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CARBON STORAGE AND SEQUESTRATION UNDER DIFFERENT LAND USES WITH A FOCUS ON BIOMASS CROPS

By

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Abstract

Climate change is caused by rising quantities of greenhouse gases, particularly CO_2, in the atmosphere, largely through consumption of fossil fuels. There is interest in sustainable energy generation from renewable resources, particularly biomass crops to reduce reliance on fossil fuels. A key advantage of such energy systems is that they assimilate atmospheric CO_2 and thus help mitigate climate change. Soil represents one of the largest pools of C in the biosphere and there is potential to use soil as a sink to sequester C to mitigate climate change. The aim of this project was to investigate soil C storage and sequestration in short rotation coppice (SRC), which is one of the major biomass cropping systems in temperate climates. 14 year old plantations of willow and poplar established at Rothamsted Research in Harpenden were used to investigate how the quantity and quality of organic matter under the SRC compare to that under adjacent land uses, including arable cropping, set-aside grassland and natural woodland. It was shown that change in land use to SRC led to increased C storage in soil relative to alternative agricultural systems, while conversion to set-aside had no effect on soil C stocks. There was no difference in C storage under different poplar or willow cultivars. Differences in C storage between arable, SRC and set-aside plots reflected changes in C stocks at 0-30 cm depth with no change occurring to C at 30-60 cm. The quality of C was investigated by analysis of the light fraction organic matter pool which acts as an early indicator of long term changes in total soil organic matter. The work showed that the free LFOM pool responded to land use change while the physically protected intra-aggregate LFOM pool did not. While changes to amounts of LFOM following land use change occurred in soil at 0-30 cm depth, there were no changes at 30-60 cm depth. Fourier Transform Infra Red spectroscopy showed that the chemical composition of free and intra aggregate LFOM was different, and that composition of both pools was affected by land use. Fourier Transform Ion Cyclotron Resonance Mass spectroscopy was shown to provide fine level resolution of the composition of soluble organic matter, and demonstrated that the aromaticity and chain length of C was higher in woodland than arable soil. The potential to sequester C in soil as biochar is a promising option to promote long-term sequestration of C in soil. The potential to use a fluidised bed reactor to produce biochar was investigated. Life Cycle Analysis showed that 470^0C was the optimal temperature to promote retention of C in biochar during pyrolysis, while minimising losses of C as bio-oil and gases. Addition of steam during pyrolysis was shown to increase the surface area of the resulting biochar. Addition of biochar to soil reduced net C mineralisation of soil organic matter and litter at a rate of 2 % w/w, but not at 0.5 % w/w. However both concentrations of biochar affected net mineralisation of N. Phospholipid fatty acid analysis showed that both concentrations of biochar altered microbial community structure, with fungal biomass in particular promoted by the addition of biochar. Overall the work demonstrates that SRC promotes significant storage of C within soil organic matter, which has implications for the overall energy budget of SRC biomass. Furthermore, conversion of biomass to biochar for land application provides opportunities for sequestration of C within soil. Application of biochar to soil would provide further benefits to the overall energy balance of SRC by reducing mineralisation rates of native soil organic matter and litter.

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Declaration

The work presented in this thesis is original, and has not been published or presented for any other degree.

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List of Abbreviations

ANOVA	:	Analysis of Variance
APPI	:	Atmospheric Pressure Photoionization
BD	:	Bulk Density
BET	:	Brunauer Emmet Teller
BFB	:	Bubbling Fluidized Bed
CO_2	:	Carbon dioxide
CPMAS-NMR	:	Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance
CVA	:	Canonical Variate Analysis
DOM	:	Dissolved Organic Matter
DBE	:	Double Bound Equivalent
DNA	:	Deoxyribonucleic acid
ECS	:	Energy Crop Scheme
EDX	:	Energy Dispersed X-ray
ESI	:	Electrosprayer Ionization
FAME	:	Fatty Acid Methyl Ester
fLFOM	:	Free Light Fraction Organic Matter
fPOM	:	Free Particulate Organic Matter
FTICR	:	Fourier Transform Ion Cyclotron Resonance
FTIR	:	Fourier Transform Infrared
GC/MS	:	Gas Chromatography/Mass Spectrometry
GHG	:	Greenhouse Gases
GPP	:	Gross Primary Production
H	:	Hydrolysable

HCl	:	Hydrochloric acid
H_2SO_4	:	Sulfuric Acid
iLFOM	:	Intra-aggregate Light Fraction Organic Matter
LCA	:	Life Cycle Assessment
LC-MS	:	Liquid Chromatography Mass Spectrometry
LFOM	:	Light Fraction Organic Matter
MANOVA	:	Multivariate Analysis of Variance
NaI	:	Sodium Iodide
NaOH	:	Sodium Hydroxide
NaPt	:	Sodium Polytungstate
NEXFAFS	:	Near Edge X-ray Absorption Fine Structure
NH	:	Non Hydrolysable
NMR	:	Nuclear Magnetic Resonance
NOM	:	Natural Organic Matter
NPP	:	Net Primary Production
NT	:	No Tillage
oPOM	:	Occluded Particulate Organic Matter
PCR_1	:	Principal Component Regression
PCR_2	:	Polymerase Chain Reaction
PLFA	:	Phospolipid Fatty Acid
PLSR	:	Partial Least Square Regression
POM	:	Particulate Organic Matter
py-GC/MS	:	Pyrolysis Gas Chromatography/Mass Spectrometry
RA	:	Relative Absorbance
RMSE	:	Root Mean Square Error

RNA	:	Ribonucleic acid
SEM	:	Scanning Electron Microscopy
SIC	:	Soil Inorganic Carbon
SMLR	:	Stepwise Multiple Linear Regression
SOC	:	Soil Organic Carbon
SOM	:	Soil Organic Matter
SRC	:	Short Rotation Coppice
SWT	:	Soil Water Tension
TRLFP	:	Terminal Restriction Fragment Length Polymorphism
WHC	:	Water Holding Capacity
XPS	:	X-ray Photoelectron Microscopy

Chapter 1

General Introduction

1.1. Global warming and its impact on the environment

Global warming has become a crucial topic, since the temperature of the Earth's surface and oceans is predicted to increase over the coming decades and centuries, with massive implications for human society. The greenhouse gases (GHG) effect is the main case of global warming with water vapour, carbon dioxide (CO_2), methane (CH_4), and other atmospheric gases absorbing outgoing infrared radiation resulting in the raising of the Earth's temperatures (Florides and Christodoulides, 2009). CO_2 is the main factor causing the greenhouse effect as it is the most important anthropogenic greenhouse gas (IPCC, 2007a; Florides and Christodoulides, 2009). Climate change caused by greenhouse gas emissions is predicted to increase the mean global temperature by 1.0 to 3.5°C in the next 50–100 years (Rustad *et al.*, 2001).

The increases in global temperature and anthropogenic carbon dioxide emissions over the period of 1855 to 2005 are presented in Figure 1a and 1b. The small incremental increases of global temperature were observed until the period of 1915-1970 before greater increases were recorded beyond this period following rapid increases of anthropogenic CO_2. In 2005, global average temperature reached 14.8^{o}C which was higher by 1°C than values in 1855. Moreover, the annual anthropogenic CO_2 emission reached 7.5 Gt C in 2005, which was nearly four times higher than those value in 1955 (2 Gt C) (Alexiadis, 2007).

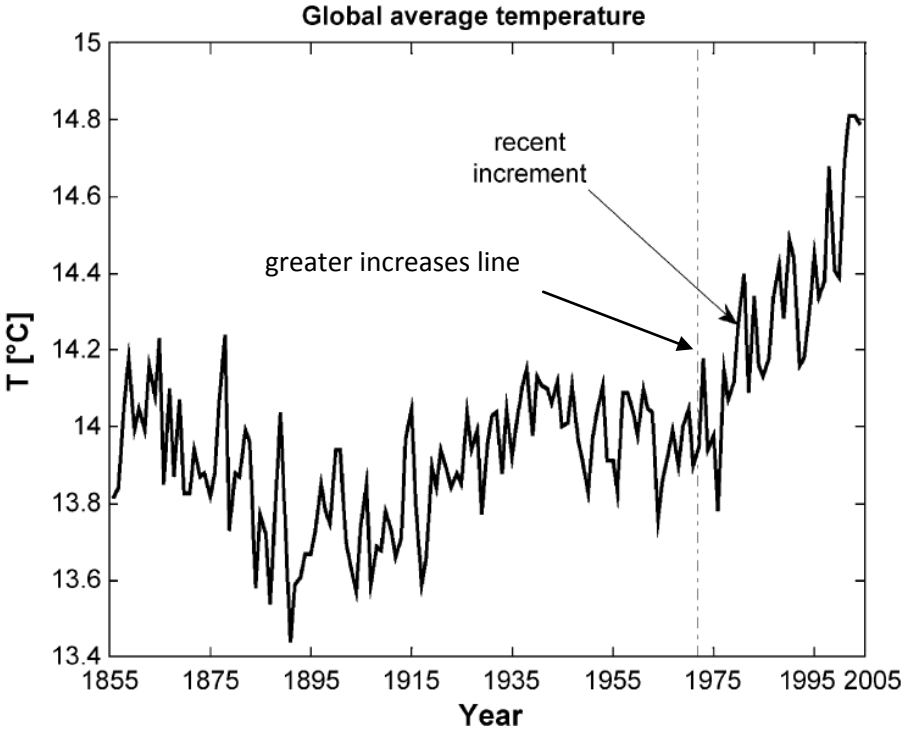


Figure 1a. Global mean temperature (^{o}C) over the period of 1855 to 2005 (adapted from Alexiadis, 2007)

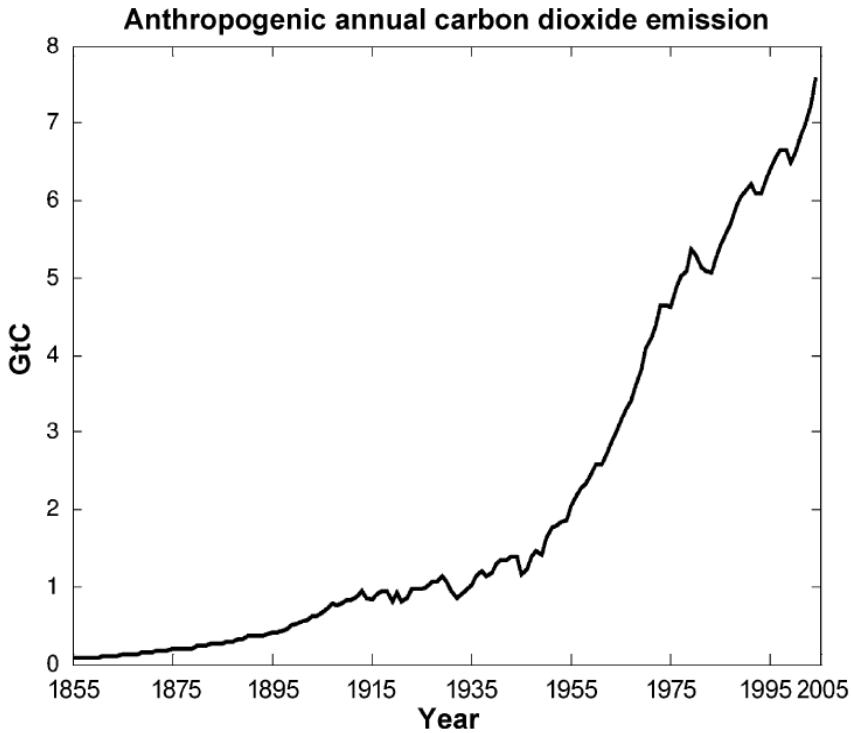


Figure 1b. Global anthropogenic carbon dioxide emission (Gt C) over the period of 1855 to 2005 (adapted from Alexiadis, 2007)

The raising atmosphere CO_2 concentration can influence global climate pattern, net carbon exchange, soil moisture, nutrition, air and soil temperature, solar radiation and air pollutants, as well as the rate of plant growth (Dhakhwa *et al.*, 1997). A doubling in the concentration of CO_2 from pre-industrial revolution levels is predicted to lead to an increase of 1.5 to 4.5^{o}C in mean global temperature (Houghton *et al.*, 1992).

Early studies on the potential effect of global warming on the sub Saharan-African climate (e.g. annual rainfall, temperature, sea level) has been reported by Glanzt, (1992) who predicted that evapotranspiration in this region will increase following a change in global temperature of 1-3°C, and precipitation rate will decline by 10%. Although there will be changes in the spatial distribution of rainfall, with some areas are getting drier, and others will be wetter. Similarly, the effect of global warming on climatic conditions in Greece has been studied by Mattyasovszky *et al.*, (1995). The study predicted a 2-3°C increase in daily mean temperature in autumn and winter, but not for spring. Moreover, there were strong relationship between temperature and precipitation. Evapotranspiration responses to global warming have been studied by Goyal, (2004). The study in an arid zone of Rajasthan, India showed that increasing temperature from 20 to 24°C would be followed by a 15% increase in evapotranspiration rate. Furthermore, a 1°C global temperature rise was predicted to increase global precipitation by 4% (Labat *et al.*, 2004), with North America as the region which is the most sensitive to this changes.

The implications of global warming for the hydrological cycle in the UK and Europe have been reviewed by Goudie, (2006). Generally, these regions will receive higher overall rainfall and lower summer rainfall (particularly in southeast) and the study predicted that by 2050, there will be the following situation: (1) Increases in

annual runoff in the north of Britain between 5 to 15%, (2) Declines in annual run off between 5 and 15% in the south, but could reach 25% in the southeast, (3) Increases in seasonal variation of surface runoff and higher total runoff during winter, (4) Increasing surface run off in the north catchment and lower surface run off in the south. Moreover, Scotland will become wetter that at present and surface runoff become more frequent. In mainland Europe flooding may increase, whilst in southern Europe the annual runoff will decrease. (Menzell and Burger, 2002; Goudie, 2006).

The regional increase in temperature in Sweden, at 4°C over the next 100 years may improve annual forest production by 33%. Biomass production of different forest tree species will be differentially impacted with growth of spruce showing a reduction in yield initially before increasing after 30 years of cultivation (Poudel *et al.,* 2011).

Climate change and global warming will not only impact climatic conditions and the hydrological cycle, but it will also affect the survival of coral reef in the ocean (Crabbe, 2008); tropical biota (Laurance *et al.,* 2011); maize growth (Dhakhwa *et al.,* 1997); infectious diseases (Kurane, 2010); (Khanis and Nettleman, 2005); soil biota (e.g collembolan community (Jucevica and Melecis, 2006): crop productivity (Tan and Shibasaki, 2003) and ocean depth, surface salinity and density (Hirst, 1999).

1.2 Global CO_2 concentration and carbon cycle

1.2.1 Global CO_2

There are concerns about increasing atmospheric concentrations of greenhouse gases (GHG) that are driving global climate change (IPPC, 2007b), particularly carbon dioxide (CO_2) where concentrations have now reached 381.2 ppm (WMO, 2006 in Lal, 2009). This concentration is higher by 30% than the concentration of

CO_2 recorded in 1850 (280 ppm), as a consequently of the increasing of human population following the onset of the industrial revolution around 1850 (Lal, 2008). It is predicted that CO_2 concentrations may reach 600-900 ppm in 2050 (Kessel, 2000). The sequential increasing of GHG concentrations over the past century is presented in Table 1.

Table. 1. *Globally and annually GHS gas emission (adapted from Frolkis et al., 2002)*

Year	CO_2 (ppmv)	CH_4 (ppbv)	CO (ppbv)	N_2O (ppbv)	NO_x (ppbv)
1850/60	287	840	51	285	0.11
1880	289	880	57	288	0.11
1900	296	974	64	292	0.11
1920	302	1020	66	293	0.19
1940	308	1120	68	294	0.33
1960	316	1270	73.5	297	0.57
1970	325	1421	79.9	299	0.75
1980	337	1570	87.7	303	0.98
1990	354	1700	79.7	310	1.29

From Table 1, it can be seen that CO_2 is the major GHG with smaller quantities of CH_4, CO, N_2O, NO_x, and CH_4. Increasing CO_2 concentrations can have direct and indirect impacts terrestrial ecosystem and in particular will affect the global carbon cycle (Dhakhwa *et al.*, 1997).

1.2.2. The global C cycle

In terms of the global C cycle, the annual CO_2 flux was recorded at a level of 4.1 ± 0.1 Gt year^{-1} in the period of 2000-2005, which was higher than in the 1990s (3.2 ± 0.1 Gt year^{-1}) (Matovic, 2011). Increasing atmospheric CO_2 concentration has predominantly arisen through fossil fuel emissions, deforestation, biomass burning, and land use change (Lal, 2009). The reduction of atmospheric CO_2 in order to mitigate climate change can be achieved by various options: (1) carbon capture and

storage, (2) improved energy efficiency, (3) the use of low carbon fuels, (4) use of nuclear power, (5) use of renewable energy, (6) enhancement of biological sinks and (7) reduction of non CO_2 green house gas emissions.

Of all these options, improving C uptake into biological sinks through natural absorption into renewable energy crop biomass, and offsetting carbon loss during combustion is among the most promising options to remove CO_2 directly from the atmosphere (Matovic, 2011). For example, Sartori *et al.,* (2006) proved that the production of 5EJ electricity technology from energy crop required 60 M ha area of plantation in the USA, which could sequester C approximately 5.4 Mg C ha^{-1} $year^{-1}$. This corresponds to 324 Tg C $year^{-1}$, offsetting 20% from the current CO_2 emission from fossil fuels.

The largest global carbon pool is the deep ocean (37,000 to 38,000 Gt C), (Figure 2) followed by fossil fuels (3,700 to 4,000 Gt), with 85% in coals, 5.5 % in oil and 3.3% in gas. The third largest pool is the soil pool, which is estimated at 2,300 to 2,500 Gt (Matovic, 2011, Lal 2008).

The terrestrial carbon pools are mostly derived from soil organic carbon (SOC), at 1,550 Gt, and soil inorganic carbon (SIC) at 950 Gt (Batjes 1996). The SOC pools are mainly humus and relatively inert charcoal C. This can be a mixture of: (i) plant and animal debris; (ii) secondary metabolites or breakdown products of substances produced microbiologically and/or chemically and (iii) the biomass of micro-organisms (Schnitzer 1991).

The SIC pool includes elemental C and carbonate minerals such as calcite, dolomite and gypsum, and comprises primary and secondary carbonates. The primary carbonates are derived from the weathering of parent material, whilst the secondary carbonates are formed through the reaction of atmospheric CO_2 with Ca^{2+} and Mg^{2+}

brought in from outside the local ecosystem (e.g. calcareous dust, irrigation water, fertilizers, manures) (Lal, 2008).

Carbon storage in ecosystems occurs through accumulation of C in aboveground plant biomass, particularly trees, and within organic matter in the soil (Lemus and Lal, 2005). The global carbon cycle has been described in detail by Matovic, (2011) and it is presented in Figure 2. The red line arrow with number indicates annual fluxes (Gt C year^{-1}), whilst the black line arrow correspond to the carbon deposited to the reservoir (boxes)

From the Figure 2 it can be seen that the total global carbon storage in soil and vegetation is estimated to be 2300 Gt C, while the atmosphere contains 800 Gt C. The average carbon uptake into vegetation via natural photosynthesis (GPP) is similar to that released from ecosystems through respiration (120 Gt). This is an indication that the terrestrial and atmospheric C pools interact strongly with one to another. The terrestrial C pool can be depleted by conversion from natural vegetation to cultivation and extractive farming practices based on low external inputs and soil degrading land uses. The soil pool loses 0.4–0.8 Pg C year to the ocean through erosion induced transportation to aquatic ecosystems (Lal, 2008). Between 1850 and 1998, anthropogenic emissions have been estimated to amount to 270 ± 30 Gt C released by fossil fuel combustion and 136 ± 30 Gt C released by land-use change, deforestation and soil cultivation (IPCC, 2001).

The boxes represent total carbon in each reservoir (e.g atmosphere, vegetation, soil and detritus, surface ocean, fossil fuels, etc) (Figure 2). For example, the annual carbon emissions from anthropogenic activity through use of fossil fuel and land use change are estimated to be 7.2 Gt C year^{-1} and 1.6 Gt C year^{-1} (red line arrow), respectively (Matovic, 2011).

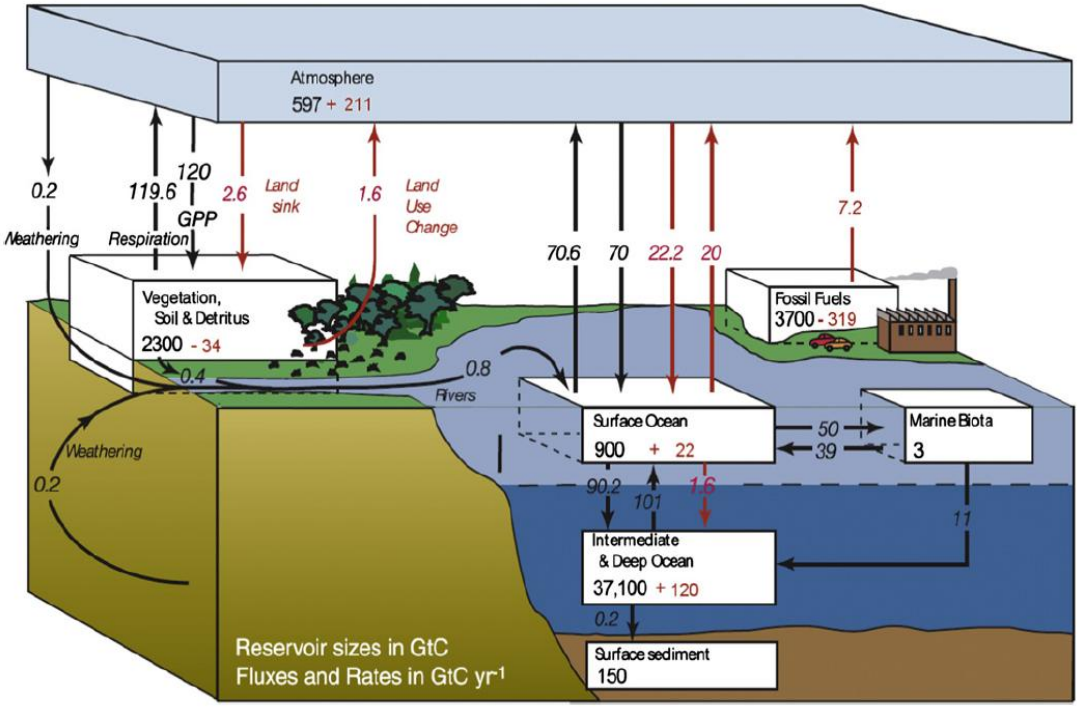


Figure. 2. The global annual carbon cycle (adapted from Matovic, 2011)

Legend:

: C fluxes (red arrow)

: C deposited (black arrow)

: C reservoir

Improving carbon sequestration in vegetation and soil within terrestrial ecosystems could be used as a tool to mitigate global warming (Sartori *et al*, 2007; Lal, 2009) and combat CO_2 emission (Lamus and Lal, 2005). The land sink has the potential to sequester carbon at a level of between 2.6 (Matovic, 2011) (red line arrow) and 5 Gt C year^{-1} (Lal, 2008) (Figure 2). The optimising of land sink C can be achieved by a variety of means, including reapplication to soil of biomass pyrolysed to charcoal (e.g biochar) or change in land use and management towards vegetation which holds larger amounts of C in aboveground biomass and promotes C storage in

SOM, and by reducing deforestation and biomass burning (Lal, 2008). The effect of biochar addition on soil respiration is one of research focus of this current work.

1.3. Soil C cycle

Lehman *et al.*, (2008) noted that soil C cycling has an important role in the global C cycle because soil organic C (SOC) stocks are almost four times greater than C in the atmosphere, and annual emissions of CO_2 from soil are one order of magnitude greater than all anthropogenic CO_2 emissions. Therefore, small uncertainties in soil processes may have large effects on climate-change predictions for those general circulation models that incorporate terrestrial biogeochemical cycles.

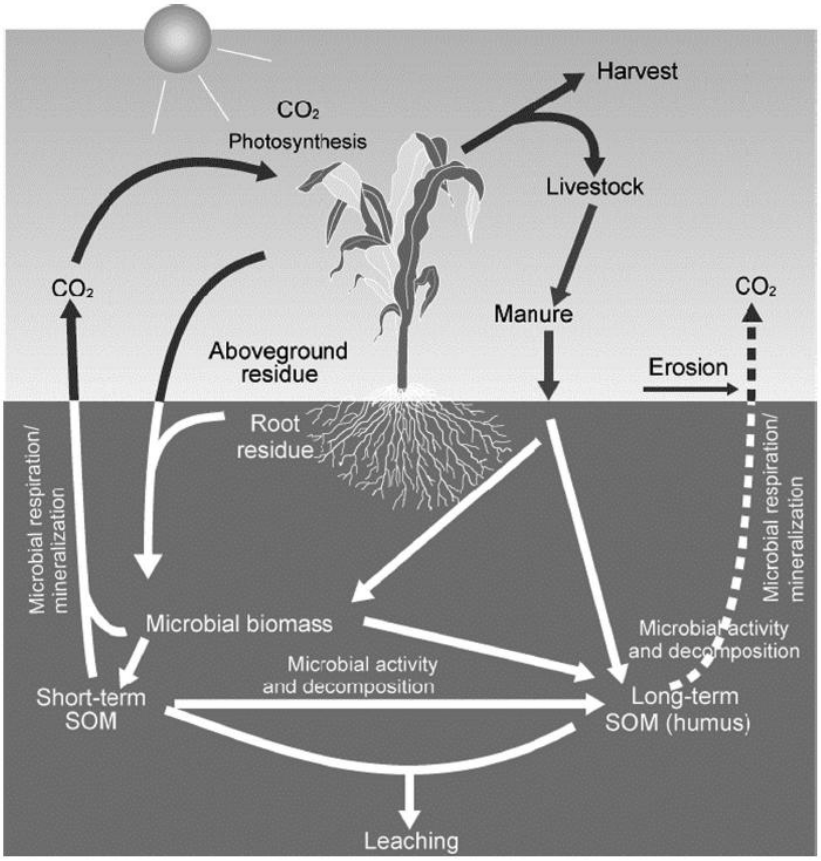


Figure 3. Soil carbon cycle (adapted from Robertson and Grandy, 2006)

Figure 3 shows that under soil C inputs can be from crop residues, cover crops, litter and soil amendment such as compost and manure. The amount of input is under the control of photosynthesis and residence time in soil is determined by the rate of SOM decomposition. SOM decomposition is largely determined by litter quality, specific climatic and soil conditions, soil disturbance and soil microbial activity. The result is short term SOM which had a quick turn over rate. The loss of carbon can be a result of erosion processes and respiration by soil microorganisms. The remaining un-degraded material as humus remained as long term SOM, while some soluble organic C can leach through the soil into aquatic systems. Respired CO_2 can be recaptured during the next photosynthetic processes.

1.3.1. Soil carbon input

Above ground biomass inputs along with root residues determine soil C storage under particular management practices. Zan *et al.*, (2001) investigated carbon storage in above and belowground biomass in a range of agricultural systems (e.g corn) including perennial bioenergy crop (e.g. switchgrass, willow), or abandoned field and forest. Above ground biomass was found to be higher in corn relative to willow in a less fertile site but not in a more fertile site. Corn had higher average shoot C (7150 kg ha^{-1}) than switchgrass (5800 kg ha^{-1}) and willow (5150 kg ha^{-1}). In contrast willow soil C (130 to 145 t ha^{-1}) was significantly higher than switchgrass (100-105 t ha^{-1}) or corn (110 to 115 t ha^{-1}). Total above ground biomass including bole, branches, twigs and foliage reflects the Net Primary Production (NPP). NPP of different vegetation type has been reviewed by several authors (Lodhiyal and Lodhiyal, 2003; Tateno *et al.*, 2004; Zan *et al.*, 2001) (Tabel 2).

From the Table 2, it can be seen that the above ground biomass varies considerably, within the range of 0.68 to 31.90 t ha year^{-1}. The *Eucalyptus* species seems to be the most promising type of vegetation to absorb a large quantity of CO_2 rapidly in short term period.

Table 2. *Aboveground biomass in different vegetation type (adapted from Lodhiyal and Lodhiyal, 2003; Tateno et al., 2004; Zan et al., 2001)*

Vegetation type	Aboveground biomass (t ha^{-1} year^{-1})	Reference
Shorea robusta (Sal)	0.70	Lodhiyal and Lodhiyal., (2003)
Quercus sp (Oak)	3.01	Lodhiyal and Lodhiyal., (2003)
Eucalyptus oblique (Australian Oak)	5.85	Lodhiyal and Lodhiyal., (2003)
Eucalyptus grandis (Rose Gum)	19.52	Lodhiyal and Lodhiyal., (2003)
Eucalyptus grandis (Rose Gum)	27.51	Lodhiyal and Lodhiyal., (2003)
Eucalyptus regnans (Tasmanian Oak)	31.90	Lodhiyal and Lodhiyal., (2003)
Eucalyptus saligna (Blue Gum)	16.23	Lodhiyal and Lodhiyal., (2003)
Eucalyptus sp	2.19	Lodhiyal and Lodhiyal., (2003)
Eucalyptus sp	10.82	Lodhiyal and Lodhiyal., (2003)
Populus deltoides (Poplar)	3.18	Lodhiyal and Lodhiyal., (2003)
Populus deltoides (Poplar)	10.54	Lodhiyal and Lodhiyal., (2003)
Dalberia sisso (Indian Rosewood)	16.40	Lodhiyal and Lodhiyal., (2003)
Dalberia sisso (Indian Rosewood)	0.68	Lodhiyal and Lodhiyal., (2003)
Fagus crenata (Japanese Beech) + Quercus crispula (White Oak)	5.6 – 8.6	Tateno *et al.*,(2004)
Salix sp (Willow)	5.92	Zan *et al.*,(2001)
Panicum sp(Switchgrass)	5.96	Zan *et al.*,(2001)
Zea mays (Corn)	5.18	Zan *et al.*,(2001)

In terms of litter input, a global biomass model of ecosystem vegetation quality and biomass allocation has been developed by Potter and Klooster (1997). Ecosystems are dynamic, and climate and biogeochemistry can modify the composition of plants and the dominance of woody and herbaceous plants, and therefore potentially to store atmospheric carbon and nutrients in biomass. A comparison of modelled leaf litter and litter fall flux from different region is presented in Table 3.

Table 3. *Leaf litter pools and litter fall flux from different ecosystems (adapted from Potter and Klooster, 1997)*

Ecosystem class	**Leaf litter mass ($g\ C\ m^{-2}$)**	**Leaf litter fall ($g\ C\ m^{-2}\ year^{-1}$)**
Tundra	382	24
Boreal forest	682	65
High latitude deciduous forest	772	68
Temperate grassland	102	55
Cropped	112	113
Temperate mixed forest	953	137
Temperate deciduous forest	838	154
Savanna and wooded grassland	955	224
Tropical evergreen forest	1300	370

From Table 3, it can be seen that the highest litter fall mass and litter fall flux was found in tropical evergreen forest at 1300 g C m^{-2} and 370 g C m^{-2} $year^{-1}$, respectively. The lowest leaf litter mass was found in temperate grassland (102 g C m^{-2}) and cropped land (112 g C m^{-2}) which was 10 times lower than tropical evergreen forest. In terms of litter fall input, tundra was the lowest amongst the ecosystems (24 g C m^{-2} $year^{-1}$).

1.3.2. Soil carbon losses

A complete understanding the contribution of C pools to the total CO_2 flux from soil is still lacking (Kuzyakov, 2006). The conceptual model of soil CO_2 flux proposed by Kuzyakov, (2006) grouped five major contributor pools as follows: (1) Root respiration, which does not involve microbial decomposition, (2) Rhizodeposits: CO_2 released from microbial decomposition root exudates, (3) CO_2 produced from microbial respiration from plant residues, (4) Priming effect: CO_2 released from existing SOM as a result of the decomposition of fresh organic materials, and (5) Basal respiration: CO_2 derived from microbial decomposition of the existing SOM (Figure 4).

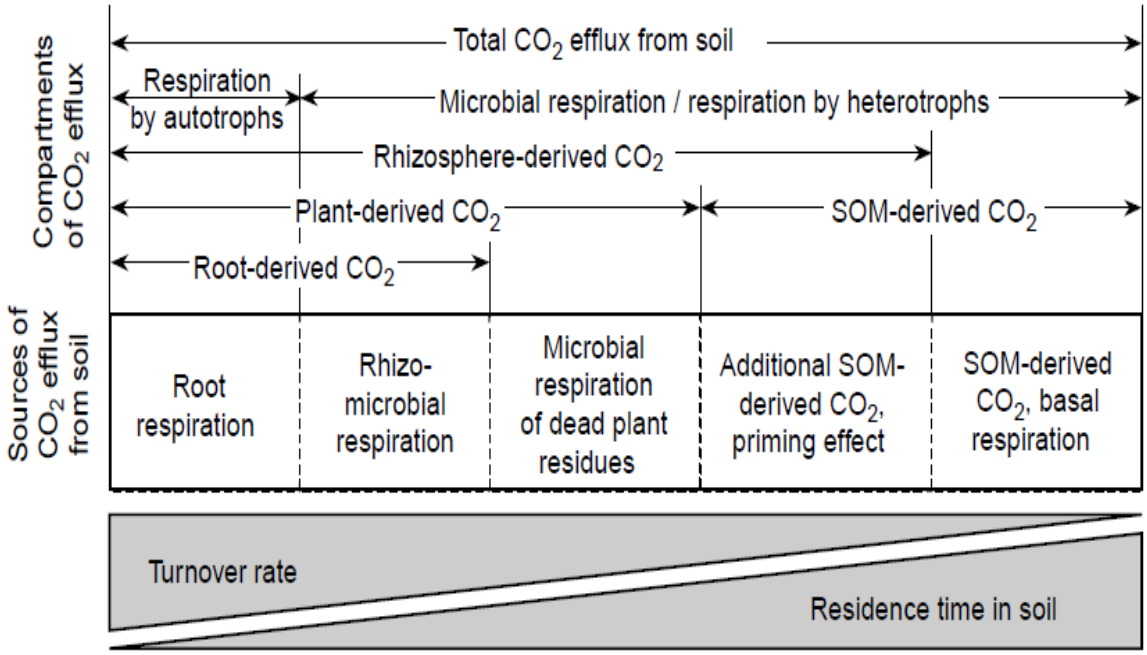


Figure. 4. Schematic conceptual diagram of total soil CO2 flux (from Kuzyakov, 2006)

Mineralization/decomposition of organic C by microorganisms is generally found to increase with warming. Thus, emission of CO_2 from soils will probably increase with increasing temperature, creating a positive climate-change feedback (Lehmann *et al.*, 2008). The SOM decomposition/mineralisation of dead biomass and humic substances has a strong relationship to the temperature. The relationship between soil respiration and temperature can be expressed as a Q_{10}-value. The amount of soil respiration (R) is as follows: $R = Q_{10}^{((T-20)/10)}$, where T is temperature (Goto *et al.*, 1994).

The Q_{10} value is varies depending on the types and quality of SOM, and ranges between 1.85 to 4.0. In a comparison of various ecosystems, the highest Q_{10} value was found in subalpine coniferous (Table 4).

Table 4. *The Q_{10} value of soil decomposition in various terrestrial ecosystems (adapted from Goto et al., 1994).*

Type of vegetation	**Q_{10}**
Evergreen broadleaf	2.86
Deciduous broadleaf	2.51-2.66
Beech	2.18
Beech and beech-fir	2.0
Subalpine coniferous	2.5-4.0
Evergreen oak forest	2.14-2.94
Mature akamatsu	2.45
Deciduous broadleaved	1.85-2.33

Global soil carbon loss by erosion has been reviewed by Lemus and Lal, (2005), which indicated that soil degradation is mainly the result of deforestation, overgrazing and cropping activities. The largest amount of erosion was found in Asia (311 Mha) which was 3 to 4 fold higher than erosion in Australia, Europe, N.America and S.America. This may be due the higher precipitation, faster land use conversion and less soil cover (Table 5).

Table 5. *Global soil degradation over different regions (adapted from Lemus and Lal, 2005)*

Region	Land area (M ha)	Soil degradation (M ha)					Erosion (M ha)
		Deforestation	Overgrazing	Cropland	Total	%	
Africa	2964	58	238	113	409	14	254
Asia	4376	292	194	200	686	16	311
Australia	811	13	83	8	104	13	86
Europe	1002	83	46	60	189	19	87
N.America	1832	17	38	92	147	8	76
S.America	2053	100	63	59	222	11	62
Total	13038	563	662	532	1757	13	900

1.4 Carbon emission and sequestration in the UK

1.4.1 Current UK carbon dioxide emissions

Current UK government policy is to reduce CO_2 emissions by an average of 12.5% between 2008-2012, compared to 1990 levels (Brandao *et al.*, 2011). The UK was reported to be the 7th largest CO_2 emitter in 1995. The 1st place was occupied by the US which emits carbon dioxide 5475 M t CO_2, 10 times higher than the UK (Table 6). However, CO_2 emissions per capita in the UK were recorded at 9.29 t CO_2, which was similar with the values in Germany (10.24 t CO_2). Percapita CO_2 in the UK was lower by 50% than that of the US (Kessel, 2000).

Table 6*. The greatest contributors of global* CO_2 *in 1995 (adapted from Kessel, 2000)*

Country	Total emission in million tonnes CO_2	Percapita emissions in tonnes CO_2
United stated (US)	5475	20.52
China	3196	2.68
Russia	1820	12.26
Japan	1126	9.03
India	910	0.9
Germany	833	10.24
Great Britain (UK)	**539**	**9.29**
Ukraine	437	8.48
Canada	433	14.84
Italy	411	7.19
South Korea	370	8.33
Mexico	359	3.93

Recently, the third annual report to the UK Parliament on the progress made in reducing emissions under the Climate Change Act has been submitted. This report summarised the latest data on progress in reducing emissions in the UK. CO_2 emissions increased by 4% in 2010, mainly due the activities of power plants, reflecting increased demand of energy, including energy consumption for heating and new developments (Committee on Climate Change, 2011). Similarly, there was also a

temporary increase in CO_2 emission in the period from 1995 to 1996 (Figure 5), despite a long term trend of reduced emissions since 1990.

Non-CO_2 emissions declined by 2% in 2010 were due to lowering activities in the agriculture sector and reductions in waste. The 3% increase in GHG emissions in 2010 (500 M t CO_2-e) followed a 9% reduction in 2009 (490 Mt CO_2-e). The reduction in 2009 was largely due to the impact of the recession, resulting in emissions which were well below the first carbon budget.

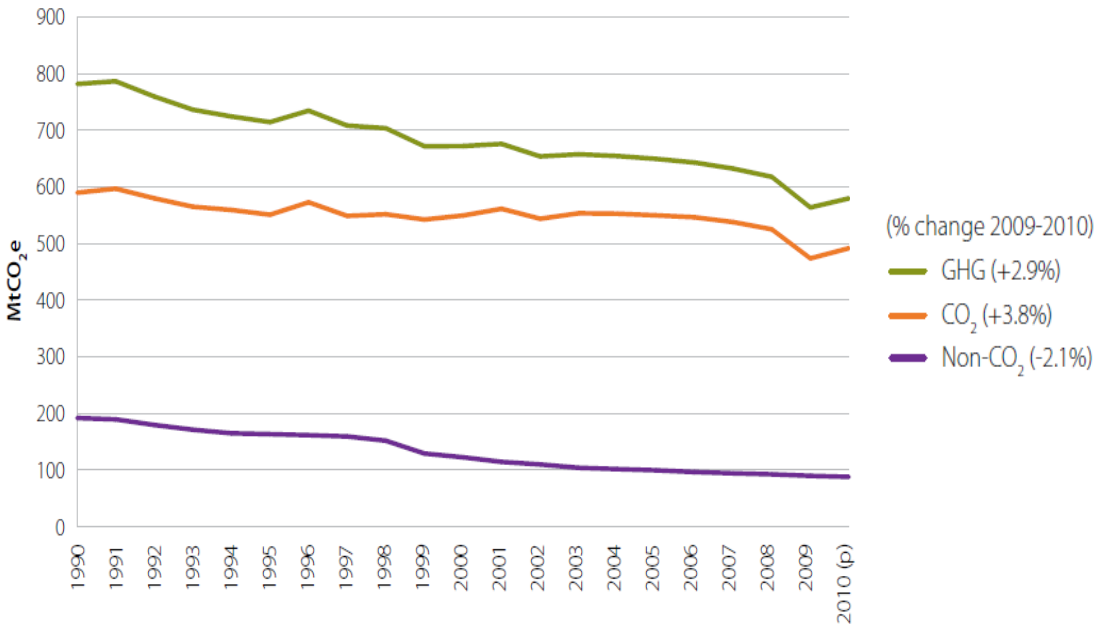


Figure 5. The changes of UK GHG over the period of 1990 – 2010 (adapted from Committee on Climate Change, 2011)

Most of the non-traded sector (i.e. direct emissions from buildings and non-energy-intensive industry) and most non-CO_2 emissions (including from agriculture and waste) show reduced emission since 1990. In 2008-2009 showed the non-traded sector emissions were well below what is required to meet the first carbon budget. The analysis suggested that this was largely due to the impact of the recession, rather than the implementation of abatement measures. The biggest CO_2 contributor was from power station which increased by 3.9% in 2010 (155 M t CO_2-e) relative to 2009

(150 M t CO_2-e), followed by transport (120 M t CO_2-e), industry (110 M t CO_2-e), residential building (85 M t CO_2-e) and non residential building (20 M t CO_2-e) (Figure 6).

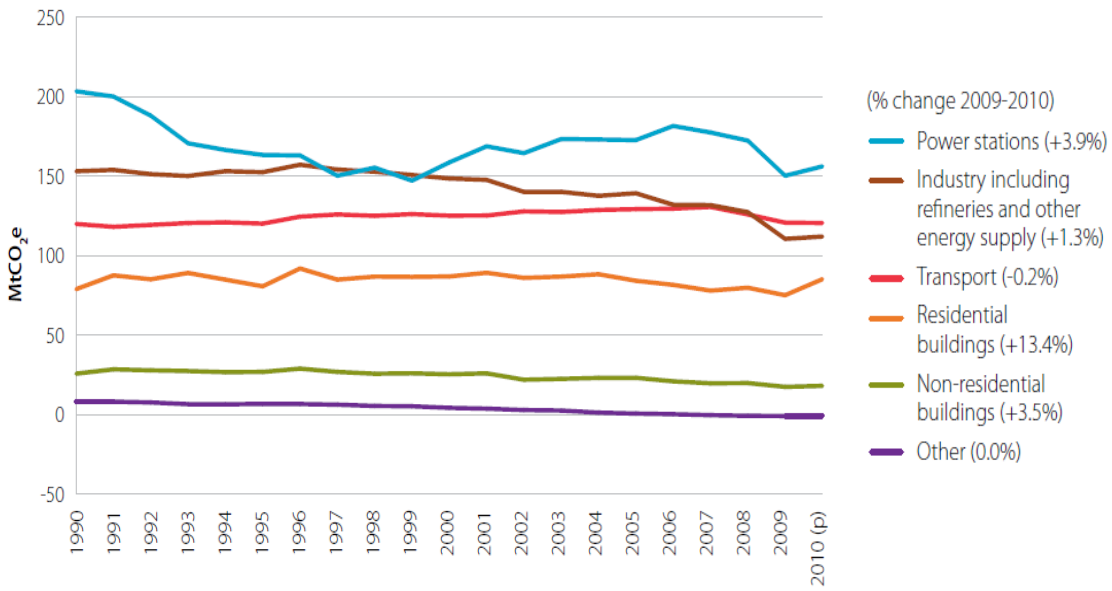


Figure 6. The major contributor sectors of UK GHG emission over the period of 1990-2010 (adapted from Committee on Climate Changes, 2011).

To achieve the target for lowering CO_2 emissions in the future, it has been recommended to develop a new electricity market arrangements, based on long-term contracts which are need to be established under competitive tariffs and cost of delivering low-carbon investment. Government should strengthen incentives for the take-up of energy efficiency measures. The government should insulate all lofts and cavity walls by 2015, as well as 2 million solid walls by 2020. Using mortgage finance where possible would reduce funding costs, therefore easing energy bills and fuel poverty impacts along with renewable heat technology and developing low carbon emission cars (Committee on Climate Changes, 2011). However, the recommendation on reduction carbon emission by biological sink using bioenergy

biomass crops sequestration as a cheap and sustainable approached were not clearly stated in this report.

1.4.2 UK present land use and management practices

Land use activities are associated with energy consumption and CO_2 emissions, from agriculture, buildings and the transport network. Changes in land use will have profound impacts on GHG fluxes, and land management is an important mechanism for climate change mitigation. Identification of existing land uses and long term prediction of the impact of management practices are key factors which could be used to reduce CO_2 emission in the future.

Table 7 shows that UK land use is dominated by agriculture. In June 2008, about 75 per cent of the total land area of the UK (24,251 million ha) was used for agriculture, with most being grassland and rough grazing (51.52%) and the rest was for crops and bare fallow (18.90%). Forest land occupied 11.65% of the UK land area. (DEFRA, 2008; Bibby, 2009)

Table 7. *The distribution of UK land use in 2008 (adapted from Bibby, 2009)*

	Percentage of country (%)					Area (1,000 ha)	
	Agricultural land						
	Crop and bare Fallow	Grasses and rough grazing	Other (set aside, yard, gardens, etc)	Forestry and woodland	Other land	Total land	Inland water
England	30.05	37.08	5.13	8.59	19.15	13,028	76
Wales	3.17	72.29	0.96	13.80	9.79	2,073	13
Scotland	7.07	66.42	1.93	17.12	7.45	7,792	169
Great Britain	19.80	50.26	3.66	11.97	14.32	22,893	258
Nothern Ireland	3.79	72.85	0.70	6.26	16.39	1,358	64
United Kingdom	**18.90**	**51.52**	**3.50**	**11.65**	**14.43**	**24,251**	**325**

The total area of agricultural land has decreased from 19.8 million ha in 1961 to a little less than 17 million ha in 2005 (www.fao.org) a fall of about 14 per cent (Figure 7) (DEFRA, 2008).

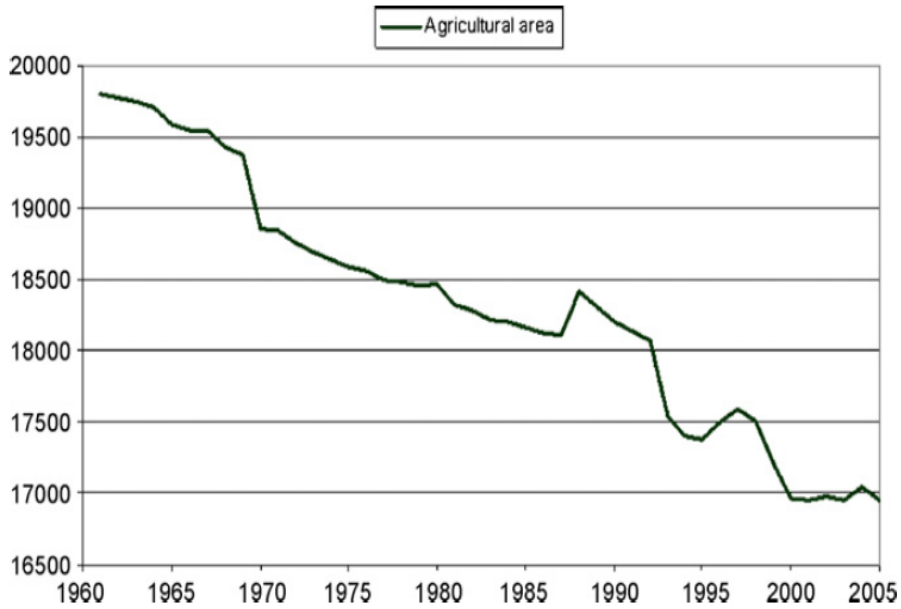


Figure 7. The trends of UK agricultural area over the period 1960 to 2005

CO_2 emissions from cropland in the UK are estimated to be 15.3 million tonnes in 2006 or 2.8 per cent of total UK CO_2 emissions, a slight decline from 1990 (DEFRA, 2006). Conversion of arable land to non-agricultural use has the potential to reduce GHG emissions because of reduced nitrogenous fertiliser use, and consequently reduced nitrous oxide emissions, and the sequestration of more organic carbon, or a reduced rate of organic carbon loss, in soils that are no longer subject to disturbance (Bibby, 2009).

1.4.3 UK Carbon sequestration policy

In 2020, renewable sources are predicted to contribute 10% to the UK total energy consumption (Climate Act, 2009; Ostle, 2009). Consequently, there is a

requirement for land use changes to establish bioenergy crops in order to accommodate renewable energy generation. This may also offset emission of CO_2 from fossil fuels. In the UK several bioenergy crops have been promoted such as willow (*Salix sp*), poplar (*Populus, sp*), *Miscanthus*, switchgrass (*Panicum varigatum)* and red canary grass (*Phalaris arundinacea*) (Ostle *et al.*, 2009). Powlson *et al.*, (2005) assumed that conversion of 80% set aside land in the UK into bioenergy crops will cover 3% of the demand for energy feedstock. Along with this conversion, 3.5 million tonnes C can be sequestered annually, equating to 2.2% of the 1990 level of UK CO_2 emissions (Smith *et al.*, 2000). However, the impact of bioenergy crops on soil carbon stocks is poorly understood (Ostle *et al.*, 2009).

Recently, incorporating of charcoal produced under low temperature pyrolysis, termed biochar, into soil has been promoted as a means to sequester carbon in soil for hundred to thousand years. However, more studies are necessary for examining the benefits and environmental risk of this material on soil biogeochemical process (Ostle *et al.*, 2009).

1.5 General Aims and Objectives of the project

The general aims and objectives of the work in this thesis was to examine the impact of land conversion from arable cropping to alternative land uses on overall soil carbon storage and sequestration with a focus on short rotation coppice (SRC) biomass crops. Biomass crops have the potential to sequester CO_2 from the atmosphere as organic C not only in above-ground biomass, but also in soil organic matter.

The amount of C stored in soil under biomass crops, and the quality of the SOM produced, was investigated. The addition of biochar produced from pyrolysis of biomass crops is believed to improve soil and provide long term sequestration of C in soil.

1.6. Structure of the thesis

Chapter 2. In this chapter the quantity and quality of SOM was compared between arable, set-aside, poplar and willow short rotation coppice (SRC) and ancient woodland plots. The set-aside and SRC plantation were converted from arable cropping 14 years prior to sampling. SRC had been managed according to conventional practice and comprised three genotypes of each species: Jorunn (*Salix vinimalis*), Germany (*Salix burjatica*) and Q83 (*Salix vinimalis vs Salix triandra*) for willow, and for poplar: Beaupre (*Populus trichocarpa vs Populus deltoides*), Ghoy (*Populus deltoides vs Populus nigra*) and Tricobel (*Populus trichocarpa*). Set-aside land was dominated by mixed grass, while the woodland was characterised by mixed deciduous tree species. The aboveground biomass within SRC plantations was determined using an allometric equation and validated using field measurement. Carbon storage was determined by measuring C stocks at 0-30 and 30-60 cm depths. The belowground carbon pool in soil microbes was also determined.

Chapter 3. Using the soil characterised in Chapter 2, the distribution of carbon between SOM fractions was examined using a physical density fractionation technique to separate free and intra aggregate Light Fraction Organic Matter (LFOM). The quality of SOM in the extracted pools was determined by Fourier Transform Infrared spectroscopy (FTIR). Canonical Variate Analysis (CVA) analysis was used

to compare organic matter quality in soil from the different land use types and different depths. Preliminary work was also conducted to investigate the potential of Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry to inform on the quality of dissolved organic matter from contrasting soil sample.

Chapter 4. Biochar derived from willow feedstock was produced at different temperatures of pyrolysis (440°C, 470°C and 530°C) and time of pyrolysis (5, 10 and 15 minute) to investigate the extent to which pyrolysis conditions affect biochar yields and losses of C as bio-oil and gases. The chemical and physical properties of the biochar formed were also compared using elemental analysis, FTIR and Scanning Electromagnetic Microscopy (SEM). A mass and carbon balance calculation as a simple Life Cycle Assessment (LCA) analysis during energy conversion was used to compare the C balances of biochars produced under different pyrolysis conditions.

Chapter 5. The effect of biochar produced in Chapter 4 on soil and litter decomposition processes was investigated. Biochar was applied to soil at different rates (0.5 and 2.0% w/w) with and without SRC willow litter at a rate of 1%. Net C and N mineralisation were measured over a period 90 days. The impact of biochar on the structure and dynamics of the soil microbial community was investigated using phospolipid fatty acid (PLFA) analysis.

Chapter 6. This Chapter summarises and integrates work in the experimental Chapters. The overall findings are placed in the context of optimizing C storage and sequestration under biomass crops. The implementation of this concept under different land use types such as palm oils plantation is discussed.

Chapter 2

The impact of land use change on aboveground biomass and soil carbon storage

2.1 Introduction

2.1.1 Carbon storage in terrestrial ecosystems

The role of terrestrial ecosystems as carbon (C) sinks has been attracted global awareness of the possibility to mitigate anthropogenic CO_2 releases by the promotion of C storage within soil (Gang *et al.*, 2011). The total accumulation of C fixed within plant biomass by photosynthesis represents a gross primary production (GPP). Approximately half of GPP is converted back into atmospheric CO_2 by plant respiration (Prentice, 2001). The remaining biomass acts as C input to soil where it is converted to soil organic matter by the activities of soil microorganisms (Lemus and Lal 2005; William *et al.*, 2004).

Soil C stocks positively affect soil structure, water and plant nutrient availability, and soil biodiversity. They also reduce the risk of soil erosion and degradation, and control the overall productivity of agricultural land (Lal, 2009). Across different ecosystems worldwide, soil C stocks are the highest in wetlands (723 t ha^{-1}), followed by boreal/taiga, tropical, tundra and temperate biomes at 343, 123, 105 and 96 t ha^{-1} respectively (Lal, 2005a). Converting land use from native vegetation to arable cropping systems reduces soil C pools by as much as 60% to 75% in temperate and tropical regions, respectively (Lal, 2004a).

2.1.2 Factors controlling soil C storage

The concept of C sequestration in soil has been proposed previously (Lemus and Lal, (2005); (Ingram and Fernandez, 2001). A range of factors control the size of C stocks within soil. These include the quantity and quality of organic matter inputs, environmental factors such as rainfall and temperature which determine GPP and the rate and extent of soil decomposition processes, and the physico-chemical properties of the soil itself such as pH and clay content which control microbial activity and the potential for protection of organic matter against decomposition.

These factors can be classed as determining the potential, attainable and actual C storage capacity of soil (Figure 8). The most important factors controlling potential C storage are the soil properties which determine the amount of C which can reside in soil, including depth and mineralogy. Attainable C storage is determined by the quantity and quality of plant biomass inputs and factors which control decomposition processes, particularly climate. The actual C storage is influenced by management factors which influence soil mineralisation processes. Changes to land use and management practices therefore represent important strategies for optimising soil C sequestration (Tan and Lal, 2005).

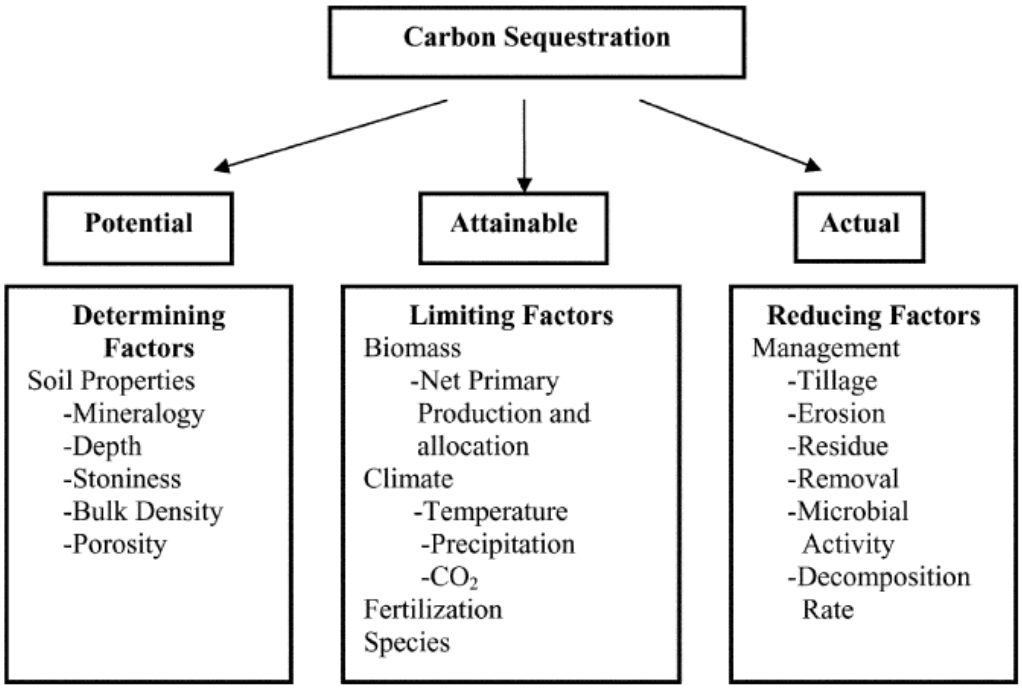


Figure 8. Factors affecting soil C sequestration (adapted from Lemus and Lal, 2005)

Within the landscape, soil under different land uses and habitats can have widely different C contents. Management of land use could therefore provide a tool to promote storage of C within the landscape. Furthermore within land uses, specific management practices can affect the balance between decomposition and protection of organic inputs, thereby altering C storage (Lal, 2004b; Ostle *et al.*, 2009). In order to understand how land use and management can be used as tools to store C in soil, there is a need to understand the extent of differences in C stocks under different types of soil, land uses and management practices, and to understand timescales involved in changes to soil C stocks and the underlying mechanisms by which soil C stocks are altered (Lemus and Lal, 2005).

2.1.3 C input into soils

Soil C inputs to soil largely reflect aboveground and belowground biomass. The annual productivity of the aboveground biomass is usually expressed as the Net Primary Production (NPP). This is recognized as the plant dry matter rate, which is the difference between accumulation of C fixed during photosynthesis and plant respiration (Goudrian, 1995; Huiqing *et al.,* 2008). For agricultural sites, NPP is determined by the sum of the total crop biomass and annual root turn over (Arevalo *et al.,* 2011). The NPP of various terrestrial ecosystems have been reviewed by Mohamed *et al.,* (2004) and is presented in Table 8. NPP was considerably carried out under different types of land uses across tropical regions to northern latitudes.

Table 8*. Global NPP in various land use types (adapted from Mohamed et al., 2004)*

Land use type	Productivity rate (t C ha^{-1} $year^{-1}$)
Tropical evergreen forest	10.19
Tropical deciduous forest	7.11
Temperate forest	6.57
Boreal forest	3.79
Woodland	4.89
Savannah	7.87
Temperate grassland	3.52
Tundra, alpine	0.95
Semi desert	0.62
Desert	0.04
Cultivated land	4.25
Swamps, marshes and coastal land	16.50
Bogs, and peat land	4.67
Lakes and stream	2.00
Human area	1.00

The highest annual vegetation productivity was found in swamps, marshes and coastal land followed by tropical evergreen and deciduous forest, whilst deserts had the lowest productivity amongst all the land use types. Surprisingly, productivity of cultivated land was reported to be similar to that of woodland, bogs and peat land Mohamed *et al.,* (2004), so that the accuracy of current estimates of NPP are not robust. Moreover, studies on C pools and its annual productivity under bioenergy biomass plantation are limited.

2.1.4 C storage in aboveground biomass of bioenergy crops

Compared to native vegetation, bioenergy crops above ground biomass production such as poplar or willow grow quickly, providing rapid biomass accretion over a short time period. There is interest in the use of biomass to replace fossil fuel energy dependancy, while reducing atmospheric CO_2 (Fang *et al.,* 2007). Promoting the use of biomass energy crops such as short rotation coppice (SRC) plantations to mitigate climate change and improve soil quality have become UK government policy (Brandao *et al.,* 2011). Because of its rapid growth rate short rotation coppice (SRC) has the potential to absorb large amounts of C from the atmosphere and thus contribute to the reduction of greenhouse emissions (Aylott *et al.,* 2008). Short rotation coppice plantations can receive financial support from the Forestry Commission Woodland Grant Scheme (WGS) (http://www.forestry.gov.uk/ewgs), which provides up to £4800 ha for the establishment of new plantations.

SRC crops such as poplar *(Populus* spp) and willow *(Salix*, spp) can be harvested over long periods of time (15-30 years), and because of their short rotation time, they can become an important alternative source of wood to replace forest resources (Klasnja *et al.,* 2002). Growing SRC is simple, as preparation and

propagation of the planting materials is cheap, the crops are mostly adaptable to any type of UK soil (Askew, 1997; Armstrong, 2000; Grogan and Matthew, 2001) and SRC has good disease resistance (Lemus and Lal, 2005). Furthermore, SRC crop biomass represents an ideal cheap material for bioenergy plant feedstocks (Hougton, 2006; Gruenewald *et al.,* 2007). SRC plantations can provide a sustainable energy source, as a part of transitional planting management practices between arable and woodland (Gruenewald *et al.,* 2007). Productivity of SRC can be maintained for up to 25-30 years before declining (Goor *et al.,* 1998; Brandao *et al.,* 2011).

In the UK, the estimated area suitable for SRC is 658,900 ha (Grogan and Matthews, 2001). At present, Sweden has 14,000 ha of established SRC willow. The establishment of SRC energy is becoming more popular in the UK and Europe (Martin and Stephen, 2008). Willow has been recognised as having the greatest potential for C capture in the UK (Grogan and Matthews, 2001). Generally, willow has a similar ability to sequester C in biomass as poplar (Lemus and Lal, 2005). In the future, willow is predicted to become a dominant bioenergy crop since it only needs one year to become established and can be harvested every three years (Brandao *et al.,* 2011).

Research on willow short rotation biomass crop was still in the early stages in the UK (Ledin, 1996). For example, growth and willow performance were monitored at the University of Aberdeeen, Loughall Horticulture and Plant Breeding Station, Long Aston and Forestry Commission in small scale experiments (Ledin, 1996). Recently, Aylott *et al,* (2008) reported that there were 49 sites of poplar and willow experimental sites across UK, planted with various number of promising genotypes such as Baupre, Boleare, Columbian River, Fritzi Pauley, Gaver Ghoy, Gilbecq, Hazendanz, Hoogvorst, Raspalje, Tricobel, and Unal for poplar, whilst for willow a

number of genotype were also recorded (e.g Bebbianna, Bjorn, Dasyclados, Delamere, Germany, Jorr, Jorunn, Orm, Q83, Spaethii, Stott, Tora and Ulf).

The establishment of SRC and *Mischanthus* bioenergy crop in the UK were only recorded at 4,600 ha and 5,400 ha, respectively, between 2000 to 2006 (Aylott *et al.,* 2008). This was far from the area of willow plantations in Sweden, which has been recorded at 17,000 ha within the period of 1991 to 1996 (Rosenqvist *et al.,* 2000). Under the grant of Biomass Power for Rural Development Program, 200 ha of willow planted in New York, USA. (Heller *et al.,* 2003). Ideally for achieving sustainable energy supply, 1 M ha of energy crops should be established in the UK by 2020, which were equivalent to 17% of the total arable land, but only 350,000 could be realized. Moreover, it has been recommended by DEFRA for converting 600,000 ha of set aside land into short rotation coppice bionergy crops (DEFRA, 2007).

Different genotypes of willow and poplar can have different amount of above ground biomass accumulation. The total above ground biomass of various genotype/cultivars of SRC has been reviewed many times, but the effect of this genotype/cultivar on soil C has never been explored and reported in detail. The dry matter biomass in the first rotation of willow (4 years) can reach 15.3 t ha^{-1} (Hytonen, 1995). In the UK, the willow genotype Q83 has been recorded to achieve the highest annual yield 10.71 t ha^{-1} $year^{-1}$ followed by Germany (7.46 t ha^{-1} $year^{-1}$) and Jorrun (9.15 t ha^{-1} $year^{-1}$) (Aylott *et al.,* 2008). Furthermore, biomass yields of willow *(Salix minimalis* and *Salix discolor*) in Canada were between 15 and 25 t ha^{-1} $year^{-1}$ (Labrecque and Teodorescu, 2003).

Poplar has become a popular SRC crop in Central Europe (Kauter *et al.,* 2003) and in the USA (Sartori *et al.,* 2006; 2007). On average, Poplar has been reported to accumulate aboveground biomass as much as 4.90–9.70 t ha^{-1} $year^{-1}$ across the UK,

depending on its genotype and soil properties. The greatest biomass has been found in Tricobel (9.70 t ha^{-1} $year^{-1}$) followed by Ghoy, which yields about 5.85 t ha^{-1} $year^{-1}$, while Beaupre can yield 4.90 t ha^{-1} $year^{-1}$ (Aylott *et al.,* 2008). This is lower than the average of poplar biomass in the USA (12 t ha^{-1} $year^{-1}$) (Sartori et al., 2007). In comparison, poplar yields in Canada reached between 9.2 and 13.6 t ha^{-1} $year^{-1}$ (Zabek and Presscott, 2006). The rates at which poplar and willow sequesters C in soil have been the subject of many studies (Zabek and Prescott, 2006; Aylott *et al.,* 2008; Verwijt and Nordh, 1992). However there have been no comprehensive studies on how management practices and genotypes of short rotation crops can affect C storage in soil (Fang *et al.,* 2007)

2.1.5 Effect of management on bioenergy crop biomass production

SRC yield can be dependent on the management strategy employed (Armstrong and Johns, 1997). Reducing spacing or increasing plant density can affect total SRC biomass production in cultivated plots (Armstrong and Johns, 1997). Moreover, the application of herbicides has been demonstrated to increase total willow and poplar yields by 10% (Clay and Dixon, 1997), whilst controlling plant nutrition and water availability reduces the risk of pest and disease in willow cultivars (Hodkinson *et al.,* 1998). The application of fertilizer increased average willow and poplar biomass production in the US from 10.7 t ha^{-1} to 11.3 t ha^{-1} (Kopp *et al.,* 1997), while there were no significant differences in the effects of irrigation on willow cultivars (Adegbidi *et al.,* 2001). The coppicing cycle impacted maximum and minimum root diameters and stem diameters of willow and poplar bioenergy crops. Poplar had a larger stem and maximum root diameter than willow of the same age,

under similar management. Additionally, poplar tends to produce a shallow root system when cultivated in wetter soil (Crow and Houston, 2004).

Planting willow in a mix system has been recommended for reducing damage by pest or disease (Wilkinson *et al.,* 2007). McCraken *et al.,* (2001) noted that an intra species mixture of willow can significantly diminish the incidence rust pathogen *Melamspora epitea* var *epitea* particularly when genotypes were planted in planted up to six components. The yield of willow in 100 m^2 increased, from 102.7 to 210.1 kg, following a 5 to 20 mixture of willow genotypes (McCraken *et al.,* 2001).

The use of a diversity of clones and species in the production of bio-energy crops of willow and poplar are required in order to decrease the risks incurred by diseases and insects was also becoming an important issue in southern Quebec-Canada. For more than 10 years the increasing risk of epidemic disease become an important issue of SRC plantation in this region. Labreque and Teodorescue, (2005) examined the growth and pest resistance in a mixture planting of two clones of poplar and 10 clones of willow. New clones which shown the best performance and tolerate to insect and disease attack were examined and identified. The best performances in willow SRC were obtained from clone SX64 (*Salix miyabeana*) and SX61 (*Salix sachalinensis*) and clones NM6 and NM5 (*Populus maximowiczii x Populus nigra*) for poplar. The worst willow performance was shown in SVQ (*Salix viminalis*) clone which is mainly planting in Quebec region, due to substantial attack by willow leaf beetles (*Plagiodera versicolora* Laichartegand *Disonycha alternata* Illiger) and potato leafhopper (*Empoasca fabae* Harris). This damage were frequently observed on willow leaves (Labreque and Teodorescue, 2005).

In the UK willow mixture experiment, the most susceptible willow genotypes to *Melamspora* rust was found in Ashton Stott (*S.vinimalis x S.burjatica*) clones

which is also less attacked by beetles (*Coleoptera, Chrysomelidae*) (Wilkinson *et al.*, 2007).

As there was a raising interest on planting poplar hybrids in mixture in Sweden, *Populus tremula* and *Populus tremuloides* genotypes were planted along with the existing hybrids (e.g *Populus trichocarpa, Populus deltoides, Populus nigra,* and *Populus maximowiczii*). Previous study on mixing of different poplar genotype was aimed to investigate the tree growth potential, monitor tree phenology, evaluate their resistance to stem crack, stem cancer, frost damage, leaf rust (*Melamspora*), cimbicid sawfly (*Cimbexlutea*), poplar leaf beetle (*Melasoma populi*), willow beetle (*Phratora vulgatissima*) and the hardiness of the trees (Christersson, 2006). The study compared three different poplar genotypes, which were *Populus trichocarpa*, hybrid *Populus trichocarpa* x *Populus deltoides* and hybrid *Populus tremula* x *Populus tremuloides*. The result showed that pure genotypes of *Populus trichocarpa* was more resistance to stem cancer than the hybrids (Christersson, 2006).

2.1.6 Estimation of C storage in bioenergy crop biomass

Biomass estimations in SRC plantations are essential for determining the highest potential yield and optimum harvesting period (Verwijst and Telenius, 1999; Verwijst and Nordh, 1992). Developing specific allometric equations for specific sites for willow and poplar SRC is necessary, as a reliable and effective method for upscaling approaches from small to large plot areas without the destructive sampling of stands (Nordh and Verwijst, 2004). In order to extend the application of these methods, more complete equations and datasets with which to develop more reliable biomass estimates are needed. This may include different scenarios on how much plant density affects yield per cultivated area, the impact of different hybrids or

genotypes on yield and how the specific location for SRC establishment affects the total biomass production (Zabek and Prescott, 2006).

In order to estimate SRC yields, a suitable non-destructive method is necessary in order to develop a suitable allometric equation. This is used to determine the relationship between biomass dry weight and tree diameter (Nordh and Verwijt, 2004). Several studies have successfully developed allometric equations to predict SRC biomass (Guidi *et al.,* 2008; Arevalo, 2007; Koop *et al.,* 2001). However, the previous studies have focused on young SRC plantations (2-3 years after planting), whereas there has only been limited research using older plantations, which could potentially store greater quantities of C per kg of biomass (Grogan and Matthews, 2001), through a greater production of leaves and therefore enhanced surface litter accumulations. All of these aboveground inputs will influence C storage.

