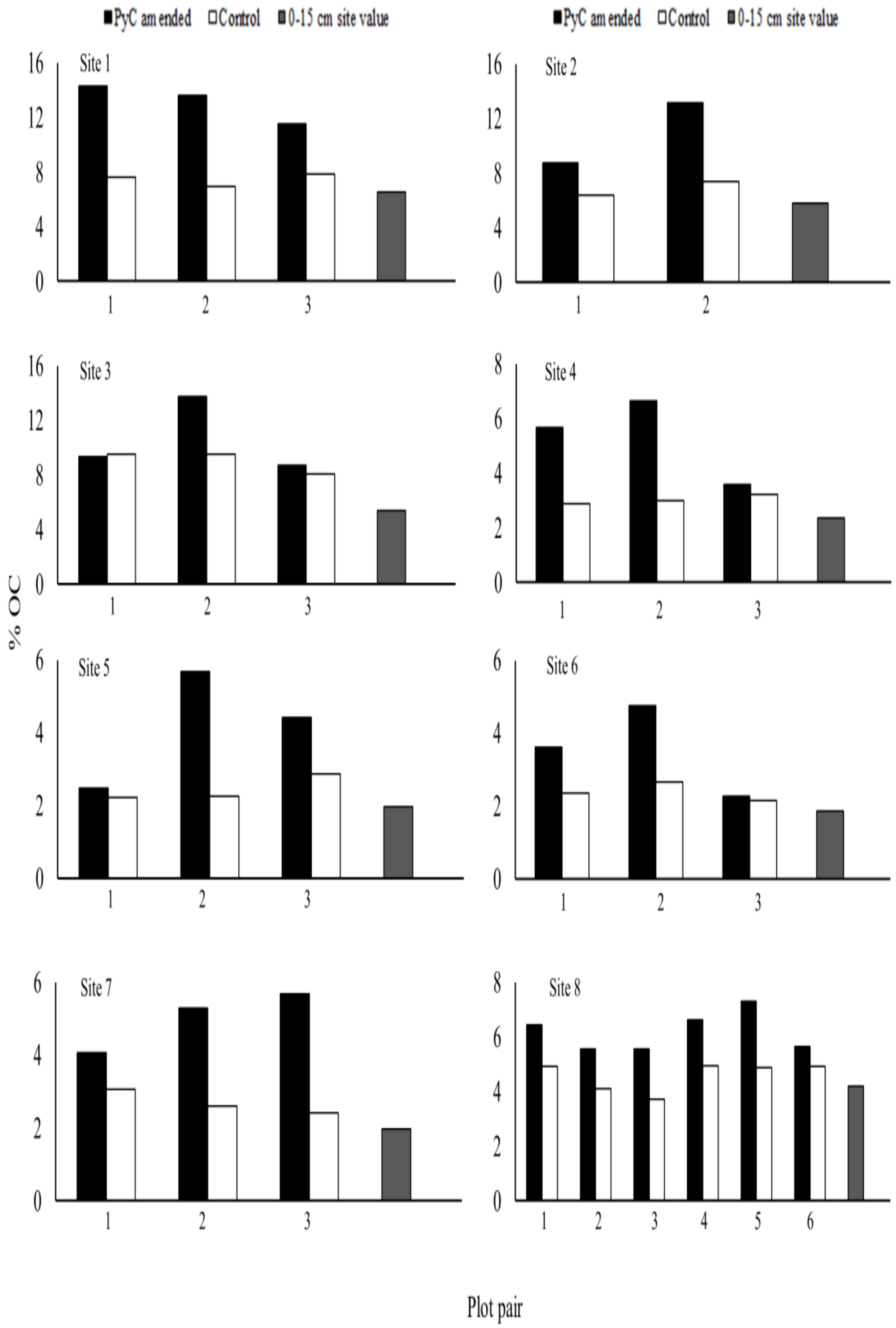

Figure 5.5 Comparison by site of the measured OC (%) for the amended and control plots and for the 0-15 cm soil sample taken from across each site. Note different y-axis scales.

5.4 Conclusion

Statistical models were developed and evaluated for the purpose of estimating soil BC content using more easily obtainable and available data. This study demonstrates that soil BC content can be reliably and accurately estimated using a simple MLR equation with only two predictor variables, OC and TN. Neither NLR nor ANN model approaches improved model performance, indicating that a linear model was most appropriate for estimating soil BC from C and N data. Predictive accuracy was high despite the range in OC status between sites. Despite the good fit, the negative bias associated with the model is potentially problematic for deriving BC stocks. This simple statistical model provides a first step towards developing a low-cost practical indicator of soil BC which, following further calibration on more soil data, could assist in rapidly developing a more accurate assessment of global soil BC stocks. Further model development is required to enable application to different soil depths.

Chapter 6 General discussion

This chapter synthesises the main findings of Chapters 2–5 to address the overall aim of the thesis. First, an assessment is made of the general short term effects of LUC from conventional agriculture to lignocellulosic biomass crop production on SOC stocks in Britain. This is then followed by a discussion of how this LUC could be better managed in future using two possible approaches: (i) the potential to devise a more targeted LUC strategy is considered by taking into account the effects of LUC under a range of conditions, and; (ii) the potential for PyC to reduce LUC-induced losses of SOC by negative priming is also assessed. Lastly, the implications of these results for policy makers and recommendations for further research are discussed.

6.1 The impact of commercial deployment of lignocellulosic biomass crops in Britain

The principal aim of this study was to assess the overall impact of commercial deployment of lignocellulosic biomass crops on SOC stocks in Britain. To do this soil was sampled from a chronosequence of 93 commercial biomass crop plantations in England and Wales to develop empirical models of SOC trajectory following LUC (Chapter 2). SOC stocks were calculated for each site using a fixed sampling depth of 30 cm and changes were estimated for each LUC by comparing against typical pre-conversion SOC stocks obtained using the National Soils Inventory (NSI). Since soil bulk density (BD) is used to convert SOC from a mass to an area basis, it was first necessary to develop a pedotransfer function (PTF) to derive estimates of 15–30 cm BD for each of the 93 biomass cropland soils (Chapter 3). Although a wide range of PTFs have been developed using soil data from various land-uses, these have not been evaluated in the context of biomass crops, which is a relatively novel land-use in Britain. To ensure an accurate assessment of the impact of LUC to biomass crops on SOC stocks, the performance of PTFs developed using biomass crop specific soil data were compared with a range of published PTFs and the best-fitting model was used to calculate SOC stocks.

For estimation of soil BD, a simple linear equation with SOC as the only independent variable performed equally as well or better than a range of models developed using multiple linear regression and curve fitting. Since this model was the simplest of the best-fitting developed and published PTFs, it was used to calculate SOC stocks. However, all of the developed and published regression models performed poorly during validation, with the highest model efficiency (EF) of 0.15. It is possible that non-parametric methods could have provided greater predictive accuracy. However, these methods have not been widely tested for estimating soil BD and one study reported no improvement in prediction accuracy using a selection of these methods compared to models developed using regression analysis (Tranter *et al*., 2007). Since the soil data used in the present study were obtained from sites in transition, this may have impacted the performance of PTFs. Although a range of other variables relating to specific site characteristics and geology have previously been reported to relate to soil BD and may have improved the model fit (Harrison and Bocock, 1981; Leonaviciute, 2000; Calhoun *et al*., 2001; Jalabert *et al*., 2010), the collection of such data is time and resource-consuming. This study demonstrates one of the main limitations of using regression analysis for estimating soil BD and, therefore, for the most accurate assessment of SOC stocks, directly measuring soil BD is recommended.

