The Biochemical Impact of Biochar in Soil Environments

A thesis submitted to Newcastle University in partial fulfilment of the requirement for the degree of Doctor of Philosophy (Integrated) in the Faculty of Science, Agriculture and Engineering

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Declaration

Except where acknowledged, the content of this thesis is the work of the author. No part of the material presented has been submitted previously for a degree or other qualifications in this, or any other University anywhere.

Sani Mu'azu Makarfi

(July, 2014)

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Glossary

ANOVA	Analysis of variance
APS	Ammonium per sulphate
ATR	Attenuated total reflectance
BD	Bulk density
BR	Basal respiration
CEC	Cation exchange capacity
DEA	Denitrification enzyme activity
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
EDTA	Ethylene diammine tetra-acetic acid
ess	Edinburgh biochar produced from ≤10mm Sitka spruce wood chips
ess400	Edinburgh biochar produced at 400^{o}C
ess600	Edinburgh biochar produced at 600^{o}C
ess800	Edinburgh biochar produced at 800^{o}C
FAO	Food and agriculture organisation
FC	Fixed carbon
FTIR	Fourier-transform infrared
GC-MS	Gas chromatography mass spectrometry
GHG	Greenhouse gas
HTT	Highest temperature of treatment
ibc	Interreg biochar
IPCC	Intergovernmental panel on climate change
Kbc800	Previous project biochar produced at 800^{o}C
NH_4-OAc	Ammonium acetate
OM	Organic matter
PAH	Polycyclic aromatic hydrocarbons

PCR	Polymerase chain reaction
QMS	Quadrupole mass spectrometry
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SA	Surface acidity
SB	Surface basicity
SEM	Scanning electron microscopy
SE	Standard error
ss	Lancashire biochar produced from ≤2mm saw dust of Sitka spruce wood
ss400	Lancashire biochar produced at 400^{o}C
ss600	Lancashire biochar produced at 600^{o}C
ss800	Lancashire biochar produced at 800^{o}C
TAE	Tris-acetate EDTA
TC	Total carbon
TCE	Trichloroethylene
TEMED	N, N, N', N'- tetramethylenediamine
TG	Thermogravimetry
TG-DSC	Thermogravimetry differential scanning calorimetry
TG-DSC-QMS	Thermogravimetry differential scanning calorimetry and quadrupole mass spectrometry
TGGE	Temperature gradient gel electrophoresis
T_{max}	Maximum decomposition temperature in the course of pyrolysis
TOC	Total organic carbon
UK	United Kingdom
US	United States
UV	Ultraviolet
VM	Volatile matter
WHC	Water holding capacity

Abstract

Biochar, a product of thermochemical conversion of biomass, is a way to sequester carbon and mitigate climate change, improve soil agronomic properties and enhance crop production. However, such uses can only be valuable if the biochar does not negatively impact on normal soil microbially mediated processes that are important to soil health. The physical and biochemical characterization of biochar products is therefore important. One of the two central factors that affect the physicochemical properties of biochar is the production temperature (or highest temperature of treatment, HTT); the other being nature of the feedstock.

A study of existing literature on biochar research reveals a lack of a systematic and rigorous approach focused on individual feedstock or HTT. It is to fill this gap that this study aimed to rigorously examine: the characteristics of biochar in a systematic way that focusses on a single feedstock source while varying the HTT from two different treatment facilities. It also aimed to assess the impact these biochars had on soil properties to which they (biochars) were added. The specific objectives were:

to synthesize from the same feedstock six biochars, three from each of two different production processes (Batch and Continuous) over a range of pyrolysis temperatures and then subject the biochars to rigorous characterization;

to investigate the influence of the synthesized biochars on a range of soil processes, and microbial diversities;

to assess how the addition of the synthesized biochars to two soil types affects the physicochemical properties of the amended soil and influences plant growth.

Sitka Spruce (*Picea sitchensis*) wood was pyrolysed at 400, 600 and 800^{o}C. Experimental methods used included; titrimetric analyses, combined thermogravimetry – differential scanning calorimetry – quadrupole mass spectrometry, Fourier transform infrared spectroscopy and gas chromatography – mass spectrometry. In order to assess the impact of the biochars on the soil environments, a fully replicated and systematic plant growth trial was done. The post-harvest amended soils were then used to measure soil processes and also determine microbial community diversity against chosen controls.

Results obtained from this study showed altered physicochemical properties of the biochars (increases in pH and total organic carbon; decreases in cation exchange capacity and water holding capacity), confirming the first hypothesis that biochemical and physical properties of the biochar are systematically altered with increasing HTT. However, there was very little difference between the properties of the biochars from the different production processes indicating that uniformity can likely be predicted based on HTT.

Biochar addition to soil enhanced its basal respiration rate in the low pH soil but suppressed it in the near neutral soil, suppressed denitrification enzyme activity in the near neutral soil and these effects were to some extent affected by HTT. Biochar addition raised the total organic carbon content and lowered bulk density in both the acid and near- neutral soils and also increased the pH in the acid soil but not in the near neutral soil. The significant alteration of these soil properties was also influenced by changing the HTT. Biochar addition also influenced leek growth compared to the controls only in the acid soil. However, altering the biochar HTT had no significant effect on leek growth in both soils.

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Chapter 1 Introduction

1.1 Background

The term 'Biochar' is applied to the solid product of the thermal decomposition of biomass in oxygen-limited environment (Mašek *et al.*, 2013; Wang *et al.*, 2013b) and is classed under the wider term 'black carbon' which simply refers to the product of burnt biomass (Ascough *et al.*, 2011). These materials actually represent a continuum embracing the carbon-rich products of incomplete biomass combustion with no agreed clear-cut boundaries between products (Bird *et al.*, 2008). The products of burning range from slightly charred biomass produced at low combustion temperatures through to char, charcoal, soot and graphitised black carbon which are formed at progressively higher temperatures (Masiello, 2004).

Figure 1.1 (a) Sitka spruce wood chips (right) and its biochar (left), (b) biochar products from various feedstock sources such as rice husk, corn cobs, wheat straw, saw dust and chicken manure.

Source for (b): carbon-negative US

Biochar has variously been referred to as 'charcoal' (Glaser *et al.*, 2002; Bell and Worrall, 2011), 'char' (Chun *et al.*, 2004), 'agrichar' (Lehmann and Joseph, 2009a), 'carbonized biomass' (Ogawa *et al.*, 2006) or even 'carbonaceous material' (Gartler *et al.*, 2013). These names and their variety depend on the context under which they are defined. However, a general definition that seems to be gaining acceptance is one that defines biochar as the carbon-rich product of biomass decomposition during pyrolysis, that is produced and applied to soil with the intention of improving fertility (Lehmann and Joseph, 2009b; Verheijen *et al.*, 2009; Enders *et al.*, 2012). Technically, the definition mentions the 'limited' or 'no-oxygen' conditions under which biochar is produced (Lehmann and Joseph, 2009a; Wang *et al.*, 2013b) at temperatures below 700^{o}C (Lehmann and Joseph, 2009a; Taghizadeh-Toosi *et al.*, 2011). Though, other authors differ on this temperature ceiling (Wang *et al.*, 2013b) and go on to suggest a maximum pyrolysis temperature of 700^{o}C to optimise some properties of crop straw biochar.

1.1.1 Interest in biochar

There has been a surging interest in biochar research in the last decade primarily due to reports of its potential uses in agriculture (based on the known property of the Amazonian *terra preta* soils on which crops grow better compared to surrounding soils), climate change mitigation, other environmental applications such as polluted land remediation and as a tool for organic solid waste management/disposal. The 'bio' in 'biochar' differentiates it from charred materials from non-biological sources such as plastics (Lehmann and Joseph, 2009a). Verheijen *et al.* (2009), provided an illustration of the growing interest in 'biochar' compared to the terms '*terra preta*' and 'black earth' using a Google trends search result for a 5-year period (figure 1.2)

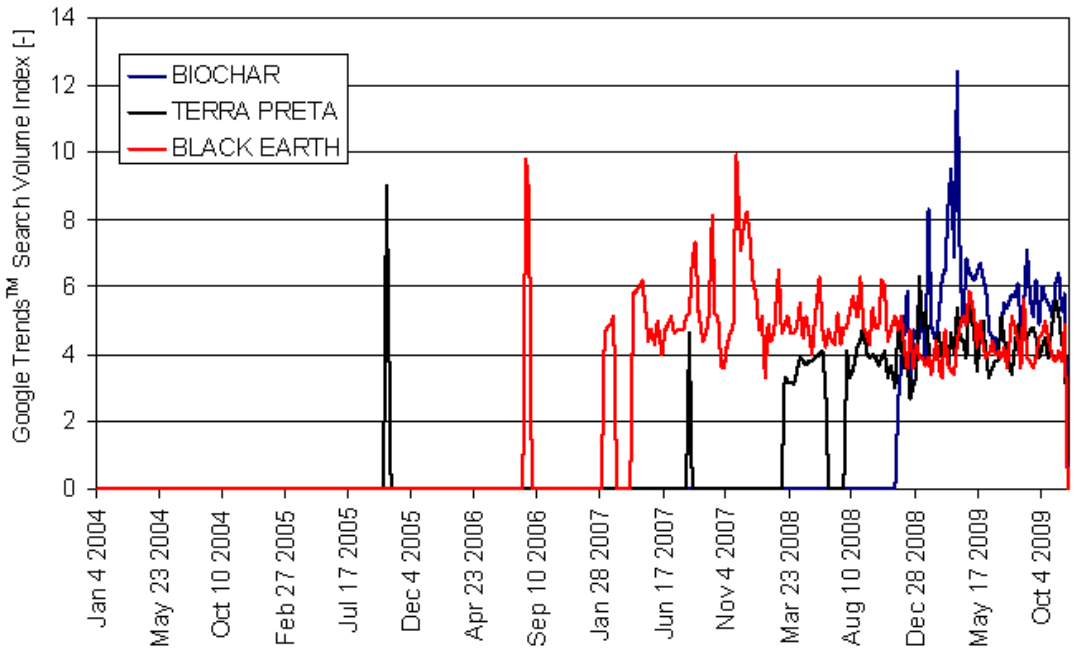

Figure 1.2 Google Trends™ result of "biochar", "*Terra Preta*" and "black earth" search for a 5-year period. Adapted from Verheijen et al., (2009)

1.1.2 Biochar and climate change

For the average temperature of the Earth to remain stable over long periods of time, incoming energy in form of solar radiation from space have to be equal to outgoing energy radiated from the Earth's surface as thermal infrared back to space (http://earthobservatory.nasa.gov/Features/EnergyBalance/page6.php, accessed on 31/07/14) . The Earth is kept warm because greenhouse gases (GHG), mainly water vapour and carbon dioxide (CO_2), trap the outgoing heat energy. This is the so called natural greenhouse effect. Other major GHG are methane (CH_4) and nitrous oxide (N_2O) (Solomon *et al.*, 2007). Referring to a report by the Intergovernmental Panel on Climate Change (IPCC), Steinbeiss *et al.* (2009) mentioned increases in atmospheric CO_2 at a yearly rate equivalent to 4.1 x 10^9 t of C. The concentration of CO_2 which is the primary greenhouse gas in the atmosphere is increased by human activities in the form of fossil fuel combustion and deforestation. For example in 2011 CO_2 amounted to ~84% of all GHG emission in the US, with electricity generation and transportation as the biggest contributors; similarly in the UK, 2012 estimates show CO_2 amounting to about 82% of all GHG emissions again with energy supply and transport contributing the most (Figure 1.3).

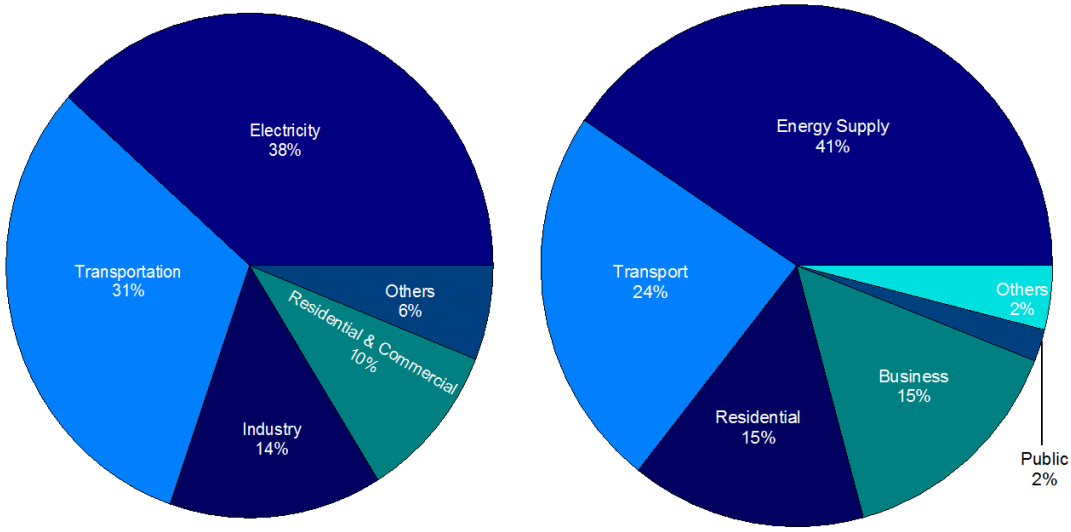

Figure 1.3: Sectoral contributions to carbon dioxide emissions in the US (left) and the UK (right)

Sources: http://www.epa.gov/climatechange/ghgemissions/gases/co2.html, accessed on 31/12/2013 and https://www.gov.uk/government/publications/final-uk-emissions-estimates, accessed on 25/03/2014.

This increase in amounts of CO_2 in the atmosphere raises the average global temperature unleashing a chain of events (Climate Change): melting snow and ice cover; raising water levels in oceans; flooding coastal areas; exposed darker areas under snow and ice absorbing more solar radiation further heating the Earth; and extreme weather scenarios (heavy rains and droughts). Hence, mitigating climate change primarily should involve the removal of the excess CO_2 out of the atmosphere into a more stable sink or reservoir.

This is where biochar comes in as a channel through which the C in CO_2 can be 'captured' in biochar and 'sequestered' or stored in soil (Bell and Worrall, 2011). Ogawa *et al.* (2006), reported a 35-year project on E*ucalyptus* plantation management that could sequester CO_2 equivalent to over 1 million t C, about 53% of which will be in the form of biochar produced from the wood residues. Other authors reported the possibility of adding large amounts of biochar (~60 t/ha) to soil without detrimental effects to crop yield (Vaccari *et al.*, 2011). Carbon sequestration using biochar has potential with multiple advantages of long term C storage (biochar is recalcitrant) and, soil quality improvement (agronomic value), and provides a good way to manage plant and animal waste. Otherwise, such waste is left to decompose in the natural way releasing in the process more green-houses gases to the atmosphere (CO_2 in aerobic

decomposition and CH_4 in anaerobic decomposition). Biochar-amended soils have also been reported as showing suppressed GHG emissions (Spokas *et al.*, 2009; Yaghoubi and Reddy, 2011; Yoo and Kang, 2012; Harter *et al.*, 2013). This adds to the climate change mitigating credentials of biochar.

1.1.3 Biochar and food security

A publication of the Food and Agriculture Organisation (FAO) of the United Nations counsels on the need to increase agricultural production by as much as 60% in the next four decades in order to cope with a 39% rise in world population (FAO, 2012). With such projected growth in world population coupled with dwindling arable land resources, restoring fertility to degraded soils is vital to enhanced global food security (Spokas *et al.*, 2012). Biochar has the potential to contribute in achieving this goal through improved soil physico-chemical properties that result in enhanced fertility. Though biochar has only limited direct nutrient value (Asai *et al.*, 2009; Chan and Xu, 2009; Sukartono *et al.*, 2011), it indirectly increases fertility through improved fertiliser-use efficiency (Chan and Xu, 2009; Hossain *et al.*, 2010; Sohi *et al.*, 2010). It does this by enhancing properties like cation exchange capacities (CEC) of amended soils which help in retaining nutrients and making them available to plants (Sanchez *et al.*, 2009). Other properties that improve productivity include increased organic carbon contents of soils (Sukartono *et al.*, 2011; Gartler *et al.*, 2013), lower bulk density (Vaccari *et al.*, 2011), ameliorating soil acidity (Masahide *et al.*, 2006; Hossain *et al.*, 2010), and plant water availability (Masahide *et al.*, 2006; Van Zwieten *et al.*, 2010).

1.2 Research gap and justification

The literature survey done and reported in the next chapter revealed many studies and reports on biochar lacking in rigor and systematic approach. This gap is evident in the way biochars from so many feedstock sources produced at many different pyrolysis temperature values are studied together, using various types of procedures and reporting in different units. Hence, repeating procedures, comparing results and most importantly identifying individual biochar suitability for specific applications (such as its impact on soil environments) based on its properties become difficult. It also reflects a general lack of a standard for biochar characterization and documentation (Spokas *et al.*, 2012).

The research work undertaken and reported in this thesis was therefore intended to contribute in filling this gap. To achieve that, a multidisciplinary approach was adopted with the objectives of assessing the biochemical and agronomic impact of the synthesized biochars on soil environment and plant growth. The approach fixes one of the most important factors affecting biochar properties (feedstock) and varying another (the pyrolysis temperature). The multidisciplinary approach involves a detailed physico-chemical characterization of the biochars, using them in a fully replicated experimental plant growth trials for agronomic impact assessment followed by biochemical study of soil processes and molecular biological determination of microbial diversity. An extended presentation of this section is at the end of Chapter 2.

1.3 Aims

- To produce and characterize different biochars from a single feedstock based on different production processes.
- To measure their impact on the biochemical and agronomic properties of the soil environments.

1.4 Objectives

The objectives this research project set out to achieve and test the accompanying hypotheses were:

- **Objective 1: Biochar synthesis and characterization**

Synthesize from the same feedstock a set of six biochars, three from each of two different production processes (Batch and Continuous) over a range of pyrolysis temperatures (400, 600 and 800^{o}C) and then rigorously subject the biochars to a range of biochemical and physical characterization.

- **Hypothesis 1:** Biological, chemical and physical properties of the biochar such as fixed carbon, pH, functional group chemistry, water holding and cation exchange capacities, are altered with increasing pyrolysis temperature.

- **Hypothesis 2:** The biological and physico-chemical properties of biochars are altered differently based on the production process used.

- **Objective 2: Biochemical impact of biochar in the soil environment**

Investigate and compare the influence of biochars synthesized at different temperatures from different production processes on a range of soil processes, and microbial diversities relative to chosen controls.

- **Hypothesis 3:** Increasing pyrolysis temperature progressively alters biochar's ability to influence the selection of resultant microbial communities and microbial mediated processes e.g. respiration, and nitrogen cycling in soil environments.

- **Objective 3: Impact of biochar amendment on the agronomic properties of the soil environment**

Assess how the addition of biochar produced at different pyrolysis temperature from different production processes to two soil types (low and near-neutral pH) affects the physicochemical properties of the amended soil and influence plant growth in both soils compared to unamended control. To achieve this objective, controlled fully replicated pot experiments were conducted in a greenhouse using Leek (*Allium porrum*) as test plant. The biochar was applied to the test soils at three amendment rates; 1, 5 and 10% w/w equivalent to 10, 50 and 100 t/ha.

- **Hypothesis 4:** Different biochar pyrolysis temperatures and their application rates will significantly alter the pH, total organic carbon (TOC) contents, bulk density (BD) water holding (WHC), and cation exchange capacities (CEC) of soils to which the biochar was added.
- **Hypothesis 5:** Different biochar pyrolysis temperatures and their application rates influence biochar's ability to impact on the growth rate of leek plant in amended soils compared to control soils.

1.5 Thesis structure

Eight chapters are presented in this thesis. Introductory notes, aims, objectives and research hypotheses are given in Chapter 1. Chapter 2 presents a review of the literature on research work around biochar; its production, properties, applications and impacts in areas such as agriculture and climate change mitigation. A fuller and

clearer research gap and thus justification for this research work emerge at the end of Chapter 2.

The methodologies adopted and equipment used in measuring the biochemical and agronomic parameters studied are presented in Chapter 3. This chapter also gives details of sample sourcing, the pre-pyrolysis handling of the Sitka spruce wood chips, and the pot experiment design that includes growing the leek seedlings and then establishing them in the amended soils in the pots. Results for the fresh biochar characterization are presented and discussed in Chapter 4; a similar format is followed for the agronomic properties of the amended soil and its impact on leek growth in Chapter 5. The results for microbial studies consisting of microbial mediated soil processes and microbial community diversity are presented and discussed in Chapter 6. Each of the three Chapters (4, 5 & 6) is ended with a conclusion.

Chapter 7 gives an overall discussion centered on the trend of changes in the various measured parameters with the main variable in this study, the pyrolysis temperature and also with the biochar amendment rates. Chapter 8 is the final chapter of this thesis and hence contains the overall conclusions with some recommendations on future work that could be done to further enhance documented scientific information on the samples studied in this research project.

Chapter 2 Literature review

2.1 Introduction

The definition of biochar is somewhat fluid depending on the intent for its production. The names char and activated carbon are preferred in fuel/energy and sorption applications, while the broader term of black carbon is used in soil science/carbon sequestration discussions. Figure 2.1 adapted from Schimmelpfennig and Glaser (2012) depicts the various forms of pyrogenic carbon within the black carbon continuum.

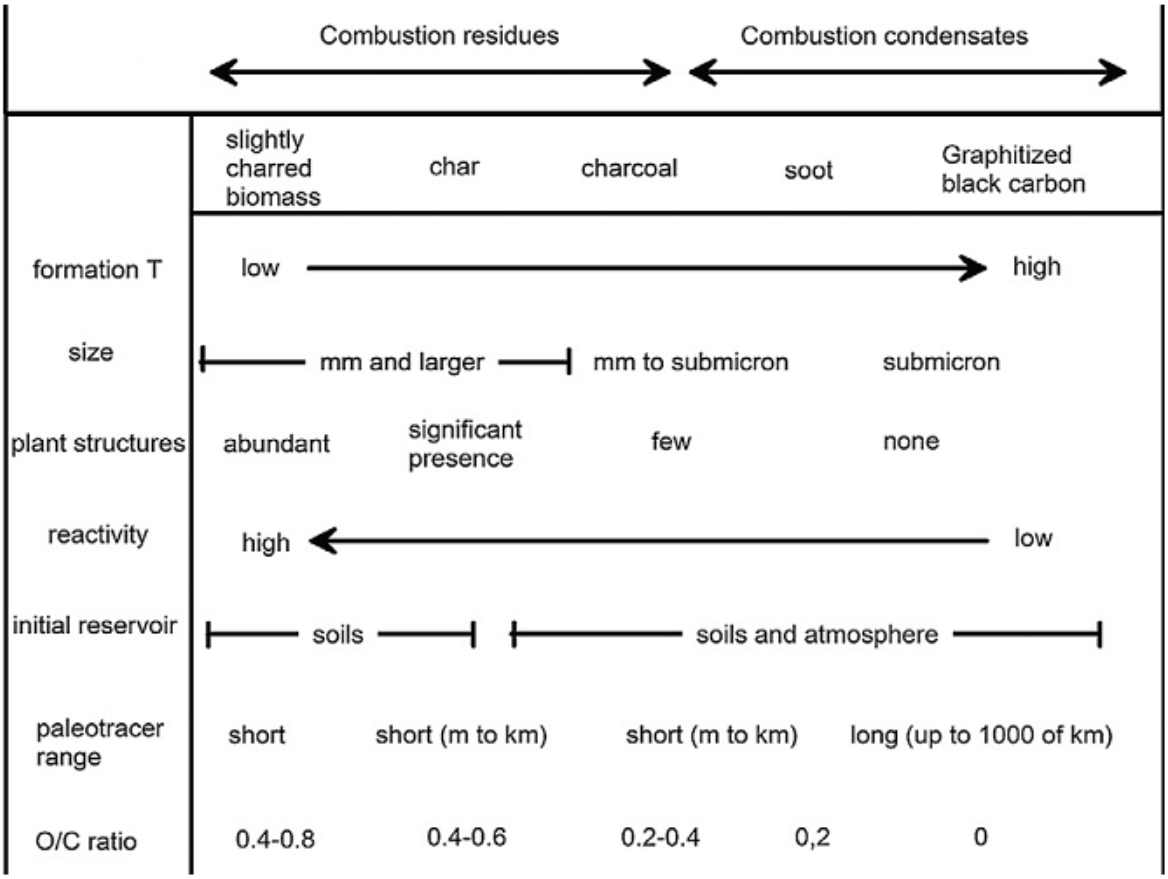

Figure 2.1: The various forms of pyrogenic carbon in the black carbon combustion continuum. Adapted from Schimmelpfennig and Glaser (2012).

It could therefore be inferred that while all biochar is black carbon, not all black carbon is biochar (Spokas *et al.*, 2012). Based on intended use, a recent publication defines 'biochar' as charred organic matter that is deliberately produced and applied to soil with the aim of improving soil properties (Lehmann and Joseph, 2009a). What relates all these terms is the fact that all are used to refer to the solid residue of partial

combustion or pyrolysis from a single precursor - any type of biomass. The properties of biochar are highly dependent on the nature of biomass and production conditions such as pre- and post-production treatment, presence or absence of oxygen, residence time in the kiln or oven (Wang *et al.*, 2013b) and especially the highest temperature of treatment (HTT) (Chan and Xu, 2009; Ahmad *et al.*, 2012). Biochar can be produced using traditional mud ovens or industrial pyrolysers as in Figure 2.2.

Figure 2.2: Manual oven (A) and Industrial pyrolysis unit (B) for Biochar production

Source: (A) Marris, E. (2006); (B) biochar-international.org

Chemically, the structure of biochar is considered to be highly aromatic (Bird *et al.*, 2008) due to the structure of the plant material feedstock which typically contains lignin, which is a complex racemic aromatic polymer as depicted by the structure of lignin in Figure 2.3 (Shen *et al.*, 2010) and its various possible monomers in Figure 2.4 (Thevenot *et al.*, 2010).

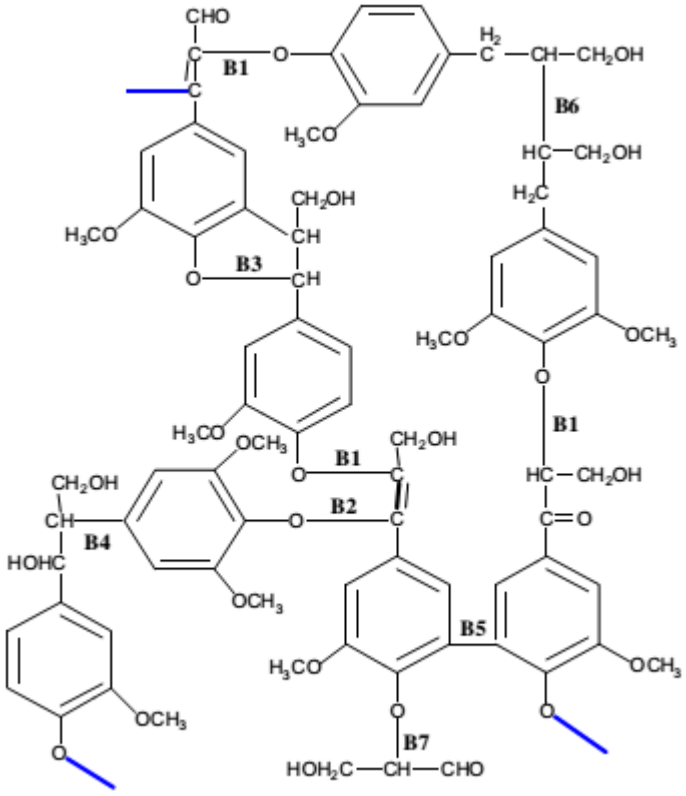

Figure 2.3 The chemical structure of wood-derived lignin. Adapted from Shen et al. (2010).

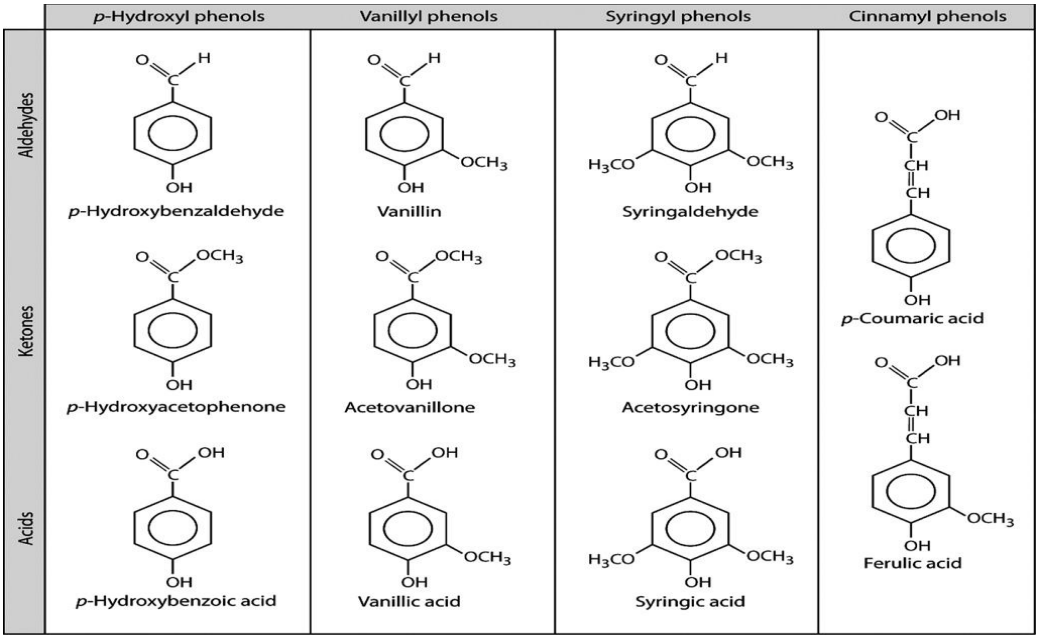

Figure 2.4 Lignin monomers: H-type, V-type, S-type and C-type phenols (Thevenot et al., 2010)

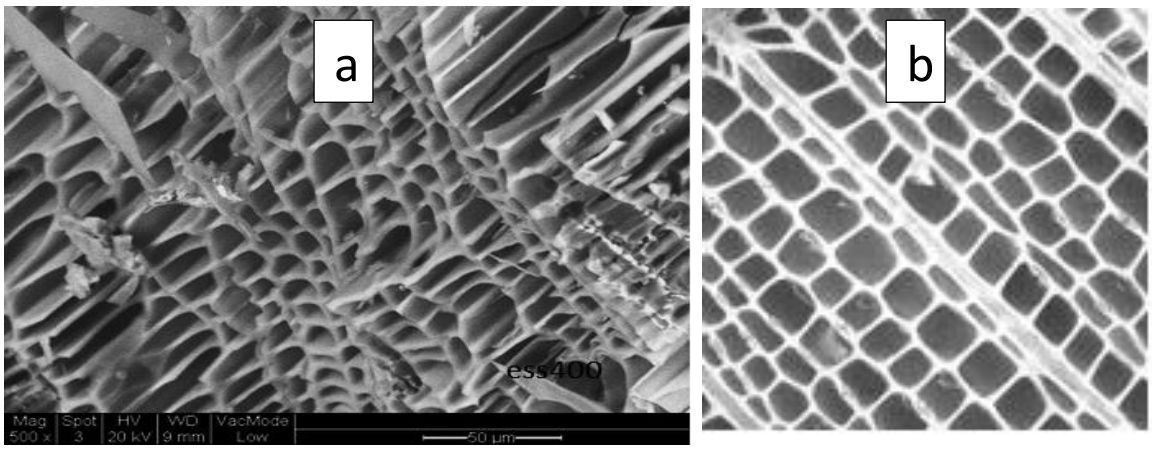

Figure 2.5: Scanning electron microscopy image of the fresh Sitka spruce biochar produced at 400°C (a) and structures in a Sitka spruce wood (b) taken from Moore (2011).

At low pyrolysis temperatures (400°C) the biochar in this study (Figure 2.5a) retained some of the annual ring structures of a Sitka spruce wood as recorded in Figure 2.5b (Moore, 2011). These porous structures could be conduits for the flow of nutrient-containing soil solutions and could also serve as havens for soil microbes (Bird *et al.*, 2008).

Research work around biochar is said to be motivated by four themes: soil improvement, climate change mitigation, energy production and waste (solid) management (Lehmann and Joseph, 2009a). These topics have recently received the attention of researchers but to varying extents. However, it should be noted that the motivations for production and usages of biochar are sometimes mutually exclusive (such as when biochar simply results as an insignificant end product in the production of bio-oil) while some are mutually inclusive (such as when biochar is produced to serve as a soil improver as well as a way to sequester carbon). This literature review will discuss these different research themes under the following headings that also include physico-chemical characterization since all potential applications to which biochar could be deployed will depend on its physico-chemical properties:

1. Solid waste management.
2. Fuels/Energy.
3. Sorption applications.
4. Carbon sequestration/Greenhouse gas emissions.

5. Agronomic impact (Soil improvement/Plant growth).

6. Biochar and soil microbial systems.

7. Physico-chemical Characterization.

2.1.1 Solid waste management

Biochar production and deployment to soil (see 2.1.5) is seen as a very positive way of managing both animal, crop and other agricultural wastes that would otherwise be dumped in open-air sites or landfills and constitute sources of both surface and groundwater pollution (Lehmann and Joseph, 2009a). This understanding has led to lots of efforts towards producing biochar from coconut shells (Amuda *et al.*, 2007), saw dust (Sun and Zhou, 2008), rice and wheat straw (Qiu *et al.*, 2009), orange peels (Chen and Chen, 2009), bagasse (Inyang *et al.*, 2010), poultry litter (Uchimiya *et al.*, 2010), waste water sludge (Hossain *et al.*, 2011), rice husk (Enders *et al.*, 2012), and various types of wood (Titiladunayo *et al.*, 2012; Liu and Balasubramanian, 2013; Mukherjee and Zimmerman, 2013).

2.1.2 Fuels/Energy

The production of bio-fuels involves pyrolyzing biomass at high temperature to obtain liquid fuel, gaseous fuel in the form of syngas (a mixture of CO, H_2 and some CO_2) and a solid carbon-rich residue (biochar). Most studies on energy from biomass (Ozcimen and Karaosmanoglu, 2004; Ozcimen and Ersoy-Mericboyu, 2008; Grierson *et al.*, 2009; Sanchez *et al.*, 2009; Agblevor *et al.*, 2010), focus heavily on maximizing bio-oil and syngas production. Hence, pyrolysis design has been geared towards minimal production of char as it is considered a low value waste product (Sohi *et al.*, 2010; Montanarella and Lugato, 2013) and consequently the little biochar produced is sometimes gasified (Melchior *et al.*, 2009), or assessed based on its heating value (Agblevor *et al.*, 2010) rather than any agronomic value. An important finding from these energy studies that relate to biochar production is that slow pyrolysis (low temperature and low heating rate) is noted to maximize biochar yield (Demirbas, 2004) while fast pyrolysis maximizes bio-oil/syngas output as Figure 2.6 illustrates. Figure 2.7 shows the schematic outlines of the two processes.

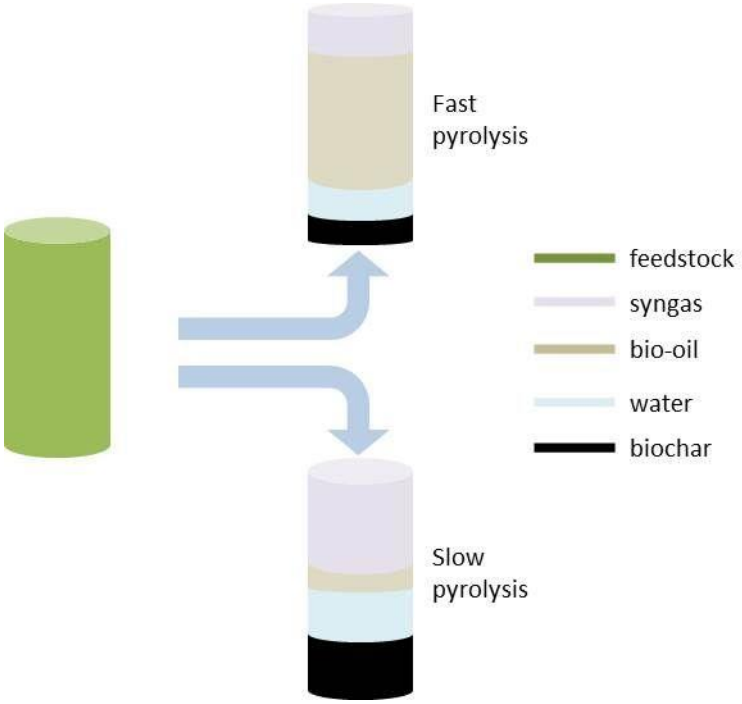

Figure 2.6: Relative output proportions from fast and slow pyrolysis processes.