2.1.7 Factors determining retention of C within soil

C deposited in soil can be lost by natural factors (e.g. changes in rainfall patterns affected by El Nino, melting ice or the raising of the ocean level, flooding or fire, decomposition) or human induced disturbance (e.g. land management practices which lead to soil erosion, deforestation etc). Numerous natural factors modulating soil carbon emission or sequestration have been recognized. Environmental variables such as soil temperature, soil water content, air temperature, photosynthetically active radiation (PAR) and air humidity significantly affect ecosystem CO_2 exchange (Lloyd and Taylor, 1994; Davidson *et al*., 1998).

Soil moisture deficit, for example, decreases root respiration by up to 17% (Burton *et al*., 1998). Another factor that significantly affects soil CO_2 emission is soil temperature, however there is a strong interaction between soil temperature and soil

moisture. The seasonality of soil temperature was consistent with the seasonal patterns of global radiation and precipitation, and these factors associated with seasonal CO_2 flux in a forest ecosystem in Guangdong Province, China (Yue-Lin *et al.*, 2008).

Soil erosion is a main contributor to C losses, and is predicted to remove 5.7 Pg of SOC worldwide annually (Jacinthe *et al.*, 2001; Martinez-Mena *et al.*, 2008), but potentially as much as 0.6 to 1.5 Gt C year^{-1} could be re-sequestered through deposition and burial processes (Lal, 2005a; Smith *et al.*, 2001; Stallard, 1998). Table 9 describes the annual regional losses of SOC. The highest total erosion and soil C loss was found in Asia, with the lowest in Oceania (Lal, 2003).

Table 9. *Total erosion and SOC loss in different region (adapted from Lal, 2003)*

Region	**Total erosion (x10^9 t year^{-1})**	**Mean erosion rate (t ha^{-1} year^{-1})**	**SOC displaced by erosion (Pg C)**
Africa	38.9	12.9	0.8-1.2
Asia	74.0	16.6	1.5-2.2
South America	39.4	22.1	0.8-1.2
North America	28.1	12.3	0.6-0.8
Europe	13.1	13.4	0.2-0.4
Oceania	7.6	9.5	0.1-0.2

Soil erosion involves the dispersal of low density particles such as those from SOM fractions including silt and clay which are then removed to a different landscape position. Eroded soil has lower aboveground biomass storage due to a reduction in soil and rooting depth, reductions in water and nutrient availability for plants, and disturbances in the hydrological cycle (Lal, 2005b). Furthermore, on site experiments have shown that SOC losses by soil erosion in various types of vegetation (i.e forest, abandoned land and olive soil) at 0 to 5 cm soil depth could result in losses of 50%

from initial values. This might be because organic fractions are highly affected by soil erosion processes which are increased by cultivation (Martinez-Mena *et al.*, 2008).

C losses during decomposition depend on several environmental factors including temperature, moisture, microbial community structure and the composition of plant residues. In general, the composition of plant residues includes cellulose, hemicelluloses, proteins, lipids and polyphenols. C and N concentrations usually determine residue quality (Martens, 2000; Leifeld and Kogel-Knabner, 2005). Zeng *et al.*, (2010) reported that the quality of short rotation coppice residues of poplar can be significantly different to other varieties (e.g. peanut roots, peanut straw, maize roots, maize straw). The results showed that there were significant differences in C mineralization due to differences in the nitrogen, phosphorous contents and the C/N ratios in each substrate. The poplar leaves mineralized 30% of the applied C (1285 µg CO_2-C g^{-1} soil) which was similar to maize roots and maize straw which recorded values of 31% (1299 µg CO_2-C g^{-1} soil) and 35 % (1388 µg CO_2-C g^{-1} soil), respectively. These were significantly lower than the mineralized C of peanuts roots and straw which were 60 % (1852 µg CO_2-C g^{-1} soil) and 44 % (11498 µg CO_2-C g^{-1} soil), respectively (Zeng *et al.*, 2010).

2.1.8 Effect of environmental factors on C storage

Climatic conditions are major factors influencing C storage. In particular precipitation and temperature control vegetation type and its productivity and thereby the amount and quality of organic C returned to soil. Furthermore soil water content and temperature control the activities of the soil biota, hence controlling decomposition processes.

Increases in precipitation and temperature can be positively correlated with rises in NPP (Mohamed *et al.,* (2004) and Gang *et al.,* (2011)). The effect of temperature and precipitation on the NPP of a Chinese *Larix* forest (*Larix gmelini, Larix sibirica,* and *Larix principiruppprechtii*) has been reported by Zhou *et al.,* (2002). An average temperature of -1.4^{o}C and a precipitation rate of 450 mm produced an NPP of 5.70 t dry matter year^{-1} which was 50% lower than the NPP at a site with an average temperature of 6.3^{o}C and a precipitation rate of 848 mm (10.96 t dry matter year^{-1}) over 10 years following planting (Zhou *et al.,* 2002).

In addition, NPP can have a positive correlation with the rate of N mineralization (Tateno *et al.,* 2004). Net C storage in soil is reliant on the rate of C addition via plant biomass and/or organic residues against that lost by crop removals (harvesting), soil and plant respiration, SOM decomposition, soil erosion and redistribution (Spargo *et al.,* 2008).

Climatic conditions can influence the decomposition and mineralization of organic matter by controlling the activities of soil microbes. Most soil organisms grow optimally within a temperature range of 25 to 35°C and 50 to 75% soil water availability (Parton *et al.,* 1987). In subtropical soils containing large amounts of SOC (>20% e.g. wetland and peatland), the accumulation of SOC is caused by poor drainage, which results in the development of anaerobic conditions, combined with low temperatures, which slows down microbial activity, and therefore mineralization. Differences in climatic conditions therefore affect soil C sequestration. Of the climatic parameters, soil moisture and temperature have the greatest influence on soil sequestration (Six *et al.,* 2002b). Warmer temperatures and moist soil conditions increase SOM decomposition rates (Robertson and Grandy, 2006).

Soil C accumulation in humid temperate regions reached 0.1 to 0.5 t C ha^{-1} $year^{-1}$, which is higher than semi arid and tropical areas (0.05 to 0.2 kg C ha^{-1} $year^{-1}$) (Pretty and Ball, 2000). Similarly soil C stock in boreal forest soil has been reported to be as much as 296 t ha^{-1}, which is higher than either tropical or temperate forest which accumulated C at 122 t ha^{-1} (Lal, 2005a).

Franzluebber (2010) showed that annual soil C accumulation under US conservation tillage is climate dependent. Soil C stocks in Texas were reported to be 0.36 t C ha^{-1} $year^{-1}$, which was lower than in Alabama (0.67 t C ha^{-1} $year^{-1}$) and Georgia (0.58 t C ha^{-1} $year^{-1}$), but higher than in Maryland (0.16 t C ha^{-1}). This might have been due to lower precipitation in Texas (625 to 993 mm) than Alabama (1391 to 1652 mm) and Georgia (1146 to 1308 mm), along with lower average temperatures in Maryland (13°C), than in Alabama (11.9 to 18.3^{o}C) and Georgia (16.0 to 18.9^{o}C), which may have acted together to affect soil C storage (Franzluebber, 2010).

Moreover, different climatic zones in Himalayan regions were associated with different soil C stocks at a soil depth of 0-30 cm. The highest concentration of soil C was found in a temperate region (101 t ha^{-1}), followed by an alpine region (81 to 85 t ha^{-1}) and a sub-tropical region (37 t ha^{-1}). The temperate region had the highest precipitation at 1171 mm, followed by sub-tropical (908 mm) and alpine (278 mm) (Singh *et al.*, 2011). Factors including low soil temperature (28°C), high vegetative growth, rapid root proliferation and decomposition of SOM provided ideal conditions for soil C sequestration in the temperate region.

2.1.9 Soil biophysical factors and land management

2.1.9.1 Impact of soil type

There is evidence that soil C stocks can be influenced by soil type and/or texture. Soil texture may regulate the retention of SOC following crop residue decomposition (McConkey *et al.*, 2003). Clay soils usually contain more organic matter than sandy soils when similar quantities of organic material are returned (Jenkinson, 1988). Soil containing sandy materials (Haplic Kastanozem; Hatton fine sandy loam) can have a lower soil C stock (8.1 t C ha^{-1}) than a silty soil (Haplic Kastanozem; Swinton silt loam) (13 t C ha^{-1}) or a clay enriched soil (Vertic Kastanozem; Sceptre clay) (12.1 t C ha^{-1}) (McConkey *et al.* 2003). The influence of soil texture on C stock has also been reported by Gami *et al.* (2009), who recorded that increases in silt and clay content were followed by increases in soil C storage, particularly at a soil depth of 0 to 30 cm. In Quarztipsamment (sandy soil), soil C sequestration ranged from 16.37 t ha^{-1} to 29.71 t ha^{-1}, which was lower than for Oxisol (clay soil) which recorded soil C value of 29.71 to 74.51 t ha^{-1} at 0-30 soil depth (Maia *et al.*, 2010).

Franzlueber, (2005) also observed a strong relationship between soil type, the age of cultivation, management practices and soil C stocks. Soil C stocks in the Typic Paleudult soil were between 23.3 and 43.7 t ha^{-1}, which was higher than for Typic Hapludult (13.3 to 29.9 t ha^{-1}) and Rhodic Paleudult (7.2 to 8.4) t ha^{-1}. This might be the result of lower SOM inputs. In addition, the soil organic C pool has been associated with soil order in the US, with Histosols accumulating higher amounts of soil C in the top of 20 cm (248 t ha^{-1}), followed by Mollisols (71.2 t ha^{-1}), Inceptisols (53.9 t ha^{-1}), Ultisols (42.4 t ha^{-1}), Alfisol (45.6 t ha^{-1}) and Entisols (36.9 t ha^{-1}) (Tan and Lal, 2005).

2.1.9.2 Effect of soil management

Management practices can determine the levels of C present in soils. Tillage practices (i.e. ploughing and harrowing) can manipulate SOC storage by mixing plant residues and soil particles (Chivenge *et al.,* 2007). This can lead to a reduction in the amount of SOC present as protected pools within soil aggregates. It has been extensively documented that conventional tillage may result in SOC losses (Chatterjee and Lal, 2009). This can be the result of exposure of SOC to the atmosphere, and thereby increased soil temperature and aeration, which accelerates the microbial oxidation of SOC (Spargo *et al.,* 2008). However, a no tillage system can result in no mixing, and therefore lower SOC degradation (Roscoe and Buurman, 2003). No tillage practices may positively impact soil aggregation and biological activity as well as increase SOC sequestration (Chatterjee and Lal, 2009).

2.1.9.3 Previous research on the impact of tillage practices

The effects of different tillage management practices on soil organic C storage have been widely reviewed (Maia *et al.,*2010; Spargo *et al.,*2008; Tan and Lal, 2005 ; Wright and Hons, 2005). Maia *et al.,* (2010) observed changes in soil organic C storage under different agricultural systems including no tillage and conventional tillage/full tillage compared with native vegetation (forest) and perennial cropping (e.g coffee and cacao). Conventional tillage/full tillage had 6% lower SOC than native vegetation (forest), whilst non tillage was only 1% higher. In comparison, perennial crop tillage had 2% less SOC than native vegetation (forest).

Significant differences in SOC between tillage systems have been observed in a number of experiments under different conditions (i.e no tillage, conventional tillage and reduced tillage). However, no significant differences between no tillage and

reduced tillage systems were reported by Alvarez, (2005). The study compiled existing datasets for the evaluation of management practice effects on C sequestration. Paired data from 137 sites with varying nitrogen rates and 161 sites with contrasting tillage systems were analysed. Eighty-five paired data sets comparing no-tillage versus conventional tillage (i.e mouldboard plough or disc plough), 53 comparing no-till versus reduced tillage (i.e chisel tillage, disc tillage or sweep tillage), and 23 comparing reduced tillage versus conventional tillage were included. Under no tillage, the average SOC content was 2.1 t ha^{-1} and 2.2 t ha^{-1} for reduced tillage, which was greater than for conventional tillage (Alvarez, 2005).

Different types of tillage system (i.e no tillage, plow tillage, ridge tillage) did not significantly affect soil C at a soil depth of 0-10 cm, even after crop residue application at a concentration of 16 t ha^{-1}. Soil organic C under no tillage system was higher (15.4 g kg^{-1}) than for ridge tillage (14.9 g kg^{-1}) or plough tillage (11.5 g kg^{-1}) (Duiker and Lal, 1999).

SOC sequestration from conventional tillage to no tillage ranged from 0.325 to 0.48 t C ha^{-1} $year^{-1}$ in both tropical and temperate soils (Six *et al.,* 2002a; West and Post, 2002). The annual sequestration of C in the 0–10 cm layer of tropical soils was almost four times greater than in temperate soils (0.43 versus 0.16 t C $ha^{-1}year^{-1}$). However in that study, only soil C in the 0–10 cm surface layer was analysed. In fact, ploughing is often performed to a depth of 15–25 cm and soil C is preferentially deposited below this depth. As no tillage potentially reduces soil C losses by erosion, it is also known that microbial biomass and earthworm abundance (along with fungal and bacterial populations) is higher under no tillage than under conventional tillage systems (Six *et al.,* 2002a). Total SOC sequestration under no-till was predicted to reach from 2.9 to 3.5 t C ha^{-1} (Paustian *et al.,* 1997; Six *et al.,* 2002a). In other studies,

annual C accumulation under no tillage practices has been reported to be as much as 0.42 to 0.46 t C ha^{-1} $year^{-1}$ (Franzluebber, 2005; West and Post, 2002), which was higher than the estimates for soil C sequestration at 63 sites in the US under no tillage cropping systems (0.308 t C ha^{-1} $year^{-1}$) (Spargo *et al.*, 2008).

Inconsistencies in the results of research into the impacts of tillage and non tillage systems on soil C sequestration illustrates that a comprehensive understanding of the factors influencing C storage measurement is still lacking. Discrepancies between studies could reflect variability in sampling protocols (e.g sampling depth), particularly since tillage affects root distribution within soil profiles, with zero tillage promoting root growth at shallower depths (Spargo *et al.*, 2008). Other aspects such as the length of an experiment, plot size, and number and frequency of sampling points could also help to explain differences between studies (Franzluebbers, 2010).

2.1.10 Effect of changing land use on soil C storage

In the UK, C storage in vegetation is estimated at 113 million Tons (Milne *et al.*, 2001), which equates to 5 percent of the total UK land C (Ostle *et al.*, 2009). Total soil C is predicted to be 9.8 billion Tons. The highest soil C stocks in the UK are found in bog ecosystems (259 t C ha^{-1}), whilst the lowest are found in arable soils (43.02 t C ha^{-1}) (Ostle *et al.*, 2009).

Other land uses (e.g. grassland, woodland, heath and swamp) can store between 61 and 82.3 t C ha^{-1}. Dewar and Channel, (1992) estimated that living forest trees can contribute as much as 40 to 80 t C ha^{-1}, whilst 15 to 25 t C ha^{-1} could be stored as litter and 70 to 90 t C ha^{-1} deposited as soil organic matter. Bioenergy crop systems have been reported to sequester C at the same rate as woodland (0.62 t C ha^{-1}

year^{-1}) which is higher than for organic farming systems (0.52 t C ha^{-1} year^{-1}) and set aside (0.38 t C ha^{-1}).

Changes in land use can impact the soil C cycle and affect C storage. Land use changes reduce the vegetation cover and physical protection of SOM (Arevalo *et al.,* 2011; Tan and Lal 2005). On the other hand, reverting degraded/abandoned land back to native vegetation could potentially reverse these effects by encouraging the accumulation of SOM inputs (Ross *et al.,* 2002), improving soil fertility and restoring soil functions (Franzluebber, 2010). The C pool in terrestrial biomass vegetation is four times lower than in soil ecosystems, however this pool plays an important role as a major source of SOM and as an integral part of the global C cycle (Sartori *et al.,* 2006). The potential of various land use management systems to sequester C has been reviewed by Smith, (2004).

Reversion of land from arable cropping to native ecosystems (e.g forest) can influence total soil C stocks, as reported previously by Poulton *et al.,* (2003). Conversion from arable to forest 120 years before sampling increased soil C from 29 t ha^{-1} in 1883 to approximately 105 t ha^{-1} in 1999 (Poulton *et al.,* 2003). Annual C accumulation following conversion from cropland to native woodland has been estimated be 0.62 t C ha^{-1} year^{-1} (Smith, 2004). In addition, the conversion of arable cropping into forest increased C accumulation over 30 years at a rate of 3.8 t C ha^{-1} year^{-1} (Vesterdal *et al.,* 2002).

Converting arable cropping to alternative land use managements such as set aside or field margin management (i.e grass margin, hedgerows, tree strip) can increase soil C stocks by 2.3 t C ha^{-1} (Falloon *et al.,* 2004; Smith *et al.,* 2002). In comparison, converting arable land into grassland over 6-8 to 24 years sequestered soil C at rate of 2.1 to 2.96 t C ha^{-1} year^{-1} (Omonode and Vyn., 2006; Don *et al.,*

2008). This means that conversion of arable systems to setaside or grassland systems can have a positive impact on soil C sequestration.

Studies monitoring soil C stocks following changes in land use have been reviewed in detail by Guo and Gifford, (2002) using meta-analysis techniques. Over 537 observation from 74 published study studies were used from various regions around the world (e.g. America, Brazil, Australia, etc). The parameters used in the meta-analyses included land use change, the effect of soil depth and precipitation levels. Generally, the results showed that soil C stocks decreased after native vegetation was converted into plantation or arable cropping, whilst increases in soil C stocks resulted from the conversion of arable cropping to forest, pasture or plantation. The greatest loss of soil C was observed when pasture was changed into cropping (59%), followed by forest into cropping (42%), and forest to plantation (13%). Changing land use from cropping into forest increased soil C content by 53%. This was more than six times higher than the conversion of native forest to pasture (8%). These effects might be observed within a period of between a decade and a century (Gou and Gifford, 2002).

Potential alterations in soil C stocks associated with land use changes can be related to precipitation and soil depth. The effects of climatic conditions (e.g precipitation/rainfall) on these changes were reviewed by Guo and Gifford, (2002). The study revealed that after conversion from pasture to cropping, soil with 400 to 500 mm of annual precipitation lost a greater amount of soil C (-75%) than soil with 300 to 400 mm (-54%). Soil depth also influenced the soil C status. Following land use changes from crop to pasture, the upper layer (0-20 cm) accumulated more soil C than the deeper layer. Similar trends have been seen in grassland (Don *et al.*, 2008) and arable soils (Duiker and Lal., 1999; Franzluebbers, 2010). However, the effects of

land use changes from arable to other native ecosystems (e.g willow or poplar bioenergy crop) on soil C accumulation have been rarely studied.

2.1.11 Soil C storage under bioenergy crops

There is increasing interest in growing bioenergy crops on agricultural land as energy feedstock. There are assumed to be C neutral energy sources since they accumulate C from the atmosphere and can sequester additional C in soil in the form of litter and SOC accumulations (Lehman, 2007a)

During conversion from arable cropping to bioenergy crops, soil C sequestration under poplar can reach at the rates of 1.63 t C ha^{-1} $year^{-1}$ (Hansen, 1993) to 4.7 t C ha^{-1} $year^{-1}$ (Sartori *et al.,* 2007) or even 5.5 t C ha^{-1} $year^{-1}$ in the 0-30 cm soil layer (Jug *et al.,* 1999) (Table 10). This was higher than the average annual C sink for other energy crops such as switchgrass, which is believed to be a sink for an estimated 0.5 to 2.9 t C ha^{-1}, annually (Al-Kaisi *et al.,* 2005; Ma *et al.,* 2000; Sanderson, 1999). The potential available C sink in the top soil for *Mischantus* and *Eucalyptus sp* plantation is less than poplar, with have recorded accumulation rates of 1.22 t C ha^{-1} $year^{-1}$ (Kahle *et al.,* 2001) and 0.22 t C ha^{-1} $year^{-1}$ (Lima *et al.,* 2000), respectively (Table 10). Plantation age can determine soil C sequestration under poplar plantations. Young poplar plantations can sequester soil C at a rate of 0.51 t C ha^{-1} $year^{-1}$ (Arevalo, 2009) at a soil depth of 0-50 cm, which was lower than for an older (10-year-old) poplar plantation (Pellegrino *et al.,* 2011).

***Table 10**. Soil C accumulation in bioenergy crops compared with the other land uses*

Land use	Age (years)	Soil depth (cm)	Soil C stock ($t\ ha^{-1}\ y^{-1}$)	Reference
Poplar (converted from agricultural crop)	12-18	0-30	1.63	Hansen, (1993)
Poplar (first rotation-converted from agricultural crop)	4-5	0-50	4.7	Sartori *et al.*, (2007)
Poplar (second rotation-converted from agricultural crop)	8-10	0-50	2.6	Sartori *et al.,(*2007)
Poplar (converted from arable land)	10	0-30	3.5-5.5	Jug *et al.*, (1999)
Poplar (converted from agricultural land)	10	0-10	1.2–1.6	Pellegrino *et al.*, (2011)
Poplar (converted from agricultural crop)	9	0-50	0.51	Arevalo *et al.*, (2009)
Poplar (converted from grassland, wheat, corn)	6-15	0-100	0	Grigal and Bergusson, (1998)
Eucalyptus (converted from pasture)	34	0-20	0.22	Lima *et al.*, (2000)
Eucalyptus (converted from sugarcane)	10-13	0-100	0	Bashkin and Binkley, (1998)
Switchgrass	10	0-15	1.2	Al-Kaisi *et al.*, (2005)
Switchgrass	10	0-30	0.5	Ma *et al.*, (2000)
Switchgrass	5	0-30	2.9	Sanderson, (1999)
Bermuda grass	5	0-6	0.5-1.4	Franzluebber, (2001)
Miscanthus (conversion from arable)	16	0-100	5.68	Hansen *et al.,(*2004)
Miscanthus (conversion from agricultural)	4-9	0-25	1.2	Kahle *et al.,(* 2001)

2.1.12 Methodological issues for quantifying soil C stocks

Soil C stocks are a function of both soil C content and bulk density (BD) (Don *et al.*, 2007; Lee *et al.*, 2009). Soil bulk density can vary spatially and temporally within soil (Don *et al.*, 2007), and it is dependent on soil properties (Gami *et al.*, 2011) and management practices (e.g application of organic fertilizer) (Purakayastha *et al.*, 2008). It can be determined using a pedotransfer function, in which the relationship between soil bulk density and soil organic C follows the relationship of $BD = 1.71\ e^{-0.013\ SOC}$ (Franzluebber, 2010) or by direct measurement (Don *et al.*,

2007). However, BD can be affected by land management because the upper layer is usually disturbed during cultivation, thus soil equivalent mass approaches are required to correct the total soil C stock calculations (Lee *et al.*, 2009).

2.1.13 Soil Microbial Biomass

SMB is labile pool of soil organic matter which had a faster turnover rate compared to plant residues. SMB can immobilize nutrients thereby acting as a temporary sink for plant accessible nutrients (Rizt and Robinson, 1988) and mineralize plant nutrients so that they are available to plants (Lovell *et al.*, 1995), Various factors can control amounts of microbial biomass, including the structure of above ground biomass, litter quality and quantity, root exudates, existing soil organic matter, soil structure and texture, and soil moisture and temperature (Aponte *et al.*, 2010). SMB C was significantly affected by different cover crops and residue management in vegetable crops following three years of treatment (Tian *et al.*, 2011). Moreover, 5 years of rotation (e.g. continuous wheat, wheat-sown pasture, wheat-beans, wheat-fallow) and differences in plant residue management (e.g. burned, incorporated and surface retained) had affected SMB C and organic matter in Australian soil. The ratio between SMB C and total soil C can be a good indicator of changes to soil organic matter input due to changes in management practices (Sparling, 1992) and may prove valuable to monitor soil carbon turn over (Ladd *et al.*, 1994).

2.2 Aims and Objectives

The work in this chapter used a 14 year old field trial at Rothamsted Research to compare the amount of C stored in aboveground biomass of willow and poplar, and the extent to which this varied between different crop genotypes.

2.2.1 Aims

The aims was for investigating the extent to which conversion of land from arable cropping to SRC affected soil C storage relative to other land uses. Lastly, differences between SRC crops and genotypes with respect to C storage were also investigated.

2.2.2 Objective

- To compare C storage in above ground biomass of different cultivars of willow and poplar relative to native woodland.
- To elucidate the effect of change from arable cropping to alternative land uses (setaside, SRC, woodland) on soil C storage.
- To investigate the extent to which different SRC cultivars alter soil C stocks.

2.3 Hypotheses

- Storage of C in aboveground biomass of SRC will be lower than that of native woodland.
- There will be no difference in aboveground biomass between different SRC genotypes.

- Soil C storage will have increased 14 years following conversion from cropping to setaside and SRC.
- Belowground soil C storage is affected by genotype of willow and poplar.
- The soil C stock under bioenergy crops will have reached the same levels as native woodland 14 years following conversion from arable.

2.4 Methods

2.4.1 Site Location

The site of investigation was the Rothamsted experimental farm. The elevation is recorded at 95-134 m above sea level and the soil steepness varies between 8-15% (Avery and Catt, 1995) with average rainfall intensity of 697.8 mm annually. The highest average rainfall is found in October, recorded at 74.4 mm, whist the lowest is observed in July (41.2 mm).

The maximum temperature is 13.4^{o}C and 5.6^{o}C for the minimum temperature. The climatic data covered the period of observation between 1970 to 2000 (http://www.metoffice.gov.uk/climate/uk/averages/19712000/sites/rothamsted.html).

Willow and poplar plantation plots were used together with an arable field, and established woodland and set aside plots (Figure 9a). Willow, poplar and set-aside fields were located in the Dell Piece field experiments (Latitude: 5.1802 ; Longitude: -0.3826) (Forest Research, 2003b), while the arable soil was situated in Claycroft fields and Knot Wood was used as the woodland sites. The plots are adjacent and each parcel of land use was split into 9 areas for sampling purposes. The SRC and set-aside plots had been converted from arable cultivation 14 years prior to soil sampling.

The site was established as a part of Forest Research Commission experiment to study performance of SRC across the UK (Forest Research, 2003b)

Vegetation type in each land use :

1.Arable: Wheat rotation

2.Setaside: mixed grasses

3.Woodland: dominated by Beach, Oak

4.Poplar: Genotypes of Tricobel, Beaupre, Ghoy

5.Willow: Genotypes of Q83, Germany,

Figure 9a. Location of land use plots at Rothamsted Institute

The soil of the Rothamsted site is clay loam, according to the Soil Survey of England reported by Poulton *et al.*, (2003). The arable, set aside, woodland, willow and poplar experimental sites were located in Dell Piece plot, west middle part of Rothamsted field. Dell Piece plot were bordered by Little Knot and Great North in the north, Knot Wood and Furze Field in the west, Claycroft in the south and Bones Close in the east (Figure 9b). In general Rothamsted field were developed over Chalk on the dipslope of Chiltern Hills, about seven miles south-east of the steep scrap slope at Dunstable and eight to nine miles north-west of the Tertiary outcrops (Reading Beds and London Clay) on the north-western margin of London Basin (Avery and Catt, 1995).

Soil at Dell Piece plot is classified in the hierarchical soil classification scheme as follows; Soil groups: typical paleo-argilic brown earth (Avery, 1980); Soil series: Winchester clayey over lithoskeletal chalk (Clayden and Hollis, 1984); Soil Subgrous: Typic (or Vertic) Hapludalf (Soil Survey Staff, 1992); Soil Unit: Chromic (or Vertic) Luvisol (FAO, 1990). The Rothamsted soil map including Dell Piece plot is presented in Figure 9b. Detailed characteristics of the soil (Profile no.5) are

described as follows: (0-10 cm) Ah: Very dark greyish brown (10 YR3/2) flinty silty clay loam; (10-23 cm) Eb: Brown flinty clay; (23-38 cm) 2Bt: Strong brown (7.5 YR5/6-8) flinty clay with paler brown ped faces; (38-66 cm) 2Bt; Yellowish red (5 YR5/8) flinty clay with brown ped faces; (66–76 cm) 3Bt: Yellowish red (5YR5/6) clay with large unbroken flints and black mangniferous segregations; (76+) 4Cu: Fragmentary Chalk with clayey inclusions.

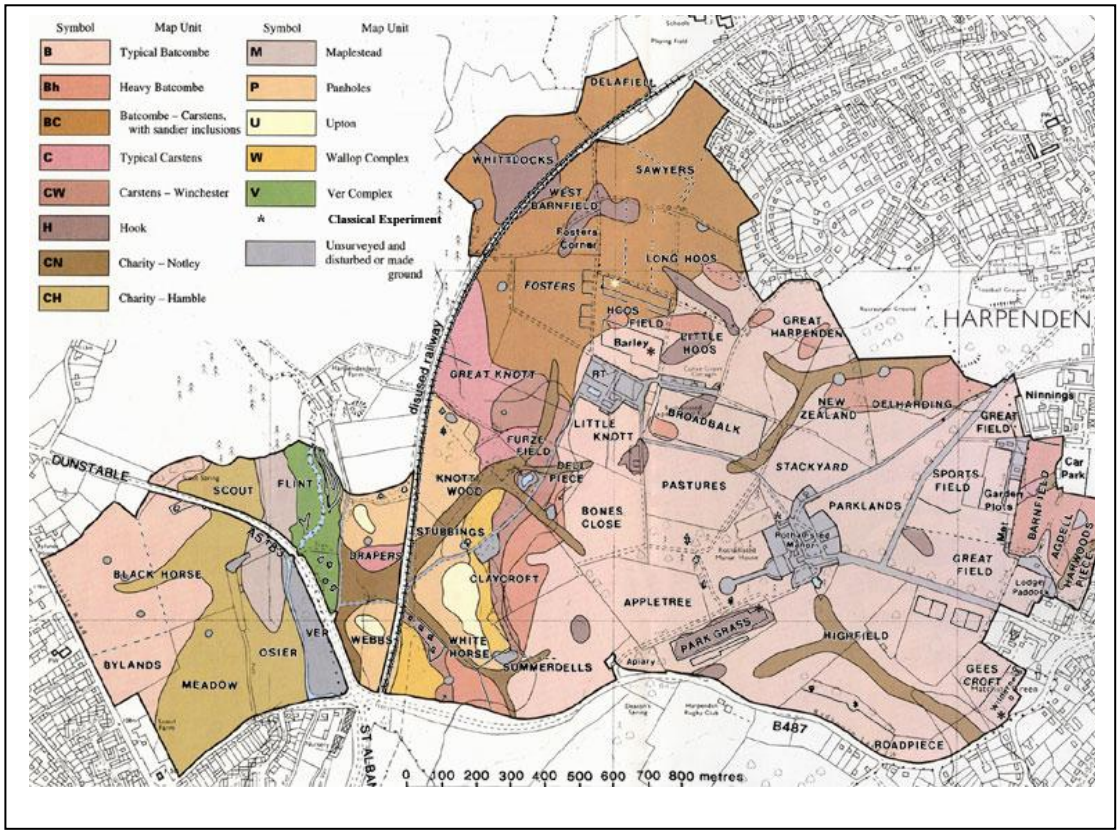


Figure 9b. Soil type and their distribution at Rothamsted field experiment (adapted from Avery and Catt, 1995)

Soil series was classified CW which is belongs to the Carstens – Winchester soil group and is a flinty silty clay loam with >27% clay; mainly well drained; subsoil unmottled heavy clay with flints within 30 cm mainly over chalk within 80 cm, having a soil pH between 4-9 to 6.5. This series was a discontinuous belt of sloping land extending southward of Great North (Avery and Catt, 1995).

2.4.2 Experimental area

The experimental design presented a unique opportunity to study the impact of land use change from arable land to alternate management practices within the same soil type. This study evaluated the effect of land use conversion from arable to short rotation forestry, compared with set aside (grassland) and adjacent natural vegetation (woodland).

The coppice plots had been managed according to standard procedure from Forest Research guideline (Tuby and Armstrong, 2002). Prior to establishment of SRC, the land had been used for arable cropping. There is no record of fertilizer addition after planting. The last wheat cultivation was in 1993. Soil was ploughed in August 1994, and Glyphosate was applied in February 1995, before cultivation in March 1995.

There were 3 genotypes of willow available: Jorunn (*Salix vinimalis*), Germany (*Salix burjatica*) and Q83 (*Salix vinimalis* x *Salix triandra*). For poplar, the cultivars available were Beaupre (*Populous deltoides* x *Populous trichocarpa)*, Ghoy (*Populous deltoides* x *Populous nigra)* and Tricobel (*Populous trichocarpa)* (Figure 10). Planting of willow and poplar cultivars was done in 3 blocks and each cultivar was replicated 3 times.

The willow and poplar were each planted in a single block, each of which was split into 9 plots in a randomized block design. The size of the each plot was 121 m^2. In poplar, trees were planted in a spacing of 1 m x 1 m, resulting in a maximum density of 10,000 trees $ha^{-1.}$ Willows were placed in double rows with 0.5 m in between tree within eachrow separated by 0.75 cm from trees within the second rows and 1 m between the doble rows giving a maximum density of 2 trees per m^2 or the equivalent of 20,000 trees ha^{-1}. However the maximum density of willow and poplar was checked by a field survey to assess the actual above ground density. In poplar, this was done using a 11 m x 9 m sampling plot = 99 m^2. In willow smaller plots (5.5 m x 4.5 m) were used, representing an area of sampling approximately 25 m^2. As willow and poplar had been coppiced several times, each tree can consist of more than one stem/stool. For achieving optimum yield, it has been recommended that willow is cultivated at the density of 15,000 stems ha^{-1} (Wilkinson *et al.*, 2007).

The average density of poplar genotypes Beaupre: *P.tricocharpa x P.deltoides*, Ghoy: *P.deltoides x P.nigra*, and Tricobel: *P.tricocharpa* were 61, 55 and 96 trees respectively in 99 m^2 sampling plots or approximately a a density of 6100, 5500 and 9600 trees ha^{-1}, respectively. Of willow genotypes Jorrun: *S.vinimalis*, Germany: *S.burjatica,* Q83: *S.triandra x S.vinimalis*, the average density in 25 m^2 sampling plots was 38, 32, 40 tress, equating to 15200, 12800 and 16000 trees per ha^{-1}.

The adjacent woodland is dominated by Oaks *(Quercus sp)*, Beech *(Fagus sylvatica L)* and Maple *(Acer pseudoplatanus L)* with the woodland age estimated to be 300 years (Keith and Poulson, 2003). The arable plots were adjacent to the SRC plots, and were predominantly managed as a mixed rotation of wheat, beans and other crops. In order to compare uncultivated management, a permanent set aside plot were

also included in this study. This field was converted to permanent grassland 14 years ago. In line with recommended practice, the field was mown yearly.

Figure 10. Poplar and willow genotypes used in the field experiment

2.4.3 Experimental design

Pair sampling approaches have been widely used to investigate the effect of land use change on soil C and SOM fraction (Cambardella and Elliot, 1992;1993;1994). The underlying assumption of this method were due to the similarity of soil C content, as the the pair plots (set aside, willow and poplar) has been converted form arable cropping in the same time (14 years ago). The values of soil C in this soil type were recorded at 1.5 % (Avery and Catt, 1995). Moreover, there were no records of external input to the set aside, willow and poplar such as organic waste or manure. Thus the

increasing of soil C in this pair experimental plot were mainly due to the differences of above ground SOM input quantity (e.g. leaves, branches) and below ground pools (e.g. root turn over or LFOM decomposition).

Within each land use type, plots of 11 m x 11 m were marked out. There were 9 plots section within the arable, set-aside and woodland areas. Within the SRC each of the 3 replicates for each genotype were sampled, giving a total of 9 plots for poplar and willow (Figure 11).

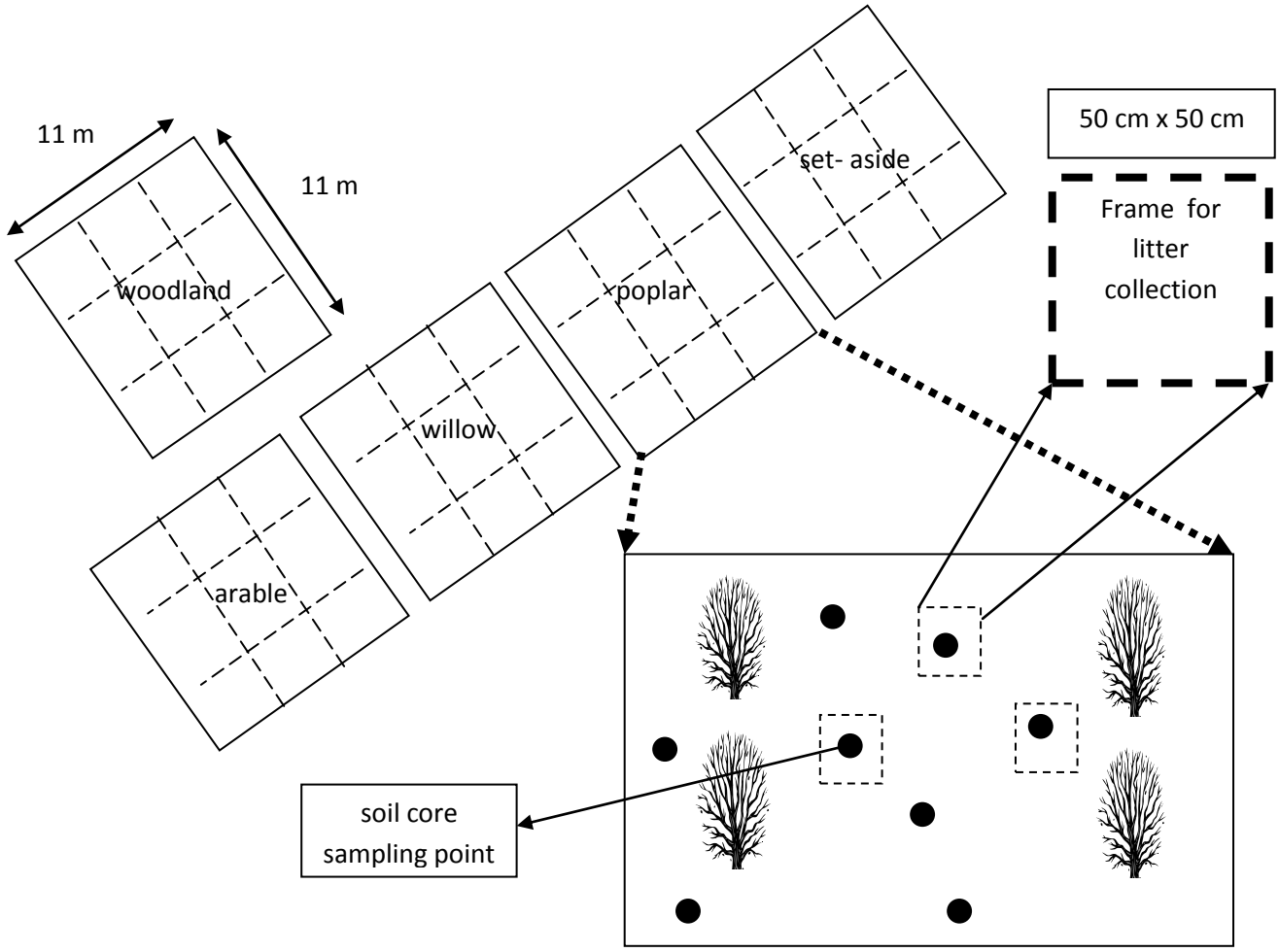


Figure 11. Soil and litter sampling points in field plots

Soil was sampled at 0-30 and 30-60 cm depths by taking cores from 9 random sampling points from within each land use plot in autumn 2008 using a hand auger (Figure 11). These were pooled. This provided a total of 9 composite samples for each land use type at each depth, giving 90 samples altogether.

Soil was collected in a zipped plastic bag. After partial drying overnight, the soil was sieved and passed through a 5 mm sieve, then stored at $4^{o}C$ until use. Bulk density was estimated using at total weight and volume of cores within each from each plots (Cambardella and Elliot, 1992; 1993).

2.4.4 Quantifying above ground biomass

The above ground biomass of the 3 different genotypes of willow and poplar, and the woodland trees was compared. The method for quantifying tree biomass followed a modified protocol from Nordh and Verwijt (2004) and was combined with the procedure of biomass sampling of the Forestry Commission (Forest Research, 2003a) and the plot experiment is a part of Forest Commission experimental plot to study the performance of selected SRC genotype across UK (Forest Research, 2003b).

The work was carried out initially by non destructive sampling using a digital calliper (Masser 2000 digital model, Finland) to measure the girth diameter of the willow or poplar stems. This was then corrected following field destructive sampling to estimate above ground biomass. The above ground biomass of arable and set aside was not measured as is more seasonal in nature and not comparable to tree based system (SRC or woodland), but information from published report of above ground of arable and set aside (grassland) have now been discussed. The measurement of SRC biomass were taken before the above ground biomass were harvested

Sampling was performed within a 11 m x 9 m area for poplar, a 5.5 m x 4.5 m for willow and 10 m x 10 m for woodland sites. The differences of plots size were due to the same number of stems being sampled and to represent tree diameter variability. Within each replicate plot all tree diameters at a defined height (1.0 m for poplar or woodland tree and 0.55 m for willow from soil surface) were measured (Telenius, and Verwijst, 1995).

Field measurement data was obtained using digital callipers for willow and poplar, whilst a measuring tape was used to determine tree diameter of those trees whose diameter was larger than the maximum measurable using the callipers. Then, the tree diameter was converted to total dry biomass using existing allometric equations for trees, as presented in Table 11.

Table 11. *Allometric equations available to predict above ground biomass*

Published by	Tree species	Allometric equation
Arevalo *et al.*, 2007 (Eq.1)	Willow	$W = 0.078 (D)^{2.847}$
Guidi *et al.*, 2008 (Eq.2)	Poplar	$W = 0.0914737 \times D^{2.30334}$
Zianis, 2008 (Eq.3)	Forest biomass (mixed trees species)	$W = 0.1464 (D)^{2.3322}$
D = Diameter of tree **W = Dry weight biomass**		

The destructive sampling was done by selecting 3 different diameters from each genotype of poplar and willow and cutting the stools at the root base. Accurate measurement of the girth diameter was conducted using a digital calliper. The calliper software was used to store a large amount of data before transferred to a computer. The biomass of each different diameter was weighed for the determination of fresh and dry weight following oven drying for 48 hours at 60°C (Uniterm drier-patent 658305,UK).

In order to determine the relationship between tree diameters (mm) and shoot dry weight biomass (kg), where W is the dry weight of biomass after drying in an oven at 60°C for 48 hours (Uniterm drier-patent 658305, UK).

The Root Means Square Error (RMSE) approach was used to calculate the difference between the predicted values of aboveground biomass from the existing models, and the actual destructive measurements from the Rothamsted field plots following a formula suggested by Arevalo *et al.,* (2007):

$$\% \text{ RMSE} = \frac{\sqrt{\sum_{i=0}^{n} (Wio - Wip)^2/n} \ x\ 100}{Wao}$$

Where, the *Wio* = observed dry weight biomass, *Wip* = predicted dry weight biomass, *Wao* = average observed dry weight biomass, and n = number of samples.

2.4.5 Quantification of surface litter

Litter was defined as the organic material above the mineral soil (i.e. organic layer equivalent to O horizon). The sampling was carefully performed in order to avoid contamination with minerals as much as possible (Vesterdal *et al.,* (2002). Surface litter was collected in woodland, willow and Poplar plantations by destructively sampling a 50 cm x 50 cm quadrat area, using a modified method from Johnson *et al.,* (2003). The quadrat was placed between the trees rows randomly. Surface litter was collected from the soil surface and fresh weight determined. Thereafter, the dry weight was determined by drying sub samples in a 60°C oven for 48 hours (Uniterm drier-patent 658305, UK). Plant root biomass was not measured as they may become a part of SOM fraction. The litter samples was collected and quantified in 3 replicates and collection was in October 2008.

2.4.6 Total organic/inorganic soil C, N, pH and bulk density.

2.4.6.1 Total C and N

Total soil C was determined by the dry combustion method using a LECO machine (CB-2000, LECO-Michigan, USA). Before analysis, soil was milled using a ball mill then 0.5 g samples were directly combusted. The samples were sent to Rothamsted Research for analysis. Inorganic C was determined using a Calcimeter (Eikelkamp P1.85-1, Netherland).

2.4.6.1.1 The principle of carbon analysis

Principally the LECO machine was used as a combustion method for measuring carbon and nitrogen with samples combusted in a combustion chamber. The chamber is heated to 1100°C and is filled with oxygen gas. The combustion process converts any elemental nitrogen into N_2 and NO_x and any elemental carbon to CO_2.

The analytical steps involve the combustion gases being swept out of the combustion chamber into a ballast tank. After the tank is filled the gas is released through an Infrared cell. The infrared source consists of nichrome wire heated to 850°C. The source radiates visible light and all wavelengths in the infrared (IR) spectrum. CO_2 absorbs IR energy at a precise wavelength. IR energy is absorbed as the gas passes through the cell, preventing it from reaching the detector. All other IR energy is filtered out by a bandpass filter. As CO_2 concentration increases the voltage output from the detector decreases.

2.4.6.1.2 The principle of nitrogen analysis

The principal concept of nitrogen analysis is similar to carbon analysis. After the tank is filled the gas is released through an aliquot loop and then swept by carrier gas (helium) through a catalyst (copper) heater where NO_x gases are reduced to N_2. CO_2 and H_2O are removed by passing through adsorbents leaving N_2 and helium to flow through one side of a Thermal Conductivity cell. Pure helium is fed through the other side of the cell and the difference in thermal conductivity between the two sides of the cell results in an output voltage which is measured. Before the analysis was performed, a calibration steps were conducted.

2.6.4.1.3 Calibration

The analyser is calibrated using EDTA (41.02% C and 9.57% N) and the baseline is also corrected for drift using blank samples.

2.4.6.1.4 Sample analyses

Sample analyses were performed following the determination of five blank samples and 2 EDTA calibrated samples (0.3 g) and the helium gas along with oxygen were supplied continuously to the machine at the pressure of 40 psi. Ceramics boats were weighed in a balance (Mettler AB54) before EDTA calibrated samples were added. EDTA filled boat ceramic and five blank samples were inserted into the LECO sample rack prior to calibration and analysis. Following calibration the LECO machine was ready for analysing a sample. The ceramic boat was filled with 0.3 g oven dry milled soil sample and inserted into LECO rack and the same step for measuring EDTA and blank samples were repeated.

2.4.6.1.5 $CaCO_3$ (inorganic C) determination

5 g finely ground soil was placed into polythene cup and transferred into a 100 ml conical flask. 5 g was recommended for low carbonate soil and 1 g for high soil carbonate concentration. Buffer vessels were adjusted to 3 ml using the burrette scale as a starting point. 20 ml deionised water was added to the conical flask, whilst at the same time 7 ml of 4 M hydrochloric acid (HCl) was placed into a small glass test tube and using tweezers carefully inserted inside the reaction vessel.

The silicon stoppers were dampened and the conical flask was sealed by turning the lever to the measuring position. By tilting the reaction vessels, the HCl flowed from the test tube over the material to be analysed and the reaction was initiated. The full reaction was 60 minutes, and flask should be swirled every 15 minutes to allow the reaction to become completed. After 60 minutes readings were taken from the burrette scale. The initial 3 ml reading and blank values were subtracted from the final reading using a callibation curve of $CaCO_3$ at different concentrations in soil. The determination of % $CaCO_3$ in soil followed the formula described below:

$$\% \ CaCO_3 \text{ in sample} = \frac{\textbf{amount CaCO3 in soil x 100}}{\textbf{weight of soil sample}}$$

$$\text{Inorganic \% C in sample} = \frac{\textbf{\% CaCO3 (determined) x C (12.011)}}{\textbf{CaCO3 (100.0872)}}$$

2.4.6.2 Soil pH

Soil pH was determined using a pH meter (AR50-Accument Research, USA) and a ratio of H_2O and soil of 1:2.5. All soils were air dried and sieved to 2 mm. 10 g

soil was weighed into a 50 ml centrifuge tube. 25 ml r.o. water was added and shaken for 15 minutes into a Griffin bottle shaker (Equipment No SHA122). The pH was measured immediately, without filtering. For calibration, pH standard solution at 4 and 7 were used. When the regression line of pH meter showed a value above 0.99, the calibration lines was accepted. pH was determined from each individual soil sample and were therefore replicated 9 times for each land uses.

2.4.6.3 Bulk Density

Samples were taken for soil bulk density (BD) analysis from different position of the plot experiment to cover variability. After collection soil was weighed, coarse root, plant residue and stones were removed. As stone can have significant effects on soil BD, their volume was determined by placing it in a glass volumetric (500 ml) after 250 ml water was added. The total stone volume was determined by determining the volume of water displaced by the stones. The soil BD assay was determined by following a method developed by Don *et al.*, (2007), as below:

$$\textbf{BD fine soil} = \frac{mass\ soil\ sample - mass\ stone}{volume\ soil\ sample - (mass\ stone)/(\rho\ stone)}$$

The ρ stone = density of stone (mass volume^{-1}) value was estimated at 2.6 g cm^{-3}. The BD was used to determine total soil organic carbon (SOC) stock. The *i* is referred to the soil depth (cm). Thus, the total SOC stock was derived as follows:

$$\textbf{SOC}_{\textbf{stock}} = \sum_{i=1}^{n} BD\ fine\ soil, i * SOC, i * depth\_volume\ \ , i$$

Bulk Density value was determined for each land use type by taking the average of the 9 measurement taken from each soil depth (0-30 cm) and (30-60 cm).

2.4.7 Soil microbial biomass C and N

Before determining microbial biomass, the soil water status was determined based on a soil water tension measurement following a protocol developed by Deka *et al.*, (1995). Total water holding capacity (WHC) of the soil was determined by soaking a 100 g soil sample for 12 hours in a plastic jar (diameter = 50 mm and height = 65 mm) with 0.2 mm nylon cloth over the base. 3 replicates from each upper horizon (0-30 cm) and lower horizon (30-60 cm) were analysed.

The soils were drained overnight before being measured for saturated water content by drying in the oven for 3 days at 60°C. Soil water tension (SWT) was determined by measuring the water content of the filter paper after 6 days of incubation. A linear regression was used to derive a soil water tension value from the soil water content.

Microbial biomass was measured using the fumigation-extraction method described by Joergensen and Brookes (1990). Approximately 20 g of soil was fumigated with chloroform for 24 hours, then the soil was extracted using 2 M K_2SO_4 before shaking using a centrifugal end over end shaker (custom made-MEM single phase motor-2SPS, UK) for 30 min and filtering through a Whatman No. 42 paper. Non-fumigated samples were extracted at the same time as the fumigated soil. The soil extract was collected in sterilised 50 ml plastic tubes and stored at -20°C before use.

For measuring microbial biomass N, ninhydrin reactive N was measured according to Joergensen and Brookes (1990), as modified by Bending and Turner

(2009). Standard curves were developed using different concentrations of ninhydrin solution (Sigma Aldrich, UK). The absorbances were detected spectrophotometrically using a UV VIS spectrophotometer (ATI-UNICAM 5625, USA) at a wavelength of 570 nm. A conversion factor (3.1) was used to determine microbial biomass N (Joergensen and Brookes, 1990). The microbial C assessment was modified from Wu *et al.,* (1990). 4 ml of soil extract was mixed with 4 ml sodium hexametaphosphate buffered at pH 2. The solution was filtered through a Millipore 45 µm filter before 1 ml of solution was directly injected to the total organic carbon (TOC) analyser (Dohrmann-80) at the Scottish Agricultural College (SAC), Scotland. A conversion factor (2.6) was used to determine biomass C (Vance *et al.,* 1987a).

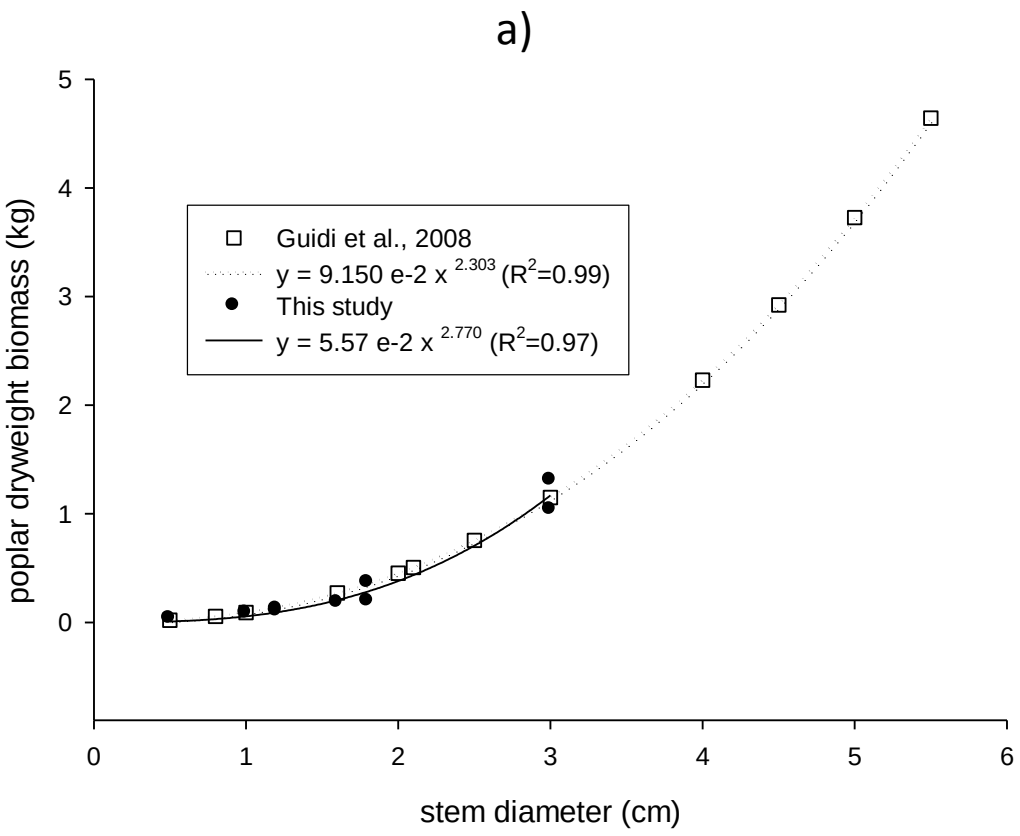
2.4.8 Statistical analyses

All results were analyzed using analysis of variance (ANOVA) in (Gentstat-Release 11, VSNI-UK) to determine the significance of differences in aboveground biomass and soil parameters between land uses. Before statistical analyses, the normality of the results were checked and plotted in two dimensional graphs. Least square difference (LSD) was used to determine any significant differences between treatments.

2.5 Results

2.5.1 Validation of the allometric equation

Both of field and estimated poplar/willow biomass measurement from the equation were plotted in 2D graph (Figure 12a and 12b). The Root Means Square Error (RMSE) approach was used to calculate the difference between the predicted values of aboveground biomass from the existing models, and the actual destructive measurements from the Rothamsted field plots. The average RMSE values for poplar and willow were 22.43 and 69.30%, respectively. The low RMSE values in poplar (<30 %) indicated that the data fitted well with the existing published model by Guidi *et al.*, (2008) and those equation was acceptable for UK condition.



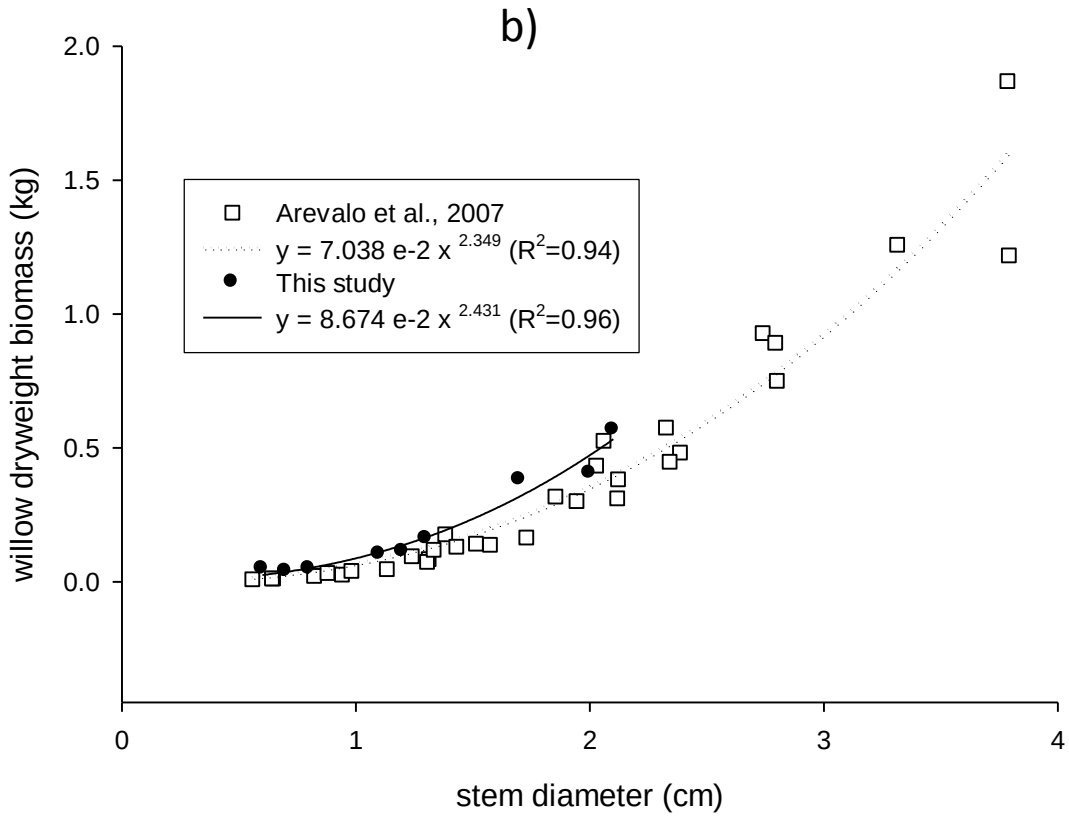


Figure. 12. The relationship between diameter and dry weight of the above ground biomass of Poplar (12a) and Willow (12b)

Since high RMSE values were observed in willow, a new equation for field measurements was required based on the actual measurement taken in this study as the use of existing equations by Arevalo *et al.*, (2007) seemed to result in underestimated above ground willow biomass.

2.5.2 Above ground biomass and litter accumulation

The results of 2-way ANOVAs showed that land use significantly affected the amount of above ground biomass ($P<0.05$) (Figure 13a and 13b).

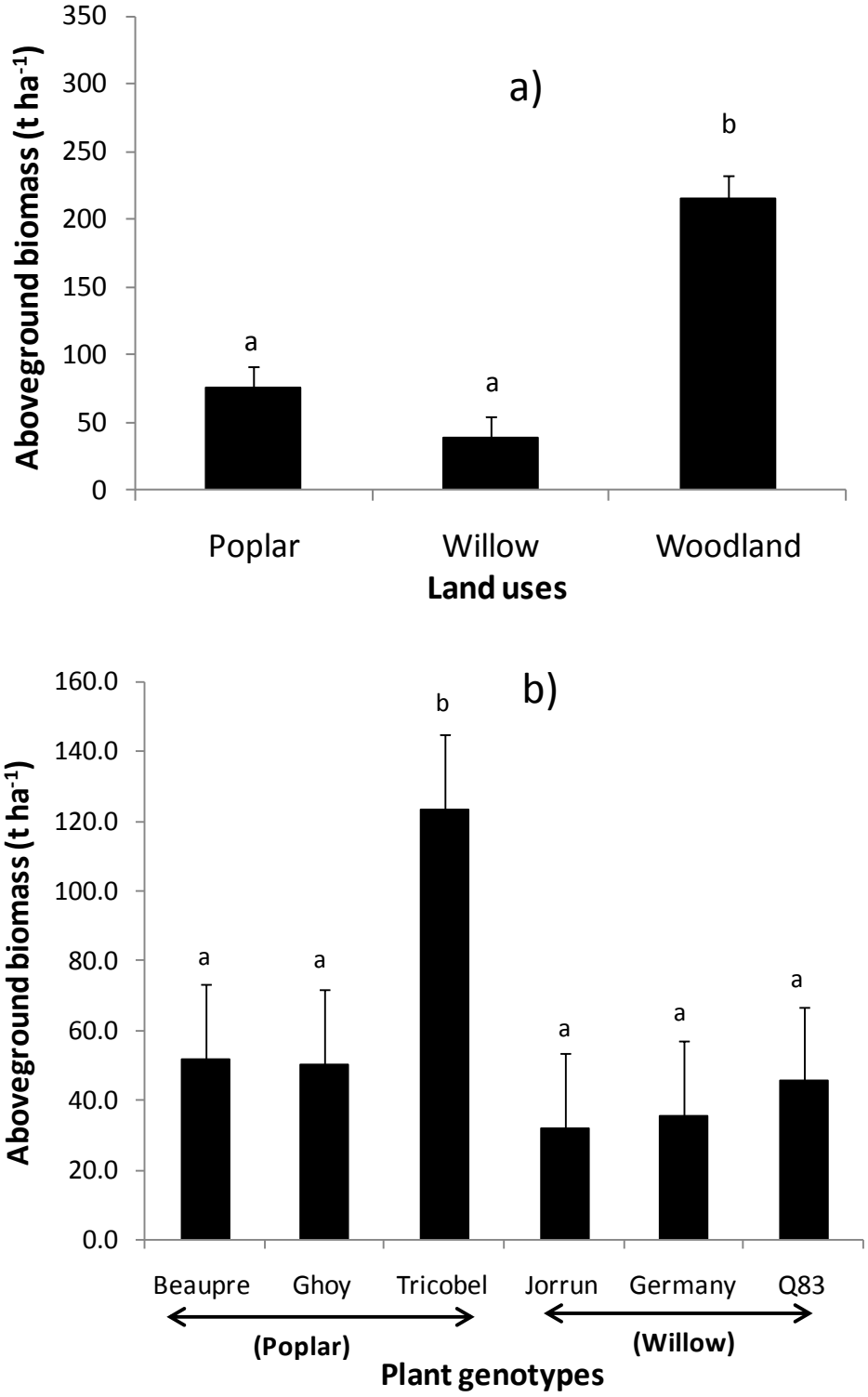


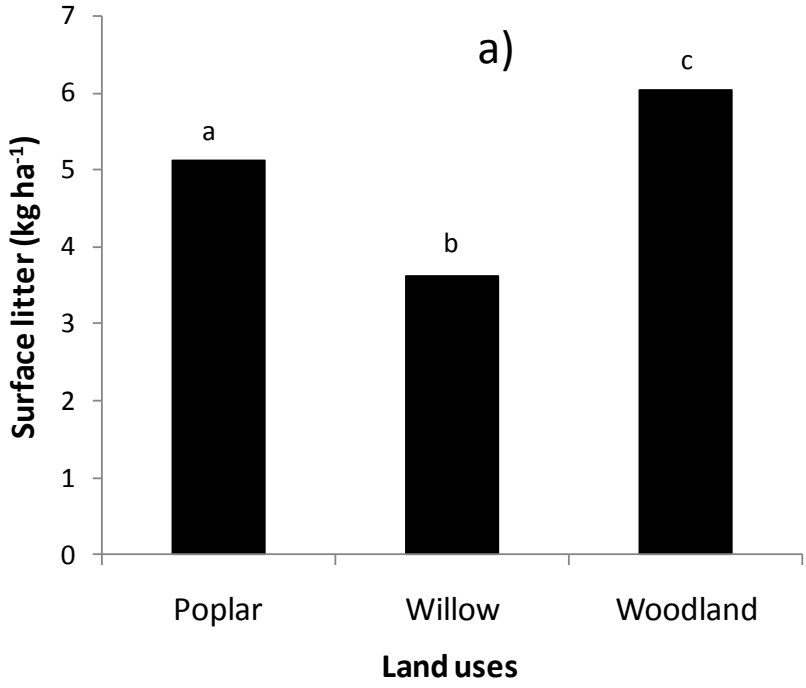
Figure 13. Above ground biomass under different land uses (13a) (LSD (1) = 33.38 and between poplar willow genotypes (13b) (LSD (1) = 43.11. Different letters denote significant differences (P<0.05)

Woodland (which was dominated by Beech and Oak species) accumulated the highest amount of aboveground biomass at 215.60 t ha^{-1}, whilst the lowest amount of biomass was found in willow at 37.85 t ha^{-1} and biomass of poplar was 75.03 t ha^{-1}.

The data also illustrated significant differences in above ground biomass between poplar genotypes ($P<0.05$) (Figure 13b). Of the poplar genotypes, Tricobel accumulated above ground biomass at 123.33 t ha^{-1}, which was significantly higher than the amount of aboveground biomass for Beaupre (51.66 t ha^{-1}) and Ghoy (50.09 t ha^{-1}). However, there were no significant differences between the willow genotypes (Jorrun, Germany and Q83), accumulated above ground biomass at 31.85, 35.58, and 45.36 t ha^{-1}, respectively.

Unfortunately the harvesting schedule of poplar and willow was not recorded precisely. Informal communications with Ian Shield (Rothamsted-Research) revealed that some of the Poplar genotypes were coppiced at least 9-10 years ago, whilst for willow coppicing was only performed 4-5 years ago. It is therefore not possible to conclude that poplar can sequester more carbon in above ground biomass than willow unless annual carbon sequestration is determined.

The previous equations (Arevalo *et al.,* 2007) for predicting willow aboveground biomass seemed to be lower estimated by 10 to 20% depending on the cultivar.



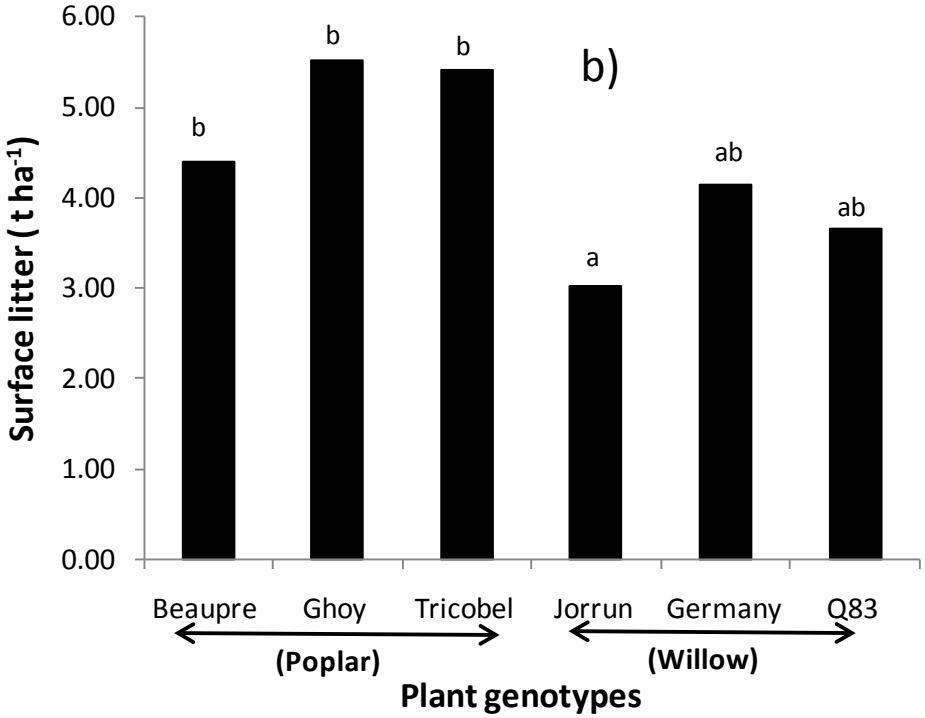


Figure 14. Surface litter accumulation under different land use (14a) (LSD=706) and between poplar and willow genotypes (14b) (LSD = 1255.3). Different letters denote significant differences (P<0.05)

There were significant differences (2-way ANOVA; P<0.01) in surface litter accumulation between woodland, poplar and willow plots (Figure 14a). There was greater litter accumulation in woodland compared to poplar or willow. The amount of litter present in woodland was 15 and 40% higher than for poplar and willow, respectively.

Figure 14b shows that there were significant differences in litter accumulation between poplar and willow genotypes (P<0.01). Ghoy and Germany produced the greatest amount of litter for the poplar and willow genotypes with 5.5 t ha^{-1} and 4.1 t ha^{-1}, respectively. Of the willow genotypes, even though Q83 accumulated the highest aboveground biomass, it did not produce the highest amount of surface litter. Similarly for the poplar genotypes, the highest aboveground biomass was found in Tricobel, but this cultivar did not produce the greatest amount of litter.

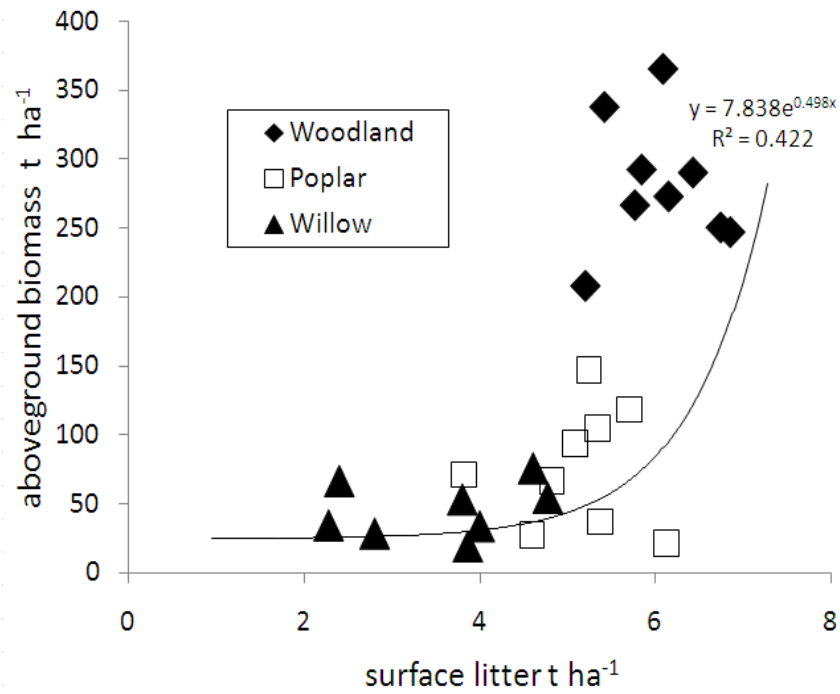


Figure. 15. The relationship between surface litter accumulation and above ground biomass

There was a significant correlation (R^2 = 0.4229; P<0.05) between above ground biomass and surface litter, when data from woodland, poplar and willow plots were combined (Figure 15). Increases in surface litter were influenced by increases in aboveground biomass.