The results presented here indicate that only LUC from arable crops to SRC willow demonstrated an overall increase in SOC stocks, by an estimated 15.3 ± 2.2 t C ha^{-1} (± 95% confidence intervals) after 14 years and 68.8 ± 49.4 t C ha^{-1} after 22 years. LUC from arable crops to *Miscanthus* and LUC from grassland to both SRC willow and *Miscanthus* demonstrated no overall net effect on SOC stocks. However, this does not suggest that, of the four LUCs investigated here, only LUC from arable crops to SRC willow has any potential to increase SOC stocks. Nor is it suggested that the largest accumulation of SOC is likely to occur following this LUC. Rather these results indicate that only the commercial planting of SRC willow on arable land appears to have caused an overall net increase in SOC stocks. While the other LUCs had no demonstrable net effect on SOC stocks there is evidence to suggest that both increases and decreases in SOC have occurred under contrasting conditions.

These results partially corroborate those of paired plot studies but with some apparent discrepancies. As expected, in most cases an initial loss of SOC is indicative of the first few years following LUC. These results also support the assertion that LUC from low C input arable soils to lignocellulosic biomass crops has the potential to increase SOC stocks (Smith *et al*., 2000). However, a net increase in SOC stocks was only observed following the establishment of SRC willow on arable land and not *Miscanthus*. Paired plot studies typically infer an increase in SOC following LUC from arable crops to *Miscanthus* (Hansen *et al*., 2004; Dondini *et al*., 2009). Therefore, an overall increase might have been expected here, due to an anticipated reduction in soil disturbance and increased C inputs to the soil from both above- and below-ground (Kuzyakov and Domanski, 2000; Christian *et al*., 2006; Dondini *et al*., 2009). Reasons why the expected SOC increase was not detected in this research include poor *Miscanthus* crop growth, which was observed at many sites but not quantified. It has previously been suggested that poor crop performance may relate to inexperience and inefficient management of a newly emerging crop (Lewandowski *et al*., 2000). It is also possible that the performance of *Miscanthus* in trials using experimental sites does not adequately reflect that of commercial planting which, due to economic factors, may be more likely to occur on lower grade land. Further research is required to confirm these effects.

Based on the results of previous studies, an overall loss of SOC might have been expected following LUC from grassland, at least to SRC willow (Jug *et al*., 1999). Although all of the available biomass crops established on permanent grassland were sampled and even supplemented with others established on set-aside grassland, there remains a large uncertainty around these estimates. This uncertainty is not related to the inclusion of set-aside sites since less of the variance was explained by the model when these sites were excluded. While SOC stocks measured for most of the SRC willow and *Miscanthus* plantations established on grassland fall below the NSI average (Figure 2.2), the true population mean lies within a broad range of values and, therefore, does not provide clear evidence for an overall net change in SOC stocks. A larger number of study sites would have been required to more accurately assess the impact of commercial planting of lignocellulosic biomass crops on grassland. The objective here was to determine the general effect of LUC by sampling a large number of sites, but this was not achievable for LUC from grassland due to the limited number of appropriate sites available. Due to inherent variability associated with the sensitivity of SOC trajectory to a range of factors, the empirical models require large retrospective datasets to reduce this uncertainty and provide useful estimates of SOC stock changes. It has been suggested that combining datasets from different studies has the potential to form an 'improved reporting scheme' (Poeplau *et al*., 2011). However, additional biomass crops that have been established on grassland may be limited and such studies also require considerable time and resources to complete. In the absence of large retrospective datasets, process-based models could be used to estimate the potential effects of LUC and the data presented here may be able to assist in the validation of these models.

Another limitation with using a space-for-time substitution methodology is that estimated SOC stock changes may not be as reliable as using repeated inventories. In this study, estimates of SOC stock changes were inferred for each LUC by comparing against typical pre-change SOC stocks obtained using the NSI database for which standard errors were not available. Therefore, while these models provide a useful indication of the general trajectory of SOC stocks following the commercial deployment of lignocellulosic biomass crops in Britain, estimates of SOC changes should be used cautiously. Furthermore, the estimates provided by the CRF_{gen} models are for an overall net effect which is a statistical abstraction rather than an estimate of the likely incurred changes in SOC under any particular set of circumstances. This provides a useful indication of what the general impact of commercial deployment in Britain is likely to have been and what the future short term net effect on SOC stocks could be if biomass crop planting were to continue on similar types of land. Due to economic factors, in recent decades commercial planting of biomass crops is more likely to have occurred on lower grade land. Therefore, this study may better reflect the impact of targeting lower rather than higher grade agricultural land, which has been suggested as a more sustainable option to limit the impact of biomass crops on food supply (Hastings *et al*., 2009; Hillier *et al*., 2009; Tilman *et al*., 2009).

6.2 Implications for an improved future management of land-use change for lignocellulosic biomass crop production

6.2.1 Assessing the potential for a targeted land-use change strategy

The poor fit of the CRF_{gen} models indicates that 'time since conversion' explains only a small amount of variance in SOC stocks, which is largely due to the additional effects of other superimposed explanatory variables on SOC trajectory. To investigate the influence of soil texture and climate as controlling factors on SOC changes following biomass crop establishment, multivariable CRF_{spec} models were created. Here the potential for these models to assist in devising a more targeted LUC strategy by providing more region-specific estimates of SOC changes following LUC is discussed. In most cases the addition of explanatory variables improved the model fit suggesting that SOC trajectory following LUC is sensitive to these factors. However, in only a few cases did the model fit improve sufficiently to provide a basis for suggesting improved future management of LUC for lignocellulosic biomass crop production with respect to increasing SOC stocks.

These results suggest that, following LUC from arable crops to SRC willow, SOC accumulation may be greater for less clayey soils and/or in warmer regions. EF was improved from 0.08 for the CRF_{gen} to 0.51 for the CRF_{spec}, comparable to reported model fits for other LUCs in studies with larger datasets and measuring SOC stocks up to around 100 years after LUC (Poeplau *et al*., 2011). However, this model fit includes the effect of 'sampling season', which had the most significant effect on

SOC, with higher SOC stocks measured in soils that were sampled later in the year. This suggests that soils from sites sampled later in the year may appear artificially high in SOC, possibly due to the accumulation of fine roots, not all of which may have been removed prior to soil analysis. Nonetheless, clay content and mean annual temperature improved the model fit and simultaneously reduced the overall prediction error. Although these results indicate greater SOC accumulation in less clayey soils it is possible that more clayey soils may be slower to respond to changes and may have a greater C storage capacity in the longer term. For the LUC arable crops to SRC willow, the variance explained by the CRF_{spec} suggests that there may be some basis for an improved future management of LUC for SOC stocks by targeting certain land areas.

In contrast, the results for the LUC of planting SRC willow on grassland indicate that rather than targeting less clayey soils and those in warmer regions for LUC, these sites may be better avoided. The results suggest that, for this LUC, SOC losses may be accentuated or are more likely to occur in sandier and warmer soils. However, more data would be required to confirm this trend as the model fit is only slightly improved by the addition of explanatory variables with an EF of 0.17. While SOC losses appear greater for certain soil types than others, unlike for LUC from arable crops to SRC willow, none of the explanatory variables had a positive effect on SOC trajectory. Although the high variance suggests that increases in SOC stocks may occur under certain conditions, these cannot be elucidated by the measured variables. Considering the lack of predictability and the potential for SOC accumulation from planting on arable land, it may not be prudent to recommend SRC willow establishment on grassland. However, in certain circumstances avoiding sandier and warmer soils may be considered as a form of 'damage limitation'.