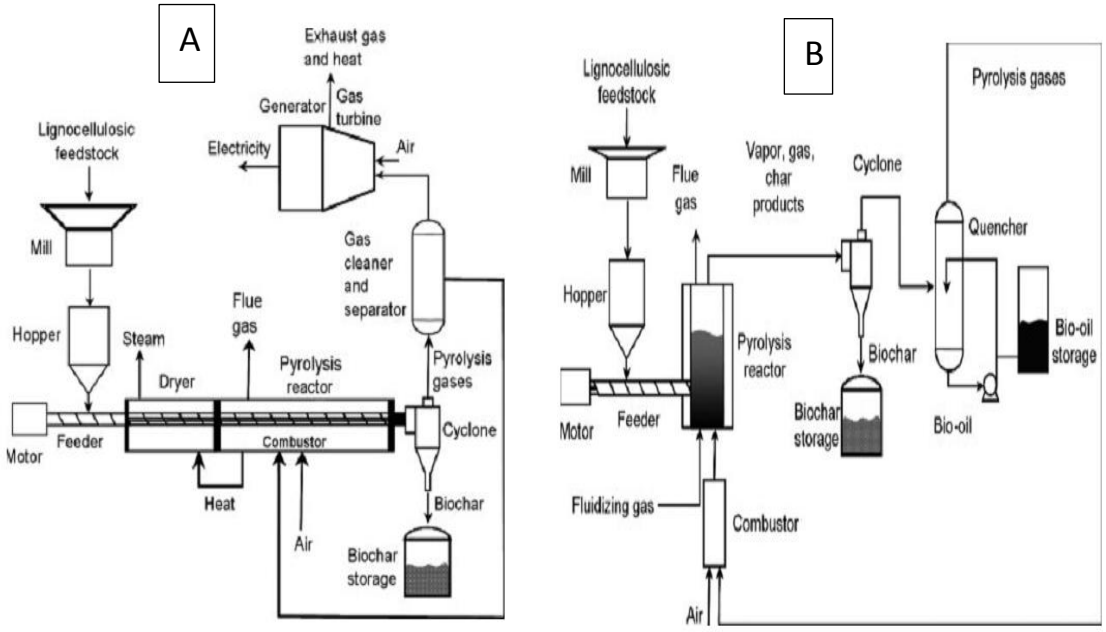

Figure 2.7 Schematic diagrams of slow (A) and fast (B) Pyrolysers; Source: (Laird, 2009)

Regardless of the yield of biofuel obtained from pyrolysis of biomass, biochar itself has been identified as a combustible fuel (Laird *et al.*, 2009), albeit a solid one with a heat energy content of about 20 MJ/Kg. However, other workers have reported higher calorific values for biochar; 25.3 MJ/Kg for biochar from rapeseed cake (Ozcimen and Karaosmanoglu, 2004), 25.96 MJ/Kg and 32.62 MJ/Kg for biochar from coconut fibre

and pine wood respectively (Liu and Balasubramanian, 2013). Such high calorific values (>20MJ/Kg) satisfy the generally held opinion for a solid fuel to ensure auto-thermal combustion (Liu *et al.*, 2012), and this calorific yield is consistent with low moisture and fixed-carbon content (Ozcimen and Ersoy-Mericboyu, 2008), low ash and oxygen content (Sanchez *et al.*, 2009). Titiladunayo *et al.* (2012), concluded that the negligible sulfur content of some hard wood biochar is a characteristic that makes this biochar environmentally friendly in terms of SO_x emissions.

2.1.3 Sorption applications

Activated carbon, made from carbon-rich biomass (Kalderis *et al.*, 2008) is used industrially as an adsorbent or filter for various volatile organic compounds (Fletcher *et al.*, 2007) and heavy metals (Amuda *et al.*, 2007). This is due to its very large surface area coupled with proper micro-porous structure, two properties that are also found in biochar (Qiu *et al.*, 2009). Thus, there is a growing interest in deploying biochar as a cost-effective adsorbent in place of activated carbon for organic compounds like dyes (Qiu *et al.*, 2009), polycyclic aromatic hydrocarbons (Chen and Chen, 2009; Beesley *et al.*, 2010), pesticides (Spokas *et al.*, 2009; Yu *et al.*, 2009) and heavy metals like arsenic (Hartley *et al.*, 2009), chromium (Wang *et al.*), copper (Uchimiya *et al.*, 2011) and lead (Liu and Zhang, 2009; Namgay *et al.*, 2010). Qiu *et al.* (2008), found Pb(II) adsorption to be higher in rice straw- and wheat straw-derived biochar than in commercial activated carbon. Biochar may also remove odorants from air (Laird *et al.*, 2009) and, toxins from water, food and drugs (Peterson *et al.*, 2013), and is effective in retaining nutrients and making them available to plants (Sanchez *et al.*, 2009) while reducing the bioavailability of both inorganic and organic contaminants to plants (Beesley *et al.*, 2010). The sorptive potentials of biochar may probably be due to electrostatic attractions between positive metal ions and negative surface functional groups on the biochar (Qiu *et al.*, 2008) which could be carboxylic in nature (Uchimiya *et al.*, 2012). Inyang *et al.* (2012), reported effective removal of heavy metals from aqueous solutions by biochar sourced from anaerobically digested diary waste and sugar beet, further suggesting the use of anaerobic digestion to biologically 'activate' biochar as a means of improving its sorptive properties.

2.1.4 Carbon sequestration/Greenhouse gas emissions

Biochar as a product of pyrolysis contains a high percentage of carbon which has been found to be stable, and 'inert' or 'recalcitrant' in the environment (Spokas *et al.*, 2009). This property makes biochar when added to soil a potential tool for carbon capture and storage (Montanarella and Lugato, 2013) in climate change mitigation. Additionally, biochar reduces the emission of greenhouse gases from amended soils. The recalcitrance of biochar is ascribed to its resistance to both chemical and microbial degradation (Lehmann and Joseph, 2009a) and an index to estimate this property (see section 4.3.2 of this thesis) has recently been suggested (Harvey *et al.*, 2012). Biochar is estimated to have a mean residence time of 2000 years in temperate soils (Kuzyakov *et al.*, 2009), and some researchers suggest biochar may have a half-life in the order of thousands of years (Forbes *et al.*, 2006). However, its rate of degradation in soil remains controversial (Bird *et al.*, 2008). Nevertheless, biochar appears to provide a channel for the removal of carbon from the short-term bio-atmospheric carbon cycle and sequestering it into the long-term geological carbon cycle (Bird *et al.*, 1999; Forbes *et al.*, 2006; Yoo and Kang, 2012). Vaccari *et al.* (2011), reported the possibility of high rates (60t/ha; 5% w/w) of application of biochar to soil as a way to sequester carbon without detrimental effect on crop yield.

In addition to sequestering carbon, biochar application to soil is reported to suppress greenhouse gas (GHG) emissions in the form of N_2O and CO_2 (Zhang *et al.*, 2012) further enhancing its climate change mitigation potential. Others found that laboratory incubation of a farmland top soil with biochar over a period of 100 days reduced the soil CO_2 production for all amendment levels corresponding to field application rates of 24 – 720 t/ha (Spokas *et al.*, 2009). They also found biochar additions >20% w/w significantly suppressed N_2O production just as it reduced ambient CH_4 oxidation at all levels compared to unamended soil. Anthropogenic sources are said to contribute about 6.75 million metric tonnes of N_2O emissions with 1.5 million tonnes from grazing animal excreta (Taghizadeh-Toosi *et al.*, 2011). Yaghoubi and Reddy (2011) reported over 40% improvement in CH_4 adsorption for a landfill cover soil amended with 5% biochar (w/w), while others reported an N_2O emission reduction potential of 47% for biochar at 2% application rate (Harter *et al.*, 2013). The impact of biochar on greenhouse gas emissions from soil environments are said to depend on soil and biochar types (Spokas and Reicosky, 2009; Harter *et al.*, 2013), biochar aging and

water-filled pore size (Singh *et al.*, 2010). More discussion on proposed mechanisms of GHG suppression is provided in section 6.3.2 (Chapter 6) of this thesis.

2.1.5 Soil improvement/Plant growth

A considerable amount of published work exists which describes the impact of biochar in improving soil quality in terms of enhanced retention and/or availability of nutrients (Glaser *et al.*, 2002; DeLuca *et al.*, 2009; Agblevor *et al.*, 2010), water retention and cation exchange capacities (Masahide *et al.*, 2006; Asai *et al.*, 2009; Van Zwieten *et al.*, 2010), reduced plant uptake of pesticides (Yu *et al.*, 2009), heavy metals (Hartley *et al.*, 2009) and increased microbial abundance (Masahide *et al.*, 2006; Steinbeiss *et al.*, 2009; Thies and Rillig, 2009). Application of biochar from the bark of *Acacia mangium* (brown Salwood) improved the availability of nutrients like Ca which increased from 0.79 to 5.86 $cmol_c$/Kg, Mg from 0.27 to 0.55 $cmol_c$/Kg, K from 0.07 to 0.21 $cmol_c$/Kg, and total N from 1.3g/Kg to 2.1g/Kg (Masahide *et al.*, 2006). These increases were attributed to the biochar's contents of the relevant nutrients and exchangeable cations. Glaser *et al.* (2002), reported higher levels of available nutrients in a pooled data table that showed a Ca increase from 1.00 $cmol_c$/Kg in unamended soil to 13.46 $cmol_c$/Kg in soil amended with 300g/Kg of hardwood charcoal. For the same treatment, K increased from 0.03 to 0.46 $cmol_c$/Kg, Mg from 0.17 to 0.41 $cmol_c$/Kg and, total N from 0.7 to 2.4g/Kg. Similarly, P increased from 7.0 to 37.4mg/Kg. Biochar-amended soils have lower bulk densities which reduce the tensile strength of the soils and hence give lower tillage costs (Vaccari *et al.*, 2011), just as it provides a liming effect to acidic soils (Van Zwieten *et al.*, 2010). However, biochar's impact on soil fertility varies with soil, crop type and over time (Mukherjee and Zimmerman, 2013) in addition to the intrinsic characteristics of the biochar itself.

There are reports of biochar's positive impact on plant growth and crop yields (Masahide *et al.*, 2006; Asai *et al.*, 2009; Yu *et al.*, 2009). Asai *et al.* (2009), investigated the impact of biochar application on the physical properties of soil and rice (*Oryza sativa L.*) yield. Their results show saturated hydraulic conductivity of the soil increased by 79% at 16 t/ha biochar application rate. This, they explain, suggests not only improved soil water permeability but also soil water holding capacity which translates into improved water availability to plant. Masahide *et al.* (2006), studied the effect of biochar on the chemical properties of soil and the yields of maize (*Zea mays L.*), cow

pea (*Vigna unguiculata L.*) and pea nut (*Arachis hypogaea L.*). From their results, biochar application was associated with increases in the pH value of the soil from 4.5 to 5.4; total N from 1.3 to 2.1g/Kg and cation exchange capacity from 8.85 to 12.38 $cmol_c$/Kg. A 15.8% increase in maize yield at 20 t/ha biochar amendment has been reported (Zhang *et al.*, 2012), while a waste water sludge biochar at half this amendment rate is said to have increased cherry tomato yield by over 60% (Hossain *et al.*, 2010). Vaccari *et al.* (2011) reported a 30% increase in biomass and durum wheat yield using a wood based biochar, while Major *et al.* (2010) have shown biochar amendment to have increased maize yield by 28, 30 and 140% over three consecutive years. This shows the potential of biochar for sustained positive impact on crop yield at least in the short term. Some other authors, however, reported no effect on plant growth (Hartley *et al.*, 2009), while yet others report depressed crop response (Chan and Xu, 2009; Gartler *et al.*, 2013). But some of the data on crop response to biochar addition collected in a review paper (Glaser *et al.*, 2002) seem to suggest that low amounts of applied biochar perform better compared to high amounts that have negative impact on plant growth. For instance, they report a 63% increase in biomass production at 5.0 t ha^{-1} charcoal amendment, while only 29% was obtained at 15.0 t ha^{-1} charcoal amendment for the same soybean plant. These authors reported improved soil fertility and high biomass yield at reduced tillage cost which could lower the energy cost of production for biofuel crops, making them better climate mitigation tools. Table 2.1 provides more information on crop responses to different biochar types (Chan and Xu, 2009).

Table 2.1 Crop yield responses from biochar applications (Source: Chan and Xu, 2009)

Feedstock for biochar and rate of application	*Crops/plants*	*Responses*	*Reasons for responses Given by authors*	*References*
Unknown wood (0.5 t ha^{-1})	Soybean	Biomass increased by 51%	Water-holding capacity and black colour on temperature	Iswaran et al (1980)
Unknown wood (5t ha^{-1} and 15t ha^{-1})	Soybean	Yield reduced by 37 and 71%	pH-induced micro-nutrient deficiency	Kishimoto and Sugiura (1985)
Wood for charcoal Production, unknown rates	Vegetation in charcoal hearth and non-hearth areas compared after 110 years	Tree density and basal area were reduced by 40%	Negative responses due to changes in soil properties	Mikan and Abrams (1995)
Wood for charcoal production, (2t ha^{-1})	Trees (*Betula pendula* and *Pinus Sylvestris*)	Affected only *B. pendula* and only in substrates high in phenolics	Increased N uptake by countering the effect of phenolics	Wardle et al (1998)
Bamboo, unknown rate	Tea tree	Height and volume increased by 20 and 40%	Retained fertilizer and maintained pH	Hoshi (2001)
Secondary forest wood (68t C ha^{-1} – 135t C ha^{-1})	Rice, cowpea and Oats	Biomass of rice increased by 17%, cowpea by 43%	Improved P, K and possibly Cu nutrition	Lehmann et al (2003b), Glaser et al (2002)
Bark of *Acacia mangium* (37t ha^{-1})	Maize, cowpea and peanut at two sites	Response only at one site (less fertile) with 200% increase (fertilized)	Increase in P and N availability and reduction of exchangeable Al^{3+}; arbuscular mycorrhizal (AM)	Yamato et al (2006)
Secondary forest wood (11t ha^{-1})	Rice and sorghum	Little response with biochar alone, but with a combination of biochar and fertilizer yielded as much as 880% more than plots with fertilizer alone	Fungal colonization Not stated	Steiner et al (2007)
Rice husk (10t ha^{-1})	Maize, soybean	10-40% yield increases	Not clearly understood, dependent upon soil, crop and other nutrients	FFTC (2007)
Green waste (0-100t ha^{-1})		No positive effect with biochar up to 100t ha^{-1}, but with added N fertilizer, 226% increase in dry matter	Indirect effect of improving physical properties of hard-setting soil	Chan et al (2007c)
Paper mill sludge (10t ha^{-1})	Wheat	Increase in wheat height by 30-40% in acid soil but not in alkaline soil	Mainly liming value	Van Zwieten et al (2007)

2.1.6 Biochar and soil microbial systems

Soil is a highly complex system that embodies a variety of microhabitats with different physico-chemical properties and environmental conditions that serve as havens for soil microorganisms (Insam, 2001; Torsvik and Øvreås, 2002). These microorganisms are of central importance in sustaining soil health due to the vital role they play in the release and cycling of nutrients and decomposition of organic matter which has a net effect on primary productivity (Rutigliano *et al.*, 2004). Figure 2.8 below depicts the connection between biochar properties, soil processes and soil biota (Lehmann *et al.*, 2011).

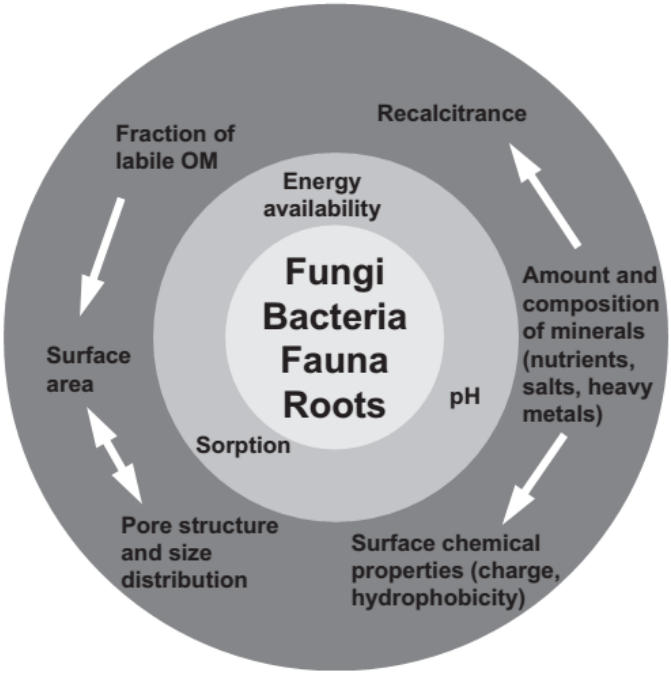

Figure 2.8 Relationships between soil biota (inner circle), soil properties biochar may influence (middle circle) and the properties of biochar (outer circle). Arrows show influence between properties. Adapted from Lehmann et al (2011)

Human activities in the form of soil amendment techniques impact on the structure, diversity and activity of microbial populations (Sheppard *et al.*, 2005). Published works report the impact of addition of sewage sludge and/or lime on ammonia oxidizing bacterial communities (Gray *et al.*, 2003), the impact of N fertilizer treatments on the diversity of ammonia-oxidizing bacteria populations (Webster *et al.*, 2002), and the impact of repeated long-term addition of anoxically digested sewage sludge on the diversity of methanogens (Sheppard *et al.*, 2005). However, the impact of biochar amendment on soil biota has been much less studied (Lehmann *et al.*, 2011) compared to its impact on the physico-chemical properties of soil.

In the case of biochar amendments, the impact can be studied in two ways, namely, the effect of biochar on the soil microbial community and how the microbes influence the biochar itself. On the one hand, addition of biochar to soil has been shown to provide pore spaces for colonization by microbes (Bird *et al.*, 2008). Biochar also possibly provides the micro-organisms access to nutrients (Brodowski *et al.*, 2005) held by the biochar within its pores. Steinbeiss *et al.* (2009), identified biochar type as the driving parameter for any effects on the microbial community. Comparing total amount of phospholipid fatty acids in soils before and after incubation as an estimate

of microbial biomass they found that while addition of glucose-derived biochar led to a significant reduction in microbial biomass, the addition of yeast-derived biochar did not have any effect. On the other hand, microbes are reported to influence the oxidation or mineralization of biochar in soil with only one study reporting no degradation as detailed in the reviews of Schmidt and Noack (2000) and Glaser *et al.* (2002). Kuzyakov *et al.* (2009), found that only between 1.5 and 2.6% of biochar C was incorporated into microbial biomass after incubation for over 89 weeks. However, all seem to agree that microbial degradation of biochar is very slow.

2.1.7 Physico-chemical characterization of biochar

All the applications to which biochar is deployed (carbon sequestration, soil improvement, sorptive potential, energy purposes) ultimately depend on biochars physical and chemical nature which in turn depend mainly on the nature of feedstock and highest temperature of treatment (HTT). Expectedly, there is a growing interest in the physico-chemical characterization of biochar especially in the last decade (Fernandes *et al.*, 2003; Ozcimen and Karaosmanoglu, 2004; Zhu *et al.*, 2005; Brown *et al.*, 2006; Bourke *et al.*, 2007; Qiu *et al.*, 2008; Chen and Chen, 2009; Song and Peng, 2010; Ascough *et al.*, 2011; Enders and Lehmann, 2012; Zhao *et al.*, 2013) . The sorptive properties of biochar for example are shown to be due to chemisorbed O_2 on the carbon surface (Boehm, 2002), while Ahmad *et al.* (2012) attributed the adsorption of trichloroethylene (TCE) by crop residue biochars to the high aromatic and low polarity nature of their surfaces. Additionally, surface area itself influenced by HTT; (Wang *et al.*, 2013b) may explain the sorption of nonpolar pollutants, as Kloss *et al.* (2012) concluded after finding higher concentration of naphthalene in woody biochars with increasing HTT (which also increased the surface area of the biochars). Straw-based biochar has also been suggested as better than wood based biochar in agriculture (Wang *et al.*, 2013b), probably due to their higher contents of soluble major and trace elements, boron apart (Kloss *et al.*, 2012). What has not received deserved attention is a systematic study focused on the main variables affecting biochar's properties (Feedstock and HTT). This is vital to weaning biochar from being considered a waste product (Sohi *et al.*, 2010; Montanarella and Lugato, 2013) to having a set of standards (Cheng and Lehmann, 2009) and properties for identifying biochar (Schimmelpfennig and Glaser, 2012) aimed at particular applications (Zhao *et al.*, 2013) especially in soil environments which require meeting regulatory constraints

(Keiluweit *et al.*, 2012) that set maximum allowable limits for certain pollutant contents in materials added to agricultural soils.

2.2 The research gap and justification

The study of existing literature on biochar research reveals a lack of a systematic and rigorous approach focused on individual feedstock and/or the highest temperature of treatment and consequent effects on the properties of biochar. It is these properties upon which biochar's potential benefits for use in climate change mitigation, pollution control and agricultural soil improvement are based. This gap is evident in the way biochar from so many feedstock sources produced at many different pyrolysis temperature values are studied together, using various types of procedures and reporting in different units. This reflects a general lack of a standard for biochar characterization and documentation (Spokas *et al.*, 2012) which makes it difficult to compare results, repeat procedures and most importantly identify individual biochars by their properties suitable for specific applications, a need that has recently been echoed by various researchers (Kloss *et al.*, 2012; Schimmelpfennig and Glaser, 2012).

Titiladunayo *et al.* (2012), for example studied biochars from three different named hard woods at five different HTT's mainly for their fuel potentials, while others analyzed biochar from two different feedstock sources at seven different HTT's for PAH content (Keiluweit *et al.*, 2012). Enders *et al.* (2012) evaluated 94 different biochars at 7 different HTT's for their recalcitrant and agronomic values, while others quantified PAH's in over 50 biochars from 22 different feedstock sources using various pyrolysis methods at numerous HTT's between 250-900^{o}C (Hale *et al.*, 2012). Another study (Beesley *et al.*, 2010) did not give any information on the identity of the hardwood used, or the HTT chosen for producing their biochar or its properties; they simply mentioned source company, a situation very similar to that of Major *et al.* (2010). Taghizadeh-Toosi *et al.* (2011), gave no information on the HTT used to produce the biochar they used to study impact on N_2O emissions. A more puzzling case is when no information is given concerning the feedstock (type or name), HTT or source of the biochar in addition to lack of any procedure used in determining the physico-chemical characteristics of the biochar used (Saxena *et al.*, 2013). Sometimes as when traditional production methods are used, only a range of HTT is mentioned (Schulz *et al.*, 2013) which obviously makes repeatability difficult.

It is to fill this gap that we aimed to rigorously study and document the characteristics of biochar in a systematic way that focusses on a single feedstock source (Sitka spruce wood) while varying the highest temperature of treatment (HTT) from two different treatment facilities. HTT is one of the two most important factors that influence biochar properties. Additionally, we aimed to assess the impact of the synthesized biochars on the soil environments using a unique multidisciplinary approach that involved applying the biochars in a fully replicated and systematic plant growth trials in multiple soils. The post-harvest soils were then used to measure impacts on soil processes (respiration and denitrification) and also employ molecular biology tools to determine microbial community diversity on the amended soils against chosen controls.

Chapter 3 Materials and methods

3.1 Introduction

This chapter presents the materials used and their sources, and the various experimental procedures and pieces of analytical equipment used to generate the results discussed in this thesis. Materials include the feedstock, biochars and soils, while analyses conducted include thermal, proximate, elemental, Fourier Transform Infra-red (FTIR), pH, soil respiration and Denaturing Gradient Gel Electrophoresis (DGGE). Table 3.1 gives details of the materials, their types and sources and production process in the case of biochars. Results of the pilot plant growth trials are also reported though they were used to understand how best to conduct the pot experiments using our synthesized biochars.

Table 3.1: Sources, types and other details of biochars and soils analysed and used for experiments

Material	Source	Type	Production process
Feedstock	Taylormade Timber Products Ltd Sherburn Hill, County Durham DH6 1PS in North East England	Sitka spruce (chips and saw dust)	Not applicable
Biochar	Interreg project (German) – (labelled ibc)	Unknown	Unknown
Biochar	Previous PhD project – (labelled kbc800)	Unknown feedstock but produced at 800^{o}C	Unknown
Biochar	Jacobi Carbons Ltd, Moss Estate, Leigh, Lancashire, WN7 3PT, UK (labelled ss)	Produced from Sitka spruce at 400, 600 and 800^{o}C	Batch
Biochar	UK Biochar Research Centre, University of Edinburgh, EH9 3JN, UK (labelled ess)	Produced from Sitka spruce at 400, 600 and 800^{o}C	Continuous
Soil	Fenton Centre, Northumberland, UK (Ordnance Survey National Grid Reference NT 966 334	Sandy (pH = 4.38)	Not applicable
Soil	Nafferton farm cottage, Stocksfield, Northumberland, UK (Ordnance Survey National Grid Reference NZ 066 657	Loamy/Clayey (pH = 6.67)	Not applicable

3.2 Biochar preparation and pre-treatment

The feedstock was sourced as detailed in Table 3.1, air dried as in section 3.2.1 and processed to produce biochar at the three chosen pyrolysis temperatures; 400, 600 and 800^{o}C using two different production processes as detailed in section 3.3 below.

3.2.1 The feedstock

Sitka Spruce (*Picea sitchensis*) saw dust and wood chips of mixed sizes were sourced from Taylormade Timber Products Ltd, (Sherburn Hill, County Durham, DH6 1PS) in North East England. These were air dried to about 10% moisture content then sieved to obtain a ≤2mm size from the saw dust and ≤10mm sized sample from the mixed wood chips.

Figure 3.1: Sitka spruce wood chips and saw dust processed to produce the biochars studied.

3.3 Biochar production

The ≤2mm wood chips were pyrolysed at Jacobi Carbons Ltd, (Moss Estate, Leigh, Lancashire, WN7 3PT, UK) in a batch system using a 10 litre horizontal electrical furnace; a residence time at maximum temperature of 30 minutes and a heating rate 10^{o}C/min, while the 10mm chips were pyrolysed at the UK Biochar Research Centre, (University of Edinburgh, EH9 3JN, UK) in a continuous flow system using a stage II continuous pyrolyser, with a residence time at maximum temperature of 30 minutes and feed rate of 0.5Kg/h. Heat up to furnace set-point took typically 50 min which gives a heating rate of between 8-16^{o}C/min for the three temperature values. Both production processes were carried out under nitrogen gas atmosphere. The biochars were henceforth labelled as ss400, ss600, ss800, ess400; ess600 and ess800 where the letters ss stand for biochar produced from Lancashire (Lancashire biochar) and ess stand for biochar produced from Edinburgh (Edinburgh biochar), while the numerical numbers stand for pyrolysis temperatures at which the biochar was produced.

3.3.1 Biochar pre-treatment for use in soil amendment and other investigations

The fresh biochar samples were gently crushed by hand to pass through a 2mm sieve and stored in sealed polythene sample bags at ambient temperature in the laboratory for use in the various analytical tests and experimental treatments.

3.4 Biochar recovery post-plant trial experiments

At the end of soil pot experiments (see section 3.6 of this chapter) sub-samples of the soil-biochar mixtures were taken for biochar recovery. One of the methods used for the recovery was a flotation method in which about a litre of distilled water was added to about 150g of soil-biochar mixture, stirred and allowed to settle after which the floating biochar particles were filtered off through a #1 Whatman filter paper. The use of tweezers (Nguyen and Lehmann, 2009) under a magnifying glass was also employed to manually pick out the biochar particles from the soil.

The recovered biochar was investigated using Scanning Electron Microscopy for evidence of possible microbial colonisation of the biochar pores (see Figure 2.9 in Chapter 2 of this thesis) similar to what other researchers have reported (Brodowski *et al.*, 2005).

3.5 Physico-chemical characterization of the freshly produced biochar.

3.5.1 Proximate analysis on the biochar samples

Proximate analysis is aimed at determining the major constituents of the biomass as a percentage of its total weight and is mostly undertaken in the energy industry to give an insight into the energy potentials of the biomass. The measured constituents are, fixed carbon (FC), volatile matter (VM), moisture content and ash content. Ultimate analysis quantifies the elemental constituents of the material which are mainly C, H, O, S and N. Other elements of interest may be determined as part of the ultimate analysis.

A Netzsch Jupiter STA449C TG-DSC (thermogravimetry-differential scanning calorimetry) system was used for the proximate analysis. About 19mg of sample was accurately weighed into an alumina crucible and heated first in helium atmosphere, then in air and then back in the inert atmosphere at a flow rate of 30ml/min. A heating rate of 20K/min was used. The equipment monitored the percentage mass loss with temperature at various stages representing moisture contents, volatile matter, fixed carbon and ash as represented in Figure 3.2.

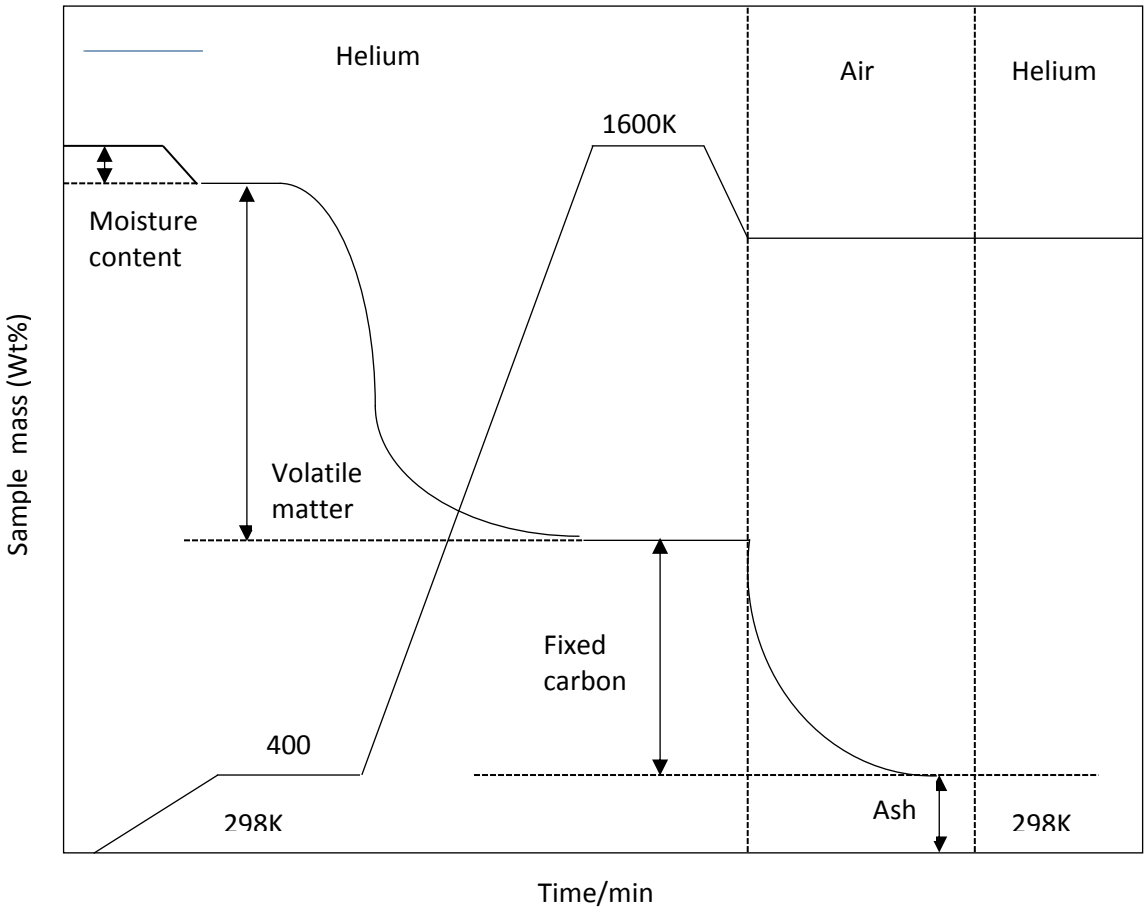

Figure 3.2: Temperature programme and mass loss profiles for the proximate analysis of the biochar samples.

3.5.2 Thermal analysis of the raw wood, freshly produced biochar and soil samples.

Figure 3.3: Coupled thermogravimetric, differential scanning calorimetric and quadrupole mass spectrometry System

Thermal analysis is defined as "a group of techniques in which a physical property of a substance and/or its reaction products is measured as a function of temperature whilst the substance is subjected to a controlled temperature programme" (Anandhan; Mackenzie, 1979). The samples in this study were analysed using thermogravimetry (TG), a thermal analysis technique that monitors the change in mass of a substance as a function of temperature or time in the course of heating the sample specimen through a controlled temperature programme

(30-900^{o}C in this study) in a controlled atmosphere (mostly inert) (http://www.perkinelmer.com/CMSResources/Images/44-74556GDE_TGABeginnersGuide.pdf), running concurrently with differential scanning calorimetry (DSC), that measures the difference in heat flow rate (mW = mJ/sec) between a sample and inert reference as a function of time and temperature (http://www4.ncsu.edu/~lalucia/courses/WPS-595B-BIOMATERIALS-CHARACTERIZATION/DSC.pdf). Samples were analysed as a fine powder, crushed using an agate pestle and mortar.

A subsample of between 17-18mg was accurately weighed into an alumina crucible and analysed using a Netzsch Jupiter STA449C TG-DSC (thermogravimetry-differential scanning calorimetry) system connected to a Netzsch Aeolos 403C quadrupole mass spectrometer (QMS) for the mass spectrometric analysis of the evolved gas. Samples were heated from 30°C to 900°C at a rate of 10°C min^{-1} in an atmosphere of 80% He + 20% O_2 (purge gas, flow rate 30 ml min^{-1}). The protective gas was helium (flow rate 30 ml min^{-1}). Adapter heads and transfer lines were heated at 150°C and 300°C, respectively. TG and DSC data were acquired and processed using Netzsch TA4 Proteus Analysis software.

3.5.3 Biochar and soil pH determination

The pH for both biochar and soil samples was measured using the British Standard method (BS7755 Section 3.2), which involves scooping out and suspending 5ml of sample in 25ml of deionised water in a 60ml plastic bottle. The bottle was mounted onto a shaker for 15minutes after which the bottles were removed and the suspensions left to stand overnight. The pH was then measured using a JENWAY 3020 pH meter.

3.5.4 Water holding capacity (WHC) for biochar and soil samples.

The water holding capacity for soil samples was determined using a slightly modified version of the British Standard method BS 7755-4.4.3:1997. The perforated base of a plastic cylinder (50mm length, 60mm diameter) was covered with a filter paper and weighed. The cylinder was then partially filled with the soil sample and introduced into a water bath at room temperature allowing water to seep through the perforated base until it submerged the soil in the cylinder. The sample was then left to soak for 3 hours at room temperature, removed from the water and then placed on a draining tray containing wet, fine quartz sand to a depth of about 20mm. The soil was left overnight

to drain after which the cylinder containing the drained wet soil was weighed. The soil was removed and dried to constant mass in an oven at 105 ^{o}C and weighed.

Water holding capacity was then calculated as a percentage using the equation;

$$WHC = \frac{m_s - m_t - m_d}{m_d} \times 100$$

Where

m_s is mass of water-saturated soil + cylinder + filter paper in grams;

m_t is mass of empty cylinder + filter paper in grams;

m_d is the mass of dried soil in grams.

Water holding capacity for the biochar samples was measured after the method of Nguyen and Lehmann (2009) which involved mixing 19 g of pure white sand (Sigma Aldrich no. 274739, -50 +70 mesh; ignited at 500 ^{o}C for 24 h) and 1 g biochar material. The two were mixed well and placed onto a previously weighed Whatman no. 1 filter paper in a funnel. The biochar–sand mixture was then saturated with deionised water. After thorough free draining, the saturated biochar-sand mixture was weighed, dried at 105 ^{o}C for 24 h, cooled in a desiccator and then weighed again. A control biochar/sand free filter paper with funnel was used to determine the mass of water held by the filter paper which was then subtracted from the water held by the biochar-sand mixture. Water holding capacity was then calculated as a percentage using the relationship;

$$WHC = \frac{m_s - m_d - m_w}{m_d} \times 100$$

Where

m_s is mass of water saturated biochar-sand mixture in grams;

m_d is mass of dried biochar-sand mixture in grams;

m_w is mass of water held by filter paper in grams.

3.5.5 Fourier-transform infrared analysis of the biochar samples.

Figure 3.4: Thermo Scientific NICOLET 6700 Fourier-transform infrared spectrometer

Fourier transform infrared (FTIR) spectroscopic measurements were done on powdered samples using a single reflectance attenuated total reflectance (ATR) method (Seredych *et al.*, 2008; Uchimiya *et al.*, 2010). A Thermo Scientific NICOLET 6700 spectrometer (Thermo Nicolet Corporation, Madison WI 53711) (Figure 3.4) fitted with a universal diamond ATR platform was used for measurements. Thirty two scans were collected for each sample spanning 550–4000 cm^{-1} at a $4cm^{-1}$ resolution. Spectra were automatically corrected for background collected with a sample free ATR crystal. Data collected were processed and analysed using OMNIC software package Version 6.1a (1992) from Thermo Nicolet Corporation. Spectral interpretation and functional group assignment were achieved through the relevant published articles as detailed in Chapter 4 (section 4.3.4) of this thesis.