2.5.3 Soil C and N stock under different land uses

Land use significantly affected the amount of soil C at both 0-30 cm and at 30-60 cm depths. At 0-30 cm, arable and set aside had significantly lower (P<0.05) levels of organic C than poplar, willow and woodland. There was no difference in soil C between willow and woodland, which were significantly higher than poplar. In sub soil, the woodland soil C content was 1.102 %, which was significantly higher (P<0.05) than the other land uses which with the exception of willow, were not significantly different (Table 12). There was no significant difference in soil C between different cultivars of willow or poplar.

Table 12. *Total soil C and N across land uses*

	Soil depth (cm)	**Arable**	**Poplar**	**Set aside**	**Willow**	**Woodland**	**LSD P<0.05**
C (%)	0-30	1.90	2.45	2.03	2.87	3.12	0.31
	30-60	0.76	0.72	0.68	0.80	1.10	0.14
N (%)	0-30	0.19	0.21	0.18	0.24	0.27	0.02
	30-60	0.09	0.08	0.08	0.09	0.11	0.01

Table 12 illustrates that there were significant differences in soil N under different land uses (P<0.01), both in the upper and lower soil layer. Soil N at 0-30 cm was higher than at 30-60 cm. The woodland site had the greatest soil N (0.27%) at 0-30 cm, whilst the lowest amounts of soil N were found in arable (0.19%) and set aside (0.18%). Poplar and set aside had lower amounts of soil N at 30-60 cm compared to other land uses.

There were no significant differences recorded in Bulk Density (BD) amongst the land use types at 0-30 cm soil depths. The significant different of soil bulk density was observed at lower depth (30-60 cm), where the highest was in arable (1.25 g cm^{-3}) and the lowest was in willow (0.93 g cm^{-3}) (Table 13).

Table 13. *Bulk Density, soil C and N stock amongst land uses and soil depth*

	Soil depth (cm)	Arable	Poplar	Set aside	Willow	Woodland	LSD (P<0.05)
BD fixed depth (g cm^{-3})	0-30	1.32	1.20	1.03	1.39	1.02	0.37(ns)
	30-60	1.25	1.15	1.21	0.93	1.11	0.17
C stock (t ha^{-1})	0-30	75.6	88.3	63.0	**119.8**	95.5	11.27
	30-60	30.11	25.95	21.15	33.42	**33.75**	5.65
Total C stock	**0-60**	**105.71**	**114.25**	**84.15**	**153.22**	**129.25**	
N (t ha^{-1})	0-30	7.67	7.60	5.78	**10.27**	8.47	0.90
	30-60	3.87	3.15	2.54	**4.03**	3.66	0.48
Total N stock	**0-60**	**11.54**	**10.75**	**8.32**	**14.3**	**12.13**	

Land use significantly affected SOC stocks at both soil depth (0-30 cm) and (30-60 cm) ($P<0.05$). The values varied from 63.0 in set aside to 119.8 t ha^{-1} in willow. Soil C stocks at 0-30 cm were higher ($P<0.05$) in willow relative to poplar by 30% and 40% to arable (Table 13).

In the lower layer, SOC stock ranged from 21.15 in set aside to 33.75 t ha^{-1} in woodland which was accounted for 30–40% that those of the SOC stocks in the upper layer (Table 13). At this depth there was no significant different of SOC stock between willow, arable and woodland.

The amount of SON stock was around 10 to 15% of the SOC stock in both layers. Willow had higher SON stocks at both depths compared to the other land uses, which were significantly different to other land uses. The lowest SON stocks in both layers have been observed in set aside.

Total C stock within 0-60 cm soil depth in arable, poplar, set aside, willow and woodland were 105.71, 114.25, 84.15, 153.22, 129.25 t ha^{-1}, respectively and 11.54, 10.75, 8.32, 14.3 and 12.13 for total SON stock (0-60 cm) (Table 13).

Net annual C sequestration (t C ha^{-1} $year^{-1}$) for each land use systems calculated based on the differences of carbon storage to arable cropping (as a reference). The land use conversion period for set aside, poplar and willow was 14 years, whilst woodland had been established over 300 years ago (Keith and Goulding, 2003). The estimation of annual C sequestration is presented in Table 14. As there was no clear information of woodland land use historical establishment, this land uses is not a good pair for the other plots and only use as a comparison plot. Woodland soil C stock can be considered as a maximum carbon and nitrogen stock that can be achieved by other land uses. The rate of soil C stock were calculated only for poplar, willow and set aside plots, as this was can be assumed as paired site experiment using arable as a reference.

Table 14. *Net annual C sequestration in set aside, willow and poplar* (t C ha^{-1} $year^{-1}$).

Land use type	Arable to set aside (14 years conversion)	Arable to willow (14 years following conversion)	Arable to poplar (14 years following conversion)	LSD (P<0.05)
C sequestration t C ha^{-1} $year^{-1}$ (0-60 cm)	-1.54	3.39	0.62	0.98

The highest annual C sequestration was found in willow (3.39 t C ha^{-1} $year^{-1}$), which was almost five times that of poplar (0.62 t C ha^{-1} $year^{-1}$). In comparison, the accumulation of carbon in set aside was negative (-1.54 t C ha^{-1} $year^{-1}$). This meant that there was a loss of C accumulation subjected to rapid SOM decomposition, erosion process or leaching. A ratio between soil C stocks in the upper layer to lower layer in this current study is presented in Table 15. This was to identify the proportion of upper and lower soil layer to total C storage.

Table 15. *Ratio between soil carbon stocks at 0-30 relative to 30-60 cm and contribution of upper soil layer C stock to total C stock amongst different land use type.*

Land use	Arable	Poplar	Set aside	Willow	Woodland
Ratio soil C stock	2.51	3.40	2.98	3.58	2.83

From Table 15, it can be seen that the ratio of soil C stock between upper and lower layer ranged between 2.51 to 3.58 and 1.98 to 2.55, respectively with the lowest ratio was found in arable soil and the highest was in willow soil followed by poplar and woodland. This result was to confirm that SRC crop of willow and poplar were substantially greatly accumulated C relative to arable, set aside or native woodland.

2.5.4 Soil pH

2 way ANOVA showed that land use significantly affected the soil pH ($P<0.05$) at both soil depths (Table 16). In the upper layer, soil pH in woodland plots was 5.79, which was the lowest among the land uses, followed by poplar (6.84). The highest soil pH was found in arable plots (7.67). In general, the soil pH of the lower

layer (30-60 cm) was higher than the upper layer and no significant differences were found amongst land uses except for woodland.

Table 16. *Average soil pH values under different land use types. Different letters denoted significant differences (P<0.05). Different letter denoted significant differences (P<0.05)*

	Soil depth (cm)	**Arable**	**Poplar**	**Set aside**	**Willow**	**Wood land**	**LSD** (P<0.05)
pH	0-30	7.67(c)	6.84(b)	7.02(b)	7.32(c)	5.79 (a)	0.46
	30-60	7.75(b)	7.69(b)	7.58(b)	7.82(b)	7.04(a)	0.47

There were no significant differences in soil pH across different genotypes of Willow or Poplar in the upper or lower soil layers.

2.5.5 Soil microbial biomass C and N

There was a significant influence of land use on soil microbial C (P<0.05) at 0-30 cm and 30 – 60 cm soil depths. In the upper layer, the lowest soil microbial biomass C was found in set aside soil (168 µg g^{-1}) which was significantly lower than woodland soil (Figure 16a), but not different to arable soil. Microbial C in the poplar and willow soils was not significantly different to the woodland soil.

In contrast, in the lower layer, poplar soil had the lowest amount of microbial C, at 38 µg g^{-1}, which was not significantly different to arable, set aside and willow soil. However, these had significantly lower microbial C levels (P<0.05) than woodland soil, which had a soil microbial C value of 81.5 µg g^{-1}. This was 4 times less than microbial C in the upper soil depths.

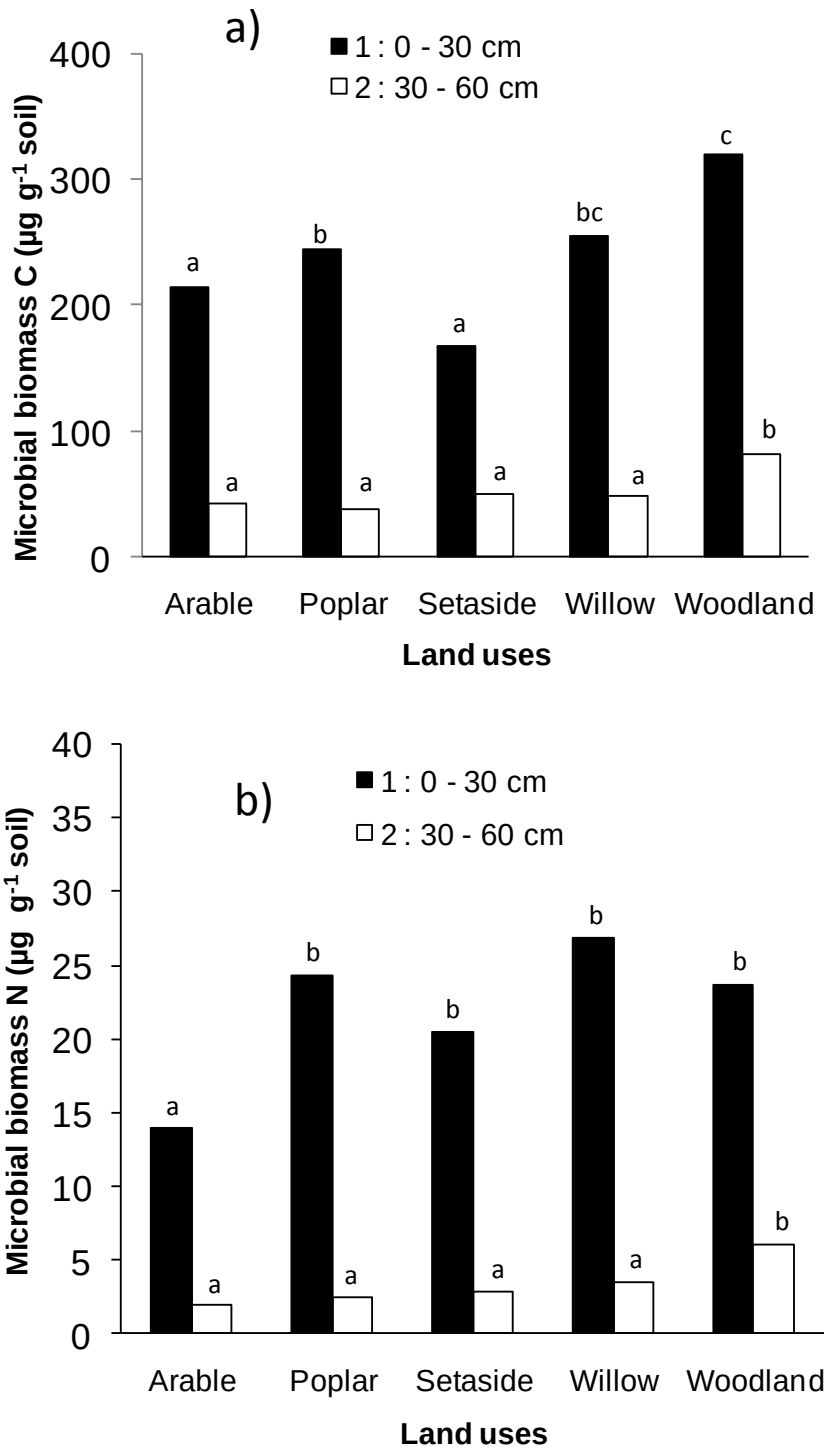


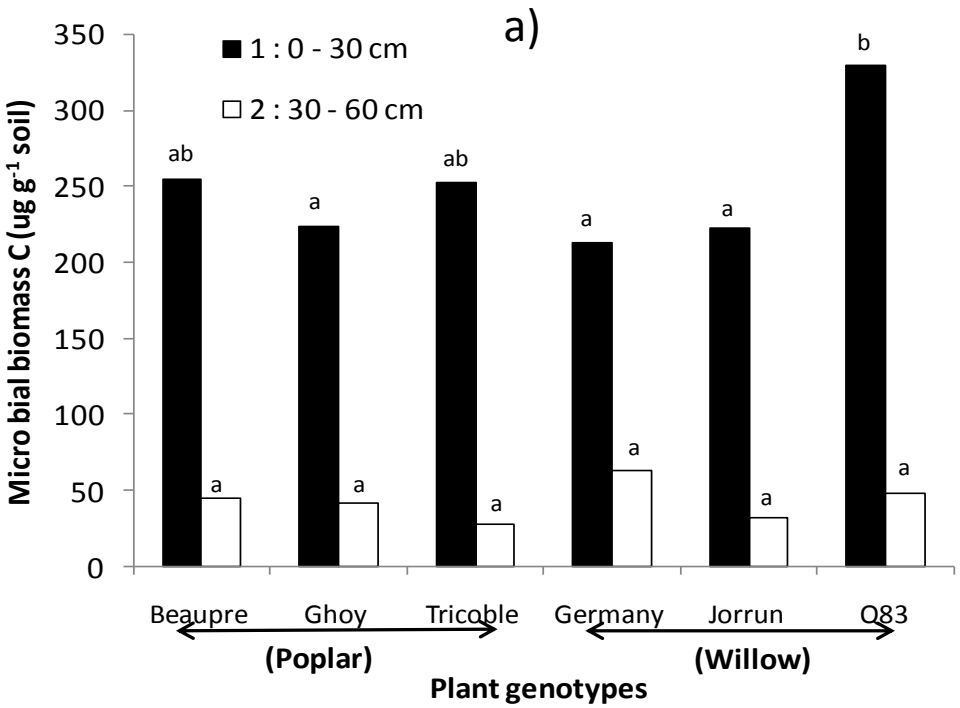
Figure 16. Soil microbial biomass C (16a) (LSD(1) = 64.00; LSD (2) =25.36) and N (16b) (LSD(1) = 7.39 ; LSD (2) = 2.45) under different land uses. Different letters denote significant differences (P<0.05)

Soil microbial N was influenced by land use (P<0.05) at both soil depths. The soil microbial N in the arable samples was 13.9 µg g^{-1}, which was significantly

lower (P<0.05) than in all of the other land uses. There were no significant differences between the other land uses (Figure 16b).

The highest microbial N in the lower layer was found in woodland soil at 5.95 µg g^{-1} which was significantly different to the other land uses. (P<0.05) The amount of biomass N in sub soil was only about 25% that of the upper layer. The distribution of microbial biomass C between different land use types was similar to that of microbial biomass N.

There were no significant impacts of poplar genotype on soil microbial C and N (P>0.05) in the upper or lower layers (Figure 17a and 17b). However, for willow cultivar Q83 had significantly higher (P<0.05) microbial biomass than the other genotypes at 0-30 cm depth.



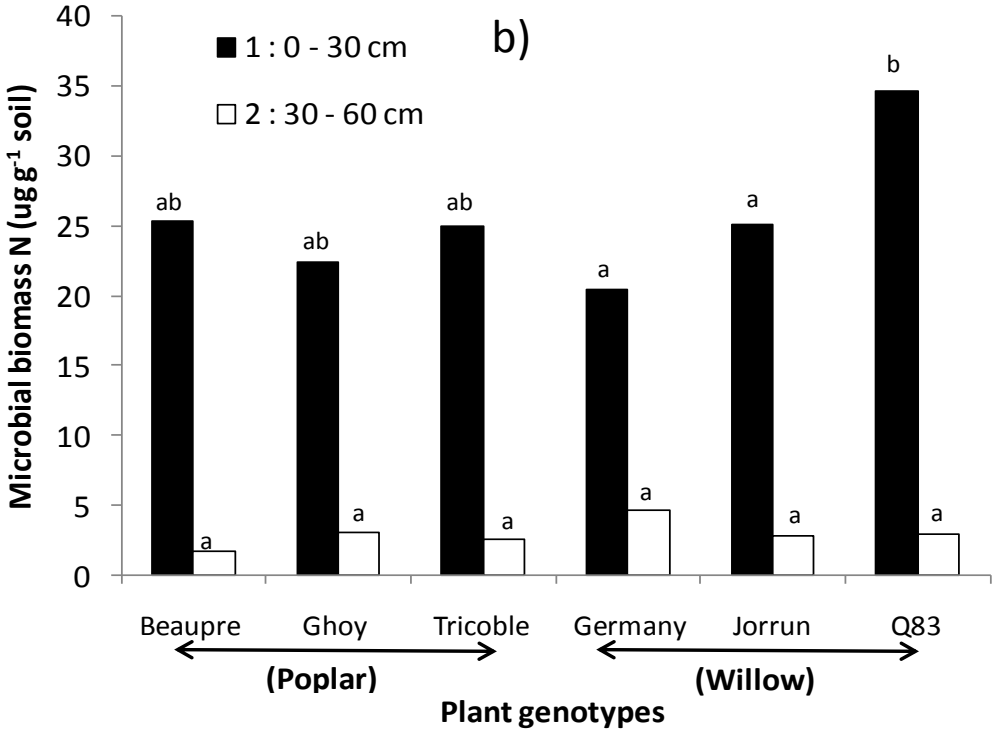
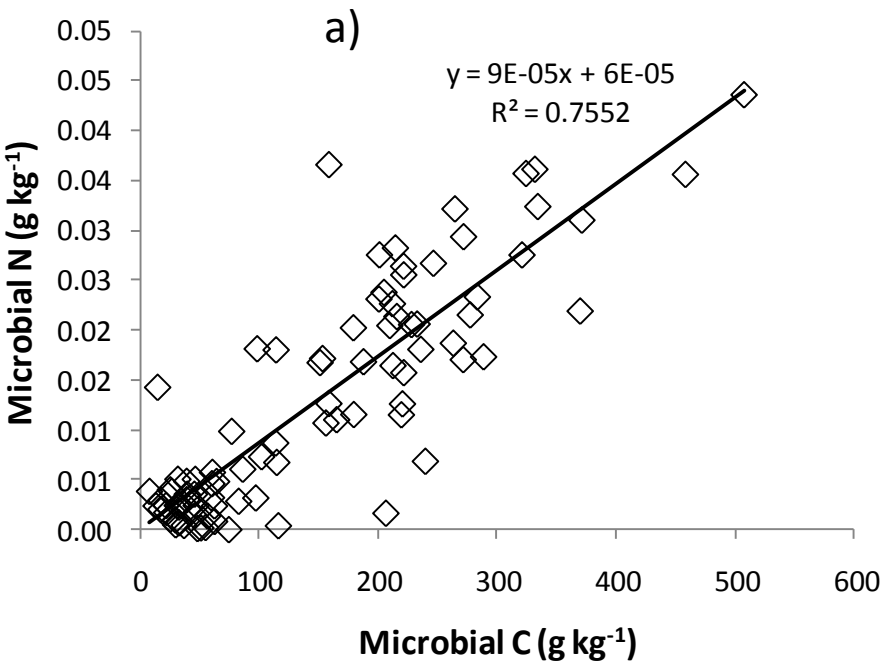


Figure 17. Soil microbial biomass C (17a) (LSD(1) = 77.1 (1) ; LSD (2) = 43.05) and N (17b) (LSD(1) = 11.04 ; LSD(2) 3.94) under different plant genotypes. Different letters denote significant differences (P<0.05)

Soil organic C was significantly correlated with soil microbial biomass C when the 90 soil samples from different land uses were combined together (R^2 = 0.93) (Figure 11a)

The relationship between microbial biomass N and C followed a linear relationship and a significant correlation (R^2 = 0.755) along with microbial C total C (R^2 = 0.84) was identified (Figure 18).



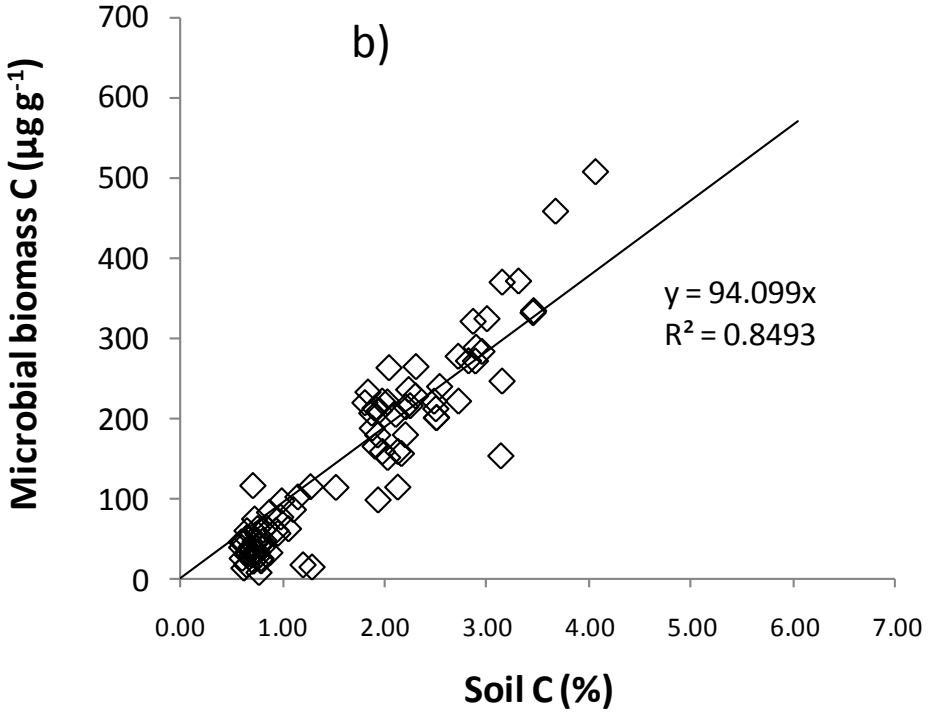


Figure 18. Relationship between soil microbial biomass C and soil microbial N (18a) and soil microbial C and total soil C (18 b)

The ratio between C_{mic} and C_{org} in the upper layer compared to lower layer in this current study is presented in Table 17. The ratio between soil carbon stocks and microbial C under different land use was not significantly different. However, there were significant differences between soil depths.

Table 17. *Ratio of C_{mic} and C_{org} across different land use and soil depths.*

Ratio C_{mic} to C_{org}	Arable	Poplar	Set aside	Willow	Woodland	LSD
0–30 cm	1.12	0.99	0.82	0.88	1.00	0.159 ($P<0.05$)
30–60 cm	0.56	0.51	0.72	0.58	0.64	

The positive correlation between the C_{mic} : C_{org} and soil N across different land uses and soil depths is shown in Figure 19 (n=90 data points). These results indicated a relationship between microbial growth and substrate/nutrient availability, particularly with regards to the use of soil N sources by soil microorganisms.

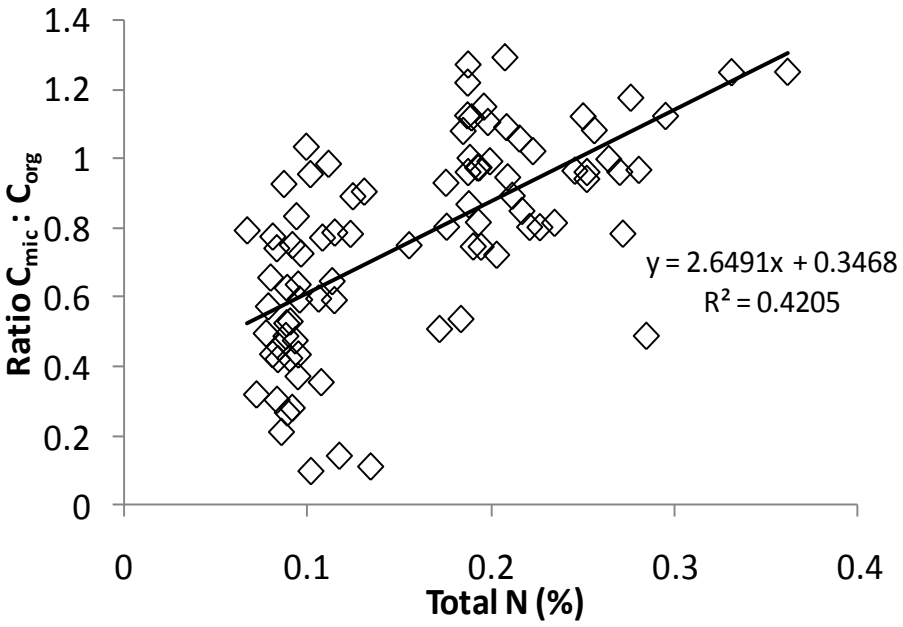


Figure 19. Linear regression between ratio C_{mic} and C_{org} and total N

2.6 Discussion

2.6.1 Aboveground biomass equation

Methods for biomass estimation for willow (*Salix spp*) (Hytonen, 1995; Telenius and Verwijst., 1995; Arevalo *et al.,* 2007) and poplar (*Populus spp*) (Zabek and Prescott *et al.,* 2006; Guidi *et al.,* 2008) bio-energy crops have been developed by other authors using non-destructive sampling. Much research has been undertaken estimate and validated these methods using destructive sampling, but this is time consuming and laborious (Verwijst and Nordh, 1992).

Most of the existing non destructive sampling equations have been constructed using young plantations of bioenergy crops of willow and poplar (e.g 2 year old *Salix dasyclados* and 3-4 years old *Salix vimimalis* (Telenius and Verwijst, 1995; Nordh and Verwijst, 2004), 2 years *Populus deltoides* (Guidi *et al.,* 2008). These allometric equations were developed under different climatic conditions and geographical locations (e.g. *Populus trichocarpa* x *Populus deltoides* in Canada (precipitation rate = 2201 mm year^{-1}) (Zabek and Prescott, 2006), or *Populus deltoides* in Italy (precipitation rate = 900 mm year^{-1}) (Guidi *et al.,* 2008)).

It has been hypothesized that in older coppice systems, there is a reduction in biomass yield after several cycles of coppicing which prevents the optimum biomass quantity for being obtained (Arevalo *et al.,* 2007).

Furthermore, non destructive methods (such using digital calliper) for biomass estimation of specific cultivar/genotypes, might be not applicable to others, because of differences in stem shape or the branching pattern of the above ground biomass (Verwijt and Nordh, 1992). In addition, published equations span across a range of densities, ages, and management practices (Arevalo *et al.,* 2007). It is therefore unclear whether these existing allometric equations are applicable for UK conditions.

In this study, the performance of the existing equations reported in the literature to a mature bioenergy crop (14 year old) to estimate carbon storage were tested. Using of the equations developed by Guidi *et al.,* (2008) a good estimation of above ground poplar biomass was obtained. This equation produced a similar value of above ground biomass to those values from field measurement. Different results were found for willow biomass estimation, using an exponential equation from Arevalo *et al.,* (2007). There was a lower estimation of aboveground biomass at tree diameter of 30 cm by 10-20 %. Therefore, the regression coefficient of the existing equation from Arevalo *et al.,* (2007) was not use for determining willow biomas in this current and alternatively a new allometric equation from field measurement was employed, as a better relationship was yielded (R^2=0.96).

The discrepancy between the predicted and observed biomass might have been due to differences in tree population, diameter classes, and tree height. The bias of several published equations ranged from 1 to 45% (Zabek and Prescott, 2006). To improve the accuracy of biomass yield determination, the equation should accommodate other parameters such as: tree height (H) or diameter at breast height (DBH)/H ratio, tree geometry or wood density (Campbell, 1985; Zabek and Prescott, 2006).

2.6.2 Bioenergy crop growth and biomass

The aboveground yield of willow is generally higher than poplar (Aylott *et al.,* 2008), which is comparable to the results of this study if we assume that the rotation time for poplar was longer (8 to 10 years) than willow, in which biomass can be harvested within a shorter period (3 to 4 years). Current data sets for above ground

biomass in bioenergy crops are mostly from the first and second year of the rotation with only a few from older plantations (Kauter *et al.,* 2003).

2.6.2.1 Poplar biomass

Different cultivars and geographic locations influence above ground poplar biomass quantity. Hybrid poplar cultivated in the USA, yielded 21-35 t ha^{-1} $year^{-1}$ (Scarascia-Mugnozza *et al.,* 1997), whilst in Scotland the yield of 5 year old hybrid poplar (*Populus balsamifera*-Michauxii x *Populus trichocarpa*-Hastata) was 51 to 81 t ha^{-1}, with average production approximately 10 t ha^{-1} $year^{-1}$ (Proe *et al.,* 2002). In China similar 6 year old plantations of three different poplar cultivars (i.e I-69: *Populus deltoides* Bartr. cv. Lux; I-72: *Populus euramericana* Dode Guinier cv. San Martino; NL-80351, a hybrid clone of I-69 x clone I-63 *Populus deltoides* Bartr cv. Harvard) were reported have aboveground biomass values between 10.5 and 13 t ha^{-1} $year^{-1}$, under a planting density of 1111 stems ha^{-1}, where the average stem diameter was 18 cm for NL-80351, 17.4 cm for I-69 and 17.0 cm for I-72 (Fang *et al.,* 1999). In this study the average polar biomass was recorded at 75.03 t ha^{-1}, which was equated to 9.37 t ha-1 year, in which within the range of previous study (Zabek and Prescott, 2006; Swamy *et al.,* 2006)

As a comparison, hybrid poplar from a 12 year old plantation in British Columbia-Canada, which was dominated by *Populus trichocarpa*-Torr x *Populus deltoides*-Marsh has been reported to accumulate from 9.2 to 13.6 t ha^{-1} $year^{-1}$ (Zabek and Prescott, 2006). In India (a humid tropical region), the production of aboveground biomass by five cultivars of introduced poplar (*Populus deltoides*) was reported to be between 48.5 and 62.2 t ha^{-1} 6 years after planting and showed biomass accumulation of between 8 and 10 t ha^{-1} $year^{-1}$ (Swamy *et al.,* 2006).

Planting density also influences poplar plantation biomass production (Fang *et al.*, 2007). After 10 years, the highest total plantation carbon storage in poplar plantations was at a density of 1111 stems ha^{-1}, in which biomass was 72.0 t ha^{-1}. This was 5.4%, 11.9% and 24.8% higher than in plantations with 833, 625 and 500 stems ha^{-1}, respectively (Fang *et al.*, 2007).

Due to the wide range of poplar and willow genotypes and their adaptability, further studies on aboveground biomass production on other sites and with other genotypes are necessary.

2.6.2.2 Willow biomass

The differences in above ground willow biomass accumulation reported in the literature could result from several environmental factors (e.g. climate, geographical position, soil, etc). Site specific conditions such as water holding capacity, soil depth, soil fertility and weed competition can also affect aboveground biomass accumulations (Arevalo *et al.*, 2007). The best growth performance of two willow cultivars (*Salix discolor* and *Salix vinimalis*) in Quebec, Canada was found when they were cultivated in clay soil compared to sandy soil. The biomass production was more than doubled (61.97 vs 28.73 t ha^{-1}), with average yields of 20.65 t ha^{-1} $year^{-1}$ for clay soil and only 9.57 t ha^{-1} $year^{-1}$ for sandy soils (Labrecque and Teodorescu, 2003).

A study by Nordh and Verwijst, (2004) revealed that the aboveground biomass of 11 cultivars from 3 year old willow plantations (*Salix vinimalis*) in Sweden, yielded between 23.6 to 37.2 t ha^{-1}. This equated to an average biomass accumulation of 10.13 t ha^{-1} $year^{-1}$.

Other than soil type, the other factor that influences the total aboveground biomass is temperature (Tahvanainen and Rytkonen, 1999). The biomass production of a 3 year old willow plantation in Southern Finland (9.2 to 16.28 t ha^{-1}) was higher than northern part (North Carelia) (0.86 to 4.51 t ha^{-1}). The significant differences in the biomass accumulation were due to frost damage which can reduce the total above ground biomass by nearly 100%.

Meanwhile, fertilizer improved willow aboveground biomass (*Salix discolor* and *Salix vinimalis*) by 10% and the application of fertilizer (e.g. N, P, and K) at different concentrations, improved the volume of 3 poplar cultivar stems (*Populus balsamifera* x *Populus trichocarpa*, *Populus balsamifera* x *Populus maxmimowiczii*, *Populus maxmimowiczii* x *Populus balsamifera*) by 41% (Guillemette and DeRochers, 2008). The amounts of nitrogen, potassium and organic matter present in soil had an effect on the biomass production and survival of various willow cultivars (*Salix viminalis*) in Finnish mineral soils (Tahvanainen and Rytkonen, 1999). Furthermore, there was a positive correlation between the content of organic matter and the number of willow stems. The most sensitive cultivar to soil chemical properties was found in cultivar 81-0217 (*Salix vinimalis*), whilst soil nitrogen content only slightly affected the biomass production of cultivar 78-021 (*Salix viminalis*).

A study from Hytonen and Kaunisto, (1999) revealed that the application of either P and K fertilizer, or ash significantly increased the nutrient foliar concentration (i.e. Ca, P, Mg) and above ground biomass of a mixed willow and birch plantation. After 14 growing seasons, the biomass was higher under P and K fertilization (61.8 t ha^{-1}) than ash application (61.4 t ha^{-1}) and those of the control stand (37.6 t ha^{-1}) (Hytonen and Kaunisto, 1999). In Denmark, experiments on the effects of nitrogen on

dry matter above ground biomass in willow have been reported by Mortensen *et al.*, (1998).

The application of nitrogen fertilizer in 1994 at concentrations of 0 and 74 kg N ha^{-1} only gave a significant effect at the Foulum site, but not in plots at Jyndevad. The willow above ground dry matter biomass in Foulum sites under 0 N fertilizer application was 2.68 t ha^{-1} which was lower than in the 75 N applications (3.71 t ha^{-1}). The 0 N fertilizer application of Jyndevad plots resulted in a willow dry matter biomass of 3.52 t ha^{-1}, which was not significantly different to the 75 N application (4.94 t ha^{-1}) (Mortensen *et al.*, 1998).

In this study, based on the average biomass accumulation, willow could accumulate carbon at 12.61 t ha^{-1} $year^{-1}$, whilst for poplar this value was 9.37 t ha^{-1} $year^{-1}$. This indicated that willow could potentially accumulate a greater amount of aboveground biomass than poplar at this site. The average biomass in poplar was consistent with a previous study by Kauter *et al.*, (2003) and the amount of willow biomass supported the results of Aylott *et al.*, (2008). These studies showed the accumulation of 7.7 to 10.7, and 4.9 to 9.6 t ha^{-1} $year^{-1}$ in willow and poplar grown in the UK. It can be concluded that willow is a more promising option for planting in UK soils than poplar since it has faster biomass accumulation.

2.6.3 Below ground pools: soil carbon storage

2.6.3.1 Bioenergy crops soil carbon stock

In this study, significant differences in total soil C in the upper soil layer (0-30 cm) were found under different land uses. Meanwhile, at the lower soil depth, with the exception of the woodland soil, no significant differences between land uses were observed. It was shown that 14 years post conversion from arable cropping soils under SRC willow had sequestered carbon more than those levels to native woodland by 10% and had accumulated almost twice as much C as arable cropping. This indicates a positive impact of land use conversion from arable cropping to bioenergy crop plantations. The annual rate of soil carbon stock sequestration (0-60 cm) in willow and poplar plantation was estimated to be 3.39 and 0.62 t C ha^{-1} $year^{-1}$ respectively, which was close to the amount of soil C stocks which accumulated under poplar plantation reported by Hansen, (1993) and Sartori *et al.*, (2007), but lower than those in a mixed plantation of willow and poplar (Jug *et al.*, 1999).

2.6.3.1.1 Soil organic C under Poplar plantations

Previously, there was little published data on soil carbon changes under short rotation crops (Dowell *et al.*, 2009). The annual increase in soil carbon stocks in poplar (0.62 t ha^{-1} $year^{-1}$) was lower than that of a previous study by Hansen, (1993) which reported an increase of about 1.63 t C ha^{-1} yr^{-1}. Similarly, Sartori *et al.*, (2007), recorded that over a 10 year planting period, a poplar plantation grown on former arable land in the Columbian plateau-Oregon-USA, had a total soil C stock at a depth of 0-30 cm of 18.75 t C ha^{-1} and an accumulation of 1.87 t C ha^{-1} $year^{-1}$. Soil organic carbon increased up to 4 years after converting from arable land before decreasing

after this period. This might have been due to a low soil clay contents and thus a lack of aggregation that limited soil C storage capacity.

Similar results were reported by Coleman *et al.,* (2004), which indicated no difference in soil carbon of the top 32 cm of soil under different ages of poplar plantation (1-12 year old) in North Central USA which were from different positions along a flood plain, adjacent to arable sites. There was no evidence for a change in soil C content relative to adjacent agricultural crops (Coleman *et al.,* 2004). Moreover, Mao and Zeng, (2010) also concluded that there were no significant changes in soil organic matter in the 0-15 cm layer in the first 15 years following poplar cultivation in former agricultural soils. Arevalo *et al.,* (2009) suggested that lower organic C inputs than outputs in early stage of poplar plantations (2 and 9 years), explained why there were no significant differences in soil C under poplar compared to arable and grassland sites. The outputs may include loss through soil respiration and rapid decomposition of SOM during preparation and cultivation.

In contrast, soil C stock reductions of 4.05 t ha^{-1}, over 5 years for a poplar plantation (*Populus deltoides*; *Populus deltoides* x *Populus nigra*) at a depth of 0-50 cm was reported by Dowell *et al.,* (2009). This reduction might have been caused by increased C mineralization following tillage of the organic matter rich pasture. Total soil carbon accumulation in a top soil 10 years after conversion to mixed poplar and willow plantations on former arable land in Germany (Jug *et al.,* 1999) was between 35-55 t ha^{-1}, giving an average carbon accumulation from 3.5 to 5.5 t ha^{-1} $year^{-1}$. This value was more than double that of the average annual soil carbon accumulation in poplar but almost similar to willow plots in this current study.

2.6.3.1.2 Soil organic C under Willow plantations

Changes in soil carbon accumulation under willow systems have been rarely described. No change in soil carbon stock was observed at a depth of 0-60 cm when grass/scrubland was converted into willow (*Salix dasyclados*) 4 years previously (Ulzen-Appiah *et al.*, 2000). Similarly, a 3 year conversion of arable crop (corn-alfalfa) to willow (*Salix alba*) did not significantly change soil carbon at a depth of up to 60 cm at two different sites in Quebec-Canada. A decrease in soil carbon was detected due to the lack of fertilization in short rotation willow plantations. The level of soil carbon loss in willow was between 9 to 15 t ha^{-1} $year^{-1}$ (Mehdi *et al.*, 1998). In this study total soil organic at 0-30 cm under willow plots was 2.87%, which was almost twice than those in arable (1.90%), and this value was 20% higher than in poplar (2.45%). Within 0-60 cm SOC under willow plantation in this study was accumulated at the level of 3.39 t C ha^{-1} $year^{-1}$.

2.6.3.2 Comparison of soil organic C under SRC to other bioenergy crops

In a trial in south western Quebec-Canada, carbon storage of two perennial biomass energy systems, switchgrass (*Panicum virgatum L.*) and short-rotation willow (*Salix alba x glaffelteri L.*) at 2 adjacent sites was compared, along with a corn plot, a 20-year-old abandoned field, and a mature hardwood forest. Soil carbon results showed that willow was associated with significantly greater soil carbon levels than switchgrass at the more fertile site, but at the less fertile site, no significant differences in soil carbon were detected between the various plant systems examined. This indicates that when bioenergy crops are grown on relatively fertile soils, they have a potential to substantially increase soil carbon levels compared to cultivation in less fertile soils (Zan, 1998).

Borzecka-Walker *et al.,* (2008) revealed that the three-year average net soil carbon sequestration for another bioenergy crop, *Miscanthus*, was 0.64 t C ha^{-1} $year^{-1}$ while for coppice willow it was 0.30 t C ha^{-1} $year^{-1}$. In another trial Bradley and King, (2004) reported a carbon storage rate under *Miscanthus* cultivation of 0.13 to 0.20 t C ha^{-1} $year^{-1}$.

Clearly there are potentially many factors controlling soil organic matter dynamics and stocks under bioenergy crops, such as: previous land use, land management, time scale of observation, soil chemical properties, tree genotypes, climatic and geographic position (Laganiere *et al.,* 2010). Thus, the continuation of a long term monitoring programmes studying the effect of reverting arable cropping to bioenergy crops on soil carbon sequestration is needed so that the overall C budget of these cropping systems can be determined. In conclusion SRC crops, in particular willow accumulated more C than other bioenergy crops.

2.6.3.3 Grassland and Set aside

Grassland can benefit soil carbon sequestration by providing greater litter and root biomass inputs than arable crops. However, significant differences in soil C stocks are inconsistent and many studies of C stocks were mainly only focused at 0-30 cm soil depth (Omonode and Vyn, 2006). For example, soil C stocks at 0-15 cm soil depths under native grassland (i.e. *Panicum varigatum, Andropogon gerardi, Sorghastrum nuttans, Andropogon scoparius*) in Robertson plots were significantly higher (0.59 t ha^{-1}) than cropland (0.53 t ha^{-1}), but on the other sites (e.g Loop) soil C stocks under cropland were higher than native grassland at 0.67 t ha^{-1} and 0.59 t ha^{-1}, respectively (Omonode and Vyn, 2006). In this study 14 years after conversion, the set aside plot showed a significant reduction in soil organic carbon at 0-30 cm depth,

compared to arable soil. Total soil carbon in set aside plots was 84.15 t C per ha (at a depth of 0-60 cm), which equated to an accumulation of -1.54 t C ha^{-1} $year^{-1}$

It has been reported that the soil carbon sequestration in permanent set aside might result in a C accumulation of 0.4 t C ha^{-1} $year^{-1}$ (Freibauer *et al.,* 2004) to 0.5 t C ha^{-1} $year^{-1}$ (Vleeshouwers and Verhagen, 2002) or even lower at 0.38 t C ha^{-1} (Smith, 2004). In order to estimate how much C could be sequestered in soil under set aside, comparison of set aside to similar ecosystems such as grassland or pasture is necessary. The amount of soil carbon sequestration in set aside plots in the current study was negative compared to the soil C stocks in other set-aside plots, as reported by Freibauer *et al.,* (2004); Vleeshouwers and Verhagen, (2002) and Smith, (2004). This meant that SOC in set aside was lost during conversion and the immobilization was occurred. The annual soil carbon sequestration in set aside plots of this current study (-1.54 t ha^{-1} $year^{-1}$) was lower than the soil carbon stocks (0-30 cm) of a grassland site in Germany, which reached 52.51 t C ha^{-1} 30 years after conversion from arable land, which equated to 1.75 t C ha^{-1} $year^{-1}$. However the initial value of the soil C stocks under arable cropping was not clearly reported in Don *et al.,* (2007) study.

The C sink saturation of a similar texture soil (silty clay loam) from a grassland system at Rothamsted, UK has been evaluated. The soil reached equilibrium at a level of 2.25%, after 100 years. Beyond this period there were no further changes and this seems to indicate that carbon sink saturation had been achieved (Freibauer *et al.,* 2004).

Furthermore, annual soil organic carbon sequestration under grassland establishments across 35 locations in the US had an average value of 0.84 t C ha^{-1} $year^{-1}$ (Franzuelebbers, 2010). This was slightly higher than the carbon accumulation

rate in switchgrass (0-30 cm), which has been recorded as 0.5 t ha^{-1} $year^{-1}$ (Ma et al., 2000) during a 10 year period in Alabama. This was lower than the carbon accumulation in the 0-6 cm layer under Bermuda grass in Georgia (1.4 t ha^{-1} $year^{-1}$) (Franzluebbers *et al.,* 2001). Managing grassland under low grazing pressure/unharvested conditions can improve soil carbon stocks (Franzluebbers and Stuedemann, 2009)

In another grassland experiment, Omonode and Vyn, (2006) reported significant differences in soil organic carbon at a depth of 0-30 cm under native grasses (2.24%) compared with cropland (1.98%), but not in switchgrass (2.24%) or tall grass (2.14%), planted after 6-8 year after an arable crop (corn-soybean rotation system). This was associated with a more intensive rooting system, greater root diameter and litter biomass in native grassland.

2.6.3.4 Native vegetation (woodland)

In this study, annual woodland carbon sequestration was estimated to be 0.43 t C ha^{-1} $year^{-1}$ assuming that the woodland has been established for 300 years (Keith and Poulson, 2003). In woodland trial in Denmark, 28/29 year old Oak and Spruce plots had above ground biomass carbon of 75 and 105 t C ha^{-1}. These amounts of carbon equate to annual biomass accumulations of 2.67 t C ha^{-1} $year^{-1}$ for Oak and 3.62 t C ha^{-1} $year^{-1}$ for Spruce (Vesterdal *et al.,* 2002). Furthermore, the study showed that soil carbon under Oak and Spruce plots was 3.8 t ha^{-1} for 0-5 cm; 7.1 t ha^{-1} for 5-15 cm and 8.4 t ha^{-1} for 15-25 cm, giving a total soil carbon accumulation over 28/29 years of 19.3 t ha^{-1}, equating to 0.69 t ha^{-1} $year^{-1}$ (Vesterdal *et al.,* 2002).

This value was lower than in previous studies from Johnson and Tood, (1998) and Johnson *et al.,* (2003), who examined the soil carbon dynamics under Oak forest

and Pine plantations, and recorded values was 82.2 t ha^{-1} and 47.52 t ha^{-1} respectively. It was recorded that the annual carbon stock increase was 1.8 t ha^{-1} $year^{-1}$ for the Oak forest and 0.2 t ha^{-1} $year^{-1}$ for the Pine plantations.

Soil carbon sequestration under woodland plots at 0 to 60 cm soil depth in this current study was recorded as 129.25 t ha^{-1}. This value was lower than for a similar woodland site at Rothamsted research as reported by Poulton *et al.,* (2003), where his study of a 200 year old woodland site on former arable land revealed that the soil carbon had accumulated at concentrations between 236 t ha^{-1} and 407 t ha^{-1}, at the soil depth tested (0-69 cm). This equated to annual accumulations of 1.18 to 2 t C ha^{-1} $year^{-1}$.

The *in situ* forest floor input over 30 years in Oak was approximately 2 t ha^{-1} and 9 t ha^{-1} for Spruce in Norway (Vesterdal *et al.,* 2002). Forest inputs beneath tree plantations of sweetgum, sycamore and loblolly pine were recorded as 5.25 t ha^{-1} to 34.28 t ha^{-1} (Garten, 2002). In this study the accumulation of litter in woodland plots was within these ranges (approximately 6 t ha^{-1}), but this was lower than the amount of litter at the Geesscroft plot (11 to 17.4 t ha^{-1}) (Poulton *et al.,* 2003). Woodland surface litter accumulation was significantly higher than adjacent poplar (5 t ha^{-1}) or willow (3.8 t ha^{-1}) plantations. This means that in terms of carbon accumulation in surface litter the poplar and willow were able to gain 80 and 65% of the values recorded in the woodland site during the 14 years following planting. We hypothesised that soil carbon saturation levels had almost been reached in woodland but not in the SRC plots.

2.6.4 The influences of soil properties on total soil carbon

Research has shown that not only litter biomass and root turn over can affect total soil carbon. Soil type can influence the level of soil carbon stock. Don *et al.*, (2007) recorded that soil carbon stocks in Vertisols (rich in clay) were twice (86 t ha^{-1}) that in Arenosols (dominated by sandy particles) (48 t ha^{-1}) at 0-60 cm soil depths. Leifeld *et al.*, (2005) examined different levels of soil carbon in grasslands which were differentiated by clay content and altitude. They studied various locations in Switzerland at 0-20 cm soil depth. At a clay content above 40%, the soil organic carbon reached 3.17% which was significantly different to soils with clay contents <20% and between 20 to 40%, where the soil carbon recorded was 1.8% and 2.67% respectively. However, the grassland located at an altitude >1000 m above sea level contained almost twice as much soil carbon (5.20%) as those locations <1000 m (2.91%), demonstrating the importance of climate in determining the balance between mineralization and storage of soil C.

2.6.5 Effects of bioenegy crop genotypes (willow and poplar) on soil C

The roles of SRC genotypes of willow and poplar for improving soil carbon storage have been rarely studied. Previous studies on SRC genotypes have largely involved predicting above ground biomass production and performance (Guillemette and DesRochers, 2008; Dowell *et al.*, 2009; Weih and Nordh, 2002; Stolarski *et al.*, 2008). Biomass production by SRC genotypes of willow and poplar have been recorded to varying extents across different studies. Kopp *et al.*, (2001), studied biomass production in five willow cultivars and one hybrid poplar during ten successive annual harvests in the USA. Biomass production estimated using a model were ranged from 12.4 t ha^{-1} (*Salix dasyclados* : SV1) to 9.5 t ha^{-1} (*Salix alba*

var.sanquinea : SA2). Hoewever, among the tested cultivars, the highest yields were recorded for SV1 (16.3 t ha^{-1}) under a N, P, K (20:11:11) fertilized treatment (Koop *et al.*, 2001).

The height and stem diameter of *Salix vinimalis* cultivars was higher than those *Salix discolor*, which produced higher numbers of shoots than *Salix vinimalis* following fertilization with of 18.8 t ha^{-1} DM of waste water sludge, equates to 100 kg N ha^{-1} (Labreque and Teodorescu, 2003). Under this treatment, the first rotation of biomass production of *Salix vinimalis* in sandy and clayey site reached up to 24.88 and 45.28 t ha^{-1}, respectively, which was increase 55 to 89% in the second rotation. This was higher than the amount of first rotation biomass production in *Salix dicolor*, yielded 15.78 t ha^{-1} in sandy soil and 30.66 t ha^{-1} for clayey soil. The production was increase by 2.7 to 55.5 % in the next rotation cycle (Labreque and Teodorescu, 2003).

In the UK, biomass production by the cultivar *Salix vinimalis* x *Salix udensis*-Ashton-stott gave an average annual yield of 6.28 t ha^{-1}, which was significantly higher than *Salix vinimalis* x *Salix schwerrinii*-Torhild, *Salix vinimalis* x *Salix schwerrinii*-Sven and *Salix vinimalis* x *Salix schwerrinii*-Tora which had a recorded values of 2.69 t ha^{-1}, 4.95 t ha^{-1},4.85 t ha^{-1}, respectively, over two cutting cycle periods (McKenzie *et al.*, 2008). This experiment proved the significant effect of different cutting periods on willow biomass production, with the second cutting cycle producing more biomass than the first one.

In another trial in Wales-UK, 19 willow varieties were studied for biomass accumulation, planted in a density of 13,330 tree per ha (Jones and Valentine, 2008). In the first trial experiment (2003), willow biomass were ranged from 8.5 to 13.2 t ha^{-1} $year^{-1}$ and this increased following the second rotation due to an increase in stem number and diameter. Tordis (*Salix vinimalis x Salix schwerrinii*) was the most

productive variety, yielding in the excess of 10 t ha^{-1} $year^{-1}$ biomass amongst those varieties. Meanwhile, in the second trial (2004), greater willow biomass productions were found in Discovery, Endeavour and Inger (Jones and Valentine, 2008).

In the study of Linderson *et al.,* (2007) *Salix vinimalis-Jorrun* had the lowest biomass and total production compared to the other cultivars (e.g. *Salix vinimalis-L78183*, *Salix vinimalis-Jorr*, Salix *dasyclados/Salix burjatica-Loden*, *Salix vinimalis-Raap*, *Salix vinimalis x Salix schwerinii-Tora*). The average diameter for Jorrun was recorded as 13 mm with the maximum value recorded as 28 mm. The average stem height was 4.3 m. This agrees with the current study in which *Jorrun* accumulated the lowest aboveground biomass. Meanwhile, *Salix triandra x Salix vinimalis-Q83* could achieve a maximum stem diameter of 44 mm over a 3 year period (Crow and Houston., 2004). This is one of the reasons why *Q83* biomass production is greater than in *Jorrun*.

For poplar, a previous study from Swamy *et al.,* (2006) revealed that for 5 *Populus deltoides* cultivars, the biomass accumulation ranged from 9.56 to 13.1 t ha^{-1} $year^{-1}$, with the variety *Populus deltoides-D121* recording the greatest yield (13.1 t ha^{-1} $year^{-1}$) followed by *Populus deltoides-G3* (11.3 t ha^{-1} $year^{-1}$) ; *Populus deltoides-65/27* (11.1 t ha^{-1} $year^{-1}$); *Populus deltoides-S7C1* (10.41 t ha^{-1} $year^{-1}$) and *Populus deltoides-G48* (9.56 t ha^{-1} $year^{-1}$). Evidence that there was a significant difference in the shoot and root ratio among these cultivars, might have been due to the different allocation of aboveground to belowground components in poplar cultivars.

In the USA, the productivity of monoclonal plot poplar varieties were much higher ranging from 11 to 18 t ha^{-1} $year^{-1}$ (Debell *et al.,* 1997). Biomass yields of *Populus trichocarpa-7/75* and *Populus trichocarpa*-8/81 were compared to two other hybrid varieties (*Populus trichocarpa x Populus deltoides-11/11* and *Populus*

trichocarpa x Populus nigra-D/01). The results showed that D/01 was significantly smaller and had a smaller basal diameter, whilst the highest cultivars values were found in cultivars 11/11. This was affected by the different shoot, leaf development and growth phenology (e.g stomata control and drought resistance) as well as weather conditions.

For poplar genotypes over a 3 year cultivation period, *Populus trichocarpa x Populus deltoides-Beaupre* had a similar maximum diameter to *Populus trichocarpa-Trichobel*, at around 66 mm and 64 mm, respectively. In addition, *Populus trichocarpa-Ghoy* had a maximum diameter of only 48 mm, but produced a higher root biomass than other cultivars (Crow and Houston., 2004). In this study, the highest amount of biomass accumulation recorded for poplar genotypes was found in *Trichobel*.

2.6.6 Do differences in SRC above ground biomass affect belowground C storage?

There are few publications recording the belowground biomass production or carbon sequestration under specific SRC genotypes. Significant differences in soil carbon sequestration under hybrid poplars were reported by Hansen, (1993). On the other hand, a study by Sartori *et al.,* (2007) revealed that there was no clear trend for increased soil carbon sequestration in poplar system under different ages.

Soil carbon sequestration in SRC was significantly higher than under arable soil, but was still lower than in native forest, as reported by Boman and Turnbull, (1997) and Baum *et al.,* (2009). The SRC soil contained 35 t ha^{-1} of carbon, 10 t ha^{-1} higher than arable and 10 t ha^{-1} lower than woodland, whilst its establishing litter was estimated to be 5 t ha^{-1}. The highest litter accumulation was found in woodland (15 t ha^{-1}) and the lowest was in arable (0.5 t ha^{-1}) (Boman and Turnbull, 1997; Baum *et*

al., 2009). This information clearly indicated that the above ground C inputs can affect belowground pools.

The increased C concentrations in soils under SRC can be explained by: i) the non-tillage practices and litter inputs (Verwijst and Makeschin, 1996; Boman and Turnbull, (1997), (ii) The external mycelium of mycorrhizal fungi is expected to be the dominant pathway through which C enters the soil organic matter (SOM) pool, and this input exceeds the litter and fine root turnover under poplar in SRC (Godbold *et al.,* 2006), (iii) relatively low levels of aeration relative to annual deep ploughing in arable soil (Mathews, 2006) (iii) recalcitrance of molecules from plant litter, rhizodeposits, microbial products and humic polymers (von Lutzow *et al.,* 2006; Marschner *et al.,* 2008).

2.6.7 Sub soil vs top soil carbon storage

C accumulation after conversion of commercial arable sites to SRC was concentrated in the topsoil (Makeschin, 1994; De Neergaard *et al.,* 2002; Dowell *et al.,* 2009). Soil is a key component of the global carbon cycle (Don *et al.,* 2007), and acts as reservoir for terrestrial carbon as organic matter (Franzluebber, 2010). Most studies have focused on studying C storage in the upper soil layer (0-30 cm) with few studying the sub soil (Conant and Paustian, 2002). Generally soil organic carbon declines with depth and coefficient variation increases (Franzluebber, 2010). Table 18. compares the soil organic carbon at different depths from different ecosystems (including bioenergy crops).

***Table 18**. Comparative analysis of how soil depth affects soil carbon status.*

Reference	Land use	Age (years)	Soil depth (cm)	Soil carbon (%)
Jug *et al.*, (1999)	Poplar	10	0-5	1.6 -1.9
			10-20	0.9-1.3
			20-30	0.6-1.0
Omonode and Vyn., (2006)	Grassland	6-8	0-15	1.19-3.4
			15-30	0.5-3.10
			30-60	0.2-2.1
	Cropland	6-8	0-15	1.2-3.2
			15-30	0.5-2.6
			30-60	5.4-2.28
Franzluebbers, (2010)	Pasture	6-50	0-5	2.8
			5-12.5	0.9
			12.5-20	0.5
	Cropland	4	0-5	1.8
			5-7.5	1.0
			7.5-15	0.7

However, Arevalo *et al.*, (2009) found no significant difference in soil C between 2 and 9 year old poplar plantations and adjacent agriculture land and grassland. However native aspen had higher soil C at the 0-20 cm and 20-50 depths relative to the other land uses. Soil carbon stocks ranged from 55.28 t ha^{-1} under native aspen to 78.23 t ha^{-1} for the 9 years old poplar plantations at 0-20 cm depths. At 20-50 cm depths, the soil carbon stock was lower, with values between 38.02 t ha^{-1} for grassland to 47.27 t ha^{-1} for native aspen being recorded.

In this experiment, soil carbon stocks at the 0-30 cm depth were higher than in the above study. The soil carbon stocks ranged from 63.0 t ha^{-1} (set aside) to 119.8 t ha^{-1} (willow), whilst at the 30-60 cm layer the values ranged from 21.15 t ha^{-1} (setaside) to 33.75 t ha^{-1} (woodland). This may have been influenced by the different quantity and quality of soil organic matter (e.g. litter or root decomposition), as well as longer periods of conversion and the depth of soil sampling along with dissimilar spatial distribution of soil physical and chemical properties across the field experiment.

2.6.8 Microbial biomass C and N under bioenergy crop

In the past, studies on microbial biomass C and N have been performed in woodland (Castellazzi *et al.,* 2004; Malchair and Carnol, 2009; Vance *et al.,* 1987b); pasture (Franzluebber *et al.,* 2000); arable (Hargreaves *et al.,* 2003; Pietri and Brookes, 2008; Aslam 1999); setaside (Cooper *et al.,* 1995; Hamer 2008); and fallow sites (Weigand *et al.,* 1995). The contribution of microbial C and N to total C content under bioenergy crop systems of willow or poplar is less well known (Pellegrino *et al.,* 2011).

Under native vegetation (e.g. woodland) soil microbial biomass C has been reported as 161 µg g^{-1} soil at 0-23 cm depth and 4600 µg g^{-1} at the organic layer (Castellazi *et al.,* 2004). In other studies, soil microbial C and N in woodland systems ranged from 1674 to 3169 µg g^{-1} soil and 121 to 442 µg g^{-1} soil, respectively. In an arable soil, microbial biomass C and N was lower than in woodland due the lower inputs of organic material, with the mean value of 263 µg g^{-1} soil being recorded (Hargreaves *et al.,* 2003). Following a fallow period in a former arable crop, soil microbial C ranged from 84 to 548 µg g^{-1} soil (Weigand *et al.,* 1995) and 175 to 190 µg g^{-1} soil in a set aside plot (Hamer *et al.,* 2008).

Recently, Pellegrino *et al.,* (2011) examined the soil microbial C under poplar bioenergy crops (10 years old) compared with an uncultivated soil, and intensive agricultural activity based on a corn and wheat rotation system. Soil microbial biomass C under the intensive arable system was significantly lower (91.20 µg g^{-1} soil) than for poplar (109.97 to 148.365 µg g^{-1} soil), but not significantly different to the uncultivated soil (94.46 µg g^{-1} soil). Furthermore, the ratio of C_{mic} to total C_{org} did not differ significantly (0.88 to 1.12%). The ratio of C_{mic} to total C_{org} in this current

study fell within the range of C_{mic} to total C_{org} in pine plantations and native forest in south eastern Australia, from 0.8 to 1.5% in Delatite plots and between 1.3 to 1.6% at Namadgi sites (Kasel and Bennet 2007). Under poplar plantation in Italy C_{mic} to total C_{org} was constantly less than 2% (Moscatelli *et al.*, 2008).

The level of soil microbial C under the poplar system was lower than willow or woodland in this study which at around 250 µg g^{-1} soil, but higher than arable or set aside plots. The lowest value was found in set aside (168 µg g^{-1} soil). This may due to the difference in organic matter input quantity and quality as litter (i.e poplar and willow accumulate 5 and 3.8 t ha^{-1} litter respectively), environmental factors sand land management (i.e soil water availability, soil temperature or soil pH) (Pietri and Brookes, 2008).

2.6.9 Bioenergy crops for climate change mitigation and ecological benefit

Bioenergy crops such as willow and poplar provide a number of ecosystem services as they could produce large quantities of biomass feedstock in a short period of time and sequester carbon both in aboveground (e.g stem, leaves, litter,) and belowground pools (e.g. soil carbon, microbial biomass, root development).

Under bioenergy crops, the carbon cycle will reflect a closed system as is the case in forest ecosystems (Sartori *et al.*, 2006). Bioenergy crops require minimum cultivation, low maintenance during the period of growth, and low application of fertilizer. All organic residues remain in the soil surface. The results of this study showed that more carbon was stored under bionergy crops (willow or poplar) compared with arable cropping or set aside. Higher accumulations of plant residue input may have allowed for rapid storage despite higher mineralization losses. At the same time, root development could contribute to the belowground carbon pools and

increase soil microbial activities (e.g mycorhiza, fungi, bacteria etc). There were indications that increases in soil microbial biomass C and N were related to the rising levels of soil carbon storage, particularly in the upper soil layer (0-30 cm).

Furthermore, bioenergy crops are likely to benefit soil ecology due to the following mechanisms: (1) continuous litter input intercepting rainfall and reducing erosion; (2) root developments that reach deep into soil and build up soil aggregation, allowing nutrient uptake and reducing nutrient loss (Mann and Tollbert, 2000; Baum *et al.,* 2009).

2.6.10 Conclusion

Carbon pools in above ground biomass of short rotation coppice of poplar and willow following 14 year conversion from arable land, reached 30 and 17%, respectively, of the total above ground of woodland which had been established for 300 years. No significant difference in above ground biomass was seen between the poplar and willow genotypes except for Trichobel. The reason why short rotation coppice did not achieve similar above ground biomass as woodland was due to coppicing activity which occurred every 4-5 years for willow and 8-9 years for poplar. However, SOC in the upper layer (0-30 cm) under willow and poplar was higher than under arable cropping and set-aside, and reached up to 92 and 79% of the SOC content in woodland, respectively. This means that the level of SOC in woodland may have reached saturated levels, whist in poplar and willow SOC was still increasing linearly. The decrease in SOC with depth seen in all treatments is usually attributed to reduced C input at lower depth. In this layer there were no significant differences in SOC between treatments with the exception of woodland, which suggests that C changes at depth take longer to occur than those in the surface layer

In this study a greater accumulation of soil carbon was observed under willow than poplar. This indicates that willow is a more promising biomass energy crop for mitigating future climate changes by means of soil C storage.

It can be concluded that reverting arable to short rotation coppice crops which produce large amounts of aboveground biomass can increase soil carbon and be a feasible option for improving carbon sequestration. The effect of land use change not only affects carbon accumulation in above ground biomass but also to the belowground carbon/nitrogen pools. Generally, both poplar and willow may suitable for biomass production in the UK, however up scaling area of the existing plantation is necessary, with genotypes selected not only on yield of above ground biomass but also for their impact on belowground carbon storage.

Chapter 3

The effect of land use change on soil organic matter quality

3.1 Introduction

3.1.1 Current conceptual understanding of soil organic matter pools

Soil Organic Matter (SOM) content is strongly affected by land use (Tan *et al.,* 2007; Ellerbrock *at al.,* 1999; Garcia and Masera, 2004; Six *et al.,* 2000a; Hagedorn *et al.,* 2001), largely via effects of the amount and quality of organic inputs (Six *et al.,* 1999; John *et al.,* 2005). Total soil carbon changes following land use conversion or changes in management practices are relatively difficult to monitor due to small changes in relative quantities compared with the initial value (Powlson, 1996). This means that decade long time scales are typically needed to monitor SOM dynamics.

The concept of using SOM characteristic as indicators of trends in SOM dynamic has become popular, since SOM is formed by a range of biochemical processes, and consequently has many different pools which may respond differently to management practices or land use change (von Lutzow *et al.,* 2007). Powlson (1996) noted that the measurement of defined SOM pools represents a better approach for examining the effects of land use changes compared to total SOM analysis, since some pools change more quickly than total SOM content (Leifeld and Kogel-Knabner, 2005).

Current conceptual models recognize four physically defined SOM pools which reflect a gradient of organic material formed during the decomposition processes (Figure 20). These are: (1) The unprotected pool (free Light Fraction

Organic Matter (fLFOM or free Particulate Organic Matter (fPOM)), which represents organic matter which is not bound by soil minerals, (2) Material physically protected by association with soil minerals, via aggregation and adsorption processes (e.g. intra-aggregate LFOM (iLFOM or occluded POM (oPOM)). This includes protection by chemical or physico-chemical binding by clay and silt particles, and (3) Biochemically protected SOM corresponding to non-hydrolyzable complex organic materials (i.e. aromatic humified components and wax-derived long chain aliphatics. (Six *et al.*, 2002a; De Nobili *et al.*, 2008; Stewart *et al.*, 2008).

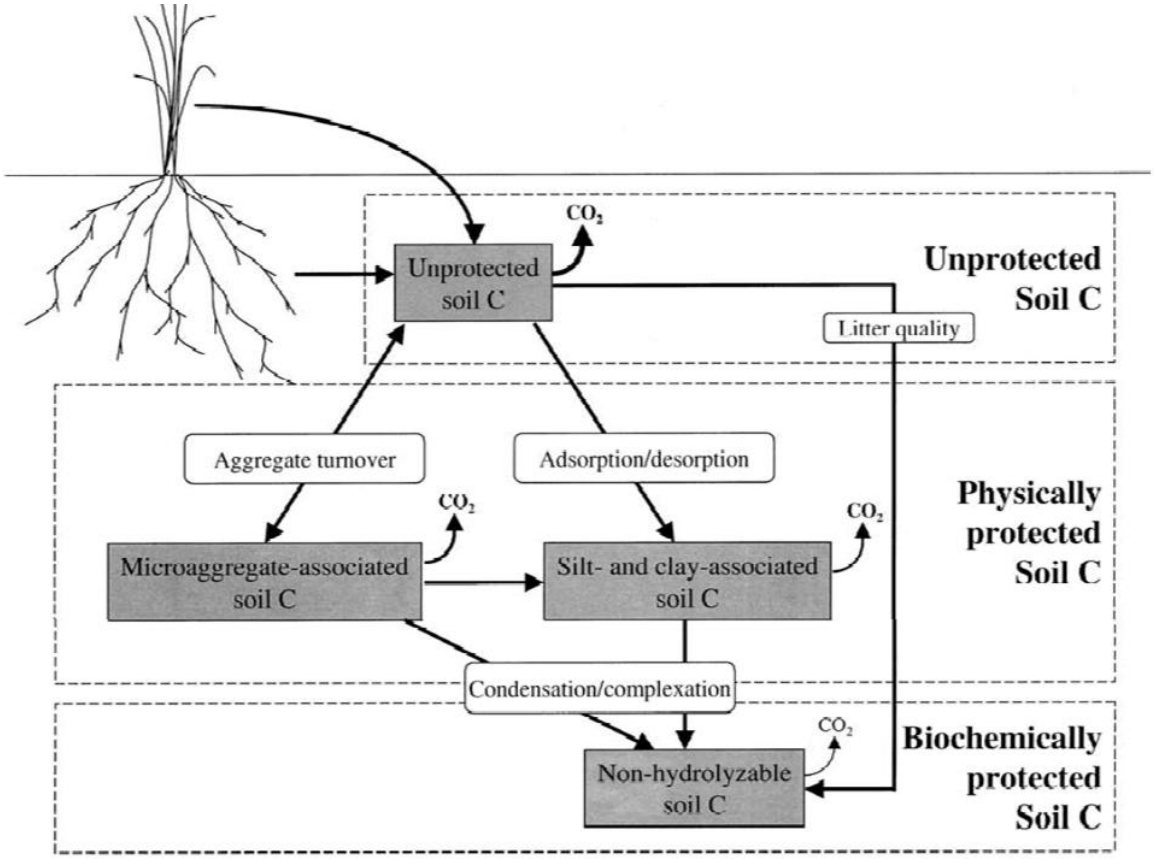


Figure 20. Conceptual model of SOM by Six et al., (2002a).

3.1.2 Physical SOM Fractionation

The characterization of SOM pools is fundamental for understanding terrestrial C dynamics and many published physical SOM fractionation methods have been developed (Liang *et al.,* 1998; Oelbermann and Voroney, 2007). The separation of SOM pools based on the conceptual model described in Figure 13 involves several steps (Figure 14). First, soil aggregates are air dried and sieved to below 2 mm to collect coarse, unprotected particulate organic matter (LFOM or POM) carbon. The remaining particles (53-250 µm) are classified as fine unprotected pools and the micro-aggregate fractions, which are separated by density fractionation. Several methods have been developed to separate this fraction from soil mineral particles (e.g. extraction with NaPt (sodium polytungstate) (Strickland and Sollins, 1987) or NaI (sodium iodide) (Sohi *et al.,* 2001)).

Six *et al.,* (2002a) considered that LFOM and POM are conceptually similar, even though in fact that there are some differences between these fractions. LFOM and POM are isolated by density fractionation and size respectively. Unprotected LFOM is collected after density flotation after minimum soil particle disruption, and equates to unprotected POM within a particle size of 53-2000 µm. Meanwhile, the protected LFOM is released by sonication or other physical disruption (e.g. using a shaker in combination with glass beads), and is similar to an occluded/protected POM with a particle size of 53-250 µm. In this study the term free LFOM and free POM refer to the unprotected soil C pool, whilst the term intra-aggregate or occluded POM refers to protected soil C fractions (Figure 21).

Following dispersion, the silt and clay associated C pools are isolated, before biochemically protected C is determined by acid hydrolysis (Six *et al.,* 2002a; Stewart *et al.,* 2008).