For LUC from arable crops to *Miscanthus* none of the explanatory variables improved the model fit. Again the high variance suggests there may be a potential for an increase in SOC stocks under certain conditions, but these cannot be elucidated using the measured variables. It is possible that inefficient management practices and/or poor performance on lower grade land is having a confounding effect. Consequently, this dataset does not provide a basis for targeting certain types of arable land for planting *Miscanthus*. Explanatory variables did improve the model fit for LUC from grassland to *Miscanthus* with an EF of 0.47. In this case, SOC losses may be greater or more likely to result from LUC for sandier soils and/or in warmer and wetter regions. Therefore, a possible strategy could be to target cooler regions with more clayey soils although it seems counterintuitive to recommend planting *Miscanthus* on grass instead of arable land, which is generally expected to have a greater potential for SOC accumulation (Smith *et al*., 2000).

Although the addition of explanatory variables improved the model fit in most cases, there remains limited potential to derive any meaningful implications from this dataset alone for an improved future management of LUC for lignocellulosic biomass crop production. The potential incorporation of any results from this research into a targeted LUC policy at present also seems highly problematic. Since SOC forms a reversible C store, it is questionable whether the benefits would warrant the long term commitment in policy and financial incentives that would be required to maintain SOC stocks following LUC. Furthermore, even with improved predictability, the land that is available in Britain may not produce the intended benefits. Aside from the many difficulties associated with incorporating considerations of SOC into policy formation and implementation, there are also many challenges facing the promotion of lignocellulosic biomass crops more generally. Although a substantial amount of land has been identified in Britain as 'available' for lignocellulosic biomass crop production, any future expansion is likely to be contingent upon increased social acceptance, economic feasibility and, for the production of biofuels, technological advancements. To address important sustainability criteria, it may be more favourable to pursue a planting strategy that utilises lower grade agricultural or unproductive land to limit the impact on food production (Hastings *et al*., 2009; Hillier *et al*., 2009; Tilman *et al*., 2009). Although a more targeted LUC policy that incorporates the potential effects on SOC seems unlikely, an improved understanding of the short and longer term impact of LUC under different conditions is important nonetheless, even if this proves more useful for C accounting than for C abatement purposes. This dataset does at least provide some preliminary indications of the short term effects of LUC under different conditions on SOC stocks.

6.2.2 Investigating the potential for pyrogenic carbon to reduce land-use change induced losses of soil organic carbon

It has been suggested that the long term C abatement potential of biomass crops could be enhanced if combined with PyC production and use as a soil amendment (Case *et al*., 2014). This could be achieved either by the direct application of PyC to biomass crop soils or the production of PyC from lignocellulosic feedstock for application elsewhere. In both cases, most of the C abatement that is potentially available derives from the C that is sequestered in the stable C core of PyC (Roberts *et al*., 2010). The structure of purposefully produced PyC, as with other forms of black carbon (BC), is composed mainly of condensed aromatic ring configurations, which provides a high level of chemical recalcitrance (Schmidt and Noack, 2000). This stable C provides a more reliable form of increasing SOC stocks than LUC since it is not easily reversible and does not depend on non-modifiable risk factors such as soil texture and climate. The formation of BC, both naturally and anthropogenically, and its subsequent deposition and persistence in soils represents an important sink in the terrestrial C cycle (Schmidt and Noack, 2000). However, the magnitude of this sink is unclear and there are considerable uncertainties associated with quantitative estimates of BC fluxes and stocks (Krull *et al*., 2008). To assist in developing a global assessment of soil BC stocks, statistical models were developed and evaluated for the purpose of estimating soil BC content using more easily obtainable and available data (Chapter 5).

In addition to the C abatement derived from the stable C fraction, studies indicate that PyC has the potential to interact with and stabilise native SOC, a process termed negative priming, which could further augment SOC stocks (Singh and Cowie, 2014). However, such effects are unpredictable with observations of both positive and negative priming following PyC application (Kuzyakov *et al*., 2009; Spokas and Reicosky, 2009; Liang *et al*., 2010; Jones *et al*., 2011; Keith *et al*., 2011; Zimmerman *et al*., 2011). As Chapter 2 of this thesis has demonstrated, LUC for lignocellulosic biomass crop production can often incur a loss of SOC, at least in the short term. Although relatively few studies have assessed the impact of PyC on soil GHG emissions from lignocellulosic biomass crops (Case *et al*., 2012; Prayogo *et al*., 2013; Case *et al*., 2014), none have directly investigated the potential for PyC to reduce LUC-induced losses of SOC by negative priming. Negative priming has often been observed in long term incubation experiments using fresh PyC (Singh and Cowie, 2014). However, these studies do not account for the effects of environmental weathering on PyC-SOC interactions or the effects of leaching and continuous C inputs that would occur in the field. This study therefore aimed to assess the impact of environmentally weathered PyC on native SOC mineralisation at different points in LUC from arable crops to SRC willow (Chapter 4).

Rather than reducing native SOC mineralisation, results from controlled incubation experiments presented here demonstrate a positive priming effect for the surface 5 cm of soil. Despite this, no net effect on soil-surface CO_2 flux was observed in the field suggesting this increase in CO_2 flux from the surface was offset by a contrasting PyC-induced effect at a different soil depth or that different effects were observed under different conditions in the laboratory and the field. Results presented here also support those of Prayogo *et al*. (2013), which documented no net effect on CO_2 using a similar PyC application rate on a 14-year old plantation (Prayogo *et al*., 2013). However, in that study negative priming was observed for an increased application rate which also warrants further investigation to determine any effect on SOC mineralisation during LUC. For the PyC type and application rate used in this study, the results do not suggest that PyC can reduce LUC-induced SOC losses through negative priming.

6.3 Implications for land-use policy

To help meet legally binding emissions reduction and renewable energy targets, biomass could provide 5–11% of the UK's electricity and 6% of heating by 2020

(DECC, DEFRA, DfT, 2012). Lignocellulosic biomass crops are expected to form a significant contribution to the growth of the UK's domestic biomass supply (DECC, DEFRA, DfT, 2012). It is suggested that lignocellulosic biomass crops have the potential to offset anthropogenic CO_2 emissions through soil C sequestration as well as fossil fuel substitution (Smith *et al*., 2000; Hillier *et al*., 2009). With the potential role for LUCs, and subsequent alterations of the SOC pool, to form either a source or a sink for atmospheric CO_2 now recognised at international level under the Kyoto Protocol, it is also important to consider these effects at a national level for emerging UK land-use policy. This could be achieved by including strategies for preventing or reducing SOC losses and/or by offering options for SOC accumulation through 'carbon conscious planning' (Ostle *et al*., 2009). However, to inform policymakers of the potential benefits of LUCs, such as for lignocellulosic biomass crop production, knowledge gaps concerning SOC impacts need to be addressed for accurate and reliable C accounting.

The results presented here indicate that, following commercial planting on former arable land, LUC-induced effects on SOC stocks did not diminish and may even have contributed to the overall GHG mitigation potential of lignocellulosic biomass crops. Based on the limited dataset in this research, the impact of commercial planting on grassland is unclear and a more accurate assessment would have required sampling a larger number of study sites. Since other studies have observed site-specific decreases in SOC stocks following establishment of biomass crops on grassland (Makeschin, 1994; Jug *et al*., 1999), from a 'carbon conscious planning' perspective as well as for the provision of other ecosystem services such as biodiversity (Aylott and McDermott, 2012), planting of lignocellulosic biomass crops on arable land appears a preferable land-use policy to planting on grassland. Furthermore, the present study suggests SRC willow may provide greater additional soil C sequestration benefits compared to *Miscanthus*. For such benefits to be realised, changes in policy support would be required to address market constraints and provide the incentives that are necessary for biomass crop production (Wilson *et al*., 2014).