3.5.6 Surface acidity/basicity of the biochar.

Boehm neutralization titrations were employed to measure the two surface amphoteric properties (acidity and basicity) of the biochar samples (Boehm, 2002; Cheng *et al.*, 2006; Fletcher *et al.*, 2006; Cheng and Lehmann, 2009). For surface acidity, about 0.15 g of biochar was added to 15 mL of 0.1 M NaOH solution and shaken with an end-over-end shaker for 30 h. The resulting biochar slurry was then filtered using a Whatman No. 42 filter paper. An aliquot (5 mL) of the NaOH filtrate was transferred to a 10-mL 0.1 M HCl solution that neutralized the unreacted base. The solution was then back-titrated with 0.1 M NaOH to an endpoint determined by phenolphthalein indicator. The adsorbed base was then converted to surface acidity content (mmol/g) of biochar. Surface basicity was measured in a similar manner to surface acidity, but in this case an aliquot (5 mL) of the HCl filtrate was directly titrated with 0.1 M NaOH. The adsorbed acid was converted to surface basicity content in (mmol/g) of biochar.

3.5.7 Elemental analysis of the CHN contents of the biochars.

Figure 3.5: Carlo Erba 1108 elemental analyser

Powdered samples were analysed for Carbon, Hydrogen and Nitrogen contents using a Carlo Erba 1108 Elemental Analyser (Figure 3.5) controlled with CE eager200 software, run in accordance with manufacturer's guidelines and weighed using a certified Mettler MX5 micro balance. The equipment was calibrated with acetanilide Organic Analytical Standard (batch No. 151853). Oxygen was determined by a difference calculation. Samples were also analysed for carbon, nitrogen and sulphur using VarioMAX V7.0.5 16.Nov. 05, CNS elemental analyser with Sulfadiazine used as the calibration standard.

3.5.8 Analysis for total organic carbon (TOC) contents of the biochars and soils.

Figure 3.6: Leco CS244 Carbon/Sulphur analyser

The total organic carbon for both biochar and soil samples was determined using the British Standard method (BS7755 section 3.8, 1995). Approximately 0.1 g of each sample was accurately weighed into a porous crucible on a tray with numbered positions, 1 mL of 4 M hydrochloric acid was then added drop wise to remove inorganic carbon contents (i.e. carbonates). The crucibles were removed from the tray and placed in a fume cupboard on a drainage platform to let the acid drain away for about 4 hours. The crucibles and contents were then dried overnight in an oven at 65°C. The tray was then removed from the oven, covered with aluminium foil to protect against possible contamination or loss of sample, allowed to cool and organic carbon content was then determined on a Leco CS244 Carbon/Sulphur Analyser (Figure 3.6). An empty crucible was also prepared and processed as a procedural blank.

3.5.9 Cation exchange capacity (CEC) for soil and biochar

Cation Exchange Capacity for the samples was determined according to the method of Enders *et al.* (2012) with some modifications. The modifications include using manual in place of mechanical vacuum extractor and Millipore filter paper (Type GV 0.22 microns) in place of filter pulp. Briefly, 1.00 +/- 0.05 g of biochar was added to 40 mL of pH 7.0 buffered ammonium acetate solution (NH_4-OAc) and shaken overnight in 60 mL glass vials. Contents were transferred using an additional 10 mL NH_4-OAc into extractor syringes prepared with Millipore filter paper (Type GV 0.22 microns) supporting a bud of glass wool. Syringes were mounted in clamps and used to manually extract a total of 50 mL NH_4-OAc solution over approximately 2 h. CEC was determined by adding 60 mL of 95% EtOH to the sample syringes to remove NH_4-OAc not adsorbed to exchange sites. Following this, 50.0 mL of 2 M KCl was added and left overnight to displace NH_4^+. Samples were then extracted over 2 h and an additional 40 mL of 2 M KCl was added to the sample syringes and extracted a second time. The two extractions were pooled and brought to 100 mL volume with 2 M KCl. Ammonium was quantified in the extracts on Spectroquant Pharo 300 spectrophotometer using MERCK's ammonium test kit (Merck KGaA, 64271 Damstadt, Germany). CEC was calculated according to the following relationship:

$$\text{CEC mmol}_c\text{ /Kg} = \frac{\text{NH}_4\text{ concentration (mg/L)} \times \text{total extract (mL)}}{\text{Biochar (g)} \times \text{NH}_4\text{ (g/mol)}}$$

3.5.10 Scanning electron microscopy (SEM)

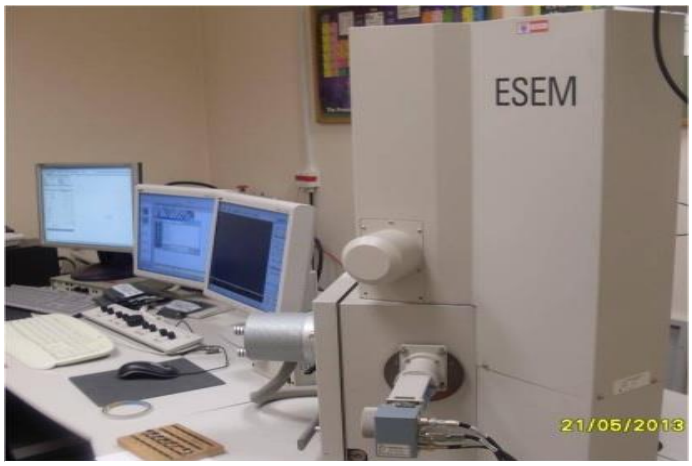

Figure 3.7: Environmental scanning electron microscope

Fresh biochar crushed to pass through a 2mm sieve was used for SEM measurements. Small amounts of the samples were mounted on aluminium stubs using carbon double sided adhesive tabs. Images were recorded using an Environmental Scanning Electron Microscope-Field Emission Gun (FEI XL30 ESEM-FEG) with a back scattered

electron detector at 20KeV. Biochar samples recovered from pot soils were prepared for images that could possibly reveal micro-organisms on the surface or inner cavities of the biochar crystals. Sample preparation involved fixing the specimens overnight in a solution of 2% gluteraldehyde ($CH_2(CH_2CHO)_2$) in Sorensons phosphate buffer (a mixture of mono and disodium hydrogen phosphates made to pH 7.4), followed by two rinses for 15 minutes each with fresh volumes of the same buffer. The samples were then dehydrated by soaking for 30minutes each in 25%, 50%, and 75% ethanol, followed by soaking in 100% ethanol twice for 60 minutes each. Final dehydration was achieved using carbon dioxide in a Baltec Critical Point Dryer. The samples were then mounted on aluminium stubs with Acheson Silver Dag, dried overnight and then coated with a standard 15nm gold layer using a Polaron SEM Coating Unit. The specimens were finally examined using a Stereoscan 240 Scanning Electron Microscope (housed within the Electron Microscopy Research Services, Newcastle University) and the digital images were collected with Orion6.60.6 software.

3.6 Leek growth pot trials in soils amended with biochars

3.6.1 Introduction

The improvement of agricultural soils by addition of biochars has been reported by various researchers. It appears that biochars improve crop yields (Masahide *et al.*, 2006; Sukartono *et al.*, 2011) by improving soil fertility status through enhanced water availability to plants, soil organic carbon contents and reduced leaching of applied N fertilizers (Berglund *et al.*, 2004; Spokas *et al.*, 2009; Taghizadeh-Toosi *et al.*, 2011) and changing physical properties (Asai *et al.*, 2009). In this study pot based growth trials were aimed at assessing the impact of different biochar amendments on the growth characteristics or yield of leek (*Allium porrum*); as well as on soil properties such as pH, water holding capacity, CEC, basal respiration and nitrogen dynamics. Microbial community composition of the amended soil from the pots has also been investigated. Biomass yield was monitored by measuring the above ground stem diameter and the leek plant owing to its morphology provided a good model for that purpose.

The pot experiments consisted of a series of three different trials to investigate the growth of a test plant (leek) in two different soils amended at different rates with four different biochars as detailed in Table 3.2. The pilot experiment using two types of biochars (see Tables 3.1 & 3.2) was designed to develop the right approach taken in

the subsequent systematic trials using our synthesized biochars in the two test soils. Results of the pilot experiment are reported and discussed along with others in the relevant chapters. Artificial lighting was used in the pilot experiment because it ran in the winter period that normally has short daylight period with diminished sunshine. The results from the pilot experiment pointed to the need for fertilizer application to the pot soils before transplanting the leek seedlings, and to address irrigation frequency (every other day with deionised water), with early potting of the leek seedlings before they overgrow in the growth chamber. The results demonstrated the need to investigate the leek growth pattern and biochar impact in a near-neutral soil, taking pot soil samples at the beginning of the experiment and at four other intervals through the course of the experiment and freezing the samples for microbial community analysis.

Table 3.2 Details on the pilot and the two main pot experiments established in a greenhouse within the indicated periods of time.

Trial	Pilot	1st Set	2nd Set
Parameter			
Trial duration	Jan-April, 2012 (14 weeks)	May-Aug, 2012 (14 weeks)	March-June, 2013 (12 weeks)
Biochar used	ibc & kbc800	ss (400, 600 & 800°C)	ess (400, 600 & 800°C) ss (400, 600 & 800°C)
Soil used	Sandy (pH = 4.38)	Sandy (pH = 4.38)	Loamy/clayey (pH = 6.67)
Amendment rate (wt %) (t/ha equivalent)	1, 5 and 10 (10, 50 and 100t/ha)	1, 5 and 10 (10, 50 and 100t/ha)	1, 5 and 10 (ess); 5 (ss) (10, 50 and 100t/ha)
Mean Day/Night Temp. (°C)	33/21	34/21	31/21
Photoperiod (hr)	13	Not applicable	Not applicable
Mean light intensity ($\mu molm^{-2}s^{-1}$)	135.0	Not applicable	Not applicable
Fertilization rate (Kg/ha)	None	N:150, K:275, P:300	N:150, K:275, P:300

Note: Control soil was in each trial treated same as other pots except it did not have biochar added.

Table 3.3: Biochar sample codes and their meanings

Biochar sample code	Meaning
ss	Lancashire biochar produced from ≤2mm sized saw dust of Sitka spruce wood
ss400	Lancashire biochar produced at 400°C
ss600	Lancashire biochar produced at 600°C
ss800	Lancashire biochar produced at 800°C
ess	Edinburgh biochar produced from ≤10mm sized Sitka spruce wood chips
ess40	Edinburgh biochar produced at 400°C
ess600	Edinburgh biochar produced at 600°C
ess800	Edinburgh biochar produced at 800°C
ibc	Biochar from the European interreg project
Kbc800	Biochar from a previous PhD project produced at 800°C

3.6.2 Seeding leek to obtain seedlings for the pot experiment

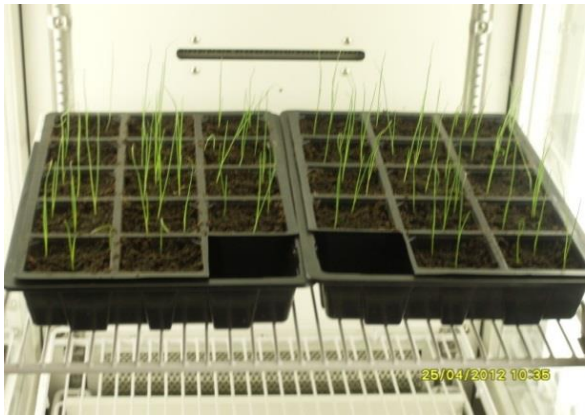

Figure 3.8 Leek seedlings in a growth chamber

All pot trial experiments were set up with transplanted seedlings. To prepare the seedlings, leek seeds 'VEG049 MUSSELBURGH' (purchased from http://www.nickys-nursery.co.uk) were sown in compost within perforated-base plastic trays contained in an outer plastic trough used to irrigate the seedlings from beneath and placed into a growth chamber (Figure 3.8) at 21^{o}C ±1 with 11 hour artificial lighting (7am – 6pm). Initially a pilot pot experiment using the sandy low pH soil was set up in which the leek seedlings at about 15cm in height were transplanted into 250grams of soil in 4" (10cm) plastic pots (one plant per pot) in a greenhouse at the Moorbank Botanic Garden under artificial lighting. At the time of this study, Moorbank Botanic Garden (Claremont Road, Newcastle NE2 4NL) was a teaching and research facility under the School of Biology, Newcastle University which maintained living collections of plants.

Figure 3.9 Potted Leeks in the near-neutral soil.

The experimental design contained replicated (x3) treatments of 1%, 5% and 10% by weight biochar/soil mixtures using two biochar types: ibc and kbc800. A triplicate control containing unamended soil only was also included. In the pilot experiment, no fertilizer was added before or in the course of the experiment and potable water was used to automatically irrigate the plants daily. The pots were appropriately labelled, randomly arranged on a garden table and the experiment lasted for 15 weeks (see Table 3.2). Plant growth was monitored on weekly basis by measuring the diameter of the plant at its base just above the soil. A digital calliper (Fisher Scientific, 0 – 150mm, accurate to within 0.01mm) was used for the diameter measurement. At the end of

the experiment, the plants (including all large bits of roots) were carefully removed from the pots and the soil, and discarded. The soil was transferred to the laboratory, air dried and stored in polythene sample bags for further analysis.

A second set of pot experiments was subsequently started with the same replicated experimental design as the pilot. However, in this case biochar treatments included the use of Sitka spruce biochars obtained from Jacobi Carbons (Lancashire biochar) and deionised water for irrigation (every other day). No artificial lighting was used as these experiments were conducted during the summer, and fertilizer was added to the same type of soil (sandy, low pH) as in the pilot experiment at the rate of 275 Kg/ha (0.1375 g/Kg soil) for potassium; 300 Kg/ha (0.15 g/Kg soil) for phosphorous and 150 Kg/ha (0.075 g/Kg soil) for nitrogen (Defra, 2010). The experiment lasted for 15 weeks with an average day/night greenhouse temperature of 34/21^{o}C.

A third set of pot experiments with a similar experimental design as those conducted previously was again started. However, in this case in addition to using mainly Sitka spruce biochar from Edinburgh (ess) at the three amendment rates as the pilot, a parallel set of pots was introduced in which the Lancashire biochar (ss) was used but only at the 5% amendment rate. Moreover, on the basis of results from our pilot experiment, a near-neutral soil (see Table 3.2) was used in this set of experiment which lasted for 13 weeks with an average day/night greenhouse temperature of 31/21^{o}C.

Pot soil samples were randomly taken at the beginning and (from the same pots) at four other intervals during the course of the second and third set of experiments and frozen for microbial community analysis. At the end of the experiments the leek plant was harvested and pot soil recovered as for the pilot experiment.

3.7 Soil process assays and molecular biological analysis

3.7.1 Basal respiration (BR)

Basal respiration of soils was determined according to ISO 16072 (2002). Triplicate samples of 10 g air dried soil were adjusted to 60 % water holding capacity in 100 ml glass serum bottles (Wheaton science products, USA). Bottles were then sealed with butyl rubber septa and crimp closed (Sigma-Aldrich, UK). Gas samples were taken at 0, 20 and 24 hours using a 100 µl gas-tight syringe (Hamilton, Switzerland) with CO_2

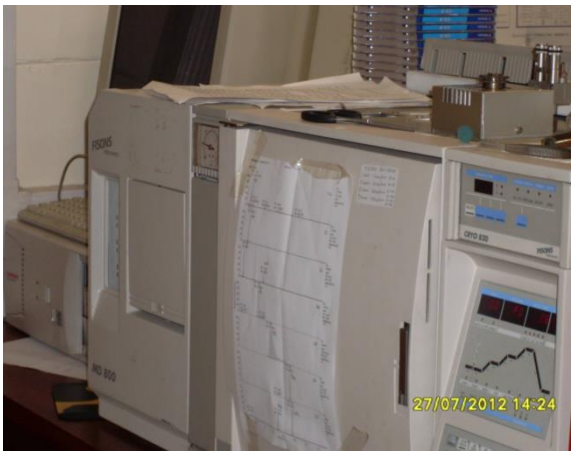

Figure 3.10: Fisons Gas chromatograph-mass spectrometer

production measured by GC-MS. Analysis of headspace CO_2 by GC-MS was conducted on a Fisons 8060 GC (Figure 3.10) using split injection (150°C) linked to a Fisons MD800 MS operated at electron voltage 70eV, emission current 150µA, source current 600µA, source temperature 200°C, multiplier voltage 500V, and interface temperature of 150°C. The acquisition was controlled using Xcalibur software in full scan mode (1.0-151.0 amu/sec). An equal volume of 100µl headspace sample gas from each serum bottle was injected in split mode through the column and the GC programme and MS data acquisition commenced. Separation was performed on a HP-PLOT-Q capillary column (30m x 0.32mm i.d) packed with 20um Q phase. The GC was held isothermally at 35°C with Helium as the carrier gas with a flow rate of 1ml/min, pressure of 65kPa, and open split at 100ml/min. Chromatogram peaks of m/z 44 corresponding to CO_2 gas were integrated and quantified in Xcalibur and saved as Excel files for further processing. Calibrations were carried out by injecting 100, 80, 60, 40, and 20 µL of a 1% CO_2 standard gas (Scientific & Technical Gases Ltd, UK), based on which $\%CO_2$ in the head space of sample bottles was determined.

CO_2 values (% in headspace) were converted to $mgCO_2/g$ dry soil, using the ideal gas equation PV = nRT rearranged to determine n, the number of moles of CO_2 produced. The product of the number of moles and molar mass of the gas gives its mass in grams.

In the ideal gas equation:

P = pressure of the gas standards (1 atm)

R = universal gas constant, 82.05746 $atm*ml*(mol*K)^{-1}$

T = absolute temperature, in this case 298K

V = volume of the gas in head space in ml, calculated as ($\%CO_2$ read off the calibration curve * head space volume in ml)$*(100)^{-1}$

BR rates expressed in mg $CO_2*g^{-1}*h^{-1}$ dry soil were then determined from the slopes of the linear regression of plots of CO_2 production against sampling times.

3.7.2 Denitrification enzyme activity (DEA)

Denitrification enzyme activity was determined via a miniaturised acetylene block method as described by Patra *et al.* (2006) and Wertz et al. (2006) with a few minor modifications (McCann, 2013).

Field moist soil equivalent to 2 g of oven dried soil was placed in 10 ml serum bottles (Wheaton, Sigma-Aldrich, UK) and amended with 2 ml of distilled water containing potassium nitrate, KNO_3 (200 µg NO^{-3}– N g^{-1} dry soil), glucose (0.5 mg C g^{-1} dry soil) and glutamic acid (0.5 mg C g^{-1} dry soil). The original experiment was carried out in 150 ml plasma flasks (Wertz *et al.*, 2006), however, with a reduction in soil mass it was decided to reduce vessel volume to 10 ml in order to further miniaturise the experiment. Supplementary water was added when necessary to achieve 100 % WHC in all soils. Bottles were sealed with butyl rubber stoppers and then the headspaces of bottles were flushed with oxygen free nitrogen (N_2) gas (BOC Gases, UK) followed by 1 % acetylene (C_2H_2) in N_2 (CK Gas Products Ltd, UK) and crimp closed (Sigma-Aldrich, UK). This provided inhibition of N_2O-reductase activity and ensured anaerobic conditions. In the original method of Wertz and colleagues, a 90:10 He–C_2H_2 mixture was used to flush headspace. However, this was unfeasible to employ due to safety reasons related to the stability of acetylene in such a gas mixture. Instead a stable and safe mixture of 1 % acetylene C_2H_2 in N_2 was used in all assays within this study.

Experimental controls were carried out in triplicate, using the same weight of soil, but only flushed with N_2 to determine natural levels of N_2O emissions. Bottles were incubated at room temperature and headspace gas samples were measured after 4 and 6 hours to determine N_2O production by GC-MS.

As for the assessment of BR (section 3.7.1), 100 µl of headspace gas was extracted with a gas-tight syringe (Hamilton, Switzerland), flushed with N_2. Concentrations of N_2O were determined using the major ion fragment of NO^+ at a mass to charge ratio (m/z) of 30. The parent ion of N_2O (m/z 44) was not used due to interference with any discharged CO_2 which also has the same mass to charge ratio of 44. Calibration and linear response was checked using a gas standard of 0.988% N_2O in N_2 (Scientific & Technical Gases Ltd, UK) injected with volumes of 100, 80, 60, 40, 20 and 10 µL. GC-MS equipment and operational parameters were as for BR measurements and the mass of N_2O produced was calculated as for CO_2. Similarly, DEA rates were determined from the slopes of the linear regression of plots of N_2O production against sampling times

and expressed as µg $N_2O*g^{-1}*h^{-1}$ dry soil following calculating rates based on N_2O accumulation over time.

3.7.3 Microbial community structure analysis using PCR-DGGE

Introduction

Pot soil samples taken in the course of plant growth trials (see section 3.6.2) were used to assess changes in the bacterial community composition over time resulting from biochar addition. This preliminary assessment involved comparing the DGGE profiles of PCR-amplified Bacterial 16S rRNA gene fragments from DNA extracts of amended and control soils as detailed in the sections below.

Soil DNA extraction

Soil DNA was extracted in replicates (x3) from 0.25 grams each of individual biochar-amended pot soil samples and control using the 'Experienced User Protocol' provided with PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., 2746 Loker Ave West, Carlsbad, CA 92010).

PCR-amplification of bacterial 16S rRNA genes

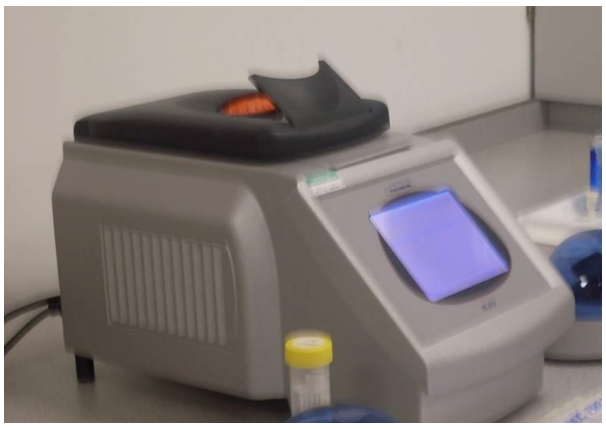

Figure 3.11: Polymerase chain reaction thermal cycler

Polymerase Chain Reaction (Torsvik and Øvreås, 2002) or Polymer Chain Reaction (Liu *et al.*, 2006) is a culture-independent technique that facilitates the investigation of the almost 99% of microorganisms that could not be routinely cultured in the laboratory (Torsvik and Øvreås, 2002; Ghazanfar *et al.*, 2010; Hirsch *et al.*, 2010). The 16S rRNA gene fragments from the DNA extracts were amplified after which the PCR products were separated using the Denaturing Gradient Gel Electrophoresis (DGGE).

DNA extracts were PCR amplified using a TECHNE TC-512 thermal cycler (TECHNE Inc. Burlington NJ, USA). Reaction mixtures contained 0.5 µL of template DNA, 23.5 µL of MegaMix-Blue (Microzone, Haywards Heath, West Sussex, UK) containing Taq polymerase (recombinant) in 1.1 PCR reaction buffer (2.75 mM $MgCl_2$) with 220 µM of deoxynucleoside triphosphates, blue agarose loading dye with stabilizer and 0.5 µL

each of primers 2 (5'-ATTACCGCGGCTGCTGG-3') and 3. Primer 3 contained a GC clamp (a 40-nucleotide GC rich sequence) attached to the 5' end of the following sequence 5'-CCTACGGGAGGCAGCAG-3' (Muyzer *et al.*, 1993). The PCR thermal profile went through 24 cycles of an initial denaturation at 95^{o}C for 10minutes followed by 65^{o}C for 30s, 54^{o}C for 30s and 72^{o}C for 30s. PCR products were identified in aliquots (7µL) by gel electrophoresis using an ethidium bromide stained, 1% (w/v) agarose gel. 3µL of hyper ladder marker was added into each of the two gel lanes that border the sample lanes. The resolved PCR products were visualized under UV light in a UVP iBox In vivo imaging system.

Denaturing gradient gel electrophoresis

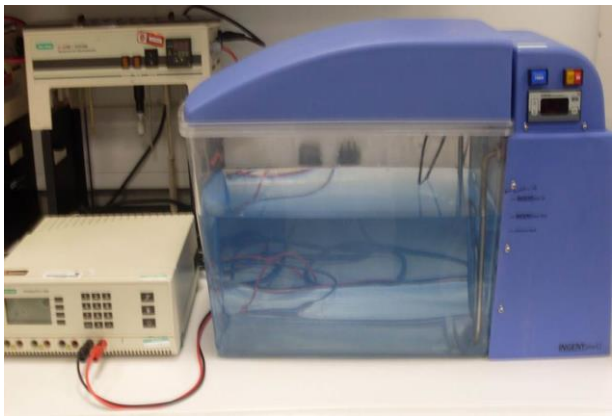

Figure 3.12: INGENY denaturing gradient gel electrophoresis tank

Denaturing Gradient Gel Electrophoresis as a method for investigating microbial diversity uses different strengths of chemical denaturants as a gradient to separated DNA (Liu *et al.*, 2006). The principle is similar to that of Temperature Gradient Gel Electrophoresis (TGGE), but in TGGE the gradient is temperature in place of chemical denaturants (Gray *et al.*, 2003; Liu *et al.*, 2006). The DGGE is considered reliable in its ability to follow changes in microbial populations and it allows for gel analysis of multiple samples in a single run (Muyzer, 1999) using markers that can be compared across gels.

DGGE was conducted using a 0.75mm thick 10% polyacrylamide denaturing gel. The denaturant gradient ranged from 30% (L) to 70% denaturant (H) (100% denaturant is 7 mol L^{-1} urea plus 40% (v/v) deionised formamide in 1 TAE (Tris-acetate EDTA)). To cast the denaturing gel, a vertical clamp cassette (Ingeny International BV) was assembled holding two thin-walled glass plates between which a 32 teeth plastic comb was inserted to form wells at the top of the gel. Twenty four millilitres each of the L and H denaturing solutions per gel was then mixed with 50µL of 20% aqueous solution of ammonium per sulphate (APS) and 10µL of N,N,N',N'-tetramethylenediammine (TEMED). The low and high solutions were mixed in a BIO RAD gradient former (model

485) and poured between the glass plates in the cassette up to a level just below the comb teeth using a peristaltic pump. The polymerised gel was left to set for 2 hours. A stacking gel was prepared by adding 60µL APS and 6µL TEMED to 6mL of 0% aqueous solution of buffer and 40% Bis/Acrylamide per gel. This was introduced through a syringe on top of the polymerised gel to generate the wells needed.

After an hour, the comb was gently removed from the set stacking gel and the cassette holding the gel was immersed in a 1 x TAE buffer (1 x TAE is 40 mM Tris-acetate plus 1 mM EDTA (pH 8.3)) in a 10L electrophoresis tank (Figure 3.12) at 60^{o}C and the electrical terminals connected. The flow tube was also connected to the top of the cassette to allow for TAE flow round the gel. Into fresh PCR tubes, 15µL each of the sample PCR products and loading dye were mixed and added into the wells with the tank at low voltage (LV) and the TAE flow halted. Sample lanes were regularly interspersed with gel lanes to which a marker PCR product was added. A BIO RAD 3000 PowerPac was then set at 100V, the DGGE tank at high voltage (HV) and started for about 5 minutes after which the TAE flow through the cassette was restarted. The electrophoresis was operated for 16 hours, after which the gel was carefully removed from between the glass plates and stained in a solution of 20µL SYBR green in 200mL of 1 x TAE for 1 hour and then visualized under UV light in a UVP iBox In vivo imaging system.

3.8 Statistical analyses

All replicate data sets were statistically analysed on untransformed data by ANOVA using IBM SPSS statistical software (IBM SPSS statistics version 21, 1989-2012, New York, NY 10022, US). For the soil microbial function trials the effects of biochar treatment and biochar pyrolysis temperature were tested through a two-way ANOVA. The tests were done using the general linear model Univariate (LSD, post hoc) analysis with the relevant microbial function indicator (rates of CO_2 production for basal respiration or rates of N_2O production for denitrification enzyme activity) as dependent variable, while amount of biochar added to the soil and pyrolysis temperature of the biochar were the fixed factors. For the plant growth trials in both the low pH and near neutral soils, the rate of leek growth was the dependent variable while amounts of biochar added to the soils and biochar pyrolysis temperature were the fixed factors. However, in the case of the pilot experiments (in the low pH soil) only the amount of biochar added was the fixed factor due to lack of information on

pyrolysis temperature for the ibc biochar. Fisher's least significant difference post hoc test was used to compare means and unless otherwise stated, mean differences were significant at the 0.05 level.

Chapter 4 Characterization of the freshly synthesized biochars from the batch and continuous processes

4.1 Introduction

Critical to all the possible functions of biochar (see Chapters 1 and 2) are the physico-chemical characteristics of individual biochars. For instance, pre- and post-pyrolysis composition determine elemental composition and what surface functional groups exist, which in turn determine to a large extent pH, and cation exchange capacity (Guo and Rockstraw, 2007). Surface area and pore size of the biochar determine its water holding capacity (which improves the plant available water holding capacity of the biochar amended soils) and also sorption abilities (Amuda *et al.*, 2007; Karhu *et al.*, 2011).

Most biochar characteristics ultimately depend on two key factors: the temperature at which the biochar is produced, so called highest temperature of treatment (HTT) and the nature of feedstock (Enders *et al.*, 2012; Kloss *et al.*, 2012; Zhao *et al.*, 2013). A third factor that is sometimes considered is production processes (Schimmelpfennig and Glaser, 2012).

The bulk of research work on biochar appears to be too broad with biochars from multiple feedstock sources considered together but confoundingly and unsystematically produced at variable production temperatures. In addition these biochars may only have been studied with respect to a few physical and chemical parameters. Calvelo Pereira *et al.* (2011), for example studied biochar from three different feedstock at two different pyrolysis temperatures to measure properties like pH, thermal gravimetry, volatiles and elemental contents. In another study, close to twenty different feedstock sources were used in a similar fashion to produce 94 biochars with four different HTT's to determine pH, elemental and proximate analysis (Enders *et al.*, 2012). The authors aimed at investigating stability properties and agronomic values of this large number of biochars in addition to examining the effect of feedstock source and HTT on biochar composition. These many factors and variables make such a study unsystematic and difficult to easily distinguish the effect of HTT from feedstock source. In a similar fashion a total of 66 biochars from 16 different feedstock sources produced at 7 different HTT's using 5 different production processes

(that include ‘others’ i.e. unspecified) were analysed (Schimmelpfennig and Glaser, 2012) with the stated aims of investigating material properties and setting analytical properties for biochar identification. The authors found it necessary to state in their conclusion the need for further research to better separate biochar characteristics due to feedstock and production processes. Hence, to efficiently separate the two principal effects on biochar quality there is a great need for rigorous characterization studies focussed either on biochars from single feedstock source and the effect of varying pyrolysis temperature or production process, or on biochars produced from multiple feedstock sources but rigorously produced at the same HTT and using the same production process. The gap that this research project seeks to fill is the former: the need for rigorous characterization focussed on biochars from a single feedstock source while varying the highest temperature of treatment from two different production processes.

It is beyond the scope of this study to investigate the effects of varying both pyrolysis temperature (HTT) and feedstock sources simultaneously as this is likely to be cumbersome and thus becloud the individual effects of one from the other. This approach is necessary if an advocated characterization data library (Enders *et al.*, 2012) and/or analytical guideline values (Schimmelpfennig and Glaser, 2012) for biochar are to be realised.

4.2 Results

Results on the characterization of freshly synthesized biochar from the same feedstock (Sitka spruce) using the batch and continuous production processes are presented and discussed. Each of the two production techniques were used to produce a set of three biochars at three different pyrolysis temperatures of 400, 600 and 800^{o}C from the same feedstock (see Chapter 3, section 3.3 and Table 3.1). Figure 4.1 shows biochar samples produced using the continuous process.

Figure 4.1: Biochar samples from continuous process (ess) and their temperature of production

4.2.1 Proximate analysis of biochars from the two production processes

The proximate analysis results in Table 4.1 show moisture content fluctuated from a large decrease between 400^{o}C to 600^{o}C and a smaller increase between 600^{o}C to 800^{o}C biochar in both the batch and continuous products (such duplication indicates the slight rise at higher temperature is reproducible). Volatile matter (VM; - material lost at high temperature in the absence of air) shows a consistent decrease with rise in pyrolysis temperature (R^2 = 0.91, Figure 4.2 and Table 4.1) for both biochars (values for ess used in plotting Figure 4.2), with consistent values for the same temperature of production for the two biochar production processes especially between ss/ess600; and ss/ess800 (Table 4.1). Fixed carbon (FC) showed a linear increase with rise in HTT (R^2 = 0.93, Figure 4.2 and Table 4.1). Thus, FC and VM show an inverse relationship as shown in Figure 4.3. The trend in the ash content differs between the two biochars; a drop and rise for the ss (Lancashire) biochar and a rise and drop for the ess (Edinburgh) biochar.

Table 4.1: Proximate analysis results for the fresh biochar samples

Biochar	Parameters			
	Moisture content (%)	Volatile matter (%)	Fixed carbon (%)	Ash content (%)
ibc	1.09	14.67	67.70	16.54
Kbc800	1.72	18.31	35.33	44.63
ss400	1.29	28.91	64.38	5.43
ss600	0.19	8.83	88.82	2.16
ss800	0.29	3.82	92.89	3.00
ess400	0.98	25.93	69.87	3.22
ess600	0.19	8.87	86.31	4.64
ess800	0.35	3.66	92.02	3.97

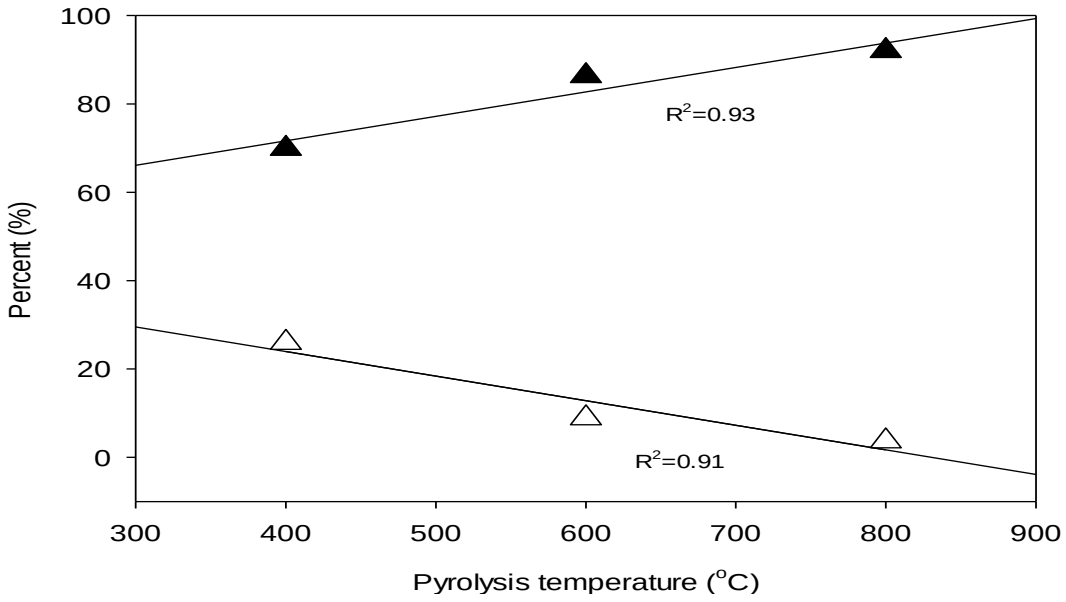

Figure 4.2: Correlation between fixed carbon (filled triangle), volatile matter (empty triangle) and pyrolysis temperature for the ess biochar.

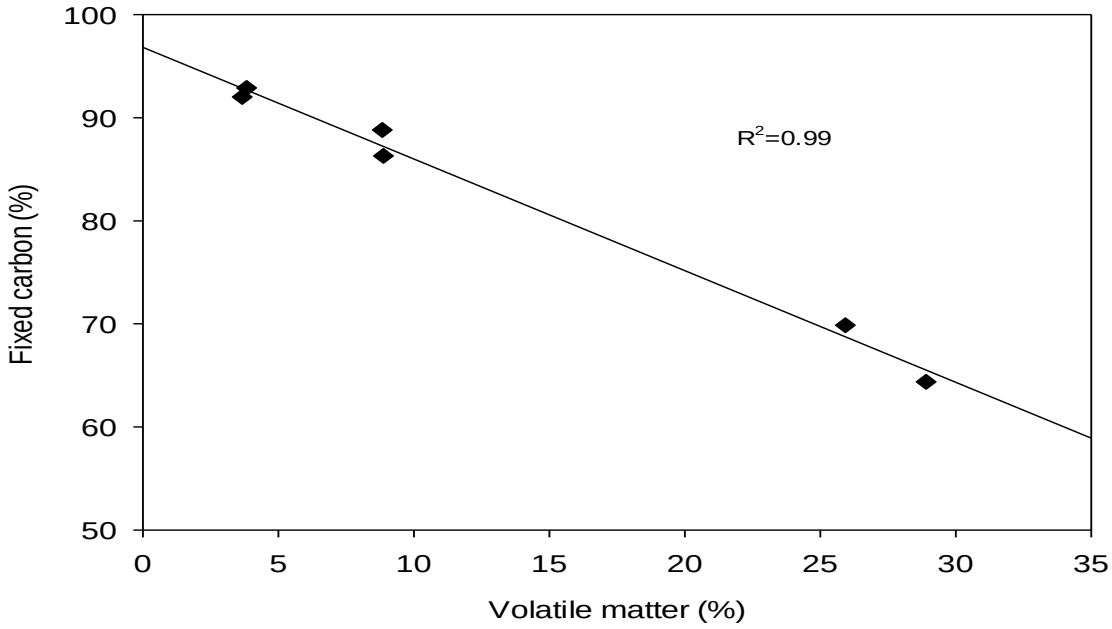

Figure 4.3: Correlation between fixed carbon and volatile matter contents of the fresh biochar samples.