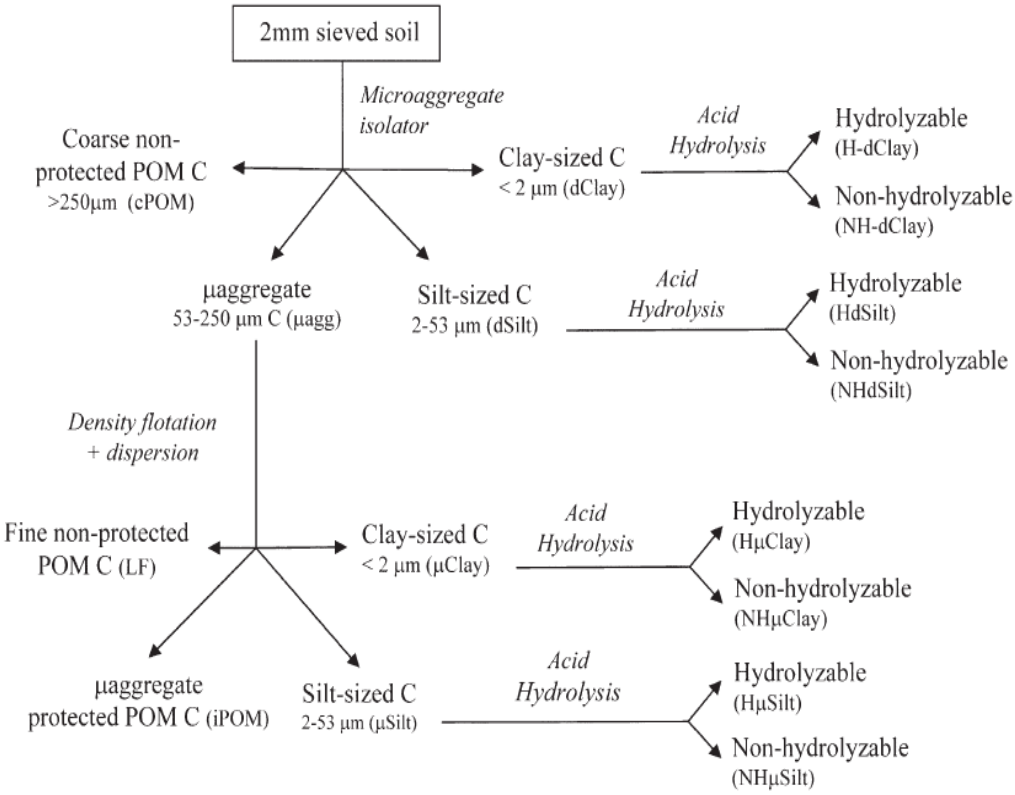


Figure 21. Schematic diagram of SOM fractionation (Stewart et al., 2008)

Acid hydrolysis can be performed in isolated silt and clay fractions (Stewart *et al.*, 2008) by refluxing at 95°C for 16 hours in 6 M HCl followed by filtering and the determination of carbon in the residue after oven drying at 60°C. This process can be used to determine non hydrolysable C pools (non hydrolysable (NH)-dsilt, non hydrolysable (NH)-clay, non hydrolysable (NH)-µsilt, non hydrolysable (NH)-µclay), and hydrolysable C pools (hydrolysable (H)-dsilt, hydrolysable (H)-clay, hydrolysable (H)-µsilt, hydrolysable (H)-µclay) by determining the differences between the total C concentration and the C concentration in the non hydrolysable C fractions (Stewart *et al.*, 2008).

3.1.3 Unprotected SOM pools

3.1.3.1 Unprotected SOM pool characteristics

Unprotected C responds rapidly to changes in land use and so could serve as an early indicator of long term trends in total SOM (Bending and Turner, 2009; Bending *et al.,* 2004; Leifeld and Kogel-Knabner, 2005). Unprotected C could therefore be a useful parameter with which to predict changes to total soil carbon stocks (Leifeld and Kogel-Knabner, 2005; Yamashita *et al.,* 2006). This fraction consists of litter at the first stage of decomposition and conversion of plant debris into stabilized C (Leifeld and Kogel-Knabner, 2005; Grandy and Roberstson., 2007). This fraction is enriched with vanillyl (V), syringyl (S) and cinnamyl (C) groups, has a low acid to aldehyde ratio [$(ac/al)_v$] and a high syringyl to vanillyl ratio (S/V), reflecting a high lignin content (Six *et al.,* 2002a; 2002b).

Unprotected C not only consists of fresh recently incorporated material, but also contains seeds, microbial debris and charcoal. Amounts of this pool respond rapidly not only to land use, but also to soil management (Yamashita *et al.,* 2006; Bending and Turner, 2009; Six *et al.,* 2002b). Additionally, unprotected OM has a quick turn over (Gregorich *et al.,* 1996), and since it comprises 1-25% of SOC it represents an important source of energy for microorganisms and turn over of this pools make a major contribution to nutrient cycling (Whalen *et al.,* 2000). The size of this pool has shown to be associated with the pattern of C and N mineralization processes, (Janzen *et al.,* 1992).

Using Nuclear Magnetic Resonance (NMR) equipment (Liefeld and Kogel-Knabner, 2005) reported that the unprotected C pool in arable soil contains a significantly higher proportion of O-alkyl-C and a lower percentage of carboxyl-C and alkyl-C than the fine mineral (heavy fraction). The proportion of alkyl C to O-

alkyl-C can be used as an indicator for the extent of OM decomposition. The unprotected OM has higher alkyl C to O-alkyl-C content than other fractions, showing that it has a lower degree of decomposition (Liefeld and Kogel-Knabner, 2005).

3.1.3.2 The effect of land use on the quantity of unprotected SOM

The amount of unprotected C from different land use types are presented in Table 19. The amount of unprotected C ranges from 0.3 to 13.4 g kg^{-1} soil. The highest amount of unprotected C relative to total SOM was found in woodland soil, whilst soil depth also influenced the amount of unprotected C. The conversion of native vegetation to agricultural cropping systems can be associated with a decline in the amount of unprotected C. Tan *et al.,* (2007) examined the effects of land use change on the quantity of unprotected C. This research showed that in forest soil unprotected C represented 8.8 g kg^{-1} soil. (Table 19).

A further study of the effect of land conversion from native forest to annual cropping (e.g. maize, wheat, faba bean, sorghum) showed that the unprotected C in cropped soil was 10 times lower than that in woodland soil (Ashagrie *et al.,* 2007). Low amounts of unprotected C in cropped soil might be caused by lower amounts of organic input and faster decomposition of unprotected pools under agricultural management via changes in soil temperature and moisture and tillage.

Table 19. *The unprotected C pool status in different land use types*

Landuse	Soil depth (cm)	SOM fraction and density of extraction liquid	(C org) g kg soil	Reference
Arable	0-3	NaPt <1.8 g cm^{-3}	3.1-4.8	Leifeld and Kogel-Knabner, (2005)
Arable	0-20	NaPt <1.8 g cm^{-3}	0.3	Ashagrie *et al.*, (2007)
Arable (maize)	0-30	NaPt <1.6 g cm^{-3}	0.5	Helfrich *et al.*, (2006)
Arable	0-5	NaPt <1.85 g cm^{-3}	5.4	Tan *et al.*, (2007)
Arable	0-20	NaI <1.7 g cm^{-3}	0.5	Liu *et al.*, (2010)
Grassland	0-3	NaPt <1.8 g cm^{-3}	2.1	Leifeld and Kogel-Knabner, (2005)
Grassland	0-10	NaPt <1.6 g cm^{-3}	0.8	Helfrich *et al.*, (2006)
Grassland	0-20	NaI <1.7 g cm^{-3}	1.0	Liu *et al.*, (2010)
Woodland	0-20	NaPt <1.8 g cm^{-3}	3.8	Ashagrie *et al.*, (2007)
Woodland	0-5	NaPt < 1.85 g cm^{-3}	8.8	Tan *et al.*, (2007)
Woodland (spruce)	0-30	NaPt <1.6 g cm^{-3}	13.4	Helfrich *et al.*, (2006)

Leifeld and Kogel-Knabner, (2005) found similar C concentrations in unprotected and protected pools, and in the ratio of unprotected pools to total soil C when grassland sites were converted to arable cropping, or arable plots were converted to grassland. The C content of unprotected C pools in arable plots converted from grassland ranged from 276 to 298 g kg^{-1}, which contributed from 3.1 to 3.8 % of the total soil C. The grassland plots converted from arable cropping recorded values of 239 to 289 g kg^{-1}, which represented 3.0 to 4.9 % of the total soil C (Leifeld and Kogel-Knabner, 2005).

3.1.3.3 The effect of management practices on the quantity of unprotected SOM

The unprotected pool is sensitive to changes in management practices. However, the impacts were varied dependent on different management practices (Tan *et al.*, 2007).

There were no significant difference in the amount of unprotected C pools under conventional and no tillage corn systems. The amount of non-protected pools under conventional tillage ranged from 0.5 to 0.7 g C kg^{-1}, which represented 4.5 to 5.4 % of the total soil C. This was similar to the no tillage system (0.6 g C kg^{-1}) in which non-protected C contributed 3.0 to 4.6% of the total soil C (Haile-Mariam *et al.,* 2008).

In contrast, different management practices (e.g. native vegetation, and conventional tilled and no-till soybean/wheat or soybean/maize) significantly influenced the amount of unprotected C at 0-5 cm soil depth (Zotarelli *et al.,* 2007). It was recorded that the quantity of unprotected pools in native vegetation (*Araucaria* woodland) was greater than under either conventional tillage on no-till management. The average amount of unprotected C in no-till soil was significantly higher than under conventional tillage for the aggregate size of 250-2000µm and >2000µm (Zotarelli *et al.,* 2007).

Bending *et al.,* (2004) noted differences in unprotected C between organic and conventionally managed arable soil 5 years following conversion. The highest concentration of C was found in organic arable plots which was significantly higher than in the other farming systems investigated (e.g organic vegetables, organic vegetable – cereal, organic ley, and conventional cereal).

3.1.4 Physically protected pools

3.1.4.1 Properties of the protected SOM pools : 1. Microaggregate associated soil C

Physically protected SOM resulted from the protection of particulate organic matter inside soil micro-aggregates which may prevent microbial degradation and hence decomposition (De Nobili *et al.,* 2008; Helfrich *et al.,* 2006). Physically protected SOM or occluded POM within soil aggregates has a longer turn over time than free SOM pools (Marriot and Wander, 2006), and responds more slowly to changes in land use. Physically protected SOM can be separated from unprotected pools using either density fractionation (NaPt >1.6, 1.8 or 2 g cm^{-3}) with physical disruption or sieving (>53 µm) (Marriott and Wander, 2006; Yamashita *et al.,* 2005: John *et al.,* 2005).

Protected C has a smaller amount of O-alkyl C and higher alkyl–C, aromatic and carbonyl-C compared to unprotected C pools (Leifeld and Kogel-Knabner, 2005; John *et al.,* 2005; Golchin *et al.,* 1994). Golchin *et al.,* (1994) estimated that protected C pools under pasture systems had a longer turn over (41 years) than unprotected pools (27 years).

3.1.4.2 The effect of land use change on protected SOM pools: 1. Microaggregate associated soil C

Grunewald *et al.,* (2007) identified the effects of tree age in mixed species plantations on the carbon concentrations of protected C pools. The systems were dominated by non woody species such as *Hymenolobus procumbens* (L) Nutt, *Spergularia media* (L) C.Presl, *Aster tripolium* (L), *Puccinellia distans* (Jacq), *Calamagrotis epigejos* (L) in the early stages of natural succession, followed by woody species of *Betula pendula* Roth, *Populus x canadanensis* Moench (poplar),

Salix alba (L) (willow), and *Myricari germanica* (L) Desv at later succession stages. The study observed that vegetation age, along with species shifts from non woody to woody species impacted the relative proportion of free and protected C pools. There was little variation in C concentration in the protected fraction. The highest C concentrations were found in protected C (intra-aggregate fractions), which ranged from 251-393 g kg^{-1}. The proportion of unprotected (free fraction) and protected fraction (intra-aggregate fraction) C concentrations has been reported increased with site age (from 15 to 70 years).

John *et al.*, (2005) compared SOM fractions (physically protected vs unprotected fractions) under grassland and other land use types (i.e. arable (wheat/maize), woodland). The contribution of unprotected C under grassland (0-30 cm) to total soil C was reported to be 3.7 to 5.9%, which was higher than in arable systems (3.3–4.1%), but lower than in woodland (14.6 to 33.6%). The contribution of protected C to total SOC in arable systems reached 0.5 to 1.0%, which was lower than in grassland (1.2 to 1.8%) and in woodland (1.4 to 2.8%).

Furthermore, Leifeld and Kogel-Knabner, (2005) found that there were no significant differences in amounts of physically protected C in arable and grassland systems, which were 310 g kg^{-1} and 320 g kg^{-1}, respectively. This fraction contributed 4.6 to 11% of total C. The effect of land use on the size of physically protected OM is presented in Table 20. It can be seen that the greatest C concentrations were found in woodland.

Table 20. *The size of the physically protected C pool across different land use systems*

No	Land use	Soil depth (cm)	SOM fraction and density of extraction liquid	Corg (g kg^{-1} soil)	Reference
1.	Arable	0-25	shaker dispersion + NaPt >1.6 g cm^{-3}	2.00	Marriot and Wander, (2006)
2.	Arable (wheat) (1000-2000 µm)	0-30	shaking glass bead centrifugation + NaPt < 1.6 g cm^{-3}	1.61	Yamashita *et al.*, (2005)
3.	Arable (maize) (1000-2000 µm)	0-30	shaking glass bead centrifugation + NaPt < 1.6 g cm^{-3}	2.40	Yamashita *et al.*, (2005)
4.	Arable (wheat)	0-30	centrifugation + NaPt <1.6 g cm^{-3}	0.5	John *et al.*, (2005)
5.	Arable (maize)	0-30	centrifugation + NaPt <1.6 g cm^{-3}	1.0	John *et al.*, (2005)
6.	Grassland (1000-2000 µm)	0-10	shaking glass bead centrifugation + NaPt < 1.6 g cm^{-3}	3.10	Yamashita *et al.*, (2005)
7.	Grassland	0-10	centrifugation + NaPt <1.6 g cm^{-3}	1.2	John *et al.*, (2005)
8.	Woodland (1000-2000 µm)	0-7	shaking glass bead centrifugation + NaPt <1.6 g cm^{-3}	7.94	Yamashita *et al.*, (2005)
9.	Woodland	0-40	centrifugation + NaPt <1.6 g cm^{-3}	1.4-2.5	John *et al.*, (2005)

Yamashita *et al.*, (2005) reported that the amount of protected C was 50% lower compared to woodland systems at 0-7 cm soil depth, and similar result was also observed by Marriott and Wonder, (2006). The amount of protected C under arable cropping ranged between 0.5 and 2.40 g kg^{-1} soil, whilst in grassland the protected C amounted to between 1.2 to 3.10 g kg^{-1} soil. Protected C in woodland can reach 7.94 g kg^{-1} soil (Table 20).

3.1.4.3 The effect of management practices on protected SOM quantity: 1. Microaggregate associated soil C

Marriott and Wander, (2006) examined the effect of different farming systems on the quantity of physically protected SOM. The results showed that organic farming

systems increased the amount of protected SOM, and the amount of soil C was three times higher than unprotected SOM. Significant differences were found between manure + legume based organic farming, legume based organic farming and conventional systems.

Furthermore, soil depth and different management practices can significantly influence the size of the physically protected pool, as reported by Stewart *et al.*, (2008). The C content of physically protected OM in grassland was reported to be 64.9 g C kg^{-1} soil, which was more than double values for conventional (29.2 g C kg^{-1} fraction) and non tillage arable cropping (32.6 g C kg^{-1} fraction) at a depth of 0-5 cm. In the lower layer (5-20 cm), amounts of physically protected C declined to 18.9, 19.6 and 23.2 g C kg^{-1} grassland, conventional and non tillage arable soil, respectively (Stewart *et al.*, 2008).

3.1.4.4 Physical properties of the unprotected SOM pools: 2. Silt and clay associated soil C (chemically protected pools)

The chemical protection of SOM involves binding to soil minerals, particularly silt and clay fractions (De Nobili *et al.*, 2008; Lorenz *et al.*, 2008; Lorenz *et al.*, 2006). The dispersal and characterization of this pool can be achieved through hydrolysis with HCl (Stewart *et al.*, 2008) (Figure 14) or stepwise chemical digestion with cold and hot H_2SO_4 (von Lutsow *et al.*, 2007). This reaction effectively removes carbohydrate and protein from soil minerals, with recalcitrant alkyl and aryl substances remaining intact along with organic polymers such as suberins, cutins, and waxes (von Lutsow *et al.*, 2007).

Acid hydrolysis has been employed to separate resistant and active C fractions. The active fractions are mostly comprised of protein, nucleic acids,

polysaccharides and some carboxyl C, while resistant fractions are comprised of aliphatic, aromatic and residual carboxyl groups. The fraction resistant to HCl (biochemically protected SOM) is relatively older than the hydrolysable fraction (chemically protected/silt and clay associated C) (Silveira *et al.,* 2008).

3.1.4.5 The effect of management practices on the amount of silt and clay associated C (chemically protected pools)

Total SOM associated with silt and clay (chemically stabilised fractions) has been shown to be higher under no tillage with added manure in a corn system, than in no tillage corn (Lorenz *et al.,* 2008). Comparison of grassland and arable plots at two different soil depths (0-5 cm and 5-20 cm), showed that the hydrolysable SOM fractions (hydrolyzable silt (H-dsilt) and hydrolysable clay (H-dclay) under grassland systems were significantly higher than under conventionally tilled and no tillage arable cropping (i.e wheat and corn). At a depth of 0-5 cm H-dsilt fractions in grassland was 20 ± 2.1 g C kg^{-1} which was lower than H-dclay (38.9 g C kg^{-1}). At lower depths it was reduced by more than 50% relative to the upper layer, at 7.8 and 16.5 g kg^{-1}, respectively (Stewart *et al.,* 2008).

In the upper layer (0-5 cm), the H-dsilt fractions under conventional tillage and no tillage were 19.6 and 17.5 g C kg^{-1}, respectively which was significantly higher than in the lower layer (13.0 and 11.4 g C kg^{-1}). The H-dclay fraction at this depth was 36.2 g C kg^{-1} for tillage and 34.1 g C kg^{-1} for non tillage. In the lower depth the value declined significantly to 23.1 g C kg^{-1} and 24.5 g C kg^{-1} for conventional tillage and non tillage, respectively (Stewart *et al.,* 2008)

3.1.5 Biochemically protected SOM

Biochemical protection or stabilization of SOM is due to condensation and complexation of the decomposing residue (Six *et al.,* 2002a). This involves the production of macromolecular organic material which is resistant to decomposition by microorganisms (De Nobili *et al.,* 2008; Tan *et al.,* 2004). This pool is referred to as the passive pool (Hayes and Clap, 2001; Parton *et al.,* 1987) and as non-hydrolyzable fraction (NH) (Paul, 1997; Six *et al.,* 2002a; Stewart *et al.,* 2008). It represents C remaining following acid hydrolysis (Stewart *et al.,* 2008). Acid hydrolysis removes protein, nucleic acids, and polysaccharide, whilst aromatic humified components or wax derived long chain aliphatics remain intact (Six *et al.,* 2002a; De Nobili *et al.,* 2008). Using ^{14}C Paul *et al.,* (1997; 2001) estimated that this pool has been established in soil for 1300 to 5420 years and has a slow turnover rate (Six *et al.,* 2002a; von Lutsow *et al.,* 2007).

The chemical composition of the non-hydrolysable pools (biochemically protected C) has been examined from the residue following acid hydrolysis using HCl or alternatively organic solvents such as n-hexane, chloroform (Schnitzer and Schuppli, 1989) or dichloromethanol/methanol (Wiesenberg *et al.,* 2004), which extract non-water soluble organic compounds from the SOM. These includes alkenes, fatty acids, long chain alcohols, waxes, lipids, cutins and suberins which may constitute 25% of the SOM in soils. These components may be stabilized in a range of OM stabilization mechanisms (e.g aggregation, encapsulation, intercalation hydrophobicity and interactions with mineral surfaces) (von Lutzow *et al.,* 2006).

3.1.5.1 The effect of management practices on the amount of biochemically protected pools

Different management practices (e.g. grassland, conventional tilled and non tilled arable) and soil depths (e.g 0-5 cm vs 5-20 cm) can affect the amount of carbon in the biochemically protected pool (Stewart *et al.,* 2008). Grassland systems have almost 50% higher non hydrolysable silt (NH-dsilt) than non hydrolysable clay (NH-dclay) (17.6 and 36.6 g C kg^{-1}, respectively), at 0-5 cm soil depth. The NH-dsilt and NH-dclay concentration at the lower layer (5-20 cm) were 11.9 and 22.8 g kg^{-1}, respectively.

In arable system (conventional and non tillage), the NH-dsilt concentration ranged between 15.6 to 14.9 g kg^{-1} fraction (0-5 cm) and between 11.6 and 13.4 g C kg fraction for the lower layer (5-20 cm) in arable systems. Meanwhile, the NH-dclay concentration in this system can reach 27.5 to 28.5 g C kg^{-1} in the upper layer (0-5 cm and 18.2 to 23.0 for the lower soil depths (5-20 cm).

3.1.6 Soluble SOM pools

The extraction of dissolved organic matter (DOM) has been the subject of much research, and can be performed using cold/hot water (von Lutzow *et al.,* 2007), or other stronger aqueous solvents (e.g. methanol) (Koch *et al.,* 2005; Sleighter and Hacther, 2008). DOM is defined as the SOM fraction < 0.45µm in solution and various extractants have been used to collect this pool. The DOM pool can account for 0.25 to 2% of the total SOC (Haynes, 2005) and consists of a range of substances from small defined molecules, to complex substances. For example, hexose and pentose compounds can indicate the presence of carbohydrates derived from microbial activity (van Lutzow *et al.,* 2007). DOM from terrestrial ecosystems

contains aromatic, carboxyl and hydroxyl compounds, whilst DOM from marine (water) ecosystems mostly consists of carbohydrates, amino acids, and lipids (Sleighter and Hatcher, 2008).

Classical chemical extraction of humic substances can be performed using NaOH or $Na_4P_2O_7$ which extracts 30 to 80% of soil organic matter (Stevenson, 1994). To determine the presence of non water soluble chemical compounds a stronger organic solvent needs to be applied (e.g. n-hexane for removing alkenes and fatty acids, chloroform for extracting fatty acids, long-chain alcohol and wax ester or dicholomethanol/methanol for lipids).

3.1.7 Methods for characterising SOM quality

A variety of analytical methods have been used to investigate the chemical composition of soil organic matter in order to understand factors controlling its quality. Also, a number of methods have been used to investigate the quality of free or protected particulate organic matter (POM). These methods have included, ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (CPMAS-NMR) spectroscopy (Rumpel *et al.,* 2000), ^{13}C nuclear magnetic resonance (NMR) (Sohi *et al.,* (2001); Poirier *et al.,* (2005), and pyrolysis gas chromatography/mass spectrometry (py-GC/MS) (Poirier *et al.,* (2005); (Vancampenhout *et al.,* 2009). For example, using py-GC/MS, Vancampenhout *et al.,* (2009) successfully identified the chemical composition of extractable SOM. The products released consisted of several types of lipids (alkenes, fatty acids, methyl ketones, alchohols), aromatic fractions (benzenes, toluenes, styrene), and polysaccharide derived fractions (furans and furaldehydes) along with lignin and phenolic compounds such as coniferyls and synapyls.

Furthermore, other non destructive techniques have been employed to detect SOM quality such as: scanning electron microscopy (SEM), energy dispersed x-ray (EDX) analyses, x-ray photoelectron microscopy (XPS) (Solomon *et al.,* 2005) and Near Edge X-ray Absorption Fine Structure (NEXAFS) spectroscopy (Lehman *et al.,* 2005). However, these techniques are time consuming (both in terms of sample preparation and data analysis) and expensive.

IR spectroscopy can be used as a tool to study SOM chemistry. For example, it has been used to detect carboxyl and hydroxyl groups in SOM extracts from two different management practice soils (fertilized with cattle manure, and the application of straw with mineral N) (Ellerbrock *et al.,* 1999).

IR has some advantages compared with other methods of soil analysis. The technique involves rapid sample preparation, and represents a less expensive and more efficient way to process a large number of samples. Furthermore, this technique avoids the use of any harmful chemicals. This method is also very sensitive at detecting organic or inorganic substances (Rossel *et al.,* 2006).

A comparison between FTIR and other methods such as NEXAFS and NMR, and their abilities to predict biochemical compounds in organic material is presented in Table 21. Carbon detected by NEXAFS, FTIR and NMR are presented in different energy level units : eV, cm^{-1} and ppm, respectively. The results shown in Table 21 indicate that FTIR is able to identify a wider range of compounds within samples than NMR or NEXAFS.

FTIR has previously been used successfully to distinguish soil horizons (Haberhauer, 2000; Bardy *et al.,* 2008), litter quality (Haberhauer *et al.,* 1998); Lammers *et al.,* 2009), peat soil properties (Chapman *et al.,* 2001), humic substance

(Geyer, 1998), calcium carbonate (Reigh, 2002) and SOM fraction quality (Zimmerman *et al.*, 2007).

Table 21. *Peak assignment for C form from NEXAFS, FTIR and NMR*

Peak assignment	**Carbon form**	**Reference**
	NEXAFS, (eV)	Lehman *et al.*, (2005)
284.3	quinone-C, protonated aromatic-C	
284.9-285.5	protonated/akylated to carbonyl-substituted aromatic-C	
286.1	unsaturated-C	
286.7	phenol-C, ketone C=O	
287.1-287.4	aliphatic-C, aromatic carbonyl	
287.7-288.6	carboxyl-C, C=O, COOH, CH_3, CH_2, CH	
289.3	Carbonyl-C, alcohol	
	FTIR, (cm^{-1})	Lehman *et al.*, (2005)
3411,3339, 3192	OH stretching vibrations of H-bonded hydroxyl groups of phenol with trace of amine stretches (N-H)	
3077	CH from aromatic-C	
2922	weak CH_3, stretch vibration of aliphatic-C	
2856	Weak CH_2, stretch vibration of aliphatic-C	
1710-1690	C=O stretching mainly of carboxyl-C and traces of ketones and esters	
1630	C=O conjugated ketons and quinines	
1595	C=C of aromatic-C	
1513	CH or NH bending, characteristic of undecomposed litter	
1433	CH_2 bending	
1393	CH deformation of aliphatic-C	
1257	C-O stretching and OH deformation	
1115	OH of carboxyl-C	
1037	C-O stretching of polysaccharide	
	NMR, ppm	Helfrich *et al.*, (2006); Kogel-Knabner, (2002)
0-45	aliphatic or alkyl-C of lipids, fatty acid and plat aliphatic polymers	
45-110	derived primarily from polysaccharide (cellulose and hemicelluloses), but also from protein and side chains of lignin	
110-162	deriving from lignin and/or protein	
162-190	aliphatic esters, carboxyl groups amide carbonyl	

Quantitative spectral analysis of soil using IR requires a robust statistical technique in order to characterise significant spectral responses (Rossel *et al.*, 2006). Due to the complexity of the SOM quality spectra produced using IR, multivariate statistical analyses (e.g. PCA or CVA) have become important tools for obtaining quantitative information from the data (Sena *et al.*, 2002). Alternatively, Ben-Dor and Banin, (1995) suggested using multiple regression analysis (MRA) to examine specific IR spectra bands, whilst Shibusawa *et al.*, (2001) preferred to employ stepwise multiple linear regression analysis (SMLR) to estimate soil properties from spectra. However, the most common techniques used are principal component regression (PCR) and partial least square regression (PLSR) (Rossel *et al.*, 2006; Chang *et al.*, 2001; McCarty *et al.*, 2002; Artz *et al.*, 2008).

Recent research has led to the development of Fourier Transform Ion Cyclotron Resonance (FTICR). FTICR is a type of mass analyzer (or mass spectrometer) using a fixed magnetic field for detecting the mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions. The ions are trapped in a magnetic field with electric trapping plates, where they are excited to a larger cyclotron radius by an oscillating electric field perpendicular to the magnetic field (Marshal *et al.*, 1998). This is an advanced technique for characterising the dissolved organic matter (DOM) and natural organic matter (NOM) complexes in aquatic ecosystems (Koch and Dittmar, 2006; Stenson *et al.*, 2003).

Fourier Transform Ion Cynclotron Resonance Mass Spectrometry (FTICR-MS) has some advantages compared with other techniques. For example, Liquid Chromatography Mass Spectrometry (LC-MS) may produce larger variations between replicated samples, and is time consuming both in terms of sample preparation and analysis (Han *et al.*, 2008; Barrow *et al.*, 2009). FTICR can be used to examine the

complex structure of any organic material (Reemtsma *et al.,* 2008). The most common method for identifying DOM or NOM chemical groups using FTICR involves the use of electrosprayer ionization (ESI) (Gaspar *et al.,* 2009; Brown and Rice., 2000; Sleighter and Hatcher, 2008).

Due to its high mass resolving power and detailed mass identification, FTICR has been used to identify the complex composition of dissolved organic matter (DOM). It has a mass resolution of >200,000 m/z and a mass accuracy of <1 ppm, which allows this technique to separate complex peaks from DOM mixtures. FTICR data can be displayed in elemental plots which provide information about the double bound equivalent (DBE) of the chemicals present or the number of ring-specific chemicals related to the oxygen compound classes (Koch *et al.,* 2005). Previously FTICR revealed thousand chemical structures from fulvic acids present in the Suwanee River, USA.

The O/C and O/H ratio can reflect the aromatic or aliphatic composition in organic substance (Sleighter and Hatcher, 2008). An example of the of O/C and H/C ratios, and the double bound equivalent (DBE) values of various chemical compounds from DOM samples obtained using FTICR is presented in Table 22. The samples were taken at different sampling points along the river to the ocean at Chesapeake Bay, USA.

Table 22. *Sample property and the average magnitude of O/C, H/C and DBE from DOM samples at different sampling points (adapted from Sleighter and Hatcher, 2008).*

Site	Temperature (^{o}C)	pH	Salinity	O/C	H/C	DBE
Dismal Swamp(DS)	11.8	3.3	0	0.39	1.25	9.6
Great Bridge (GB)	15.0	7.13	11	0.35	1.29	8.5
Town Point (TP)	14.3	7.21	20	0.35	1.37	7.6
Chesapeake Bay Bridge (CBB)	15.4	7.51	22	0.35	1.40	7.4
Off Shore Coast (OSC)	16.2	8.01	32	0.33	1.43	7.2

FTICR was able to detect changes in the O/C and H/C ratios and in particular the decrease in the DBE values from the more inland locations to the coastal sampling points. The highest O/C ratio was found in Dismal Swamp sites (DS).

The magnitude of DOM peaks, detected by FTICR are presented in Figure 22 (represented by the negative ion mass). The data displayed in Figure 22 shows multiple peaks spanning an m/z range of 200 to 700, where the smallest detectable unit has the same mass as a neutron (1.000335 m/z unit). It shows expanded regions at nominal m/z values of 315, 427, 483, and 519 in the C18 extracted Dismal Swamp water (Figure 22a) and the C18 extracted offshore coastal water (Figure 22b). This shift of peak magnitude within each nominal mass indicates that the organic matter from the offshore site is oxygen poor and/or hydrogen rich when compared to the onshore sample from the Dismal Swamp, consistent with the notion that terrestrial organic matter is perhaps more aromatic while marine organic matter is likely to be more aliphatic (Sleighter and Hatcher, 2008).

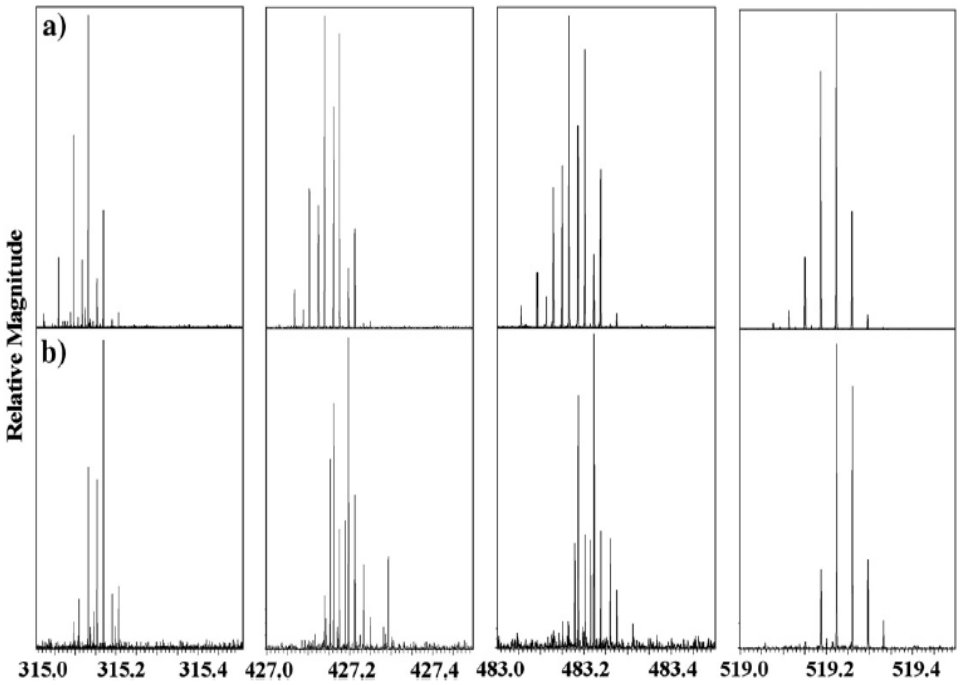


Figure 22. Negative ion mass spectrum of DOM from Chesapeake Bay Dismal swamp water (22a) and Coastal water (adapted from Sleighter and Hatcher, 2008)(22b)

Brown and Rice (2000) reviewed the advantages of FTICR compared to other elemental analytical approaches such as ^{13}C NMR or IR. Unlike FTICR, these methods were not able to identify specific components of the DOM. Instead, they only provided an average composition of the bulk material. FTICR has been used previously to indentify the components of complex chemical compounds comprising fulvic and humic acids (Brown and Rice, 2000)

Despite the great potential of FTICR, this technique still has not been used for identifying soluble SOM compounds in soil extracts from terrestrial ecosystems (e.g. soil sample).

3.2 Aims and Objectives

3.2.1 Aims

The work presented in this Chapter aimed to investigate the effect of change from arable cropping to alternative land uses (set aside, SRC, woodland), which was reported in Chapter 2, on the quality of soil organic matter.

3.2.2 Objectives

- To examine the effects of land use change on the levels of unprotected and protected (free and intra-aggregate) SOM fractions.
- To compare the chemical and spectroscopic properties of soil and light fractions under different land uses.

3.3 Hypotheses

- 14 years following conversion from arable to SRC and set aside the amount of total, free and intra-aggregate light fraction organic matter (LFOM) will increase.

- The amount of LFOM will be affected by soil depths.
- LFOM in soil under different land uses and depth has different chemical composition.
- The amount and chemical composition of total, free and intra-aggregate light fraction organic matter (LFOM) under SRC will be similar to that of native woodland.
- Free LFOM is a better and more sensitive indicator of land use change than the intra-aggregate fraction.
- The chemical compounds present in the DOM of soil under contrasting land uses will be significantly different.

3.4 Methods

3.4.1 Extraction of Free and Intra-aggregate Light Fraction of Organic Matter (LFOM)

Light fraction organic matter was extracted from soil samples collected from land uses and depths described in section 2.4 Chapter 2. A total of 180 soils samples were used. These were a combination from 5 different land use types (arable, set aside, poplar, willow and woodland), 2 soil depths (0-30 cm and 30-60 cm), 9 sampling points, and 2 OM fractions (free and intra aggregate) were isolated from each soil sample. For each soil sample LFOM were extracted from duplicate sample and pooled. Density fractionation was used to extract free and intra-aggregate LFOM. The method used was based on those described by Strickland and Sollins, (1987); Sohi *et al.*, (2001), and Bending and Turner, (2009) with some modifications.

In order to fractionate the soil, samples were partially dried by leaving them overnight at room temperature. Approximately 30 g fresh weight soil was accurately weighed into digital balance and transferred into plastic jar (100 ml). To determine soil water content, 10 g of soil from each sample was weighed and placed in oven at 60°C overnight and the dry weight recorded. The soil water content was determined gravimetrically, by subtracting oven dry weight from fresh weight to determine moisture content.

A solution of NaI was made up at a density of 1.75 g cm^{-3}, by dissolving 140 g NaI in distilled water. The density then was checked using a hydrometer (scale range: 1.6 to 1.8). The correct density was achieved by adding further NaI to obtain a higher density, or more water to achieve a lower density. The solution stored in a 4°C fridge and mixed again when it used.

For extracting LFOM, samples were transferred from plastic jar into rounded plastic centrifuged flask (120 ml). 50 ml of NaI was added to each replicate. Samples were homogenized by swirling by hand at 1 rotation per second for 30 seconds. Before centrifuged in pairs, samples were being placed on a balance for checking the weights to make sure that the variation in weight is within the tolerance of the centrifuge. Samples were centrifuged at 3600 rpm for 30 minutes using a Mistrall 2000 centrifuge.

The free LFOM material floating in the supernatant was transferred into glass beakers using a hose under the suction of a vacuum pump taking care to include all of the floating SOM clinging to the side of the tube, and the liquid containing floating material was filtered through 5 µm SWMP Millipore (10 cm in diameter) filter.

The weight of each SWMP millipore was determined before hand by placing the filter paper in oven overnight at 60°C and determining the weight using a digital balance. For each soil sample, duplicate extracts samples were pooled together to represent amount of LFOM in 60 g of dry soil. The membrane (filter paper) was washed three times with 2.5 ml 1M $CaCl_2$ to obtain a clean LFOM, free from NaI solution.

The millipore filters paper and free LFOM was dried, by placing in the oven at 60°C for overnight and then weighed. The quantity of LFOM was calculated by subtracting the weight of the filter from the weight of the filter plus LFOM. The material collected in this way represent free Light Fraction Organic Matter (free LFOM) were separated from filter paper using a fine brush and collected in a zipped plastic bag.

For extracting intra-aggregate Light Fraction Organic Matter (intra-aggregate LFOM), following extraction of the free LFOM, the pellets were dispersed with

another 50 ml of NaI. In order to break up the soil aggregates and remove physically protected LFOM, the solutions were placed on ice and then sonicated for 15 minutes at 58 watts (Soniprep 150). The samples were then centrifuged for 15 minutes at 3600 rpm. The intra-aggregate LFOM contained in the resulting supernatant were collected, washed and weighed using the method described for free LFOM, and was stored for further analysis.

The chemistry of free and intra-aggregate LFOM fractions was investigated by Fourier Transform Infra Red spectroscopy (FT-IR) and by C/N analysis. Free and intra-aggregate LFOM was pooled for analysis of C/N content, which was determined using a LECO C/N analyser. The total C content of LFOM fraction was determined by calculating LFOM C g^{-1} soil using the pooled free and intra aggregate LFOM C content.

3.4.2 Fourier Transform Infra-Red spectroscopy (FTIR)

The chemistry of the extracted LFOM pools and whole soil was determined by Fourier Transform Infra-Red spectroscopy (FTIR) combined with canonical variate analysis (CVA) to identify functional groups associated with different land uses, soil depths and pools of LFOM. The quantity of LFOM at 30-60 depth was insufficient for FTIR analyses.

FTIR (Perkin Elmer 100) was used to ascertain the spectral characteristics of samples from soil and LFOM. The dried soil samples were ground using a ball mill to a fine powder. LFOM was brushed from the filter paper into a mortar and pestle and ground to a fine powder. Samples were placed in the sample holder in the top of diamond aluminum plate of the IR detector. The transmission FTIR spectra were collected at room temperature. The background spectra were determined by

subtracting the spectra of blank air samples from the spectra of the samples. The detailed method for FTIR analysis was as described by Artz *et al.,* (2008). The scan range of the FTIR machine was 650 to 4000 cm^{-1} and the resolution was 4 cm^{-1} each. Spectra were obtained by averaging 200 scan per sample.

3.4.3 Fourier Transform Ion Cyclotron Resonance (FTICR)

Single air dried arable and woodland soil sample were used for extraction of organic matter in dichloromethane (DCM). For each land use, the soil was prepared by mixing 20 g sample from each the 9 sampling location described in Chapter 2. Approximately 50 g of these composite samples were used for organic matter extraction.

The soil sample was mixed with 200 ml 50:50 v/v dichloromethane and deionized water solution. Samples were placed in the glass soxhlet extraction apparatus and heated for 1 hour at $40^{o}C$.

The resultant solutions were then evaporated in a vacuum rotavapor and the residues were re-dissolved using acetonitrile to give 2 ml sample solutions. These samples were then sent to the Chemistry Department, University of Warwick for analysis using FTICR. The data was obtained using atmospheric pressure photoionization (APPI), as this allowed for the observation of a broader range of species than electrospray ionization (ESI).

The instrument used was a 12 T Bruker Solari-X FTICR mass spectrometer. APPI was the ionization method used and we obtained both positive-ion and negative-ion spectra during the session.

Samples for the APPI assay were dissolved to a concentration of 0.01 mg mL^{-1} in 50% acetonitrile and 50% toluene. Broadband mode was used which allowed 4

MW datasets to be acquired. 400° C nebulizing gas (nitrogen) and a flow rate of 1.8 L min^{-1} was used in order to homogenise the extracts following the use of a drying gas (nitrogen) at a temperature of 200° C and a flow rate of 5 L min^{-1}. The chemical compounds detected using this method had (m/z) within the range of 147.4–5000. There were 300 scanned datasets which were collected in total as a input for van Krevelen analysis.

3.4.5 Statistical analysis

Multivariate analyses using Canonical Variate Analysis (CVA) (Genstat ver 11) were carried out on the soil and LFOM IR spectral datasets. Each dataset consisted of 3550 parameters. CVA then separated the datasets into by chemical composition. This was achieved by minimising the within-group, and maximising the between-group variance. The CVA data was then displayed on 2D plots.

To observe relative changes, the spectra of selected peaks were compared by determining their relative absorbance (RA), which were obtained by determining the absorbance of selected peaks (e.g 2920, 1730, 1630, 1510, 1450 1370 1270 or 1050 cm^{-1}) as a proportion of total peak area (Haberhauer *et al.*, 1998).

3.5 Results

3.5.1 Light fraction organic matter (LFOM) quantity

Soil depth had a significant effect on the quantity of free LFOM ($P<0.05$) with substantially less LFOM at 30-60 cm relative to 0-30 cm (Figure 23). Land use significantly affected the amount of free LFOM at both 0-30 and 30-60 cm soil depths ($P<0.05$). Woodland had the highest amount of free LFOM in the upper layer (0-30 cm), which was significantly higher ($P<0.05$) than all of the other land uses. No significant differences were found between arable and set aside plots. Amounts of LFOM at 0-30 cm in willow and poplar plots were not significantly different, however both were significantly higher than in arable plots.

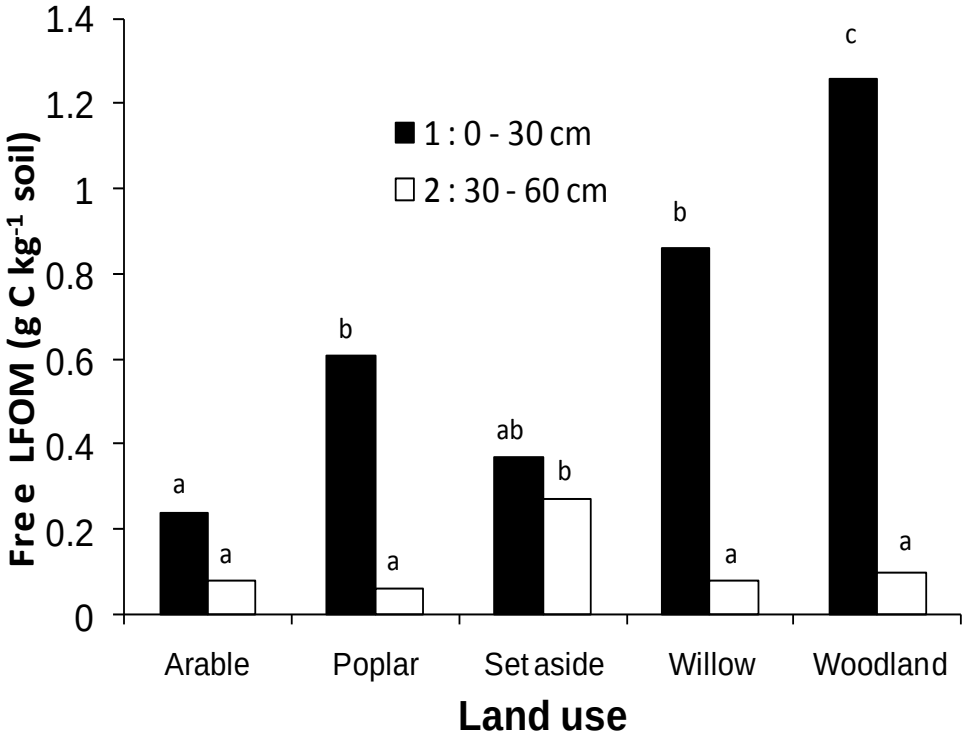


Figure 23. Free LFOM distribution in soil from different land uses (LSD (1) = 0.49 ; LSD (2) =0.16). Different letters denote significant differences (P<0.05)

In the lower layer (30-60 cm), free LFOM levels were not significantly different between the land uses except for set aside, which was significantly higher (P<0.05) than the others plots.

There was a significant difference in the amount of intra-aggregate LFOM between 0-30 cm and 30-60 cm depths (P<0.05), with substantially higher amounts of intra-aggregate LFOM at 0-30 cm. Land use did not significantly affect the amount of intra-aggregate LFOM at either 0-30 or 30-60 soil depths (Figure 24).

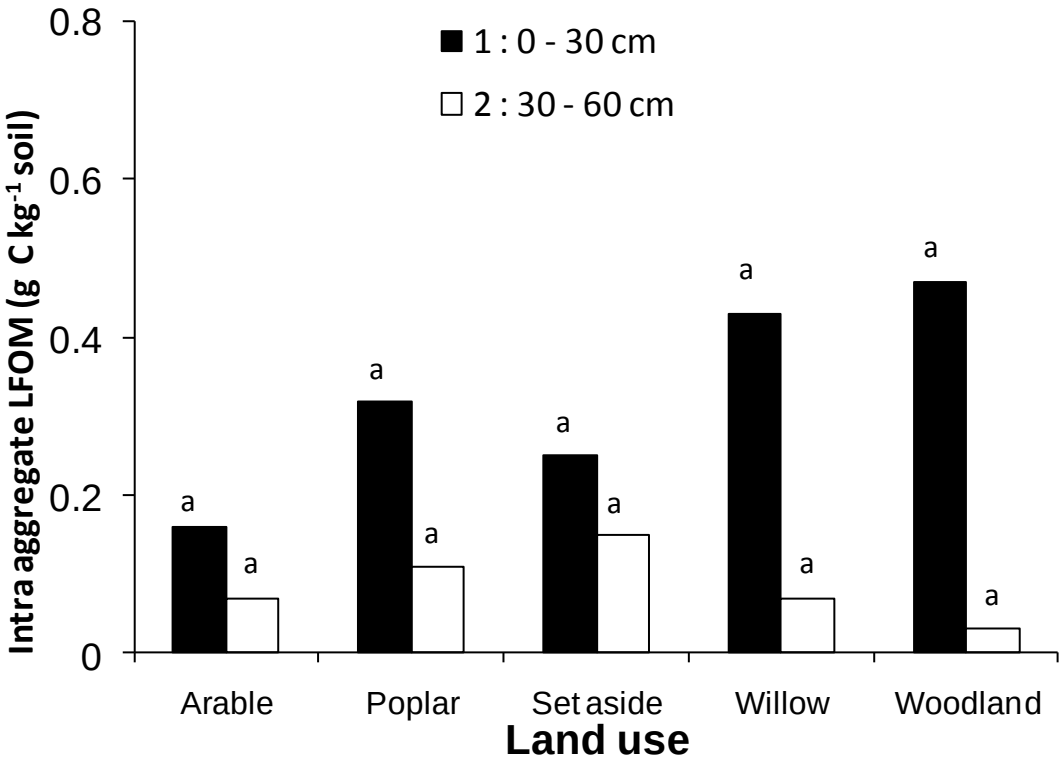


Figure 24. Intra aggregate LFOM in soil from different land uses (LSD (1) = 0.34 ; LSD (2) = 0.13). Different letters denote significant differences (P<0.05)

Free and intra-aggregate LFOM fractions were pooled to give total LFOM. There was a significant difference (P<0.05) in total LFOM between land uses. There was significantly more total LFOM at 0-30 cm than 30-60 cm depth, with an average

of 0.99 g C kg^{-1} soil and 0.20 g C kg^{-1} at 0-30 and 30-60 cm, respectively (Figure 25). There was 3-4 times less total LFOM at 0-30 cm in arable soil than in willow and woodland soil. However there were no significant differences between amounts of total LFOM at 0-30 cm in soil under the other land uses. At 30-60 cm there was significantly higher (P<0.05) LFOM in set aside soil than in soil under the other land uses, which were not significantly different from each other.

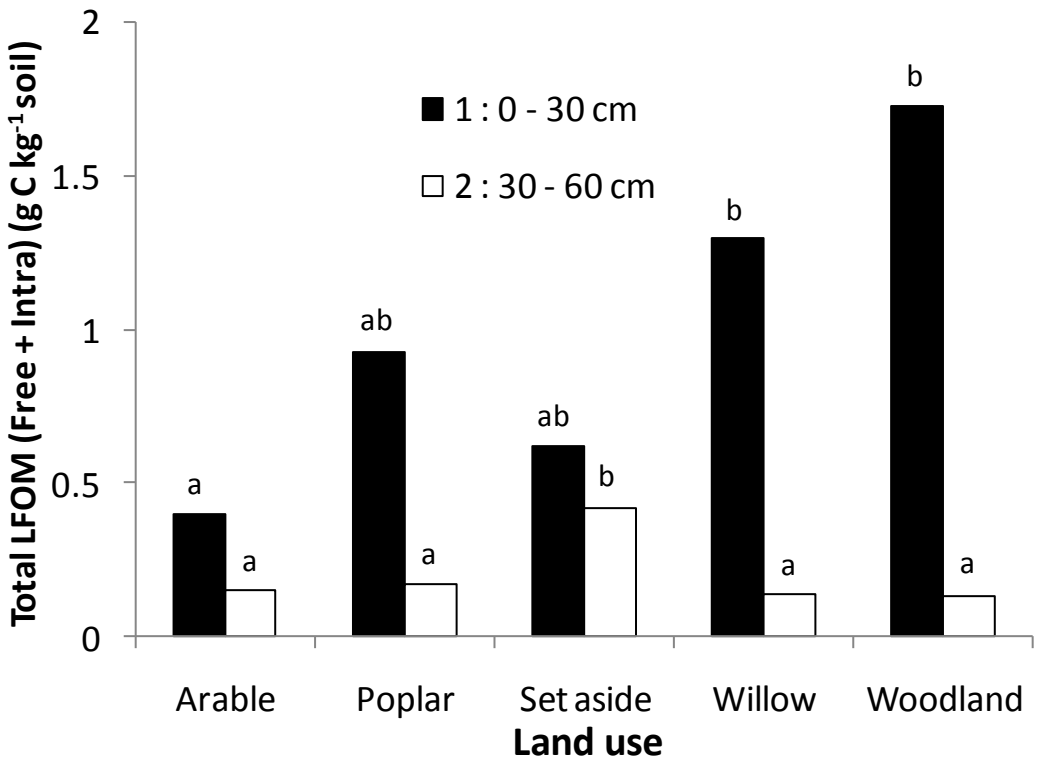


Figure. 25. Total LFOM across different land use types (LSD (1) = 0.75 ; LSD (2) = 0.26), Different letters denote significant differences (P<0.05)

Amounts of free, intra-aggregate and total LFOM were compared among different bioenergy crop genotypes. No significant differences were observed amongst the willow or poplar genotypes at either soil depth (Figure 26, 27 and 28).

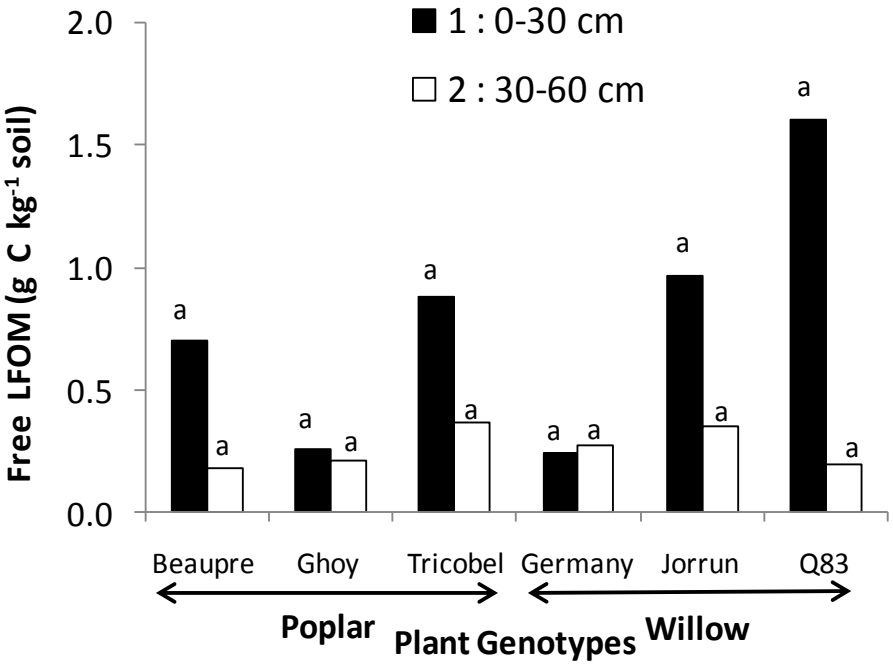


Figure 26. Free LFOM in soil under different plant genotypes (LSD (1) = 1.05 ; LSD (2) = 0.18). Different letters denote significant differences (P<0.05)

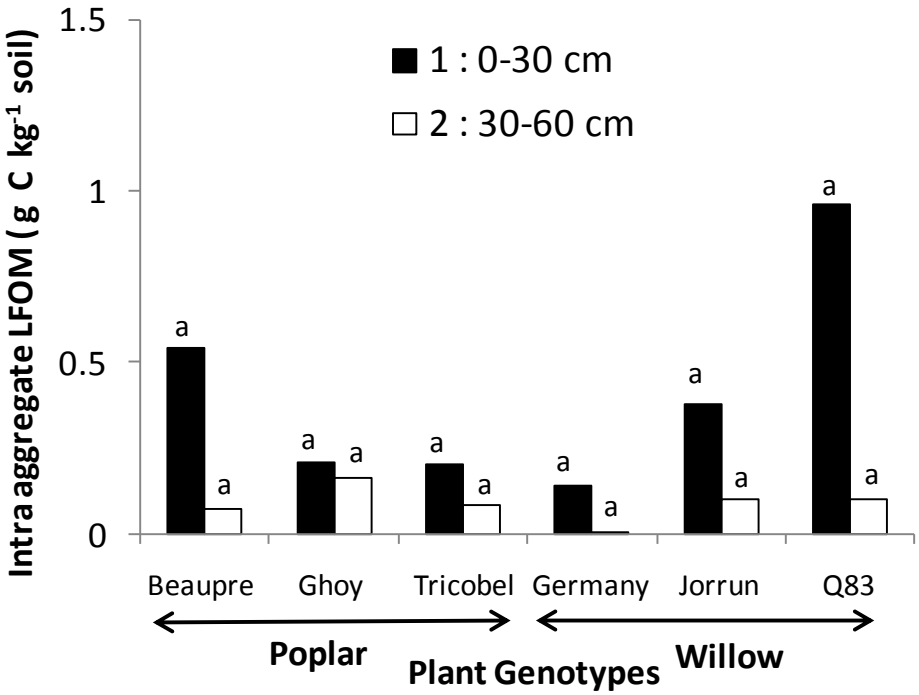


Figure 27. Intra aggregate LFOM in soil under different plant genotypes (LSD = 0.70 ; LSD (2) = 0.18). Different letters denote significant differences (P<0.05)

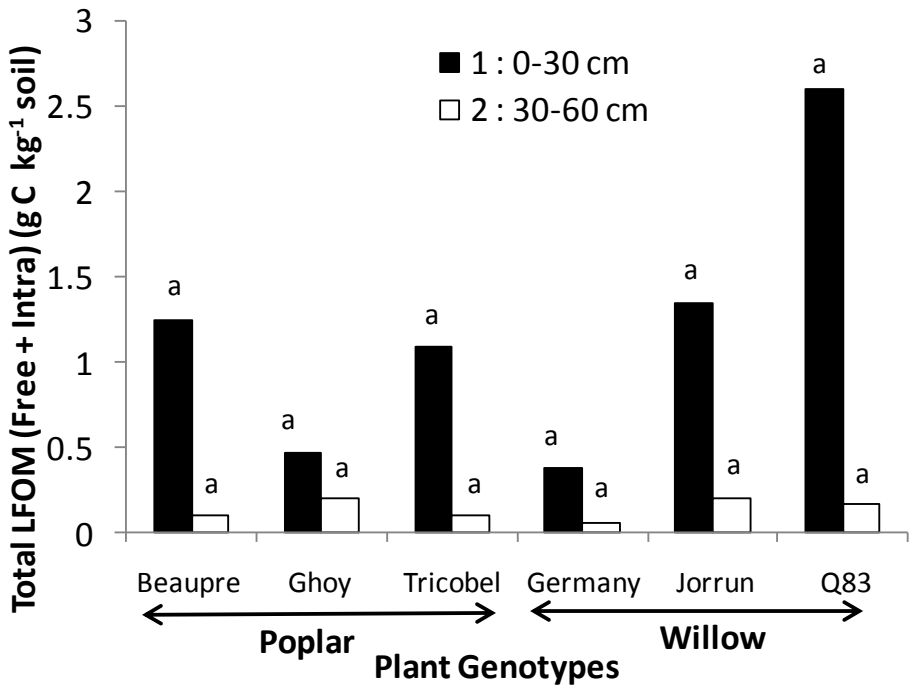


Figure 28. Total LFOM in soil under different plant genotypes (LSD (1) = 1.65 ; LSD (2) = 0.25). Different letters denote significant differences (P<0.05)

3.5.2 Whole soil mineral FTIR spectroscopy

FTIR spectra can reflect both mineral and organic soil components. Table 23 represents the inorganic material of soil mineral and was organic compound of OM functional groups presented in Table 24. Overlapping was found at a wavelength in region of 1000-1050 cm^{-1} which was represented C-O stretch of polysaccharide (Tabel 23) and at the same time correspond to O-Si-O stretch of soil mineral (Table 24). In order obtain a proper analyses, visual identification of peak intensity then were supported by statistical analysis of each wavelength.

Visual inspection of soil FTIR spectra showed noticeable differences between 0-30 cm and 30-60 cm depths particularly in the region of 1400–1800 cm^{-1} (Figure 29 and 30). Furthermore, at 0-30 cm depth there were differences in spectra between soil from different land uses, particularly in the region of 900 to 1100 cm^{-1} (Figure 29). At

30-60 cm there were differences in spectra between soil from different land uses in the region of 1400 to 1800 cm^{-1} (Figure 30).

Peaks at 650–700 cm^{-1}, which were more intense in arable than other land uses at both soil depths, are associated with inorganic materials such as clay and quartz particles (Table 23). Moreover, the peaks in the region of 800 to 874 cm^{-1}, which were more intense at lower soil depths in set aside, correspond to carbonate compounds.

Table 23. *Wavelength at which inorganic materials in soil absorb IR light*

No	Wavenumber (cm^{-1})	Functional group	Reference
1.	650-700	Clay and quartz	Haberhauer *et al.*, (1988); Orlov, (1986)
2.	800-874	Carbonate	Boke *et al.*, (2004); Reigh *et al.*, (2002)
3.	1000-1050	O-Si-O stretch	Madari *et al.*, (2006); Nguyen *et al.*, (1991)
4.	3150-3220	OH bond	Rumple *et al.*, (2001)
5.	3620-3720	Alumino silicate	Nguyen *et al.*, (1991)

Peaks in the region of 1000 to 1050 cm^{-1} which varied in intensity between land uses and depths correspond to mineral peaks of O-Si-O, which overlapped with the functional groups of polysaccharides/carbohydrates. In the upper layer, the highest transmittance of these peaks was found in arable soil followed by woodland, poplar, set aside and willow soils (Figure 29). At lower soil depths, these peaks showed lower transmittance than in the upper layer (Figure 30).

A broad peak from 1280 to 1510 cm^{-1} represents aliphatic C-H bonds (Madari *et al.*, 2006; Bardy *et al.*, 2008) or bands of lignin, cellulose, carboxylate, and amide (Nguyen *et al.*, 1991). These peaks were more intense at lower soil depths, particularly in woodland and set aside plots. However, since visual analysis is difficult, proper analysis using a statistical analysis was performed to back up those above conclusion.

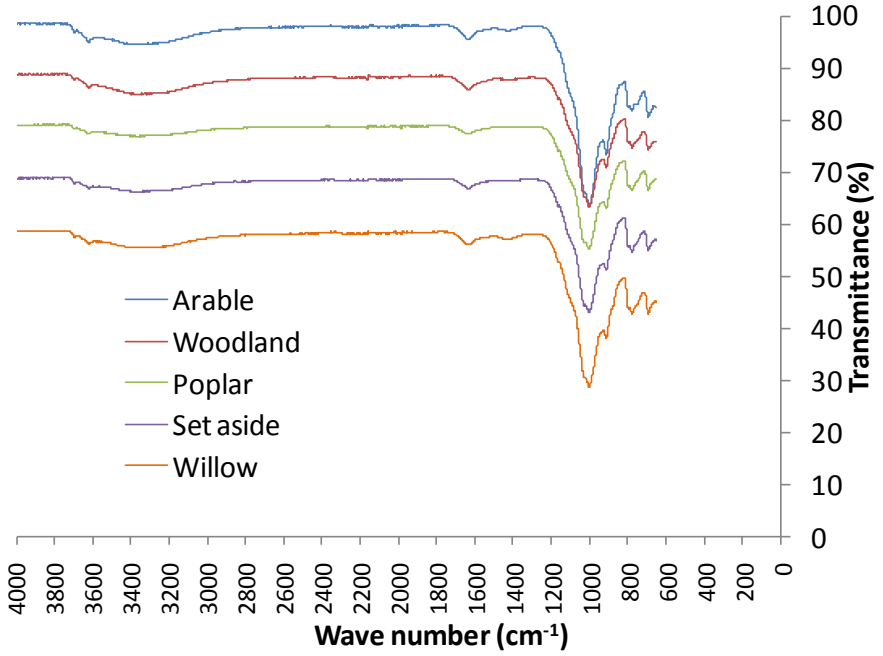


Figure 29. Soil FTIR spectra at 0-30 cm soil depth under different land uses (Data represents the mean of 9 sampling points)

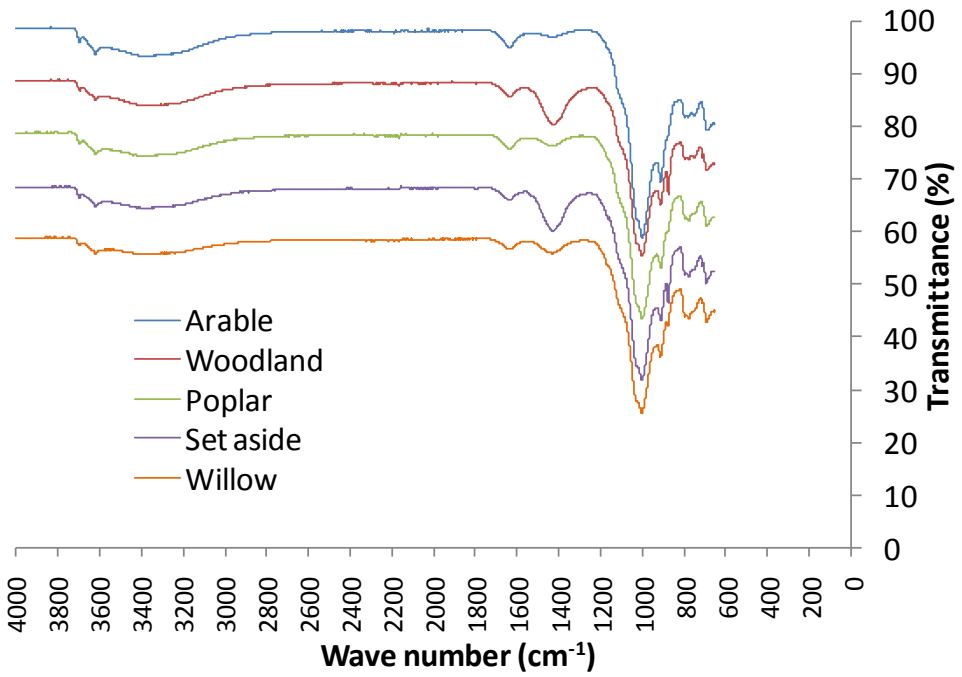


Figure 30. The average FTIR spectra at 30-60 cm soil depth under different land uses (Data represents the mean of 9 sampling points)

For both the 0-30 cm and 30-60 cm soil depths, there was a broad peak at 3150 to 3250 cm^{-1} which may represent hydroxyl bonds (Rumple *et al.*, 2001). These were next to peaks at 3620 and 3720 cm^{-1}, which may assigned to 2:1 layer alumino silicates, including smectites and illites, usually overlapped by kaolinite, water amorphous silica and many other clay minerals (Nguyen *et al.*, 1991). The intensity of this peak was similar at both soil depths.

In order to quantify differences in organic compostion of OM using FTIR spectra, a sample from different soil depths and under different land uses were analysed. Transmittance at 13 key wavelengths corresponding to define organic matter functional groups were used (Table 24) and a statistical analysis were employed

Table 24. *Wavelength at which organic materials in soil absorb IR light*

No	Wavenumber (cm^{-1})	Wavenumber for statistical analysis (cm^{-1})	Functional group	Reference
1.	1000-1100	1050	C-O stretching of polysaccharide	Gressel *et al.*, (1995); Lehman et al., (2005); Haberhauer *et al.*, (1998)
2.	1100-1200	1150	OH of carboxyl-C or S=O in sulphate	Gressel *et al.*, (1995); Lehman *et al.*, (2005); Haberhauer *et al.*, (1998)
3.	1250-1300	1270	C-C or C=O or C-O stretching and OH deformation or phenolic OH	Gressel *et al.*, (1995); Lehman *et al.*, (2005); Haberhauer *et al.*, (1998)
4.	1350-1400	1370	phenolic/aliphatic stretch or CH deformation of aliphatic-C	Haberhauer *et al.*, (1998)
5.	1400-1500	1460	carbonyl/carboxyl stretch of lignin or aliphatic C-H deformation	Haberhauer *et al.*, (1998); Gressel *et al.*, (1995)
6.	1510-1550	1510	C-H deformation or C-H or N-H bending	Lehman *et al.*, (2005)
7.	1550-1600	1590	C=C of aromatic C	Gressel *et al.*, (1995); Lehman *et al.*, (2005)
8.	1600-1650	1630	C-H deformation or C-O stretch or C=O conjugated ketons and quinine	Lehman *et al.*, (2005); Lammer *et al.*, (2009)
9.	1690-1730, 1720	1730	C=C vibration or C=O stretching mainly carboxyl–C and trace of keton and ester	Lehman *et al.*, (2005); Gressel *at al.*, (1995); Lammers *et al.*, (2009)
10.	2856	2850	Stretch vibration of aliphatic-C	Lehman *et al.*, (2005)
11.	2922, 2940	2920	C-H stretch of aliphatic compound or (CH_2 or CH_3) of fat/wax or stretch vibration of aliphatic-C	Lehman *et al.*, (2005); Gressel *at al.*, (1995); Haberhauer et al., (1998); Fuentes *et al.*, (2007)
12.	3077	3090	CH from aromatic-C	Lehman *et al.*, (2005)
13.	3411,3339, 3192	3400	OH stretching vibration of O-B bond hydroxyl groups of phenol with trace of amine stretch	Gressel *et al.*, (1995); Lehman *et al.*, (2005)

These included C-O stretching of polysaccharide, C-C or C-O bonds, phenolics, C-H deformation, C=C vibration or aromatic C, lignin carbonyl and carboxyl stretch, C-O stretch and C-H stretch of fat/waxes or aliphatic compound, OH of carboxyl-C.

The MANOVA showed that of the 13 IR spectra analysed, 9 were significantly affected by soil depth and land uses. These are presented in Table 25.

Table 25. *The effect of soil depth and land use on FTIR transmittance at wavelengths corresponding to key organic matter functional groups. Different letter is denoted for significant differences (P<0.05).*

		% transmittance at each wavelength (cm^{-1})								
		1050	**1270**	**1370**	**1460**	**1510**	**1630**	**1730**	**2920**	**3400**
Soil depth (cm)										
0-30		79.5a	98.2a	97.9a	97.8a	97.9a	96.3a	98.4a	97.7a	95.6a
30-60		75.7b	97.7a	95.5b	94.5b	97.1b	95.7a	98.3a	97.5b	94.3b
LSD		***2.39***	***0.50***	***1.69***	***1.98***	***0.63***	***0.65***	***0.22***	***0.19***	***0.92***
Landuse										
Arable	0-30	75.7b	98.0ab	97.6a	97.3a	97.5ab	95.5b	98.1b	97.2b	94.5b
Poplar	0-30	81.9a	98.6a	98.6a	98.6a	98.4a	97.2a	98.8a	98.3a	96.9a
Set aside	0-30	80.4ab	98.5a	98.3a	98.3a	98.2a	96.8ab	98.6ab	98.0ab	96.2ab
Willow	0-30	77.9ab	98.0ab	97.5a	97.1a	97.6ab	96.0ab	98.3ab	97.5ab	95.4ab
Woodland	0-30	81.8a	98.1ab	97.8a	97.8a	97.7a	95.9ab	98.2b	97.4a	95.1ab
Arable	30-60	74.4b	98.2a	97.5a	97.1a	97.5ab	94.9b	98.1b	97.2b	93.3b
Poplar	30-60	76.0b	98.1a	97.2a	96.5a	97.7a	95.7b	98.3ab	97.7ab	94.4b
Set aside	30-60	74.4b	97.1a	93.2b	91.5b	96.3b	96.0ab	98.2b	97.5ab	94.5b
Willow	30-60	76.4b	98.2a	97.1a	96.2a	97.8a	96.6ab	98.6ab	97.9ab	95.7ab
Woodland	30-60	77.4ab	97.0b	92.8b	91.5b	96.2b	95.6b	98.3ab	97.3b	93.9b
LSD		***5.28***	***1.09***	***3.6***	***4.2***	***1.3***	***1.4***	***0.50***	***0.85***	***2.0***

Wavelengths 1050, 1370, 1460, 1510, 2920 and 3400 cm^{-1} showed significantly lower transmittance (P<0.05) at 30-60 cm than at 0-30 cm. There was no effect of land use on transmittance at 1270, 1370, 1460 or 1510 cm^{-1} in soil at 0-30 cm. However, arable soil showed significantly lower transmittance at 0-30 cm than soil from other land uses at 1050, 1630, 1730, 2920 and 3400 cm^{-1}.