To support the energy crop market, various subsidies aimed at farmers and energy producers have been introduced. Under the energy crop scheme that was first introduced in 2000, farmers in England have had access to grants covering 50% of the establishment costs of SRC willow and *Miscanthus* plantations. Financial incentives have also benefitted renewable electricity and heat generators in the form of renewable obligation certificates (ROCs) and renewable heat incentives (RHIs). However, these policies have only had limited success and the planting rate has remained relatively low with some crops later being removed due to a lack of profitability (DEFRA, 2013; Aylott and McDermott, 2012). Studies suggest that the UK energy crop market is constrained by farmers' concerns over crop productivity and profitability, market risk, land suitability and a lack of knowledge regarding crop management (Sherrington *et al*., 2008; Aylott and McDermott, 2012; Lindegaard, 2013; Alexander *et al*., 2014; Wilson *et al*., 2014). With the current subsidy arrangements in flux and uncertainty over future policy commitments (Alexander *et al*., 2014), policy makers would be required to revise existing support mechanisms to ensure the commercial competitiveness of biomass crop production in the UK and/or by compensating farmers for any potential financial losses (Fischer *et al*., 2010; Aylott and McDermott, 2012).

Since profitability is directly related to crop productivity, land suitability is an important consideration for farmers. Although this study did not assess the quality of land used, the crop yield or profitability of the plantations used in the present study, communication with growers, as well as research from a study using farmers' focus groups in Britain, all suggest a tendency to select the least productive land for establishing biomass crops (Sherrington *et al*., 2008). Therefore, this study may better reflect the impact of targeting lower rather than higher grade agricultural land. This may partially explain the poor crop yields that were observed at some *Miscanthus* plantations and the lack of SOC accumulation that has been observed in other studies following establishment of *Miscanthus* on arable land (Hansen *et al*., 2004; Dondini *et al*., 2009).

Some studies have questioned the economic potential of planting on less productive land, citing the insufficient monetary value from poor crop yields of Jatropha plantations established on marginal land in India (Ariza-Montobbio and Lele, 2010; Action Aid, 2010; Shortall, 2013). However, before the successful expansion of SRC willow in Sweden, the early period of planting was also characterised by large areas of low productivity (Mola-Yudego *et al*., 2014). Yields were successfully increased by genetic improvements through breeding programmes and improvements in crop management and cultivation practices (Mola-Yudego *et al*., 2014). This case study demonstrates the importance of stable policies and long term contracts for farmers, as well as breeding programmes and training in crop management practices to develop economies of scale and reduce the gap between actual and potential yields in commercial biomass crop plantations (Mola-Yudego *et al*., 2014).

It is possible, therefore, that increased policy support in general terms would increase crop yields and lead to greater profitability and C abatement potential of lignocellulosic biomass crops. In terms of more specific policy implications that can be derived from this study, there are few meaningful implications for policy makers regarding the potential methods for improved future management of LUC that were investigated here. Although the CRF_{spec} models indicate that more specific targeting of land according to local soil and climatic conditions may be beneficial from a 'carbon conscious planning' perspective, further research would be required to improve model predictability to enable consideration for emerging land-use policy. While PyC amendment to biomass crops did not induce a negative priming effect to reduce SOC losses following LUC, it is certain that the stable aromatic C fraction would offset any such losses of SOC and would increase the GHG mitigation potential of biomass crops. However, since there is currently no mechanism for attributing a monetary value to this stable C and no obvious route for how this would be achieved (Shackley *et al.*, 2011), PyC amendment in a LUC context seems unlikely to be commercially viable at present, particularly as farmers already perceive lignocellulosic biomass crops as an economic risk (Wilson *et al.*, 2014).

Chapter 7 Summary and recommendations for further research

7.1 Summary

7.1.1 The impact of commercial deployment of lignocellulosic biomass crops on soil organic carbon stocks in Britain

Of the LUCs investigated here, only LUC from arable crops to SRC willow demonstrated an overall net change in SOC stocks. Compared to the NSI average of 77 t C ha^{-1} for arable land, there was an increase of 15.3 ± 2.2 t C ha^{-1} (± 95% confidence intervals) after 14 years and 68.8 ± 49.4 t C ha^{-1} after 22 years. LUC from arable crops to *Miscanthus* and LUC from grassland to both SRC willow and *Miscanthus* demonstrated no overall net effect on SOC stocks, with no difference observed between SOC stocks under biomass crops and the NSI averages for arable and grassland. However, there remains a large uncertainty around estimated SOC trajectory following LUC from grassland to lignocellulosic biomass crops. This was due to the limited number of biomass crop plantations that were established on grassland. Following commercial planting on former arable land, LUC-induced effects on SOC stocks did not diminish and may have contributed to the overall GHG mitigation potential of lignocellulosic biomass crops. However, the soil C sequestration benefits of commercial *Miscanthus* plantations do not reflect that of experimental field trials.

In most cases the addition of explanatory variables improved the model fit suggesting that SOC trajectory following LUC is sensitive to soil texture and climate.

Clay density improved the model fit for LUC from arable to SRC willow with a negative effect on model trajectory indicating lower SOC accumulation and possibly a slower rate of change for more clayey soils. Sand and silt density improved the model fit for LUC from grassland to SRC willow and *Miscanthus* with both variables having a negative effect on the response function. This indicates that less clayey soils may be more susceptible to SOC losses due to a higher proportion of mineral and aggregate bound SOC in clayey soils, which is more resistant to decomposition than the particulate SOC that is more abundant in sandy soils. Climatic factors also improved the model fit with potentially greater SOC losses and/or less accumulation in warmer and wetter regions following the conversion of grassland. Greater SOC accumulation may have occurred in warmer regions following the establishment of SRC willow on arable land. This indicates SOC losses may be accentuated or are more likely to occur in warmer and wetter regions where conditions favour microbial activity. Where SOC accumulation occurs the C inputs may have a greater effect on the SOC balance than decomposition, with larger inputs in warmer regions due to higher net primary production.

7.1.2 Priming potential of pyrogenic carbon

For the surface 5 cm of soil, mean cumulative CO_2 flux was 21% higher from soil incubated with weathered PyC than the control soil. Due to the high aromaticity and stability of the PyC, this increase in CO_2 flux is unlikely to relate to decomposition of the PyC indicating that a positive priming effect has been observed. Of the potential mechanisms that have been proposed to explain positive priming, the increase in C mineralisation observed here may relate to prior adsorption and subsequent mineralisation of labile C compounds during incubation. Positive priming may be partially explained by PyC-induced changes in soil physicochemical properties, such as increased soil pH or reduced water availability, through the alleviation of constraints on C utilisation. However, these effects are unlikely to be the driving mechanism for positive priming for all sites since: (i) soils with a range of pH were used in this study and positive priming was not significantly related to pH and; (ii) other studies have also observed a reduction in water availability following PyC amendment but did not report positive priming. Positive priming observed in the laboratory incubation was not sensitive to changes in soil properties that follow LUC from arable crops to SRC willow.

Despite the increased CO_2 flux observed under controlled conditions, no net effect on CO_2 flux was observed in the field. It is possible that any increase may have been offset by a contrasting PyC-induced effect such as a reduction in either root respiration or SOC mineralisation in the deeper soil layers. Increased sorption of labile C in the surface layer may have reduced the delivery of labile C to the subsoil which would otherwise activate the mineralisation of slower-cycling C in the deeper soil layers. For the PyC and application rate used in this study, results suggest that PyC does not reduce LUC-induced SOC losses through negative priming.