4.2.2 Thermal analysis of biochars from the two production processes

Percentage mass losses for thermally unstable components of the biochars are given in Table 4.2 along with the temperature ranges within which they occur. The first temperature range (59-152^{o}C for Lancashire biochar and 26-146^{o}C for Edinburgh biochar) encompasses free moisture loss through evaporation. The other temperature ranges represent the loss of labile carbon. The values for moisture loss support the trend in proximate analysis, a decrease between 400^{o}C to 600^{o}C HTT and an increase between 600^{o}C to 800^{o}C HTT biochar in both the batch and continuous products. Mass loss between 152 and 430^{o}C represents the release of labile carbon from the decomposition of mostly cellulosic material (Yang *et al.*, 2007) corroborated by the identifiable shoulders and peak (at 421^{o}C for the feedstock) on the Differential Scanning Calorimetry (DSC) curves in Figure 4.5 (a & b). The last three temperature ranges in Table 4.2 cover the loss of recalcitrant carbon from the biochars which decompose at different final temperatures as can be seen in the spaced Thermal Gravimetric (TG) traces in Figure 4.4 (a & b). Similarly Table 4.3 and Figure 4.5 (a & b) show increasing T_{max} (maximum decomposition temperature in the course of pyrolysis) with increase in HTT for the different biochars and the feedstock. T_{max} and HTT

correlate well with R^2 = 0.86 as shown in Figure 4.6. Fuller explanations will be given under the discussion section of this thesis.

Table 4.2: Thermal gravimetry parameters and estimated proportions (%) of thermally unstable components of the biochars at pyrolysis temperature intervals

	59-152°C	152-430°C	430-543°C	430-580°C	430-625°C
ss400	2.50	22.73	70.65		
ss600	0.39	3.47		92.77	
ss800	0.47	1.35			94.46
	26-146°C	**146-400°C**	**400-552°C**	**400-592°C**	**400-640°C**
ess400	1.30	14.26	82.07		
ess600	0.37	1.78		91.86	
ess800	0.58	0.74			94.61
	52-114°C	114-480°C	480-583°C		
Feedstock	4.79	66.43	23.48		

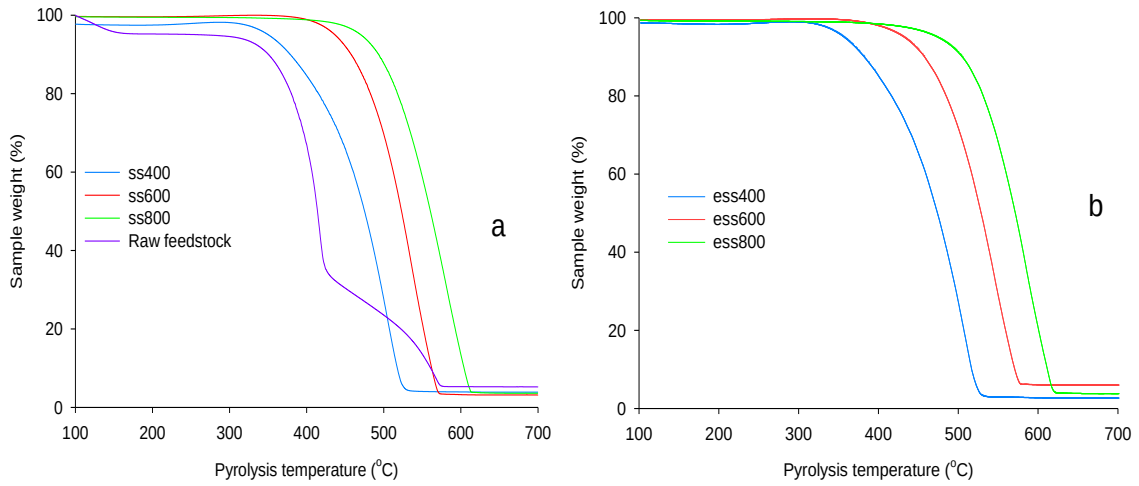

Figure 4.4: Stacked thermal gravimetry plot for Lancashire (a) biochar with raw wood and Edinburgh (b) biochar

Table 4.3: Differential scanning calorimetry parameters and temperature range of peaks and their maximum temperatures (T_{max})

	1st exotherm (°C)	T_{max} (°C)	2nd exotherm (°C)	T_{max} (°C)
ss400	150-430 (s)	348	430-540 (b)	508
ss600	n.d.	n.d.	430-580 (b)	538
ss800	n.d.	n.d.	430-620 (b)	581
ess400	150-430 (s)	351	430-550 (b)	511
ess600	n.d.	n.d.	430-590 (b)	559
ess800	n.d.	n.d.	430-640 (b)	581 & 613
Feedstock	150-480 (b)	421	480-582 (b)	569

S: shoulder; b: broad; n.d.: not detected

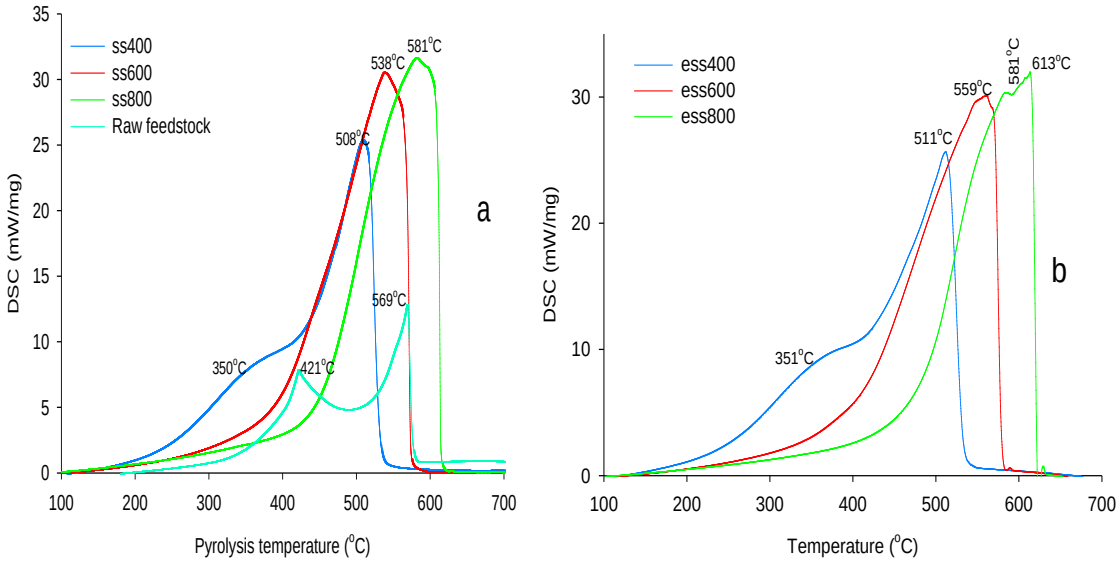

Figure 4.5: Stacked differential scanning calorimetry plot for Lancashire (a) biochar with raw wood and Edinburgh (b) biochar

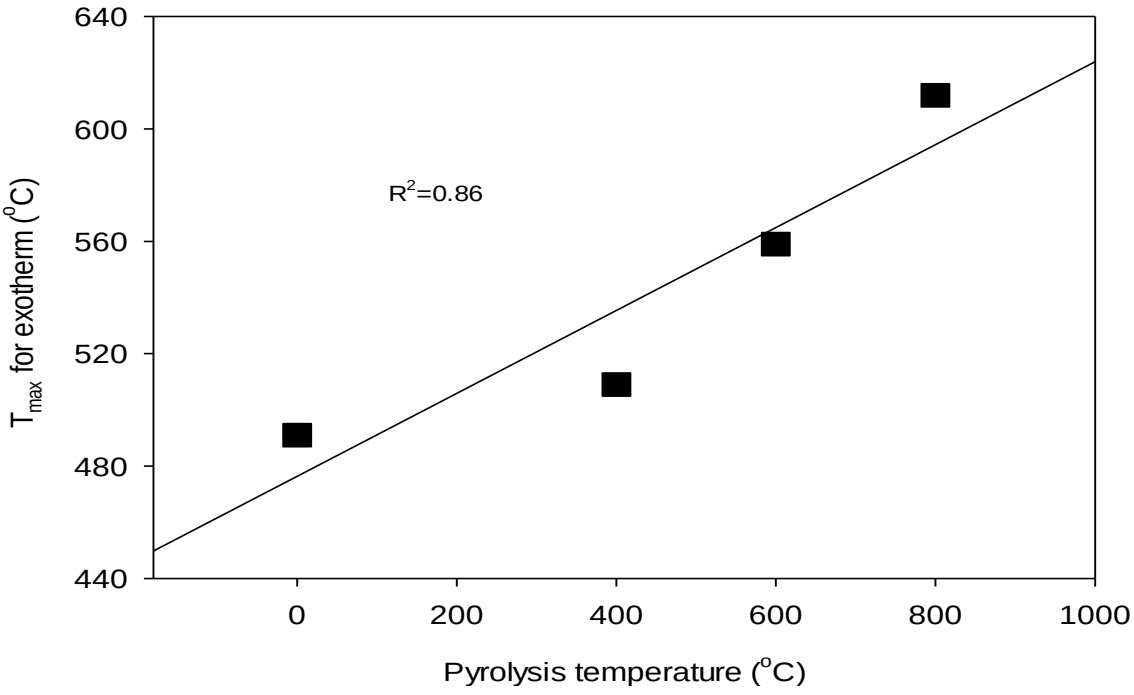

Figure 4.6: Correlation between highest temperature of treatment and maximum temperature of decomposition.

4.2.3 Elemental and other chemical analyses

The results for elemental analysis, pH and other chemical characteristics are recorded in Table 4.4 below. The pH increases with increase in pyrolysis temperature of the biochars (R^2 = 0.90) from acidic in the 400°C through near neutral in the 600°C to a basic character in the 800°C for both streams of biochar. Total carbon (TC) also

increases with rise in pyrolysis temperature and quite similar values for both biochars. The proportion of hydrogen in the biochars drops with rise in HTT, while that of nitrogen increases though with small margins. Oxygen determined by difference (Calvelo Pereira *et al.*, 2011; Enders *et al.*, 2012), also drops with rise in production temperature for the biochars. With higher proportions for total carbon and low proportions for both of hydrogen and oxygen with rise in HTT, the elemental ratios (O:C, H:C and (O+N):C) decreased from the 400^{o}C to the 800^{o}C HTT biochars for both production streams. The significance of these changing ratios and their relationship to pyrolysis temperature will be elaborated in the discussion section. Total organic carbon (TOC) increases with rise in pyrolysis temperature (R^2 = 0.74) and is essentially the same as the total carbon (TC) due to the absence of inorganic carbon (see Figure 4.7 and section 4.3.3), while both cation exchange capacity (CEC) and water holding capacity (WHC) decrease with rise in production temperature. In line with the trend in H content, the surface acidity (SA) of the biochars measured in mmol/g drops with rise in HTT; while surface basicity (SB) increases except for the ss600 biochar which shows a negative value for SA and a fairly big spike for SB compared to the ess600.

An important point to note is that regardless of differences in production processes (Batch for Lancashire biochar and Continuous for Edinburgh biochar), Table 4.4 shows the 400^{o}C biochars to stand well apart from their higher temperature counterparts in many properties such as TC/TOC, WHC, Oxygen, elemental ratios and to some extent even CEC. A similar pattern is observed with fixed carbon (see Table 4.1).

Table 4.4: Elemental composition and chemical characteristics for the fresh biochars

	ibc	Kbc800	ss400	ss600	ss800	ess400	ess600	ess800
pH	8.94	7.85	5.53	6.44	8.13	5.03	6.48	9.37
C (%)	71.89	45.65	75.30	94.08	95.26	83.00	94.18	95.68
H (%)	1.45	1.13	3.08	2.21	0.74	3.62	2.23	0.61
N (%)	0.26	0.65	0.05	0.06	0.07	0.09	0.13	0.17
S (%)	n.d.	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ash (%)	14.49	42.35	3.84	3.13	3.61	2.86	2.48	3.01
O (%)	11.91	10.07	17.73	0.52	0.32	10.43	0.98	0.53
O:C	0.17	0.22	0.24	0.01	0.01	0.13	0.01	0.01
H:C	0.02	0.02	0.04	0.02	0.01	0.04	0.02	0.01
N:C	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
(O+N):C	0.17	0.23	0.24	0.01	0.00	0.13	0.01	0.01
TOC (%)	66.29	40.96	74.73	89.51	92.41	82.53	93.63	95.35
CEC ($mmolcKg^{-1}$)	n.d.	n.d.	7.25	4.63	3.11	10.34	5.66	2.43
BD (gcm^{-3})	0.32	0.28	0.12	0.12	0.14	0.14	0.14	0.13
WHC (%)	41.50	41.00	45.79	35.95	36.09	56.94	44.03	47.63
SA ($mmolg^{-1}$)	0.2	0.4	0.47	-0.20	0.00	0.07	0.00	0.00
SB ($mmolg^{-1}$)	0.33	0.87	0.00	0.13	0.07	0.07	0.07	0.20

Note: TOC: total organic carbon; CEC: cation exchange capacity; BD: bulk density; WHC: water holding capacity; SA: surface acidity; SB: surface basicity; n.d.: not detected.

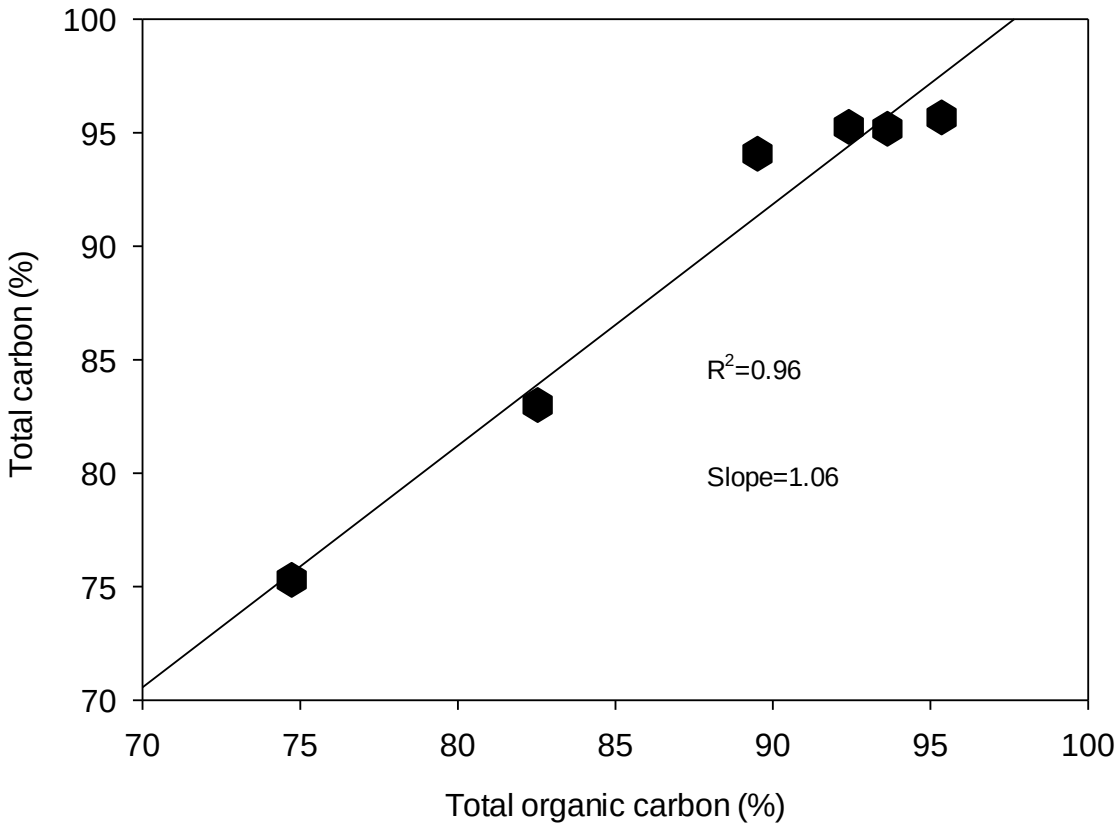

Figure 4.7: Correlation between total carbon and total organic carbon for the biochar

4.2.4 Fourier-transform infra-red (FT-IR) analysis

The wavenumbers of prominent spectral absorbance bands identified from the results of FTIR measurements on the biochars under investigation are marked in Figure 4.8 and recorded along with assigned functional groups in Table 4.5. Possible functional groups assigned based on the absorbance wavenumbers include hydroxyls of water and alcohols, carbonyls of carboxylic acid and ketones, and aliphatic and aromatic ethers.

Table 4.5: Main functional groups assignment for the recorded Fourier-transform infrared spectral bands of the biochars (Chen and Chen, 2009; Cheng et al., 2006; Shen et al., 2010; Yang et al., 2007; Zhao et al., 2013).

Wavenumber (cm^{-1})	Types of bonds, vibrations and compounds
700-900	C-C stretching; Aromatic C-H
1024	Aliphatic C-O stretching of R-OH in an alcohol
1035	C-O stretching in polysaccharides
1200	Aliphatic C-O-C stretching as in Pyranose ring
1232	C-O-C stretching in Aryl-alkyl ether linkage
1370	CH_2, alkyl C-CH_3 bending
1430	O-H bending of an acid; CH_2 and CH_3 bending
1600	-COO anti-symmetric stretching of amino acids; C=O stretching of ketone and carbonyl
1690	O-H bending in H_2O

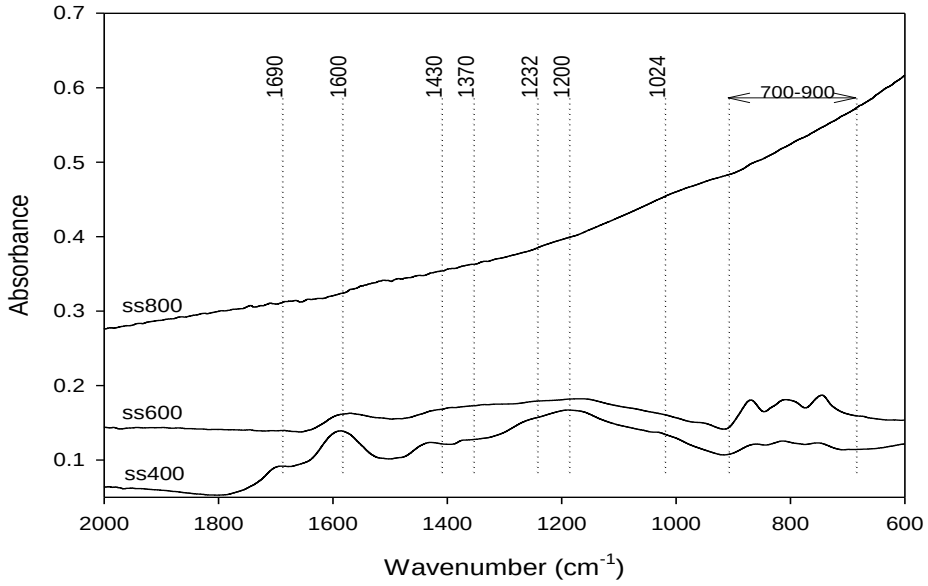

Figure 4.8: Fourier-transform infrared spectral traces for the ss (similar to that for ess) biochar.

4.3 Discussion

4.3.1 Proximate analysis

The moisture content is largely contributed by the loss of free water by evaporation at low temperatures (Chen and Chen, 2009) which may explain the drop with rise in pyrolysis temperature. The rise in moisture content from 600^{o}C to 800^{o}C is confirmed by the values for moisture content from the thermogravimetry plots (see Table 4.2).

The volatile matter (VM) contents decrease with rise in pyrolysis temperature, while fixed carbon content increases with pyrolysis temperature. A similar trend has been reported by Titiladunayo *et al.* (2012) who studied woody biochars produced at temperatures that included 400, 600 and 800^{o}C. The two parameters of FC and VM have been used by Liu and Balasubramanian (2013) to calculate the fuel ratio (FR) which is defined as the ratio between fixed carbon and volatiles (FC:VM); a characteristic value for solid fuels. The higher the fuel ratio the better the fuel quality for the solid fuel in addition to an indication of lower volatiles, hence reduced emission of air pollutants (Liu and Balasubramanian, 2013). These authors determined a fuel ratio of 1.85 for pine wood biochar produced at 350^{o}C. Comparing the biochar samples in this study with one of the biochars studied by Titiladunayo *et al.* (2012) the calculated fuel ratio (FC:VM) increased from 2.23 (for ss400) to 10.06 (for ss600) by over 350% while the Iroko biochar they used increased by just about 100% from 3.65 (for the 400^{o}C biochar) to 7.41 (for the 600^{o}C biochar). These results may indicate better fuel quality for the biochar derived from Sitka spruce. Volatile matter has also been suggested in addition to other parameters as a measure of carbon sequestration potential; a volatile matter content exceeding 80% shows such biochar has no C sequestration value (Enders *et al.*, 2012). All the biochars investigated in this study have less than 30% volatile matter contents (see Table 4.1).

4.3.2 Thermal analysis

The total weight loss during the thermogravimetric analysis reflects loss of water, CO_2 and other volatile products of heating the sample. The weight loss (TG) curves in Figure 4.4 show all the biochar samples losing weight within a minimum of three temperature intervals as recorded in Table 4.2. The mass loss in the first interval (59-152^{o}C) is attributed to free water (Chen and Chen, 2009); while the second interval (152-430^{o}C) could be due the thermal decomposition of the solid residues resulting

from the pyrolysis of hemicellulose and cellulose in the original plant material (Yang *et al.*, 2007) which is Sitka spruce wood in this project. It is worth noting here that temperature intervals for mass loss in TG curves overlap as is obvious from the literature. For example, hemicellulose and cellulose or other labile carbon contents are said to be lost within 200-350^{o}C (Lopez-Capel *et al.*, 2005), 300-350^{o}C (Lopez-Capel *et al.*, 2006) and 220-400^{o}C (Yang *et al.*, 2007). Table 4.2 further shows the labile carbon content of the chars decreases with rise in pyrolysis temperature and this is confirmed by the fact that while in Figure 4.5, a & b (DSC curves) a clear first exotherm (shoulder) is seen for the 400^{o}C biochars, none is discernible for the higher temperature samples and this is held as a characteristic of highly condensed black carbon materials (Harvey *et al.*, 2012). The largest mass loss is recorded from temperatures >403^{o}C which is attributed to the decomposition of mainly aromatic recalcitrant carbon (Lopez-Capel *et al.*, 2006).

Figure 4.4 and Table 4.2 show different upper limits for the third mass loss interval, getting higher with rise in HTT for the biochar samples which likely point to the breakdown of strong aromatic C=C bonds. Table 4.2 also shows the proportion of recalcitrant C contents which are mainly aromatic (Lopez-Capel *et al.*, 2006) increasing with rise in HTT as is also supported by the DSC curves in Figure 4.5 which show increasing T_{max} (maximum decomposition temperature) values with rise in pyrolysis temperature. This observation is in line with the report of Enders *et al.* (2012). The T_{max} values in Figure 4.5 and also in Table 4.3 indicate increased thermal stability of the recalcitrant carbon fractions in the biochar samples with rise in HTT; the higher the T_{max} the more the thermal stability of the fraction. Hence, recalcitrance of the biochars as indicated by T_{max} is directly related to aromatic character of the biochar as shown in Figure 4.9 (R^2 = 0.92). For a single feedstock as the one under investigation therefore, the sole influence on the recalcitrance character of the biochars may be the HTT since both aromatic character and T_{max} increase with increasing HTT as is clear from the R^2 values in Figure 4.10.

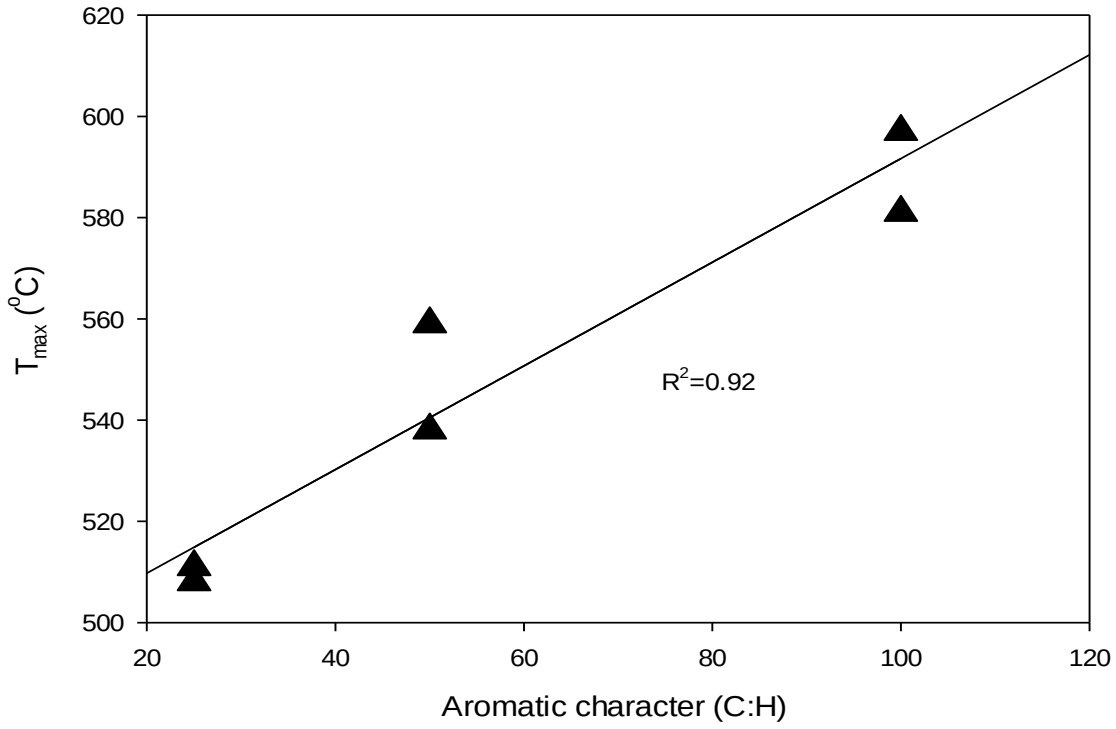

Figure 4.9: Correlation between aromatic character and highest temperature of decomposition (Tmax) for the fresh biochars

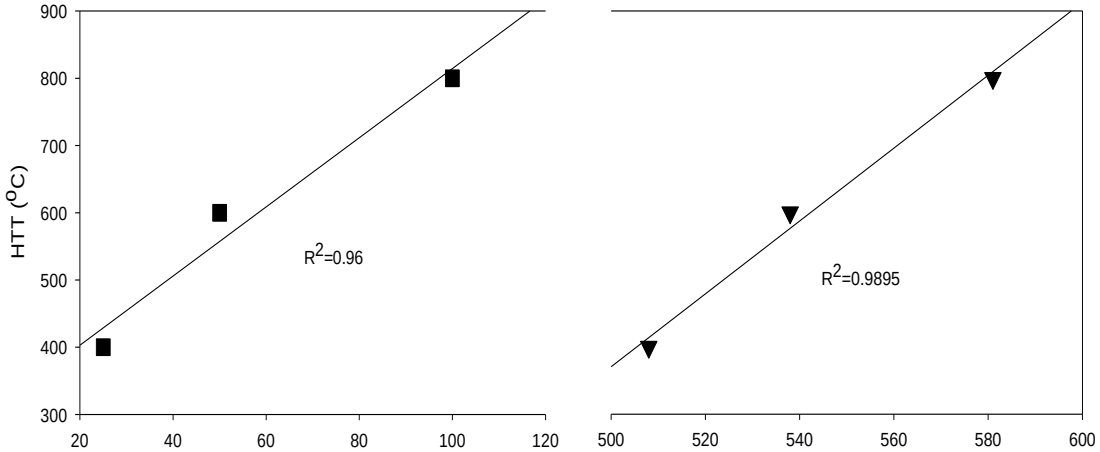

Figure 4.10: Correlations between aromatic character, highest temperature of decomposition (T_{max}) and highest temperature of treatment (HTT) for the biochars.

The T_{max} values for all samples show those for Edinburgh biochar to be higher than the corresponding samples from Lancashire which probably is a factor of difference in production process; continuous for Edinburgh and batch for Lancashire. The higher of

the two T_{max} values for both ss800 and ess800 in the DSC plots (Figure 4.5) may likely be due to the decomposition of some of the ligneous content since it decomposes over a wide range of temperature (Lopez-Capel *et al.*, 2006; Yang *et al.*, 2007; Shen *et al.*, 2010). Such decomposition may then probably explain the increase in moisture content in the 800^{o}C biochars above that for the 600^{o}C biochars as recorded in both Tables 4.1 and 4.2. The increased recalcitrance of the biochars assumed from their enhanced thermal stability can be further evaluated by applying the recently developed (Harvey *et al.*, 2012) method of calculating the recalcitrance index (R_{50}). The recalcitrance index is calculated using the equation:

$$R_{50,\ x} = T_{50,\ x} / T_{50,\ graphite}$$

Where $T_{50,\ x}$ and $T_{50,\ graphite}$ are the temperature values at which half of the weight of the carbon material and graphite are respectively lost through oxidation/volatilisation. The two parameters are directly obtained from the TG thermograms of x (the individual biochar samples in this project) and graphite that have been corrected for moisture and ash. Using the T_{50} values from our corrected TG curves and the T_{50} value of 886^{o}C for graphite as determined by Harvey *et al.* (2012) the calculated R_{50} values for our biochar samples are: ss400 (0.54); ss600 (0.59); ss800 (0.62); ess400 (0.52); ess600 (0.59); and ess800 (0.64). These values place all the biochars into class B ($0.50 \leq R_{50} < 0.7$) on the sequestration potential scale which is an intermediate level above that of the uncharred biomass, $R_{50} < 0.5$ and below that comparable to graphite, $R_{50} \geq 0.7$ (Harvey *et al.*, 2012). Examples of other reported (Harvey *et al.*, 2012) class B biochars produced at 650^{o}C under nitrogen atmosphere include those from loblolly pine (R_{50} = 0.58), eastern red cedar (R_{50} = 0.56) and swamp oak (R_{50} = 0.52). These examples show the biochar under investigation as more recalcitrant (see section 4.1) and hence having better potential as a tool for carbon sequestration since its R_{50} values even at 600^{o}C are higher than those for the compared wood biochars prepared at 650^{o}C (a higher HTT). The recalcitrance index values also add to the conclusion based on their characteristics that the 400^{o}C biochars stand apart from the higher temperature ones as observed earlier.

4.3.3 Elemental and other chemical analyses

Table 4.4 shows pH values increasing with rise in pyrolysis temperature and this agrees with other reports (Pereira *et al.*, 2003; Enders *et al.*, 2012). Increased basic character

(decreased H content) with rise in HTT is likely due to depleted concentration of mainly carboxylic acid functional groups (Pereira *et al.*, 2003), a reason that is also associated with decrease in cation exchange capacity (Enders *et al.*, 2012; Kloss *et al.*, 2012) as the correlation plot in Figure 4.11 shows. Hence, a direct relationship between CEC and H contents exists in the biochars under investigation (Figure 4.12, R^2=0.91).

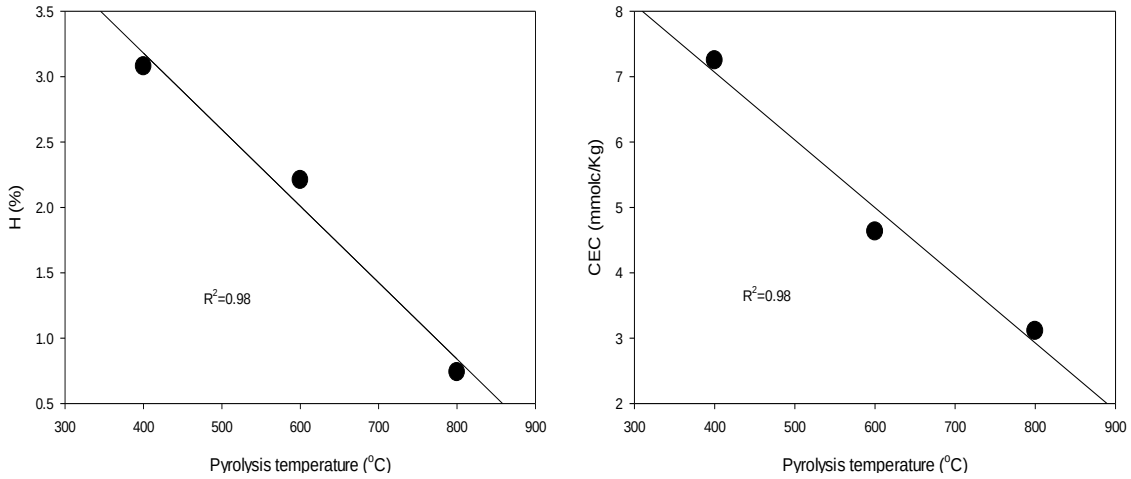

Figure 4.11: Correlations between highest temperature of treatment versus hydrogen; and cation exchange capacity for the biochars under investigation.

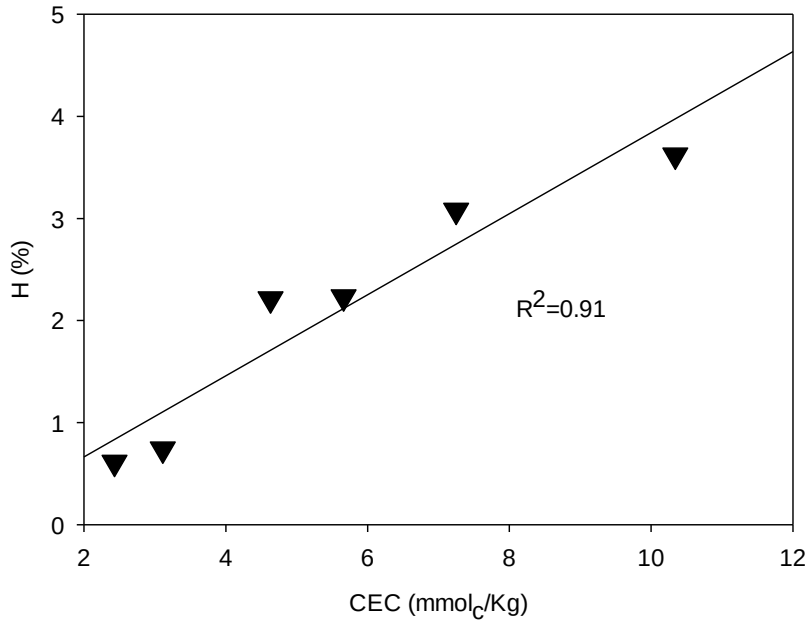

Figure 4.12: Correlation between cation exchange capacity and hydrogen contents of the biochars

Decrease in concentration of carboxyl groups with rise in HTT is supported in our biochars by the disappearance of FTIR absorption bands associated with these groups in the traces for the higher temperature biochars (see Figure 4.8 and Table 4.5). The ultimate analysis (elemental composition) results in Table 4.4 show the C content increasing with temperature, a trend that agrees with other reports (Chen and Chen, 2009; Enders *et al.*, 2012; Kloss *et al.*, 2012). The C content values for the 600 and 800^{o}C biochars almost attain absolute values (100%) and compares well with biochars at these temperatures produced from Apa and Iroko woods (Titiladunayo *et al.*, 2012). Nitrogen also shows some enrichment with rise in pyrolysis temperature and this trend is supported by other reports (Calvelo Pereira *et al.*, 2011; Enders *et al.*, 2012; Kloss *et al.*, 2012; Titiladunayo *et al.*, 2012). The proportions of both H and O decrease with rise in pyrolysis temperature as shown in Table 4.4 which is in line with the observations of many authors (Chen and Chen, 2009; Enders *et al.*, 2012; Titiladunayo *et al.*, 2012). The loss of these two elements with rise in HTT may point to the loss of functional groups as indicated by the flat line FTIR trace for the 800^{o}C biochars (see Figure 4.8).