There was an effect of land use on transmittance of all wavelengths at 30-60 cm. At this depths, woodland soil showed lower transmittance at 1270, 1370, 1510, and 2920 cm^{-1} than other land uses. Set aside showed transmittance as lowest as woodland at 1460 cm^{-1}. Arable soil showed lowest transmittance at 1730 cm^{-1} than soil from other land uses and had a similar transmittance to set aside at 1050 cm^{-1}, but lower than woodland at 2920 cm^{-1}.

Canonical Variate Analysis (CVA) using the full 355 wavelengths confirmed that the spectra of 0-30 and 30-60 cm depth of soil were distinct with clear separation along CV2 (Figure 31). The first canonical variate analysis axis (CVA 1) accounted for 41.11% of the variance which was higher than the second axis (CVA 2) which represented 14.72% of the variation. Differences in spectra between depths and land uses were shown to be significantly different using Chi squared and F test analysis ($P<0.05$). In particular the arable soils formed a distinct group which were clearly separated from the other soil at both depths.

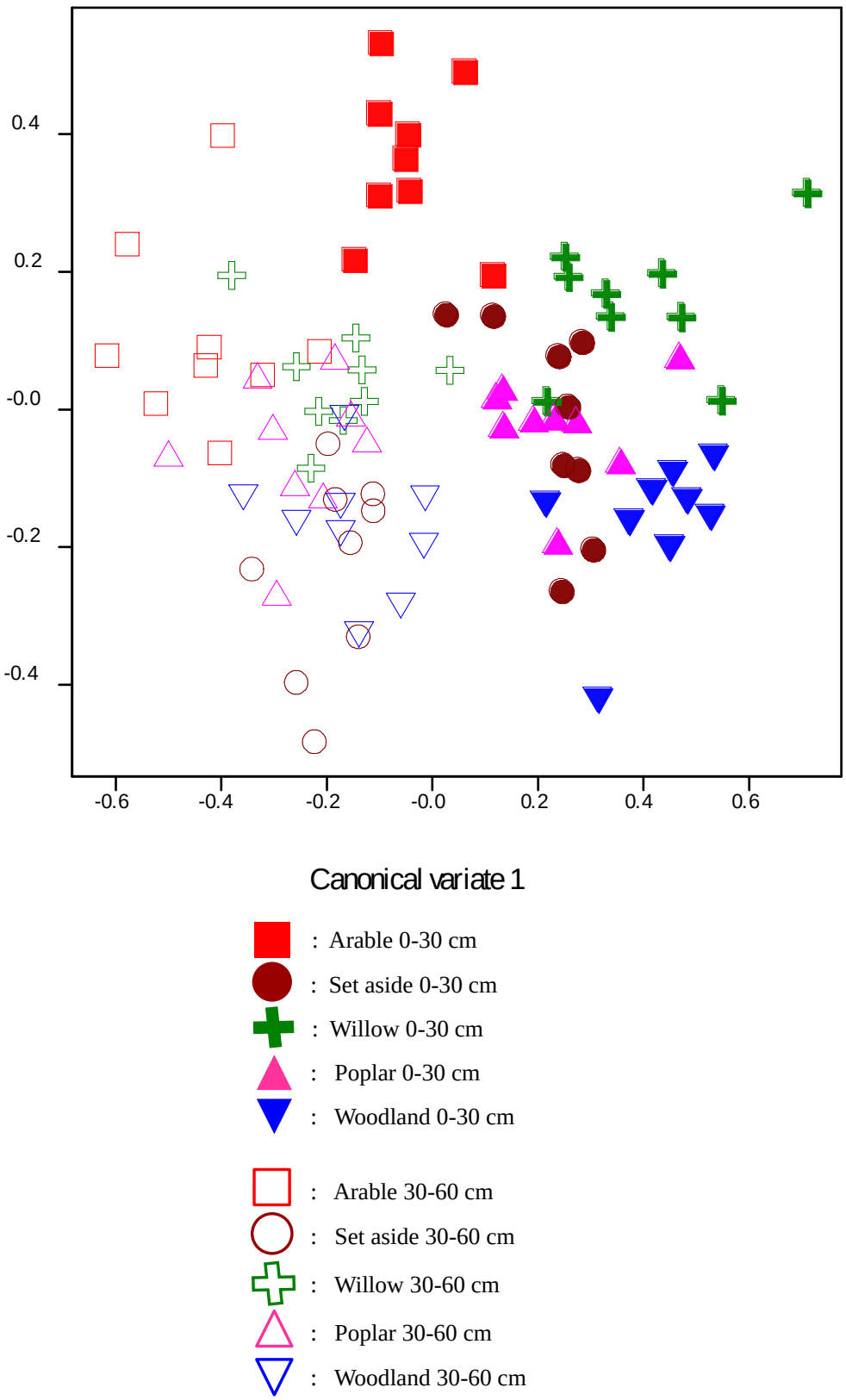


Figure 31. Canonical variate analysis of soil IR spectra using 355 references wavelengths

3.5.3 LFOM spectroscopic analyses

Visual inspection of IR spectra of free and intra-aggregate LFOM pools indicated that they were different (Figure 32 and 33). Most FTIR peaks in the LFOM represented organic compounds rather than soil mineral particles. The peaks for carbonyl, carboxyl stretch, C=O stretch of COOH or R-COO-R (ester), carboxylate, COOH, aromatic C=C stretching or symmetric C-O stretch in COO- (Bardy *et al.*, 2008; Lammers *et al.*, 2009; Artz *et al.*, 2008), which appeared between 1650 and 1730 cm^{-1} were more intense in the intra-aggregate LFOM than in the free LFOM.

Furthermore, intra-aggregate LFOM and free LFOM FTIR spectra showed clear differences in intensity between 2800 and 2900 cm^{-1} suggesting differences in the amount of fats, waxes or lipids (aliphatic/alkyl groups), and between 3160-3420 cm^{-1} relating to alcohols, phenols and organic acids (Fuentes *et al.*, 2007), or O-H stretching of cellulose (Artz *et al*, 2008). There were indications of differences in FTIR spectra in soil from different land uses. In the free fraction, the polysaccharide groups at 1000-1090 cm^{-1} was more prevalent in poplar and set aside soils than in the other land uses.

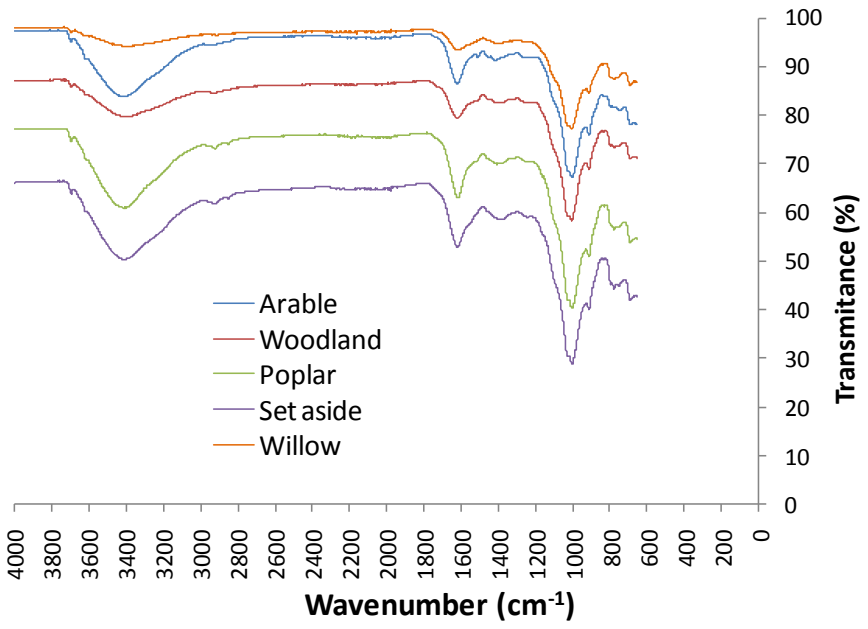


Figure 32. The average free LFOM FTIR spectra in different land uses (Data represents the mean of 9 sampling points)

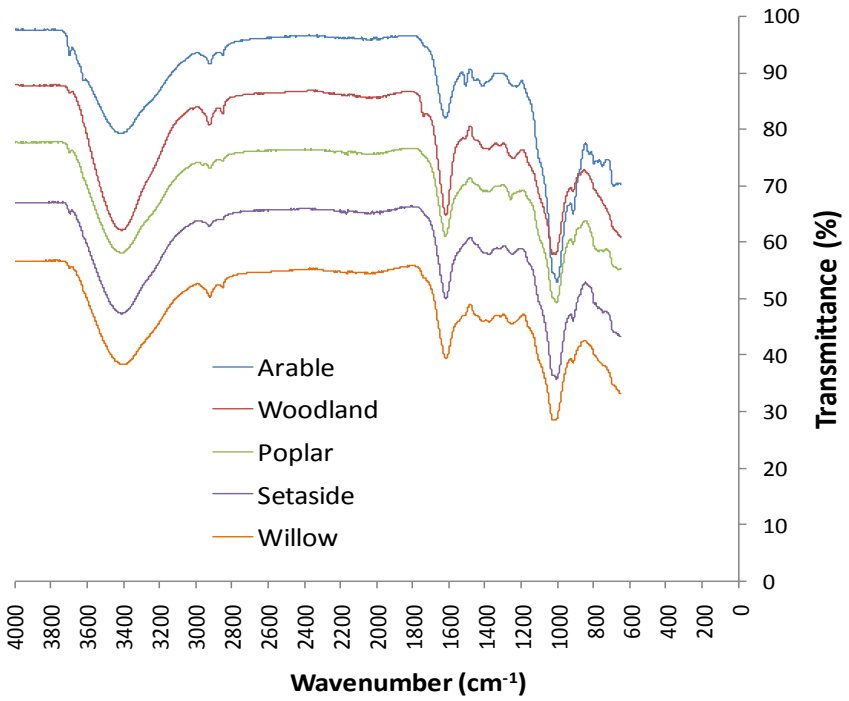


Figure 33. The average intra LFOM FTIR spectra in different land uses (Data represents the mean of 9 sampling points)

Furthermore, within free OM from willow soil there was a greatly diminished transmittance at 1634 cm^{-1} which relates to C-H deformation or C-O stretch.

Meanwhile, within intra-aggregate LFOM there was lower transmittance at 1000 to 1050 cm^{-1} in arable relative to other land uses, which indicates differences in polysaccharides. The highest intensity of the 3160-3420 cm^{-1} intra aggregate peaks was found in woodland soil (Figure 32 and 33).

The effect of LFOM fraction and land use on the 13 selected IR functional groups, using MANOVA, is presented in Table 26.

There was significantly greater transmittance in free relative to intra-aggregate LFOM at 1150, 1270, 1370, 1460, 1510, 1590, 1730, 2850, 2920, 3090, and 3400 cm^{-1} with the reverse true at 1630 cm^{-1}. Land use significantly affected transmittance of free LFOM at all wavelengths. In general transmittance of free LFOM in willow was higher than other samples, but the reverse was true for intra-aggregate LFOM. Between land uses there were significant differences in transmittance of free LFOM at all wavelengths except 1460 and 1730 cm^{-1}. Mostly, the lowest transmittance of free LFOM IR spectra was found in set aside at 1050, 1150, 1270, 1370, 1460, 1510, 1630, 1730, 2850, 2920, 3090 and 3400 cm^{-1}. Willow has been occupied the greatest transmittance in most of wavelength.

Generally, there were significant differences in transmittance of intra-aggregate LFOM between land uses except at 1050, 1270, 1510, 1590, 2920, 3900 and 3400 cm^{-1}. The lowest transmittance of intra aggregate LFOM has been observed in willow at 1370, 1510, 1630, 1730, 2850, and 2920 cm^{-1}, whereas woodland employed the weakest transmittance at 1150, 1270, 1460, and 1590 cm^{-1}.

The lowest transmittance means that there was a higher absorbance of the functional groups and it was indicate a stronger quantity of an existing of a specific chemical compound. The absorbance was calculated from the difference between total transmittance with the detected transmittance intensity.

***Table 26**. The effect of LFOM fraction and land use on FTIR transmittance at wavelengths corresponding to key organic matter functional groups. Different letter is denoted for significant differences (P<0.05).*

	% transmittance of each wavenumber (cm^{-1})												
	1050	**1150**	**1270**	**1370**	**1460**	**1510**	**1590**	**1630**	**1730**	**2850**	**2920**	**3090**	**3400**
LFOM													
Free	73.8a	88.7a	92.1a	91.7a	92.5a	92.1a	89.0a	87.5b	95.7a	94.7a	97.0a	93.1a	85.9a
Intra	71.2a	85.2b	87.4b	87.7b	80.5b	88.8b	83.5b	94.1a	88.6b	93.2b	91.7b	91.5b	77.1b
LSD	***4.3***	***2.09***	***1.8***	***1.8***	***2.76***	***1.66***	***2.8***	***2.42***	***1.34***	***1.14***	***1.36***	***1.38***	***4.75***
Landuse													
Arable free LFOM	75.3ab	89.6ab	92.0ab	91.9ab	92.1a	91.8ab	89.0ab	86.9b	95.5ab	95.1ab	94.4ab	93.0b	84.0ab
Poplar free LFOM	69.4b	86.7bc	90.8b	90.2b	91.2ab	90.6b	86.3b	84.0bc	95.2ab	94.1b	93.1b	92.1b	81.1b
Set aside free LFOM	67.5b	84.9bc	89.5b	88.6b	90.3ab	89.9bc	85.4bc	83.5c	94.5ab	92.9bc	91.7bc	90.5c	80.5b
Willow free LFOM	81.9a	92.4a	95.2a	94.9a	95.5a	95.3a	93.8a	93.6ab	97.2a	96.6a	96.4a	96.1a	94.2a
Woodland free LFOM	74.9ab	89.6ab	92.8ab	92.6ab	93.4a	93.0ab	80.7c	89.6bc	95.9ab	95.0ab	94.5ab	93.9ab	89.8ab
Arable intra LFOM	64.1b	84.9bc	88.5bc	88.8bc	82.5b	88.6bc	85.2bc	94.6a	87.9bc	93.0bc	91.5bc	91.4b	79.4b
Poplar intra LFOM	75.0ab	87.1b	88.8bc	89.1bc	82.0bc	90.3bc	85.0bc	95.0a	90.2b	94.2ab	93.0bc	92.0b	78.3b
Set aside intra LFOM	71.8b	85.9bc	88.4bc	88.0b	81.3bc	89.8bc	84.0bc	94.8a	89.7bc	94.0bc	92.9bc	91.7b	77.4b
Willow intra LFOM	72.9ab	84.5bc	85.8bc	86.0c	80.4bc	87.3c	82.3bc	93.5c	87.0c	91.8c	90.3c	90.5b	78.3b
Woodland intra LFOM	72.4ab	83.5c	85.7c	86.6bc	76.5c	88.1bc	80.7c	92.7ab	88.2bc	92.8bc	90.7bc	91.9b	72.3b
LSD	***9.8***	***4.4***	***3.8***	***4.0***	***6.1***	***3.6***	***6.1***	***4.9***	***2.9***	***2.3***	***2.8***	***2.9***	***10.3***

From the observation under a microscope, there were differences in visual characterisation of free and intra aggregate LFOM. It can be seen that free LFOM were similar to plant material since it was only partially degraded and had a brighter colour. Meanwhile, the intra aggregate LFOM is more decomposed, smaller in size and had a darker colour relative to free LFOM (Figure 34)

(a)

(b)

.

Figure 34. Visual characterization of free (a) and intra aggregate LFOM (b)

Detailed comparison of the relative abundance of the 13 selected OR wavelengths described in Table 27. 13 peaks were chosen in this study to identify difference in the chemistry of free and intra aggregate LFOM between land uses. These selected peaks were as follows: 1050 cm^{-1} (C-O stretching of polysaccharide), 1150 (OH of carboxyl-C), 1270 cm^{-1} (C-C or C=O deformation), 1370 cm^{-1} (C-H deformation of phenolic/aliphatic stretch), 1460 cm^{-1} (carbonyl/carboxyl stretch of lignin), 1510 cm^{-1} (C-H deformation), 1630 cm^{-1} (C-H deformation of keton and quinine), 1730 cm^{-1} (C=C vibration), 2850 cm^{-1} (stretch vibration of aliphatic C), 2920 cm^{-1} (C-H stretch of aliphatic compound (CH_2 or CH_3) of fat/wax, 3090 cm^{-1} (C-H from aromatic C), and 3400 cm^{-1} (OH stretch of phenol or amine).

The greatest changes in relative absorbance were seen at 1050, 1630, 1730 which represented 21, 9 and 5% of the total absorbance in free LFOM and 6, 3, 10% of total absorbance in intra aggregate LFOM. The effect of LFOM fraction and land use on the relative absorbance of the 13 selected IR functional groups is presented in Table 27.

There was significantly greater relative absorbance in free relative to intra-aggregate LFOM at 1050, 1150, 1630, 2850 and 3090 cm^{-1} with the reverse true at 1270, 1370, 1460, 1590, 1730, and 3400 cm^{-1}. Land use significantly affected relative absorbance of free LFOM at all wavelengths, except the peak at 1370, 1460, 1730, 2850 and 2920 cm^{-1}. No significant differences in intra-aggregate LFOM relative absorbance between land uses was seen at 1150, 1510, 1630, 2850, 2920, and 3400 cm^{-1}, with significant differences between land uses at the other wavelengths.

Table 27. *The effect of LFOM fraction and land use on the relative absorbance of key wavelengths corresponding organic matter functional groups. Different letter is denoted for significant differences (P<0.05).*

	% relative absorbance of each wavelength (cm^{-1})												
	1050	**1150**	**1270**	**1370**	**1460**	**1510**	**1590**	**1630**	**1730**	**2850**	**2920**	**3090**	**3400**
LFOM													
Free	21.5a	9.0a	6.1a	6.4a	5.8a	6.0a	8.2a	9.2a	3.3a	4.1a	4.6a	5.2a	10.0a
Intra	16.4b	8.3b	7.0b	6.8b	10.6b	6.2a	9.0b	3.3b	6.3b	3.9b	4.7a	4.7a	12.3b
LSD	***1.5***	***0.4***	***0.3***	***0.3***	***0.6***	***0.2***	***0.4***	***0.5***	***0.3***	***0.4***	***0.4***	***0.2***	***1.5***
Landuse													
Arable free LFOM	19.7b	8.2b	6.2bc	6.3b	6.1c	6.3a	8.3b	9.8a	3.1c	3.9a	4.3a	5.4ab	11.8a
Poplar free LFOM	21.0ab	8.8b	5.9c	6.2b	5.7c	6.0a	8.5a	9.7a	3.2c	4.0a	4.6a	5.1a	10.8ab
Set aside free LFOM	19.6b	8.8b	5.9c	6.5b	5.6c	5.8b	8.3b	9.6a	3.2c	4.2a	4.9a	5.5a	11.3a
Willow free LFOM	23.9a	9.9a	6.1bc	6.5b	5.8c	6.1a	8.0b	8.2b	3.6c	4.3a	4.5a	5.0b	7.6b
Woodland free LFOM	23.1ab	9.3a	6.2bc	6.3b	5.7c	6.0a	8.0b	8.9a	3.5c	4.3a	4.7a	4.1c	8.5b
Arable intra LFOM	20.2b	8.5b	6.2b	6.1b	9.5b	6.3a	8.0b	3.0c	6.9a	3.9a	4.8a	4.7b	11.3a
Poplar intra LFOM	15.8cd	8.1b	7.1a	6.7a	11.1a	6.0a	9.3ab	3.0c	6.0b	3.5a	4.3a	5.0b	13.5a
Set aside intra LFOM	17.2cd	8.5b	6.8ab	7.1ab	10.3ab	6.0a	9.0a	3.1c	6.1b	3.8a	4.4a	4.9b	12.2a
Willow intra LFOM	14.5d	8.1b	7.4a	7.3a	10.4ab	6.6a	9.3ab	3.4c	6.8a	4.3a	5.1a	4.9b	11.4a
Woodland intra LFOM	14.4d	8.5b	7.3a	6.7a	11.5a	5.9b	9.4a	3.8c	5.9b	3.9a	5.0a	4.1c	13.2a
LSD	***3.1***	***1.0***	***0.7***	***0.7***	***1.5***	***0.6***	***1.0***	***1.0***	***0.7***	***0.9***	***1.1***	***0.4***	***3.2***

Using the full 355 wavelengths, canonical variate analysis in conjunction with Chi square and F test showed that free and intra-aggregate LFOM had different composition and that this was also affected by land use. Free and intra aggregate LFOM were separated along CV1, which contributed 28.31% of variance, and CV2, which represented 22.98% of the variance (Figure 35).

Free LFOM from different land uses were separated along CV2 while intra aggregate LFOM from different land uses showed a far greater spread, with separation along both CV1 and CV2. Samples from within land uses clustered together for both free and intra-aggregate LFOM, although there was relatively little separation between land uses for free fraction compared to intra aggregate LFOM (Figure 35).

Furthermore, for arable and set aside soils, there was little distinction between intra-aggregate and free LFOM samples, whilst for poplar, willow and woodland free and intra-aggregate LFOM were clearly differentiated. A distinct separation between woodland and the other land uses were found along with CV2 in the intra aggregate LFOM.

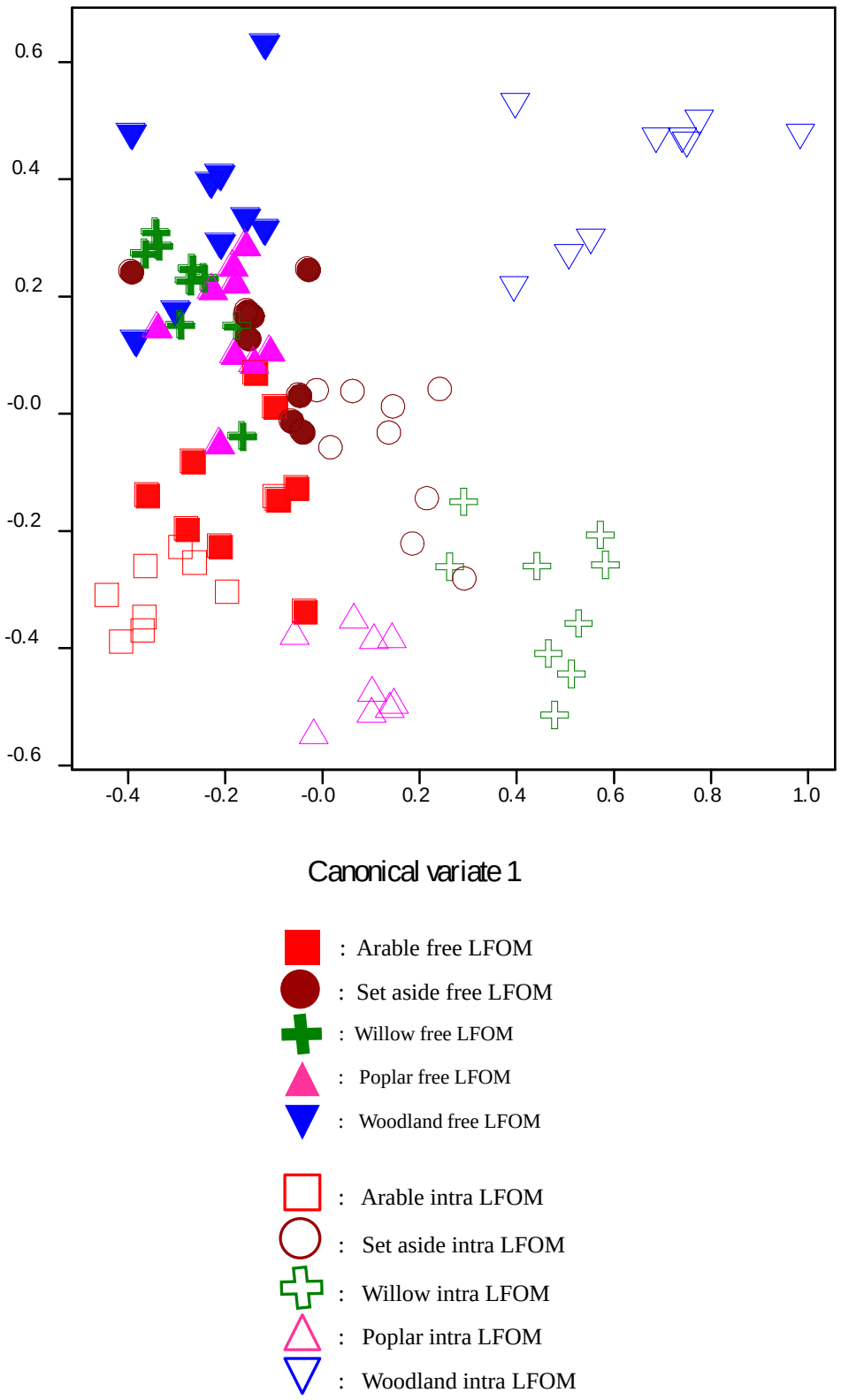


Figure 35. Canonical variate analysis of LFOM IR spectra using 355 reference wavelengths

3.5.4. LFOM as an indicator of land use change

Soil organic carbon (SOC) was significantly correlated with both free and intra aggregate LFOM C, when the average of SOC of the 45 soil samples at 0- 30 cm from each land uses were plotted against C content in free LFOM. The relationship between soil organic C and both free and intra aggregate LFOM C were in a linear relationship, more C in free LFOM were followed by the increasing of C in soil (Figure 36a and 36b).

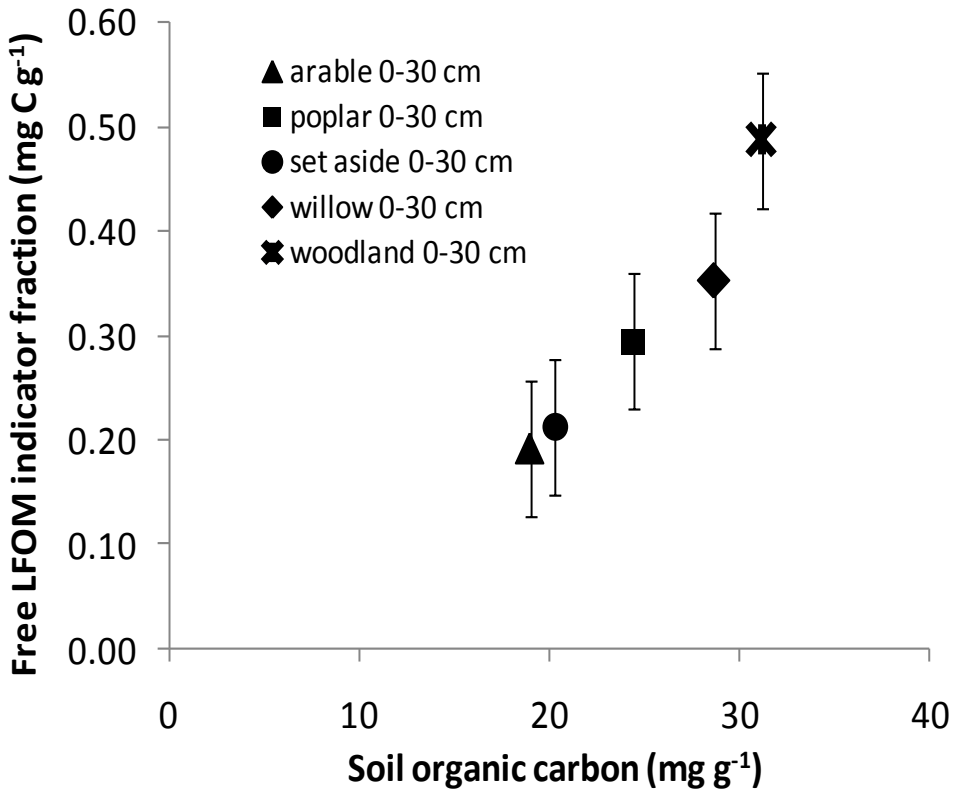


Figure 36a. The relationship between SOC and free LFOM C across different land uses at 0-30 cm soil depth (Error bar shown least square difference; P<0.05).

The gradient slope of free LFOM was steeper than intra aggregate LFOM and the error bar was smaller. The error bars represent least significant different at (P<0.05).

This indicated that free LFOM may be a better indicator of change in land use than intra aggregate LFOM.

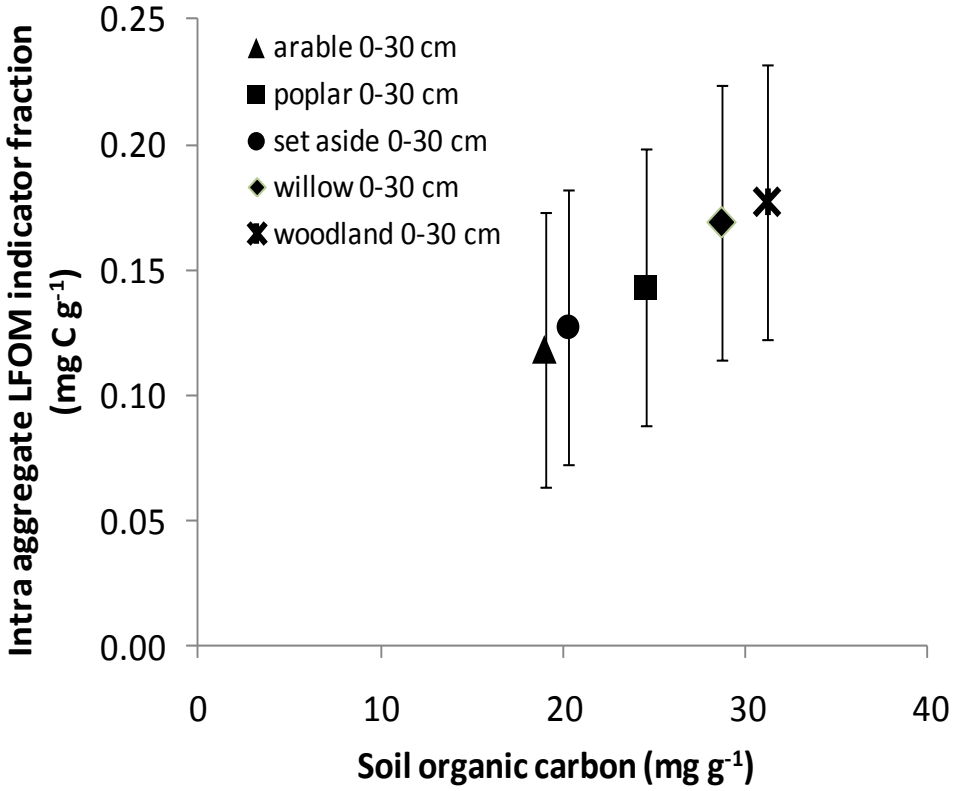


Figure 36b. The relationship between SOC and intra aggregate LFOM C across different land uses at 0-30 cm soil depth (Error bars shown least square differences P:<0.05)

The contribution of C in LFOM to the total soil C under different land uses was determined (Table 28).

Table 28. *Contribution of carbon in LFOM to total soil C (%) at the depth of 0-30 and 30-60 cm soil*

	Arable	**Poplar**	**Set aside**	**Willow**	**Woodland**	**LSD**
Free LFOM 0-30 cm	1.01	1.22	1.04	1.24	1.60	± 0.57
Intra aggregate LFOM 0-30 cm	0.61	0.57	0.61	0.63	0.58	± 0.50
Total LFOM 0-30 cm	1.63	1.79	1.66	1.88	2.20	± 0.85
Free LFOM 30-60 cm	1.14	1.02	2.55	1.18	0.93	± 1.32
Intra aggregate LFOM 30-60 cm	0.88	1.74	1.28	1.04	0.40	± 0.96
Total LFOM 30-60 cm	2.02	2.76	3.83	2.23	1.32	± 1.79

There were no significant differences in the percentage contribution of carbon in free, intra aggregate or total LFOM to total soil C between land uses at either soil depth. The contribution of free LFOM to total soil C was higher than intra aggregate LFOM at 0-30 cm (ranging between 1.01 to 1.60 %), whilst there was little difference between the proportion of soil C contained in free and intra aggregate LFOM at the lower soil depth.

The contribution of C concentrations in LFOM to the bulk soil under different poplar and willow genotypes is shown in Table 29. There were no significant differences on the percentage contribution of carbon in free, intra aggregate or total LFOM to total soil C under different SRC genotypes.

Table 29. *Contribution of carbon in LFOM to total soil C (%) at the depth of 0-30 and 30-60 cm soil under different SRC genotypes*

	Poplar			Willow			
	Beaupre	**Ghoy**	**Tricobel**	**Germany**	**Jorrun**	**Q83**	**LSD**
Free LFOM 0-30 cm	1.28	0.70	1.69	0.75	1.48	1.64	± 1.1
Intra aggregate LFOM 0-30 cm	0.90	0.42	0.39	0.56	0.50	0.95	± 1.0
Total LFOM 0-30 cm	**2.18**	**1.12**	**2.08**	**1.30**	**1.98**	**2.58**	**± 1.7**
Free LFOM 30-60 cm	0.52	1.11	1.41	0.93	1.47	1.12	± 2.7
Intra aggregate LFOM 30-60 cm	1.50	2.27	1.45	0.23	1.46	1.62	± 1.8
Total LFOM 30-60 cm	**2.02**	**3.38**	**2.86**	**1.16**	**2.93**	**2.75**	**± 2.8**

3.5.4 FTICR analysis of soil extracts

Two contrasting soil extracts were examined (arable vs woodland) as they were most different in term of their soil quality indicator characterization. This was based on SOC content, LFOM quantity (free and intra aggregate fraction), and IR spectral functional groups (as shown by a greatest separation following multivariate analyses (CVA).

The FTICR showed that a soil extract from arable and woodland mainly were dominated by oxygen (O) and nitrogen (N) compound classes. Therefore, these compound classes became the focused discussion in this present work.

The O and N compound classes were plotted in 2 dimensional bar charts, where the Y axis represented the percentage contributions of each ion (Figure 37a and 37b). All negative and positive ions were presented in bar charts. The negative and positive ions were used to represent each compound class (i.e. H or O). The labels “[H]” or “[O]” on the bar charts represented a protonated ion species (e.g. $C_2H_2O_2$), rather than

radical ions, and classes were labelled according to their heteroatom content. For example, "O_2" referred to $C_cH_hO_2$, where c is the number of carbon atoms, h is the number of hydrogen atoms along with two oxygen atoms. The label "CH" means there were no hetero atoms, only carbon and hydrogen.

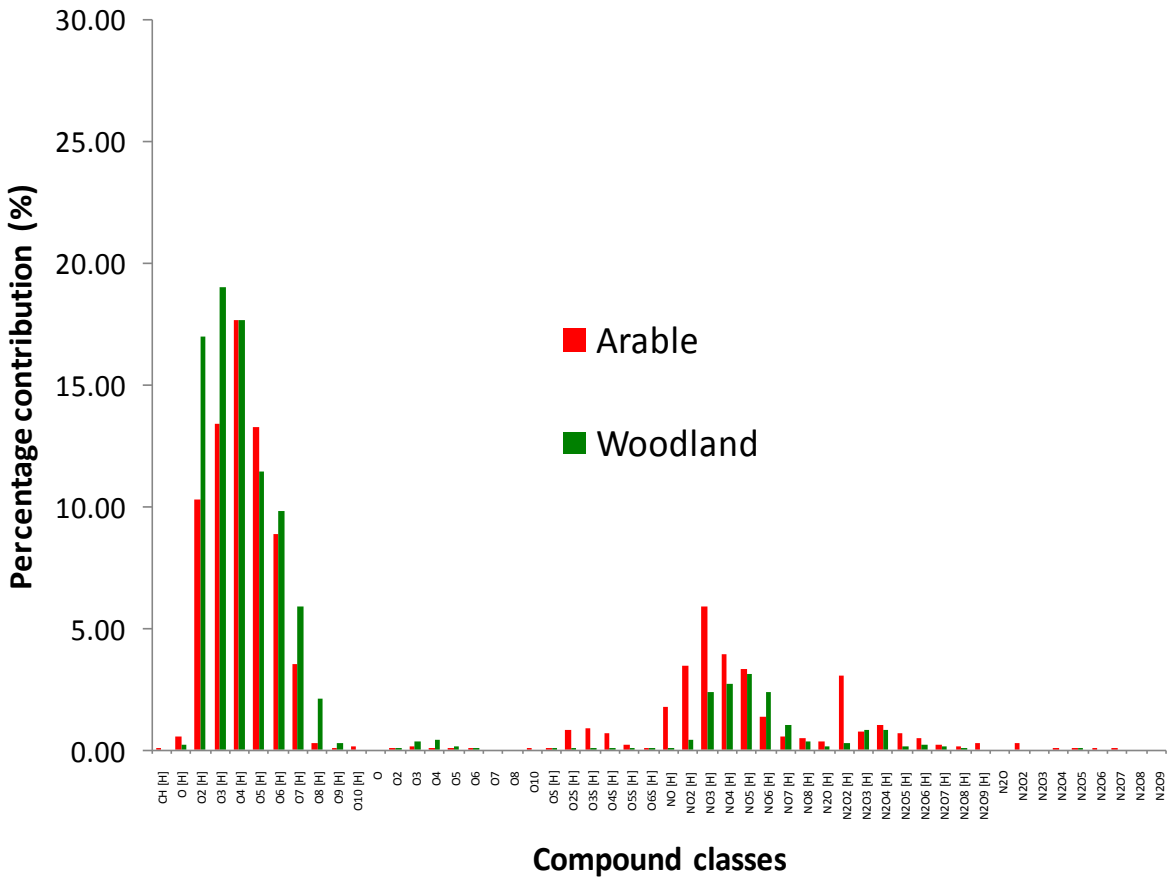


Figure 37a. Negative ion compound classes in soil extract from arable and woodland soil

The protonated nitrogen (N_2O_9 to NO) compound classes in both negative and positive ions contributed 20 to 30% to the total compound classes observed. The most dominant compound classes were protonated independent oxygen compounds (O_6 to O_2), which contributed 70 to 80% of the total ions.

Of the oxygen compound classes, woodland extracts had a higher percentage contribution than arable extracts in both negative and positive ion mode, with the exception of O_4 compound classes in positive ion mode.

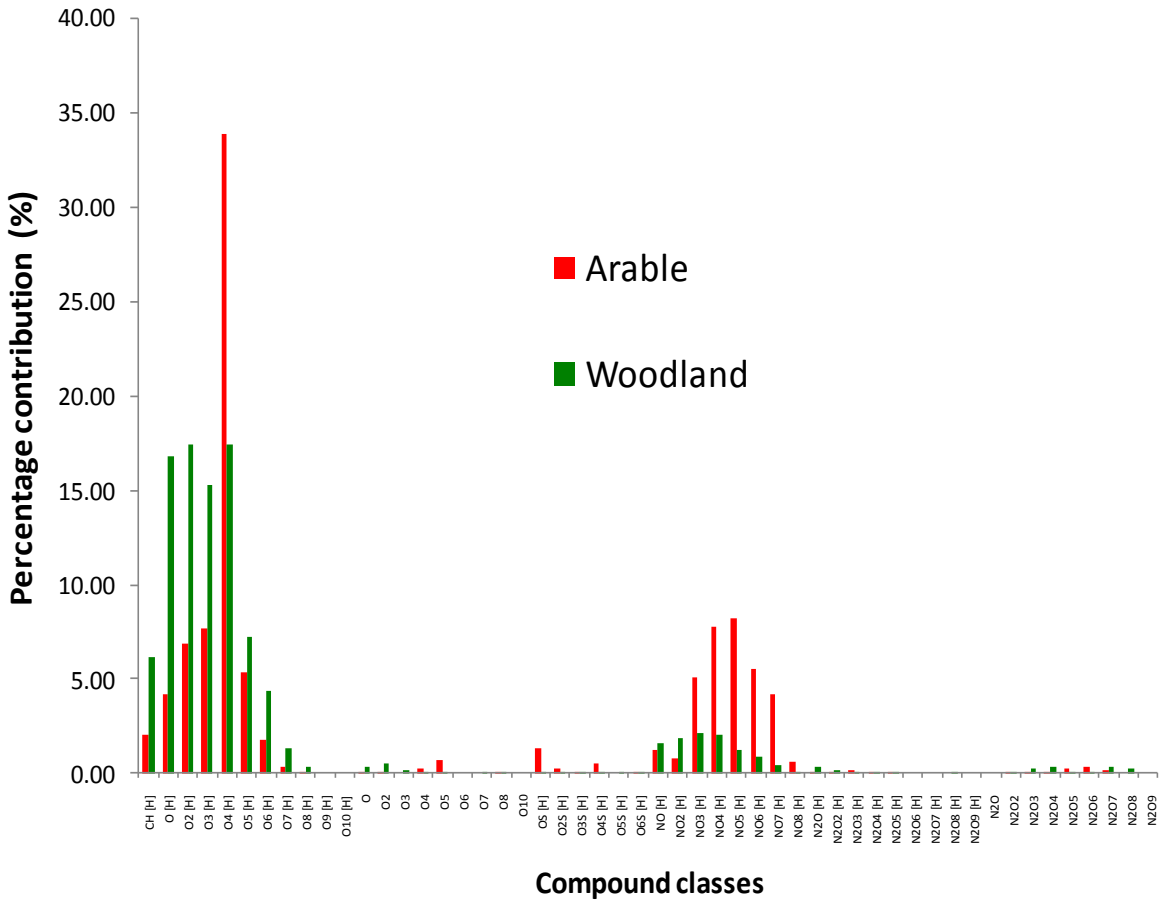


Figure 37b. Positive ion compound classes in soil extract from arable and woodland soil

This might be due to a contamination during extraction. O_2 to O_4 were the most dominant oxygen compound classes of the negative ion and positive ion, and NO_2 to NO_5 for nitrogen compound classes in the positive ion mode, while NO_4 and NO_5 dominated nitrogen in the negative ion mode. The nitrogen and oxygen bonds (N_2O_9 to NO) showed a different trend to O, with the contribution of arable compound classes being much greater than those in woodland.

Negative and positive ions of soil extracts were plotted using van Krevelen diagrams (Figures 38). These was to represent the ratios of hydrogen content to carbon content, and oxygen content to carbon content, which were plotted against each other. The advantage of this technique was that it allowed the grouping of ions along diagonal/straight lines. This allowed the identification of distinct compound classes and the associated relative intensity of each species.

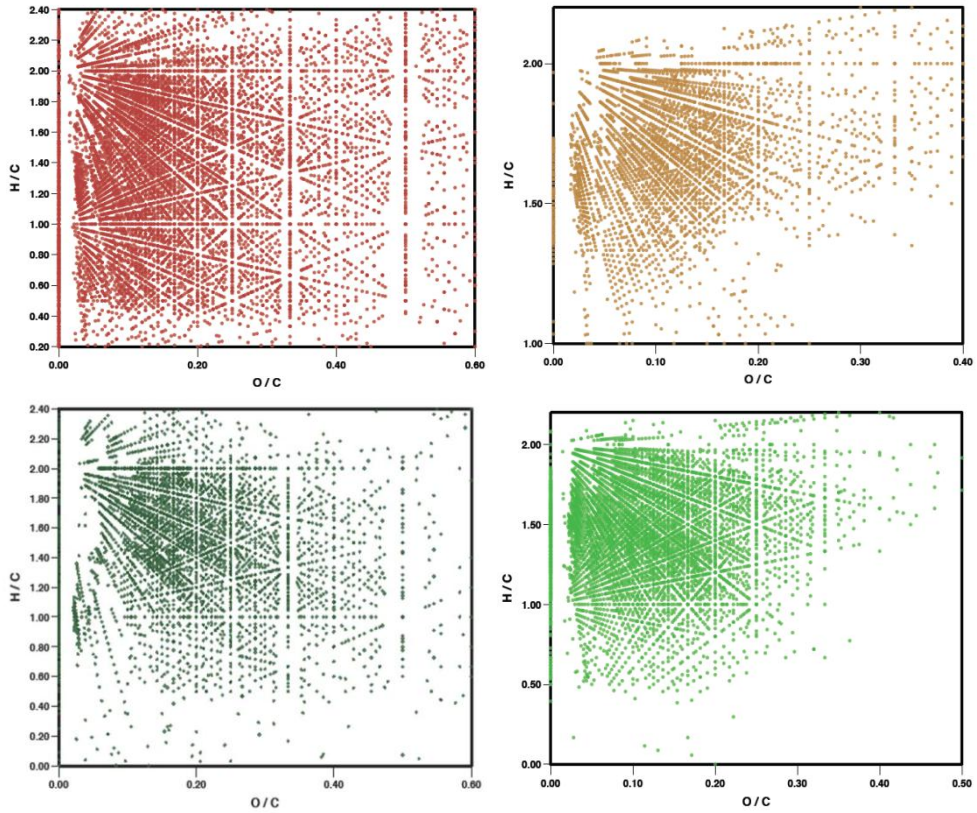


Figure 38. van Krevelen diagram of negative ions (upper left) and positive ions (upper right) of arable soil extract and negative ions (lower left) and positive ions (lower right) of woodland soil extracts.

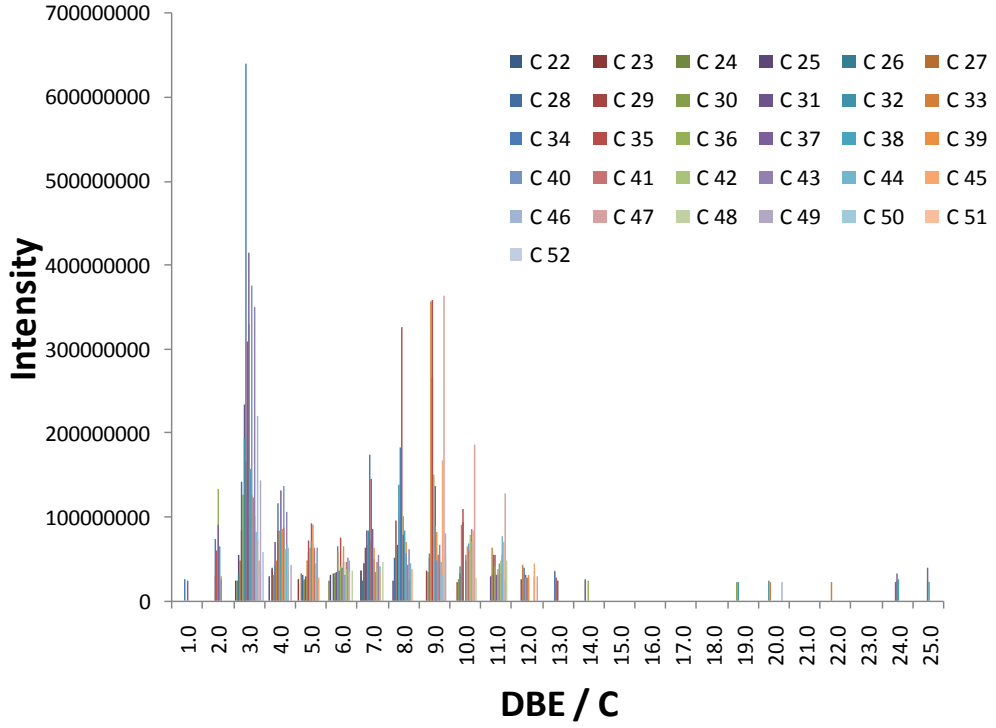
The arable soil extract contained a higher O/C and H/C ratio than the woodland extract. This indicated the presence of chemicals compounds corresponding to amino acids, lipids or carbohydrate. In contrast, lower O/C ratio or H/C of soil which represent lignin, long aromatic structures or condensed hydrocarbons was found in the woodland extract. The differences in the O/C ratios for negative and positive ion mode are presented in Table 30.

The average O/C ratio for negative ions in arable extracts was around 0.32, which was significantly higher ($P<0.05$) than in woodland (0.25), whilst there were no difference in positive ion modes. Similarly, the average H/C ratio in the arable extract (1.64) was significantly greater than in woodland (1.43).

Table 30. *Average of O/C and H/C ratio of both negative and positive ions mode from arable and woodland soil extract*

Compound classes	**O/C**	**H/C**	**Intensity**
Arable negative ions	0.32	1.36	23753450.6
Arable positive ions	0.13	1.64	106097154.6
Woodland negative ions	0.25	1.50	62121479.7
Woodland positive ions	0.12	1.43	40902573.3
LSD	±0.02	±0.02	±10895116.6

Because of the extremely complex nature of the OM extract, analysis by FTICR-MS produces a large data set with thousands of peaks, major compound classes were selected to understand the structure of the chemical compounds observed. For nitrogen, a positive ion mode compound classes of NO_3 and positive ion mode compound classes of O_4 for oxygen from arable and woodland extract were selected for comparison. The DBE value refers to double bond equivalent which indicate the total number of double bound and ring in a molecule.



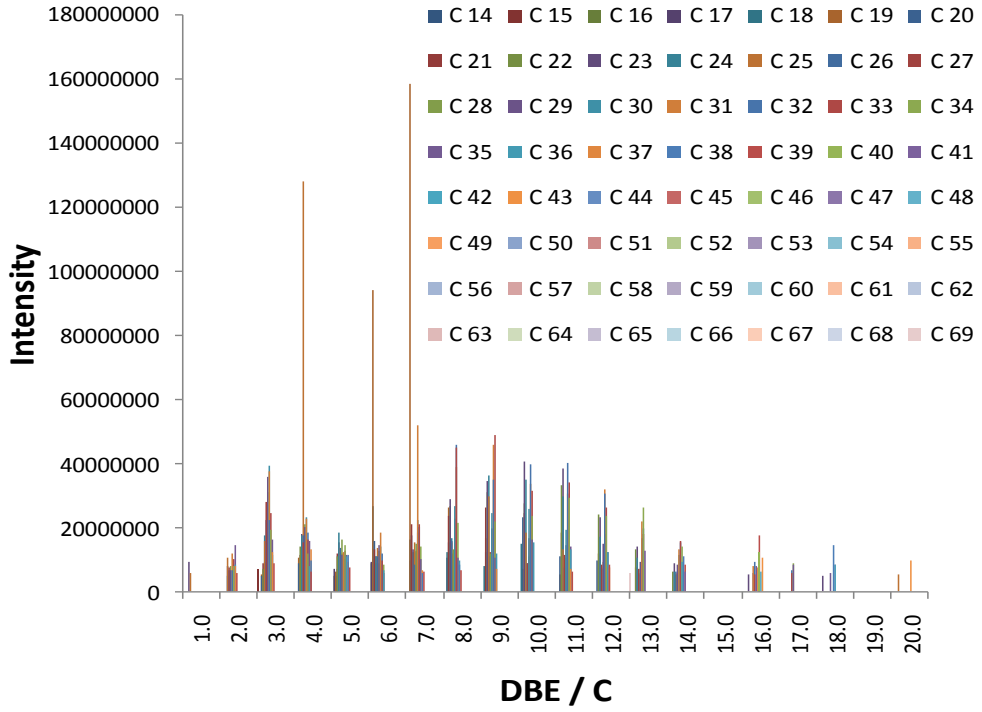


Figure 39. DBE versus intensity of positive ions mode NO_3 compound classes in arable (upper)(39a) and woodland soil extracts (lower) (39b).

Figure 39 shows that in terms of the NO_3 positive ion mode, there was a distinct pattern in the distribution of DBE/C value. In arable sample, most of the DBE/C values were predominantly observed within the range of 2.0 to 12.0, with the major DBE/C observed at 3. In woodland the range of the values was much bigger (2.0 to 16.0), but generally there was a lower intensity than in the arable extract. NO_3 compound classes which were mainly found in the arable extract may derive from the application of nitrogen fertilizer to this soil. However, the number of carbon attached to this compound was longer (C_{14} to C_{69}) in woodland than those in arable (C_{22} to C_{52}).

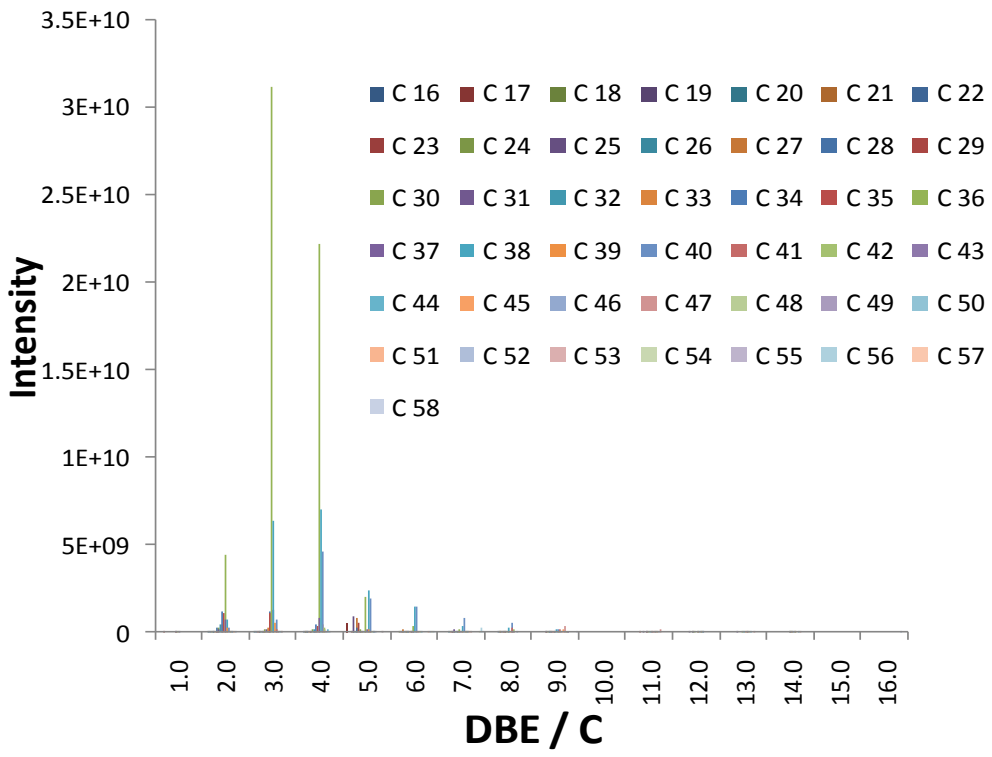


Figure 40a. DBE versus intensity of positive ions mode O_4 compound classes in arable

Positive ion O_4 compound classes in arable and woodland were also compared. The number of carbon atom attached to these compounds in woodland and arable sample extract were similar (C_{12} to C_{59}). However, the intensity of this compound class was higher in woodland that arable extract. The DBE/C value in woodland (4.0 to 19.0) was also greater compared to that in arable (2.0 to 8.0) (Figure 40a and 40b).

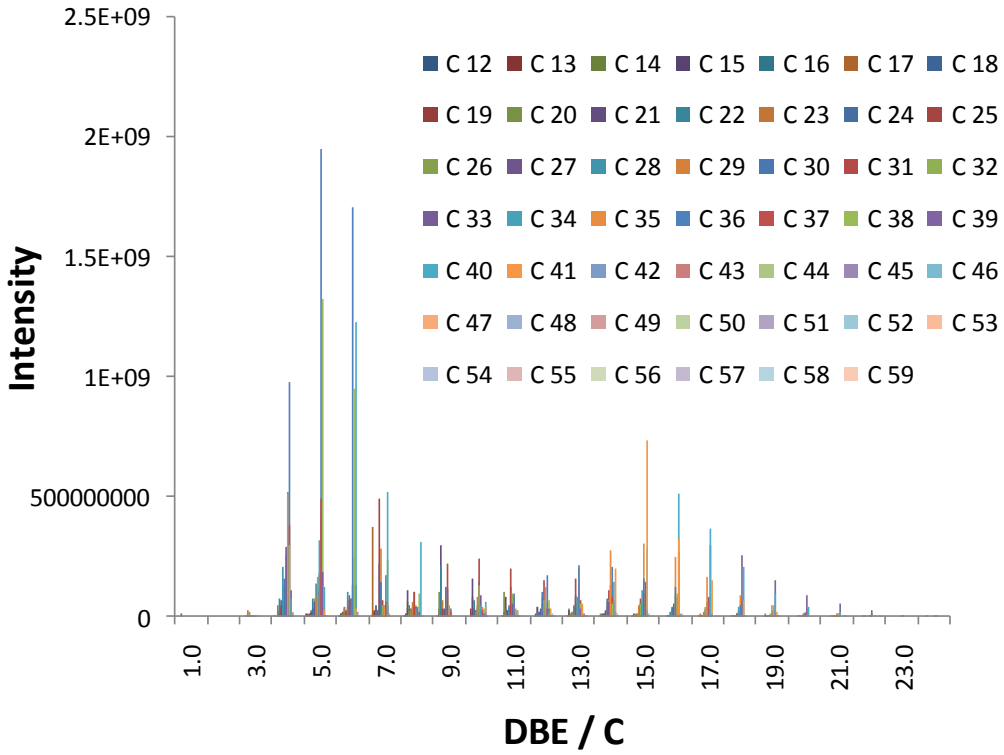


Figure 40b. DBE versus intensity of positive ions mode O_4 compound classes in woodland soil extracts

3.6 Discussion

3.6.1 The effect of land use change on free and intra aggregate organic matter

Previously, much research has focussed on investigating the effect of inter conversion between arable cropping, grassland and woodland on LFOM dynamics (John *et al.,* 2005, Yamashita *et al.,* 2005). However, there is currently limited data available describing the effect of conversion from arable cropping to short rotation coppice plantations. Instead, existing publications studying the impacts of land use conversion to short rotation cropping have mostly focused on changes in total soil carbon stocks (Hansen, 1993; Sartori *et al.,* 2007; Arevalo *et al.,* 2009), or the effects of management practices (e.g cutting cycle, fertilising) on the production of SRC crop above ground biomass (Labrecque and Teodorescu, 2005; Stolarski *et al.,* 2008; Walle *et al.,* 2007). These effects have been discussed in detail in Chapter 2.

The work in this chapter has shown that 14 years following conversion from arable cropping to SRC willow and poplar there had been 3 and 4 fold increases in the amount of free LFOM at 0-30 cm, respectively. However, amounts had not reached those in adjacent woodland. In contrast conversion from arable cropping to set aside had not resulted in change to free LFOM content.

While amounts of free LFOM at 0-30 cm were found to alter with change in land use, at 30-60 cm there had been no change in free LFOM content following land use changes. Furthermore, there was no difference in intra aggregate LFOM content between soil under arable cropping and woodland.

The results demonstrate that the free LFOM responds to land use change in top soil. Sub soil free LFOM was unresponsive to land use change as was intra aggregate LFOM in both top soil and sub soil.

C sequestration in crop biomass and soil LFOM pools in hybrid poplar plantations has been studied by Teklay and Chang, (2008), who used a chronosequence of 2 to 13-year-old stands. They found that amounts of LFOM declined two years following conversion to SRC, but remained steady thereafter, suggesting no trajectory for increase in LFOM over time. However, in their study conversion to SRC did not result in a change to total soil C content and it was not clear what the land use prior to SRC was. Additionally, the C and N concentrations (g kg^{-1} of fraction) in the SOM density fractions followed the order: LFo>LF>HF, whilst the C/N ratio followed the sequence: LF>LFo>HF. The contributions of carbon in the LF, LFo, and HF to total soil C 2 years after conversion were recorded as 0.3, 0.2 and 2.1%, respectively and the value for 5 years were recorded at 0.25, 0.15 and 2.2%. There was no change in the contribution of LFOM to total C until 11 years when the proportion of C in LFOM increased, although no difference was seen after 13 years.

The result of the current work has shown that land use change affected amounts of free LFOM, although this depended on the type of land use change. While conversion of arable cropping to broadleaved tree plantations resulted in an increase in free LFOM after 14 years, there had been no changes in free LFOM following change from arable cropping to set aside.

Similarly, studies by Helfrich *et al.,* (2006) and John *et al.,* (2005) found that conversion from arable cropping to woody tree plantations had resulted in large increases in LFOM after several decades, while conversion from arable cropping to set aside had resulted in minor changes to LFOM content. The results can be explained by quantitative and qualitative differences in the nature of organic matter inputs in the different systems.

In the case of Helfrich *et al.,* (2006) carbon concentrations of free fraction POM (fPOM) at a density of <1.6 g cm^{-3} under spruce (0-7 cm), grassland (0-10 cm) and an arable system (maize plot) (0-30 cm) were recorded at 214, 228, 320 g kg^{-1} fraction, respectively. The carbon concentration in fPOM was slightly higher than that of in the occluded POM (oPOM) or intra aggregate fraction which were 216 g kg^{-1} for spruce, 191 g kg^{-1} for grassland and 197 g kg^{-1} for arable cropping.

In the USA, grassland plots accumulated as much as 0.47 g LFOM kg^{-1} soil, which was within the range of the amount of grassland LFOM in New Zealand (0.09 to 0.42 g kg^{-1} soil) (Strickland and Sollins, 1987; Boone *et al.,* 1994). The amount of total LFOM in set aside which is assumed to be similar to grassland in this study was recorded at 0.9 and 0.7 g kg^{-1} soil for free LFOM at 0-30 and 30-60 cm soil depths, respectively. In term of the intra aggregate fraction, the values were 0.58 for 0-30 cm and 0.49 g kg^{-1} soil for 30-60 cm soil depths. These values were higher than the amounts of LFOM in grassland systems which were reported by Strickland and Sollins, (1987) and Boone *et al.,* (1994).

The C concentration of fPOM in a maize plot (35 years cultivation) was recorded 13 g kg^{-1} soil which contributed 4.2% of total soil C. This value was higher than the contribution of fPOM to total soil C which as 3.7% under a grassland system (John *et al.,* 2005). For them intra aggregate fraction, values of 1.0% have been recorded in maize, which were shown to lower than grassland (1.2%). In addition, total fPOM and oPOM of grassland plots have been reported to contain 6.15 g C kg^{-1} soil. The contribution of these pools to total soil C was 7.4 and 7.8%, respectively (Baisden *et al.,* 2002).

Change in land use from forest to pasture altered the percentage of carbon in fPOM and oPOM <1.6 g cm^{-3} to total soil C from 5 and 4% to 7.6 and 3.8% 35 years following conversion (Golchin *et al.,* 1994). It can be concluded that the contribution of carbon in fPOM and oPOM to total soil C across land uses is typically between 1 to 10%. In this study free and intra aggregate LFOM at 0-30 cm depth in the arable plot contributed 1% and 0.60% carbon to the total soil C, respectively, which is lower than previous studies (John *et al.,* 2005; Baisden *et al.,* 2002; Golchin *et al.,* 1994). Relative to top soil the proportion of total soil C as intra aggregate LFOM was higher in sub soil.

Bu *et al.,* (2012) compared the quantity of LFOM in soil under different types of native vegetation (i.e. evergreen broad-leaved forest, coniferous forest, dwarf forest and alpine meadow). The contributions of LFOM to total soil C across vegetation types ranged from 1.25 to 2.55% at a depth of 0-10 cm. The highest contribution of LFOM to total C was found in alpine meadow plots. This may have been due to the greater contribution of litter fall input and root biomass. However, environmental factors (e.g. altitude, annual temperature and rainfall may have affected the rate of SOM decomposition (Bu *et al.,* 2012).

Decline in the quantity of OM during cultivation can lead to losses in particulate organic matter or LFOM pools, which are rapidly affected by different management practices (Compton and Boone, 2002). The amount of LFOM in arable cropping systems may reach only to 30-60% of the levels present in environments under permanent vegetation (e.g. woodland) (Compton and Boone, 2000). Generally, LFOM in woodland plots can be as much as 60% of the total soil C, whilst in cultivated land it can range from 1-25% (Bu *et al.,* 2012; Janzen *et al.,* 1992; Boone, 1994).

At 0-30 cm depth, there had been no changes in the LFOM content of set aside relative to arable soil 14 years following conversion. A major difference between permanent set aside and arable cropping in term of management practice is tillage. Total amounts of LFOM (free and intra aggregate fractions) have been reported to be significantly affected by different tillage regimes.

For example, Haynes (1999) investigated LFOM accumulation under range of management regimes including: (1) low tillage arable-barley (*Hordeum vulgare*) (LT arable), (2) continuous grass/clover-perennial rygrass (*Lolium perenne* L. cv Supernui) and white clover (*Trifolium repens* L cv Huia) (cont grass/clover), (3) continuous grass – perennial ryegrass (Cont grass), (4) conventional tillage grass – perennial grass + ploughing (CT grass), and (5) zero tillage grass – perennial grass + glyphosate (ZT grass). The highest quantity of total LFOM was recorded in continuous grass/clover-perennial ryegrass at 15.9 g C kg^{-1} soil followed by ZT grass (5 g C kg^{-1} soil), cont grass/clover (4 g C kg^{-1} soil), cont grass (3.8 g C kg^{-1} soil), CT grass (3.5 g C kg^{-1} soil) and LT arable (2.8 g kg^{-1} soil).

3.6.2 The effect of depth on free and intra aggregate LFOM

In this study, conversion from arable cropping to set aside resulted in the amount of free LFOM at 0-30 cm almost doubling, but amounts were 50% lower than those fractions in woodland. However, in the lower layer LFOM was 4 times higher in set aside than in arable soil. Accumulation of LFOM will be determined by interplay between input of organic matter and decomposition processes. Litter and root biomass will predominantly accumulate at 0-30 cm depth across all land uses, with inputs at 30-60 cm depth relatively minor.

Within sub soil temperature is likely to be higher throughout the year than in top soil which will favour more rapid decomposition. However, conversely sub soil supports relatively smaller microbial populations than top soil and lower aeration may reduce decomposition processes (Rumpel and Kogel-Knabner, 2011).

In contrast to free LFOM, land use changes had no effect on intra aggregate LFOM even when arable soil was converted to SRC plantation. Furthermore, there was no difference in intra aggregate LFOM between woodland and arable soil. Sohi *et al.*, (2010) found a linear relationship between the total soil C content and intra aggregate LFOM. Similarly, John *et al.*, (2005) found that occluded LFOM was higher in forest relative to arable soil 30 years following conversion. Sohi *et al.*, (2010) suggested use of intra aggregate LFOM as an indicator fraction which could be applied to investigate soil status in term of stability or susceptibility to changes. Clearly, results presented in this chapter suggest that this fraction may not be universally useful in such a capacity, although free LFOM may be a suitable indicator. Furthermore, free LFOM has been shown to respond rapidly to land use and management changes. Much less data for response to land use change of occluded LFOM relative to free LFOM is available, and more evidence is required before the use of the two pools as indicators can be determined.

In contrast to soil from 0-30 cm depth, at 30-60 cm there had been no changes in LFOM content under SRC relative to arable cropping. Furthermore at this depth, the amount of LFOM in soil under woodland was not different to that in arable soil. Generally, studies for examining LFOM quantities and quality in various land use types (i.e arable, grassland or woodland) have focused on the upper soil layer (<30 cm) (Yamashita *et al.*, 2006; Helfrich *et al.*, 2006; John *et al.*, 2005), with very limited information being available about the LFOM status below this depth.

The concentration of fPOM in grassland at 0-10 cm depth was reported to be 1.5 times higher than at 10-30 cm, whilst the concentration of oPOM at this depth was more than 3.5 times lower than in the upper layer (Yamashita *et al.,* 2006). In woodland fPOM (free fraction) and oPOM (intra aggregate fraction) were more than 5 times lower in a sub soil (7-25 cm) compared to the upper layer (0-7 cm) (John *et al.,* 2005).

Significant differences in free and intra-aggregate SOM quantity were observed between native vegetation (woodland) and arable cropping at different soil depths (e.g. 0-5 cm, 5-10 cm, 10-20 cm) (Freixo *et al.,* 2002). The study showed that in woodland the amount of free fraction was 14.6 g kg^{-1} soil at 0-5 cm soil depth, and declined to 1.7 g kg^{-1} soil at 5-10 cm and 0.7 g kg^{-1} soil at 10-20 cm. However in arable soil, the average amounts of free LFOM declined from 2.5 g kg^{-1} soil at 0-5 cm to 0.5 g kg^{-1} soil at 5-10 cm and 0.2 g kg^{-1} at 10-20 cm soil depth.

Similarly, John *et al.,* (2005) found that after 35 years following conversion from arable cropping to spruce there had been no change to LFOM content at a depth of 7 to 45 cm, although there had been an increase at 0-7 cm depth.

3.6.3 The effect of genotype of SRC on free and intra aggregate LFOM

The work in this chapter has shown that the accumulation of free and intra aggregate LFOM at 0-30 cm was not affected by SRC willow and poplar genotype (Beaupre, Ghoy, Tricobel, Germany, Jorrun, Q83), 14 years following conversion from arable cropping. This may have been due to similar quality and quantity of OM inputs and hence decomposition between genotypes of the same species.

Amounts of LFOM under SRC genotypes ranged between 0.18 to 0.52 g C kg^{-1} soil for free LFOM and 0.09 to 0.29 g C kg^{-1} soil for intra aggregate LFOM, which had reached 25 to 75% of the value in adjacent woodland.

Boone, (1994) stated that total LFOM pools in Pine stands represented 14% of the total soil C, whilst for Maple stands this value was 4.60%. This was due the accumulation of LFOM at concentrations of 0.160 g kg^{-1} soil in Pine and 0.062 g kg^{-1} soil for Maple. However, these previous studies did not compare the LFOM quantity of the adjacent land use system to monitor the changes in LFOM and they only focused on the upper layer. In comparison, the oak species in woodland plots in this study accumulated total LFOM at the level of 2.5 g kg^{-1} soil at 0-30 cm depth which was 5 times higher than ar lower soil depths (0.5 g kg^{-1} soil).