7.1.3 Statistical models for estimating soil properties

Soil studies often require data for properties that are time-consuming or difficult to directly measure. In these circumstances, statistical models, or pedotransfer functions (PTFs), can be used to estimate such properties using more easily obtainable and available data. PTFs were developed using regression analysis for the estimation of bulk density (BD) of biomass cropland soils in Britain for the purpose of SOC stock calculations. The performance of these regression models was then compared with a range of published PTFs also developed using regression analysis. A simple linear equation with SOC as the only independent variable provided the best fitting model. Predictive accuracy of PTFs was not improved either by considering SRC willow separately to *Miscanthus* or by considering lignocellulosic biomass crops separately to other land-uses. However, all of the regression models that were developed in this study as well as published models performed poorly when validated for all of the biomass crop soil data, with the highest EF of 0.15. This demonstrates one of the main limitations of using regression models for estimating soil BD and directly measuring soil BD is recommended.

Statistical models were also developed and evaluated for the purpose of estimating soil black carbon (BC) content using regression analysis as well as artificial neural networks (ANNs). A multiple linear regression (MLR) equation with only two predictor variables, organic carbon and total nitrogen, provided the best fitting model. Predictive accuracy was high with an EF of 0.92 during validation. Despite the good fit, the negative bias associated with the model is potentially problematic for deriving BC stocks. This simple statistical model provides a first step towards developing a low-cost practical indicator of soil BC which, following further calibration on more soil data, could assist in rapidly developing a more accurate assessment of global soil BC stocks.

7.2 Recommendations for further research

- Further research is recommended to investigate *Miscanthus* growth and biomass productivity on marginal land and in response to different management practices.

Based on the results of single-site paired plot studies, an overall increase in SOC stocks might have been expected here following planting of *Miscanthus* on arable land. This is due to an anticipated reduction in soil disturbance and increased C inputs to the soil from both above- and below-ground. However, the CRF_{gen} model projects a downward trend in SOC stocks over time, with the appearance of greater SOC accumulation under younger than older plantations, and with no overall net change in SOC stocks 13 years after establishment. Since the variance in the data could not be explained using the environmental conditions of climate and soil texture, this trend may relate to changing management practices and their effect on crop growth. Although crop yield data could not be obtained for these commercial plantations, patchy *Miscanthus* crop establishment was observed at some sites. To assess the effects of improvements in propagation and planting practices, such as storage and mechanisation of rhizome establishment, this information could be collected and included as nominal variables in the CRF_{spec} models.

- The uncertainty in model estimates is high, particularly for LUC from grassland to biomass crops. To reduce this uncertainty and provide a more accurate assessment of the impact of biomass crop planting on grassland, targeted sampling of additional field sites could be carried out and combined with datasets from different studies to form an ‘improved reporting scheme’, as others have previously suggested (Poeplau *et al.*, 2011). Reducing the uncertainty of the CRF_{gen} models would provide a better understanding of the overall net effect of commercial biomass crop planting on a landscape scale.

- For more accurate region-specific estimates of SOC changes, the CRF_{spec} model fits could also be enhanced by further sampling and data collection. Additional data should include information on factors affecting SOC, in particular soil and climate. For example, in this study climate data was summarised by mean annual precipitation and temperature for the nearest Met Office weather station to each study site. It is possible that more site-specific data, that includes additional variables such as slope aspect, at a higher spatial resolution could improve the model fits.
- For more reliable longer term predictions of SOC trajectory, the experimental data presented here could be used in conjunction with process-based models. For example, site-specific testing of simulations derived from process-based models could be evaluated against the generality of a statistical model. Process-based models can then be used to more confidently extrapolate beyond the time-frame of observational data, where for novel LUCs only relatively short term effects can be directly examined using the CRFs. In this instance, such models may be particularly useful for determining if any future increases in SOC stocks are likely to occur as any losses incurred by LUC are usually rapid and should have been captured by the present study.
- Further laboratory incubation experiments are recommended to investigate the mechanism(s) responsible for the increase in CO_2 flux that was observed from the surface 5 cm of soil following PyC amendment. To confirm this is a priming effect of PyC on SOC decomposition, stable isotope analysis can be carried out since the PyC used in this study was produced from *Miscanthus* straw, which has a distinct $\delta^{13}C$ signature from the SRC willow-derived SOC. Analysing samples

by isotope ratio mass spectrometry from the headspace of chambers incubated with PyC-amended and control soils would confirm if the increased CO_2 derives from accelerated native SOC decomposition or from PyC mineralisation.

- Since the PyC-amended and control soils in this study were adjusted to equalised gravimetric moisture content, it is possible that the PyC has reduced water availability which could have enhanced aerobic activity. Considering the observed increase in water filled pore space (WFPS) with PyC amendment, it is possible that positive priming may have been caused by the removal of these controls on soil respiration in the laboratory compared to *in-situ* conditions. The effect of moisture availability and subsequent aeration could be assessed by incubating PyC-amended and control soil that has been adjusted to equalised WFPS and maintaining this WFPS for the duration of the incubation experiment.
- It is possible that increased sorption of labile C in the surface layer has reduced the delivery of labile C to the subsoil, which would otherwise activate the mineralisation of slower-cycling C in the deeper soil layers. Further research is required to assess the impact of PyC on the distribution and mineralisation of SOC throughout the soil profile. For this purpose, soil could be collected and incubated from different depth increments. Measuring additional soil properties could also help to elucidate PyC-induced effects at different depths. By measuring soil microbial community structure it may be possible to identify shifts in functional taxa that are sensitive to certain conditions e.g. soil pH, and/or are associated with different substrate quality. Carrying out nutrient extractions on PyC-amended and control soil could help to determine if the increase in CO_2 flux relates to enhanced nutrient availability.

- Since the presence of active plants can be expected to alter priming dynamics between PyC and SOC, field flux measurements are essential to determine the priming potential of PyC under field conditions. In the present study, it was only possible to measure field-flux measurements on a single day for each site. However, studies have observed that the priming impact of PyC varies temporally and a larger number of measurements are required to capture seasonal variability. Therefore, continuous flux measurements could be made using manual or automated chambers at a selected number of study sites. Simultaneous measurements could be made for CO_2 as well as CH_4 fluxes, which may be affected by the increase in WFPS following PyC amendment.
- It is possible that a reduction in root respiration has occurred in the field subsequently offsetting a positive priming effect of PyC on SOC. Therefore, additional field experimental plots could be established to assess the effects of PyC amendment on plant root respiration. Root exclusion methods could be combined with isotope measurements to enable accurate and reliable source-partitioning of CO_2 fluxes. In this way, the contribution of PyC-, SOC- and root-derived CO_2 could be quantified to determine the impact of: (i) PyC on root respiration; (ii) the priming effect of PyC on SOC and; (iii) any priming effect of living roots on PyC mineralisation. Experimental plots could also be established with varying rates of PyC application, since other studies have observed no effect at low application and negative priming at higher rates of PyC application. This could help to establish a threshold effect for priming mechanisms.

- Further research could also be carried out to build on the statistical models that have been developed here. For estimation of soil BD, non-parametric methods of model development as well as more robust methods of model validation could be evaluated for the purpose of PTF development. This may improve the predictive accuracy of PTFs which might be required for the use of pre-existing datasets with missing BD data. However, for future soil studies, directly measuring soil BD is recommended. For estimation of soil BC, the model developed here should be validated using a larger independent dataset consisting of different soil types and with a broader range of SOC content. This would help to assess its general applicability for the development of a database for global soil BC stocks.