Increased C content coupled with decrease in both H and O contents of the biochars with rise in temperature means elemental ratios H:C, O:C and (O+N):C decrease with rise in temperature. These decreased values point to increased aromaticity with rise in temperature (Lopez-Capel *et al.*, 2006; Kloss *et al.*, 2012) as can be exemplified with the H contents of cyclohexane (an alicyclic compound with 6 C atoms and 12 H atoms) and benzene (an aromatic compound with 6 C atoms but only 6 H atoms). These elemental ratios have variously been applied in assessing the potential of biochars as a tool for C sequestration, soil amendment and sorption applications. Enders *et al.* (2012) propose that a biochar with volatile matter content < 80%, and O:C_{org} < 0.2 or H: C_{org} < 0.2 may indicate high sequestration value. The values in Table 4.4 therefore show a pattern of increasing sequestration potential with increasing pyrolysis temperature for the biochars in this study especially since the total carbon essentially equals the total organic carbon (see Figure 4.7 with a slope of 1.0645), a fact supported by the absence of any endotherm (carbonate decomposition) beyond 600^{o}C in the DSC thermogram (Figure 4.5) for the biochars which would have indicated the presence of inorganic carbon. Decreased O:C and H:C may also indicate fewer surface functional groups

(Schimmelpfennig and Glaser, 2012) which will hence point to increased aromatic character. Disappearance of surface functional groups with rise in HTT is evident in our samples from the FTIR traces of the higher temperature biochars especially at 800^{o}C where no absorption bands are discernible. The recorded (see Table 4.4) decrease in Cation Exchange Capacity (CEC) for the investigated biochars with rise in pyrolysis temperature (see Figure 4.11a) may also be due to the diminished amount of functional groups (Kloss *et al.*, 2012). The (O+N):C is a measure of polarity of the surface groups (Chen and Chen, 2009) which decreases with rise in temperature for the biochar samples in this study. Decreased polarity coupled with significant surface area (not measured in this project) in high temperature biochars may enhance retention of non-polar pollutants (Kloss *et al.*, 2012). The elemental analysis reported zero readings for S (not tabulated) which may indicate its absence in our samples and this probably makes these biochars environmentally friendly in terms of SO_x emissions. Titiladunayo *et al.* (2012), claimed such environmental friendliness for the biochars they investigated for industrial applications even though they contain S concentrations of up to 0.30%. The water holding capacity for the biochar samples under investigation as recorded in Table 4.4 compares with the value of 34% reported for oak wood biochar at 600^{o}C (Nguyen and Lehmann, 2009).

There seems to be no clear trend in the values for surface amphoteric properties especially for the Lancashire biochar as shown in Table 4.4. Edinburgh biochar though shows a decrease in surface acidity and increase in surface basicity with rise in HTT. This is in line with our pH and FTIR results and agrees with the reports of (Chun *et al.*, 2004). Negative results for an amphoteric surface property has been reported (Pereira *et al.*, 2003) and may indicate high values for the opposing property since surface acidic and basic sites co-exist and seem to be inversely related (Boehm, 2002).

4.3.4 FT-IR

The bands between 700-900cm^{-1} (see Figure 4.8) are assigned to aromatic structures that include C-H bonds (Yang *et al.*, 2007; Kloss *et al.*, 2012) and their broadening in ss600 indicates a shift to more condensed carbon and an increase in aromatic nature with rise in HTT which agrees with the findings of a number of authors (Chen and Chen, 2009; Kloss *et al.*, 2012). This helps to confirm the observed decrease in the concentrations of H with rise in pyrolysis temperature (see Figure 4.11a). Kloss *et al.*

(2012) observed that a band at 875cm^{-1} may also indicate vibrations due to carbonates, but this is discounted for the samples here since no endotherm exists on the DSC curves in Figure 4.5. The band in the region 1024-1035cm^{-1} is assigned to aliphatic C-O stretching of alcohols (Shen *et al.*, 2010) and polysaccharides (Cheng *et al.*, 2006) which are cellulosic materials. The loss of these bands in the higher temperature biochars (600 and 800^{o}C) again indicates shift to more recalcitrant carbon as soft carbon fractions are eliminated (Chen and Chen, 2009). The band around 1200cm^{-1} in the 400^{o}C biochars is assigned to aliphatic ethers while the one at 1232cm^{-1} in the 600^{o}C biochars represents an aryl-alkyl C-O-C linkage (Yang *et al.*, 2007). This transformation may also be another indication of developing aromatic character with rise in HTT. The band in the region 1310-1370cm^{-1} is assigned to a methylene group (Chen and Chen, 2009) and aliphatic C-CH_3 bending vibration (Shen *et al.*, 2010) and that band is greatly diminished in the 600^{o}C biochars; and it along with all other aliphatic absorption bands completely disappears in the 800^{o}C biochars, again indicating gradual dehydrogenation leading to formation of aromatics at higher temperatures. This conforms with the observation of Zhao *et al.* (2013) who explained such disappearance of aliphatic groups results from the dehydration of cellulosic and ligneous components. The absorption band at 1430cm^{-1} is assigned to O-H bending vibration of an acid (Yang *et al.*, 2007) and this group appears removed in the trace for the 600^{o}C biochars. This goes to support the observed increase in pH with rise in pyrolysis temperature and is in line with the observations of Chun *et al.* (2004), that rise in temperature reduces surface acidity. Pereira *et al.* (2003) also reported temperature programmed desorption (TPD) results that showed the removal of acidic oxygen groups at 700^{o}C. The strong absorption band at about 1510-1600cm^{-1} is assumed to be due to anti-symmetric stretching vibration of amino acids (Zhao *et al.*, 2013) and C=O stretching of ketones and carbonyls (Yang *et al.*, 2007). This band appears diminished in the 600^{o}C biochars to about a third of its size in the 400^{o}C biochars which may indicate decarboxylation of acidic groups that could further support increase in basic character with rise in HTT. The final spectral band for our samples is around 1690cm^{-1} and is assigned to O-H bending vibration in water molecules (Shen *et al.*, 2010). The band clearly disappears in the 600^{o}C biochars which supports measured decreased moisture content and also increased basicity with rise in pyrolysis temperature.

4.4 Conclusion

The totality of the results considered goes a long way in addressing the first objective and hypothesis put forward in this study; that physico-chemical properties of biochar are progressively altered with increasing pyrolysis temperature. The progressive changes in properties recorded in this Chapter may provide a framework of understanding the trend of biochar amendment impact on plant growth and soil processes. With the feedstock source fixed in this study, the results clearly show that temperature of production is an important factor that alters both physical and chemical properties of biochars (Wang *et al.*, 2013a), a position supported in the case of C content by the report of Enders *et al.* (2012).

It could also be concluded that as noted in this thesis the 400^{o}C biochar stands well apart from the higher temperature biochars which show only minor differences between themselves in properties like all proximate analysis (except ash content), pH, TC, TOC, thermal behaviour, elemental ratios and CEC. These minor differences in many properties between the two higher temperature biochars mean depending on the desired property it could be cheaper to produce and use the 600^{o}C biochar rather than the 800^{o}C. Production process may also influence some biochar properties as shown by identifiable differences, even though small, between the Lancashire and Edinburgh biochars produced from the same feedstock and at the same temperatures. The Sitka Spruce biochar may have both high fuel quality and C sequestration potential as evidenced by its satisfactory fuel ratio and recalcitrant index values.

Chapter 5 The impact of biochar amendments on plant growth and the physico-chemical properties of amended soils.

5.1 Introduction

Considerable research effort (see Chapters 1 and 2) has gone into determining the potential of biochar as a vehicle to sequester atmospheric CO_2 and hence fight climate change. To properly establish the importance of biochar as a soil additive for this purpose it must not negatively affect the bio-physicochemical properties of the soil which are critical in maintaining soil health. A healthy soil is one that possesses qualities which make it fit to provide many important ecosystem goods and services (Haygarth and Ritz, 2009) that include supporting the growth of food crops, livestock and space for building and recreation (Kennedy and Smith, 1995). The potential impact of biochar on the agronomic properties of a given soil could be assessed by determining how biochar application impacts on the growth and yield of plant material in the amended soil, which will be a reflection of improvements in the bio-physico-chemical properties of the soil. These properties and how biochar application to soil affects them are crucial in determining the health status of the amended soil.

Masahide *et al.* (2006) for example reported on the impact of biochar amendments on the chemical properties of an acid soil and yield of maize, cowpea and peanut crops in a field experiment, but they used a biochar with no definite pyrolysis temperature (260-360 ^{o}C) and an application rate of 10 Lm^{-2} both of which create difficulty in results comparison. Asai *et al.* (2009) monitored soil physical properties and grain (rice) yield on various soils with pH range of 5.2-8.3 amended with biochar (at 4, 8 and 16t/ha) from wood wastes in a field experiment. The authors however, used a commercial biochar with no information on its highest temperature of treatment (HTT) which makes the investigation unsystematic and results difficult for comparison. Biochar from waste water sludge (HTT, 550^{o}C) was used in a greenhouse experiment to study the bioavailability of metals and the yield of cherry tomatoes in an acid soil (Hossain *et al.*, 2010). The authors monitored plant height as an indicator of plant growth. Sukartono *et al.* (2011), reported on the effect of biochar amendment (in a field experiment) on soil fertility and maize yield in a sandy loam soil, though they used three non-wood feedstock sources and a biochar production method that only gave a

range for HTT (200-300^{o}C) again making it unsystematic and raising difficulties in comparing results. Other researchers worked on the possible effect of biochar addition to bio-fortify some crops with zinc metal (Gartler *et al.*, 2013) using a large number of crops (11) ranging from above ground shrubs such as lettuce to underground tubers like carrot. The authors used weight of dry biomass as a measure of amendment impact on plant growth. However, making a definite statement on the impact of biochar on the agronomic properties of an amended soil is not easy as it depends on the nature of the soil-crop-climate trio (Enders *et al.*, 2012). Hence in this study, in addition to using a single feedstock source to produce the test biochar, a single plant (leek) had been used within a uniform climatic condition in form of the greenhouse, all in an effort to make the result of impact clearer. Plant growth in this investigation was monitored by measuring the ground level diameter of the leek plant which grows upwards as a single unbranched shoot (Hay and Kemp, 1992) making diameter measurement easy. The diameter measurement gives a more dependable linear dataset compared to monitoring biomass weight, which is prone to errors resulting from handling during harvest. Diameter measurement also allows continuous monitoring of plant growth dynamics and the impact of biochar throughout the experimental period while biomass estimation happens only at the end of the experiment.

Figure 5.1: Potted leek plants in the greenhouse

The influence of biochar on soil fertility and plant growth/yield have been assessed using both pot experiments (Hartley *et al.*, 2009; Graber *et al.*, 2010; Hossain *et al.*, 2010; Gartler *et al.*, 2013) and field studies (Masahide *et al.*, 2006; Asai *et al.*, 2009; Sukartono *et al.*, 2011). Parameters measured to monitor plant growth or crop yield range from fresh biomass weight (Masahide *et al.*, 2006; Gartler *et al.*, 2013), dry biomass weight (Hossain *et al.*, 2010), weight of harvested crop (Asai *et al.*, 2009; Sukartono *et al.*, 2011), and weight/height of shoot (Khan *et al.*, 2013; Schulz *et al.*, 2013). Similarly, various types of crops have been used by researchers as test plants and these in most cases include maize (Masahide *et al.*, 2006; Major *et al.*, 2010;

Sukartono *et al.*, 2011; Zhang *et al.*, 2012; Gartler *et al.*, 2013), rice (Asai *et al.*, 2009; Khan *et al.*, 2013), wheat (Van Zwieten *et al.*, 2010; Vaccari *et al.*, 2011) and tomatoes (Graber *et al.*, 2010; Hossain *et al.*, 2010), with a few reporting the use of beans (Van Zwieten *et al.*, 2010) and leek (Gartler *et al.*, 2013) amongst others. For the purpose of this work, pot experiments were employed using leek (*Allium porrum*) as the test plant in two different soils; sandy of low pH and loamy/clayey of near-neutral pH. Leek growth was monitored by measuring the diameter of the above ground part of the stem. A preliminary (pilot) pot experiment was conducted as part of method development (see Chapter 3, section 3.6 and Table 3.2) for the two subsequent experiments. Available data from the pilot experiment are also given here for comparison to those from the main experiments.

The specific objective this chapter sought to achieve was to assess how the addition of biochar produced at different pyrolysis temperatures using different production processes to two soil types (low and near-neutral pH) affects the physicochemical properties of the amended soil and influences plant growth in both soils compared to unamended controls. These biochars have already been shown to possess systematically variable properties (see Chapter 4) many of which are likely to affect the properties of soils to which they could be added. It was hypothesized (see hypotheses 4 and 5 in Chapter 1 section 1.4) that:

- Different biochar pyrolysis temperatures and their application rates will significantly alter the pH, total organic carbon (TOC) contents, bulk density (BD) water holding (WHC), and cation exchange capacities (CEC) of soils to which the biochar was added.
- Different biochar pyrolysis temperatures and their application rates influence biochar's ability to impact on the growth rate of Leek plant in amended soils compared to control soils.

5.2 Results

The results concerning the impact of biochar addition on the agronomic properties of the two types of amended soils (low pH and near-neutral) are graphically presented under section 5.2.1 and in Table 5.1. The first section is on the biochars' impact on the physico-chemical properties of the amended soils (pH, TOC, BD, WHC and CEC) while a second section (5.2.2) is on the impact of biochar on leek growth (presented as rates)

in the two soils against relevant controls. Under both sections, relevant ANOVA statistical tables are provided.

Table 5.1: Summary of the investigated agronomic properties of the amended soils. The sandy acid soil was amended with ss biochar and the loamy/clayey was amended with ess biochar.

Biochar HTT (°C)			400			600			800		
			Amendments (%)			Amendments (%)			Amendments (%)		
Parameter	**Soil Type**	Control	1	5	10	1	5	10	1	5	10
pH	sandy	4.30	4.45	4.46	4.44	4.34	4.42	4.60	4.45	4.76	4.77
	loamy/clayey	6.54	6.46	6.28	6.41	6.50	6.50	6.52	6.53	6.55	6.99
	sandy (pilot)	5.20	5.42	5.53	6.25				5.50	6.34	6.65
TOC (%)	sandy	2.31	2.81	4.13	6.50	2.94	6.22	7.83	2.99	5.45	7.95
	loamy/clayey	2.19	2.75	5.41	9.02	3.09	6.74	6.01	3.53	6.23	10.84
	sandy (pilot)	2.69	2.69	4.58	7.23				2.69	4.02	6.54
BD	sandy	1.15		0.90			0.87			0.90	
($g\ cm^{-3}$)	loamy/clayey	1.16		0.78			0.94			0.92	
%CEC incr	sandy		21.52	35.18	47.70	21.14	30.25	25.58	18.67	21.75	26.86
WHC (%)	sandy	53.53	52.89	73.89	105.36	60.42	75.68	90.07	32.03	79.70	146.77
	loamy/clayey	52.55	54.19	62.48	75.40	54.34	59.64	71.55	56.05	60.02	72.51
	sandy (pilot)	54.54	60.45	64.64	70.34				56.78	62.71	66.74

TOC: total organic carbon; BD: bulk density; CEC: cation exchange capacity;

WHC: water holding capacity (replicated measurements taken only in the acid soil of the pilot experiment.

Note: HTT 400 does not apply to values for the pilot experiment since no HTT is available for the ibc biochar.

5.1.1 Impact on soil properties

Figures 5.2, 5.3a and 5.3b show the impact of the various levels of biochar amendments (1, 5 and 10% ; 10, 50 and 100t/ha) on the pH of amended soils. Figure 5.2 is for the acid soil amended with ibc and kbc800 biochars used in the pilot experiment while Figures 5.3a and b are for acid soil amended with ss biochar in the first experiment and near neutral soil amended with ess biochar in the second experiment respectively. Comparing the controls (unamended soils) with the amended soils, statistical treatment of the data (Table 5.2) shows the 5 and 10% amended soils in the pilot and first experiments differ significantly from their respective controls (Univariate ANOVA, Post Hoc Tests, p = 0.000), while the 1% amendment in both cases is not significantly different from the controls.

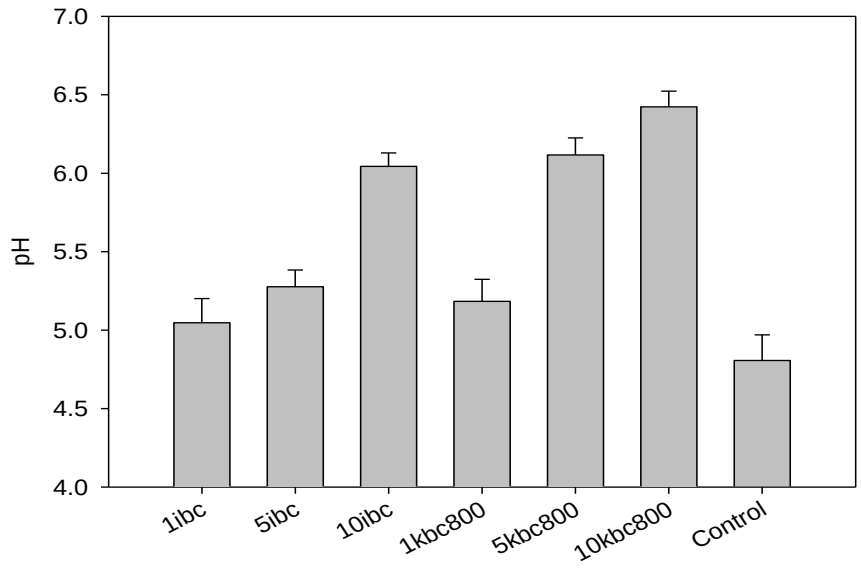

Figure 5.2: Impact of ibc and kbc800 biochars on the pH of the acid soil used in the pilot experiment. The initial numbers in the sample codes represent weight percent of added biochar. Error bars represent ±SE of the means.

ibc: an interreg biochar; kbc: biochar from a previous research project (see Chapter 3 Table 3.3).

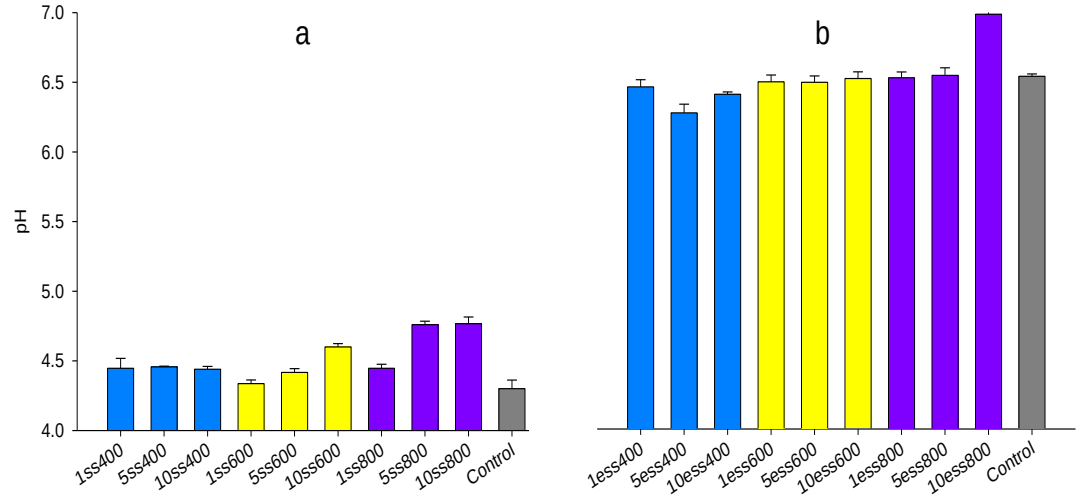

Figure 5.3: Impact of different levels of amendments using biochar at the different highest temperature of treatments on soil pH for (a) low pH and (b) near-neutral soils. Error bars represent ±SE of the means.

1ss400, 1ess400: 1% amendment with 400^{o}C Lancashire and Edinburgh biochar respectively.

For the near neutral soil (Figure 5.3b) none of the pH values of the amended soils significantly differed from the control; p values are 0.521 for the 1%, and 0.138 for each of the 5% and 10% amendment levels (Table 5.2).

Table 5.2: Analysis of variance results comparing p values between controls and factors (amendment level and highest temperature of treatment) for the amended soil properties investigated. This goes to test the fourth hypothesis of this study.

	Experiment	Pilot			1st			2nd		
	Soil type	acid						Near neutral		
Variable	Amendment level (%)	1	5	10	1	5	10	1	5	10
pH	Control x	0.124	0.000*	0.000*	0.059	0.000*	0.000*	0.521	0.138	0.138
BD		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CEC		n.d.	n.d.	n.d.	0.000*	0.000*	0.000*		n.d.	
TOC		0.991	0.011*	0.000*	0.501	0.007*	0.000*	0.346	0.001*	0.000*
WHC		0.009*	0.000*	0.000*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	HTT (°C)	400	600	800	400	600	800	400	600	800
pH	Control x	n.d.	n.d.	0.000*	0.014*	0.012*	0.000*	0.025*	0.612	0.033*
BD		n.d.	n.d.	n.d.	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
CEC		n.d.		n.d.	0.000*	0.000*	0.000*		n.d.	
TOC		n.d.		0.006*	0.031*	0.003*	0.004*	0.003*	0.003*	0.000*
WHC		n.d.		0.000*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

*mean difference significant at the 0.05 level

n.d.: not determined; for the pilot experiment due to lack of information on the HTT for one of the biochars (ibc).

BD: bulk density (measured only for 5% amended soils in both test soils).

CEC: cation exchange capacity (measured only in the first experiment); TOC: total organic carbon.

WHC: water holding capacity (replicated measurements taken only in the pilot experiment).

1st experiment is the first set of experiments after the pilot where ss biochar was used to amend the acid soil (see Chapter 3 section 3.6 Table 3.2)

2nd experiment is the second set of experiments where ess biochar (at all amendment levels) and ss biochar (only at 5% amendment level) were used to amend the near neutral soil (see Chapter 3 section 3.6 Table 3.2).

Two factor ANOVA was done on all the soil properties data (Table 5.3) to determine differences between the amendment levels and the biochars' highest temperature of treatment (HTT). For the pH, the three amendment levels significantly differ from each other in the pilot experiment (Univariate ANOVA, Post Hoc Tests, p = 0.002 (1x5); p = 0.000 (1x10) and p = 0.004 (5x10)), while in the first experiment there is no significant difference between the 5 and 10% amendment levels (Univariate ANOVA, Post Hoc Tests, p = 0.153) and no significant difference between the 1 and 5% amendment levels for the pH in the near neutral ess amended soil (Univariate ANOVA, Post Hoc Tests, p = 0.221). The pH in both the acid and near neutral soils shows significant differences between the three HTT's except for the insignificant difference between the 400 and 600°C biochars in the acid soil (Table 5.3; Univariate ANOVA, Post Hoc Tests, p = 0.933). This reflects the major trend of increased pH values for the biochars with rise in HTT (see Chapter 4, section 4.2.3 and Table 4.4).

Table 5.3: Analysis of variance results comparing p values within factors (Amendment levels and highest temperature of treatment) for the amended soil properties investigated.

	Experiment	Pilot			1st			2nd		
	Soil type				acid			Near neutral		
Variable	Amendment level (%)	1	5	10	1	5	10	1	5	10
pH	1		0.002*	0.000*		0.002*	0.000*		0.221	0.006*
	5									
	10		0.004*			0.153			0.000*	
BD	1									
	5		n.d.			n.d.			n.d.	
	10									
CEC	1					0.013*	0.001*			
	5		n.d.						n.d.	
	10					0.062				
TOC	1		0.004*	0.000*		0.003*	0.000*		0.000*	0.000*
	5									
	10		0.000*			0.005*			0.000*	
WHC	1		0.001*	0.000*						
	5					n.d.			n.d.	
	10		0.001*							
	HTT (°C)	400	600	800	400	600	800	400	600	800
pH	400					0.933	0.000*		0.014*	0.000*
	600		n.d.							
	800					0.000*			0.001*	
BD	400					0.236	1.000		0.000*	0.000*
	600		n.d.							
	800					0.236			0.117	
CEC	400		n.d.			0.010*	0.001*			
	600								n.d.	
	800					0.284				
TOC	400					0.083	0.140		0.992	0.040*
	600		n.d.							
	800					0.753			0.040*	

*mean difference significant at the 0.05 level

n.d.: not determined; for the pilot experiment due to lack of information on the HTT for one of the biochars (ibc).

All amended soils show increased TOC contents compared to the controls both in the pilot (Figure 5.4) and the other experiments (Figure 5.5). However, the increases in the 1% amended soils are not significantly different from the controls in all of the pilot experiment (Univariate ANOVA, Post Hoc Tests, p = 0.991), the first experiment (Univariate ANOVA, Post Hoc Tests, p = 0.501) and the second experiment (Univariate ANOVA, Post Hoc Tests, p = 0.346) (Table 5.2). In comparison with the controls all the HTT's of the biochars show significant influence on the TOC contents (Table 5.2) and this agrees with the trend of increasing biochar TOC contents with HTT (R^2 = 0.74) (see Chapter 4, Table 4.4).

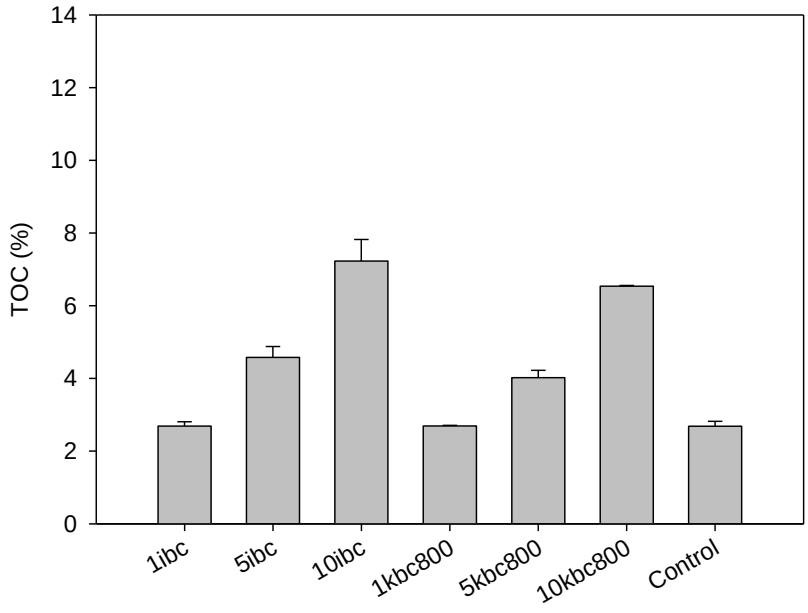

Figure 5.4: Impact of ibc and kbc800 biochars on the total organic carbon contents of the acid soil used in the pilot experiment. The initial numbers in the sample codes represent weight percent of added biochar. Error bars represent ±SE of the means.

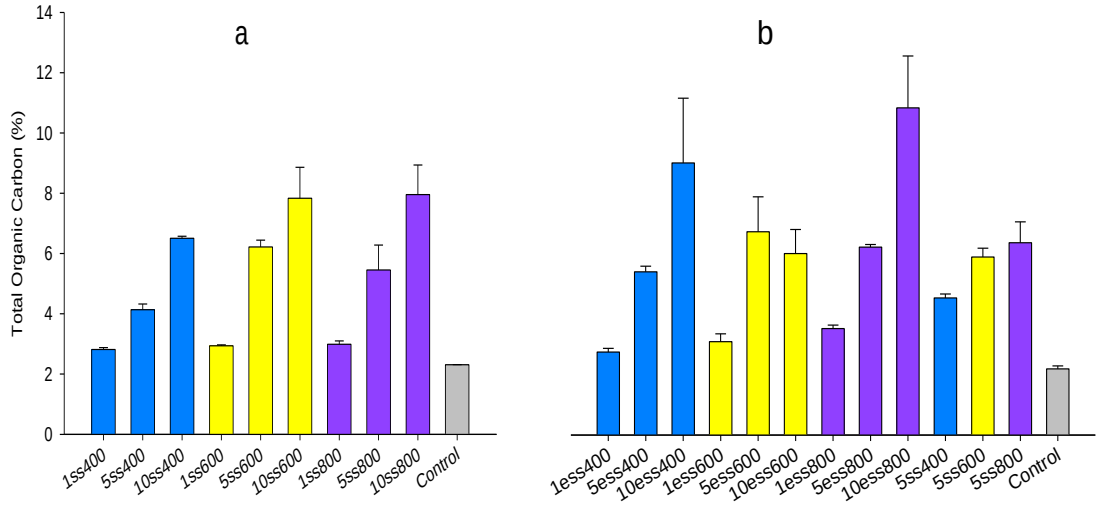

Figure 5.5: Impact of the different biochars used at different amendment levels on the total organic carbon contents of (a) the low pH soil and (b) the near-neutral soil. Error bars represent ±SE of the means.

Comparison between the amendment levels shows they have significantly different impacts on the TOC contents of the amended soils (Table 5.3). But a similar comparison between the HTT's shows significantly different influences on TOC contents only between the 400/800 (Univariate ANOVA, Post Hoc Tests, $p = 0.040$) and 600/800^{o}C (Univariate ANOVA, Post Hoc Tests, $p = 0.040$) biochars in the near neutral

soil (Table 5.3). The increases in the TOC contents of the amended soils with both amendment levels and HTT's of the biochars is a reflection of the trend in TOC contents of the biochars (Chapter 4, section 4.2.3, Table 4.4).

Figure 5.6 shows all bulk densities of the 5% amended soils are lower than the respective unamended controls. There were no post hoc tests for amendments on bulk density data (Tables 5.2 and 5.3) because the property was determined on the same amendment level (5%) in both soil types. But compared to the control, the different biochar HTT's show a significant lowering effect on the bulk density (Univariate ANOVA, Post Hoc Tests, p = 0.000) (Table 5.2) in both soil types. However, between the HTT's significantly different influence on the BD exist only between 400/600 and 400/800^{o}C biochar pairs in near neutral soil.

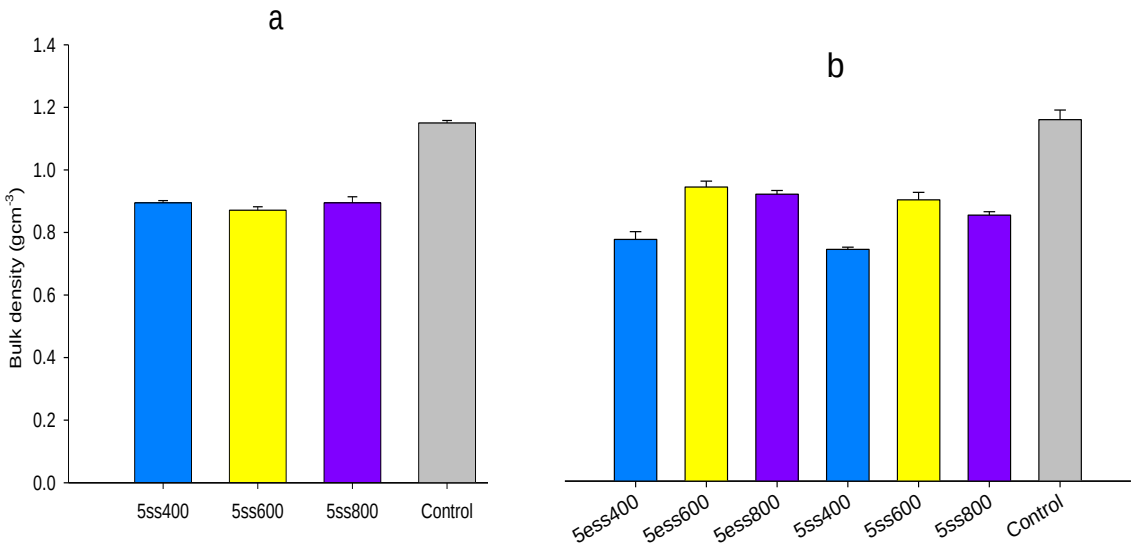

Figure 5.6: Impact of the different biochars at 5% amendment rate on the bulk density of (a) the low pH soil and (b) the near- neutral soil. Error bars represent ±SE.

Cation exchange capacity (CEC) was determined only for the ss amended acid soils and is presented as percentage increase in CEC over the control unamended soil (Figure 5.7). All the amended soils have significantly different CEC from the control at all three amendment levels and for all biochar HTT's (Univariate ANOVA, Post Hoc Tests, p = 0.000) (Table 5.2). However, comparing p values within the factors (amendment level and biochar HTT's) shows no significant CEC difference between the 5% and 10% amended soils (Univariate ANOVA, Post Hoc Tests, p = 0.062) (Table 5.3) just as there is no significant difference influencing the CEC between the 600 and 800^{o}C biochars (Univariate ANOVA, Post Hoc Tests, p = 0.284) (Table 5.3).

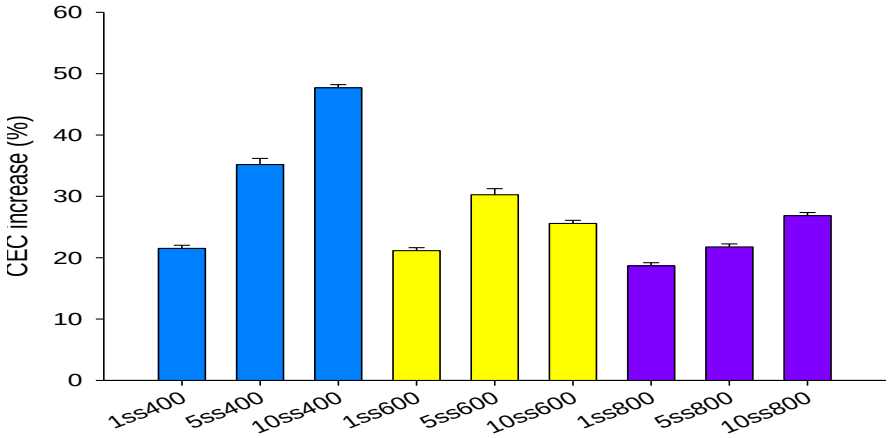

Figure 5.7: Percentage increase in cation exchange capacity for the ss amended low pH soils over the control. Error bars represent ±SE of the mean.

Figure 5.8 shows changes in water holding capacity (WHC) of the amended acid soil from the pilot experiment while Figure 5.9 shows the impact of amendment on the acid and near neutral soils. Replicate measurements were only taken on the pilot amended soils hence the only ones for which statistical treatment of data are available (Tables 5.2 and 5.3). Compared with the control, all the amended soils have significantly different WHC values (Table 5.2). The amendment levels also have significantly different influences between them on the WHC of the amended soils (Table 5.3; p values, 0.000 and 0.001). These ANOVA results could be assumed to be true at least for the amended soils of the first experiment (Figure 5.9a) where the same soil as the pilot was used.

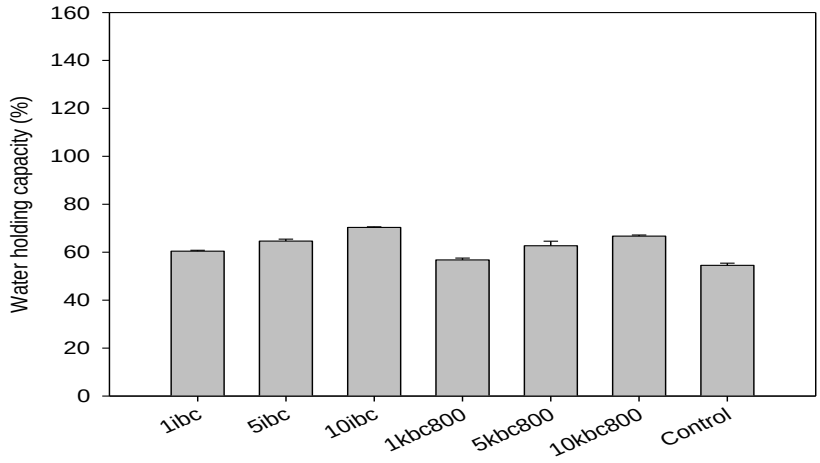

Figure 5.8: Impact of the different levels of biochar amendments on the water holding capacity of the low pH soils from the pilot experiment. Error bars represent ±SE of the mean and those not visible have too small values.

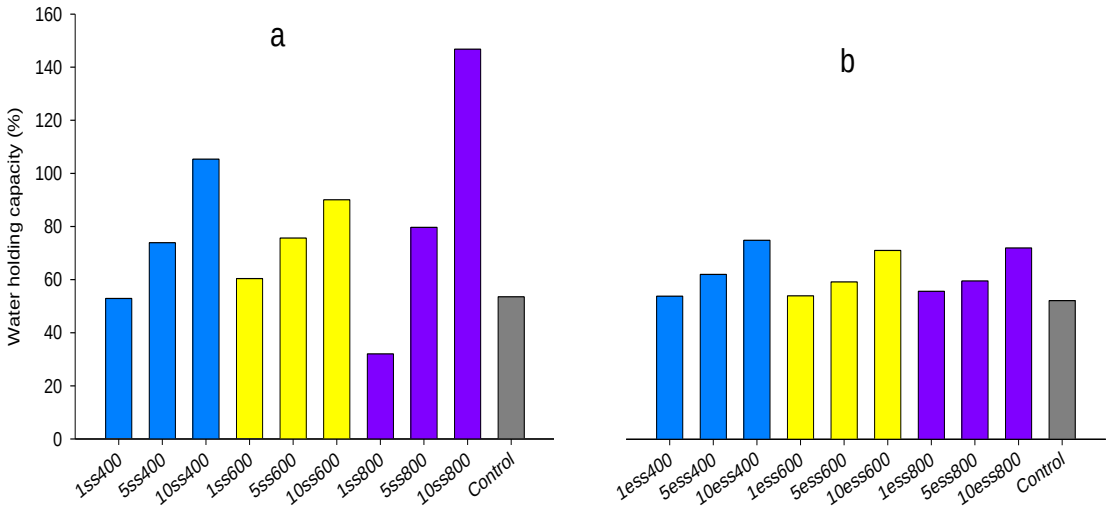

Figure 5.9: Impact of the different levels of biochar amendments on the water holding capacity of the (a) low pH and (b) near-neutral soils.