Laik *et al.,* (2009) reported that different types of plantation (i.e *Pongamia pinnata, Albizia procera, Acacia lenticularis, Eucalyptus tereticornis, Swietenia mahogany, and Terminalia arjuna*) influenced the LFOM dry weight and the proportion of LFOM to total soil C. The greatest amount of LFOM at a depth of 0-15 cm was observed in *T.arjuna* plots (27 g kg^{-1} soil), which was significantly higher than the other species (i.e. *P.pinnata* (18 g kg^{-1} soil), *A.procera* (25 g kg^{-1} soil), *A.lenticularis* (23 mg kg^{-1} soil), *E. tereticornis* (26 g kg^{-1} soil), *S.mahogany* (21 mg kg^{-1} soil) and control (no plantation) (12 g kg^{-1} soil). In addition, the amount of LFOM in the lower layer (15 – 30 cm) was less than the upper layer. This significant difference in total LFOM was thought to be due to differences in tree growth performance and rooting behaviour. LFOM represented 10 to 12% of the total soil C (Laik *et al.,* 2009).

Similarly, in various tree species in the USA (i.e Conifer, Alder, Douglas fir), Sollins *et al.,* (1984) recorded that the quantity of LFOM at 0-12 cm soil depth, ranged

between 0.64 to 1.47 g kg^{-1} soil, which was equivalent to between 6.4 to 14.7% of total soil C.

3.6.4 LFOM and soil spectroscopic analyses

The value of analysing the biochemical characteristics of LFOM to inform on its quality is still under debate (Poirier *et al.,* 2000), and there are limited studies on the effects of land use on the chemical composition of SOC in aggregates and soil density fractions (Helfrich *et al.,* 2006).

Solid state CPMAS ^{13}C NMR spectroscopy has been successfully applied in studies looking at differences in SOM composition between soil fractions (Sohi *et al.,* 2001; Helfrich *et al.,* 2006). The O-alkyl-C content reduced in the order of: fPOM (54%) > oPOM (44.9%), whereas the alkyl-C content raised in the order of: oPOM (40.1%) > (22.7%) (Helfrich *et al.,* 2006). In addition, the aromatic, phenolic and carboxyl peaks of fPOM (free LFOM) were lower than in oPOM (intra-aggregate fraction) amongst three different soil textures (e.g sandy loam, silty clay and heavy clay) (Sohi *et al.,* 2001).

Furthermore, Sohi *et al.,* (2001) combined NMR approaches with FTIR spectra within a wavelength range of 1430 and 4000 cm^{-1} to investigate the composition of free and intra aggregate LFOM from a range of soils. The intense peaks at 1620-1600 cm^{-1}, which represent aromatic C (C=C stretching) and carboxylic anions (-COO asymmetric stretching) appeared in both fractions of the different soils. Aromatic C and C-H stretching on aromatic structures at 1525 and 3030 cm^{-1} were less intense in free than in intra aggregate LFOM, while the peak at 1450 cm^{-1}, corresponding to aliphatic hydrocarbons (C-H stretching), and at 2900 to 2940 cm^{-1} for the C-H aliphatic chain were more pronounced in intra aggregate than in free LFOM (Sohi *et al.,* 2001).

However, although the results showed a greater intensity of several peaks in the intra-aggregate pools which indicated that there was greater microbial transformation than in the free fraction, there were no statistical analyses performed to confirm these differences.

However, Poirier *et al., (*2005) was unable to detect any large difference in chemistry between unprotected (free light fraction organic matter) and protected POM C (intra-aggregate light fraction organic matter) using FTIR. In this study, the intensities of a range of bands were similar across fractions. However, absorption maxima in the free SOM fractions was higher than the intra aggregate fraction, which indicated less degradation of organic material in free than intra aggregate pools. Moreover, no statistical analyses were performed to support these conclusions.

Considering the complexity of IR spectra, robust and integrated analysis is required to draw definite conclusions on the significance of differences in spectra between samples. In this study, MANOVA and multivariate analyses (CVA) were used to determine the peaks that contributed most to the overall variations between the free and intra-aggregate fractions.

The contributions of these selected peaks to the total IR spectrum intensity were determined by their relative abundances (Haberhaurer, 1998). The intra-aggregate fraction had less intense peaks of polysaccharide (1050 cm^{-1}), C-H deformation or C-O stretch (1630 cm^{-1}), but had higher carbonyl/carboxyl stretches of lignin (1460 cm^{-1}) and C=C vibrations (1730 cm^{-1}) compared to the free fraction.

A previous study by Ellerbrock *et al.,* (1999) showed that the carboxyl group within the FTIR spectrum differentiated SOM from different types of management practice. The intensity of the carboxyl band at 1710 cm^{-1} increased in the sequence: liquid manure with low mineral nitrogen (LM) > wheat straw mixed with mustard as green manure with high mineral nitrogen (S + N) > high mineral nitrogen (N) > liquid manure with high mineral nitrogen (LM + N). It was concluded that the application of liquid manure resulted in the highest content of carboxylic groups within the organic matter.

FTIR provided a good indicator of decomposing SOM in soil horizons under pine (*Pinus halepensis*) and oak (*Quercus calliprinos*) forest (Gressel *et al.,* 1995). The organic layer under oak forest contained more polysaccharide (1080 cm^{-1}) indicating an early stage of SOM decomposition. A sharp decline in the aromatic peak at 1600 cm^{-1} and increases of the peaks at 1460, 1410 and 870 cm^{-1} were found at lower soil depths. This suggested that aliphatic groups accumulated, whilst the aromatic structures were oxidized.

A similar result was found in this study, whereas the peaks intensities from 1280 to 1510 cm^{-1}, assigned for aliphatic C-H bonds, increased at lower soil depths (30-60 cm), particularly in woodland, set side and willow soils.

A previous study from Chapman *et al.,* (2001) noted that the characteristics of organic layers from peat (*Calluna vulgraris, Sphagnum spp*), bog (*Trichoporum cespitosum*) and woodland (*P.sylvestris*) sites can be distinguished using IR spectroscopy. Relative to bog and moorland, in forest, higher peaks intensities were found in carboxylate (1601 cm^{-1}) and carboxylic acid (1710 cm^{-1}) functional groups and lower peak intensities was observed in the polysaccharide/carbohydrate region (1063

cm^{-1}). Bog and moorland samples were differentiated by peak intensity in the regions of 2924 cm^{-1}, which corresponds to wax, and 1624 cm^{-1} for carboxylate.

Haberhauer *et al.,* (1998) compared FTIR spectra of different soil horizons in contrasting sites and climatic regions. The organic layer (L), fermentation horizon (F), humified layer (H) and mineral soil (Ah) showed a similar peaks in the L to H horizons across sites and climatic zones. There was an increasing peak intensity at 1630 cm^{-1}, which represents carboxylic and aromatic functional groups and decreasing intensity at a region of 1510 to 1230 cm^{-1}, which represents amide II or aromatic C=C vibration. It might be concluded that these peaks can be used litter decomposition stages indicator.

The CVA analysis of free and intra aggregate LFOM could separate the samples from different land use types and different SOM fractions, whilst CVA analysis of the whole soil could only separate soils from different depths (0-30 cm vs 30-60 cm). It was concluded that the combination of IR spectra with multivariate analyses could be a rapid and sensitive tool for identifying the quality of SOM fractions.

PCA analysis has been reported to differentiate soil samples from grass clover ley restoration mining sites, taken across different years of sampling (e.g 1967, 1972 and 1985) as the level of SOM were decreased. The peaks related to organic matter functional group which may influence the separation of the PCA plots across time of observation were at 3060 to 3080 cm^{-1}, 2910 to 2940 cm^{-1}, 2800 to 2850 cm^{-1} and 1150 cm^{-1} (Elliott *et al.,* 2007).

3.6.5 Compound classes in soil extracts

The high resolution of FTICR can reveal complexity in the composition and structure of OM based on the DBE/C value. van Krevelen diagram can cluster molecules based on compound composition derived from complex biochemical pathways (e.g. methylation, hydrogenation, hydration, redox, carboxylation, etc) (Sleighter and Hatcher, 2008). Several studies have used van krevelen diagrams to investigate coposition of samples from aquatic ecosystems (Stenson *et al.*, 2003; Reemtsma *et al.*, 2008; Sleighter and Hatcher, 2008). The distribution of molecules from Dismal Swamp DOM was described by Sleighter and Hatcher, 2008) (Figure 41). Lower H/C and O/C indicated the presence of lignin, condensed aromatics, and unsaturated hydrocarbons which were also major components of the woodland extract in the current study.

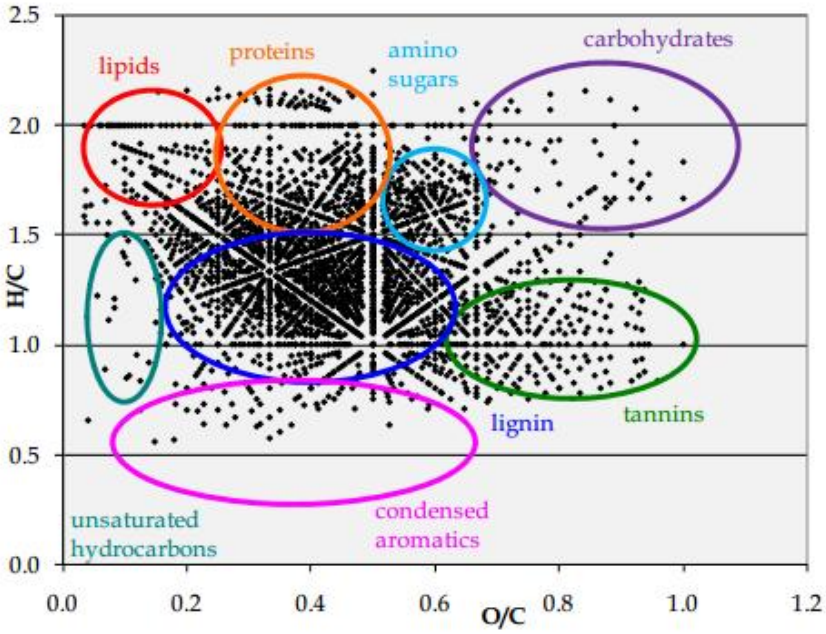


Figure 41. van krevelen diagram for dismal swamp DOM (from Sleighter and Hatcher., 2008).

Based on the DBE value, a large database of chemical compounds based on compound classes can be obtained. For example, various types of carboxylic acid can be verified and classified according to the carbon atom number and attachment of aromatic rings to specific compound classes (e.g NO_3 or O_4). Generally, the O/C value of soil extracts was much lower than the Dismal Swamp sample, but had similar range of H/C values (Sleighter and Hatcher, 2008).

3.6.6 Conclusion

The result showed that quantifying the amount of free and total LFOM can be useful tools for monitoring long term changes of carbon an nitrogen pools following 14 years conversion from the former arable land into alternative management practices (e.g. set aside, willow poplar and woodland). The data supported the hypothesis that reverting land use from arable cropping into SRC willow and poplar exceed the quantity of total LFOM by 3 and 2, respectively, compared to the amount of LFOM in arable soil at 0-30 cm depths. For willow, the quantity of free and total LFOM reached 70-80% of those amount of total LFOM in woodland, whilst for poplar the value was lower (50-60%). The lower input of SOM in arable and set aside resulted in low accumulation of free and total LFOM in this plot.

There was no significant different between treatments in the amount of free and total LFOM in the lower layer (30-60), except in set aside plot. This was due to the contribution of intensive hairy root development under grassland system. In term of intra-aggregate LFOM, no significant differences between soil layer was observed. Less activity of soil biota and lower SOM input in lower depth (30-60 cm) resulted in the reduction of total LFOM by 10-80%. Moreover, SRC genotype of willow or poplar did not affect on the amount of LFOM in both soil layer.

Soil and LFOM properties, based on the IR-spectrals which may represent typical functional groups of mineral or OM were clearly impacted by land use changes. Lower transmittance at 30-60 cm than at 0-30 cm, indicated the raising of the numerous mineral functional group (e.g O-Si-O bond, alumino silicate). In general, arable and willow soil had a lower IR spectral trasmittance than other land use at wavelengths 1050, 1630, 1730, 2920 and 3400 cm^{-1}.

In terms of LFOM IR-spectra, there was a distinct on the OM fingerprint (e.g C-O stretching of polysacharide, O-H of carboxyl C) between free and intra aggregate fraction. The result of FTIR analyses indicated that intra-aggregate LFOM was in a greater state of decomposition stages than free LFOM in most land use types. It can be seen from the higher absorbance on 3400 cm^{-1} (O-H stretch of phenol or amine), 1270 cm^{-1} (C-C or C=O or O-H deformation), 1730 cm^{-1} (C=C vibration of carboxyl ot trace of keton and ester), 1590 cm^{-1} (C=C aromatic C), 1460 cm^{-1} (carbonyl/carboxyl stretch of lignin) and lower absorbance in 1150 cm^{-1} (O-H or carbonyl C), 1050 cm^{-1} (C-O stretching of polysaccharide), 1630 cm^{-1} (C-H deformation or C-O stretch or C=O conjugated ketons and quinines) and 2850 cm^{-1} (stretch vibration of aliphatic C).

Multivariate analysis on soil IR spectra revealed a clear separation of land uses at 0-30 cm and less separation at 30-60 cm. At 0-30 cm greater separation was observed between arable and woodland samples, whilst willow, poplar and set aside clustered together.

Molecular elemental composition obtained using FTICR is suitable for distinguishing complex OM fingerprint from arable and woodland. Arable sample had a higher O/C and lower H/C ratio in negative ion mode. In term of positive ion mode, arable had a similar O/C ratio but higher H/C ratio. Lower O/C ratio and H/C in woodland indicated the presence of lignin, condensed aromatic and unsaturated

hydrocarbon. Detail relative intensity of selected compound classes (e.g. NO_3 and O_4) showed that the number of C atom in woodland is longer than those in arable. This means that organic compound in woodland are less degraded than arable and much more diverse. This was to backup the hypothesis that under contrasting management practices (arable vs woodland) the organic chemical present were not similar.

Chapter 4

Optimizing Carbon Sequestration during Pyrolysis of SRC biomass *(Salix sp)*: Biochar Properties and Carbon Balance Assessment

4.1 Introduction

4.1.1 Why are Willow SRC *(Salix, sp)* important as biomass feedstock?

The potential of biomass crops as viable renewable sources of energy has been widely recognized (e.g. Bridgwater, 2003; Ozcimen and Karaosmanoglu, 2004; Di Blasi, 2005; Faaij, 2006; Van de Velden *et al*., 2008 and 2010). Several crops have been tested as energy feedstocks such as willow short rotation coppice (SRC) (Volk *et al*., 2004; Park *et al*., 2004; Valmari *et al*., 1998); green waste (Chan, 2007); rapeseed cake (Karaosmanoglu, 2000); sugarcane bagasse (Inyang *et al*., 2010); and *Mischanthus*-grass (Clifton-Brown *et al*., 2004).

The rapid growth rate of SRC eg. Willow *(Salix sp)* has the potential to absorb a large amount of carbon from the atmosphere, and mitigate GHG-emissions (Aylott, *et al*., 2007; Walle *et al.*, 2007), thus contributing to the long term sustainability of biomass feedstock (Sartori *et al*., 2007; 2006). In terms of electricity production, Life cycle GHG-emissions willow biomass have been reported to be substantially lower than other conventional feedstock materials (e.g. peat and coal or even *Mischantus*) (Styles and Jones, 2007).

Willow has been considered as an important bioenergy crop as it has a high growth rate, quick rotation period (3 years), and the plant density can reach up to 15,000 ha^{-1}. However, most current studies of the potential use of short rotation coppice as a

biomass feedstock have only focused on poplar (Tharakan *et al*, 2003; Ozyurtkan *et al*, 2008). Biomass yields and production sustainability are major considerations when choosing feedstock (Lehman *et al.*, 2006). Willow biomass can reach 13.6 t ha^{-1} dry weight (Heller *et al*., 2003).

Willow feedstock was reported to have a calorific value of 22.57 MJ kg^{-1} (Klasnja *et al.*, 2002), which is higher than many other feedstock materials (e.g. rapeseed straw stalk at 16.37 to 17.64 MJ kg^{-1} (Karaosmanoglu, *et al*., 2000); apricot stone, hazelnut shell, grapeseed) at 19.28, 18.33, and 20.51 MJ kg^{-1} respectively (Ozcimen and Mericboyu, 2010); or even *Miscanthus* at 18.5 MJ kg^{-1} (Melligan *et al.*, 2011). Higher calorific values may lead to higher energy production during pyrolysis.

A comparison of the physical and chemical stem characteristics of willow and poplar bioenergy crops is presented in Table 31.

Table 31. *The physical and chemical stem characteristics of willow and poplar(adapted from Tharakan et al., 2003).*

Characteristic	Willow	Poplar
N (g kg^{-1})	3.79	4.06
P (g kg^{-1})	0.53	0.56
K (g kg^{-1})	1.56	2.04
Na (g kg^{-1})	0.13	0.13
Ca (g kg^{-1})	5.97	5.32
Mg (g kg^{-1})	0.36	0.39
Ash (g kg^{-1})	19.97	18.70
Moisture percentage (%)	0.53	0.55
Specific gravity (g cm^{-1})	0.41	0.35
Bark percentage (%)	5.88	4.83

As can be seen from Table 31, the authors found that willow had significantly higher Ca concentration, specific gravity and bark percentage, whereas poplar had significantly higher concentration of K.

The higher bark percentage of willow may be important because bark was found to contain higher concentrations of inorganic elements. Lower concentrations of elements in willow correspond to lower nutrient requirements for growth, whilst higher nutrient levels may require additional inputs through fertilization. Higher N concentrations in stem biomass are potentially emitted more NO_x during pyrolysis (Tharakan *et al*., 2003). The residual ash from willow biomass pyrolysis may still contain several major elements which can improve soil quality when applied as a supplement. The higher specific gravity of willow (0.41 g cm^{-1}) compared with poplar (0.35 g cm^{-1}) results in higher yield production and energy output (Table 31). In contrast, higher bark percentage composition can reduce energy output during combustion (Tharakan *et al*., 2003). Lower elemental or ash concentrations have been found in willow feedstocks compared to the other energy crops (e.g switchgrass or corn stover), which allows the production of cleaner energy (Tharakan *et al*., 2003; Brewer *et al.*, 2009). The need for fertilizer (N,P,K) application to willow exists only in the early stages of each rotation cycle (Styles and Jones, 2007). This means that using willow feedstock is more efficient as the need for external inputs are minimized. However, the application of organic manure to obtain optimum biomass production are commonly observed in willow plantation (e.g. waste water (Labreque *et al.*, 2001), pig slurry manure (Cavanagh *et al.*, 2001), wood ash (Lazdina *et al.*, 2011).

Expanding wildlife habitats and improving soil quality can also be significant effects of willow energy crops (Heller *et al*., 2003). All of the above aspects illustrate why willow feedstocks were used in this study to produce biochar following clean energy concepts.

4.1.2 The concept of Pyrolysis and Biochar production

The reduction of fossil fuel energy use is a major strategy for minimising green house gas emissions. Additionally, research has analysed the possibility of withdrawing carbon dioxide from the atmosphere through long term, rapid, low risk and accountable carbon storage mechanisms such as biochar sequestration. (Lehman, 2007a). The concept of biomass pyrolysis as described by Lehman, (2007a) is presented in Figure 42. Biomass pyrolysis for producing carbon neutral oil, gas and char is a type of technology that potentially breaks away our dependency on fossil fuels resources, by providing alternative fuels and chemicals (Mulligan *et al.*, 2010).

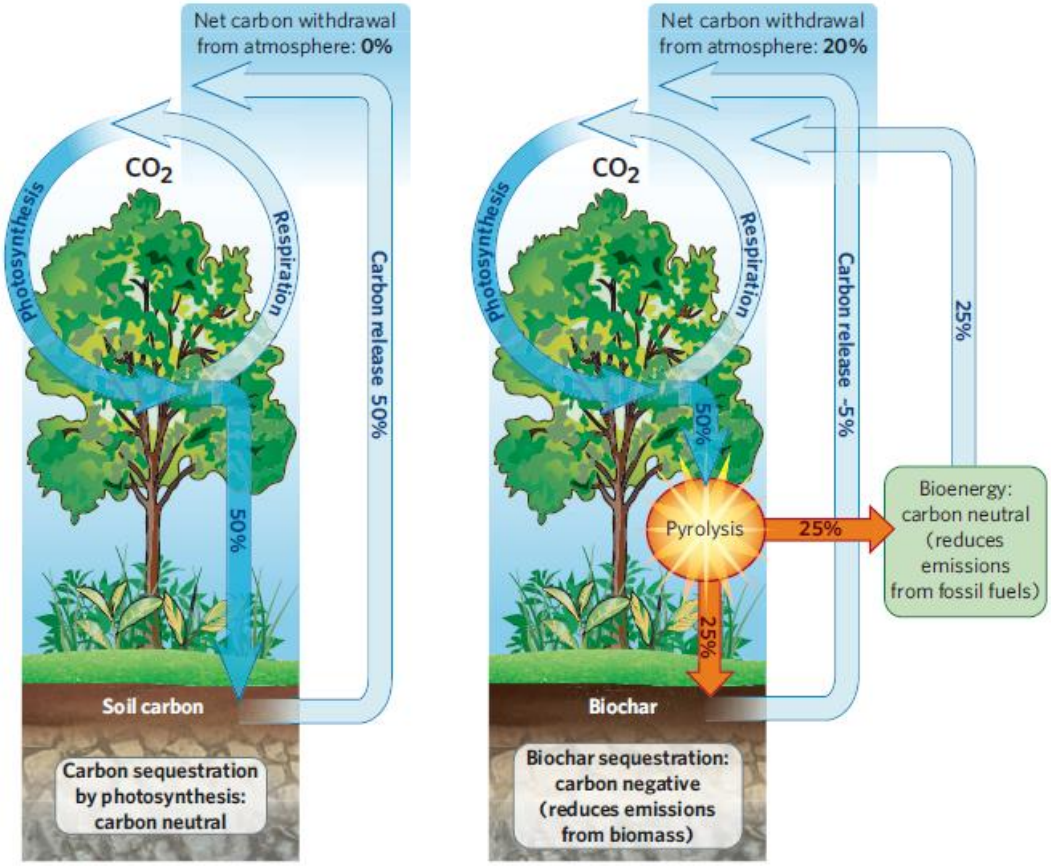


Figure 42. Conceptual carbon sequestration by biomass pyrolysis (biochar) (from Lehman, 2007a)

From Figure 42, it can be seen that under natural conditions repiration and decomposition release the same amounts of carbon dioxide (e.g plant respiration, SOM

decomposition) that are removed during photosynthesis. Conversion of biomass to biochar produces a material that retains significant amounts of C which are resistant to decay when added back to soil.

The term biochar refers to a fine-grained and porous substance, similar in its appearance to charcoal which is form by a specific process (e.g certain temperature or pressure), known as slow pyrolysis. There are a wide variety of char products produced industrially from different substrates and for a range of applications (Figure 43). For applications such as production of activated carbon, char may be produced at high temperatures (e.g 600°C), under long heating times and with a controlled supply of oxygen.

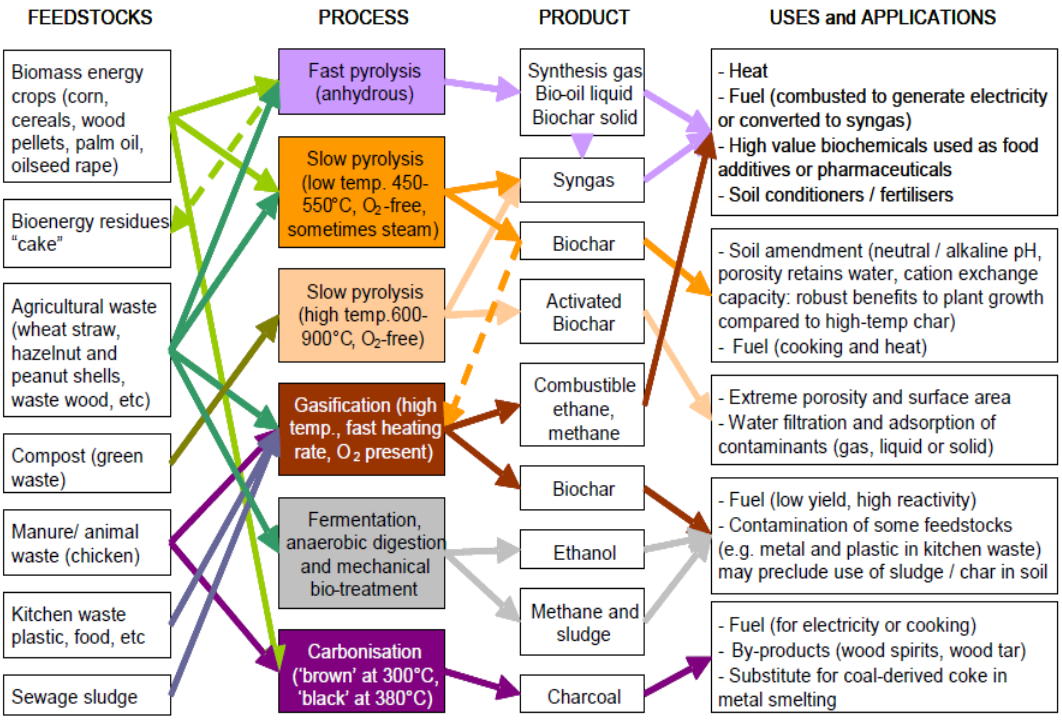


Figure 43. Schematic diagram of char products based on production process and their application (from Sohi et al., 2009)

In contrast, basic techniques for manufacture of charcoal (such as clay kilns) tend to function at a lower temperature (e.g 300°C), and without tightly controlled conditions. Therefore, traditional charcoal production is widely used to provide material for heating, cooking or producing electricity (Sohi *et al.,* 2009).

Unlike SOM, biochar is more stable and contains two times the amounts of carbon as ordinary SOM per kg^{-1} of dry weight, along with a longer duration of decomposition (hundreds to thousand years). By withdrawing organic carbon from the cycle of photosynthesis and decomposition, biochar sequestration directly removes carbon dioxide from the atmosphere and prevents the rapid decomposition of carbon from the plant biomass (Lehman, 2007b). Biochar can be accredited as a soil amendment or fertilizer, including soil conditioning, by improving cation exchange capacity, pH, water retention, sorption of pollutants and sequestering toxic metals and gradually releasing nutrients (Bailey *et al.,* 2011 ; Uchimiya *et al.,* 2010).

Pyrolysis is the thermal decomposition of a substrate in the complete absence of an oxidizing agent (air or oxygen), or with such a limited supply that combustion or gasification does not occur to any appreciable extent. The process is typically divided into 3 different types (slow, intermediate and fast) (Bridgwater, 2006). It is the most popular thermal conversion process, particularly in Europe (Ozcimen and Karaosmanoglu, 2004).

Different types of pyrolysis during biochar production affect the composition of the products formed (Table 32). Under fast pyrolysis (moderate temperature, shorter vapour residence time), the most dominant product produced is bio-oil, whilst gasification (higher temperature, longer residence time) maximises the production of gasses (Sohi *et al.,* 2009).

***Table 32**. The product release under different temperature and residence time of pyrolysis (from Sohi et al., 2009)*

Process	Liquid (bio-oil)	Solid (biochar)	Gas (syngas)
FAST PYROLYSIS Moderate temperature (~500 °C) Short hot vapour residence time (<2s)	75% (25% water)	12%	13%
INTERMEDIATE PYROLYSIS Low-moderate temperature, Moderate hot vapour residence time	50% (50% water)	25%	25%
SLOW PYROLYSIS Low-moderate temperature, Long residence time	30% (70% water)	35%	35%
GASIFICATION high temperature (>800 °C) Long vapour residence time	5% tar 5% water	10%	85%

The material used and the specific pyrolysis conditions (e.g. temperature, pressure, heating time) determine the physiochemical structure of the biochar formed (IEA, 2007; Spokas *et al.*, 2009; Czimczik and Masiello, 2007). The conditions can be modified to produce biochar with specific characteristics (e.g high surface area, condensation grade, particle size, etc) (Titirici *et al.*, 2007).

Although using pyrolysis to produce energy and/or biochar is recognised as a key technology in energy valorisation of SRC biomass, at present most of the experiments performed to study the production and use of biochar have been small-scale (Liu *et al*, 2010; Karaosmanoglu *et al.*, 1999; Durenkamp *et al.*, 2010; Inyang *et al.*, 2010; Zhang *et al.*, 2010a), and data from medium to large scale experiments is unfortunately lacking.

4.1.3 Fluidized Bed Technology for producing Biochar

Fluidized bed is a typical slow model of pyrolysis involving a low to medium temperature (450 to 650°C) (Sohi, *et al* 2009), which targets the production of biochar, whilst fast pyrolysis mostly targets the production of bio-oil using higher temperatures (>600°C) (Kwapinski *et al*., 2010).

Biochar production under slow pyrolysis can reach 35%, which is higher than the average amount of biochar produced by fast pyrolysis (12%) (Table 32). However, fast pyrolysis can yield 75% bio-oil which is more than twice that of slower methods (30%) (Winsley, 2007). It has been shown that the production of biochar for maximizing carbon capture requires slow pyrolysis. Much concern is currently focused on fast pyrolysis (Khodier *et al.,* 2009; Liu *et al.,* 2010; Brewer *et al.,* 2009) using higher temperatures (600 to 1000°C), whilst the application of slow pyrolysis in biochar production using energy crop feedstocks has rarely been studied.

In a conventional bubbling fluidized bed pyrolyzer, the char particles will mostly remain in the bed material, while the pyrolysis vapours and gases are carried out of the reactor. The advantages of fluidized a bed pyrolyzer are its ease of operation and simple design, which allows the system to be scaled up for producing biochar in greater quantities (Liu *et al*., 2010; Bridgwater and Peacocke, 2000). In addition the fluidized bed is a flexible technology, with high combustion efficiency and low environmental impact (Scala *et al.,* 2003). The disadvantages are concerned with operational problems such as: mixing/segregating the feedstocks and char or spatial burning profile of the reactor, collection of volatile organic material and slagging/fouling/agglomeration of the ash component.

Furthermore, Gerber *et al.,* (2010) reported that the fluidized bed reactor is the most efficient way to extract energy from biomass due to high particle heating rates and good mixing processes. The study showed that the bed material (e.g sand) will allow the temperature to remain constant and evenly distributed, preventing direct contact between the biomass and the combustor/heater. This was supported by the evidence that low segregation of char particles and equal densities were observed throughout the reactor (Gerber *et al,* 2010).

The difficulty of predicting spatial heating in a fluidized reactor has been successfully determined using a Euleraian dynamic movement model, developed by Gerber *et al.,* (2010). The study showed that the formations of bubbles at the bottom of a fluidized bed reactor were relatively small compared to the upper part. Thermal image analysis showed that the heat was distributed along the reactor walls. This means the feedstock will receive heat evenly and be completely combusted at the same time throughout the reactor (Gerber *et al.,* 2010).

To better elucidate the potential use of willow as a feedstock for biochar production, a detailed assessment of the effects of low temperature on the properties of biochar produced using a fluidized bed reactor is required as no information is currently available.

4.1.4 Methods for assessing Biochar quality

The quality various types of biochars have been indentified using Fourier Transform Infra-red spectroscopy (FTIR). (e.g apricot stones, hazelnut shells, grape-seeds and chestnut shells (Ozcimen and Mericboyu, 2010), wheat straw (Mulligan *et al.,* 2010), rapeseed (Karaosmanoglu *et al.,* 2000), orange peels (Chen and Chen, 2009) and *Miscanthus* (Luo *et al.,* 2011).

FTIR methods are known as simple and quick approaches for observing changes in functional groups of any organic material. More advanced techniques (e.q. NMR) need longer preparation times, are more expensive, and require skilled technicians to operate the equipment and interpret the results. Archaeological deposits of black carbon (BC)/biochar have been characterized using FTIR in combination with NMR. Additionally, scanning electron microscopy (SEM) (for examining visual properties) and X-ray diffraction along with the Brunauer-Emmet-Teller (BET) machine (for examining surface area) have been used to identify the properties and the characteristic of switchgrass biochar (Boateng, 2007). Visual characterization of biochar produced from corn stover at the pyrolysis temperature of 550°C has been observed using SEM, showing that the surface area of the product was increased by pyrolysis (Fuertes *et al.,* 2010).

The chemical composition of willow biochar produced in a laboratory scale reactor at 550°C has been indentified using GC/MS and GC/FTIR/FID (Ingemarsson *et al.,* 1999). Using IR spectroscopy the functional groups and peak intensities of wheat straw biochar produced at temperatures of 500°C and 1000°C were shown to be different to the raw material (Mulligan *et al.,* 2010).

The wheat straw data was dominated by a peak in the region of 1030 cm^{-1} which represented primary alcohols or ethers in the lignin structure. A lower intensity of this peak was found at 500°C compared to the raw material and it was completely lost at the higher temperature (1000°C).

A similar pattern was also recorded for peaks at 3300, 1420 and 1368 cm^{-1} which represent aromatic –OH groups. Moreover, aromatic rings corresponding to peaks in the region of 1620 and 1514 cm^{-1}, C=O vibration at 1730 cm^{-1}, stretching of C-O or C-O-C at 1200 to 1400 cm^{-1}, asymmetric/symmetric CH_2 bond at 2920/2848 cm^{-1} in the raw material were mainly detected weakly at 500°C, with no peaks remaining at 1000°C (Mulligan *et al.,* 2010). This meant that temperature affects biochar quality and their properties.

Different pressures during pyrolysis have been reported to affect the visual characteristics of switchgrass biochar under SEM imaging (Boateng, 2007). Using similar techniques, Luo *et al.,* (2011) noted that more of the plant cell structures of *Miscanthus* biochar were collapsed when heated to 750°C compared with biochar produced at a lower temperature (350°C).

IR spectroscopy has also been used for identifying black carbon (BC)/biochar deposit properties from a “slash and burn” farming system in Kenya over a period of 100 years (Nguyen *et al.,* 2008). Significant changes in several functional groups were observed between samples from slash and burn and unburned areas. The reductions in relative peak intensities of BC was found in the regions of aromatic groups (C=C) (1640 cm^{-1}), aliphatic C-H (1389 cm^{-1} and 2920 cm^{-1}) and hydroxyl groups (O-H) (3370 cm^{-1}), whilst a peak representing carbonyl groups (1700 cm^{-1}) increased. In addition, there was a 30% reduction in soil C and N over the 100 year period (Nguyen *at al.,* 2008).

Using IR, the ratio between absorption intensity at 2490 cm^{-1} (aliphatic C-H) and 1600 cm^{-1} (aromatic C=C) declined from 1.18 to 0.80, when the *Miscanthus* biochar was produced at temperatures of 350°C and 750°C. This means that there was an increased contribution of aromatic structures in the higher temperature products (Lou *et al.,* 2011).

Total content of residual nutrients in biochar depend on the pyrolysis temperature. Increasing pyrolysis temperature of wastewater biochar from 300°C to 700°C reduced the concentration of N by 55%, but increased the concentration of P by 43% and other nutrients such as Ca, Fe, Mg, S, Cu and Zn (Hossain *et al.,* 2011).

4.1.5 Greenhouse gasses (GHG) released during pyrolysis

Determination of the amount of GHG formed during pyrolysis has been the subject of many studies (Chen *et al.,* 2010; Detourney *et al.,* 2011). The production of GHG during pyrolysis depends on various factors (e.g temperature, quantity and quality (chemical compostion) of feedstocks, pressure, water content etc). The most dominant gasses released are CO, CO_2, CH_4, H_2 and NO_2 (De Bari *et al.,* 2000). The nature of gases formed during pyrolysis in previous studies is presented in Table 33.

From Table 33, the H_2 concentration ranges from 1.66 to 23.1%, CO from 18.5 to 55.3%, CO_2 from 5.5 to 75.0%, CH_4 from 4.4 to 13.2 % and C_2H_4 from 0.5 to 53.36%.

Table 33. *Total gas produced from various feedstocks under different temperatures of pyrolysis*

Feedstock	Temperature (^oC)	Type of gas (mol %)					Reference
		H_2	CO	CO_2	CH_4	C_2H_4	
Pine sawdust	500	1.66	26.22	14.77	4.99	52.36	Chen *et al.*, (2010)
Oak wood	855	19.3	53.2	8.4	13.2	5.8	Detourney *et al.*, (2011)
Oak wood	900	18.7	55.2	7.2	12.7	5.6	Detourney *et al.*, (2011)
Oak wood	940	21.6	55.3	6.3	11.9	6.4	Detourney *et al.*, (2011)
Conifer wood	855	18.5	54.5	8.0	12.8	6.2	Detourney *et al.*, (2011)
Conifer wood	900	21.0	54.0	6.7	11.8	6.4	Detourney *et al.*, (2011)
Conifer wood	940	20.6	53.0	5.5	11.0	6.6	Detourney *et al.*, (2011)
Conifer wood	980	23.1	53.5	6.0	10.2	5.2	Detourney *et al.*, (2011)
Wheat straw	500	0	18.5	75.0	4.4	0.8	Mulligan *et al.*, (2010)
Wheat straw	1000	3.2	33.8	55.6	5.9	0.6	Mulligan *et al.*, (2010)
Malle residue	500	0.1	23.6	70.2	4.7	0.7	Mulligan *et al.*, (2010)
Malle residue	1000	3.4	33.9	56.2	5.5	0.5	Mulligan *et al.*, (2010)

4.1.6 Secondary pyrolysis product (Bio-oil)

Associated products of biochar production such as bio-oil can contribute economically to the operation. The bio-oil product is estimated to be 20-40 wt % of charcoal/biochar dry weight (Boateng, 2007).

Bio-oil from different feedstocks materials (e.g almond shells, oak wood) consisted of several chemical derivatives such as: toluene, ethylbenzene, *p*-xylene, styrene, phenol, indene, *o*-cresol, *p*-cresol, naphthalene, 1-methylnapthalene, 2-methylnapthalene, acenaphthyle, flourene, phenatrene, anthracene, flouranthene, pyrene (De Bari *et al.*, 2000).

The chemical composition of willow bio oil obtained from a small scale ceramic tube reactor (550°C) have been reported by Ingemarsson *et al.*, (1999), as shown in Table 34.

Table 34. *Organic compounds formed during pyrolysis of willow wood at 550°C (from Ingemarsson et al., 1999)*

Retention time	Compound	Formula	Molecular weight
3.62	Acetic acid	$C_2H_4O_2$	60
4.34	1-Hydroxy-2-propanone	$C_3H_6O_2$	74
7.75	Acetic acid,metyl ester	$C_3H_6O_2$	74
8.18	Propanal	$C_3H_6O_2$	58
8.5	Acetoacetic acid	$C_4H_6O_3$	102
13.75	Cyclohexanone	$C_6H_{10}O$	98
17.48	2-Hydroxy-3-metyl-2-cyclopenten-1-one	$C_6H_8O_2$	112
19.52	Guaicol (2-Metoxyphenol)	$C_7H_8O_2$	124
22.63	4-Metylguiacol	$C_8H_{10}O_2$	138
25.23	4-Ethylguiacol	$C_9H_{12}O_2$	152
25.24	4-Ethenylguiacol	$C_9H_{10}O_2$	150
25.89	Syringol (2,6-Dimethoxyphenol)	$C_8H_{10}O_3$	154
25.99	4-(1-Propenyl) guiacol	$C_{10}H_{12}O_2$	164
26.84	4-(2-Propenyl) guiacol	$C_{10}H_{12}O_2$	164
27.44	4-Methylsyringol	$C_9H_{12}O_3$	168
27.48	4-(1-Propenyl) guiacol	$C_{10}H_{12}O_2$	164
28.08	Levoglucosan	$C_6H_{10}O_5$	162
29.07	4-Ethenylsyringol	$C_{10}H_{12}O_3$	180
30.76	4-(1-Propenyl) sryingol	$C_{11}H_{14}O_3$	194

Table 34 showed that GC/MS can detect high levels of smaller oxygenated compound from willow feedstock such as: acids, aldehydes, ketones, furans, guaiacols and syringols compounds.

4.1.8 Carbon balance analyses in Biochar production system

Carbon balance analyses can be detected using the LCA (Life Cycle Assessment), unfortunately in order to obtain full LCA in biochar production is challenging. LCA is one of the best approaches as the guidelines have been standardized (Fan *et al.*, 2011) including the process in biochar production system. This was to examine the environmental impacts, energy consumption, resource depletion and other impacts of the production system.

The LCA technique can determine how a system interacts with the environment. Some of the analytical tools available for use as part of an LCA include environmental accounting, multiple criteria analysis, environmental auditing, energy and material analysis and the application of waste reduction technology (Mohee and Beeharry, 1999). However due to a wider range of feedstock for biochar production system, a specific study on carbon balance analyses using a sort rotation coppice biomass crop feedstock is necessary.

An LCA has been previously been performed for the use of a willow biomass based feedstock model for producing electricity (Heller *et al*., 2003), whilst LCA applications for sugarcane bioenergy (Mohee and Beeharry, 1999) and poplar biomass feedstock (Rafaschieri *et al*., 1999) have also been performed. However, the energy conversion stage in full LCA calculation in different temperature of pyrolysis was remained absent. This energy conversion stages can be described as a process of extracting energy from biomass during pyrolysis and potentially could released a certain amount of CO_2 to the atmosphere. However this information was limited.

A full investigation of the dependence of biochar properties and carbon sequestration potential on the different temperature and duration of pyrolysis has not yet been systematically performed, and is the subject of the present chapter, using SRC willow as the target biomass. This study was to examine carbon balance assessment to fulfil a gap information during pyrolyses of willow biomass biochar production system.

4.2 Aims and Objectives

To better understand the environmental impact of biochar production, the present study presents carbon balance analysis during energy conversion as an integral part of a whole LCA for mitigating GHG emissions using willow-biochar slow pyrolysis system, produced at different temperatures of pyrolysis. The present research used willow as the biomass feedstock and investigated: (1) the use of a bubbling fluidized bed pyrolysis reactor, operated in an isothermal mode, (2) the effect of operating conditions (temperature and time) on the biochar yield, (3) the composition of condensable organics and gasses formed during pyrolysis, and (4) the full characterization of the biochar with elemental analysis, BET and variation of functional groups.

To finally verify that the willow-based biochar production is a carbon negative process, a carbon balance was established in-line with previous studies (Walle *et al*., 2007). The carbon balance assessment summarized in the last section of the present chapter, will provide more complete information of the environmental impacts, energy consumption and resource depletion of the process.

4.2.1 Aims

- To elucidate the effects of changes in temperature and the pyrolysis duration on biochar quality and structure.
- To optimize carbon sequestration potential during biomass SRC pyrolysis

4.2.2 Objectives

- To compare the chemical, visual and spectroscopic properties of biochar produced under different temperatures and pyrolysis durations.
- To examine the effects of temperature and the pyrolysis duration on levels of biochar yield and the degree of losses.
- To identify the GHG evolved during pyrolysis of willow biochar
- To monitor the carbon sequestration value of pyrolysed willow biomass using carbin balance analysis

4.3. Hypotheses

- The quantity and quality (chemical composition) of willow biochar will reduce following increases in temperature and the period of pyrolysis.
- A low temperature and short period of pyrolysis can reduce GHG emission during biochar production.

4.2. Methods

4.2.1 Feedstock Material

Biochar was produced from fresh willow (cultivar Q83) obtained from Rothamsted Research, UK. The willow was chopped into 1-2 cm long chips with a maximum thickness of 3 to 4 mm, then air dried to approximately 10 % moisture content. In the present study, the use of a typical bubbling fluidized reactor (BFB) was investigated, whilst the biomass feedstock was placed separately on the top bed material (quartz sand) using a metal basket in order to achieve effective recovery of biochar weight.

4.2.2 Fluidized Bed Pyrolysis reactor

The pyrolysis experiments were performed in a bubbling fluidized reactor. The experimental set-up is illustrated in Figure 44.

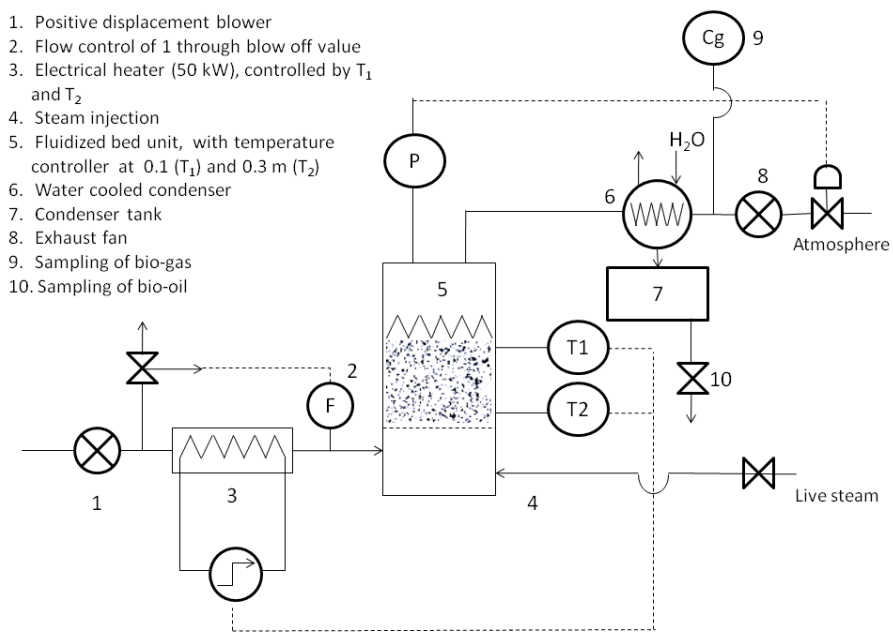


Figure 44. Schematic diagram of the pyrolysis set-up

The current study used a medium scale slow fluidized bed pyrolysis system to produce willow biochar. The equipment was manufactured by Torver Engineering, Manchester-UK. The bubbling fluidized bed was manufactured from Incoloy steel, and externally insulated. It consisted of 15 kg of quartz sand (150-300 µm, 2600 kg/m³), for a total static bed height of approximately 35 cm.

The air flow rate was controlled by a flow meter. The heat-input from the electrical heater (50 kW) was set by the bed temperature. Vapours, pyrolysis gases and fluidization gas were extracted by an induced draft fan, and passed through a water-cooled condenser and a bio-oil collection tank. Live steam (110 °C, 5 bar) could be added in the windbox of the fluidized bed, i.e. below the distributor plate, built as a sintered metal plate with a pressure drop of about 3000 Pa at the operating flow rate and temperature (Figure 44).

The air flow rate was maintained at 10 to 15% of the stoichiometric air flow needed for willow combustion (Equivalence Factor, EF, 10-15%), and the velocity in the bed varied from 2 to 4 times the minimum fluidisation velocity ($U_{mf} = 5$). For some of the experiments, a steam dilution was also applied, reducing EF to below 5%.

A known weight (between 0.3 and 0.5 kg) of willow wood chips were placed into a perforated basket. The perforations enabled the fluidized bed sand to flow within the voids of the willow chips.

To collect the products, biochar was sieved from the bed material after the reaction and the vapours were condensed. Gases were exhausted to the atmosphere, and analysis of CO, CO_2, H_2 and CH_4 was performed. Biochar was produced at temperatures of, 440, 470, 530 and 550°C, for 15 minutes and for different durations at 470 °C (5, 10 and 15 minutes).

In additional experiments, the biomass was either soaked with de-ionized water prior to pyrolysis, or live-steam (110°C, 5 bar) was directly injected directly into the fluidization air flow, further reducing the partial pressure of the O_2 present. All experiments were repeated in duplicate under identical operating conditions, and the average results were used.

4.2.3 Product characterization and Carbon balance inventory

4.2.3.1 Elemental and functional analysis

Analysis of the carbon and nitrogen content of the biochar was performed using a LECO analyzer (CB-2000, LECO Coorporation, Michigan, USA), and exchangeable cations were measured using an inductively coupled plasma (ICP) spectrophotometer. Before analysis, the biochar was homogenized and powdered using a ball mill. The functional groups present in the biochar were detected by Fourier Transformed Infrared (FTIR) spectrometry (Perkin Elmer-100), whilst the visual characteristics were imaged and identified using Scanning Electromagnetic Microscopy (SEM) **(**Cambridge S200 Scanning EM)**.**

To understand volatility of elements under the combustion conditions, relative enrichment (RE) factors are calculated as follows (Hossain *et al.*, 2011):

RE = (Elemental concentration in biochar/ Elemental concentration in willow chips) x (biochar yield/1000)

Relative enrichment factors help to identify the degree of enrichment of elements in the biochar and reveal the volatility of trace elements. RE factors in biochar greater than 1 indicate larger enrichment of the trace element in the biochar, while when RE factors are less than 1 then the elements exhibit volatilisation (Hossain *et al.,* 2011).

4.2.3.2 Visual characterization of biochar using SEM

Biochar samples (0.5 to 1 cm long) were glued into the stand of an ion sputter machine in order to create a thin conductive layer on their surface. Samples were transferred from the ion sputter machine using a pin set and they were then inserted into the object chamber of microscope and observation the surfaces can be started. The electrons pass the distance between electron gun and specimen stage through the column and interact with the sample substance. Selected magnifications of the objects were chosen (e.g 200 µm). As result different types of radiation were generated and displayed on the monitor.

4.2.3.3 Specific surface area

The specific surface area of the different biochar samples was measured using a Micromeretics, Tristar II 3020 analyser as outlined by Jones *et al.,* (2011). The instrument features a thermally stable dosing manifold (M), a three-port sample manifold, a dedicated tube for measuring saturation pressure (Po), and a rapid response servo valve (S).

Principally, N_2 gas absorption was used at 77° K. This allows the free space to be determined at the analysis temperature. Once the free space analysis is finished, the saturation pressure of the adsorptive is determined using the Po tube. Typically nitrogen is dosed into the tube above atmospheric pressure.

The nitrogen is allowed to condense and the vapour pressure of the nitrogen is easily monitored by a transducer throughout the analysis. The 0.2 g biochar sample was degassed under vacuum at 150°C for 2 hours prior to volumetric dosing of N_2 to a pre-set partial pressure in the range of 0.02-0.20 P/Po (biochar). The BET method was employed for calculating surface area using a measured Po value and N_2 molecular cross sectional area of 0.162 nm^2. The measured adsorption of P/Po were recorded for obtaining a BET transform plot which has a correlation coefficient of 0.99 or higher, derived from 5 absorption points.

4.2.3.4 Composition of the condensable pyrolysis products

Condensed liquids were collected and analysed by a GC-MS 8130 (Voyager 8000 Fisons Instruments) following the method described by Wachholz *et al.,* (1995). Using a FISONS gas chromatograph GC 8130 the sample was separated on an Anabond-225 column (50 m x 0.32 mm; 1 gm film thickness). The helium (5.6) gas flow was 1.2 ml min-1. 1 µl of the sample mixture, diluted 1:5 with dichloromethane, was injected with a split ratio of 1 to 24. The GC oven temperature program was initially 80°C (1 min isothermal), then programmed at 12°C min^{-1} to 250°C, with 20 min isothermal at 250°C for removing the last components from the column. The GC was connected with the MS by a heated transfer line held at 280°C. Spectral search from library spectra measured in the condensed phase were used to indentify chemical compounds in bio-oil.

4.2.3.5 Biochar carbon balance

Biochar carbon balance assessment was determined, although a lack detail of carbon losses during energy conversion stages prevented a Life Cycle Assessment (LCA) being done. However the results complement a sort rotation willow biomass LCA developed by Heller *et al.,* (2003). The target was to achieve a comprehensive systems-based analysis of the energy and environmental performance of biochar production from willow biomass.

In Heller *et al.,* (2003) a LCA model was developed for a willow biomass based electricity production system in New York-USA. Heller *et al.,* (2003) covered all steps of the energy performance and net greenhouse gas emissions of the biomass feedstock production system such as field preparation, planting, mechanical and chemical weed controlling, coppice, fertilizing, and harvesting except the energy conversion stages (Figure 45).

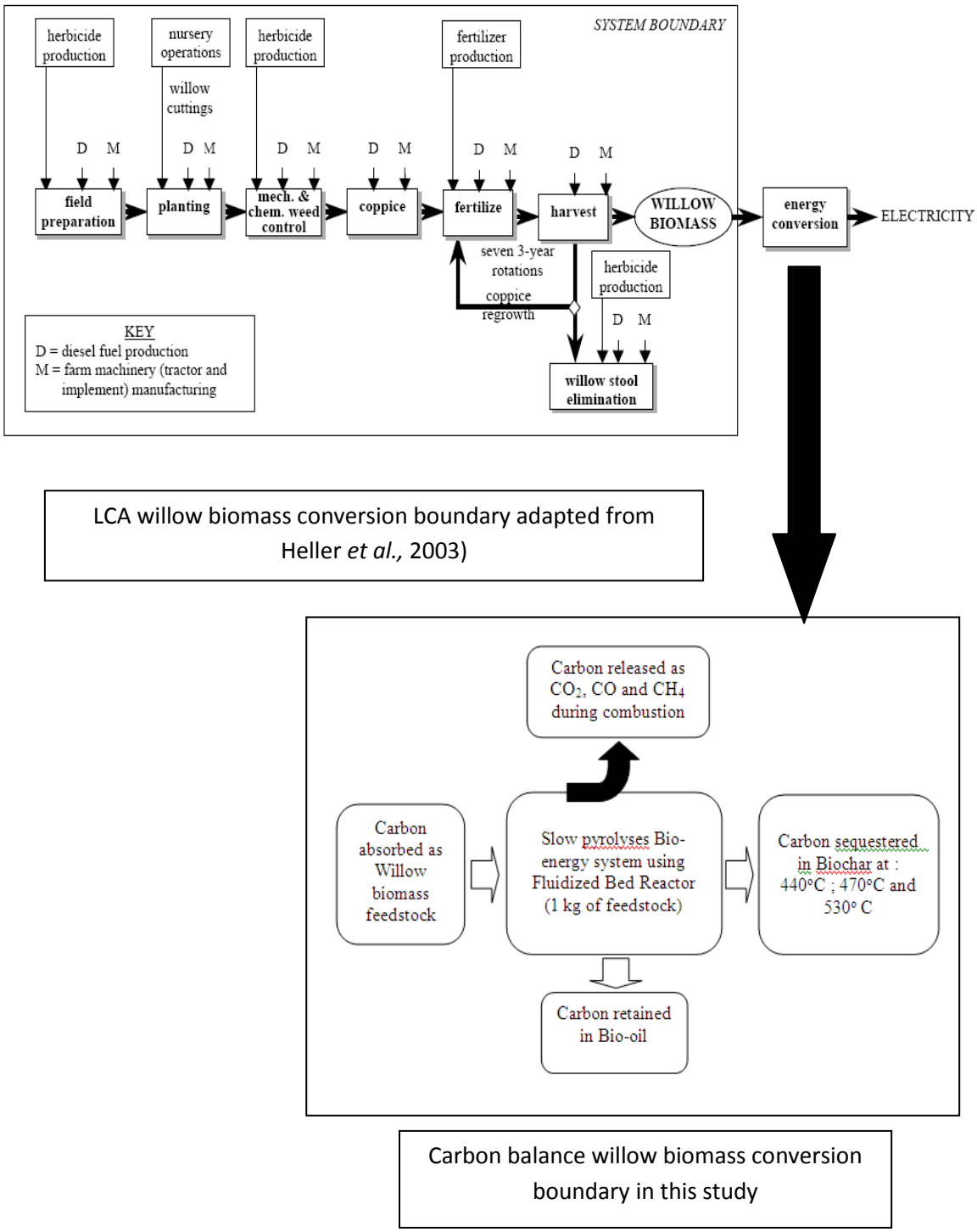


Figure 45. Schematic diagram contribution of carbon balance during energy conversion stage to overall LCA

In the Heller *et al.*, (2003) study there was no information about carbon sequestration in soil or carbon emissions during the energy conversion stage (Table 35). It can be seen from Table 35 that the total net carbon emissions under a willow energy crop was +3.7 Mg CO_2 ha^{-1}. The greatest loss of CO_2 was recorded from litter decomposition (+7.28 CO_2 eq ha^{-1}), whilst the biggest carbon sequestration was observed in belowground biomass (e.g root biomass) (Heller *et al.*, 2003). More detailed information is necessary for completing a full LCA investigation including carbon losses during energy conversion under different temperatures and time of pyrolysis, since this information was rarely reported.

Table 35. LCA on carbon equivalent gas flow per ha^{-1} willow rotation system (adapted from Heller *et al.*, (2003)

	CO_2 (Mg)	Other GHG Mg CO_2 eq. ha^{-1}	Total
Carbon Emission			
1.Diesel fuel	+ 3.12	+0.06	+3.18
2.Agricultural input	+2.97	+0.40	+3.37
3.N_2O from applied N		+3.97	+3.97
4.N_2O from leaf litter		+7.38	+7.28
5.CO_2,CH_4 and CO during pyrolysis at different temperature (440^oC,470^oC and 530^oC)	was determined in this current study		
Carbon sequestration			
1.Below ground biomass	-14.10		-14.0
2.Soil carbon	0		0
Net Total	-8.01	+11.7	+3.7
Harvested biomass	-499.2		-499.2

4.5 Results

4.5.1 Biochar yield

The biochar yields for different pyrolysis temperatures are given in Figure 46. The data illustrated that increases in treatment temperature decreased biochar yields. The decomposition reaction was incomplete at 350°C, with raw willow chips still visible in the product. The corresponding yield for this was therefore not included. A similar observation was made at 440°C, although the carbonization process was nearly complete. At higher temperatures, a carbonaceous residue was completely formed. At 530°C an average of 190 g kg^{-1} was produced, which equated to 19 % of the raw willow feed. The average yields at 440°C and 470°C were 407 g kg^{-1} and 273 g kg^{-1} respectively.

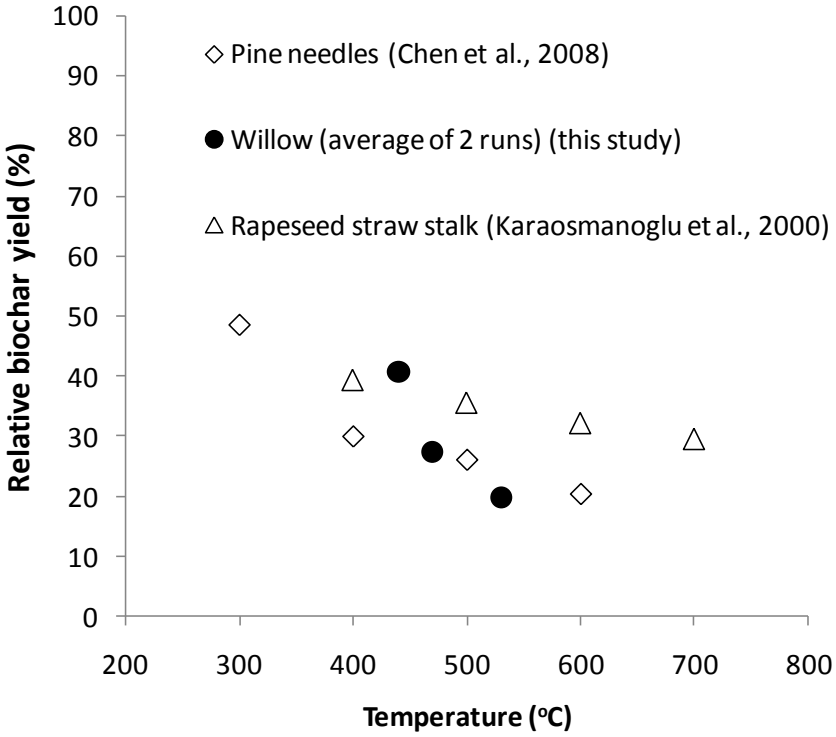


Figure 46. Biochar yield versus pyrolysis temperature, for a pyrolysis duration of 15 minutes (average value of two experiments)

In a second series of experiments, the effects of the thermal decomposition time on biochar yield were studied at a single temperature of 470°C, which was selected as the ideal temperature for maximising the biochar yield and properties.

The pyrolysis times used were 5, 10 and 15 minutes. The relationship between biochar yield and heating time is presented in Figure 47. Increasing the heating time from 5 minutes to 15 minutes considerably reduced the biochar yield. For a 5 minute treatment, the biochar yield was close to 50%. The lowest biochar yield (27%) was obtained for a treatment at 15 minutes (Figure 47).

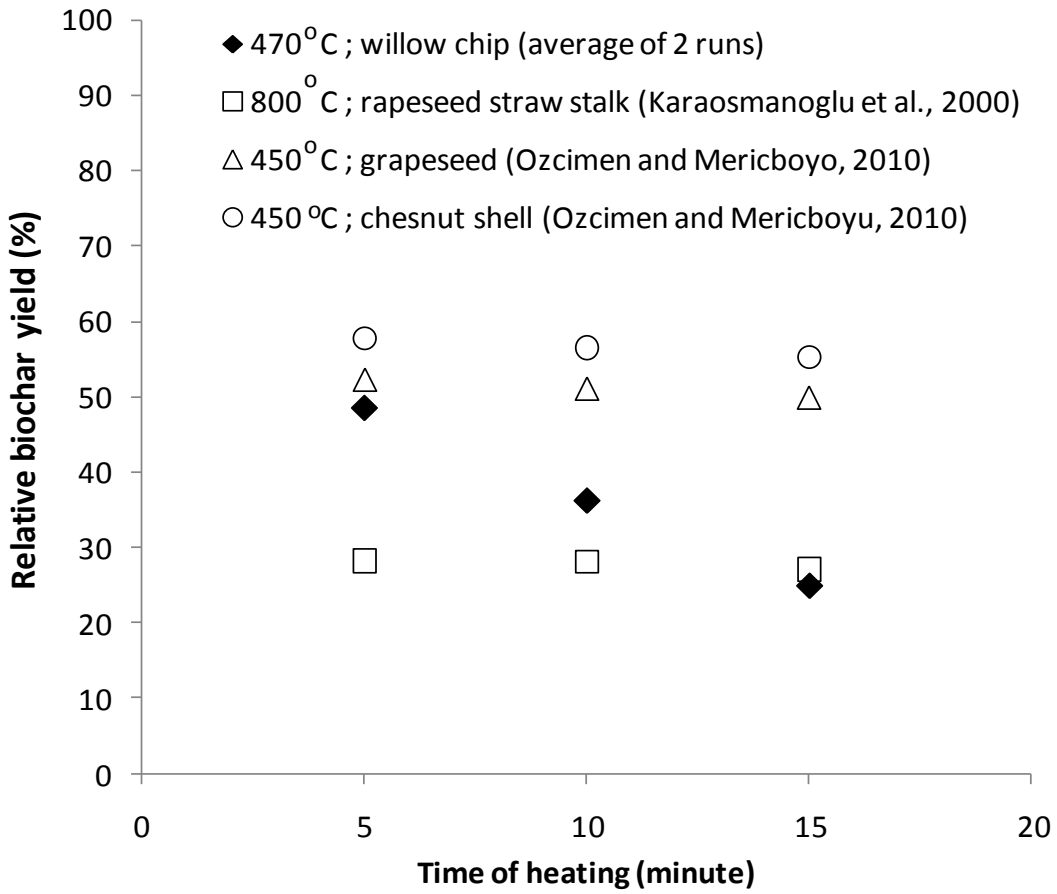


Figure 47. Relationship between time of pyrolysis and biochar yield (average value of two experiments)

Experiments were also conducted using wood chips pre-soaked with water, nd when live steam was directly added to the fluidized bed. The yield at 470°C and 15 minutes was about 50%, nearly twice the yield of biochar production without water vapour being present. It was therefore evident that steam pyrolysis enhanced the biochar yield.

4.5.2 Biochar characteristics

The structures of the willow wood chips, and of the biochar produced at different temperatures, are shown in Figure 48. SEM identified the structural and morphological differences of both the feedstock and biochar produced. Whereas the willow feedstock had large pores and contained some debris, the biochar had a higher porosity, with significantly smaller pore sizes for high temperature biochar, thus offering an increased potential for the adsorption of organic or mineral soil compounds.

At 440°C, the plant cells were only partially degraded. At higher temperatures (470°C and 530°C) a large number of narrow pores were created. Whereas 440°C produced a partially developed biochar, higher production temperatures produced the required effect of increased porosity.

A temperature of 470°C was preferred to 530°C as similar morphologies were produced but resulted in considerably higher biochar yields. A clear sequential process of plant cell shrinkage and the formation of large pores within the biochar were observed. This has never been reported in previous research (Figure 48).

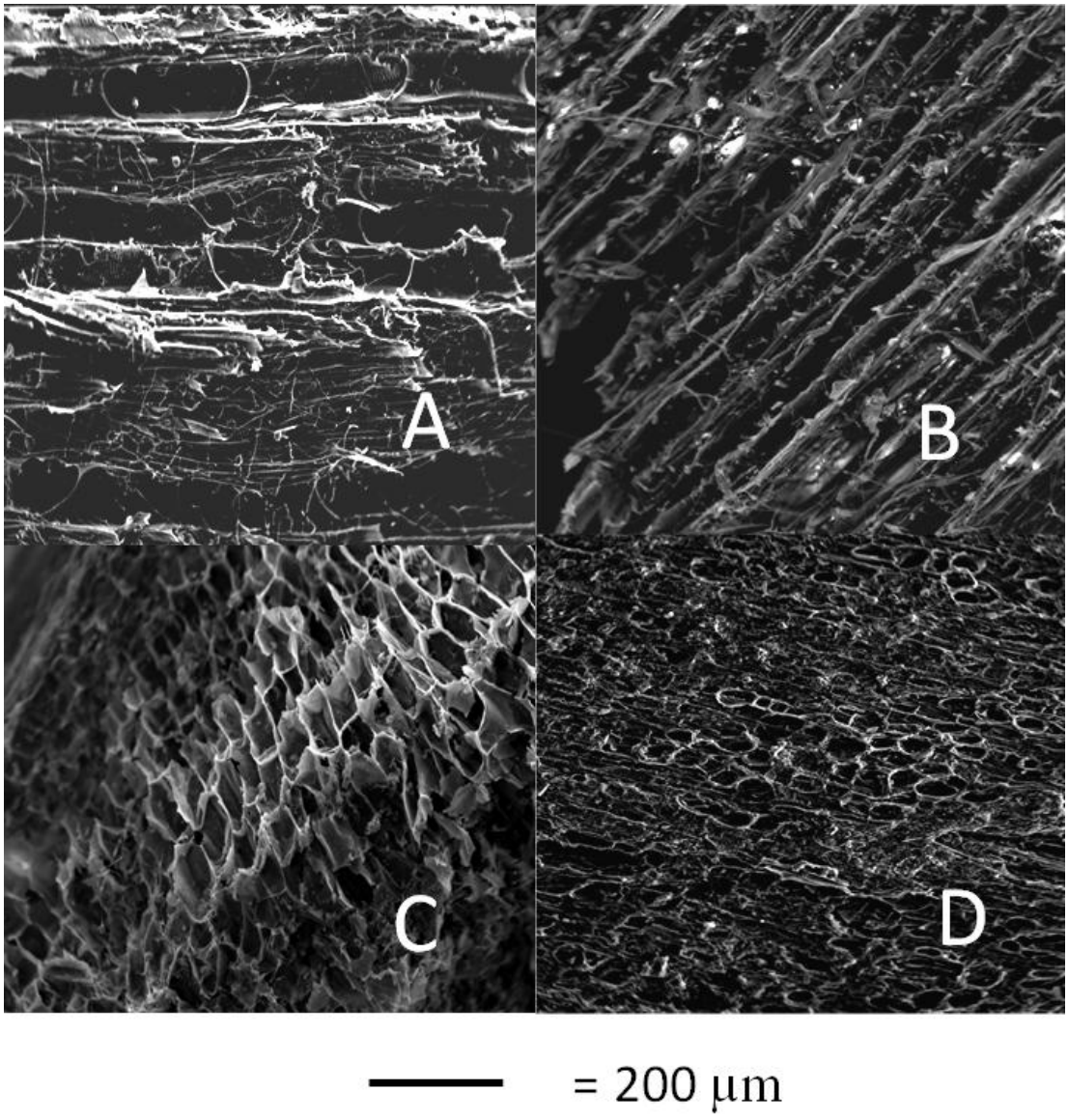


Figure 48. SEM-images of (A) willow chip feedstock (B) biochar produced at 440°C (C) 470°C and (D) 530°C

The increased porosity and reduced pore sizes were also reflected in the specific surface area values (measured as BET-unit). The values ranged from 62 to 84.4 m^{-2} g^{-1} for 530°C-biochar, or even in excess of 142 m^{-2} g^{-1} for steam-assisted pyrolysis at 530°C. Furthermore, this surface area was nearly double that of the non-steamed biochar.

4.5.3 Biochar elemental analysis

The laboratory analyses of 13 elements in biochar, produced by thermal treatment during 15 minutes but different temperatures, are given in Table 36, together with the reference analysis of the willow chips.

***Table 36**. Elemental composition of dry willow feedstock and biochar produced at different temperatures, in wt%, or ppm* (Na, B, Cu, Fe, Mn and Zn)*

	Willow chips	**Biochar 440 ^{o}C**	**Recovery 440 ^{o}C**	**Biochar 470°C**	**Recovery 470 ^{o}C**	**Biochar 530 ^{o}C**	**Recovery 530 ^{o}C**
pH	5.8	6.5	0.46	9.1	0.43	10	0.33
C	51.5	51.3	0.41	54.6	0.29	43.7	0.16
N	1.5	1.0	0.27	1.0	0.18	0.8	0.10
Ca	2.4	1.0	0.17	2.3	0.26	1.9	0.15
K	0.1	0.8	3.26	1.5	4.10	1.1	2.09
Mg	0.2	0.2	0.41	0.4	0.55	0.4	0.38
P	0.1	0.2	0.81	0.6	1.64	0.5	0.95
S	0.1	0.1	0.41	0.1	0.27	0.1	0.19
Na*	0.0	0.0	0.00	0.1	0.00	0.1	0.00
B*	39.3	24.2	0.25	48	0.33	35.4	0.17
Cu*	9.9	11.6	0.48	24.7	0.68	27.8	0.53
Fe*	366.3	242.1	0.27	439.9	0.33	601.7	0.31
Mn*	445.3	173.8	0.16	397.7	0.24	320.5	0.14
Zn*	345.1	212.7	0.25	476.8	0.38	438.5	0.24

The pH of the biochar-water suspensions substantially increased with increasing pyrolysis temperatures, changing from 5.8 for the wood chips to 10 for 530°C-biochar as a result of increased concentrations of alkaline elements. Also, the concentration of other elements (except C and N) increased with increasing temperatures (from 440°C to 530°C), due to an increased release of volatiles with increasing temperatures. The maximum enrichment was recorded in the 470°C-biochar samples.