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Appendix 1 Effect of sample pretreatment on soil particle size distribution measured by laser diffraction

A1.1 Introduction

Traditional methods of measuring soil particle size distribution (PSD) are based on sedimentation rates for the fine fractions and sieving for the coarse fractions (Gee and Bauder, 1986). Key disadvantages of these methods are the dependence on specific laboratory techniques and susceptibility to operator error (Syvitski *et al*., 1991) and the time-consuming nature of the sedimentation process, especially for particles <2 µm in size since they require a large sample size of at least 10–20 g for the pipette method and 40–50 g for the hydrometer method (Eshel *et al*., 2004). Furthermore, measurements for particles <1 µm in size are increasingly unreliable due to the effect of Brownian motion on the rate of sedimentation (Allen, 1981; Loveland and Whalley, 2001). These methods are therefore unsuitable for rapid, accurate and reliable analysis of a large number of samples.

Various other techniques have been developed in recent decades for measuring PSD, primarily for industrial application, and among these the laser diffraction method (LDM) has been widely adopted in soil science (e.g. Cooper *et al*., 1984; McCave *et al*., 1986; De Boer *et al*., 1987; Levy *et al*., 1993; Buurman *et al*., 1997; Muggler *et al*., 1997; Konert and Vandenberghe, 1997; Chappell, 1998; Beuselinck *et al*., 1998; Eshel *et al*., 2004; Pieri *et al*., 2006). In this method, a beam of monochromatic light passes through a sample in suspension and the diffracted light is focused onto detectors. The forward diffraction of light by the particles is used to determine their size distribution and is based on the principle that the angle of diffraction is inversely proportional to particle size and the intensity of the diffracted beam at any angle reflects the number of particles with a specific cross-sectional area (Eshel *et al*., 2004). The main advantages of this method include its suitability for rapid analysis (typically 5–10 min per sample), high repeatability, the small size of sample required for analysis and the ability to obtain detailed information on a wide range of fraction sizes which is obtained in a digital format (Beuselinck *et al*., 1998).

As with the use of sedimentation methods, pretreatment of samples prior to LDM to enhance the separation of particles is widely recommended since incomplete separation of aggregates may cause an underestimation of smaller particle size fractions (Gee and Or, 2002). Soil organic matter (SOM) is a strong binding agent (Tisdall, 1996) and the removal of SOM by chemical reagents is commonly the first pretreatment step for measuring soil PSD (Gee and Bauder, 1986). Since hydrogen peroxide (H_2O_2) was first introduced as a chemical oxidant for use in soils (Robinson, 1922), this has become the most widely used chemical reagent for SOM removal (Day, 1965; Mikutta *et al*., 2005).

Depending on the quantity of SOM present in the sample, this chemical oxidation procedure can be time and resource consuming, especially for large numbers of samples (Vaasma, 2008; Blott *et al*., 2004). Treatment typically begins at room temperature, with a strong initial reaction due to the presence of labile SOM. As frothing subsides, the sample is heated, which accelerates decomposition and shortens the reaction time (Schultz *et al*., 1999). At temperatures >70°C, H_2O_2 is rapidly consumed and additional quantities of reagent are required. The procedure therefore varies according to sample composition and complete oxidation often requires several days involving incremental additions of H_2O_2 with no reliable indicator to demonstrate completion of the reaction as frothing may continue due to decomposition of excess H_2O_2 on mineral surfaces (Mikutta *et al*., 2005).

In addition to being costly and time-consuming, studies have demonstrated an inconsistency in the effectiveness of SOM removal using H_2O_2 (Lavkulich and Wiens, 1970; Sequi and Aringhieri, 1977; Mikutta *et al*., 2005) and its effect on PSD measurements. While some studies indicate that measuring soil PSD using LDM without SOM removal can influence the accuracy of results and often causing an underestimation of the clay sized fraction (Di Stefano *et al*., 2010; Gunal *et al*., 2011), others have observed no improvement in the measurement accuracy or reproducibility of PSD measurements (Beuselnick *et al*., 1998; Blott *et al*., 2004). For example, Blott *et al*. (2004) compared PSD measurements of 15 samples consisting of sand, loess and soil both with and without SOM removal and reported no increase in measurement accuracy following treatment with H_2O_2. It was therefore suggested that this process is too time consuming for routine analysis and should be reserved for highly glutinous sediments such as lacustrine muds where the organic fraction comprises the bulk of the sediment (Blott *et al*., 2004). Similarly, Beuselnick *et al*. (1998) observed no effect of chemical oxidation on PSD of 24 quartz and 83 silty soil samples that were pretreated prior to analysis to provide a range of clay-, silt- and sand-rich sub-samples.

Following the removal of binding agents, samples must be dispersed and maintained in a dispersed state until PSD measurements (Gee and Or, 2002). Lack of standardisation in methods has led to a variety of pretreatment procedures among studies although most methods require that soil particles are dispersed in aqueous solution by both physical and chemical means (Maeda *et al*., 1977; Mikhail and Briner, 1978). Various dispersing chemicals have been used and sodium hexametaphosphate (NaHMP) $(NaPO_3)_6$ has become one of the most commonly used (Gee and Or, 2002). The American Society for Testing and Materials (ASTM) developed the standard test method for particle-size analysis of soils (D422–63), which recommends using a solution of 4% NaHMP (ASTM, 2007). However, concentrations of solutions continue to vary between studies and increasing concentrations were reported to increase dispersion in soils dominated by smectite, kaolinite and quartz (Zhang *et al*., 2005; Mishra *et al*., 2011). Concentrations used in most studies typically range between 1 and 5% with evidence of agglomeration occurring over this threshold (Blott *et al*., 2004). Several methods of physical dispersion have also been used in combination with chemical dispersion and these include turbulent mixing, magnetic stirring and ultrasonic dispersion among the most common methods (Gee and Or, 2002).

To develop a protocol for sample pretreatment to ensure the separation of aggregates prior to LDM, experiments were carried out to: (i) assess the effect of SOM removal on PSD measurements; (ii) compare the use of comparatively low (4%) and high (10%) concentrations of NaHMP and; (iii) compare the effectiveness of using ultrasonic dispersion alone with the additional use of a magnetic stirrer and rigorous shaking by hand to separate soil aggregates.

A1.2 Materials and methods

A1.2.1 Soil sampling and analysis

In this study, 186 soil samples were used for analysis and these were taken from across 93 biomass crop plantations of varying ages in Britain. Site selection and soil sampling methods are outlined in more detail in Chapter 2.2. Briefly, each field was divided into a grid with 100 intersections and 25 soil cores of 30 mm diam. were taken at random locations to 30 cm depth and divided into 0–15 and 15–30 cm layers. Samples were combined for each of the two layers to yield two samples for each of the 93 sites and 186 soil samples in total. Samples were sieved using a 5.6 mm sieve and homogenised using the cone and quarter method (Raab *et al*., 1990). A sub-sample of each was air-dried at room temperature for 7 days, before being crushed, sieved using a 2 mm sieve, milled and analysed for C and N by dry combustion using a TruMac elemental analyser (Leco, St. Joseph, MI, USA). Inorganic C was measured using an automated acidification module and coulometry (CM 5012 and CM 5130, UIC, Joliet, Illinois) and SOC was determined by subtracting inorganic C from the total C content.