5.2.2 Impact on leek growth

The rates of Leek growth in mm/week in the pilot experiment are shown in Figure 5.10 while Figure 5.11 shows the leek growth rates in the two other plant trial experiments. Compared to the controls, only 1% (10t/ha) amended acid soil shows significantly different (Univariate ANOVA, Post Hoc Tests, p = 0.018) leek growth rate in the pilot experiment which from Figure 5.10 is obviously due to the wilted growth of the leeks in the 1% amended soil. The reason for this is not clear since if it were toxicity due to amendment there should have been a greater effect in the higher amendments. Only the 5% amendment in the first experiment (Univariate ANOVA, Post Hoc Tests, p = 0.015) is significantly different from the control but none of the amendment levels in the near neutral soil of the second experiment showed any significant difference compared to the control (Univariate ANOVA, Post Hoc Tests, p > 0.05) (Table 5.4). Influence of biochar HTT on the growth rate is only significant for the 600^{o}C biochar in the acid soil of the first experiment (Table 5.4, Univariate ANOVA, Post Hoc Tests, p = 0.041). Two factor ANOVA shows no significant difference (p > 0.05) in impact on leek growth rate between any pair of amendment levels or biochar HTT's in both the first and second experiments (Table 5.5).

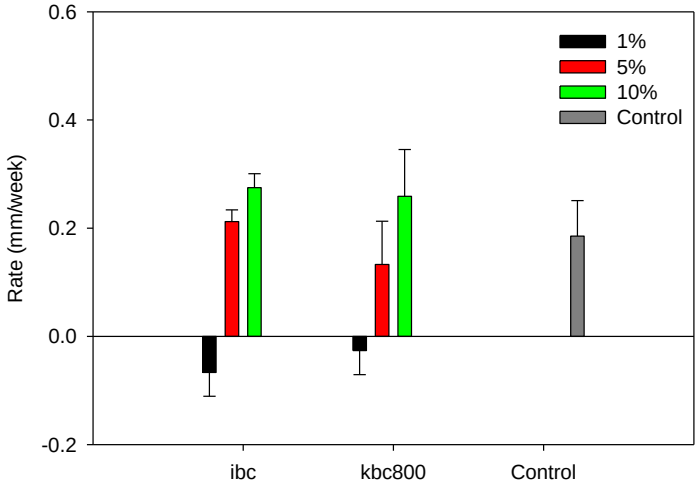

Figure 5.10: Leek growth rates in the pilot experiment. The acid soil was amended with the interreg (ibc) and previous project (kbc800) biochars. Error bars represent ±SE of the means.

Rates were calculated as slopes of linear regression lines from plots of leek diameter against sampling times for each treatment in the replicated experiments. Percentages are amendment rates and the control contained the unamended soil. Negative rate represents wilted growth.

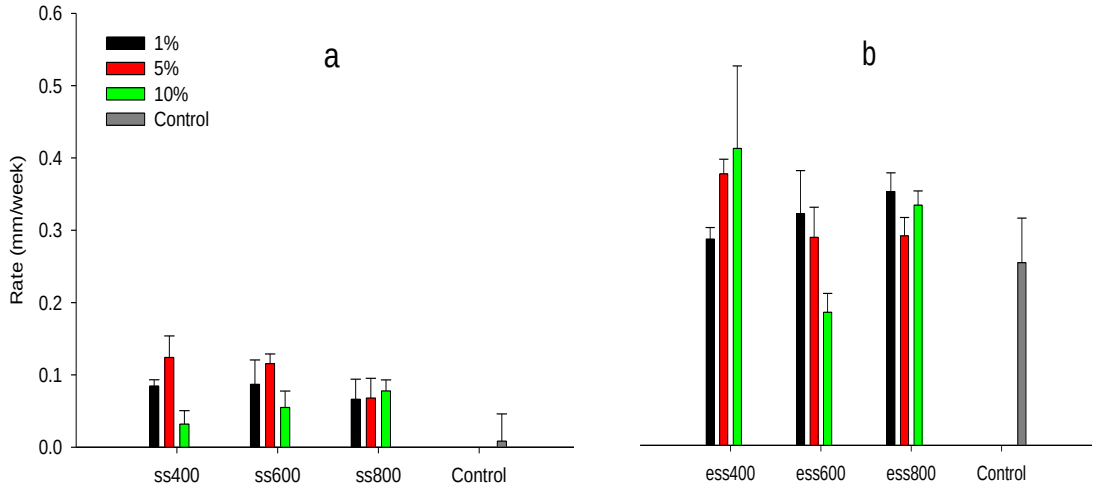

Figure 5.11 Leek growth rates in (a) the acid soil amended with Sitka spruce (ss) biochar and (b) the near-neutral soil amended with Edinburgh Sitka spruce (ess) biochar.

Rates were calculated as slopes of linear regression lines from plots of leek diameter against sampling times for each treatment in the replicated experiments. The biochars on the x-axis represent biochars used for amendment in each case while percentages are amendment rates and the control contained the relevant unamended soil in each case. Error bars represent ±SE.

Table 5.4: Analysis of variance results comparing controls with factors (amendment levels and highest temperature of treatment) for leek growth rates in the amended soils.

Experiment	**Pilot**			**1st**			**2nd**		
Soil type used	**acid**						**Near neutral**		
Amendment level (%)	**1**	**5**	**10**	**1**	**5**	**10**	**1**	**5**	**10**
Control x	0.018*	0.886	0.360	0.059	0.015*	0.205	0.361	0.370	0.436
HTT (°C)	**400**	**600**	**800**	**400**	**600**	**800**	**400**	**600**	**800**
Control x	n.d.		0.450	0.057	0.041*	0.094	0.156	0.875	0.324

*mean difference significant at the 0.05 level

n.d.: not determined; for the pilot experiment due to lack of information on the HTT for one of the biochars (ibc).

Table 5.5: Analysis of variance results comparing p values within factors (amendment level and highest temperature of treatment) for leek growth rates in the amended soils.

Experiment	**Pilot**			**1st**			**2nd**		
Soil type used	**acid**						**Near neutral**		
Amendment level (%)	**1**	**5**	**10**	**1**	**5**	**10**	**1**	**5**	**10**
1		0.008*	0.001*		0.365	0.342		0.981	0.845
5									
10		0.202			0.072			0.864	
HTT (°C)	**400**	**600**	**800**	**400**	**600**	**800**	**400**	**600**	**800**
400					0.824	0.710		0.078	0.520
600		n.d.							
800					0.553			0.243	

*mean difference significant at the 0.05 level

n.d.: not determined; for the pilot experiment due to lack of information on the HTT for one of the biochars (ibc).

Figure 5.12 shows leek growth rates in the two soils with their respective controls at 5% amendment using the ss biochar. Fixing the biochar type (ss) and amendment rate (5%) allows for a direct comparison of the influence of biochar HTT's and soil type on leek growth rates in the two different soils. Compared to their respective controls, biochar pyrolysis temperature has significant impact on leek growth rates in the acid soil ($p < 0.05$) but not in the near neutral soil ($p > 0.05$) (Table 5.6). Table 5.7 shows significant difference in leek growth rate between the two different soil types ($p < 0.05$) but not between pairs of biochar types ($p > 0.05$).

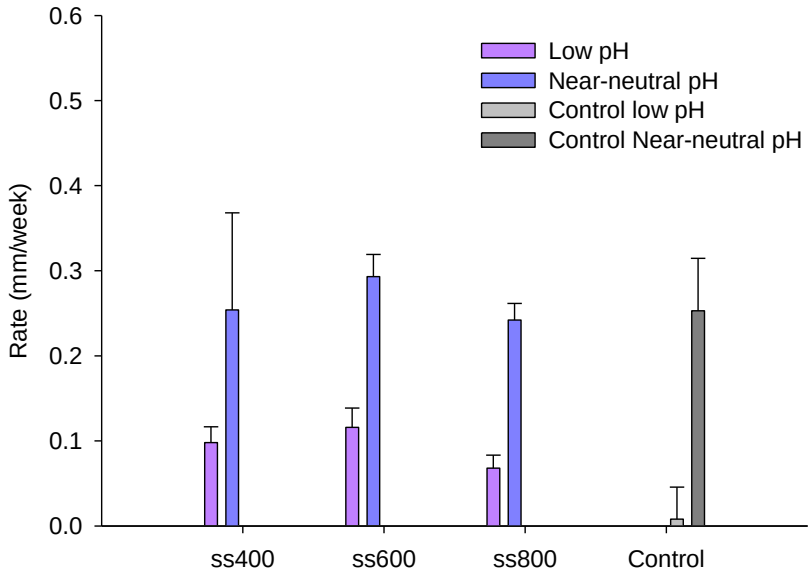

Figure 5.12 Leek growth rates at 5% ss amendment in the two soils

Table 5.6: Analysis of variance results comparing controls with factors for leek growth rates at 5% amendment in the acid and near neutral soils

HTT (°C)	400	600	800	Acid control
Acid control x	0.002*	0.001*	0.008*	
Near neutral control x	0.206	0.329	0.061	0.000*
Soil type	**Near neutral**		**Acid**	**Acid control**
Acid control x	0.000*		0.055	
Near neutral control x	0.823		0.005*	0.000*

*mean difference significant at the 0.05 level

Table 5.7: Analysis of variance results for within factors comparison for leek growth rates at 5% amendment in the acid and near neutral soils

HTT (%)	400	600	800
400		0.707	0.404
600			
800		0.233	
Soil type	Acid	Near neutral	
Acid		0.000*	
Near neutral	0.000*		

*mean difference significant at the 0.05 level

Figure 5.13 compares leek growth rates in 5% ss and ess amended near neutral soil which fixes amendment level and soil type and hence allows for assessing the impact of the two biochars from the two different production streams on leek growth rates and also the influence of HTT on leek growth. Compared to the control, none of biochar type and HTT has significant influence on leek growth ($p > 0.05$) (Table 5.8). Similarly there is insignificant difference ($p > 0.05$) in impact between the factors (biochar type and HTT) (Table 5.9)

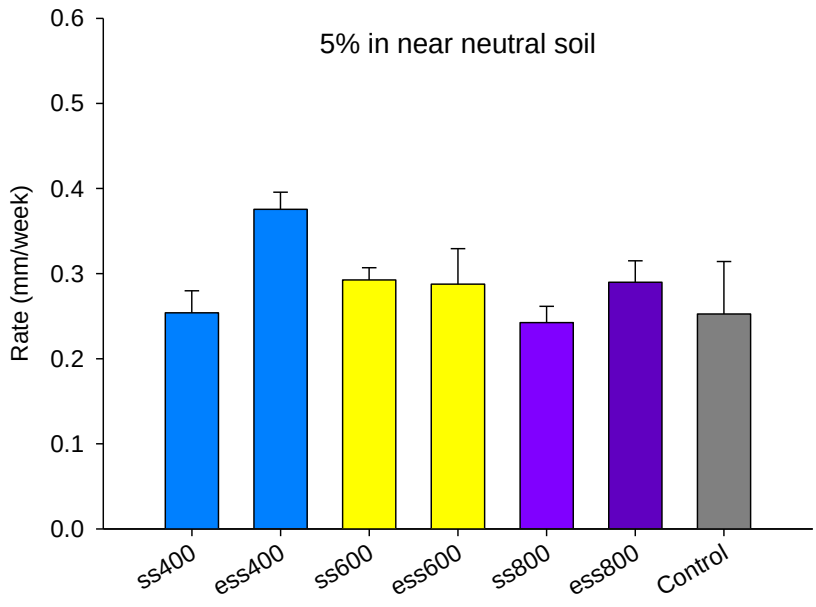

Figure 5.13: Leek growth rates at 5% ss and ess amendments in the near neutral soil

Table 5.8: Analysis of variance results comparing controls with factors for leek growth rates at 5% ss and ess amendment in the near neutral soil

HTT (°C)	400	600	800
Control x	0.236	0.468	0.791
Biochar type	**ss**		**ess**
Control x	0.830		0.191

Table 5.9: Analysis of variance results for within factors comparison for leek growth rates at 5% ss and ess amendment in the near neutral soil

HTT (%)	400	600	800
400		0.556	0.255
600			
800		0.569	
Biochar type	**ss**	**ess**	
ss		0.124	
ess	0.124		

Combined ANOVA was done on all leek growth data across the two soils comparing unamended controls with amendment levels of the amended soil, biochar HTT's and soil types (Table 5.10); comparison within the factors (Table 5.11) and interactions between the factors (Table 5.12). Significant leek growth increases relative to the control resulted from the 5% and 10% amendment levels (Table 5.10, $p < 0.05$) only in the acid soil but there is no significant difference between the two amendment levels (Table 5.11, $p = 0.583$) on influencing leek growth. The 400 and 600°C biochars have significantly different influence ($p < 0.05$) on leek growth compared to the control but compared between the HTT pairs there is no significant difference (Table 5.11, $p > 0.05$). All these confirm the results of the separate ANOVA treatments (Tables 5.4 – 5.7).

Table 5.10: Combined Analysis of variance across experiments and soil types comparing controls with factors for leek growth rates

Amendment level (%)	1	5	10
Acid control x	0.364	0.007*	0.020*
Near neutral control x	0.060	0.557	0.414
HTT (°C)	**400**	**600**	**800**
Acid control x	0.007*	0.040*	0.064
Near neutral control x	0.635	0.318	0.222
Soil type	**Acid**		**Near neutral**
Acid control x	0.944		0.000*
Near neutral control x	0.011*		0.404

*mean difference significant at the 0.05 level

Table 5.11: Combined Analysis of variance results across experiments and soil types for within factor comparison for leek growth rates

Amendment level (%)	1	5	10
1		0.003*	0.024*
5			
10		0.583	
HTT (°C)	400	600	800
400		0.294	0.117
600			
800		0.659	
Soil type	Acid		Near neutral
Acid			0.000*
Near neutral	0.000*		

*mean difference significant at the 0.05 level

Table 5.12: Interactions between factors from the combined analysis of variance on leek growth rates

Amendment x HTT	0.009*
Amendment x soil type	0.397
HTT x soil type	0.466
HTT x soil type x Amendment	0.190

*mean difference significant at the 0.05 level

5.3 Discussion

5.3.1 Soil properties

The significant increase in pH for the amended acid soils compared to the control is consistent with the high pH values of the biochars used for amendment in especially the two higher temperature biochars (see Chapter 4, Table 4.4) which agrees with other reports (Schulz and Glaser, 2012; Khan *et al.*, 2013). Schulz and Glaser (2012), reported significant increase in pH of an acidic infertile sandy soil (pH = 4.5) amended with 5% of charcoal produced at about 400°C while (Khan *et al.*, 2013) achieved an increase in pH of an acid soil (pH = 5.01) at both 5 and 10% amendment level using a sewage sludge biochar in a paddy soil. Wood biochar pyrolysed at 550°C has also been reported to raise the pH of an acidic (pH = 5.2) silty loam soil at both 30 and 60t/ha (Vaccari *et al.*, 2011). Increased pH values for the biochar amended acid soils may be due to the added biochars that have reduced acid functional groups with rise in pyrolysis temperature (see Chapter 4, section 4.3.3). For the near-neutral loamy/clayey soil, the impact of amendment on pH is insignificant compared to the control (Figure 5.3b and Table 5.2). The essentially basic nature of the soil and especially the higher temperature biochars (ess600 and ess800) intuitively explains the absence of a

significant impact on the pH of the amended soil. Similar insignificant biochar amendment effect on the pH of a non-acidic soil has been reported by Zhang *et al.* (2012) who amended a high pH (8.38) calcareous loamy soil with a wheat straw biochar (pH = 10.4) at 20 and 40 t/ha (about 2 and 4%) amendment levels. Haefele *et al.* (2011) also reported no significant effect on the pH of an anthraquic Gleysol near neutral soil (pH = 6.5) amended with 16 t/ha rice husk biochar (pH = 8.6).

The recorded two factors ANOVA on the pH data (Table 5.3) shows significant difference between pH at the three amendment levels in the pilot soils; no significant difference between 5 and 10% in the first experiment and no significant difference between 1 and 5% in the near neutral soil. Hence, 5% amendment level could then be the amendment level of choice across the two soil types dependent on the priorities of achieving change in soil properties, maximizing agronomic effects and sequestering carbon.

The significant increases in total organic carbon (TOC) contents in the low pH soil range from 79% in 5ss400 to 244% in 10ss800 and from 147% in 5ess400 to 394% in 10ess800 in the near-neutral amended soil. These compare well with the report of Zhang *et al.* (2012) who recorded 44% increase in soil organic carbon at 20t ha^{-1} (about 2%) application rate with a wheat straw biochar; other authors reported a 66.5% increase in organic carbon contents at 41.3 t ha^{-1} (about 4%) using rice husk biochar in a near neutral soil (Haefele *et al.*, 2011). Another report (Khan *et al.*, 2013) achieved a 550% increase in total carbon contents in a 5% amendment using sewage sludge biochar in an acidic paddy soil. Organic matter (OM) is comprised mainly of organic carbon, and in soils a lot of benefits are derived from OM; it serves as nutrient reservoir and source of fertility, acts as a buffer against rapid changes in pH (soil reaction), an energy source for soil microorganisms and contributes to soil aeration that is important in reducing soil compaction and increasing infiltration rate and water storage capacity (Jones *et al.*, 2005). Zhang *et al.* (2012) compared crop (maize) N usage in a fertilized unamended control and wheat straw biochar amended fertilized calcareous soil of low organic carbon contents and reported significant increase in N use efficiency (and increased crop yield) with increase in soil organic carbon due to biochar addition. Biochar addition could also give a positive priming effect on soil organic matter decomposition (Vaccari *et al.*, 2011). Hence, the Sitka spruce biochar

can potentially boost the organic carbon contents of soils leading to a more stable soil organic matter essential to enhanced nutrient availability, reduced bulk density, increased aeration and water storage which are all important for boosting crop production while simultaneously helping to sequester carbon in the soil environment and even mitigate flood risks.

Biochar amendments have been reported to decrease the bulk density (BD) of soils. Haefele *et al.* (2011), reported a 3% reduction in bulk density over the control at about 4% amendment with rice husk biochar, while others reported a 4.5% decrease in BD at 5% amendment rate using sewage sludge biochar produced at 550^{o}C in an acid paddy soil (Khan *et al.*, 2013). Results in this study (Figure 5.6 and Table 5.1) show bulk density reductions of between 22 – 24% in the acid soil and 19 – 32% in the near-neutral soil at 5% biochar amendments. The bulk density values show little difference between the amended soils as would be expected for same level of treatment and are also in line with the almost equal bulk density values of the different biochars added to the soils (see Table 4.4 in Chapter 4). But the bulk density is significantly lower than the controls in both soils for all biochar HTT's (Table 5.2). These reductions are much higher than the reported 4.5% using sewage sludge biochar (HTT, 550^{o}C) at 5% amendment (Khan *et al.*, 2013); 4% using woody biochar (HTT, 500^{o}C) at 30 t/ha amendment (Vaccari *et al.*, 2011); and 19% using wheat straw biochar (HTT, 350-55^{o}C) at 40 t/ha (about 4%) (Zhang *et al.*, 2012). The agronomic benefit of lowered bulk density in amended soils could be in the form of reduced tensile strength that offers cheaper tillage cost (Vaccari *et al.*, 2011) and potential higher yield especially for root crops like carrots and beetroot (Gartler *et al.*, 2013). Hence, the biochar under study here has the potential of enhancing the physical structure of amended soils making them amenable to the growth of tubers and easy flow of water through the system.

The Cation Exchange Capacity (CEC) of the amended sandy soils was significantly increased relative to the control (Table 5.2) across all amendments with percentages ranging from 18.67 – 47.70% (Table 5.1). The difference in influence on CEC between 5% and 10% amendment levels and between 600 and 800^{o}C biochars is insignificant (Table 5.3) which makes 5% amendment with 400^{o}C biochar a good choice for raising CEC in amended soils. A more significant impact on CEC from 400^{o}C biochar reflects the measured CEC levels for the biochars which was highest for the lowest temperature

biochar (see Chapter 4, Table 4.4 and section 4.3.3). Increased soil CEC with biochar amendment agrees with the observations of DeLuca *et al.* (2009) and Atkinson *et al.* (2010) on the impact of biochar addition to soils. Enhanced Cation Exchange Capacity helps in retention of nutrients (such as K and NH_4^+) and cycling within amended soils. Though, while some reported CEC increase of about 13% (Sukartono *et al.*, 2011) to as high as 40% (Masahide *et al.*, 2006), others reported a decrease of about the same margin (Haefele *et al.*, 2011) or no effect (Schulz and Glaser, 2012). This could be due to differences in feedstock source, HTT, application rates and/or soil nature.

The significant increase in water holding capacity (WHC) for the amended soils relative to the control in the pilot experiment (Figure 5.8 and Table 5.2) correlates well with the TOC contents of the soils (Figure 5.14). Similar correlation plots show good linear relationships for the ss amended soil (R^2 = 0.72) and ess amended soil (R^2 = 0.73). However, for the biochars the two measured properties (TOC and WHC) show a linear relationship but with a negative slope (Figure 5.15) for both ss and ess biochars (see Chapter 4, Table 4.4). A similar plot for ess biochar gave a Pearson's coefficient of R^2 = 0.85.

Increased water holding capacity for an amended soil is a positive agronomic impact since enhanced WHC translates into more water availability to plants (Asai *et al.*, 2009) and is also a possible remedy in free draining soils (Atkinson *et al.*, 2010) that are susceptible to causing the flooding of surrounding infrastructures during storm events. Recent changes in the rainfall pattern in the UK that has led to frequent flooding events point to the great importance of enhancing the soil's ability to retain more water.

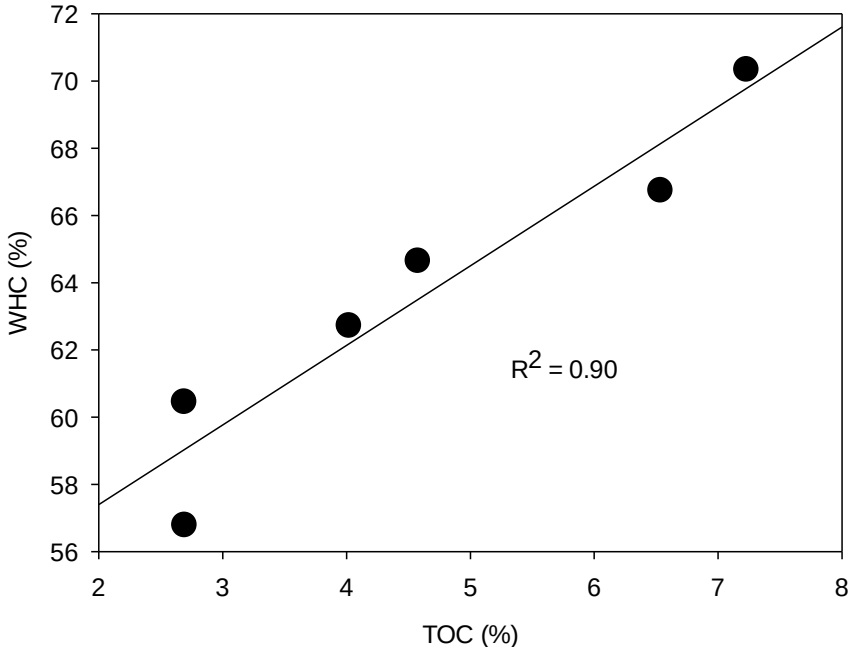

Figure 5.14: Relationship between total organic carbon (TOC) and water holding capacity (WHC) for the amended acid soil from the pilot experiment.

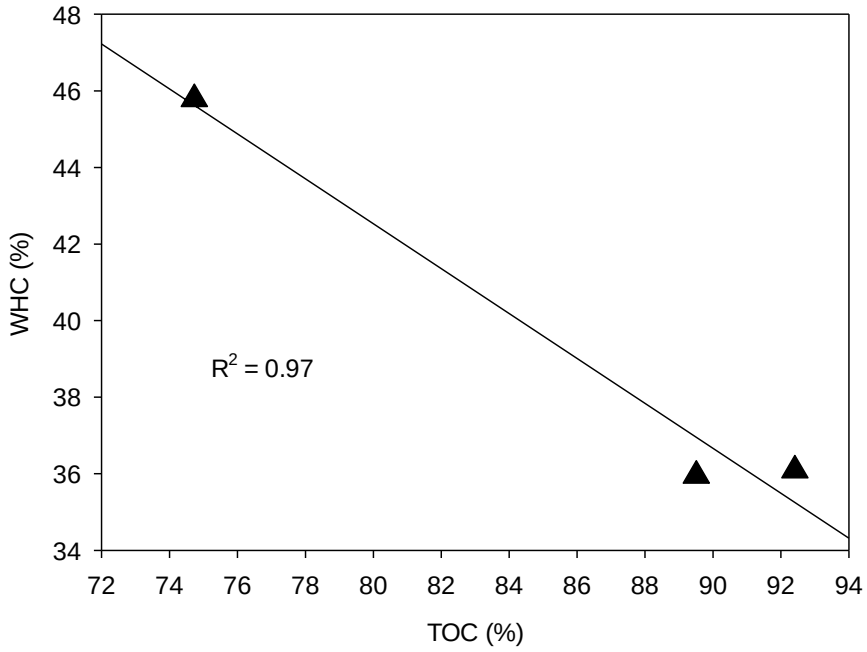

Figure 5.15: Relationship between total organic carbon (TOC) and water holding capacity (WHC) for the ss biochar used to amend the acid soil in both the pilot and first experiments.

Compared to the controls, our results show WHC increases by between 13 – 174% in the sandy soil and 3 – 44% in the near-neutral soil across the amendment rates. Higher increase margins in the amended sandy soils compared to the loamy/clayey soils confirms a possibility suggested by Atkinson *et al.* (2010) who reviewed potential mechanisms for achieving agricultural benefits through the addition of biochar to temperate soils. The authors reported works that showed sandy soils having higher water holding capacity on treatment with biochar compared to similarly amended clayey or loamy soils.

5.3.2 Leek growth

The provision of food crops is one of the most important functions of the soil (Kennedy and Smith, 1995), consequently plant growth depends on soil properties such as nutrient availability, pH, plant available water, and a functional microbial community that plays important role in soil organic matter decomposition. Deficiency and/or extremes in any of these variables could limit plant growth (Mingorance *et al.*, 2014). The impact of biochar amendment on leek growth is therefore discussed in relation to the impact of amendment on the soil properties treated in the previous section. The stated objective in this Chapter was to assess how biochar produced at different pyrolysis temperatures alters the agronomic properties of soil to which it was added and what impact that had on plant growth. To facilitate a systematic investigation, biochars used were from same feedstock; same test plant with same level of fertilization and water regimen used; and same biochar addition rates. Statistical treatment of the overall data was also used to assess interactions between the trio of soil type, biochar HTT and amendment rates across the two soil types in addition to separate treatment of leek growth data in the two soil types (acidic sandy and near neutral loamy/clayey) and three experiments (the pilot, first and second) (see Chapter 3, Table 3.2).

Significant leek growth relative to the control in the acid soil resulted only from 5% amendment (Univariate ANOVA, Post Hoc Tests, $p = 0.015$) with ss600 biochar (Univariate ANOVA, Post Hoc Tests, $p = 0.041$) (Tables 5.4 and 5.5). The growth could be due to increased TOC, WHC and decreased BD over the control (Table 5.1). TOC correlates well with WHC in the acid soil (Table 5.1 and Figure 5.14). Increased biomass growth due to biochar addition could result from positive priming on soil organic

matter decomposition leading to faster mineralisation and improved nutrient availability (Vaccari *et al.*, 2011). These authors used wheat as a test plant and applied a wood biochar (HTT, 500^{o}C) into a silty loam soil of pH 5.2. Schulz and Glaser (2012) applied barbecue charcoal (HTT, 400^{o}C) along with charcoal + compost and charcoal + mineral fertilizer at 5% amendment into a modelled infertile sandy soil planted with oat (*Avena sativa L.*) to test impact on soil fertility and plant growth and concluded that biochar addition raised soil organic matter, soil fertility and increased plant growth. However, in the near neutral soil biochar addition has no significant impact compared to the control ($p > 0.05$) at all amendment levels for all biochar HTT's (Table 5.4) even though from Table 5.1 the amended soils have increased TOC, WHC and decreased BD compared to the control. The reason could be due to the soil pH which was not greatly affected being already high which itself also may explain the better overall leek growth in the near neutral soil (Figure 5.11).

Both soil types received the same level of mineral fertilization (see Chapter 3, Table 3.2) in addition to having similar levels of TOC, WHC and BD (Table 5.1) and therefore none of these parameters could be the reason for the limited leek growth in the acid soil compared to the near neutral. The pH levels of the amended acid soils (4.42-4.77) and the near neutral soils (6.28-6.99) could explain the leek growth differences in the two soils (Figure 5.12 and Table 5.6) as the optimum pH for leek growth is estimated to be 6.0-6.8.

(http://www.extension.umn.edu/garden/yard-garden/vegetables/leeks/doc/M1230.pdf
Accessed on 19/05/2014)

Soil pH could limit plant growth in a number of ways that centre on availability or lack of it of nutrients both macro (N, P, K, Ca and Mg) and micro (Fe, Mn and Zn) and also the presence and uptake by plants of Al^{3+} that is phytotoxic in its various forms. Low soil pH solubilise Al^{3+} species in soil which go on to displace macro nutrients from the soil and cation exchange sites (Cristancho *et al.*, 2014) thus starving the plant of nutrients needed for growth. Masahide *et al.* (2006) partly ascribed low productivity of maize, cowpea and peanut in their control soils to low pH (4.1), low nutrients and high Al^{3+} (2.61 molc/Kg). Other authors also observed that close to neutral pH favours nutrient availability and increased crop yield (Vaccari *et al.*, 2011). Schulz and Glaser (2012), also reported significant increase in available K with increased soil pH.

5.4 Conclusion

The combined results in this Chapter and statistical treatment of the data positively show that addition of Sitka spruce biochar to the test soils did impact to a certain extent the growth of leek by altering some of the physico-chemical properties of the soil environment in which the plant was grown. Significant soil property changes compared to unamended controls include raising the TOC and lowering BD in both the acid and near neutral soils ($p < 0.05$); the pH in the acid soil ($p < 0.05$) but not in the near neutral soil ($p > 0.05$); and increasing the CEC and WHC ($p < 0.05$) although replicated measurements on these last two properties were only determined in the acid soil. The alteration of these soil properties due to biochar addition was also significantly influenced by changing the pyrolysis temperatures of the biochars (HTT) used for amendment which makes the fourth hypothesis put forward in this study acceptable.

Sitka spruce biochar addition to the test soils significantly influenced leek growth compared to the controls only in the acid soil and not in the near neutral soil (Table 5.10). More directly related to the fifth hypothesis in this investigation, altering the biochar HTT had no significant effect on leek growth in both soils (Table 5.11) and hence the fifth hypothesis is rejected.

Another conclusion that could be drawn from this study is that production process did not significantly influence the impact of these biochars on leek growth (Figure 5.13 and Tables 5.8 & 5.9).

From the ANOVA results in Tables 5.2 and 5.3, a suggested suitable dosage of Sitka spruce biochar could be 5% (50 t/ha) of 600^{o}C biochars for enhanced pH in sandy acid soil; 1% (10 t/ha) of 400^{o}C biochars for enhanced TOC in both acidic sandy soil and near neutral loamy clayey soil; 5% of 600^{o}C biochars for enhanced CEC in acid soil; and 1% amendment level for enhanced WHC in acid soil (no ANOVA result for near neutral soil and no HTT chosen due to lack of full information on it since replicate measurement for WHC was only determined in the acid soil of the pilot experiment). Similarly from the ANOVA results in Tables 5.10 and 5.11, a dose of 5% 400^{o}C biochars could be suggested for enhanced leek growth in the acid soil only.

Chapter 6 Soil processes and soil microbial community structure as a function of biochar amendment

6.1 : Introduction

Biochar is receiving increasing attention from researchers due to its use as a soil fertility enhancer (Zimmerman *et al.*, 2011; Dempster *et al.*, 2012; Yoo and Kang, 2012) and its effect in abating climate change through its potential for both reducing greenhouse gas (GHG) emissions (Spokas *et al.*, 2010; Yoo and Kang, 2012; Harter *et al.*, 2013) and sequestering atmospheric carbon dioxide in soils. This is due to its relative inertness and resistance to microbial degradation (Spokas *et al.*, 2009). Carbon dioxide (CO_2) is the primary greenhouse gas and anthropogenic activities in the form of fossil fuel combustion and deforestation are blamed for increases in its atmospheric concentrations. Biochar as a product which has a high concentration of carbon contributes greatly when used as a soil improver in withdrawing CO_2 from the atmosphere, in addition to its suppression of basal respiration (CO_2 emissions) from soil environments (Calvelo Pereira *et al.*, 2011).

A productive or fertile soil that is essential to sustainable agriculture invariably depends on the maintenance of healthy, diverse and functional microbial populations (Kennedy and Smith, 1995; Lehmann *et al.*, 2011) due to the pivotal role they play in organic matter decomposition, nutrient cycling and many other ecosystem services provided by the soil (Liu *et al.*, 2006; Ritz *et al.*, 2009). In an effort to cater for the growing world population, human activity has placed many ecosystems under pressure from unsustainable use of land resources through deforestation and intensive mechanised agricultural production (Kennedy and Smith, 1995). Microbial populations are quite intimate in their contact and interaction with the soil environment and are therefore very sensitive and respond to such environmental stresses (Kennedy and Smith, 1995; Bloem and Breure, 2003; Sheppard *et al.*, 2005) in terms of their structure, diversity and functions (Webster *et al.*, 2002; Gray *et al.*, 2003; Harter *et al.*, 2013). Thus, the role of biochar as a climate change mitigation tool can only be valuable if the biochar does not negatively impact on normal soil microbial mediated processes that are central to the maintenance of soil health.

The term ‘soil health’ is in some ways synonymous to ‘soil quality’ and a healthy soil should be fit for contemporary purposes that include the provision of a whole

spectrum of ecosystem goods and services (Haygarth and Ritz, 2009), such as food crops and livestock and the provision of space for buildings and recreation (Kennedy and Smith, 1995). Soils are extremely complex, heterogeneous (Haygarth and Ritz, 2009) and heavily populated by microorganisms; a gram of soil could be home to close to 10 billion microbes with a diversity running into thousands of different species (Torsvik and Øvreås, 2002).

The scope of this Chapter is limited to investigating the impact of biochar amendments in a low pH (pH = 4.38) and a near-neutral (pH = 6.67) soils, on:

- Basal Respiration (BR) measured as evolved carbon dioxide, which doubles as an estimate of microbial activity (Winding *et al.*, 2005) and as an intrinsic indicator of C cycling which is fundamental to soil function (Ritz *et al.*, 2009).
- Denitrification Enzyme Activity (DEA) measured as released nitrous oxide (N_2O), which indicates on soil function in nutrient (N) cycling (Liu *et al.*, 2006), and
- Microbial diversity that is one of the three microbiological parameters (amount of biomass, the activity and the diversity of the microbial community) that could be measured to monitor environmental stress resulting from anthropological soil management practices (Bloem and Breure, 2003).

The choice of these properties for investigation in this study is based on the very sensitive response of microorganisms to environmental stress, which makes biological parameters effective candidates as indicators for environmental monitoring and ecological risk assessment (Kennedy and Smith, 1995; Bloem and Breure, 2003). The hypothesis (see Chapter 1 section 1.4) to be tested here was,

- Increasing pyrolysis temperature progressively alters biochar's ability to influence the selection of resultant microbial communities and microbial mediated processes e.g. respiration, and nitrogen cycling in soil environments.

Laboratory-based microcosms were used to incubate biochar-amended soils recovered from 12-14 week pot experiments (see Chapter 3 for details on method) and the flux of CO_2 and N_2O in the headspace (as indicators of soil microbial activity), were measured for the biochar treated soils and controls (unamended soil and fresh biochar).

Although it is said to be recalcitrant (Forbes *et al.*, 2006; Kuzyakov *et al.*, 2009; Harvey *et al.*, 2012) and largely unavailable to soil microorganisms (Anderson *et al.*, 2011), biochar controls were considered because, it cannot be immune to degradation at some rate (Zimmerman, 2010). This degradation is reported to possibly be both abiotic (Cheng *et al.*, 2006) and biotic (Zimmerman, 2010).

To qualitatively assess biochar's impact on the indigenous microbial population, microbial diversity patterns in the amended soils were assessed. To this end, Polymerase Chain Reaction (PCR) products of bacterial 16S rRNA genes amplified from DNA extracts of the amended soils and controls were separated using Denaturing Gradient Gel Electrophoresis (DGGE). DGGE is a culture-independent molecular technique (see Chapter 3 for method), where gene fragment separation is achieved based on differences in the electrophoretic mobility of denatured (partially melted) DNA fragments through a polyacrylamide gel; sequence variants (representing individual bacterial species) melt at different temperatures and stop migrating through the gel when they melt, hence the separation to produce a barcode pattern representing the diversity of the system (Muyzer *et al.*, 1993). Critically DGGE represents a rapid if crude diversity fingerprinting method that allows a comparison of diversity patterns across the replicated experimental treatments in this study.