Carbon (C) was the predominant element followed by calcium (Ca), which was almost twice the concentration of potassium (K) in the 15 minute treatments. The carbon content of the willow and of its biochar was about 45 to 55%. The total N present in the biochar decreased considerably with increased production temperature, and was only half of the original value present in the willow chips. The phosphorus (P) content was more than doubled at the higher temperature, indicating that this element was mostly associated with the inorganic fraction of the willow chips. In contrast, the amount of sulphur (S) consistently decreased with increased temperature, indicating that some S components were volatile.

As illustrated in Table 37, Increases in the treatment duration (from 5 to 15 minutes) did not substantially affect the pH or the concentrations of P and S or alkaline cations such as Ca, K, and Mg. The concentrations of Fe, Mn and Zn increased substantially.

The recovery value of potassium and phosporous in biochar produced under different temperature and time of pyrolyses are more than 1 which means there is a enrichment of the chemical composition in the biochar compared to the original willow feedstock.

Table 37. *Elemental composition of biochar produced at 470 °C for different durations, in wt%, or ppm* (Na, B, Cu, Fe, Mn and Zn)*

	Bio char 470°C, 5 minutes	**Recovery Bio char 470°C, 5 minutes**	**Bio char 470°C, 10 minutes**	**Recovery Bio char 470°C, 10 minutes**	**Bio char 470°C, 10 minutes, soaked with water**	**Recovery Bio char 470°C, 10 minutes soaked with water**	**Bio char 470°C, 15 minutes**	**Recovery Bio char 470°C, 15 minutes**
pH	5.9	0.28	6.2	0.29	6.9	0.32	9.1	0.43
C	49.8	0.26	48.5	0.26	53.1	0.28	54.6	0.29
N	0.9	0.16	0.8	0.15	0.8	0.15	1.0	0.18
Ca	0.6	0.07	0.7	0.08	0.6	0.07	2.3	0.26
K	0.5	1.37	0.5	1.37	0.7	1.91	1.5	4.10
Mg	0.1	0.14	0.1	0.14	0.1	0.14	0.4	0.55
P	0.1	0.27	0.2	0.55	0.1	0.27	0.6	1.64
S	0.1	0.27	0.1	0.27	0.1	0.27	0.1	0.27
Na*	0.1	0.00	0.1	0.00	0.1	0.00	0.1	0.00
B*	13.8	0.10	16.5	0.11	14.9	0.10	48	0.33
Cu*	9.6	0.26	11.0	0.30	9.8	0.27	24.7	0.68
Fe*	1243	0.93	270.4	0.20	138.3	0.10	439.9	0.33
Mn*	104.1	0.06	114.2	0.07	93.4	0.06	397.7	0.24
Zn*	134.2	0.11	145.5	0.12	134.3	0.11	476.8	0.38

4.5.4 Physicochemical properties of biochar

The changing biochar properties can also be indentified from the FTIR signature. Each valley of this signature corresponds with a specific functional group. The signatures of the biochar produced at different temperatures and with different pyrolysis times were compared (Figure 49).

Increases in temperature from 440°C to 470°C eliminated several functional groups such as : the –OH bond at 3350-3400 cm^{-1}, esters at 1740-1760 cm^{-1} and aliphatic alkyl groups at 2850-2900 cm^{-1}.

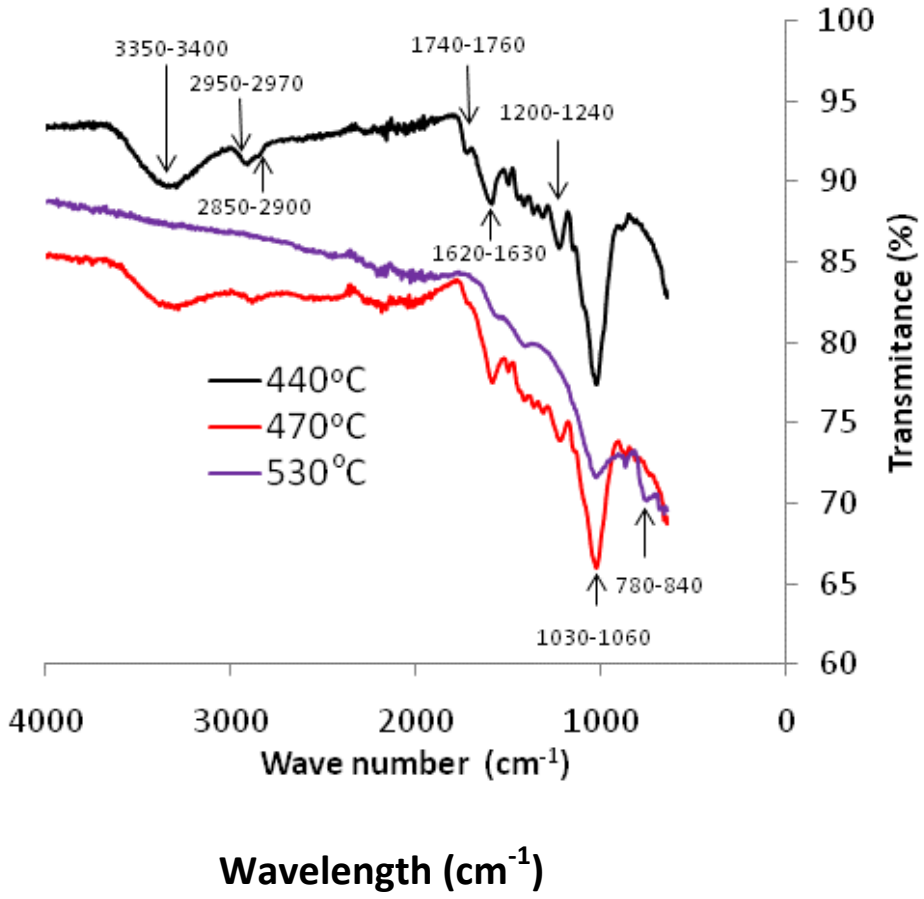


Figure 49. The FTIR signature of biochar produced at different temperature of pyrolysis.

A similar picture was observed for biochar produced at 470 °C (Figure 50), but at different pyrolysis durations all functional groups remained present, but the extent of the valleys varied slightly.

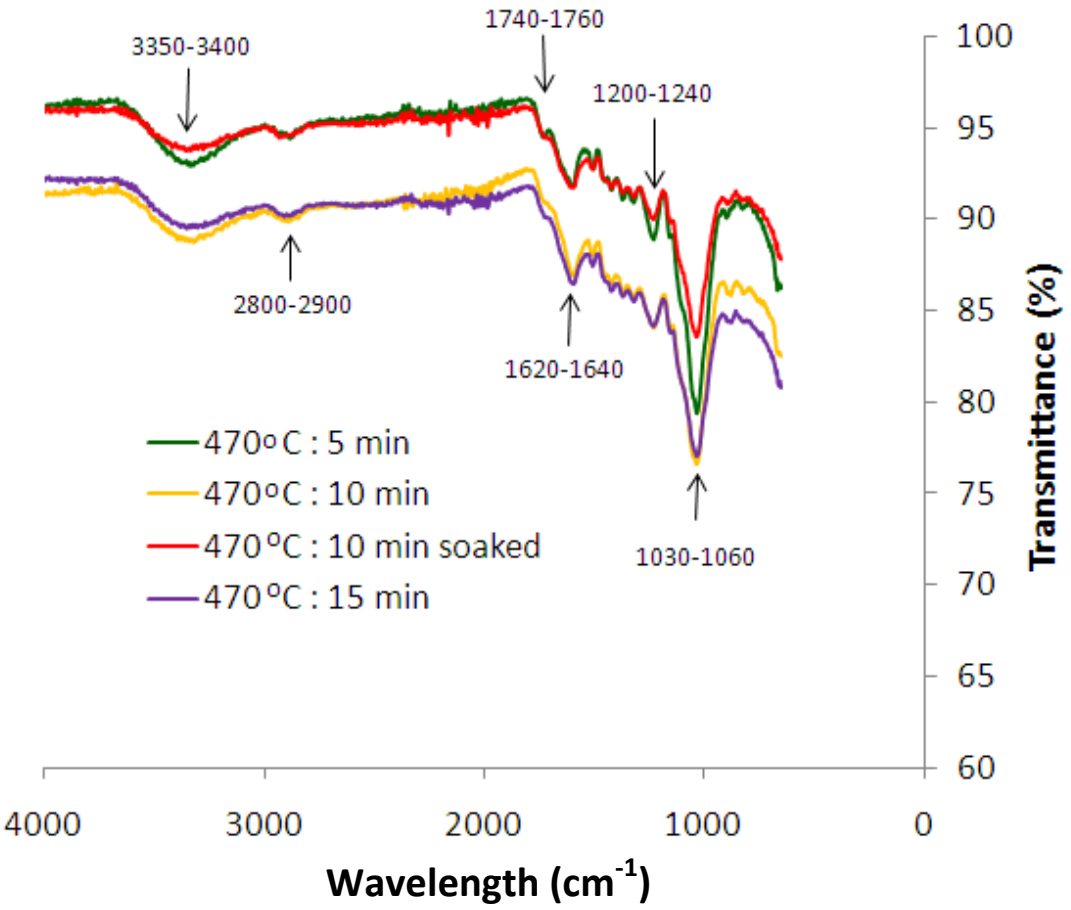


Figure 50. The FTIR signature of biochar under different duration of pyrolysis

4.5.6 Characteristics of the produced condensable liquids and gases

Samples of the condensates were collected from the condensate drum. These samples contained 16 to 20 wt% of water. The organic fraction was analyzed by GC-MS, and Table 38 listed the major identified products (non-identified compounds are not listed).

Important ligninocellulosic willow biomass derived compound were guaiacol/guaiacol TMS derivatives, phenol syringols, eugenols, acetic acids, furfurals, etc. The identified compounds formed by willow pyrolysis all contained C, H and O in their molecular structure.

Table 38. *% composition of the condensables (bio-liquid/bio-oil)*

Compound	**wt%**
Acetic acid methyl ester	2.74
Acetic acid	4.45
Acetol	2.05
Furfural	3.40
2-hydroxy-3-methyl-2-cyclopentene	3.42
Phenol	13.01
Guaiacol	7.53
Guaiacol TMS derivative	18.15
4-methyl-guaiacol	7.88
4-ethyl-guaiacol	5.14
Cis-eugenol	7.88
Syringol	3.77
Eugenol	2.40
Trans-eugenol	1.37
4-methyl-syringol	1.03

The gas stream was analyzed for CO, CO_2, H_2 and CH_4. The analysis results were approximate values, since the compounds were diluted in a 6 m^{-3} h^{-1} gas stream. Moreover, the composition of the gas stream varied with time, and the concentration of the target gases decreased steadily. Average concentrations in ppm_v converted to vol%, are given in Table 39.

Table 39. *Compositions of the pyrolysis gas (ppm$_v$ converted into approx. vol%) following a 15 minute pyrolysis period*

Compound	**440 °C**	**470 °C**	**530 °C**
CO_2	0.42	0.61	0.74
CO	0.21	0.40	0.52
H_2	0.09	0.23	0.28
CH_4	0.11	0.15	0.19
N_2O or NO_2	ND	ND	ND

ND : Not Detected (below detection limit of 10 ppm)

4.5.7 Biochar carbon balance

The total carbon balance can be determined by calculating the C available in the willow feedstock, and the sum of the C contained in biochar, gases and bio-oil. The C balance was performed on the basis of 1 kg of both feedstock and biochar, at pyrolysis temperatures of 440°C, 470 °C and 550°C. The values of the carbon balance used for providing a full LCA assessment below are shown in Table 40a-40b.

Table 40a. *Mass Balance per 1000 g biochar produced*

(figures in brackets indicate the relative proportion of gaseous C)

Biochar temp (^{o}C)	Feedstock (g)	Biochar yield (g)	CO_2 (g) (*)	CO (g) (*)	CH_4 (g) (*)	Condensable (g)
440	2457	1000	666.49 (80.16%)	140.27 (17.0%)	22.41 (2.83%)	627
470	3663	1000	1123.05 (66.7%)	465.38 (27.6%)	96.28 (0.5%)	978
530	5263	1000	1670.14 (63.2%)	805.70 (30.5%)	164.08 (0.6%)	1623

(*) estimated gaseous product based on actual measurement

Table 40b *Mass balance per 1000 g feedstock*

(figures in brackets indicate the relative proportion of gaseous C)

Biochar temp (oC)	Feedstock (g)	Biochar yield (g)	CO_2 (g) (*)	CO (g) (*)	CH_4 (g) (*)	Condensable (g)
440	1000	407	270.94 (80.16%)	57.46 (17.0%)	9.56 (2.83%)	255
470	1000	273	306.2 (66.7%)	127.04 (27.6%)	2.62 (0.5%)	267
530	1000	190	317.5 (63.2%)	153.2 (30.5%)	3.01 (0.6%)	308

(*) estimated gaseous product based on actual measurement

Studies of the mass balance for biochar using LCA show that the distribution of mass between the biochar, gases and bio-oil are highly dependent on the pyrolysis conditions. The proportion of biochar, gas released and condensables recovered at the lower temperature (440°C) was recorded in the ratio of 41%:34%:25%, for the middle temperature (470°C) it was 27%:46%:27% and 19%:50%:30% at the highest temperature.

The most important gas released during pyrolysis was CO_2 which represented 63-80 % of the total emissions, whilst the lowest recorded was H_2 which was not included in the carbon balance analysis since there was no carbon component in this gas. No detection of either N_2O or NO_2 was recorded. As pyrolysis temperature increased, more CO_2 and CO was released during pyrolysis.

For producing 1000 g biochar, greater amounts of feedstock were required as pyrolysis temperature increased. At the highest temperature (530°C), the quantity of feedstock required was more than double that at 440°C.

The carbon balance of the biochar is shown in Table 40c and 40d. This shows that the highest carbon content in biochar was found at the middle temperature (470°C) at 547 C g^{-1} biochar, which was 6% higher than the amount of carbon obtained at the lower pyrolysis temperature (440°C) (513 C g^{-1} biochar) and 20% higher than the amount of carbon at the highest temperature (530°C) (437.30 C g^{-1} biochar). Carbon released as CO_2, CO and CH_4 was recorded at 27%, 43 % and 75%.

Table 40c *Carbon balance equivalent to 1000 g biochar produced*

(figures in brackets indicate the relative proportion of gaseous C)

Biochar temp (oC)	Feedstock (g C)	Biochar yield (g C)	CO_2-C (g C) (*)(27%)	CO-C (g C)(*) (42.9%)	CH_4-C (g C) (*)(75%)	Condensable -(g) (C estimated at 50%)
440	1265.35	513	181.77	60.11	16.80	313.5
470	1886.44	547	306.36	199.45	72.21	489
530	2710.44	437	455.49	345.33	123.06	811.5

(*) estimated gaseous product based on actual measurement

Table 40d. *Carbon balance equivalent to 1000 g feedstock*

Biochar temp (oC)	Feedstock (g C)	Biochar yield (g C)	CO_2-C (g C) (*)(27%)	CO-C (g C)(*) (42.9%)	CH_4-C (g C) (*)(75%)	Condensable -C (g) (estimated 50%)
440	515	208.79	73.89	24.62	7.17	127.5
470	515	149.05	83.50	54.44	1.96	133.5
530	515	83.03	86.59	65.65	2.25	154

(*) estimated gaseous product based on actual measurement

The ratio between C in gases and feedstock/ biochar is shown in Table 41. The ratios of gas (CO_2, CO and CH_4) to willow feedstock were 0.27, 0.057 and 0.009 t t^{-1} feedstock, respectively at the lower temperature (440°C). This slightly increased following increases in the pyrolysis temperature. The ratio of gases to biochar followed a similar trend, at 0.66, 0.14, 0.022 t t^{-1} biochar for CO_2, CO and CH_4 respectively.

Table 41. *Ratio between C in gasses and feedstock/biochar*

Pyrolysis temp (oC)	CO2 (g C g-1 feedstock)	CO (g C g-1 feedstock)	CH4 (g C g-1 feedstock)	CO2 (g C g-1 biochar)	CO (g C g-1 biochar)	CH4 (g C g-1 biochar)
440	0.271	0.057	0.009	0.666	0.140	0.022
470	0.30	0.12	0.02	1.12	0.46	0.096
530	0.31	0.15	0.03	1.67	0.80	0.164

4.5 Discussion

4.5.1 Degradation kinetics of willow biomass pyrolysis

The degradation kinetics of willow biochar yield following at temperatures of pyrolysis were faster than those recorded in previous studies using pine needles (Chen et al, 2008) and rapeseed straw stalks (Karaosmanoglu *et al.,* 2000). Increasing the pyrolysis temperature from 440°C to 530°C, decreased the biochar yield by 60%. This may have been due to an increased release of volatile compounds from the willow biomass, compared to the feedstocks mentioned above, during combustion.

Tharakan *et al.,* (2003) reported that biochar quality was determined by the chemical composition of the material (nutrient/cellulosic, hemicellulosic or lignin content), heating value, and moisture content. For this experiment the factors that were also responsible for biochar quality were the gas production, collected condensable liquid (bio-oil) and remaining char.

Exponential reductions in biochar yield with increasing pyrolysis temperature were also observed in previous studies by Hossain *et al*. (2011) and Nguyen and Lehman (2009). In the current study, 50% of the feedstock had been converted to biochar after 5 minutes which is comparable to the value of 60.6% obtained from *Eucalyptus grandis* (Vital *et al*., 1986, in Lehmann *et al*. 2002).

The effects of altering pyrolysis time between 5 and 15 minutes at 470°C showed decreasing biochar yields with increasing pyrolysis time. The reduction in biochar yield with increasing pyrolysis time contrasts with biochar derived from grapeseed or chestnut shells (Ozcimen and Mericboyu, 2010) produced at 450°C, and rapeseed straw at 800°C, in which biochar yield did not decline substantially after 5 minutes of pyrolysis (Karaosmanoglu *et al.,* 2000). These differences could reflect feedstock quality or the energy content of the feedstock.

Increases in temperature and/or pyrolysis times increases the heating value, which is well known to cause yield decreases (Lehman *et al.,* 2006; Katyal, 2003; Ozcimen and Mericboyu, 2010; Karaosmanoglu *et al.,* 2000), with hemicelluloses being degraded first at temperatures of around 200 to 250°C, followed by cellulose at 250 to 350°C and the remaining lignin at temperatures of 350 to 500°C (Ozcimen and Mericboyu, 2010; Demirbas, 2007).

It can be concluded that controlling pyrolysis temperature and reaction times is essential for optimizing carbon fixation during the combustion processes and as a result, determining the quality and quantity of the biochar produced.

4.5.2 The effect of different pyrolysis temperatures on biochar quality

4.5.2.1 Chemical composition

The loss of volatile compounds, along with yield losses can be detected from the changes in the amounts of elements remaining in the biochar. Increasing the temperature from 440°C to 530°C, dramatically increased the pH to almost 10 compared to the raw material (pH=5.8). This can be associated with the fact that cationic species were remained in biochar, increasingly greater concentrations (K, Mg, P, Cu, Mn, Na and Zn) at the higher temperatures.

The pH values in this study were higher than those obtained from sugarcane bagasse (pH=7.7) (Inyang *et al.,* 2010) pyrolysed at 600°C or waste water sludge (pH=7.27) (Hossain *et al.,* 2011) at 500°C or even rice husks (pH=8.6) at uncontrolled temperatures (Knoblauch *et al.,* 2010). The consequences of this could be that willow biochar is suitable for use in soil pH improvements, as well as providing important

nutrients. Therefore, it is suggested that willow biochar could be used for application to acidic soils (pH<3 or 4).

For the macro nutrients (e.g N, K, Ca, Fe), comparisons between the willow biochar and those produced using other materials (e.g corn residue or oak wood charred at 350 and 600°C) (Nguyen and Lehman, 2009), revealed that the fluidized bed reactor used in this study successfully retained these elements. This finding showed that willow biochar could represent a higher nutrient alternative to other biochar types.

In the other experiment, a treatment temperature of 300°C or 600°C increased the amounts of Fe in corn residues and oak wood from 500 ppm to 800 ppm, and 30 to 1300 ppm respectively (Nguyen and Lehman, 2009). This was within the same range of Fe detected by the present analysis. The concentrations of Cu and Zn in biochar from waste water sludge, which mostly contained organic material, increased from 1150 to 1500 ppm, and 1675 to 2175 ppm respectively, when the treatment temperature was increased from 300°C to 700°C (Hossain *et al*., 2011). These high values are the result of the known high concentrations of Cu and Zn in sludge, which are far above the values occurring naturally in woody biomass.

The carbon values in willow biochar were however lower than the values reported for other materials, such as beech trunk, rapeseed, wood bark, cotton stalks and hazelnut shells (88 %, 67%, 85%, 72% and 96% respectively) (Sohi *et al*, 2009; Demirbas, 2004), or from other individual tree species (76 %) such as: *(Acacia mangium, Eucalyptus grandis, Eucalyptus camaldulensis, Pinus sylestris* and *Robinia pseudoacacia)* as given by Lehman *et al*., (2002). The major elemental analysis of biochar (wt %) from other feedstocks and under different pyrolysis temperatures is presented in Table 42 and compared to this study.

Table 42. *The major elemental analysis of biochar (wt %) from various feedstocks*

Feedstocks	**Temp (^oC)**	**C (%)**	**H (%)**	**N (%)**	**O (%)**	**S (%)**	**Reference**
Pine sawdust	500	78.1	3.3	0.3	18.2	-	Chen *et al.,* (2010)
Almond shell	850	45.0	5.0	0.4	48.0	0.01	De Bari *et al.,* (2000)
Oak	850	50.0	6.0	0.1	43.0	0.02	De Bari *et al.,* (2000)
Turkey oak	850	50.0	6.0	0.1	43.0	0.10	De Bari *et al.,* (2000)
Robinia pseudoacacia	850	43.9	7.8	0.02	46.78	-	Scala *et al.,* (2003)
Rapeseed cake	500	54.90	2.14	4.97	20.04	0.35	Ozcimen and Karaosmagnoglu., (2004)
Rapeseed straw and stalk	400	71.34	3.93	1.43	10.84	0.24	Karaosmanoglu *et al.,* (2000)
Rapeseed straw and stalk	500	75.03	2.62	1.41	7.79	0.24	Karaosmanoglu *et al.,* (2000)
Rapeseed straw and stalk	600	78.48	1.88	1.53	3.94	0.32	Karaosmanoglu *et al.,* (2000)
Rapeseed straw and stalk	700	79.48	1.20	1.35	3.29	0.31	Karaosmanoglu *et al.,* (2000)
Rapeseed straw and stalk	800	79.51	0.72	1.45	2.61	0.39	Karaosmanoglu *et al.,* (2000)
Rapeseed straw and stalk	900	79.89	0.42	1.57	1.67	0.36	Karaosmanoglu *et al.,* (2000)
Wheat straw	500	73.6	2.34	2.58	8.0	-	Mulligan *et al.,* (2010)
Wheat straw	1000	80.4	0.19	1.19	3.2	-	Mulligan *et al.,* (2010)
Malle residue	500	78.7	2.48	1.18	8.7	-	Mulligan *et al.,* (2010)
Malle residue	1000	84.5	0.25	0.84	2.6	-	Mulligan *et al.,* (2010)
Sugarcane baggase	600	76.45	2.93	0.76	19.83	-	Inyang *et al.,* (2010)
Willow	440	51.5	-	1.0	-	-	This study
Willow	470	54.6	-	1.0	-	-	This study
Willow	530	43.7	-	0.8	-	-	This study

From Table 42, it can be seen that the carbon, nitrogen, hydrogen or oxygen remaining in biochar is dependent on the pyrolysis temperature and the quality of the feedstock. In this study the carbon concentration increased from 52% to 55% at the pyrolysis temperatures of 440^oC and 470^oC before declining to 44% at 530^oC. These values were within the ranges of the previous studies.

4.5.2.2 IR spectroscopy analysis

Fourier Transform Infrared Spectroscopy (FTIR) analyses detailed the changes in the biochar chemical composition under different production conditions. Even though the IR technique was able to detect the general structure, the results can be hard to interpret as each functional group can be associated with several bands at different wavelengths.

In this study, the effects of a higher pyrolysis temperature (530°C), included the removal of lignocellulosic functional groups (e.g. –OH bound, ester, aliphatic alkyl, CO- and –OH), along with an increase in the presence of aromatic structures. The higher temperature produced a stronger reaction than lower ones (440°C or 470°C), releasing several volatile compounds which were associated with the loss of C and N, and the phenolic/aromatic chemicals collected in the bio-oil.

This was in line with observations by Chen *et al*. (2010) who observed the changes of pine sawdust IR spectra and by Chen and Chen (2009) who studied orange peel biochar, and reported that a strong valley at 1030-1060 cm^{-1} which was assigned to the C-O-C, or aliphatic ether C-C and alcohol C-O streching in the raw material declined at higher temperatures. At the highest temperature (550°C), most of these functional groups were completely removed, including the valley for aromatic CO- and phenolic –OH compunds at 1200-1240 cm^{-1}. The remaining valley at around 1600 cm^{-1} in our experiments represented recalcitrant compunds (aromatic C=C and C=O).

These represented similar results to Karaosmanoglu *et al.,* (2000) who studied changes in rapeseed straw and stalk biochar. Interestingly, there was an increased intensity in the valley from 780 to 840 cm^{-1} (representing the aromatic ring structure). This was also detected by Ozcimen and Karaosmanoglu, (2004) who monitored rapeseed cake biochar.

On the other hand, there were no changes in the IR spectra of biochar produced using different pyrolysis durations at the same temperature (470°C). The only effects were on peak intensities, revealing that temperature is a more crucial factor than pyrolysis time.

Using Nuclear Magnetic Resonance Spectroscopy (NMR), Brewer *et al.,* (2009) reported that the higher pyrolysis temperatures can create aromatic structures containing 17 rings per cluster, which is more than double than the 7-8 rings per cluster formed using low temperatures under slow and/or fast pyrolysis, respectively. This shows that the properties of willow biochar are different under different pyrolysis temperatures. Scanning electromagnetic microscopy (SEM) was employed to determine differences in the visual characteristics of biochar produced at different temperatures.

The pyrolysis temperature of 530°C was sufficient for complete conversion of the willow biomass to char, as shown by differences in the sequential SEM images and increases in it's surface area. SEM imaging showed that smaller pores (25 to 50 µm) were created in the biochar produced at the highest temperature. This compared to the pores of raw willow biomass feedstock, which measured 100 to 200 µm.

The spherical micro particles were five times larger than those of corn stover biochar pores (5 to 10 µm) (Fuentes *et al.,* 2010). Pyrolysis caused substantial changes to surface morphology and increased surface area. BET confirmed that increasing temperature and steam injection increased the surface area nearly 2 fold.

However the quality of biochar is not only dependant on it's surface area but also the type and quantity of nutrients, it will be provided to soil and its effectiveness for carbon sequestration. The values of surface area values reported for biochar from apricot stone, hazelnut shell and grape seed are at 11.24, 14.68 and 14.47 m^{-2} g^{-1} respectively (Ozcimen and Mericboyu, 2010), and values of biochar from sugarcane

bagasse between 14.07 to 17.66 m^{-2} g^{-1} (Inyang *et al.*, 2010). The willow biochar had a lower BET-value than the value obtained for 500°C biochar from pine needles, which was reported as 236.4 m^{-2} g^{-1} (Chen *et al*. 2008). Steam pyrolysis appears to have the highest potential, for its high biochar yields, and high BET-values.

4.5.2.3 Gas composition

The dominant gas products (e.g. CO_2, CO, H_2 or CH_4) were similar to the typical gas composition of the wood gasification processes (Gerber *et al.*, 2010) or other biochar feedstocks (e.g coal, rice husk, sugarcane baggase, rice straw, groundnut shell) (Loha *et al.*, 2010). At the highest temperature (530°C) CO_2, CH_4 or CO concentrations were almost twice those of the lower temperature (440°C or 470°C). This was supported by a clear reduction of C remaining in biochar and bio-oil.

An average measurement of gas composition during pyrolysis was obtained. Gasification involves a complex, rapid chemical transformation of the solid organic material into the gas phase (Kaushal *et al.*, 2010). In this study, the highest gas composition was found in the form of CO_2, which ranged from 42 to 50%, followed by CO, H_2 and CH_4, which were recorded at 25 to 30%, 10 to 16%, and 10 to 13%, respectively. In addition, Kaushal *et al.*, (2010), stated that at different temperatures, the gas composition remained constant except for hydrogen (H_2), which varied from 19 to 35%.

Overall the increases in gas proportions followed a linear trend with temperature. These proportions were slightly different to those compositions of gases from fluidized bed steam gasification of various materials (e.g coal, rice husks, sugarcane baggase, rice straw, groundnut shells) pyrolysed at 690°C to 770°C, which

were mainly dominated by H_2 (50-54%), followed by CO_2 (19-26%), CO (14-23%) and CH_4 (5–8%) (Loha *et al.*, 2010).

The production of biochar from willow feedstock produced a high proportion of CO_2 during pyrolysis. This may be a potential drawback of the process. Thus it is necessary to consider the application of live steam apparatus during pyrolysis. According to Loha *et al.*, (2010), the application of steam apparatus to a fluidized bed at higher temperatures (>600°C) could potentially modify the gas composition including CO_2.

4.5.3 Bio-oil chemistry

The results of the bio-oil composition analyses were more detailed than previous studies on bio-oil obtained from different substrates (Ozcimen and Mericboyu, 2010;Ozcimen and Karaosmanoglu, 2004). Several compounds (e.g phenols, acetic acids) were similar to those present in the bio-oil derived from corn straw (Liu *et al.*, 2010).

Within the liquid products obtained, there were several valuable chemicals whose derivatives can be used in medicine as expectorants, antiseptics, and local anaesthetics (e.g eugenol or guaiacol), in the food industry (eg. syringol), or even as chemical agents (e.g. acetic acid and phenol). Phenol is well known as a valuable commercial compound of raw material used for producing industrial chemicals such as aspirin, herbicides, and synthetic resins (Ross and Mazza, 2010).

In comparison, the bio-oil from various materials (eg. rapeseed cake, hazelnut, grapeseed) consisted of asphaltens, pentane, parafins, aromatics (toluene), and polar components (methanol) (Ozcimen and Mericboyu, 2010; Ozcimen and Karaosmanoglu,

2004). The chemical composition of the bio-oil in this study was much more detailed. However, due to its toxicity, it is not recommend for use as a soil amendment.

4.5.4 Carbon balance analysis

The carbon balance analysis revealed that amongst the different scenarios of biochar production, the mid range temperature (470°C) method was the most favourable option, as it offered much greater carbon storage and fixation in the form of biochar, with lower gas emissions compared to the highest temperature (530°C).

The advantages of producing biochar at mid temperature as opposed to the other treatments depended on the overall biochar characteristics achieved. This was due the greater number of residual nutrients in the final products.

The carbon balance assessment showed the worst results for the highest temperature (530°C) due to a lack of nutrients, and much greater gas phase releases, although it had a better surface area. The ratio of total greenhouse gas production (CO_2, CO, CH_4, H_2) to willow biomass feedstock at 440°C was 0.5:1, which was lower than at 470°C. This reduced emissions by 20% compared to 530°C.

5. Conclusions

Biochar produced from SRC biomass under different operating conditions in an isothermal fluidized bed pyrolysis reactor, showed that there was an impact of temperature of pyrolysis affected biochar characteristic and carbon balance assessment.

It was shown that increasing both the pyrolysis temperature from 440°C to 530°C, and the reaction time from 5 to 15 minutes, substantially influenced the yield, chemical composition and functional groups A pyrolysis temperature of 470°C and a slow reaction (15 minutes) was shown to be the optimum for both maximum biochar yield (27 wt%) and optimum functional characteristics and BET-surface area. Applying a direct injection of steam in the reactor appears to about double the amount of biochar formed, whilst the BET-value also nearly doubles. The carbon balance analysis demonstrated that this 470°C-biochar has a high carbon sequestration capacity, and that the biomass-to-biochar conversion is a carbon negative process with the assumption that all gas emission will be capture in the next period of SRC rotation.

Chapter 5

The effects of biochar application on litter decomposition and mineralization

5.1 Introduction

5.1.1 Interactions between biochar and soil biogeochemical cycling processes

Biochar can be applied to soil to enhance soil nutrient cycling and retention (e.g. C, N, P) and improve soil cation exchange capacity. Biochar application may be particularly beneficial to severe weathered and deprived soils which have low soil pH, potassium, and humus material (Lehmann *et al.,* 2003). Due to it's high chemical stability and carbon content, and it's potential to reside in soil over centuries, and even up to millennia, biochar applications could contribute to long-term carbon sequestration (Glaser *et al.,* 2002; Lehman, 2007a:2007b; Krull, 2010; Sohi *et al.,* 2009).

Biochar is stable for a long time period since it is resistant to breakdown by soil microorganisms (Winsley, 2007). However, biochar is not totally inert in soil. Biochar is comprised of labile pool which mineralises over a time period of days following addition to soil, and a recalcitrant pool which mineralises over longer time frames by abiotic and biotic processes (Smith *et al.,* 2010). The temperature under which biochar is produced is likely to influence biochar mineralisation rate. Luo *et al.,* (2011) showed that the mineralisation rate of biochar produced at 350°C was higher than that produced at 700°C, with 0.84 and 0.18% of the biochars mineralised in a pH 7.6 soil after 87 days, respectively. However, despite short term mineralisation of labile biochar components, most biochar-C is recalcitrant to decomposition. Biochar mean residence time (MRT) is predicted to reach up to 2000 years, with a half life of 1400 years (Kuzyakov *et al.,* 2009).

Biochar application to soil affects soil biogeochemical cycling processes, which can influence emission of greenhouse gasses. There are indications of significant reduction of N_2O and CH_4 after biochar application to soil (Gaunt and Lehmann, (2008); Baum and Weitner, (2006)). Rondon *et al.,* (2005) revealed that biochar addition to an acidic soil reduced N_2O emission by 50-80% depending on the management of the soil to which it was applied. There was also evidence for suppression of CH_4 emissions. Conversely, Knoblauch *et al.,* (2010) reported that application of charred rice residues increased release of CH_4 from a rice paddy soil relative to an unamended soil. However CH_4 emissions was five fold higher in soil receiving fresh rice residues, than they were in the soil to which charred residues were added.

Interactions between biochar and the decomposition of native soil organic matter were remained poorly understood and controversial. Wardle *et al.,* (2008) observed that in a field experiment over a 10-year period, mineralization rate of charcoal and humus together in litter bags was higher than each individual component, suggesting that the biochar primed mineralisation of native soil organic matter. However, Lehman and Sohi, (2008) commented that C loss from charcoal-humus mixtures could have been attributed to priming of C mineralisation from the biochar rather than the humus. Such priming effects have been reported elsewhere. Hamer *et al.,* (2004) estimated that the rate of biochar decomposition when added to sand was between 0.3 to 0.8% of the original char material over a 60 day period, with addition of glucose increasing mineralisation to between 0.6 and 1.2 % of the added biochar.

Furthermore, in the Wardle et al. (2008) study it was unclear whether the C loss was solely related to mineralization to CO_2 since loss through physical export could not be excluded. However, recently Luo *et al.*, (2011) confirmed that under laboratory conditions biochar addition caused priming of native SOM mineralisation, with water soluble components of the biochar likely to have been responsible. Furthermore it was shown that the extent of the priming effect depended on the temperature under which the biochar was prepared, with the biochar produced under 700°C having a greater priming effect than biochar produced at 350°C.

The finding that the temperature under which biochar was produced influences priming following addition of biochar to soil was confirmed by Zimmerman *et al.*, (2011) using a range of soil types and biochar materials. Biochar application had positive, negative and neutral effects on mineralisation, and the effects altered over time. However, the greatest priming effects were observed for biochars produced at 250-400°C. Furthermore biochar produced from grasses also induced greater priming than those produced from hardwood. Soil type was also important in determining the extent of priming, with the greatest priming effects seen in lower organic–C content soil to which grass biochar was added. Use of ^{13}C signatures to differentiate the source of the priming effect indicated that at early stages of decomposition, the priming effect was the result of enhanced mineralisation of biochar-C, reflecting degradation of the labile biochar pool. Similarly, Kuzyakov *et al.*, (2009), using ^{14}C labelled biochar, demonstrated that the enhanced mineralisation rate seen in soil amended with biochar and glucose, relative to soil receiving biochar only, was the result of increased mineralisation of biochar C, rather than impacts on native SOM.

However, it is important to note that biochar application to soil is not always associated with priming effects. Zavalloni *et al.,* (2009) found no evidence for priming effects when hardwood biochar produced at 500°C was added to soil together with wheat straw.

Zimmerman *et al.,* (2011) found that at month to year timescales, biochar reduced mineralisation of SOM probably via physical protection of sorbed C within biochar pores or at the surface of biochar, as occurs with organic matter sorbed onto silicaceous materials. Zimmerman *et al.,* (2010) suggested that such protection of soil C could be also be the result of greater aggregation, protecting both biochar and SOC from degradation, changes in microbial enzyme activity as a result of sorption to biochar, and toxicity of biochar components such as dioxins and polyaromatic hydrocarbons to the soil microbiota, which could reduce microbial activity, and hence decomposition of SOM.

The quantity of biochar added to soil would be expected to have a major impact on the extent to which biochar amendment will affect soil processes, including priming of SOM mineralisation and trace gas formation. In a laboratory experiment, Spokas *et al.,* (2009) found that increasing biochar application rates to soil from 1 to 30% caused progressively greater reduction of CO_2 emission, although only applications over 20% reduced formation of N_2O. Zhang *et al.,* (2012) found that biochar application to a rice paddy reduced emissions of CH_4, N_2O and CO_2, but there was no difference in the effectiveness of 10 and 40 t ha^{-1} application rates. Clearly there are thresholds at which biochar concentration affects the formation of greenhouse gases, and these thresholds may be different for different greenhouse gases. Furthermore the characteristics of biochar and soil

characteristics such as moisture content could have major influences on these thresholds.

The effects of biochar on greenhouse gas emissions from soil and the stabilisation of SOM are likely to be major contributors to the overall CO_2 abatement value of biochar (Hammond *et al.*, 2011; Shackley *et al.*, 2012). Clearly there is a need to generate robust understanding of these effects and extent to which their size and duration are affected by biochar properties and soil characteristics, so that a full understanding of the potential benefits of biochar for climate change mitigation can be developed.

5.1.2 The effect of biochar application on soil microbial communities

At present there is little knowledge about the effect of biochar addition on the activities of soil microorganisms. The application of biochar and its associated protection of C from microbial decay may have profound influences on soil biological activity, since there is evidence that ancient *Terra Preta* soils, which contain anthropogenic C added centuries ago are rich in organic matter compared to adjacent soil (Sohi *et al.*, 2009; Lehman *et al.*, 2003).

Traditionally, characterisation of microbial diversity in environmental substrates has relied on culture-dependent approaches. However, it has long been recognised that most microbes are not culturable, and in the last two decades a range of culture-independent methods have been developed to understand the structure of microbial communities inhabiting environmental matrices. This includes methods based on extraction of RNA/DNA and PCR amplification of specific components of the microbiota using group specific primers (e.g. 16S rRNA Terminal Restriction Fragment Length Polymorphism; O'Neill *et al.*,

2009), and methods based on extraction of fatty acids, such as phospolipid fatty acids (PLFA; Frostegaard *et al.*, 2011). While fatty acid based profiling does not provide detailed characterisation of microbial community composition and diversity as DNA approaches do, it has the advantage over DNA based approaches in that it allows broad taxonomic characterisation of the entire biomass, rather than defined taxa, and crucially, it provides quantification of the biomass of specific microbial groups (Zornoza *et al.*, 2009).

Use of fatty acids as a biomarker and the pathways of biosynthesis which allow specific fatty acids to act as indicators for defined microbial groups have been reviewed by Ruess and Chamberlain (2010). The major structure of fatty acids consists of a long straight carbon chain which possesses a carboxyl groups (COOH) at the one end (Figure 51a).

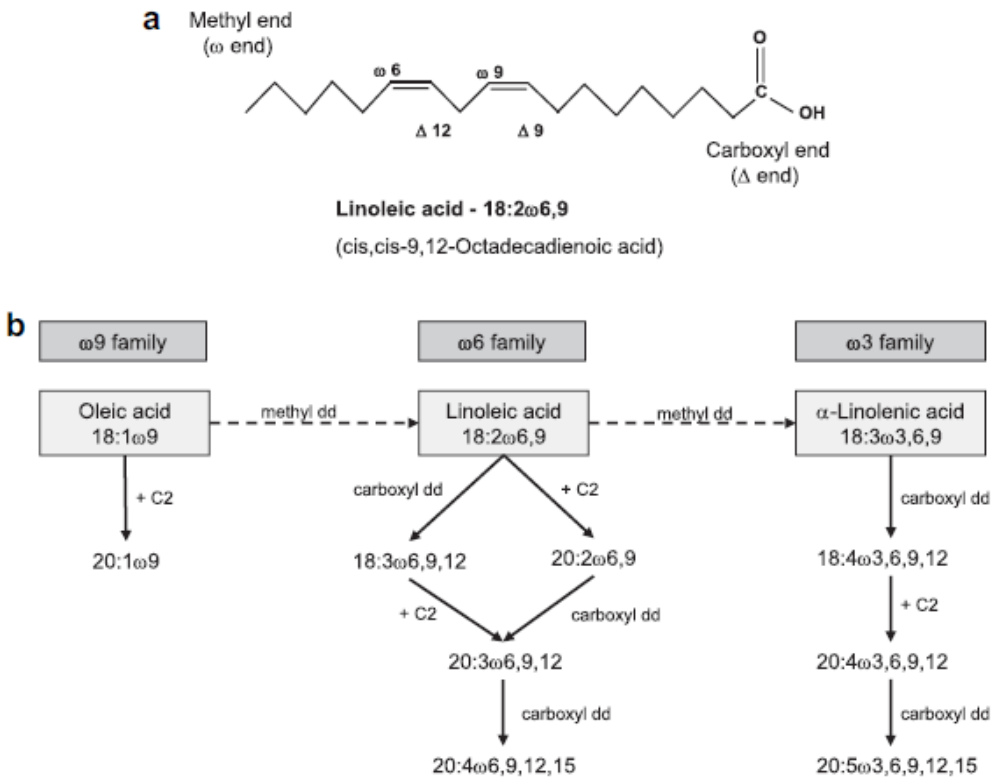


Figure 51a and 51b. The conceptual model of PLFA nomenclature

The saturated fatty acid has no double bond, whilst the unsaturated fatty acid either has one or more double bonds between carbon atoms. The classification of unsaturated fatty acids is determined by the number of carbon atoms from the carboxyl group. Unsaturated fatty acids are elongated by the addition of carbon atoms or methyl groups (ω) to the structure (Figure 42b).

Fatty acids found in common biota have been described by Zogg *et al.*, (1997); Sakamoto *et al.*, (2004); Frostergard *et al.*, (1996); Zelles, (1997:1999); Ruess and Chamberlain, (2010), and key indicator fatty acids are presented in Table 43.

Table 43. *Types of Fatty acid and their natural occurrence*

Fatty acid type	Fatty acid nomenclature	Predominant origin	Reference
A. Saturated			
Straight, >C20	20:0, 24:0	Plants	Ruess and Chamberlain, (2010)
Iso/Anteso metyl branched	i and a in C14 to C18	G(+)bacteria/bacteria	Zelles, (1997; 1999); Zogg, (1997); Frostergard *et al*, (1996)
10-methyl branched	10Me in C15 to C18	Sulphate reducing bacteria/actinobacteria	Ruess and Chamberlain, (2010)
Cyclopropyl ring	cy17:0,cy19:0	G(-)bacteria	Ruess and Chamberlain, (2010); Zogg, (1997)
Hydroxy substituted	OH in C10 to C18	G(-)bacteria /actinomycetes	Ruess and Chamberlain., (2010)
B. Mono unsaturated			
Double bond C5	16:1ω5	AM fungi/bacteria/G(-) bacteria	Ruess and Chamberlain, (2010)
Double bond C7	16:1ω7, 17:1w7,18:1ω7	AM fungi/bacteria/G(-) bacteria	Zogg (1997); Ruess and Chamberlain, (2010)
Double bond C8	18:1ω8	Methane oxidazing bacteria	Ruess and Chamberlain, (2010)
Double bond C9	18:1ω9	Fungi	Ruess and Chamberlain, (2010)
	20:1ω9	AM fungi	Sakamoto *et al*, (2004)
C. Poly unsaturated			
ω6 family	18:2ω6,9	Fungi	Frostergard *et al*, (1996); Ruess and Chamberlain, (2010)
	18:3ω6,9,12	Plants, Zygomycetes	Ruess and Chamberlain, (2010)
	20:4ω6,9,12,15	Animal	Ruess and Chamberlain, (2010)
ω3 family	18:3ω3,9,12	Higher fungi, plants	Ruess and Chamberlain, (2010)
	20:5ω3,6,9,12,15	Algae, colembola	Ruess and Chamberlain, (2010)

Fatty acid biosynthetic pathways may be conserved across animals, plant and soil microbes, producing similar types of fatty acid, preventing their use as specific biomarkers. This includes palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1ω7) and oleic acid (18:1ω9) (Ruess and Chamberlain, 2010). However, other fatty acids are specific to selected taxa. Frostergard *et al.*, (2010) reported that PLFAs 18:2ω6,9 and 18:1ω9 are common in fungi and can be recognized as a marker for this group. Furthermore the ratio between fatty acids can be used as stress or starvation indicators such as trans/cis (i.e: 16:1ω7t/16 1ω7c) or cyclo/mono unsaturated fingerprints (cy17:0/16, 1ω7c and cy19:0/18:1ω7c). Relative abundance of fatty acids which act as indicators for selected microbial groups to total PLFAs has been shown to indicate the proportion and dominance of microbial groups within the microbial community (Zornoza *et al.*, 2009).

Fatty acids represent living microbial biomass since they are rapidly degraded after cell death (Zelles, 1997) and they can be useful for detecting land use disturbance (McKinley *et al.*, 2005). Numerous studies have employed PLFA analysis to determine soil microbial structure and communities under different land use or cropping systems (e.g tall grass prairie (McKinley *et al.*, 2005); soybean (Meriles *et al.*, 2009); fallow (Kulmatiski and Beard, 2008); forest (Hackl et al., 2005); grassland (Marshall *et al.*, 2011). For example, comparison of PLFAs between forest and other cropping systems (i.e sugarcane and soybean) has been reported by Montecchia *et al.*, (2011). Soil microbial communities under agricultural cropping had a lower quantity of bacterial group fatty acids (i15:0, i16:0, i17:0, 18:1ω7 and cy19:0), actinobacterial fatty acid (10Me16:0) and fungal

fatty acid (18:1ω9c). Total microbial biomass, based on PLFA concentration was 5 fold higher in the forest than the agricultural cropping system.

However, a new finding from Buchan *et al.,* (2012) study revealed that in some circumstances there may be no correlation between PLFA and soil microbial biomass as the PLFA of microorganism still remain intact in dead microorganism biomass. Meanwhile, PLFA turn over is difficult to measure using a existing method (e.g. labelling substrate or microorganism) (Frostegard *et al.,* 2011)

Other approaches to characterise microbial communities have focussed on functional attributes such as enzyme profiles and community level physiological profiles using Biolog plates, which can be used to investigate treatment effects on community structure and functioning (e.g. Bending *et al.,* 2004). These methods have proved to be broadly comparable as indicators of community responses to agricultural management practice, although neither method can be reliably used to inform on decomposition processes. Alternative approaches to measure functioning rely on measurement of microbial processes such as nitrogen fixation or N_2O emission, isolation and enumeration of functional microbial groups, such as nitrogen fixing bacteria, or the use of DNA/RNA based approaches to investigate the structure and population size of defined functional groups using taxonomic or functional probes (Lehmann *et al.,* 2011).

Application of biochar has been shown to have a variety of effects on the soil biota. Generally biochar has been shown to increase formation of soil microbial biomass and alter the structure of microbial populations, and affect soil enzyme profiles, suggesting impacts on decomposition processes (Lehman *et al.,* 2011). These effects may be mediated by effects of biochar on soil properties, such as pH, and altered availability of organic matter and nutrients, as well as

indirectly via effects on plant growth and subsequent alteration of the quality and quantity of plant inputs to soil, although the relative importance of these effects remains to be elucidated.

Many studies have investigated differences in microbial communities between *Terra Preta* soils and adjacent pristine forest soils. The *Terra Preta* soils are 'Anthrosols' produced by application of charred biomass to the soil, presumably as a soil conditioner, in pre-Columbian times, with ages of such soils ranging from 600 to over 8000 years before present. The Anthrosols have higher organic carbon and charcoal contents than surrounding soils (Lehman *et al.*, 2006). Liang *et al.*, (2010) reported the impact of biochar on soil microbial biomass in three Anthrosols compared to adjacent forest soil. The study showed that soil microbial biomass in the biochar amended Anthrosols was higher by 43-125% relative to the adjacent soils. However, the mineralization of newly added organic matter was similar in the biochar rich Anthrosols and the adjacent soils.

Kim *et al.*, (2007) used 16S rRNA gene diversity to investigate differences in bacterial community structure in the *Terra Preta* and pristine forest soil, in the Jamari National Forest, of Rondonia, Brazil. It was shown that *Terra Preta* soil was richer in bacterial diversity, with approximately 25% greater richness of bacterial taxa in *terra preta* than forest soil. Similar results were observed in a comparison of Anthrosols and adjacent soils collected from the Amazon Basin in Brazil (O'Neill *et al.*, 2009). The Anthrosols had a dark, blackish colour down to 1 m or deeper due to the presence of black carbon (BC) which had been applied to the soil between 600 and 2300 years before present. Addition of BC had generated a higher pH, higher cation exchange capacity (CEC), and higher total P and Ca contents. Using culture-independent approaches it was found that numbers of

culturable bacteria were generally higher in the Anthrosol than the surrounding soil, and there were differences in diversity between the two soils. A broader range of taxa was found in the Anthrosol than in the surrounding forest soil, with 15 of the 19 bacterial families found in Anthrosol, and 8 found in the forest soil. Actinobacteria and Proteobacteria were more abundant in the Anthrosol than the forest soil, while the reverse was true for Firmicutes.

A range of studies have indicated that biochar application affects key functional soil taxa. For example biochar addition significantly improved biological nitrogen fixation in common bean (Rondon *et al.,* 2007), and a range of studies have found altered root colonisation by arbuscular mycorrhizal fungi, and mycorrhizal community composition as a result of biochar application to soil (Lehman *et al.,* 2011).

A range of studies have shown that application of biochar can alter levels of hydrolytic enzymes (Lehman *et al.,* 2011). For example, Bailey *et al.,* (2011) found that application of *Switchgrass* biochar to a range of soils affected the activity of four soil enzymes (β-glucosidase, β-N-acetylglucosaminidase, lipase, and leucine amino peptidase). However, the effects of biochar were soil specific. While β-N-acetylglucosaminidase increased in a sandy soil following biochar addition, both β-N-acetylglucosaminidase and β-glucosidase increased after biochar addition to a sandy-loam soil. However, lipase declined following application of biochar to both soils, whilst reduced activity of leucine aminopeptidase was recorded only after addition of biochar to the sandy-loam soil.

Steinbeiss *et al.,* (2009) used PLFA analysis to investigate how addition of yeast and glucose derived biochar affected soil community structure. The chars

had different chemical compositions, particularly N content. The chars had different impacts on microbial community structure, with yeast biochar promoting fungal biomass relative to unamended control and glucose amended soil, while glucose derived biochar can alter biomass and community structure of Gram negative bacteria.

Biomass crop plantations are likely to be an attractive option for biochar application for a number of reasons. Biochar application may improve plant growth, although the evidence is not clear-cut, with more field data needed, particularly for temperate regions (Shackley *et al.,* 2012). However, Biochar application could be a valuable soil conditioner, improving crop growth and thereby biomass production. Energy involved in the transport and spreading of biochar are likely to be major contributors to the overall energy balance of biochar use and production (Hammond *et al.,* 2011), and the proximity of biomass plantations to power stations would make transport of biochar to the plantation feasible. Lastly, pyrolysed material may contain a range of toxic materials such as dioxins and polyaromatic hydrocarbons, and application of such materials to crops for human or animal consumption may prove unacceptable until it can be proven that there would be no associated risk to human health.

5.2 Aims and Objectives

5.2.1 Aims

The aim of the work presented in this chapter was to investigate the effect of biochar application to soil from a short rotation coppice (SRC) on net C and N mineralisation. Since SRC plantations have a surface litter layer, the effects of biochar on CO_2 and mineral-N release from soil in the presence and absence of

litter were investigated. Since biochar application rate is likely to impact soil processes, experiments were conducted with a high and low application rate. Additionally the effects of biochar on microbial community biomass and composition were investigated by analysis of PLFA.

5.2.2 Objective

- To determine the quantity of CO_2 released and mineralized N (ammonium and nitrate).
- To examine the changes of soil microbial community and structure.

5.3 Hypotheses

- The addition of biochar will reduce soil and litter C and N mineralization.
- Biochar will increase microbial biomass and alter microbial community structure
- Rate of biochar application will determine the extents of the impact on mineralization and microbial communities.

5.4 Methods

5.4.1 Soil samples

Soil for this experiment was collected at Del Piece plot at Rothamsted Research Institute, Harpenden, in February 2010. This site was under 16 year old Willow SRC energy biomass crop (Genotype Q83) which had been harvested several times. The soil texture was a flinty clay loam (27% clay), developed over chalk. Soil carbon status at 0-30 cm was 2.87% and 0.80% for 30-60 cm, whilst for soil nitrogen at 0-30 cm was 0.24% and 0.09% for soil at a depth of 30-60 cm. The soil pH was 7.32 (0-30 cm) and 7.82 (30-60cm).

From each of 3 plots of the Willow Q83 genotype, a composite sample was made by collecting soil from 9 sampling point within the plot. These nine samples were collected from 0-30 cm depth with a 2.5 cm auger. Each sampling location was 0.5 m from a tree, with 3 sampling plots within 3 adjacent rows. Following collection, soils were sieved to <2 mm, and the soil from the 3 plots was pooled and mixed thoroughly prior to use. Soil water potential was determined using the filter paper technique of Deka *et al.,* (1995). Sterile Millipore water was added to the composite soil sample to bring it to a water potential of -33 kPa.

Fresh litter was collected from plots of Willow Q83. Surface litter was collected, ensuring that those leaves collected were from the previous autumn. Leaves were selected on the basis of colour and stage of decomposition, with only complete, yellow-brown leaves collected. Leaves were cut to approximately 1 cm pieces prior to use.

5.4.2 Biochar sample

Willow genotype Q83 biochar was produced by pyrolysis at 470°C for 15 minutes, as described in Chapter 4. Detailed physical properties and visual characterization of biochar has been described in detail in Chapter 4.

5.4.3 Experimental design

Soil was used without biochar or with a biochar application of 0.5 or 2% w/w. 1 Kg portions of soil was placed into plastic bags and biochar was added to provide the appropriate concentration. The soil and biochar mixtures were mixed thoroughly using a large spatula. In those treatments receiving litter, the litter was then added at a rate of 1% w/w and mixed through the soil until distribution was visibly uniform. A total of 6 treatments were set up:

1. Unamended soil
2. Soil + 1% litter
3. Soil + 0.5% biochar
4. Soil + 2.0% biochar
5. Soil + 0.5% biochar + 1% litter
6. Soil + 2.0% biochar + 1% litter

For each treatment, 6 replicates were set up by adding 100 g fresh weight soil/biochar/litter to 500 ml sealed plastic jars with a neck diameter of 22 mm (Fisher), topped with rubber suba-seal septum (Sigma-Aldrich). Soils were incubated in a random design in the dark at 15°C. Bottles were weighed every two days and water was added as necessary to maintain the water content.

5.4.4 Headspace CO_2 concentration.

Headspace CO_2 concentration was determined using a GC-MS (Agilent-6890) equipped with an MSD detector at a temperature of 250°C and a HP-DB-5 MS column was used. Helium was used as a carrier gas at 0.5 ml per minute. In order to quantify CO_2 concentration, the relationship between peak area and CO_2 concentration was determined. Gas standard (5000 mg kg^{-1} CO_2) was emptied into a 250 ml glass bottle, equipped with an inlet and outlet valve. The bottle was sealed and then 10, 20, 30, 40 and 50 µl aliquots of the headspace were removed and injected into the GC-MS. The retention time of CO_2 was 1.75 minute and the peak area was recorded. A standard regression curve between peak area and CO_2 concentration was determined in MS-Excel.

Four of the 8 bottles for each treatment were used for CO_2 analysis over a 90 day period. At regular intervals over 90 days, 50 µl aliquots of the headspace were removed from each experimental bottle for analysis of CO_2 concentration using the method described above. Headspace CO_2 concentration was collected using a 500 µl gas tight locking syringe (Hamilton, Revo Nevada) before injection into the GC-MS.

Following this, the suba-seal lids were removed and bottles were left in a fume cabinet for 4 hours to allow complete flushing of CO_2 before being resealed. Following analysis of CO_2 concentration, cumulative CO_2 mineralized per g soil per day was determined.

5.4.5 N mineralization

After 30 and 90 days 4 replicate bottles were destructively sampled for mineral-N analysis. Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) were

extracted from the sample with 50 ml of 2M KCl. Samples were shaken for 1 h and (NH_4^+-N) and nitrate (NO_3^--N) in solution were quantified using an automatic flow analyser (FIA star 5000-Flow Injection Analyser-Warrington-UK).

5.4.6 Phopholipid fatty acid analysis (PLFA)

In the bottles destructively sampled at 30 and 90 days microbial community biomass and structure in each treatment was determined using phospolipid fatty acid (PLFA) analysis. Briefly 50 g of soil was freeze dried. Soil was ball milled prior to extracting PLFAs. A modified technique from Frostegrad *et al.,* (1993) was used, which is based on the method developed previously by Bligh and Dyer (1959) and White *et al.,* (1979). Principally, lipids were extracted from soil using a mixture of chloroform: methanol: citrate buffer (1:2:0.8 v/v). The PLFA assay can be separated into several steps, which are: 1. Lipid extraction, 2. Lipid separation, 3. Lipid methanolysis, and 4. Lipid quantification and identification. All solvents and chemicals used were analytical grade. To avoid lipid contamination all glassware was soaked in 10% Decon 90 and deionised H_2O and rinsed before being placed in a muffle furnace overnight at 450°C. Pasteur pipettes and vial inserts were also incubated in a furnace prior to use. Pipette tips were autoclaved. Sample concentrator needles and silicic acid column stoppers were soaked in MeOH (Frostegard *et al.,* 1993).

a. Lipid Extraction

Firstly, 500 mg freeze dried soil from each treatment was weighed accurately to 4 decimal places into a 15 ml borosilicate glass culture tube with a PTFE-lined screw cap. Then 9.2 ml Bligh & Dyer solution was added to each sample. The sample was mixed on a vortex mixer, then left for 2 hours, before

vortexing and mixing every 30 mins. After 2 hours, the samples were vortexed again then centrifuged for 10 min at 1500 rpm and 20°C, on the Mistral 1500 centrifuge. Using a clean glass pasteur pipette for each sample, the supernatants transferred into a clean culture tube. Another 2.0 ml Bligh & Dyer solution was added to the soil residue, the sample was vortexed and centrifuged as before. Again, supernatant were transferred to the culture tube using a clean pasteur pipette.

Secondly, to the supernatant, 2.6 ml $CHCl_3$ and 2.6 ml Citrate Buffer were added, and then vortexed. Following mixing the samples on a sample rotator for 30 minutes, they were centrifuged as before. If the separation is successful, an organic layer is found at the bottom under an aqueous layer. The aqueous layer was removed and discard, using a clean pasteur pipette. Using a clean pasteur pipette, all the lower organic phase was transferred to a clean scintillation vial.

Finally, the sample was evaporated to dryness under a stream of nitrogen on a dry-block heater set at 40°C. Once the sample was completely dry, 1 ml methanol was added following evaporation the sample in to dryness under stream of nitrogen and the process was repeated again.

b. Lipid separation

Lipids were separated into neutral lipids, glycolipids, and polar lipids (phospolipid) following three steps of extraction on a silicic acid column. Silica columns with a sorbent mass of 500 mg and reservoir volume of 6 ml were used in solid phase extraction fitted with one-way stop cocks (SPE) to each column.

The column was washed with 5 ml $CHCl_3$, then the taps were closed. 400 μL $CHCl_3$ were add to the sample before vortexing twice and the samples were

transferred in to the column using a clean pasteur pipette. The vials were washed with 3 x 200 μL $CHCl_3$ and the washings transferred to the column. Those steps were repeated to the next column until all the samples were loaded to the columns.

The each tap was opened and the sample allowed to load onto the column slowly. 2 x 3 ml $CHCl_3$ were added and collected in a culture tube (at this stage neutral lipids were eluted). Later, 2 x 3 ml Acetone were added and eluted solution were collected in the collection vial (at this stage glycolipids were eluted). When the acetone has passed through the column, the rack was moved forward to clean culture tubes, and the previous collections were discarded into the appropriate waste bottle.

For collecting phospolipid fatty acid, 4 ml and 2 x 3 ml methanol were added and allowed to dry out once all the methanol had passed through the column. The collected solutions were evaporated to dryness at 40°C under a stream of nitrogen on a dry-block heater. Once these steps were completed, 200 μL Internal Standard (Methyl nonadecanoate) were added and re-evaporated to dryness under stream of nitrogen on the dry-block heater (McKinley *et al.*, 2005).

c. Lipid methanolyis

Polar lipid (phospolipid) was recognized as fatty acid methyl ester (FAME) following mild acid methanolysis into an organic solvent (iso-hexane). This was achieved as follow: 1. 1 ml MeOH:Toluene (1:1 v/v) was added to each sample and vortexed following the addition of 1 ml 0.2 M KOH to each sample and re-vortexed again, 3. The samples then were placed in a rack and incubated at 37°C (± 3°C) in a water bath for 15 minute at 37°C, 4. After methanolysis was

performed, the samples were removed from the water bath and 2 ml Iso-hexane:$CHCl_3$ (4:1 v/v) + 0.3 ml 1 M Acetic Acid + 2 ml Millipore water were added, before vortexing and placing on a sample rotator for 10 minutes, 5. The samples were centrifuged for 10 minutes at 1500 rpm on a Mistral 1500 to separate aqueous and organic phases, 6. The upper organic phase was transferred to a clean scintillation vial using a clean pasteur pipette, and the lower aqueous layer was discard, 7. A further 2 ml Hexane:$CHCl_3$ (4:1 v/v) was added to the culture tube containing the lower aqueous layer for re-dissolving the remaining organic phase, 8. The samples were vortexed and centrifuged as before and the upper layer re-transferred (using a clean pasteur pipette) to the scintillation vial containing the first washing, 9. The samples were evaporated to dryness under stream of nitrogen on the dry-block heater at 40°C.

d. Lipid quantification and identification

The samples were sent to the Macaulay Institute, Aberdeen, UK for further analysis. Principally, identification of the lipid fractions was performed by comparison to standard qualitative fungal and bacterial lipids using acid ester mix (Supelco) reagents. This covered a range of lipids between C_{11} to C_{20} (Grayston *et al*, 2001). The quantification of PLFAs per dry weight of soil was performed by GC chromatography (GC Agilent 6890-Agilent Technology, Santa Clara-USA) using a flame ionization detector (FID), split injector and HP-7673 auto sampler. A capillary gas liquid chromatography column 50 m x 200 µm x 0.33 µm film thickness, coated with 0.5% phenyl methyl siloxane was used. Helium as a carrier gas was operated with a flow rate at 0.8 ml minute^{-1} and a pressure of 35 psi. The

temperature of injector and FID detector were set up at 250°C and 300°C respectively. 2 µl samples were injected using the spitless mode of the machine.

For each sample, the abundance of individual fatty acid methyl esters was expressed as µg PLFAs g^{-1} soil. The fatty acid nomenclature used was described by Frostergaard (1996) and Zogg, (1997). The fatty acids i15:0, a15:0, 15:0, i16:0, 10Me 16:0, 16:1ω5c, 16:1ω7c, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 17:1ω8t, 18:0, 18:1ω7 and cy19:0 were chosen to represent bacterial PLFAs. The polyeonic, unsaturated fatty acid 18:2ω6 was used as an indicator of fungal biomass and 16:1ω5c was used as an indicator of arbuscular mycorrhizal fungi. The PLFAs 10Me 16:0, i15:0, a15:0, 15:0, i16:0, 16:0, i17:0, a17:0, and 18:0 were selected to represent Gram positive (+) bacteria. The monoenonoic and cyclopropane unsaturated PLFAs 16:1ω5c, 16:1ω7t, 16:1ω7c, 18:1ω7c, cy17:0 and cy19:0 were chosen for Gram negative (-) bacteria. Actinobacteria were classified as 10Me 16:0, 10Me 17:0 and 10 Me 18:0. The ratio of fungal/bacterial PLFA was used as an indicator of changes in the relative abundance of these two microbial groups. The ratio of each microbial group to total PLFA was used to represent its relative abundance within the total microbial community.

The fatty acid nomenclature used is as follows: total number of carbon atoms, number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. Cis and trans configuration are encoded by c and t, respectively. Anteiso and iso branching are designated by prefix a or i. 10 Me is a methyl groups of the 10th carbon atom of the carbonyl end of the molecule. Cy is indicated the cyclopropane fatty acid (Baath and Anderson, 2003).

5.4.7 Statistical analyses

The results of cumulative CO_2 concentration and mineral-N concentrations were analyzed using analysis of variance (ANOVA) in Genstat ver. 11, whilst MANOVA was used for determining the significance of difference ($P<0.05$) in PLFAs in each treatment. Cannonical Variate Analysis (CVA) was used to examine the effect of treatments on the structure of the microbial community. 95% confidential intervals were used to determine the significance of differences between treatments.

5.5 Results

5.5.2 The effect of biochar application on C mineralization

In all treatments the rate of CO_2 release was rapid in the first 14 days and then reduced thereafter (Figure 52). When applied at a rate of 0.5%, biochar had no significant effect on CO_2 released relative to the control (soil only) treatment. However, at a rate of 2% biochar there was significantly less CO_2 released over the 90 day period relative to the control. This amounted to a 10% reduction in mineralization.

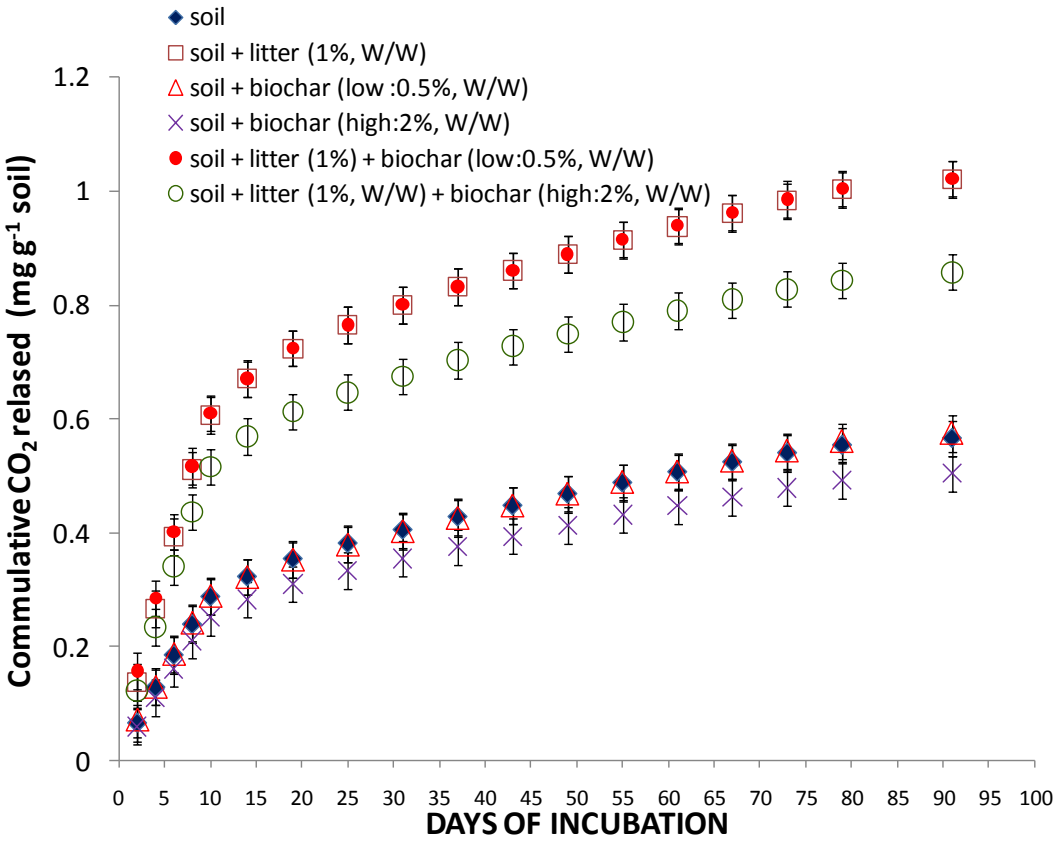


Figure 52. Cumulative CO_2 flux from treatments over the 90 day incubation. Error bars represent LSD ($P<0.05$)

Addition of litter significantly increased CO_2 mineralization relative to the unamended control. There was no significant difference in CO_2 mineralization between the treatment receiving litter only, and the treatment to which both litter and 0.5% biochar was added. However, addition of the high biochar concentration to litter reduced net mineralization of CO_2 by 20% over the 90 day period.

5.5.3 N mineralization

After 30 days there was no difference in NH_4-N between the treatments. After 90 days, addition of the low biochar concentration had no effect on NH_4-N relative to the unamended control soil, while the high biochar concentration had reduced NH_4-N by 30% relative to the control (Table 44).

Table 44. *Soil N mineral concentrations under different treatments*

Treatments	**NH_4-N (30 days after incubation) (mg kg^{-1})**	**NH_4-N (90 days after incubation) (mg kg^{-1})**	**NO_3-N (30 days after incubation) (mg kg^{-1})**	**NO_3-N (90 days after incubation) (mg kg^{-1})**
Soil	8.0	4.6	44.1	83.1
Soil + litter	9.1	6.6	39.0	77.7
Soil + biochar (L)	7.4	4.3	34.2	69.2
Soil + biochar (H)	7.8	3.3	27.5	37.6
Soil + litter + biochar (L)	9.1	3.2	34.7	59.3
Soil + litter + biochar (H)	10.3	3.0	24.5	34.6
LSD	**2.38**	**1.57 (*)**	**6.02 (*)**	**6.15 (*)**

(*) indicated that significant difference was found ($P<0.05$)

Addition of litter increased NH_4-N by 12 and 30% relative to the unamended control soil following incubation for 30 and 90 days, respectively. However when biochar was added together with litter, NH_4-N was reduced to below levels seen in the unamended control soil, with no differences between the two biochar treatments.