Abundance ratios of clay- (<2 μm), silt- (2–63 μm) and sand-sized (63–2000 μm) primary particles were determined for the soil mineral fraction using a laser diffractometer (Beckmann Coulter LS230, High Wycombe, UK). For each of the 186 soil samples, a sub-sample was pretreated for SOM removal before LDM and another sub-sample was analysed without SOM removal. In both cases, all samples with inorganic C >0.01% by weight were first treated for carbonate removal. For this, 20 g of each sample was acidified with 20 ml of 1 *M* sodium acetate (NaOAc), adjusted to pH 5 with glacial acetic acid (CH_3COOH). Acidified samples were maintained at 70°C overnight in a water bath and then centrifuged at 2500 rpm and the supernatant discarded.

After carbonate removal, 10 g sub-samples were treated for SOM removal with 20 ml of 30% w/w hydrogen peroxide (H_2O_2) and the suspension maintained at pH 5 with 0.1 *M* NaOAc buffer. This buffer was used to prevent acidic conditions which would result from the formation of acid oxidation products during chemical oxidation. It has been reported that the pH of unbuffered soil-H_2O_2 suspensions may drop by up to 3 units with final pH values between 2 and 4 (Douglas and Fiessinger, 1971; Lavkulich and Wiens, 1971; Griffith and Schnitzer, 1977). This method of chemical oxidation has been adopted since alkaline conditions and additives favouring dispersion are considered crucial for SOM removal efficiency and the pH buffer will reduce the destructive effect of H_2O_2 treatment on poorly crystalline minerals, which preferentially dissolve at lower pH (Mikutta *et al*., 2005).

The mixture was left at room temperature for 1 hour and then heated to 70°C for 24 hours using a water bath. Each residue was then rinsed three times with deionised water which assists in removing oxalate from the soil (Escudey *et al*., 1999) and oven dried overnight at 80°C (Lavkulich and Wiens, 1970; Dumat *et al*., 1997). Samples treated with and without H_2O_2 were then dispersed by treating overnight with 25 ml of 4% w/v NaHMP before being placed in an ultrasonic bath for 10 minutes and sieved (<1 mm) prior to analysis with the laser diffractometer. The >1 mm residue was isolated by vacuum filtration then oven-dried at 80°C and weighed. The volume of the >1 mm fraction was estimated using an assumed grain density of 2.65 g cm^{-3} (Freeze and Cherry, 1979) and particle size distribution calculated for the

<2 mm sample. This procedure was used to prevent particles >1 mm from damaging the lens of the laser diffractometer.

In addition, prior to sieving, four replicate sub-samples of three soil samples that were expected to have high clay contents were treated with a higher concentration of chemical dispersant to compare the use of 4 and 10% w/v NaHMP. Sub-samples of the same three soil samples were also subjected to two additional forms of physical dispersion following ultrasonic dispersion to determine if additional agitation using a magnetic stirrer or rigorous shaking by hand assisted in soil particle dispersion.

A1.2.2 Data analysis

Paired t-tests were used to assess the effects of chemical oxidation on soil PSD. A general linear model (GLM) was used to explore the relationship between various site properties and changes in PSD with chemical oxidation. Changes in the clay-, silt- and sand-sized fraction with chemical oxidation were used as the dependent variables and the following site properties as main effects: %SOC, age of plantation, former land-use (arable v grass) and biomass crop type (SRC willow v *Miscanthus*). Interaction effects were also tested between: (i) former land-use and biomass crop type; (ii) biomass crop type and age and; (iii) former land-use and age. Two-way analysis of variance (ANOVA) was used to assess the effects of chemical dispersant concentration and the physical dispersion method on soil PSD. Statistical analyses were carried out using SPSS 19 software (IBM, Armonk, USA).

A1.3 Results and discussion

Soil PSD was significantly affected by chemical oxidation with H_2O_2 (Table A1.1). Mean percentage clay abundance increased significantly ($p < 0.001$) following treatment with H_2O_2, while mean percentage silt and sand abundances decreased significantly ($p = 0.005$ and < 0.001 respectively, Table A1.1). Of the 186 samples used in this study, 58 were categorised differently depending on whether the sample was pretreated for SOM removal (Figure A1.1). Most of the soils used in this study are medium textured (15–30% clay) although many soils were categorised as silty or sandy loam without chemical oxidation and as loam or silty clay loam with chemical oxidation. This indicates the separation of silt- (2–63 µm) and sand-sized (63–2000 µm) aggregates following chemical oxidation that could not be separated by chemical and physical dispersion without the prior removal of SOM, which acts as a strong binding agent and increases aggregate stability. It has previously been suggested that chemical oxidation with H_2O_2 has no effect on the accuracy of PSD measurements (Blott *et al*., 2004; Beuselnick *et al*., 1998). However, the results presented here corroborate those of other studies that have demonstrated that measurements of PSD without pretreatment for the removal of SOM are likely to cause a significant underestimation of the clay-sized fraction (Di Stefano *et al*., 2010; Gunal *et al*., 2011).

Table A1.1 The effects of chemical oxidation on soil PSD. Results are from paired t-tests (n = 186). Data indicate mean abundance ratios ± standard error.

Dependent variable	Pre-chemical oxidation	Post-chemical oxidation	t value	p value
% clay	13.38 ± 0.35	19.04 ± 0.43	-16.66	<0.001
% silt	58.72 ± 0.75	56.59 ± 0.77	2.83	0.005
% sand	27.91 ± 0.95	24.38 ± 1.04	3.96	<0.001

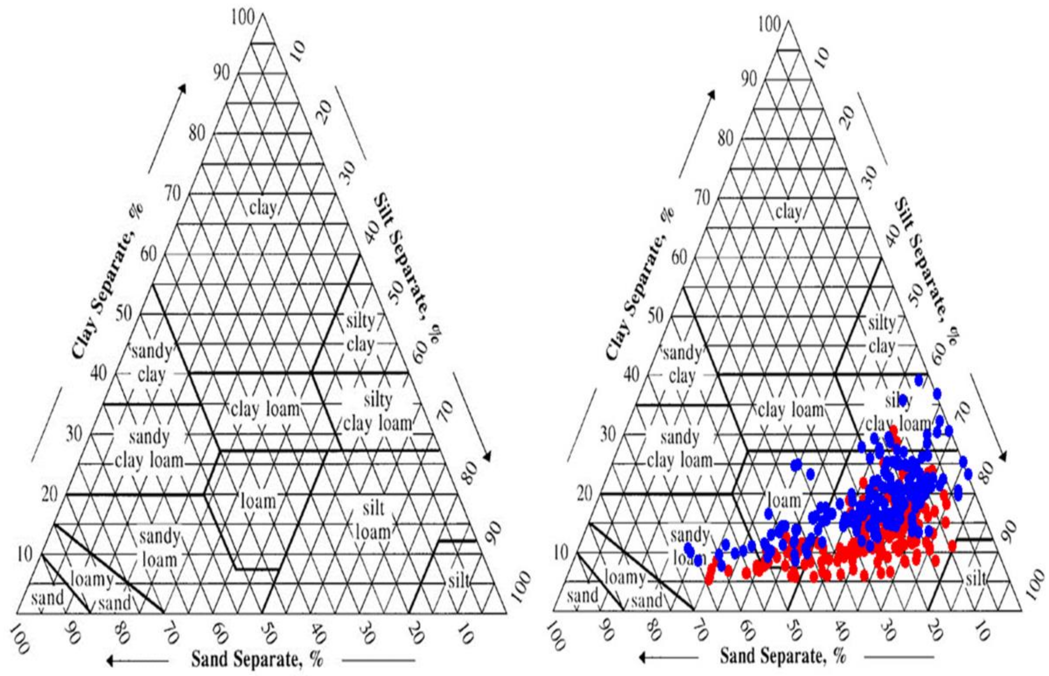

Figure A1.1 The triangle on the left illustrates labels for soil texture groups and the triangle on the right illustrates the distribution of soils within these groups. Soil samples treated with and without chemical oxidation are represented by blue and red dots respectively.