6.2 Results

6.2.1 Basal respiration (BR)

The results for basal respiration (as rates in µg CO_2/g soil/hour) measured 13 weeks after biochar amendment from the plant trial pot soils are presented in Figures 6.1, 6.2 and 6.3. For the pilot experiment, Figure 6.1 represents rates of CO_2 production in the microcosms containing the low pH sandy soil amended with ibc and kbc800 biochars at the three levels of treatment (1, 5, and 10% equivalent to 10, 50 and 100 t/ha) along with a control soil that contained no biochar. Figure 6.2 shows a similar arrangement for first experiment in which same type of soil as in the pilot was amended with ss biochar while Figure 6.3 shows rates of CO_2 production in the microcosms for the ess biochar amended near neutral soils and unamended control. In all cases, the observed data have been corrected by subtracting gas emissions from the biochar control before producing the charts (Spokas *et al.*, 2009). Additionally, all data were adjusted for actual mass of soil in microcosms to remove the effect of dilution resulting from

amendment additions. The rates were calculated from the slopes of the regression plots of CO_2 emissions over the three gas sampling times (0, 20 and 24 hours) for each of the replicated experiments.

Statistical treatment of the data presented in Figure 6.1 showed that for all biochars combined the 1% amended soil in the pilot experiment did not have significantly different rate of CO_2 production (Univariate ANOVA, Post Hoc Tests, $p = 0.118$) compared to the biochar-free control (Table 6.1), while the soil at the other two amendment levels had significantly different rate of CO_2 production (Univariate ANOVA, Post Hoc Tests, $p = 0.000$) compared to control for all biochar types. The pyrolysis temperature of the kbc800 biochar showed a significant influence on the rate compared to the control (Univariate ANOVA, Post Hoc Tests, $p = 0.000$). No information is available on the production temperature of the ibc biochar. From Table 6.2, all the three amendment levels significantly differ from one another (Univariate ANOVA, Post Hoc Tests, $p < 0.05$).

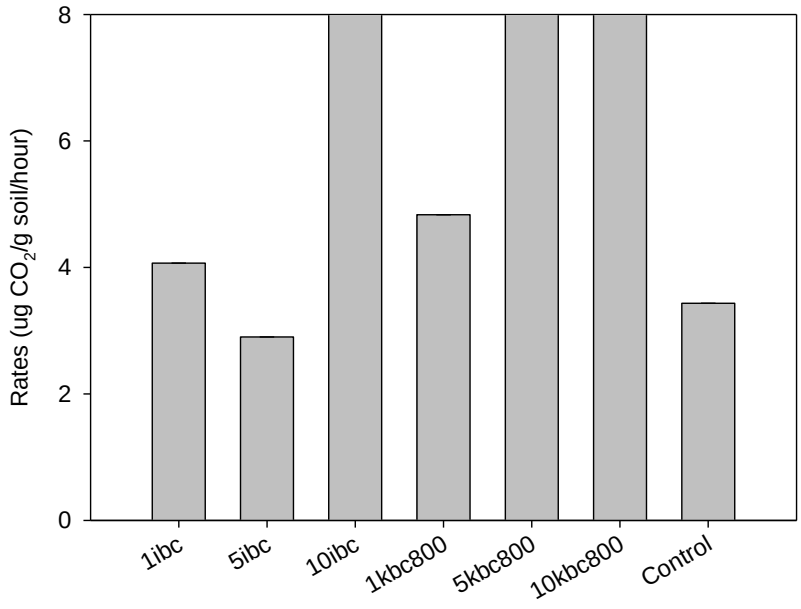

Figure 6.1: Rates of carbon dioxide production in the pilot experiment. Rates determined after subtracting carbon dioxide emissions due to biochar. Control was the unamended soil. Error bars (±SE) too small to be seen on plots.

In the main experiment (Figure 6.2), the rate of CO_2 production significantly differed from the control soil at all the amendment levels (Univariate ANOVA, Post Hoc Tests, $p = 0.000$) and the amendment levels had significantly different influences on the rate of CO_2 emissions between them (Univariate ANOVA, Post Hoc Tests, $p < 0.05$) (Table 6.2). However, while all the biochar HTT's had a significantly different influence on CO_2 emissions compared to the control (Table 6.1), the influence of ss800 is not significantly different (Univariate ANOVA, Post Hoc Tests, $p = 0.149$) from that of ss400 (Table 6.2).

Table 6.1: Analysis of variance results comparing unamended controls with factors (amendment level and highest temperature of treatment) for rates of carbon dioxide and nitrous oxide production from biochar amended soil microcosms.

	Experiment	Pilot			1st			2nd		
	Soil type used	acid						Near neutral		
Variable	Amendment level (%)	1	5	10	1	5	10	1	5	10
CO_2	Control x	0.118	0.000*	0.000*	0.000*	0.002*	0.000*	0.681	0.000*	0.000*
N_2O		0.081	0.032*	0.002*		n.d.		0.000*	0.000*	0.000*
	HTT (°C)	400	600	800	400	600	800	400	600	800
CO_2	Control x		n.d.	0.000*	0.000*	0.000*	0.002*	0.001*	0.000*	0.000*
N_2O			n.d.	0.028*		n.d.		0.000*	0.000*	0.000*

*mean difference significant at the 0.05 level; n.d.: not determined

1st and 2nd experiments are the first and second sets of experiments after the pilot where ss and ess biochars were used to amend the acid and near neutral soils respectively (see chapter 3 section 3.6 Table 3.2).

Table 6.2: Analysis of variance results comparing factors (amendment level and highest temperature of treatment) for their influence on rates of carbon dioxide and nitrous oxide production from biochar amended soil microcosms.

	Experiment	Pilot			1st			2nd		
	Soil type	acid						Near neutral		
Variable	Amendment level (%)	1	5	10	1	5	10	1	5	10
CO_2	1		0.001*	0.000*		0.000*	0.000*		0.000*	0.000*
	5									
	10		0.000*			0.036*			0.000*	
N_2O	1		0.552	0.045*					0.000*	0.000*
	5					n.d.				
	10		0.134						0.000*	
	HTT (°C)	400	600	800	400	600	800	400	600	800
CO_2	400					0.000*	0.149		0.000*	0.000*
	600		n.d.							
	800					0.000*			0.076	
N_2O	400								0.000*	0.000*
	600		n.d.			n.d.				
	800								0.027*	

*mean difference significant at the 0.05 level; n.d.: not determined

Figure 6.3 shows the rates of CO_2 production in the near-neutral soil amended with ess biochar at all amendment rates. The rates of CO_2 production show a progressive drop in CO_2 production with increase in the amount of biochar for all biochars, but generally higher rates of CO_2 produced for all treatments and controls compared to the low pH soil. However, compared to the unamended soil (control), except for 1% ess400, all other soils at all treatments produced CO_2 at rates lower than the soil control, hence biochar suppressed emission rates.

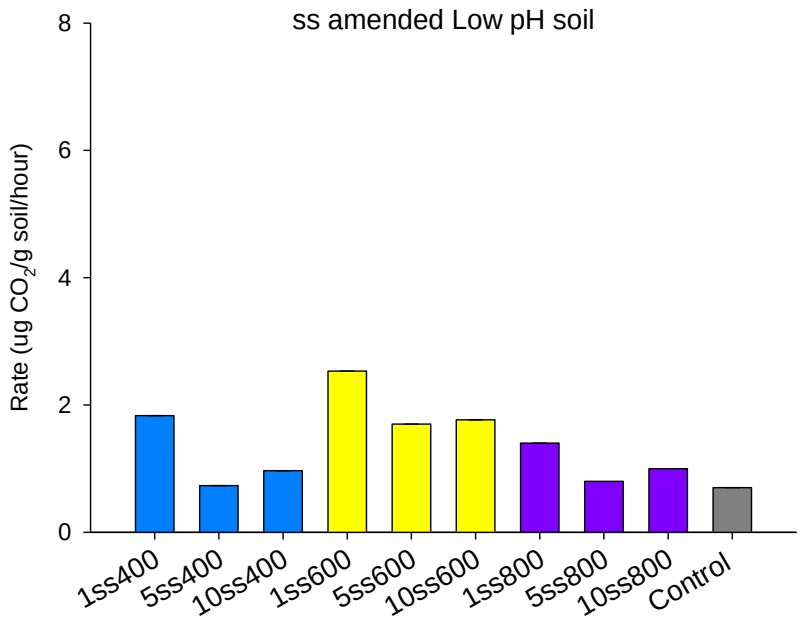

Figure 6.2: Rates of carbon dioxide evolved from ss biochar amended low pH soil. Data points represent means ± standard error (n=3). Unseen error bars due to small values of the standard errors. Rates were calculated as explained in chapter 3, section 3.7.1. Control is the unamended soil.

But considering all biochar types, the higher rate for 1% ess400 is not significantly different from the control (Univariate ANOVA, Post Hoc Tests, $p = 0.681$) (Table 6.1). The decrease in CO_2 production with increasing amendment level (Figure 6.3) is significant from Table 6.2 (Univariate ANOVA, Post Hoc Tests, $p < 0.05$). Compared to the control, the highest temperature of treatment (HTT) of the biochars show significant influence on the biochars' impact on CO_2 production (Table 6.1, Univariate ANOVA, Post Hoc Tests, $p < 0.05$) but the influence is not significantly different between ss600 and ss800 biochars (Table 6.2, Univariate ANOVA, Post Hoc Tests, $p = 0.076$).

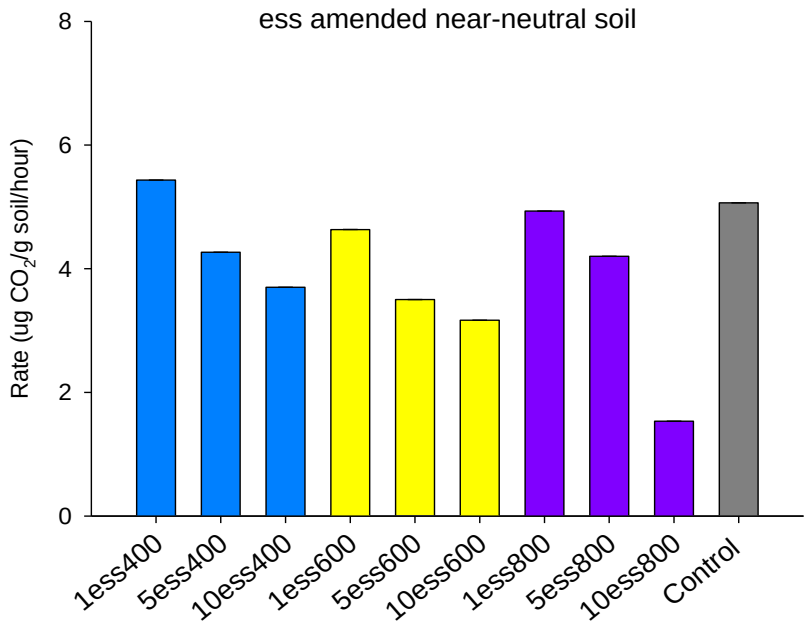

Figure 6.3: Rates of carbon dioxide evolved from ess biochar amended near-neutral soil. Data points represent mean ±standard error (n=3). Unseen error bars due to small values of the standard errors. 1ess400 means soil amended with 1% ess400 biochar. Control is the unamended soil.

6.2.2 Denitrification enzyme activity (DEA)

Results for the DEA are presented in Figures 6.4 and 6.5 as rates of N_2O production in (µg N_2O/g soil/hour). There were no detectable N_2O emissions from ss amended low pH soil regardless of biochar type or amendment rate while the pilot experiment pot amended soils showed some detectable nitrous oxide emissions (Figure 6.4) in a few samples but none from the unamended control soil. Moreover, a statistical treatment of the combined data for the two biochars showed only one of the biochar treatments (10% or 100t/ha) had a rate of N_2O production that significantly (Univariate ANOVA, Post Hoc Tests, p = 0.045) differed from zero, the rate for the unamended control (Tables 6.1 and 6.2). Nitrogen based gas emissions (N_2O, NO and N_2) are lower in acid soils compared to soils with higher pH values (Šlmek and Cooper, 2002). Khan *et al.* (2013), measured some N_2O emissions from an acidic soil (pH = 4.02), but they sampled from flux chambers enclosing pots of growing plants while the microcosms used in this study used a re-wetted field moist soil transferred from pot experiments. Additionally the soil used in this study is more acidic at pH = 4.38 because they

measured their soil pH in $CaCl_2$ solution which if converted (Little, 1992) to pH in water like in this study would give a pH > 5.0 and hence less acidic.

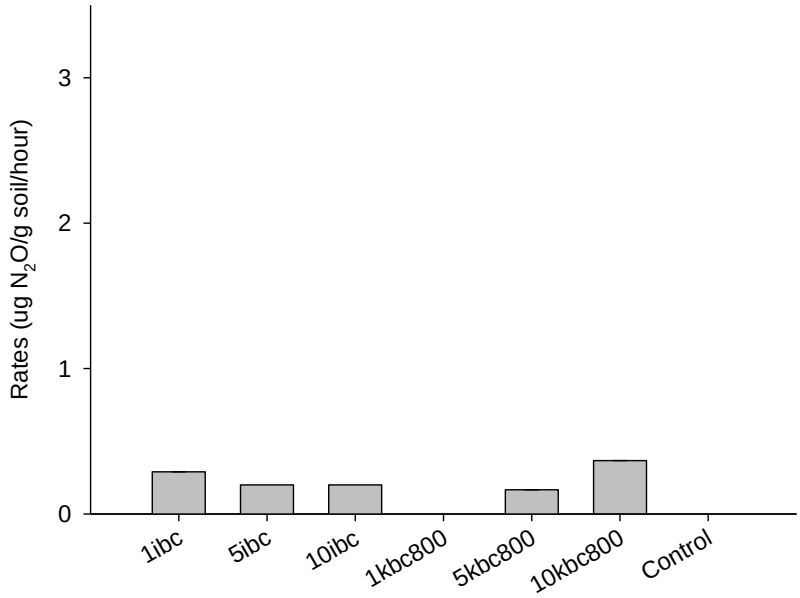

Figure 6.4: Rates of headspace nitrous oxide production in microcosms of ibc and kbc800 amended acid soils from the pilot experiment. There were no detectable nitrous oxide emissions from both the biochar and unamended controls. Error bars (±SE) too small to be seen on plots.

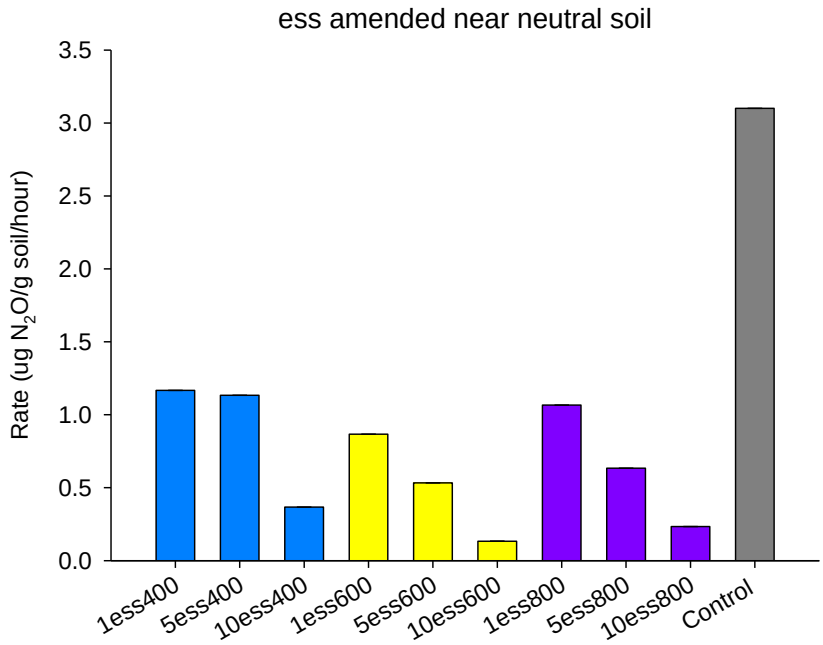

Figure 6.5: Rates of headspace nitrous oxide evolved from microcosms of ess biochar amended near-neutral soil. Error bars representing standard error of the mean (n=3) are not visible on the bars due to small values of the standard errors (order of 10^{-5}).

Figure 6.5 shows the Nitrous oxide production rates for the ess biochar-amended near-neutral soils. All the rates fall below that of the unamended control soil which shows suppressed rates of N_2O production. Compared to the unamended control, the changes in N_2O production due to amendments with biochars at the different amendment levels are significant (Table 6.1, Univariate ANOVA, Post Hoc Tests, $p = 0.000$). Similarly, the different biochar HTT's show significantly different influence (Table 6.2, Univariate ANOVA, Post Hoc Tests, $p < 0.05$) on the biochars' ability to impact on the denitrification process responsible for the N_2O emissions in this study. These reduced production rates with increasing biochar amendment level and the different influence from the various biochar pyrolysis temperatures could be directly from the changes in the denitrifying microbial community and/or other physicochemical factors as presented in the discussion section of this Chapter.

6.2.3 Microbial community structure

Microbial community analysis of the post-plant trials pot soils was carried out by comparing DGGE profiles for the ss and ess biochar amended soils (both low pH and near neutral) and the unamended control soil. PCR was done in triplicate on each of soil DNA extract and the PCR products were analysed by DGGE. All the soil samples used for the microbial community analysis were amended with 5% of the biochars except the controls that did not contain any biochar. The DGGE profile in Figure 6.6 reflects ss400, ss600; ss800 biochar amended low pH sandy soils at the end of the pot experiment (12 weeks) and related unamended control soils at the start of the pot experiments (banding patterns 1 and 2). In this preliminary analysis there is no apparent loss of bands in the banding patterns in Figure 6.6 compared to the controls which may mean biochar amendment did not adversely affect the microbial diversity of the amended soils. Figure 6.7 shows the DGGE profile for the ess biochar amended near neutral loamy/clayey soils (es4, es6 and es8) at the end of the plant trial experiment with the unamended control soils at the beginning (C0) and at the end (C12) of the experiment. The banding patterns also show no discernible shift in the diversity of the microbial community in all the amended soils for all the biochar types compared to the control soil.

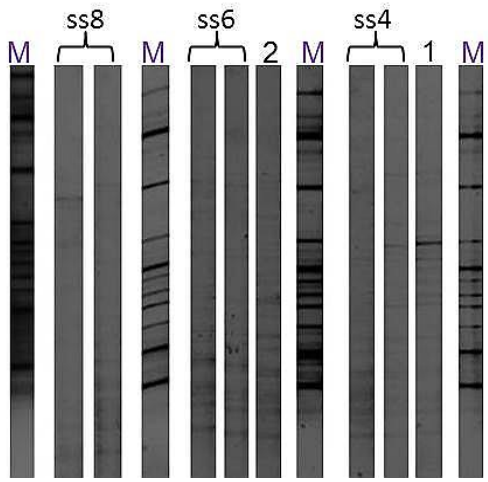

Figure 6.6: Denaturing gradient gel electrophoresis profile for the ss biochar amended soil samples and controls. The banding patterns 1 and 2 represent unamended controls at time zero for the ss400 and ss600 amended soils respectively while ss4, ss6 and ss8 represent the ss400, ss600 and ss800 amended soil samples after 12 weeks of running the plant trials respectively. The symbol 'M' represents the marker.

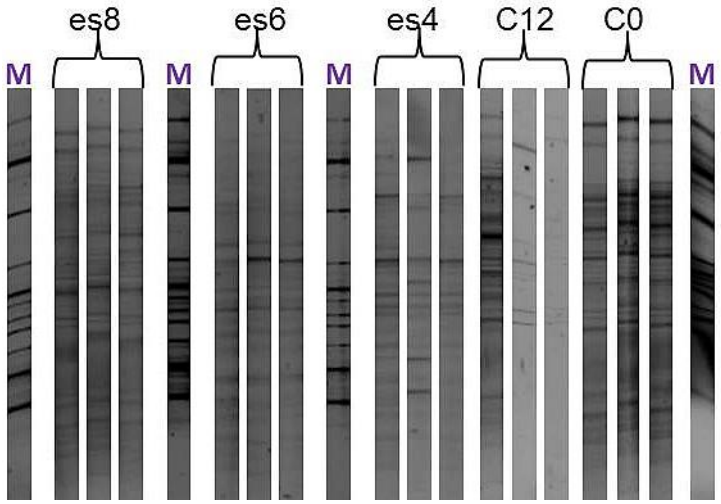

Figure 6.7: Denaturing gradient gel electrophoresis profile for the ess biochar amended soil samples and controls. The banding patterns C0 and C12 represent unamended controls at the beginning and after 12 weeks while es4, es6 and es8 represent the ess400, ess600 and ess800 amended soil samples after 12 weeks of running the plant trials respectively. The symbol 'M' represents the marker.

The addition of biochar to soil could also provide pore spaces for possible colonization by microbes as shown in the scanning electron microscope image of the ss400^{o}C biochar recovered from the pot experiment of this study (Figure 6.8).

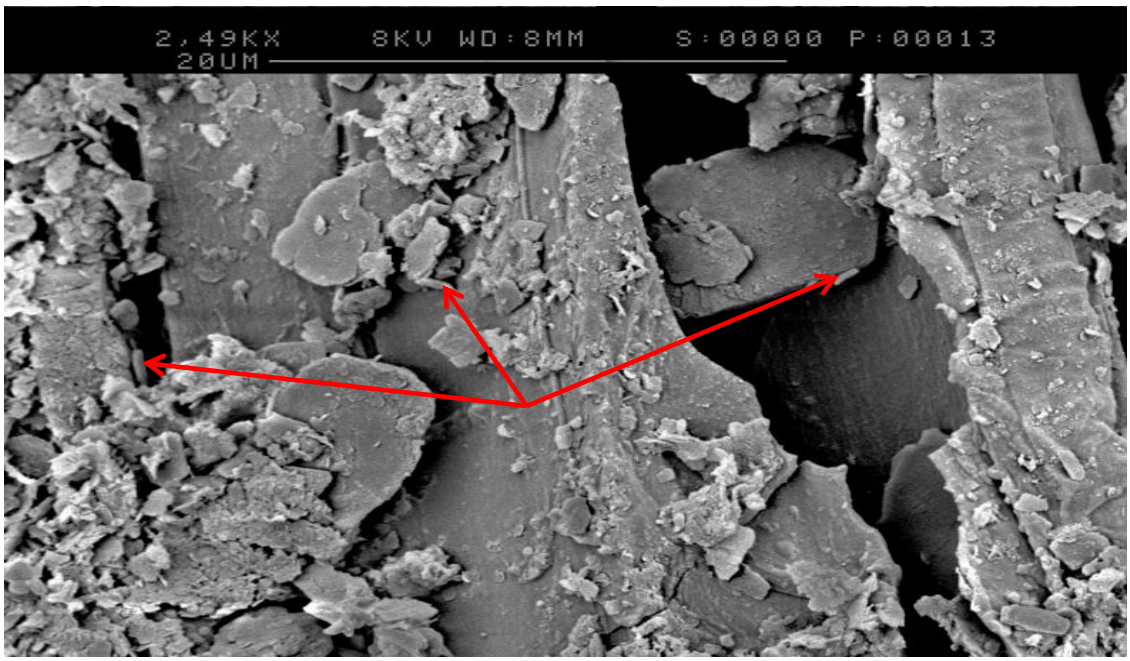

Figure 6.8: Scanning electron microscope image (x2500) showing putatively microbial cells within Sitka spruce biochar prepared at 400°C recovered from pot soil.

6.3 Discussion

The impact of biochar amendments on greenhouse gas production from soils depends on factors such as type of soil and biochar (Yoo and Kang, 2012; Saarnio *et al.*, 2013), available organic substrate (Šlmek and Cooper, 2002; Angst *et al.*, 2013), plant type in cultivated soils and environmental conditions (Saarnio *et al.*, 2013), moisture regime and biochar application rates (Yoo and Kang, 2012).

6.3.1 Basal respiration

The results for basal respiration in the low pH soil (pH = 4.38) used in both the pilot and first experiments show different responses based on the biochar used for amendment but on the whole more CO_2 was produced compared to the controls in both experiments (Table 6.3). In the pilot experiment where ibc and kbc800 biochars were used, CO_2 production was stimulated and showed strong correlation with pH and a weak one with TOC (Figure 6.8 and Chapter 5, Table 5.3).

Table 6.3: Mean rates of carbon dioxide production from the biochar amended soils and unamended controls. Low pH sandy soil was used in the pilot and first experiments, while near neutral loamy/clayey soil was used in the second experiment.

Sample ID	Experiment	Mean Rates (µg CO_2/g soil/hour)	±SE
1ibc	Pilot	4.1	0.0001453
5ibc		2.9	0.00100167
10ibc		9.4	0.00034641
1kbc800		4.8	6.6667E-05
5kbc800		10.3	0.00073106
10kbc800		10.3	0.0001453
control		3.4	0.00017638
1ss400	First	1.8	0.00013333
5ss400		0.7	3.3333E-05
10ss400		1.0	3.3333E-05
1ss600		2.5	8.8192E-05
5ss600		1.7	5.7735E-05
10ss600		1.8	0.00017638
1ss800		1.4	0.0001
5ss800		0.8	0
10ss800		1.0	0
Control		0.7	0.0001
1ess400	Second	5.4	0.00029627
5ess400		4.3	8.8192E-05
10ess400		3.7	5.7735E-05
1ess600		4.6	0.00012019
5ess600		3.5	0.0001
10ess600		3.2	3.3333E-05
1ess800		4.9	0.00013333
5ess800		4.2	0.0002
10ess800		1.5	8.8192E-05
Control		5.1	3.3333E-05

1, 5 and 10 in the amended soil sample ID's define amendment levels in percentage with the accompanying biochar ID's.

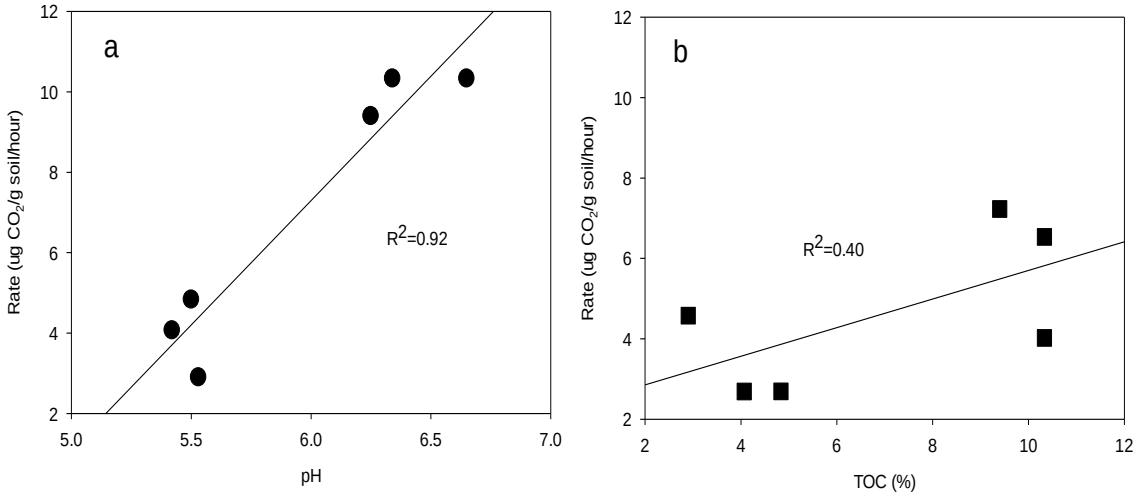

Figure 6.9: Correlation plots of carbon dioxide rate of production in µg CO_2/g soil/hour with (a) pH and (b) total organic carbon in the amended acid soils of the pilot experiment

The enhanced CO_2 production could therefore be explained more by the increased soil pH as a result of amendment than due to positive priming from increased TOC contents. The lower enhancement of CO_2 production in the ss amended soils (Table 6.3) could also be due to the marginal pH increases in the amended soils (Chapter 5, Table 5.3), though no correlation existed between CO_2 production and pH (R^2 = 0.23), TOC (R^2 = 0.12) and WHC (R^2 = 0.17). In the ess amended near neutral soil, CO_2 production showed no correlation with pH but inversely correlated with TOC (Figure 6.9) and WHC (R^2 = 0.59). The reduced CO_2 production compared to control especially with increasing amendment level (higher TOC contents) in the near neutral soil could be due to negative priming (reduced organic carbon decomposition or substrate exhaustion) as the report of Dempster *et al.* (2012) indicated. These authors noted a decrease in CO_2 evolution at 5 t ha^{-1} Eucalyptus biochar application rate but no effect at five times that amount attributing the decrease in CO_2 evolution to negative priming effect from the carbonate contents of the added biochar. Yoo and Kang (2012), proposed that negative correlation may suggest adsorption of evolved CO_2 by the biochar but expressed the need for further sorption studies to determine the underlying mechanism. Similar suppressed CO_2 production with biochar amendment (and unknown cause) has been reported by Spokas *et al.* (2010). It is however worth noting that across the two soil types (Figures 6.1-6.3) microbial carbon based respiration may not have been negatively affected as a result of biochar additions because even though CO_2 gas production is suppressed with amendment in the ess amended near neutral

soil, the rates are still higher compared to the enhanced rates in the ss amended acid soil.

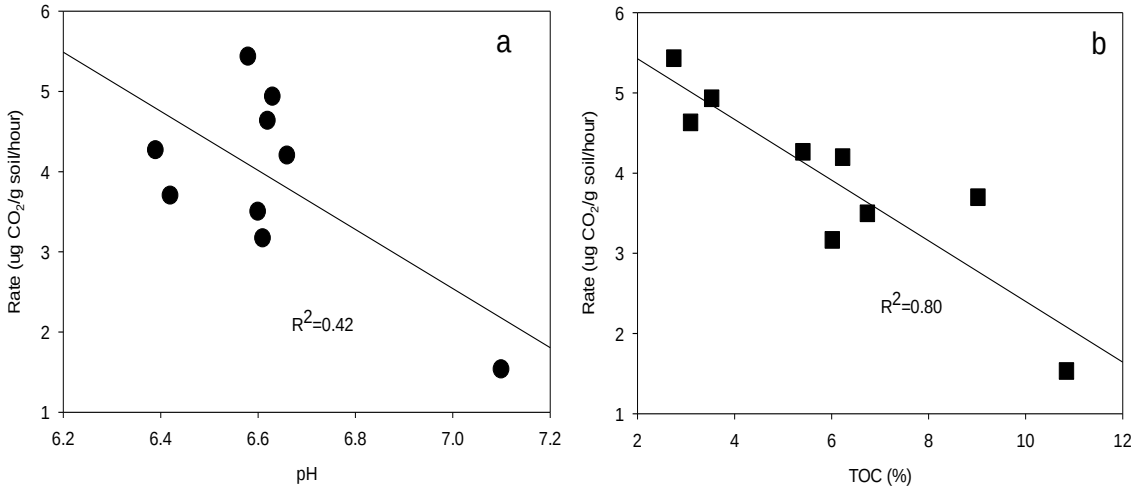

Figure 6.10: Correlation plots of carbon dioxide rate of production with (a) pH and (b) total organic carbon in the amended near neutral soils of the second experiment.

The influence of HTT on how biochar altered rate of CO_2 production is significant between 400 and 800^{o}C biochars across the two soils. For the acid soils the more stimulating effect from the ss600 biochar over the ss400 may simply be due to the higher liming effect of the former, though the ss800 biochar should have had the same liming effect based on its pH value (see Chapter 4, Table 4.4); while in the ess amended near neutral soil, the higher rate of CO_2 production from the lower temperature biochar (ess400) could be due to its higher labile carbon contents reflected in its higher volatile matter (see Chapter 4, Table 4.1) relative to the 600 and 800^{o}C biochars. Calvelo Pereira *et al.* (2011), reported decreased CO_2 evolution with increasing HTT for three different biochars, and ascribed the trend as partly due to intrinsic labile carbon contents of the biochars though they mentioned one of their low temperature samples (pine wood at 400^{o}C) did not follow that pattern probably due to its low surface area, limited liming ability, absence of carbonate and possible presence of compounds toxic to microbes. The authors however, varied both feedstock sources (3) and HTT (2) instead of focussing on only one variable as in this study where the feedstock source was fixed.

6.3.2 Denitrification enzyme activity

The trace N_2O emissions from the acid soil in the pilot experiment showed no correlation with any of pH, TOC (Figure 6.10) and WHC (R^2 = 0.31), while the suppressed rates of N_2O production with increasing biochar amendment in the near neutral soil showed no correlation with pH but had an inverse correlation with TOC (Figure 6.11) and WHC (R^2 = 0.66). The trace emission in the acid soil may perhaps be due to the intrinsically low carbon to NO_3^- ratio in these soils in addition to the low pH of the soil which are two of the three primary regulators to the synthesis and activity of the enzymes (nitrate reductase, Nar; nitrite reductase, Nir; nitric oxide reductase, Nor; and nitrous oxide reductase, Nos) responsible for the denitrification process (Cavigelli and Robertson, 2001). Having the only significant N_2O emission in the 10% amended soil could support this since the carbon to NO_3^- ratio is highest at that amendment level.

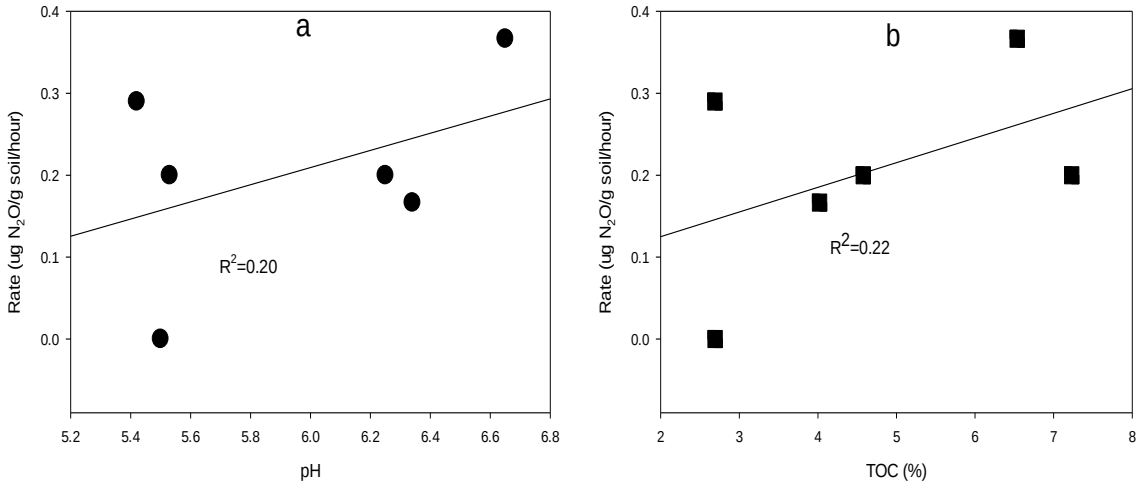

Figure 6.11: Correlation plots of nitrous oxide rate of production in µg N_2O/g soil/hour with (a) pH and (b) total organic carbon in the amended acid soils of the pilot experiment.

Reduced or suppressed N_2O production in the near neutral soil with increasing biochar additions agrees with many other reports (Spokas *et al.*, 2009; Spokas *et al.*, 2010; Taghizadeh-Toosi *et al.*, 2011; Case *et al.*, 2012; Yoo and Kang, 2012; Ameloot *et al.*, 2013; Harter *et al.*, 2013; Khan *et al.*, 2013; Saarnio *et al.*, 2013).

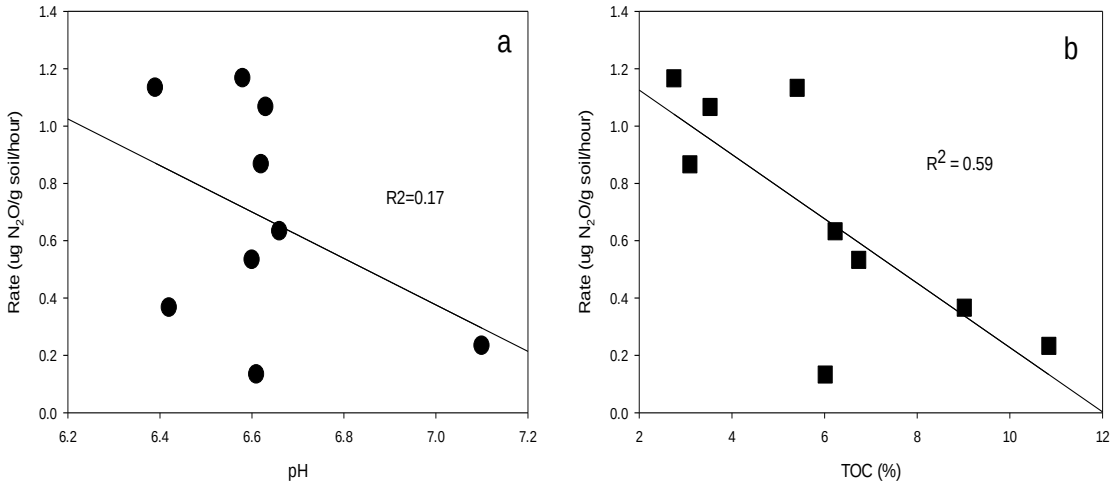

Figure 6.12: Correlation plots of nitrous oxide rate of production in µg N_2O/g soil/hour with (a) pH and (b) total organic carbon in the amended near neutral soils of the second experiment.