Addition of biochar reduced amounts of NO_3-N, with the high concentrations almost halving amounts of NO_3-N at both 30 and 90 days relative to the unamended control. Addition of litter resulted in greater amounts of NO_3-N relative to the unamended soil after 90 days. When biochar was added together with litter, there was a significant decrease in the amounts of NO_3-N at both 30 and 90 days for the high biochar treatments and after 90 days only in the low biochar treatment, relative to amounts in the litter treatment. In the case of the 0.5% application of biochar together with litter, amounts were reduced to below those found in the corresponding soil without litter.

5.5.4. The effect of biochar addition on soil pH

2 way ANOVA showed that litter and biochar significantly affected the soil pH ($P<0.05$) at both days of incubation (30 and 90 days) (Table 45). Litter addition had no significant effect on soil pH. At both time points the high rate of biochar addition had significantly increased pH relative to the control, when it was added to soil both in the absence and presence of litter.

Table 45. *Average soil pH values following the addition of litter and biochar at different level of application.*

	Soil pH	
	30 days of incubation	**90 days of incubation**
Treatments :		
Soil	7.19	7.17
Soil + litter	7.06	7.02
Soil + low rate biochar (0.5%)	7.13	7.18
Soil + high rate biochar (2.0%)	7.36	7.43
Soil + litter + low rate biochar (0.5%)	7.18	7.21
Soil + litter + high rate biochar (2.0%)	7.49	7.41
LSD (P<0.05)	**± 0.17**	**± 0.16**

When added to soil together with litter, the low biochar application rate increased pH relative to the soil and litter control treatment after 90 days only.

5.5.5 The impact of litter and biochar application on soil microbial structure and communities

There were no significant differences between treatments in the size of the microbial biomass (Table 46), so that neither litter or biochar addition affected biomass at either 30 or 90 days. However, there were significant differences between treatments in the relative abundance of specific PLFAs. In general, amounts of the PLFAs C17:0(10Me), C18:2(9,12), C18:1w10or11, C18:0, C20:1w9,C20:1 and C20:0 were higher in soil receiving litter and 2% biochar relative to most other treatments after 30 days. However, after 90 days, only the PLFA C18:2(9,12) showed a significant difference between treatments, with this PLFA being significantly higher in soil receiving litter, with the addition of biochar having no effect on its abundance.

When PLFAs were pooled to report on total bacterial, actinobacterial, Gram positive, Gram negative or fungal biomass, it was found that the only group showing a response to the treatments was fungi. After 30 days there was significantly higher fungal biomass in treatments receiving both litter and biochar relative to the treatment receiving litter alone or the treatments without litter. After 90 days there was significantly more fungal biomass in treatments receiving litter than the other treatments, although addition of biochar had no significant effect on fungal biomass.

There were significant differences in the relative proportions of the different microbial groups within the biomass after 30 days (Table 47). The abundance of actinobacteria and Gram positive bacteria reduced in treatments

receiving litter, while the relative abundance of fungi was increased by the addition of litter. After 90 days these differences persisted in the case of the fungal community, but not for the other groups. Furthermore addition of litter affected the fungus: total PLFA ratio. After both 30 and 90 days the abundance of fungi within the community increased. The same pattern emerged when fungus: bacterial ratio was determined (data not shown).

Table 46. The pattern of individual PLFAs in 30 and 90 days of incubation

PLFAs	Indicator	30 days after incubation (µg)							90 days after incubation (µg)						
		Soil	soil + biochar (L)	soil + biochar (H)	soil + litter	soil + litter + biochar (L)	soil + litter + biochar (H)	**LSD**	Soil	soil + biochar (L)	soil + biochar (H)	soil + litter	soil + litter + biochar (L)	soil + litter + biochar (H)	**LSD**
C12:0	*Unclassified*	0.24	0.26	0.14	0.22	0.21	0.20	**0.22**	0.35	0.59	0.60	0.54	0.58	0.47	**0.40**
C13:0	*Unclassified*	0.08	0.08	0.08	0.08	0.08	0.08	**0.03**	0.01	0.02	0.01	0.01	0.07	0.01	**0.07**
C14:0i	*Unclassified*	0.01	0.01	0.01	0.01	0.01	0.01	**0.00**	0.54	0.63	0.62	0.59	0.55	0.63	**0.22**
C14:0	*Unclassified*	0.43	0.52	0.45	0.39	0.47	0.50	**0.23**	0.37	0.64	0.42	0.50	0.94	0.53	**0.69**
C14:1w9c	*Unclassified*	0.22	0.17	0.25	0.20	0.25	0.17	**0.13**	0.10	0.18	0.11	0.14	0.07	0.11	**0.15**
C14:1w9t	*Unclassified*	0.01	0.01	0.01	0.01	0.01	0.01	**0.00**	0.15	0.25	0.11	0.17	0.45	0.17	**0.42**
C15:0i	Bacterial/G+	3.36	3.51	3.61	3.01	3.61	3.49	**0.94**	3.12	3.31	3.31	3.56	3.16	3.67	**0.91**
C15:0ai	Bacterial/G+	2.51	2.69	2.79	2.64	2.68	2.93	**0.95**	2.00	3.53	2.38	2.64	3.02	2.77	**1.97**
C15:0	Bacterial	0.37	0.42	0.35	0.35	0.43	0.42	**0.17**	0.40	0.64	0.44	0.61	0.70	0.64	**0.34**
C16:0br	*Unclassified*	0.15	0.17	0.15	0.16	0.21	0.18	**0.04**	0.09	0.22	0.08	0.14	0.23	0.13	**0.20**
C16:1i	*Unclassified*	0.43	0.57	0.48	0.39	0.48	0.54	**0.19**	0.36	0.58	0.37	0.47	0.47	0.52	**0.26**
C16:1w11c	*Unclassified*	0.03	0.13	0.01	0.01	0.01	0.06	**0.15**	0.21	0.37	0.17	0.27	0.33	0.18	**0.24**
C16:0i	Bacterial/G+	1.40	1.47	1.58	1.28	1.56	1.61	**0.30**	1.33	1.38	1.35	1.49	1.39	1.63	**0.34**
C16:1w11t	*Unclassified*	0.87	0.82	0.84	0.75	0.93	0.86	**0.27**	0.62	0.89	0.83	0.86	0.83	0.86	**0.36**
C16:1w7c	Bacterial/G-	3.36	3.63	3.81	3.12	3.86	3.87	**0.72**	3.30	3.33	3.55	3.76	3.36	3.76	**0.90**
C16:1w7t	Bacterial/G-	0.27	0.29	0.29	0.24	0.27	0.33	**0.06**	0.23	0.40	0.31	0.35	0.38	0.36	**0.18**
C16:1w5	Bacterial/G-	2.97	3.14	3.19	2.68	3.25	3.10	**0.61**	2.85	2.89	3.05	3.15	2.85	3.18	**0.65**
C16:0	*Unclassified*	6.65	7.31	7.33	6.85	8.37	8.19	**1.58**	5.61	6.35	6.57	8.23	7.54	8.38	**2.44**
C17:0br	*Unclassified*	0.91	1.00	0.90	0.73	0.88	0.92	**0.25**	1.12	1.53	0.97	1.42	1.42	1.34	**0.72**
C16:0(10me)	Bacterial/G+/ Actinobacteria	0.15	0.18	0.12	0.12	0.10	0.16	**0.06**	0.62	1.07	0.65	0.86	0.92	0.79	**0.51**
C17:0i	Bacterial/G+	0.71	0.81	0.63	0.57	0.66	0.66	**0.24**	1.11	1.22	0.74	1.29	1.23	1.09	**0.80**
C17:0ai	Bacterial/G+	1.52	1.64	1.59	1.32	1.54	1.70	**0.34**	1.43	1.95	1.55	1.99	1.31	1.86	**0.97**
C17:0brb	*Unclassified*	1.31	1.54	1.38	1.13	1.31	1.56	**0.45**	1.29	2.01	1.38	1.68	1.79	1.82	**0.89**
C17:1w8c	*Unclassified*	0.68	0.80	0.69	0.68	0.72	0.95	**0.26**	0.76	1.22	0.70	1.07	1.19	1.11	**0.61**
C17:0cy	Bacterial/G-	2.20	2.39	2.49	2.00	2.35	2.49	**0.43**	2.40	2.61	2.50	2.70	2.68	2.75	**0.62**
C17:1w8t	*Unclassified*	0.01	0.01	0.01	0.01	0.01	0.01	**0.00**	0.01	0.23	0.01	0.01	0.14	0.01	**0.31**
C17:1w7	*Unclassified*	0.01	0.01	0.01	0.01	0.01	0.01	**0.00**	0.46	0.64	0.50	0.66	0.66	0.73	**0.25**
C17:0	Bacterial	0.36	0.41	0.36	0.36	0.37	0.49	**0.11**	0.77	1.26	0.87	0.92	0.96	1.18	**0.57**
C17:0(12me)	*Unclassified*	0.87	0.91	0.95	0.70	0.78	1.06	**0.25**	0.29	0.45	0.27	0.32	0.37	0.41	**0.23**
C17:0(10me)	Bacterial/G+/ Actinobacteria	0.44(*)	0.50(*)	0.47(*)	0.40(*)	0.39(*)	0.64(*)	**0.15(*)**	0.37	0.91	0.41	0.50	0.59	0.60	**0.55**
C18:3(5,10,12)	*Unclassified*	0.02	0.12	0.04	0.01	0.01	0.17	**0.17**	0.49	1.03	0.76	0.77	1.69	1.11	**0.71**
C18:2(4,10or5,10)	*Unclassified*	0.01	0.01	0.01	0.01	0.01	0.19	**0.22**	0.12	0.35	0.17	0.23	0.35	0.18	**0.23**
C18:2(9,12)	Fungus	2.79(*)	3.46(*)	3.16(*)	3.64(*)	4.52(*)	4.67(*)	**1.18(*)**	1.99(*)	2.72(*)	2.54(*)	4.11(*)	4.27(*)	4.97(*)	**1.50(*)**
C18:1w9	*Unclassified*	4.92	5.18	5.39	4.59	5.60	5.94	**1.03**	3.37	4.39	4.24	5.00	5.31	5.47	**2.14**
C18:1w7	Bacterial/G-	6.31	6.92	7.25	5.94	7.35	7.50	**1.31**	4.86	6.45	6.82	7.48	6.89	7.60	**3.12**
C18:1w13	*Unclassified*	0.05	0.09	0.05	0.08	0.05	0.25	**0.20**	0.18	0.66	0.13	0.21	0.49	0.25	**0.69**
C18:1w10or11	*Unclassified*	1.13(*)	1.28(*)	1.22(*)	0.93(*)	1.07(*)	1.31(*)	**0.23(*)**	0.77	1.24	1.01	1.09	1.12	1.23	**0.44**

C18:0	*Unclassified*	1.81(*)	1.92(*)	1.85(*)	1.64(*)	1.83(*)	2.16(*)	**0.28(*)**	1.70	2.40	1.78	2.40	2.54	2.34	**1.10**
C19:1w6	*Unclassified*	0.65	0.73	0.69	0.57	0.47	1.29	**0.66**	0.43	2.14	0.43	0.71	1.67	0.76	**2.27**
C18:0(10me)	Bacterial/G+/ Actinobacteria	0.78	0.81	0.78	0.60	0.68	1.03	**0.30**	1.10	1.77	2.52	1.69	1.68	1.86	**1.31**
C19:1w8	*Unclassified*	0.30	0.36	0.23	0.25	0.16	0.50	**0.24**	0.38	0.82	0.23	0.49	0.84	0.45	**0.79**
C19:0cy	Bacterial/G-	3.32	3.75	3.64	2.76	3.33	4.09	**1.05**	2.43	4.09	3.25	3.28	3.87	3.81	**2.03**
C20:4(5,8,11,14)	*Unclassified*	0.54	0.64	0.43	0.50	0.38	0.64	**0.19**	0.39	0.85	0.34	0.52	0.76	0.60	**0.62**
C20:5w3	*Unclassified*	0.21	0.66	0.53	0.67	0.80	0.32	**0.64**	0.71	1.68	0.74	0.92	1.48	1.08	**1.19**
C20:4(6,10,14,18)	*Unclassified*	0.21	0.10	0.01	0.20	0.16	0.20	**0.28**	0.33	0.80	0.33	0.31	0.58	0.46	**0.62**
C20:4(8,11,14,17)	*Unclassified*	0.17	0.34	0.45	0.23	0.19	0.45	**0.34**	0.25	1.05	0.20	0.41	0.76	0.34	**1.04**
C20:1w9	*Unclassified*	0.67(*)	0.74(*)	0.59(*)	0.57(*)	0.48(*)	0.80(*)	**0.19(*)**	0.54	1.77	0.63	0.72	1.63	0.79	**1.74**
C20:1	*Unclassified*	0.31(*)	0.34(*)	0.26(*)	0.27(*)	0.18(*)	0.37(*)	**0.11(*)**	0.06	0.07	0.04	0.12	0.03	0.06	**0.08**
C20:0	*Unclassified*	0.46(*)	0.50(*)	0.39(*)	0.40(*)	0.26(*)	0.80(*)	**0.30(*)**	0.26	0.82	0.63	0.41	0.70	0.64	**0.57**
Cphthalate	*Unclassified*	4.61	4.79	5.07	3.98	4.95	5.06	**0.89**	4.15	4.35	4.33	4.47	4.16	4.75	**1.27**
Total PLFAs		61.9	68.3	67.2	58.0	68.4	75.1	**15.2**	70.6	80.9	74.8	76.4	81.1	80.5	**24.20**
Total bacterial PLFAs		34.6	37.4	38.1	31.0	37.4	39.6	**8.09**	32.5	41.2	38.1	40.8	39.2	42.3	**12.85**
Total actinobacteria PLFAs		1.38	1.50	1.39	1.13	1.18	1.84	**0.50**	2.10	3.75	3.59	3.06	3.20	3.26	**1.68**
Total G(+) bacterial PLFAs		15.40	16.42	16.67	13.51	16.19	17.30	**3.92**	15.24	19.50	17.25	18.52	17.48	19.02	**5.88**
Total G(-) bacterial PLFAs		18.44	20.14	20.68	16.75	20.43	21.39	**3.97**	16.10	19.78	19.49	20.73	20.04	21.46	**6.52**
Total fungal PLFAs		2.79(*)	3.466(*)	3.16(*)	3.64(*)	4.52(*)	4.67(*)	**1.18(*)**	2.19(*)	2.72(*)	2.54(*)	4.11(*)	4.27(*)	4.97(*)	**1.25(*)**

Table 47. The relative abundance microbial groups within the microbial community after 30 and 90 days of incubation

	30 days after incubation							**90 days after incubation**						
	soil	soil + biochar (L)	soil + biochar (H)	soil + litter	soil + litter + biochar (L)	soil + litter + biochar (H)	**LSD**	Soil	soil + biochar (L)	soil + biochar (H)	soil + litter	soil + litter + biochar (L)	soil + litter + biochar (H)	**LSD**
Ratio bacteria/total PLFAs	0.55	0.54	0.56	0.52	0.54	0.52	**0.02**	0.51	0.51	0.50	0.53	0.51	0.52	**0.15**
Ratio actino bacteria/total PLFAs	0.02(*)	0.02(*)	0.02(*)	0.01(*)	0.01(*)	0.02(*)	**0.003(*)**	0.03	0.04	0.04	0.04	0.04	0.04	**0.01**
Ratio G(+) bacteria/total PLFAs	0.25(*)	0.24(*)	0.25(*)	0.22(*)	0.23(*)	0.22(*)	**0.01(*)**	0.23	0.24	0.23	0.24	0.22	0.23	**0.06**
Ratio G(-) bacteria/total PLFAs	0.29	0.29	0.30	0.28	0.29	0.28	**0.01**	0.25	0.25	0.26	0.27	0.26	0.26	**0.08**
Ratio fungal/total PLFAs	0.04(*)	0.04(*)	0.04(*)	0.06(*)	0.06(*)	0.06(*)	**0.009(*)**	0.03(*)	0.03(*)	0.03(*)	0.05(*)	0.05(*)	0.06(*)	**0.02(*)**

Canonical Variate Analysis (CVA) using the 50 PLFAs analysed was used to investigate the effect of time, litter and biochar amendment on microbial community structure. The first canonical variate analysis axis (CV1) accounted for 78.2% of the variance and the second axis (CV2) which represented 10.91% of the variation (Figure 53). It was found that soil microbial community structure was clearly distinct at the two sampling points with a clear separation along CV1, whilst treatments at the 90 day, but not the 30 day harvests were significantly separated along CV2.

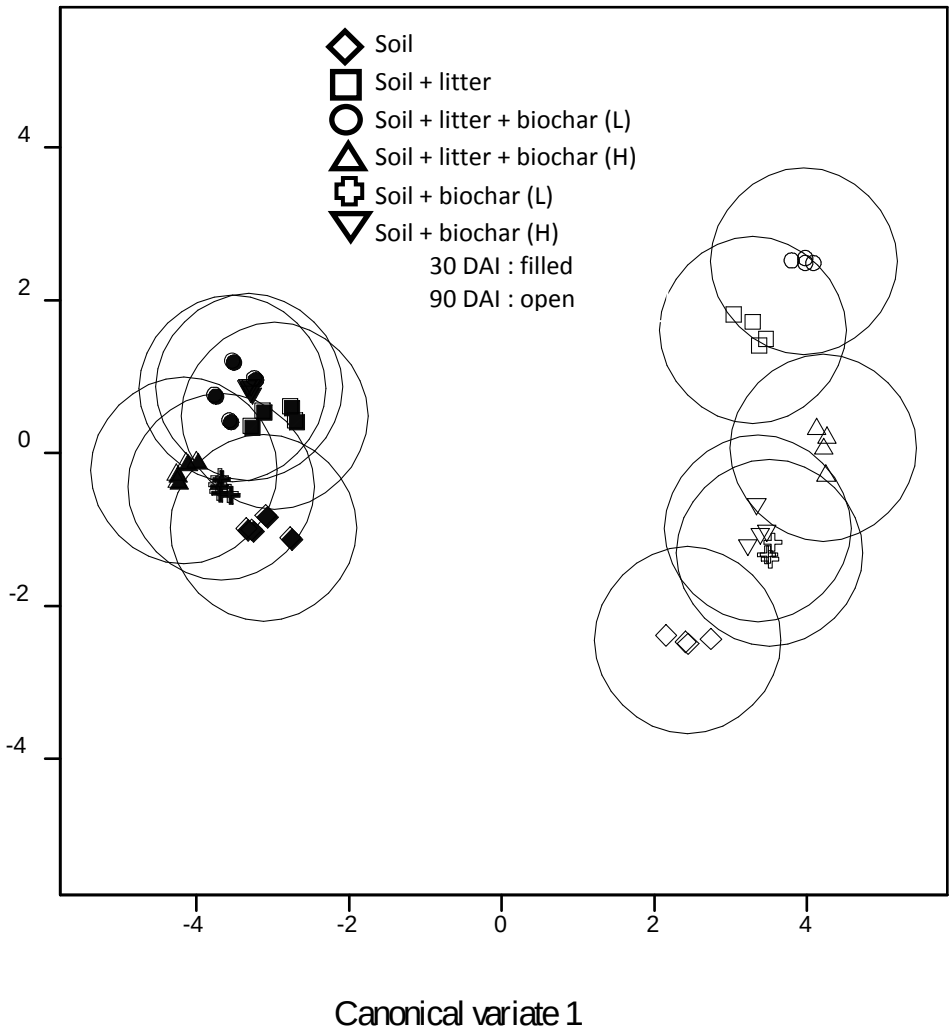


Figure 53. Canonical variate analysis of 50 PLFAs at two different days of incubation

After 90 days, there was no significant difference in community structure between unamended soil and soil amended with either rate of biochar. Similarly, biochar addition had no significant effect on microbial community structure when it was added to soil together with litter. However, there was a significant difference in community structure between the soil and soil plus litter control treatments.

MANOVA was investigated to determine which PLFAs contributed most to the separation of treatments when data from 30 and 90 days was combined (Fig 54). CV 1 contributed 99% of the variance and CV2 1% .

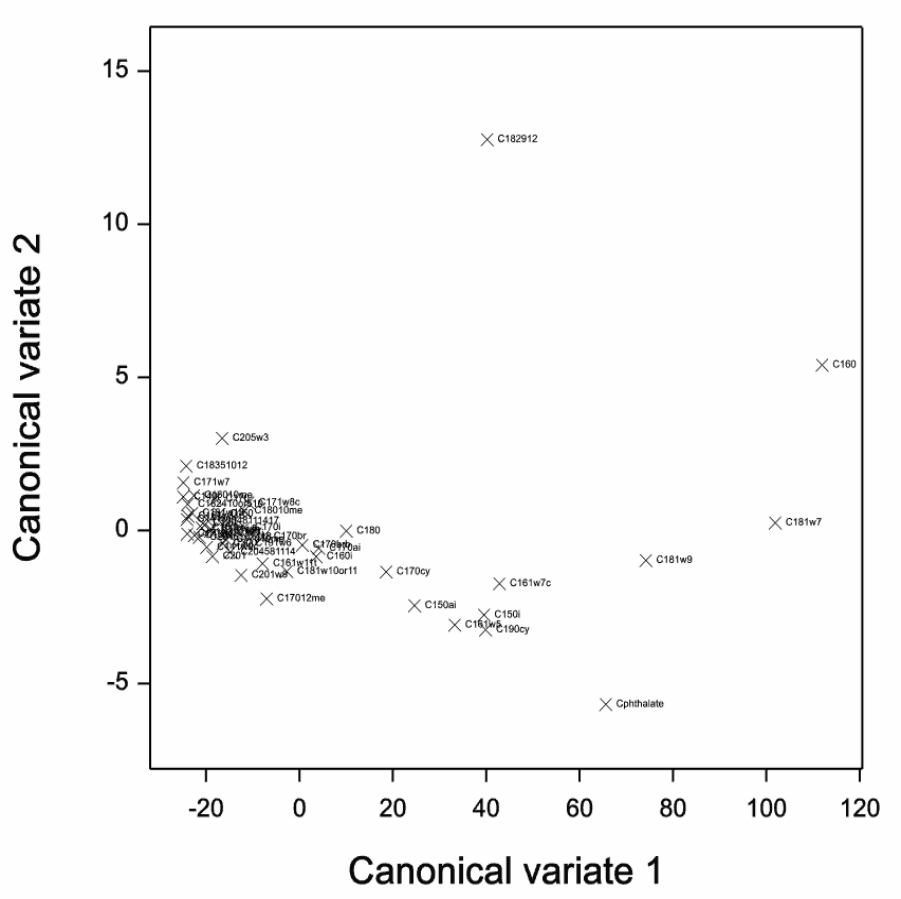


Figure 54. Canonical variate analysis of the distribution of PLFAs

It was found that most PLFAs clustered together along CV1 and CV2. Three of the most important PLFAs which separated treatments along CV1 were C16:0, Cphtalate and C18:1ω9 which cannot be assigned to specific groups. The remainder of the PLFAs which separated treatments along CV1 could be assigned to specific microbial groups. This included PLFAs characteristic of Gram negative bacteria (e.g C18:1ω7, C16:1ω7c, C19:0cy, C17:0cy), and PLFA C15:0ai and C15:0i which represent Gram positive bacteria. Separation along CV1 suggests that differences in the relative abundance of these PLFAs were important for changes in microbial community structure between 30 and 90 days. In contrast the fungal PLFA (C18:2(9,12) was separated from the other PLFAs along CV2, suggesting that changes in the relative abundance of this PLFA were largely associated with differences between treatments within each time point.

In order to understand in more detail the overall impact of biochar on soil microbial community structure, the treatments were clustered into 3 groups for CVA analysis; no biochar, biochar low rate (L), and biochar high rate (H). This analysis was to eliminate the effect of litter and solely examine the impact of biochar. The result is presented in Figure 55. The first canonical variate analysis axis (CV1) accounted for 75.45% of the variance and the second axis (CV2) which represented 24.55% of the variation (Figure 48). It was found that the treatments were separated significantly ($P<0.05$, 95% confidential interval) along CV1. This suggests that biochar applied at both low and high concentration did have a significant effect on microbial community structure.

Similarly, when treatments were blocked into with and without litter treatments (Figure 56), it was found that addition of litter significantly affected ($P<0.05$, 95% confidential interval) soil microbial community structure, with the treatments separated

along CV1 and CV2. Furthermore there was a strong time interaction, with 30 and 90 day time points for each treatment separated along CV1 and CV2. CV1 contributed 77.77% of the variation, whilst CV2 accounted for 21.38% of the variation.

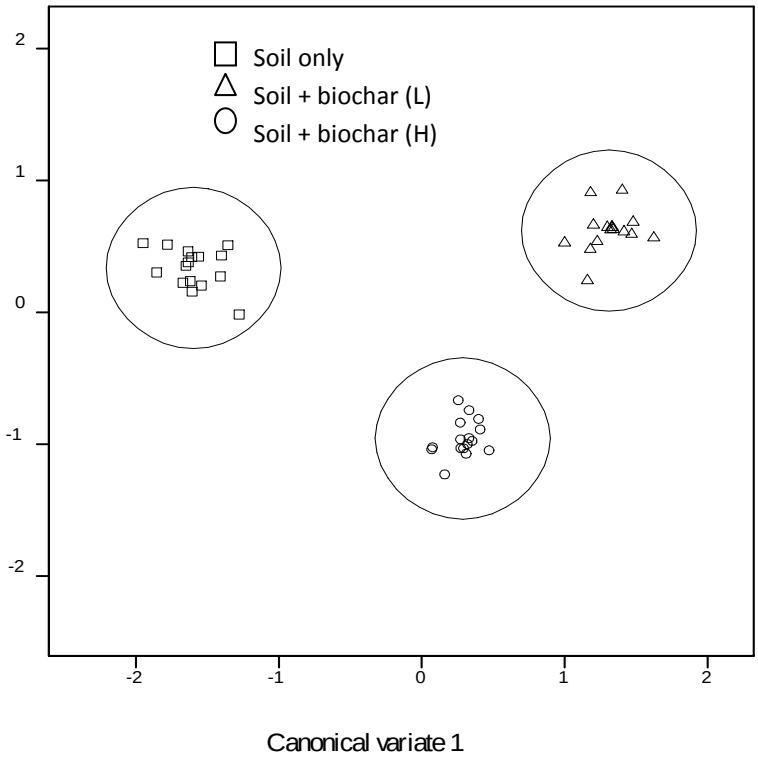


Figure 55. CVA of 50 PLFAs when the control and biochar treatments are blocked together across 30 and 90 day time points

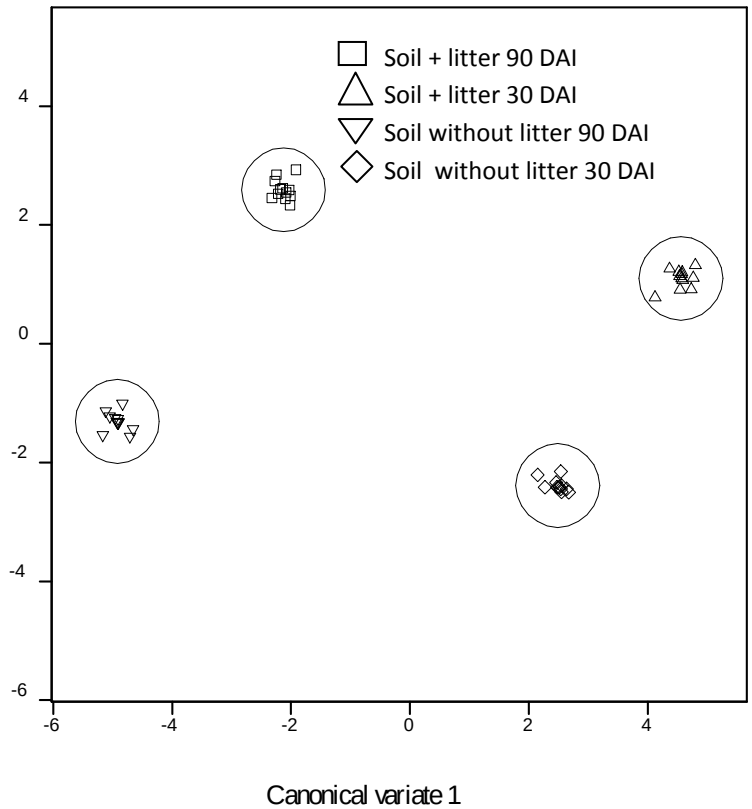


Figure 56. CVA of 50 PLFAs when treatments with and without biochar are blocked together

5.5 Discussion

5.5.1. Soil C and N mineralization

Data presented in this Chapter showed that addition of biochar to soil or soil amended with litter did not have a priming effect on CO_2 mineralisation, even at early time points of the experiment. While the low biochar amendment had no significant effect on CO_2 mineralisation, the high biochar concentration reduced CO_2 release by approximately 10% when applied to soil, and 20% when added to soil with litter.

Many studies have found that following addition of biochar to soil there is an immediate flush of CO_2 release. This effect could be the result of a range of processes, including the biodegradation biochar-C, the abiotic degradation of biochar-C and biochar induced priming of soil organic matter decomposition (Jones *et al.,* 2011). Several studies have attributed the mineralisation to biodegradation of a labile pool of C within the biochar (Kuzyakov *et al.,* 2009; Zimmerman *et al.,* 2011), although there is evidence that release of inorganic carbonate in the biochar can also contribute to this flush (Jones *et al.,* 2011).

In the case of switchgrass biochar, the flush largely appeared within the first 6 days following addition of biochar to soil, irrespective of the rate of biochar applied. The labile pool has been suggested to represent a dissolved organic pool (Jones *et al.,* 2011) while Smith *et al.,* (2010) suggested that the labile pool represents bio-oil condensates which become absorbed to biochar as it cools. However, in common with our results, other authors have found no short-term flush of mineralisation following application of some types of biochar to soil, and this has been attributed to differences in the composition of biochar as a result of different pyrolysis termperatures or starting material (Luo *et al.,* 2011; Zavalloni *et al.,* 2009).

Zimmerman *et al.,* (2011) reported that the size and duration of the early CO_2 flush following addition of biochar to soil decreased with increasing pyrolysis temperature, and was greater for grass relative to hardwood biochar.

Following the short-term flush of mineralisation on application of biochar to soil, there is concensus that biochar is stable over periods of up to thousands of years (Kuzyakov *et al.,* 2009). Furthermore there is considerable evidence that biochar inhibits mineralisation of soil organic carbon, which effectively represents a negative priming effect. The work presented in this Chapter demonstrates that the high biochar application rate, which was equivalent to 60 t biochar ha^{-1} repressed mineralisation of native organic matter by 10%, and of added litter by 20%. Negative priming has also been seen in a range of other studies, although the extent of the priming varies considerably. Cross and Sohi *et al.,* (2011) applied 10 different biochar types to three different types of soil and found that after 4 weeks, most soil/biochar mixtures had resulted in positive priming of net C mineralisation, although a small number of biochar types caused negative priming, particularly when added to a high organic matter content grassland soil. Similarly Spokas and Reicosky (2009) found that of 8 char types added to an agricultural soil, 3 chars had negative priming effects on C mineralisation while 5 had positive priming effects, while in a forest nursery soil, 3 biochar types increased C mineralisation, while 5 suppressed it.

When seen, the extent of negative priming of C mineralisation has been similar to that seen in the current study. For example, Zimmerman *et al.,* (2010) found that an application of 90 t ha^{-1} oak biochar to soil suppressed net C mineralisation by 25%, while a grass biochar applied at the same rate had a negative priming of 5% after 500 days. Similarly a sugarcane biochar applied at a rate of 80 t ha^{-1} reduced CO_2 mineralisation by 25% after 2 weeks application to soil.

Jones *et al.,* (2011) identified six mechanisms by which biochar induced negative priming could occur. These were: 1. Release of soluble phenolic materials from the biochar which bind to and inhibit degradative enzymes, 2. Sorption of extracellular enzymes onto the biochar resulting in their removal from sites of organic matter turnover, 3. Release of soluble C from the biochar which acts as a preferential C source for the soil microbiota, thereby reducing utilisation of soil organic C, 4. A shift in pH induced by application of biochar which alters microbial community structure, 5. Sorption of dissolved organic C into biochar, protecting it from decomposition and 6. Increased production of microbial biomass, so that C becomes stored in microbial tissues rather than becoming mineralised.

Currently there is little direct evidence to determine the relative importance of these mechanisms, although Jones *et al.,* (2011) suggested that the most likely mechanism was sorption of carbon into biomass and its subsequent protection from decay. In the current study PLFA analysis indicated that biochar addition to soil had no effect on microbial biomass, so mechanism 6 can be excluded as a factor contributing to the reduced C mineralisation rates following biochar application to the soil and the soil plus litter treatments.

The effect of biochar on nitrogen mineralisation has received less attention than C. Our work showed that biochar suppressed accumulation of NH_4^+ and NO_3^- when added to soil and soil amended with litter. Furthermore, the low biochar concentration significantly reduced soil NO_3 concentrations, even though it had no effect on C mineralisation. Similarly Zavalloni *et al.,* (2011) and Novak *et al.,* (2010) found that biochar application reduced soil NO_3 accumulation.

The effect of biochar on soil mineral N could occur via a number of mechanisms. Since N mineralisation is closely related to C mineralisation, the mechanisms proposed by Jones *et al.,* (2011), as described above, could all play a role in reducing N turnover. Since the reduction of NO_3 accumulation caused by biochar is not associated with increased amounts of NH_4, direct effects of biochar on bacterial and archaeal nitrifying bacteria can be excluded.

The effect of biochar application on soil microbial community are rarely informed and it's depend on biochar type (Steinbeiss *et al*, 2009), which was in some circumstances it can modify NO_2 oxidizer community and structure. However, the effect of biochar on mineral N is unlikely to reflect direct effect on NO_2 oxidizer. This was based on the evidence on this study that there were no significant effect of biochar application at different level to Gram negative PLFA (e.g. 16:1ω7c, 18:1ω7c). However there was a significant reduction in the amount of N mineral (NH_4^+ and NO_3^-), following soil incubation for 90 days. This reduction is more likely due to the absorption of mineral N to biochar surface instead of the alteration of Nitrate oxidiser microorganism community and structure.

As comparison to biochar application, liming and wood ash addition to soil under forest ecosystem for 5-6 years increased soil pH, along with the changes in Gram negative and positive bacteria, but it was not affected fungal community and structure (Frostegard *et al.,* 1993). In other experiment using ^{13}C urea, the application of labelled manure impacted NO_2 oxidizer. Their study revealed that the greatest amount of ^{13}C were found in the common fatty acid of 16:0, 16:1ω7c, 18:1ω7c, which was mainly detected in Nitrate oxidiser microorganism, such as *Nitrosomonas* and *Nitrobacter* cell membrane. These organisms can be classified as Gram negative bacteria.

5.5.2 Soil microbial community and structure

Generally, there is limited understanding about the impact of biochar on soil microbial community and structure (Lehman *et al.*, 2011). Biochar is likely to affect soil microbial communities in a number of direct and indirect ways. As discussed above, labile pools of carbon within biochar will act as a carbon source for the soil biota, and the short term flush of carbon seen following addition to soil is likely to be associated with an increase in soil biomass, and a shift in structure towards those organisms able to grow on the soluble components. Furthermore Biochar contains a range of compounds which may be inhibitory to some portions of the soil biota, such as dioxins, phenolics and polyaromatic hydrocarbons, and release of these compounds into the soil solution could cause toxicity to some components of the biota, resulting shifts in the microbial community (Zimmerman *et al.*, 2010). Additionally, alteration of soil pH following biochar application could result in shifts of microbial community structure, particularly in the ratio of fungi and bacteria, which is particularly sensitive to pH (Baath and Anderson, 2003). In the longer term, since biochar carbon is largely stable and very slowly degraded it is likely to support only small populations of microbes directly. Through it's sorption and protection of nutrients and dissolved organic C, biochar addition to soil would restrict carbon availability to the biota, and would be expected to generally reduce microbial populations. However, biochar alters soil properties, such as aggregation, water retention and aeration, all of which could alter microbial populations positively or negatively depending on circumstances, and these effects will have an influence on the size and composition of the soil biota.

Furthermore since biochar acts as a solid, porous substrate and is capable of sorbing water, nutrients and dissolved organic matter from the soil solution, it is possible that the surface of biochar could develop a biofilm, so that biochar particles become enriched in biota relative to the bulk soil. Over longer time periods, as biochar degrades, C protected from decay by sorption to biochar will become available to microbial decay and contribute to microbial biomass. Over longer time frames, biochar application may promote plant growth in some circumstances (Shackley *et al.,* 2012) and therefore biochar application may increase the input of root exudates, roots and aboveground biomass to soil, which would promote microbial biomass.

Data from the experiment reported in this Chapter found no overall effect of biochar on the size of the microbial population when biochar was added to soil or soil amended with litter. Jones *et al.,* (2011) suggested that one of the mechanisms by which biochar could reduce mineralisation of C could be the enhanced partitioning of C into anabolic versus catabolic metabolism. Data from this experiment does not support the existence of this processes as a means by which biochar reduces C mineralisation.

A range of studies have shown that ancient soils to which biochar was applied between 600 and 8000 years before present support bacterial communities which are distinct from unamended soil. However, there is far less known about the effect of fresh biochar applications on microbial communities. Lehmann *et al.,* (2011) suggested that application of biochar to soil would be expected to cause a shift in the fungus: bacteria ration, since fungi could be better placed to degrade lignin contained within biochar.

In the current study we found that fungal biomass was indeed promoted by biochar application soil amended with litter, although the effect was seen only after 30 days, and was not evident after 90 days. Similarly Steinbeiss *et al.,* (2009) found that addition of yeast biochar to soil promoted fungal biomass. Interestingly, in the current study, the shifts in fungal biomass were seen even at the low application of biochar, at which there was no effect on overall net C mineralisation. Application of biochar to soil in the absence of litter had no affect on fungal biomass. This suggests that the increased fungal biomass seen when biochar was applied to soil in the presence of litter was due to a specific interaction between biochar and litter.

Similarly, the amounts of a range of further unclassified and actinobacterial PLFAs were affected by biochar only when it was added to soil together with litter. This supports evidence in the literature that actinobacteria are promoted by addition of charred biomass to soil. Using culture-dependent approaches, O'Neill *et al.,* (2009) found that populations of actinobacteria were elavated in terra preta soils to which biochar had been added between 600 and 2300 years bp.

Similarly, Khodadad *et al.,* (2011) again using culture dependent approaches, found that numbers of culturable actinobacteria, and the diversity of actinobacteria, became enriched following addition of biochar to soil. Since actinobacteria are effective at degradation of complex aromatic materials such as lignin, this could suggest an involvement of these bacteria in degradation of biochar. However, in the current study, only one of three actinobacterial PLFAs was increased by addition of biochar, and overall there was no increase in actinobacterial biomass. Furthermore, the single actinobacterial PLFA which was increased in biochar amended soil did not remain elevated after 90 days. The data therefore only provides limited evidence for an actinobacterial response to the addition of biochar.

When PLFAs were used to derive a fingerprint of the microbial community, it was clear that time, litter and biochar all had a role in determining community structure. Treatment differences were greatest after 90 days, even though respiration was low relative to day 30 values. Interestingly, biochar had affected microbial community structure even when applied at a low rate to soil and to soil together with litter. These changes provide additional evidence to the mineral N data of biological effects of biochar application even at low doses, and in the absence of net effects on C mineralisation.

The changes in microbial community composition were associated in some, but not all cases, with shifts in pH induced by the application of biochar and litter. While application of litter significantly reduced soil pH, addition of biochar at a high concentration increased pH. Application of the low biochar level increased pH only when litter was applied together with biochar, in which case it acted to buffer the effect of litter addition, so that pH remained the same as unamended soil. Similarly, a number of other studies have found that biochar addition to soil increases soil pH by similar extents to those shown in the current work. Zhang *et al.*, (2012) found that an application rate of 10 and 40 t biochar ha^{-1} increased pH by approximately 0.2 and 0.4 units, similar to the pH increase of 0.3 units observed by Jones *et al.*, (2012) following a 50 t ha^{-1} application. Peng *et al.*, (2011) found that the extent to which biochar affects soil pH depended on both charring temperature and duration of charring, with greater increases on pH as charring duration increased.

The biological significance of the shift in pH induced by biochar is unclear. Jones *et al.,* (2011) suggested that it could cause changes in biodegradation processes and the structure of microbial communities, contributing to reduced decomposition rates. However, there is little evidence for such effects in the current study. The fact that the rate of low biochar application had no effect on pH suggests that the shifts in community composition caused by the addition of biochar cannot necessarily be attributed to a pH effect. Furthermore, bacterial rather than fungal biomass is expected to be promoted by increases to soil pH (Baath and Anderson, 2003), yet when biochar increased pH, it was associated with an increase in the fungus: bacteria ratio.

5.5.3. Conclusion

In this study, the addition of biochar high concentration (2%) had as a significant effect on cumulative CO_2 over 90 days of incubation. It can be concluded that biochar produce from willow in this experiment is inert, which is not readily available to soil microorganism. On the other hand, the application of litter was also significantly increase CO_2 production. Labile fraction in litter was preferable as energy source than biochar by soil microorganism. The interaction between litter and biochar application was rarely inform. Many publications were informed that the addition of biochar can increase CO_2 emission, which were contradict with the findings in this study. Thus, it can be claimed that the CO_2 emission under short rotation soil which were rich in SOM can be controlled by manipulating different rate of biochar application as a new alternative technique on managing carbon in biomass energy crop.

A decreasing in soil respiration following high rate application of biochar indicate that soil microbial decomposition process remarkably slowed down after 90 days of incubation as those application also significantly increased soil pH. Some soil

microorganism which may be affected by the changes of soil pH will not be able to adapt with the new condition, resulted in lowering their activity and reducing on cumulative CO_2 respired.

Similar to C mineralization, biochar application in low (0.5%) and high rate (2%) solely or in a combination with litter significantly reduced the amount of N mineral, particularly nitrate (NO_3^-) following 30 and 90 days incubation, whilst the significant effects of the amount of ammonium (NH_4^+) only detected after 90 days of incubation. The raising of ammonium quantity under the application of litter only or in the combination of biochar during 30 days of incubation was suspected a short term priming effect of soil microbial activity, which then reduced substantially after 90 days of incubation. The reduction of N mineral under biochar treatment was due to the ability of biochar to absorb soil cations including ammonium (NH_4^+) and nitrate (NO_3^-). The consequences of the high application of biochar was corresponded to the further absorption of N mineral, so it was a crucial deliberation on maintaining a certain quantity of biochar for being applied in soil to secure a sufficient amount N mineral for soil microorganism.

PLFA profile provided a sensitive and meaningful measure of microbial community composition of short rotation coppice soil under the application of biochar at different rate. In this present study, fungal PLFA biomass (C18:2(9,12)) increased following the treatment of biochar and litter relative to litter solely or non-amended litter treatment at 30 and 90 days after incubation. The effect of litter and biochar addition on Gram (+) bacterial/Actinobacteria PLFA (C17:0(10Me)) only detected at 30 days of incubation.

It can be concluded that biochar only alter a specific microbial community structure, particularly fungus. A combination treatment of higher rate of biochar application and litter can exceed a greater amount of fungal biomass. It is recommended to identify a specific fungal taxa which may influenced by the addition of biochar under soil from short rotation coppice plantation.

The use of multivariate analysis (CVA) allowed us to determine PLFA pattern in term of the effect of treatment at 30 and 90 days after incubation. Further separation in CVA was observed at 90 days after incubation relative to 30 days, which were explained of 89.11% variation. This meant the impact of the treatment were substantially much stronger following 90 days of incubation. Moreover, one of the PLFA selected for important discriminator of the effect of treatment, which were contributed to the separation was found in fungal PLFAs (C18:2(9,12)). Further analyses using blocking treatment showed that the elimination of the effect of litter and examine the impact of biochar solely or vice versa resulted in a significant separation of the each treatment.

Chapter 6

General Conclusions and Future work

6.1. General conclusions

6.1.1. Overall carbon balance under Short Rotation Coppice (SRC) bioenergy crops

Land uses investigated were 14 year plots of short rotation coppice (SRC) plantation and set aside which were converted from arable cropping. These land uses were compared to the adjacent native woodland. The aims of the experiment in Chapter 2 and 3 were to examine the impact of land use change on overall C balance in the soil. The work showed that land use change significantly affected several soil quality parameters, including soil C and N, pH, microbial biomass C and N, and the amount and the quality of free and intra aggregate Light Fraction Organic Matter. Positive impacts of land use change from arable cropping to alternative agricultural systems on soil C stocks in this current study was supported the previous study by Gou and Gifford, (2002).

In a meta-analysis of the changes in soil carbon stocks upon land use change experienced world-wide, Guo and Gifford (2002) found that two of the three largest positive changes occurred when cropland was converted to either secondary forest, or plantations. However, the impact of SRC crop on soil C stock have still received little attention (Arevalo *et al.,* 2007). Most studies of the effect of biomass crops on soil C have limited duration, sometimes unclear understanding of historical land management, and a focus on the top layer of soil, creating difficulties for making a comparison. In this current study the contribution of litter and root decomposition

under SRC crop was able to improve total C storage, particularly in upper layer (0-30 cm).

In terms of SRC, many studies have monitored growth the aboveground biomass and much less is known of changes belowground (Hansen, 1993). Generally, belowground studies on soil carbon storage under SRC have been on young plantations (2-3 times coppicing), and long term effects of SRC on overall carbon storage have rarely been made.

14 years of SRC crop management under conventional management practices led to significantly improved soil quality by increasing carbon stocks in upper soil layer (0-30 cm) to within 60 to 80% of levels found in native vegetation (woodland) and 20-30% greater than carbon stocks under arable and/or set aside. The annual C accumulation has been estimated at approximately 0.5 t ha^{-1} $year^{-1}$ under willow crops (Borjesson, 1999), which was lower by two to four times than the annual C accumulation under SRC willow and poplar in this study, which was 1.29 and 2.28 t ha^{-1} $year^{-1}$, respectively.

C storage in the lower layer was less than 20% of levels in the upper layer. However, there were no differences on carbon sequestered in soil in different land use types in the lower layer. This may due to the lower SOM input and slow mineralisation process. Sub horizon SOM is a key component of terrestrial C but poorly understood and has been largely neglected to date (Rumpel and Kogel-Knabner, 2011)

Improvements of soil C stock under SRC crop is due to its tolerance and adaptability to different soil properties, rapid biomass accumulation and large production of litter. This means that litter deposition and decomposition on the soil surface before winter time should contribute substantially to soil C sequestration in

the upper layer as it is expected that short rotation woody crops would be harvested in these periods (Bransby *et al.,* 1998).

The aboveground biomass accumulation of SRC were estimated to be at the level of and 9 t DM ha^{-1} $year^{-1}$ and 7.6 t DM ha^{-1} $year^{-1}$ for willow and poplar, respectively. The current study showed that SRC willow accumulated a greater annual aboveground biomass than poplar. Similarly this finding was previously reported by Aylott *et al.,* (2008). In comparison, Brandao *et al.,* (2011) predicted that the average mature willow biomass in the UK is 9.5 t DM ha^{-1} $year^{-1}$. However, results from this work on SRC biomass estimation using an existing equation by Arevalo *et al.,* (2007) and Guidi *et al.,* (2008) suggested that these equations overestimated biomass production. This indicated that developing a site specific site formula for UK conditions to predict carbon stocks of SRC above ground is essential.

One of the key findings in this current work has been that free Light Fraction Organic Matter (fLFOM) acts as an early indicator of long term change in total organic matter and can be used to detect the effect of management changes on SOM quality. This fact disagrees with Sohi *et al.,* (2010) who proposed that intra aggregate LFOM can be a better indicator of changes of land use or management practices than free LFOM since it has been stabilized for longer period in soil. The free and total LFOM under SRC were twice those values for set aside and almost three times higher than arable soil.

To give an overall estimate of C balance under SRC crops an estimation of annual accumulation rate of carbon in above ground biomass, litter accumulation, LFOM and microbial biomass were calculated and compared to native woodland (Table 48).

Table 48. *The comparison of annual C accumulation under SRC*

Landuse	Willow (14 year)	Poplar (14 year)
Above ground C pools:	(t C ha^{-1} $year^{-1}$)	(t C ha^{-1} $year^{-1}$)
a.Above ground biomass (C=50%)	6.30	4.68
b.Surface litter (C=50%)	0.12	0.18
Total above ground C	3.51	0.80
Belowground C pools:		
c.LFOM (0-30 cm)	0.30	0.21
d.LFOM (30-60 cm)	0.03	0.04
e.Microbial biomass C (0-30 cm)	0.05	0.05
f.Microbial biomass C (30-60 cm)	0.01	0.008
Total below ground C (c+d+e+f) (0-60 cm)	0.39	0.308
Total soil C stock (0-60 cm)	3.39	0.62

These rates of accumulation actually seem to under estimated compared with values measured by Hansen, (1993) when he compared amounts of soil organic carbon under poplar crops of various ages and adjacent areas under arable and mown grass crops, throughout Minnesota and Wisconsin in the USA. Here he calculated annualised accumulation rates in the soil (including litter) of 1.63 t ha^{-1} $year^{-1}$ ($\pm$ 0.16) and above ground biomass of 3.4 t ha^{-1} $year^{-1}$, which were higher more than twice to total soil C under poplar in this study (0.62 t ha^{-1} $year^{-1}$) (Table 48). Meanwhile, the annual poplar above ground C sequestration was 4.68 t C ha^{-1} $year^{-1}$.

In this current work annual soil C stock accumulation under willow was 3.39 t C ha^{-1} $year^{-1}$ and 6.30 t C ha^{-1} $year^{-1}$ in above ground biomass excluding the surface litter, which was higher than the annual carbon accumulation in poplar (Table 48). For optimizing carbon stocks in terrestrial systems, combustion of biomass to biochar provides a means of long term sequestration. Even though SRC biomass would eventually be burnt through pyrolysis and re-cycled to the atmosphere as CO_2, there will be a permanent standing biomass containing captured atmospheric C.

Differentiation of SOM pool quality was achieved by examining the FTIR signature from soil and LFOM samples, in order identify functional groups of

organic compounds, and this approach was supported with more detailed characterisation of the dissolved soil organic matter extract using FTICR. The result revealed that the quality of free and intra aggregate LFOM under various land use were different. It also filled a gap on information about the amount and quality of LFOM under SRC crops.

The free LFOM signature mainly consisted of C-O stretching of polysaccharide and C-H deformation or C-O stretch or C=O conjugated ketones and quinines, whilst intra aggregate LFOM was dominated by carbonyl/carboxyl stretches and stretch of phenols or amines. This supported evidence that intra aggregate LFOM had higher aromatic, phenolic and carboxyl peaks than fPOM (free LFOM) (Sohi *et al.*, 2001) using ^{13}C NMR spectroscopy. The combination of statistical analysis using ANOVA on selected functional groups along with the application of multivariate analysis was effective in differentiating the effect of land use changes on SOM quality.

New evidence of the detailed chemical composition of dissolved organic matter and its structure from two distinct land uses (arable vs woodland) using FTICR were reported in this current study. Chemical composition obtained using FT-ICR-MS is suitable to monitor differences in the structure of SOM. Short chain lengths of carbon were observed in arable soil, whilst the sample from woodland had longer carbon chains and greater DBE/C value. In this current work, the average value of O/C in arable (0.13-0.32) and woodland (0.12-0.25) were lower than those values for O/C in marine ecosystems, which were between 0.37-0.42 (Koch *et al.*, 2005). Moreover, the average value of H/C ratio in this study (1.36-1.64) and the DBE value (2-25) were recorded to be greater than H/C and DBE/C value in six marine samples, which were reported at 1.05–1.29 and 9.3 to 11.4, respectively (Koch *et al.*, 2005). Another study of Chesapeake Bay river DOM (e.g Dismal swamp water,

Great bridge water, Town point water, Chesapeake Bay Bridge water, Offshore coastal water) showed that the average O/C, H/C and DBE were 0.33 to 0.39, 1.25 to 1.43 and 7.2 to 9.6, respectively (Sleighter and Hatcher, 2008).

The FTICR technique is a powerful tool for examining terrestrial DOM under contrasting land uses. Van Krevelen diagram showed the distinct distribution of each compound class when plotted in negative or positive ion modes. A shift from a simple hydrocarbons in arable to more complex and larger compound classes was clearly observed. Koch and Dittmar, (2006) noted that the increasing number of carbon atoms within compounds leads to higher DBE value and decreasing in the presence of hydrogen lower the value. Larger molecules potentially had more double bonds and ring structures.

6.1.2 C sequestered during SRC biochar production

The potential to sequester C in soil as biochar was the focus of Chapter 4. One option for generating energy from SRC biomass is partial or controlled pyrolysis to charcoal which leaves approximately 25% of the initial biomass as carbon, which is stable for hundreds to thousands of years in soil (Lehman, 2006). The aim of Chapter 4 was to investigate how temperature and time of pyrolysis affect overall carbon balance of biochar derived from willow SRC biomass. This involved carbon balance analysis, and analysis of the changes in the quality of biochar using FTIR and SEM along with nutrient recovery analysis of the final product.

The yield of biochar decreased considerably with pyrolysis temperature in the range of 440°C to 530°C and a holding time of 15 minutes. Similarly, a reduction in biochar yield was observed when pyrolysis was conducted at 470°C but at increasing time of pyrolysis between 5, 10 and 15 minutes. The carbon content of biochar on the other hand followed the opposite trend to biochar yield, increasing considerably with

pyrolysis time. This supported the evidence from the study by Masek *et al.*, (2013), who studied biochar production at 350° to 550°C using pine wood chips at a laboratory scale.

The findings were finally used in calculating the carbon balance using a life cycle assessment (LCA). It was shown that increasing both the pyrolysis temperature from 440°C to 530°C, and the reaction time from 5 to 15 minutes significantly influenced the yield and chemical composition of biochar. A minimum temperature of >440 °C is needed to produce a sufficiently carbonized biochar. The lowest biochar yield was found at 530°C, whereas the biochar produced at 470°C is preferred to 530°C, since similar morphologies are produced but at a considerably higher biochar yield.

Applying a direct injection of steam in the reactor appears to about double the amount of biochar formed, whilst the BET-value also nearly doubled. The carbon balance analysis demonstrated that this 470 °C-biochar had the highest carbon sequestration capacity. Several nutrients were enriched in the final product, for example K and P.

6.1.3 SRC biochar affects SOM decomposition / mineralization and soil microbial community and structure

The relationship between biochar and SOM mineralization and decomposition is still not well documented. Many studies show that the addition of biochar can have a priming effect on SOM (Wardle *et al.*, 2008; Novak *et al.*, 2010), but some studies show the reverse effect (Zimmerman, 2011). The large area surface area of biochar may cause positive and negative priming effects (Luo *et al.*, 2011). The finding of this current student showed that negative priming occurred following application of biochar at a high concentration (2%) in the presence and the absence of litter. A positive priming effect may be due to the increase in microbial biomass and

extracelluar enzymes and labile organic material remaining in biochar (Hamer *et al.*, 2004), whereas negative priming may be caused by biochar toxicity (Verheijen *et al.*, 2009).

Besides reducing CO_2 production, the biochar addition had an impact on the N cycle. Lower amounts of NO_3-N and NH_4-N present in soil treated with biochar after 30 and 90 days after incubation may be due to the assimilation of these compounds or immobilisation by soil microbes. Another possibility is binding of NH_4^+ to the negative charge of the biochar surface (Novak *et al.*, 2010). The reductions of soil N after biochar application have been reported in many soils (Lehman *et al.*, 2003; Steiner *et al.*, 2007).

6.2. Future work

The concept of integrated approaches to carbon sequestration in this current study will be used and implemented in a tropical ecosystem. As Indonesia is now the leading supplier for the global market of palm oil which there is increasing planting of this crop in tropical forests, killing endangered species, uprooting local communities, and contributing to the release of climate-warming gases. A new understanding of the impact of conversion of forest to oil palm on the existing SOM and soil quality needs to be addressed. Much oil plantation in South East Asia has a low soil pH, and low content of N, P and K (Mutert, 1999).

No information on the quantity and quality of SOM under palm oil plantation is available, and the impact of change to soil C on the sustainability of palm oil production is uncertain. Furthermore understanding of changes to soil microbial community structure after forest conversion into palm oil has not been reported yet. The conversion of palm fruit bunch following removal of oil, to biochar and its incorporation into soil could benefit soil quality and C storage in these systems.

Appendix 1. Statistical analyses

a. Above gound biomass (Landuse)

Analysis of variance

Variate: Aboveground_biomass

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Landuse	2		158218.	79109.	67.52	<.001
Residual	23	(1)	26949.	1172.		
Total	25	(1)	179837.			

Message: the following units have large residuals.

units 17 71.974 s.e. 31.593

Tables of effects, contrasts and residuals

Variate: Aboveground_biomass

Landuse effects, e.s.e. 11.4100, rep. 9

Landuse	Poplar	Willow	Woodland
	-34.465	-71.644	106.109

Units residuals, s.e. 31.5930, rep. 1

units	1	2	3	4	5	6	7
	61.744	10.389	-22.186	-21.987	-11.581	-54.836	41.604
units	8	9	10	11	12	13	14
	3.626	-6.777	-57.382	-39.267	29.716	-8.494	-54.161
units	15	16	17	18	19	20	21
	43.221	-4.233	71.974	18.627	-9.155	15.076	4.018
units	22	23	24	25	26	27	
	-3.533	-19.608	3.694	0.000	-5.312	14.822	

Tables of means

Variate: Aboveground_biomass

Grand mean 109.496

Landuse	Poplar	Willow	Woodland
	75.031	37.851	215.605

Standard errors of means

Table	Landuse
rep.	9
d.f.	23
e.s.e.	11.4100

(Not adjusted for missing values)

Standard errors of differences of means

Table	Landuse
rep.	9
d.f.	23
s.e.d.	16.1362

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Landuse
rep.	9
d.f.	23
l.s.d.	33.3803

(Not adjusted for missing values)

Combined estimates

No combined estimates (design orthogonal).

Estimated stratum variances

Variate: Aboveground_biomass

variance	effective d.f.	variance component
1171.7008	23.000	1171.7008

Stratum standard errors and coefficients of variation

Variate: Aboveground_biomass

d.f.	s.e.	cv%
23	34.2301	31.3

Missing values

Variate: Aboveground_biomass

Unit	estimate
25	37.851

Max. no. iterations 2

```
52  DELETE [REDEFINE=yes] _treatform,_nt
53  AKEEP [TREAT=_treatform]
54  FCLASSIFICATION [FACT=32; NTERMS=_nt] #_treatform
55  DELETE [REDEFINE=yes] _sedm[1...#_nt]
56  AKEEP [FACT=32] #_treatform; SEDMEANS=_sedm[1...#_nt]
57  PRINT [RLPRINT=lab,int; CLPRINT=int; IPRINT=*] _sedm[1...#_nt]
```

Landuse Poplar	1	*		
Landuse Willow	2	16.14	*	
Landuse Woodland	3	16.14	16.14	*
		1	2	3

b. Above ground (Genotype)

Analysis of variance

Variate: Aboveground_biomass

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Genotype	6		169337.7	28223.0	33.19	<.001
Residual	19	(1)	16158.4	850.4		
Total	25	(1)	179837.2			

Message: the following units have large residuals.

units 1 61.744 approx. s.e. 24.463

units 6 -54.836 approx. s.e. 24.463

Tables of effects, contrasts and residuals

Variate: Aboveground_biomass

Genotype effects, e.s.e. 9.7208 - 16.8369

Genotype	Beaupre (Poplar)	Beech + Oak (Woodland)	Germany (Willow)
	-57.751	106.193	-73.824
rep.	3	9	3
Genotype	Ghoy (Poplar)	Jorrun (Willow)	Q83 (Willow)
	-59.315	-77.561	-64.050
rep.	3	3	3
Genotype	Tricoble (Poplar)		
	13.923		
rep.	3		

Units residuals, s.e. 24.4634, rep. 1

units	1	2	3	4	5	6	7
	61.744	10.389	-22.186	-21.987	-11.581	-54.836	41.604
units	8	9	10	11	12	13	14
	3.626	-6.777	-34.012	-14.333	-18.588	14.876	-29.227
units	15	16	17	18	19	20	21
	-5.083	19.137	23.670	43.561	-3.155	17.339	-3.493
units	22	23	24	25	26	27	
	2.467	-17.345	-3.817	0.005	0.688	7.311	

Tables of means

Variate: Aboveground_biomass

Grand mean 109.412

Genotype	Beaupre (Poplar)	Beech + Oak (Woodland)	Germany (Willow)
	51.661	215.605	35.588
rep.	3	9	3
Genotype	Ghoy (Poplar)	Jorrun (Willow)	Q83 (Willow)
	50.097	31.851	45.362
rep.	3	3	3
Genotype	Tricoble (Poplar)		
	123.335		
rep.	3		

Standard errors of means

Table	Genotype	
rep.	unequal	
d.f.	19	
e.s.e.	16.8369	min.rep
	9.7208	max.rep

(Not adjusted for missing values)

Standard errors of differences of means

Table	Genotype	
rep.	unequal	
d.f.	19	
s.e.d.	23.8109	min.rep
	19.4415	max-min
	13.7472X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Genotype	
rep.	unequal	
d.f.	19	
l.s.d.	49.8368	min.rep
	40.6916	max-min
	28.7733X	max.rep

(No comparisons in categories where l.s.d. marked with an X)
(Not adjusted for missing values)

Combined estimates

No combined estimates (design orthogonal).

Estimated stratum variances

Variate: Aboveground_biomass

variance	effective d.f.	variance component
850.4404	19.000	850.4404

Stratum standard errors and coefficients of variation

Variate: Aboveground_biomass

d.f.	s.e.	cv%
19	29.1623	26.7

Missing values

Variate: Aboveground_biomass

Unit	estimate
25	35.593

Max. no. iterations 4

```
65  DELETE [REDEFINE=yes] _treatform,_nt
66  AKEEP [TREAT=_treatform]
67  FCLASSIFICATION [FACT=32; NTERMS=_nt] #_treatform
68  DELETE [REDEFINE=yes] _sedm[1...#_nt]
69  AKEEP [FACT=32] #_treatform; SEDMEANS=_sedm[1...#_nt]
70  PRINT [RLPRINT=lab,int; CLPRINT=int; IPRINT=*] _sedm[1...#_nt]
```

Genotype Beaupre (Poplar)	1	*		
Genotype Beech + Oak (Woodland)	2	19.44	*	
Genotype Germany (Willow)	3	23.81	19.44	*
Genotype Ghoy (Poplar)	4	23.81	19.44	23.81
Genotype Jorrun (Willow)	5	23.81	19.44	23.81
Genotype Q83 (Willow)	6	23.81	19.44	23.81
Genotype Tricoble (Poplar)	7	23.81	19.44	23.81
		1	2	3

Genotype Ghoy (Poplar)	4	*		
Genotype Jorrun (Willow)	5	23.81	*	
Genotype Q83 (Willow)	6	23.81	23.81	*
Genotype Tricoble (Poplar)	7	23.81	23.81	23.81
		4	5	6

Genotype Tricoble (Poplar)	7	*
	7	

c. Surface litter (Landuse)

Analysis of variance

Variate: Litter_accumulation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Landuse	2	271124.	135562.	25.73	<.001
Residual	24	126431.	5268.		
Total	26	397554.			

Tables of effects, contrasts and residuals

Variate: Litter_accumulation

Landuse effects, e.s.e. 24.1935, rep. 9

Landuse	Poplar	Willow	Woodland
	19.244	-131.214	111.970

Units residuals, s.e. 68.4296, rep. 1

units	1	2	3	4	5	6	7
	3.157	-21.146	68.494	79.477	-28.682	-84.845	-63.194
units	8	9	10	11	12	13	14
	37.182	9.556	-52.734	25.082	21.866	-30.213	100.247
units	15	16	17	18	19	20	21
	58.308	-130.676	11.717	-3.597	-80.584	99.700	19.117
units	22	23	24	25	26	27	
	-132.990	25.451	116.340	34.824	38.972	-120.831	

Tables of means

Variate: Litter_accumulation

Grand mean 492.492

Landuse	Poplar	Willow	Woodland
	511.736	361.278	604.463

Standard errors of means

Table	Landuse
rep.	9
d.f.	24
e.s.e.	24.1935

Standard errors of differences of means

Table	Landuse
rep.	9
d.f.	24
s.e.d.	34.2148

Least significant differences of means (5% level)

Table	Landuse
rep.	9
d.f.	24
l.s.d.	70.6159

Combined estimates

No combined estimates (design orthogonal).

Estimated stratum variances

Variate: Litter_accumulation

variance	effective d.f.	variance component
5267.9395	24.000	5267.9395

Stratum standard errors and coefficients of variation

Variate: Litter_accumulation

d.f.	s.e.	cv%
24	72.5806	14.7

```
DELETE [REDEFINE=yes] _treatform,_nt
AKEEP [TREAT=_treatform]
FCLASSIFICATION [FACT=32; NTERMS=_nt] #_treatform
DELETE [REDEFINE=yes] _sedm[1...#_nt]
AKEEP [FACT=32] #_treatform; SEDMEANS=_sedm[1...#_nt]
PRINT [RLPRINT=lab,int; CLPRINT=int; IPRINT=*] _sedm[1...#_nt]
```

Landuse Poplar	1	*		
Landuse Willow	2	34.21	*	
Landuse Woodland	3	34.21	34.21	*
		1	2	3

d. Surface litter (Genotype)

Analysis of variance

Variate: Litter_accumulation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	6	312853.	52142.	12.31	<.001
Residual	20	84701.	4235.		
Total	26	397554.			

Message: the following units have large residuals.

units 27 -125.706 approx. s.e. 56.010

Tables of effects, contrasts and residuals

Variate: Litter_accumulation

Genotype effects, e.s.e. 21.6924 - 37.5723

Genotype	Beaupre (Poplar)	Beech + Oak (Woodland)	Germany (Willow)
	-51.964	111.970	-77.889
rep.	3	9	3
Genotype	Ghoy (Poplar)	Jorrun (Willow)	Q83 (Willow)
	59.821	-189.415	-126.339
rep.	3	3	3
Genotype	Tricoble (Poplar)		
	49.874		
rep.	3		

Units residuals, s.e. 56.0095, rep. 1

units	1	2	3	4	5	6	7
	3.157	-21.146	68.494	79.477	-28.682	-84.845	-63.194
units	8	9	10	11	12	13	14
	37.182	9.556	18.474	-15.496	-8.765	40.995	59.670
units	15	16	17	18	19	20	21
	27.678	-59.469	-18.913	-44.174	-22.383	46.375	14.242
units	22	23	24	25	26	27	
	-74.790	-27.874	111.464	-18.501	97.173	-125.706	

Tables of means

Variate: Litter_accumulation

Grand mean 492.492

Genotype	Beaupre (Poplar)	Beech + Oak (Woodland)	Germany (Willow)
	440.528	604.463	414.603
rep.	3	9	3
Genotype	Ghoy (Poplar)	Jorrun (Willow)	Q83 (Willow)
	552.313	303.078	366.153
rep.	3	3	3
Genotype	Tricoble (Poplar)		
	542.366		
rep.	3		

Standard errors of means

TableGenotype

rep.	unequal	
d.f.	20	
e.s.e.	37.5723	min.rep
	21.6924	max.rep

Standard errors of differences of means

Table	Genotype	
rep.	unequal	
d.f.	20	
s.e.d.	53.1353	min.rep
	43.3848	max-min
	30.6777X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Genotype	
rep.	unequal	
d.f.	20	
l.s.d.	110.8383	min.rep
	90.4991	max-min
	63.9925X	max.rep

(No comparisons in categories where l.s.d. marked with an X)

Combined estimates

No combined estimates (design orthogonal).

Estimated stratum variances

Variate: Litter_accumulation

variance	effective d.f.	variance component
4235.0383	20.000	4235.0383

Stratum standard errors and coefficients of variation

Variate: Litter_accumulation

d.f.	s.e.	cv%
20	65.0772	13.2

```
DELETE [REDEFINE=yes] _treatform,_nt
AKEEP [TREAT=_treatform]
FCLASSIFICATION [FACT=32; NTERMS=_nt] #_treatform
DELETE [REDEFINE=yes] _sedm[1...#_nt]
AKEEP [FACT=32] #_treatform; SEDMEANS=_sedm[1...#_nt]
PRINT [RLPRINT=lab,int; CLPRINT=int; IPRINT=*] _sedm[1...#_nt]
```

Genotype Beaupre (Poplar)	1	*		
Genotype Beech + Oak (Woodland)	2	43.38	*	
Genotype Germany (Willow)	3	53.14	43.38	*
Genotype Ghoy (Poplar)	4	53.14	43.38	53.14
Genotype Jorrun (Willow)	5	53.14	43.38	53.14
Genotype Q83 (Willow)	6	53.14	43.38	53.14
Genotype Tricoble (Poplar)	7	53.14	43.38	53.14
		1	2	3

Genotype Ghoy (Poplar)	4	*		
Genotype Jorrun (Willow)	5	53.14	*	
Genotype Q83 (Willow)	6	53.14	53.14	*
Genotype Tricoble (Poplar)	7	53.14	53.14	53.14
		4	5	6

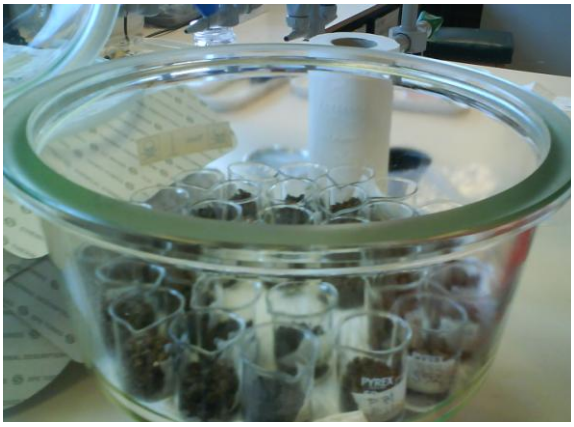
Genotype Tricoble (Poplar)	7	*
		7

Appendix 2. Field and Laboratory activity

a. Biomass estimation and litter collection

b. Soil sampling and collection

c. Soil water tension determination using filter paper technique



d. Soil microbial N using fumigation technique

e. Soil microbial C determination using TOC machine

f. Unprotected SOM extraction (free and intra LFOM) using density frationation (NaI solution)

g. LFOM quality assesment using FTIR machine

h. Extracting soluble organic matter for FTICR analysis

i. Bioconversion unit for producing Biochar from willow biomass

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