SOC content had a significant effect on the magnitude of changes in the clay- ($p < 0.001$), silt- ($p < 0.001$) and sand-sized ($p = 0.040$) fractions following chemical oxidation (Table A1.2). This indicates that a greater underestimation of the clay-sized fraction and a greater overestimation of the silt-sized fraction and, to a lesser extent, the sand-sized fraction occurred in the absence of chemical oxidation for soils with a higher %SOC content. This might have been expected since SOC forms a major role in both micro- and macro-aggregate formation and stabilisation (Six *et al.*, 2002). Of the other main effects, age of plantation had a significant effect on Δclay but not on Δsilt and Δsand, while former land-use and biomass crop had a significant effect on Δsilt and Δsand but not on Δclay (Table A1.2).

Table A1.2 The effects of various site properties on changes in PSD with chemical oxidation. Results are from a GLM (n = 186).

	Dependent variable					
	Δclay		Δsilt		Δsand	
Independent variable	F-statistic	P value	F-statistic	P value	F-statistic	P value
%SOC	21.00	<0.001	21.78	<0.001	4.26	0.040
Age	4.92	0.028	0.93	0.337	0.00	0.962
Former land-use	3.69	0.056	6.00	0.015	7.23	0.008
Biomass crop type	0.39	0.533	20.03	<0.001	14.78	<0.001
Former land-use*biomass crop type	4.08	0.045	7.91	0.005	9.12	0.003
Biomass crop type*age	6.42	0.012	2.10	0.149	0.05	0.819
Former land-use*age	0.10	0.757	0.05	0.828	0.00	0.951

For Δclay, interaction effects between former land-use and biomass crop and between biomass crop and age both had a significant effect. It is possible that this reflects the relationship between %SOC and Δclay since changes in the clay-sized fraction were greatest for arable and SRC willow (Table A1.3) and accumulation of SOC was also greatest for this land-use change scenario (discussed in more detail in Chapter 2). Age of plantation did not have a significant effect on Δsilt and Δsand, although former land-use and biomass crop type both had a significant effect. The largest changes in the silt-sized fraction occurred following chemical oxidation of soils that were sampled from *Miscanthus* plantations that were established on former grassland (Table A1.3). Conversely the largest changes in the sand-sized fraction occurred following chemical oxidation of soils that were sampled from SRC willow plantations that were established on former arable land (Table A1.3). Since land-use change from arable to SRC willow caused the greatest accumulation of SOC which is an important agent in binding micro-aggregates to form macro-aggregates (Jastrow, 1996), it is therefore possible that a greater proportion of silt-sized aggregates have been bound to form sand-sized aggregates in these soils which have then been separated by chemical oxidation to release the constituent clay-, silt- and sand-sized particles (Bronick and Lal, 2005).

Table A1.3 The effects of former land-use and biomass crop type on changes in PSD with chemical oxidation. Data indicate mean abundance ratios ± standard error.

		Dependent variable		
Independent variable		Δclay	Δsilt	Δsand
Former land-use	Arable	5.82 ± 0.42	-2.08 ± 0.95	-3.90 ± 1.13
	Grass (including set-aside)	4.82 ± 0.53	-2.26 ± 1.11	-2.56 ± 1.27
Biomass crop type	SRC willow	6.22 ± 0.56	1.26 ± 1.29	-7.48 ± 1.59
	Miscanthus	5.13 ± 0.40	-5.30 ± 0.68	0.18 ± 0.69

The concentration of chemical dispersant that was used had a significant effect on the clay-sized fraction ($p = 0.004$, Table A1.4) measured using LDM. Using a 4% NaHMP solution yielded a higher mean clay-sized fraction for the three soil samples that were tested compared to using a 10% NaHMP solution (Table A1.5). This supports the results of other studies that indicate a saturation adsorption of the

Table A1.4 The effects of chemical dispersant concentration and method of physical dispersion on soil PSD. Results are from two-way analysis of variance (*n* = 4).

	Clay		Silt		Sand	
Source of variance	F-statistic	P value	F-statistic	P value	F-statistic	P value
Concentration of chemical dispersant	8.78	0.004	1.09	0.30	0.17	0.682
Physical dispersion method	0.09	0.914	2.49	0.091	1.93	0.153
Interaction	3.09	0.052	0.34	0.716	0.08	0.922

Table A1.5 Descriptive statistics of soil PSD for each of the following pretreatment methods: (i) $_4$; 4% sodium hexametaphosphate; (ii) $_{10}$; 10% sodium hexametaphosphate; (iii) $_u$; ultrasonic dispersion only; (iv) $_{u+s}$; ultrasonic dispersion followed by rigorous shaking by hand; (v) $_{u+m}$; ultrasonic dispersion followed by agitation with magnetic stirrer (*n* = 12).

	Mean	Standard error	Standard deviation
$Clay_4$	10.51	0.39	2.36
$Clay_{10}$	9.00	0.34	2.03
$Clay_u$	9.78	0.37	1.81
$Clay_{u+s}$	9.61	0.42	2.06
$Clay_{u+m}$	9.87	0.61	2.99
$Silt_4$	74.60	0.60	3.57
$Silt_{10}$	75.60	0.77	4.62
$Silt_u$	74.44	0.70	3.45
$Silt_{u+s}$	74.24	0.72	3.55
$Silt_{u+m}$	76.61	1.01	4.96
$Sand_4$	14.98	0.80	4.78
$Sand_{10}$	15.49	0.95	5.67
$Sand_u$	16.03	0.98	4.80
$Sand_{u+s}$	16.15	0.89	4.34
$Sand_{u+m}$	13.52	1.25	6.11

dispersants on to the clay particles resulting in agglomeration (Blott *et al.*, 2004). This experiment was conducted prior to PSD analysis of the 186 soil samples for the purpose of assisting in developing a robust and accurate method for analysis.

Therefore, three soil samples were selected that were expected to contain a high clay content based on questionnaires that were sent to commercial growers of the biomass crop plantations. However, following analysis of these samples using LDM, two of these soils were classified as being 'light' textured. A larger number of samples with a broader textural range would need to be tested to determine if these results are representative of varying mineralogical composition. Neither the use of rigorous shaking nor agitation with a magnetic stirrer increased the clay-sized fraction (Table A1.4). Although it is not possible to draw a direct comparison on the effectiveness of using either of these two methods of physical dispersion instead of ultrasonic dispersion, these results suggest that neither method is more effective at achieving particle dispersion since the clay-sized fraction did not significantly increase with the addition of either method.

A1.4 Conclusion

Soil PSD was significantly affected by pretreatment for SOM removal across a range of soils with varying SOM content. Without chemical oxidation, the clay-sized fraction was significantly underestimated, while the silt- and sand-sized fractions were significantly overestimated. The magnitude of changes in PSD increased with %SOC content. Of the 186 samples used in this study, 58 were categorised differently depending on whether the sample was pretreated for SOM removal. Increasing the concentration of chemical dispersant significantly decreased the clay-sized fraction for a subsample of three soils. This supports the results of other studies that indicate a threshold for effective particle dispersion and beyond this point saturation adsorption of the dispersants on to the clay particles can cause agglomeration and underestimation of the clay-sized fraction. Neither the additional use of rigorous shaking nor agitation with a magnetic stirrer was demonstrated to increase the effectiveness of aggregate separation following ultrasonic dispersion.