Some of these reports (Case *et al.*, 2012; Khan *et al.*, 2013) show results that do not support an earlier report suggesting only biochar amendment rates >20% w/w suppress nitrous oxide emissions (Spokas *et al.*, 2010). These authors and others proposed various reasons for the sort of decreased N_2O emissions with biochar amendment observed in this study and most of them suggested reduced availability of N to denitrifying microbes. In this study, N_2O was measured to assess DEA as a microbiological indicator on soil health (section 6.1) hence a suppression of N_2O may point to decreased activity. However, reasons other than reduced microbial activity have been proposed for the decrease in N_2O emissions from biochar amended low and/or high pH soils as explained in the next paragraph.

One of the earlier suggestions ascribed reduced nitrous oxide emission to either faster rate of N_2O reduction to molecular nitrogen or a low rate of its production resulting from biochar addition to soil (Spokas *et al.*, 2009). Faster rate of N_2O reduction as an explanation is not considered in this study because such reduction is blocked by the addition of acetylene which was meant to inhibit the activity of N_2O-reductase (see Chapter 3 section 3.7.2). Angst *et al.* (2013), believe biochar adsorbs NH_4^+ thereby retarding the production of NO_3^- needed by denitrifiers for N_2O production. This view is shared by Taghizadeh-Toosi *et al.* (2011) who added that another possible explanation may be the presence of microbial inhibiting chemical compounds on biochar surface. The hypotheses of Angst *et al.* (2013) may not apply in this investigation because NO_3^- was added to the microcosms in the DEA assay. Others

however, explain the negative correlation between N_2O production and increased biochar amendment rates they obtained as due to GHG absorption by biochar (Yoo and Kang, 2012). Case *et al.* (2012), while doubting the effect of soil pH on suppressed N_2O emission, hypothesized a mechanism involving physical or biological immobilization of NO_3^- needed for denitrification to give N_2O. The authors did not discuss the mechanism for such immobilization. Šlmek and Cooper (2002), reviewed 50 years of published research work on the influence of pH on denitrification noting that overall denitrification process is affected by soil pH; the process is less in acid soil compared to neutral or slightly alkaline soils. But the authors concluded that the influence of pH on the denitrification process is likely indirect in the form of lower availability of organic carbon and mineralised N (NH_4^+ or NO_3^-) to the denitrifying bacteria and not direct effect of pH on the denitrifying enzymes. It has been hypothesized that nitrate (an anion) immobilization could be in solution within biochar pores (Prendergast-Miller *et al.*, 2011), as it cannot be due to improved Cation Exchange Capacity (CEC) as suggested by other authors (Van Zwieten *et al.*, 2010) who associated greater nutrient retention to higher CEC. Ameloot *et al.* (2013), further added the possible role of increased soil aeration to NO_3^- retention by biochar; though the reference they cited for the claim of nitrate retention (Cheng *et al.*, 2008) does not contain such information. It rather contains reference to the report of Lehmann *et al.* (2003) which showed decreased leaching of applied ammonium in biochar-containing soils. Improved plant N uptake in cultivated soils which makes the element less available to denitrifiers has also been given as a reason for decreased N_2O emissions from biochar amended soils (Saarnio *et al.*, 2013). But this does not explain reduced emissions with increased biochar addition since in this study a single test plant was planted per pot at all amendment levels and more importantly N source was supplied in the microcosms. Thus a more plausible explanation for the observed reduction of N_2O emissions in this study is adsorption and/or immobilization of NO_3^- by the added biochar which will increase with increase in added biochar leading to decreased NO_3^- availability and lower N_2O emissions hence the inverse correlation between TOC and N_2O emissions. Moreover such a mechanism does not alter the soil microbial community which the DGGE profiles in Figures 6.6 and 6.7 seem to support.

6.3.3 Microbial community structure

Soil microorganisms play a central role in organic matter decomposition and nutrient cycling (Liu *et al.*, 2006). The DGGE method is a powerful culture-independent analytical tool capable of identifying community constituents representing as low as 1% of total microbial populations (Muyzer *et al.*, 1993). Each DGGE band represents many copies of a single amplicon (Hirsch *et al.*, 2010), hence the greater the number of bands in a DGGE profile the greater the microbial diversity and the higher the intensity of a band the higher the microbial abundance or population (Torsvik and Øvreås, 2002; Bloem and Breure, 2003). However, Nakatsu (2007) cautions that number of bands only represent dominant species and not necessarily overall diversity and band intensities point to relative densities of PCR products and should not be taken as equivalent to numerical microbial abundance in the original soil community. The author further stated that the disappearance of a band may not mean complete removal of specie from the community but rather may represent a change to reduced presence (to a level below detection limit) relative to other populations within the community.

Results in this study indicate similar community diversity in both the biochar amended and unamended control soils across the soil types especially after 12 weeks except for the lower intensity bands in the low pH soils. Hence biochar addition did not alter the microbial community structure in the test soils. However, it needs to be pointed out that these microbial studies are preliminary and a lot more could be done using techniques such as quantitative PCR (qPCR) to investigate physical increase or decrease of the soil bacterial community populations as a result of biochar addition.

6.4 Conclusion

In conclusion, the results under this Chapter indicated that addition of Sitka spruce biochar to the two test soils did influence the two microbial mediated soil processes measured (BR and DEA) in different ways, enhancing basal respiration in the low pH sandy soil and suppressing it in a systematic pattern based on HTT induced biochar properties in the near neutral soil. The amendment suppressed DEA in terms of reduced N_2O production potential in the near neutral soil but had no measurable effect in the acid soil. However, the reduced N_2O production potential may not be due to directly inhibited enzyme activity as other physicochemical reasons such as nutrient

immobilisation may explain the reductions. Moreover, there seems to be no change in the microbial community structure in the test soils as a result of biochar addition. Increasing the pyrolysis temperature of the biochars used in this study did change how the biochar influenced the processes measured but not in a progressive way. Taken together therefore the hypothesis put forward in this Chapter is partly accepted (HTT alters biochars influence on BR and DEA) and partly rejected (HTT alters biochars influence on microbial community selection).

From the perspective of biochar's other applications in agriculture and the environment, the suppression of N_2O emission at higher biochar application rate may offer double advantages in using biochar as a climate change mitigation tool; solid carbon sequestration and reduction in atmospheric N_2O concentrations. Additionally, the hypothesized mechanism for reduced N_2O emission (sorption and immobilisation of NH_4^+ and NO_3^-) could be advantageous in nutrient availability and hence improved soil fertility in biochar amended soils.

Chapter 7 General discussion

7.1 Introduction

This Chapter includes a general discussion in the light of all results as discussed in the various chapters of this thesis. Discussions are centred on how biochar properties and the impact of biochar addition on soil properties change with changes in our main variable, the Highest Temperature of Treatment (HTT), and finally compare the properties of the biochar products from the two different production streams; batch process from Lancashire and continuous process from Edinburgh.

7.2 Trends in biochar properties with highest temperature of treatment

7.2.1 Proximate analysis

The parameters determined under proximate analysis of the biochar included moisture content, volatile matter, fixed carbon and ash content. The trends of changes in these properties with pyrolysis temperature are presented in Figure 7.1 (a, b, c & d).

Moisture content (Figure 7.1a) in both biochars (ss & ess) decreases with increase in highest temperature of treatment which agrees with other reports (Titiladunayo *et al.*, 2012). The little increase in moisture content with the 800^{o}C biochar means the influence of HTT on moisture contents of the biochar is not progressive since the decrease with rise in HTT is not linear.

Figure 7.1b shows a linear drop of volatile matter contents with rise in pyrolysis temperature for both biochars, while Figure 7.2c shows increasing fixed carbon content for the biochar with increase in HTT. The decrease in volatile matter and increase in fixed carbon with increasing pyrolysis temperature have both been reported by other researchers (Kloss *et al.*, 2012; Crombie *et al.*, 2013; Ronsse *et al.*, 2013).

The ash contents of our biochars show no defined pattern (Figure 7.1d) with change in pyrolysis temperature. These contrasts with other reports (Titiladunayo *et al.*, 2012; Crombie *et al.*, 2013) that indicate increase in ash contents with rise in HTT. However, reports exist (Keiluweit *et al.*, 2010; Ronsse *et al.*, 2013) that show a rise and fall in ash content with rise in pyrolysis temperature for wood biochars especially at HTT > 700^{o}C and residence time above 10 minutes.

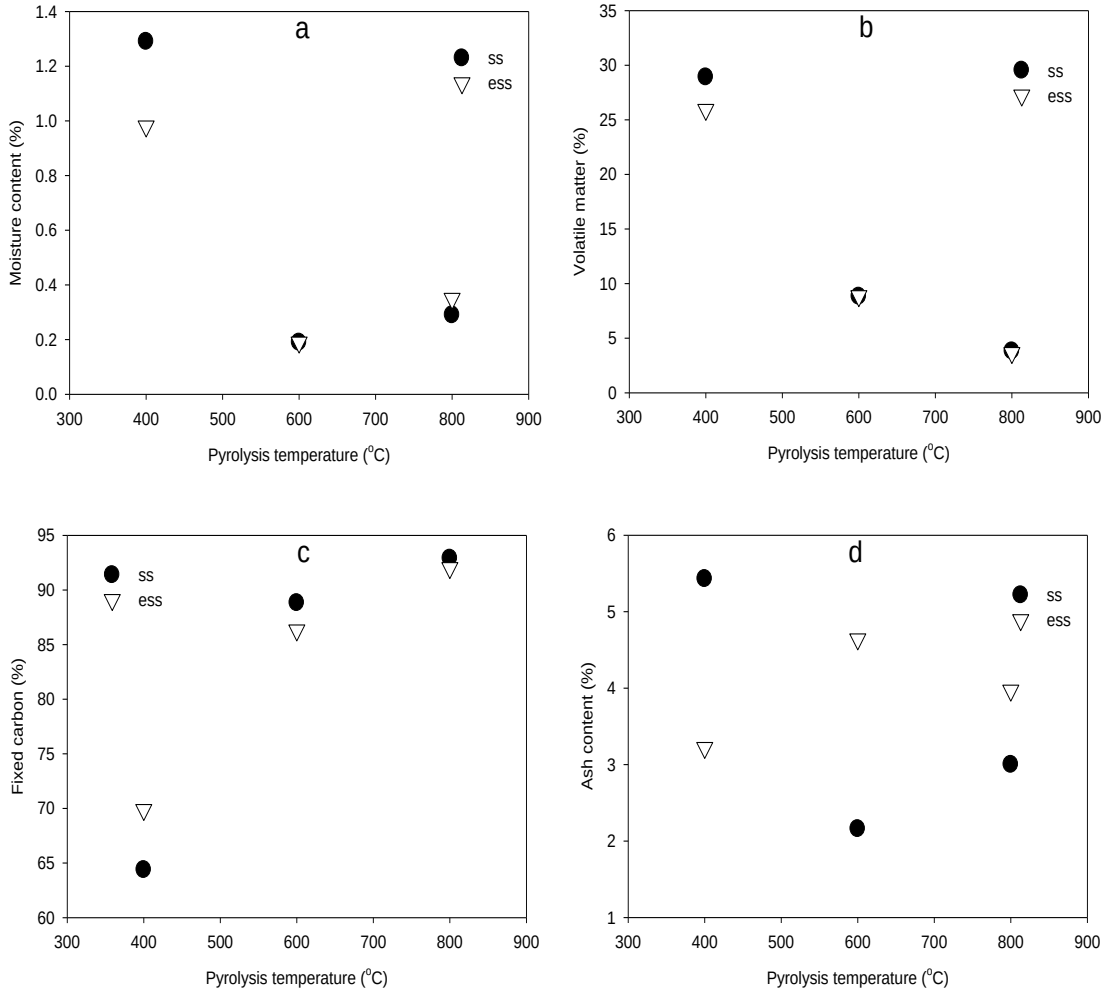

Figure 7.1: Trends of changes in proximate analysis results for the fresh biochar with highest temperature of treatment.

7.2.2 Physicochemical properties

Figure 7.2 shows the trends in physico-chemical properties of the biochars under investigation with pyrolysis temperature. Figure 7.2a shows a trend of increasing basic character for the biochars with increasing pyrolysis temperature. This conforms to several other reports (Pereira *et al.*, 2003; Enders *et al.*, 2012; Ronsse *et al.*, 2013; Wang *et al.*, 2013b). Increase in pH with HTT is associated with the loss of carboxylic acid functional groups from the biochar surfaces (Pereira *et al.*, 2003), an observation that is supported in this study by the FTIR results for the biochars (see Chapter 4, Table 4.5 and Figure 4.8).

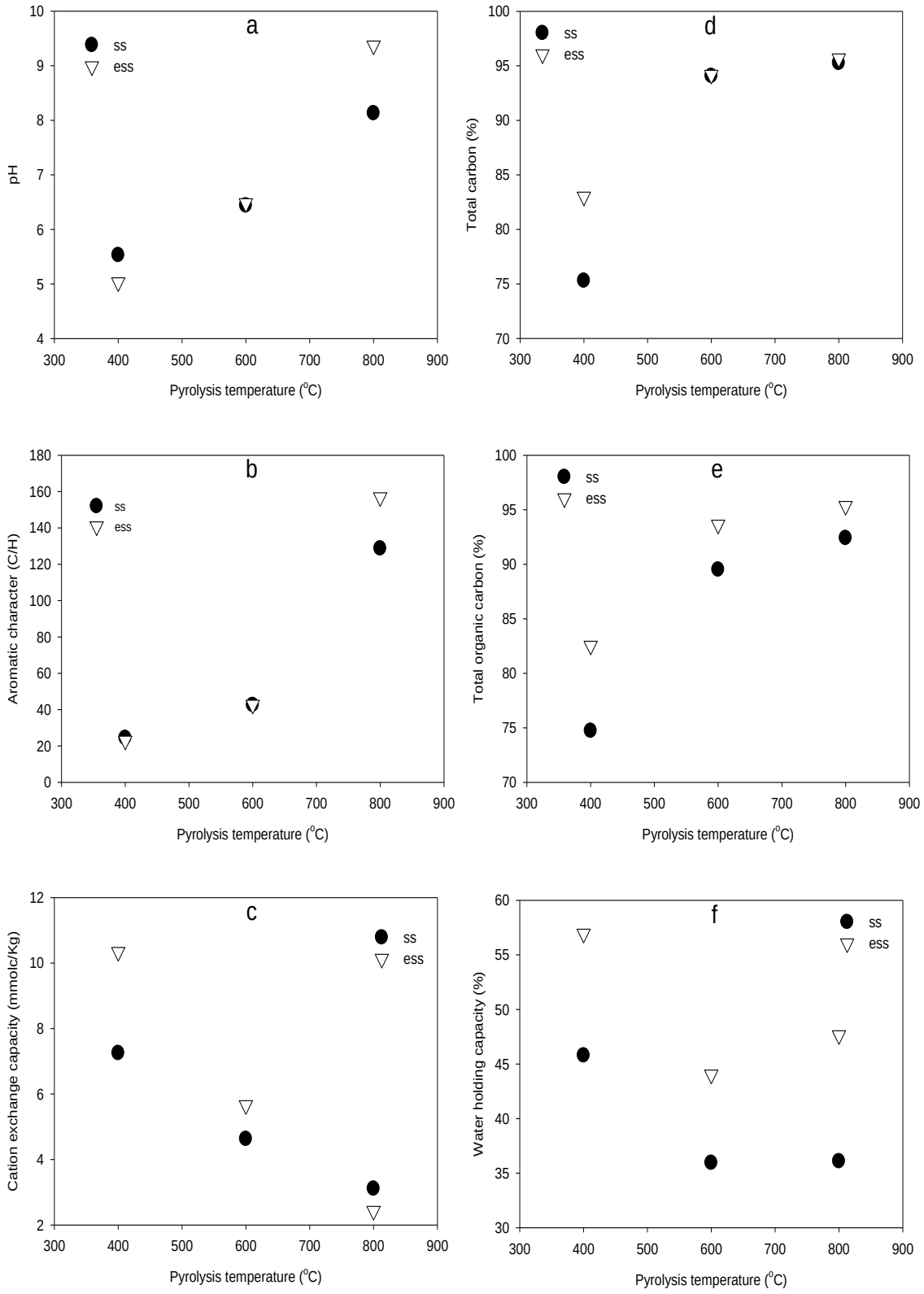

Figure 7.2: Trends of changes in the physicochemical properties of the fresh biochars with highest temperature of treatment.

An inverse relationship is reported between H/C ratio (a measure of aromatic character) and pyrolysis temperature (Kloss *et al.*, 2012). Hence, a plot of the reciprocal of this ratio (Figure 7.2b) shows a linear increase in aromatic character with

rise in HTT for the biochars produced in this study. The results agree with other reports (Kim *et al.*, 2012; Kloss *et al.*, 2012; Wang *et al.*, 2013b) and are supported by the decreased H contents of the biochars with increase in HTT (see Chapter 4, Table 4.4). Moreover, both aromatic character and pH are thought to be more influenced by pyrolysis temperature than by nature of feedstock (Zhao *et al.*, 2013).

The Cation Exchange Capacity (CEC) for our biochars shows a linear decrease with pyrolysis temperature (Figure 7.2c) which agrees with other findings (Kloss *et al.*, 2012; Wang *et al.*, 2013b). Kloss *et al.* (2012), ascribe the decrease in CEC to the removal of oxygen-containing functional groups on the biochar with rise in HTT, an observation that the FTIR results in this study also confirm (see Chapter 4, Table 4.5 and Figure 4.8).

The total carbon (Figure 7.2d) and total organic carbon (Figure 7.2e) contents of the biochars increase with increasing pyrolysis temperature in agreement with other reports (Kim *et al.*, 2012; Kloss *et al.*, 2012; Mašek *et al.*, 2013) and also supported by similar trends in fixed carbon contents of the biochars (see Chapter 4, Table 4.1). Water holding capacity (WHC) decreases with increase in highest temperature of treatment (Figure 7.2f). All the three properties in Figures 7.2 d, e & f show a much larger change (increase or decrease) between the 400^{o}C and 600^{o}C biochars compared to the change between the 600^{o}C and 800^{o}C biochars. The same observation is true with all proximate analysis results except ash contents (Figure 7.1 and Chapter 4, Table 4.1); elemental ratios (Figures 7.3 d, e & f, and Chapter 4, Table 4.4) and thermal properties (see Chapter 4, Table 4.2).

The percentage amounts of the elements H and O decrease with increase in pyrolysis temperature while N content was enhanced (Figure 7.3 a, b & c) in line with other reports (Chen and Chen, 2009; Kim *et al.*, 2012; Kloss *et al.*, 2012; Ronsse *et al.*, 2013) and supported by the evidence of gradual loss of functional groups on the biochar surfaces (see Chapter 4, Table 4.5 and Figure 4.8). However, Wang *et al.* (2013b) reported reduced N content with increasing HTT for bamboo wood which likely reflects similar observations made on the fluctuations of N and other mineral elements depending on feedstock source (Enders *et al.*, 2012; Zhao *et al.*, 2013).

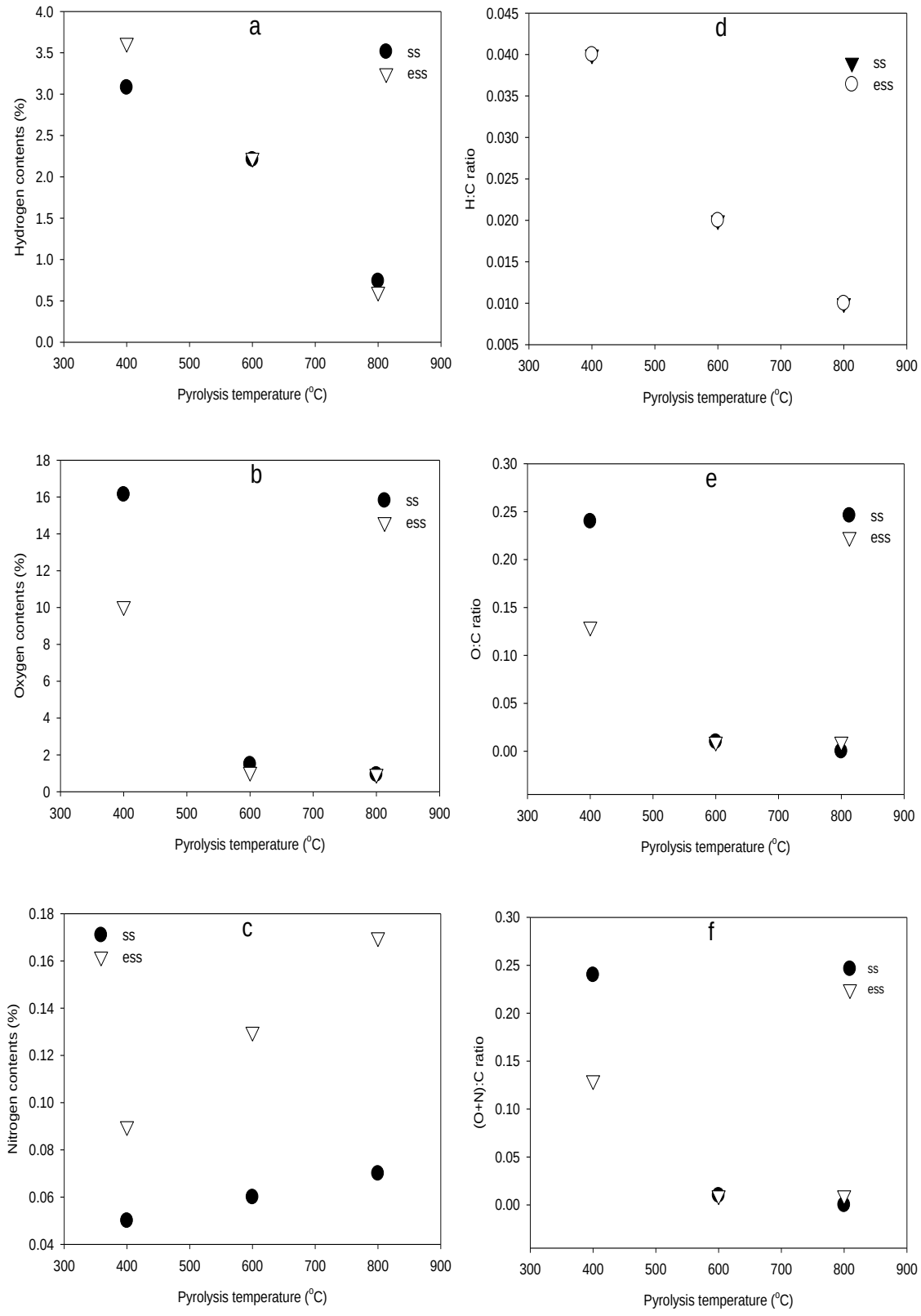

Figure 7.3: Trends of changes in the physicochemical properties of the fresh biochars with pyrolysis temperature.

The loss of H from aliphatic chains leads to increased aromatic character while its loss from protonated carbonyl groups explains the increased basic character of the biochars with rise in pyrolysis temperature. The decrease in O contents helps explain decrease in CEC with increase in HTT (Kloss *et al.*, 2012).

All elemental ratios for the biochars decrease with increasing pyrolysis temperature (Figure 7.3 d, e & f). This trend with HTT results from the increase in total C with HTT (Figure 7.2d) coupled with decreasing amounts of H and O with increasing pyrolysis temperature (Figure 7.3 a & b). Many other reports (Keiluweit *et al.*, 2010; Kloss *et al.*, 2012; Schimmelpfennig and Glaser, 2012) indicate this trend in elemental ratios for biochars.

7.3 Pyrolysis temperature and the effect of biochar amendment on soil properties

To assess how the main variable in this research project influenced the impact of the test biochars on the properties of the test soils, samples treated at 5% amendment rate were chosen for discussion because a larger number of properties were determined at this level of amendment. The plots in Figure 7.4 are used for discussion along with reference to Tables 5.2 and 5.3 from Chapter 5.

Figure 7.4a shows some influence of pyrolysis temperature of the biochars on pH of the amended soils. In the acid soil, increasing the HTT from 600 to 800^{o}C or 400 to 800^{o}C influenced a significant (Univariate ANOVA, Post Hoc Tests, $p = 0.000$) increase in pH of the biochar amended soil although there was no significant influence between the 400 and 600^{o}C biochars (Univariate ANOVA, Post Hoc Tests, $p = 0.933$). Changing the pyrolysis temperature of the biochars significantly (Univariate ANOVA, Post Hoc Tests, $p < 0.05$) influenced the biochars ability to progressively raise the pH of the amended near neutral soil.

The total organic carbon contents (Figure 7.4b) of the low pH amended soils were not significantly altered by increasing the pyrolysis temperature of the ss biochar (Univariate ANOVA, Post Hoc Tests, $p > 0.05$). However, the impact of ess biochar on the TOC contents of the near neutral soil was significant with increasing HTT from 400 to 800^{o}C (Univariate ANOVA, Post Hoc Tests, $p < 0.05$). But TOC contents for all amended soils had significant increases at all biochar amendment rates compared to

the control soil (Univariate ANOVA, Post Hoc Tests, $p < 0.05$) similar to other reports (Haefele *et al.*, 2011; Khan *et al.*, 2013).

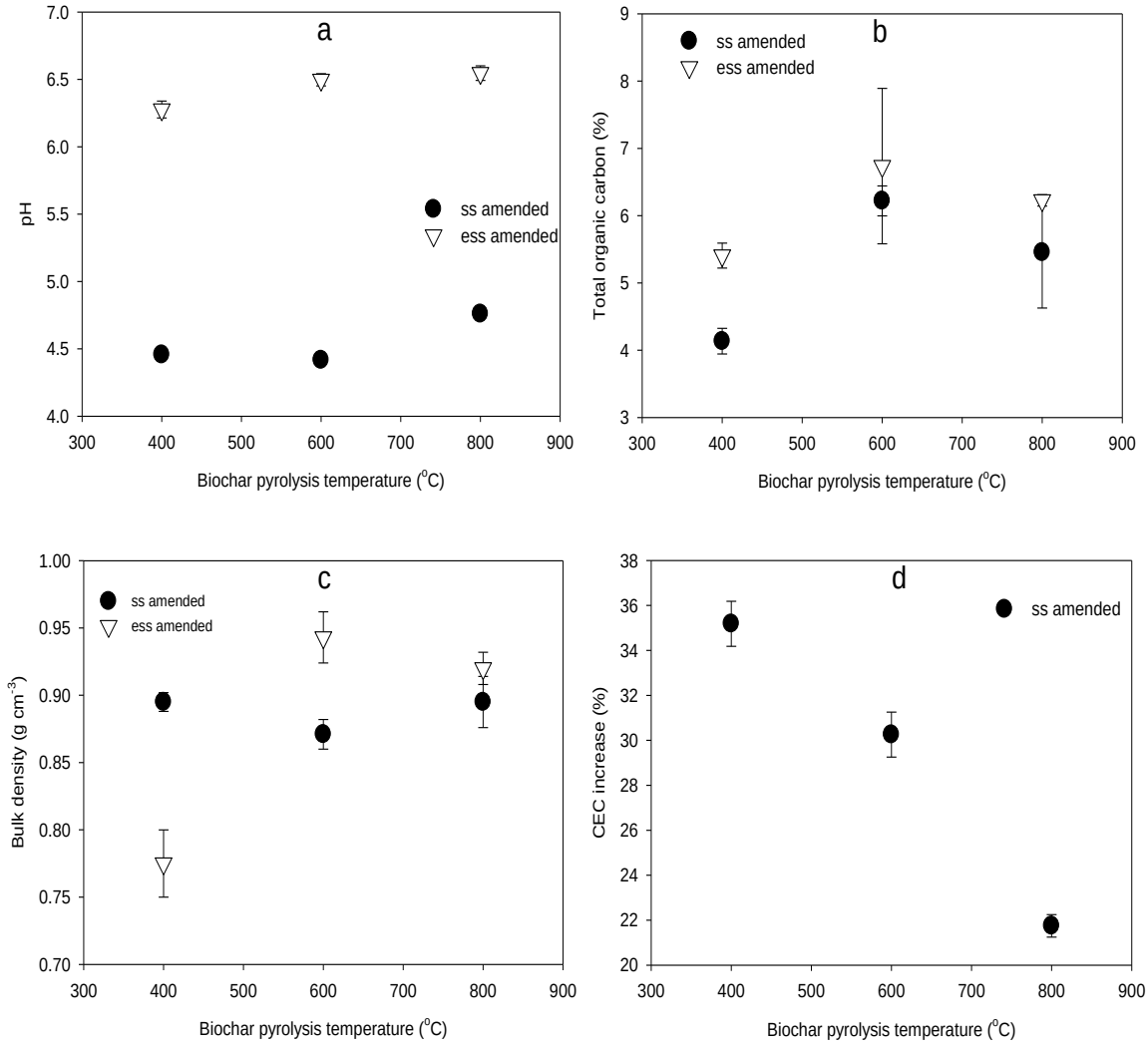

Figure 7.4: Influence of biochar pyrolysis temperature on the trends of properties change in the amended soils. Symbols represent soils amended at 5% level with the indicated biochar (ss or ess) produced at indicated temperature (400, 600 or 800°C). The ss biochar was used to amend the low pH sandy soil while the ess biochar was used to amend the near neutral loamy/clayey soil. Error bars are ±SE.

The impact of amendment on the bulk density (Figure 7.4c) of the test soil is not significantly influenced by increase in the pyrolysis temperature of the ss biochars (Univariate ANOVA, Post Hoc Tests, $p > 0.05$), while in the amended near neutral soil the influence of HTT is significant (Univariate ANOVA, Post Hoc Tests, $p = 0.000$) when increased from 400°C to either 600 or 800°C.

As mentioned earlier (see Chapter 5, section 5.2.1), cation exchange capacity (CEC) was determined only in the low pH soil. The CEC of the amended soils indicates a

dependence on the HTT's of the biochars used (Figure 7.4d). Increasing the pyrolysis temperature of the biochar from 400°C to either 600 or 800°C had significant influence (Univariate ANOVA, Post Hoc Tests, $p < 0.05$) on the impact of the added biochar on the CEC of the amended soil.

7.4 The influence of pyrolysis temperature on how biochar amendment impacts on soil processes and leek growth.

The impact of biochar amendment on soil respiration (Figure 7.5) is significantly altered with increase in HTT's of the biochars in both soils (Univariate ANOVA, Post Hoc Tests, $p = 0.000$), though the influence in both soils was for increase from 400 to 600°C since in the acid soil there was no significant difference between 400 and 800°C biochars (Univariate ANOVA, Post Hoc Tests, $p = 0.149$) and none between 600 and 800°C biochars in the near neutral soil (Univariate ANOVA, Post Hoc Tests, $p = 0.076$). There is a significant influence of increasing HTT (Univariate ANOVA, Post Hoc Tests, $p < 0.000$) on the impact of ess biochar on denitrification enzyme activity measured as rate of N_2O (Figure 7.6), though the suppression of N_2O emissions from the amended soil compared to the control was not ascribed to reduced microbial activity but rather due to other physicochemical reasons (see Chapter 6, section 6.3.2).

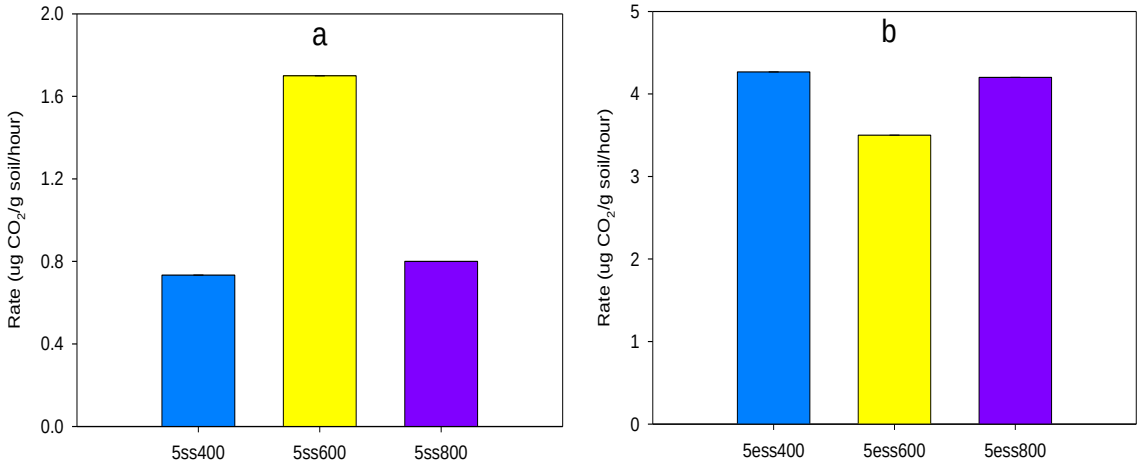

Figure 7.5: Influence of changes in highest temperature of treatment on the impact of biochar amendment on the rate of soil carbon dioxide emissions. Error bars (±SE) are not discernible due to the small values of the standard error.

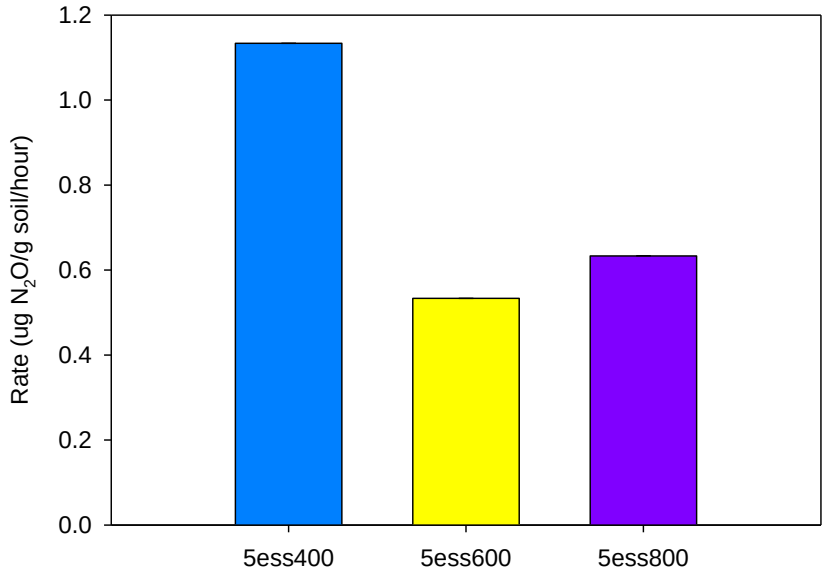

Figure 7.6: Influence of changes in highest temperature of treatment on the impact of biochar amendment on the rate of soil nitrous oxide emissions. Error bars (±SE) are not discernible due to the small values of the standard error.

From the ANOVA results on rates of leek growth in both soils (see Chapter 5, Table 5.5), the seeming influence of increasing biochar HTT's on biochar impact (Figure 7.7) is not significant (Univariate ANOVA, Post Hoc Tests, $p > 0.05$).

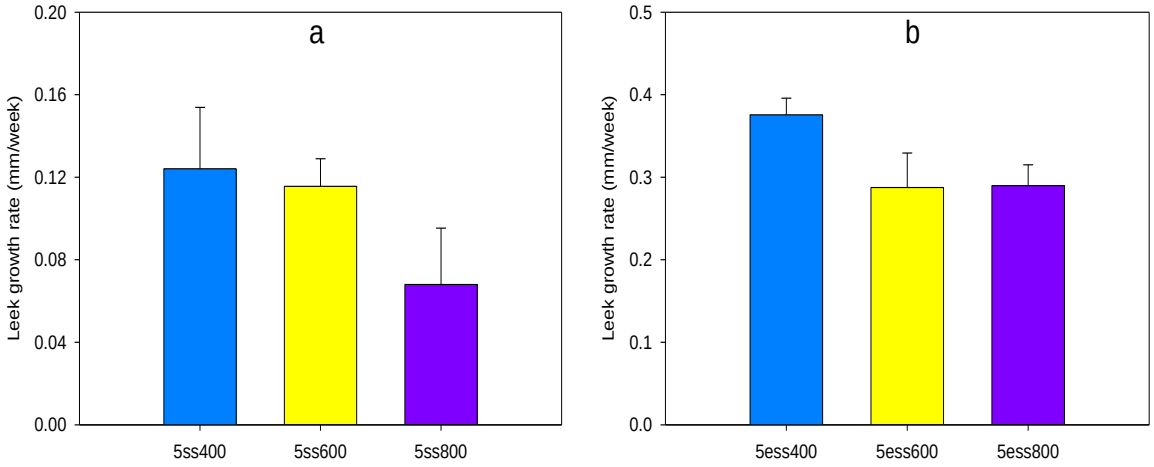

Figure 7.7: Influence of changes in highest temperature of treatment on the impact of biochar amendment on the rate of leek growth. Error bars represent ±SE.

7.5 Influence of production process

Biomass pyrolysis process parameters that could influence the properties of biochar include nature of feedstock, highest temperature of treatment (HTT), particle size of feedstock, residence time at HTT, heating rate, oven/kiln/furnace atmosphere and flow rate of purge gas, the first two being most important (Demirbas, 2004; Schimmelpfennig and Glaser, 2012; Crombie *et al.*, 2013; Mašek *et al.*, 2013; Ronsse *et al.*, 2013; Wang *et al.*, 2013b). For the biochar samples investigated in this study, feedstock type, HTT, residence time at HTT and furnace atmosphere were all the same in both the batch and continuous processes (Table 7.1). The heating rate is essentially the same, hence the only different parameters between the two production processes are particle size of the feedstock and possibly flow rate of purge gas since no information on it is available for the batch process (Table 7.1).

Table 7.1 Biochar production process conditions

Parameter	Production process	
	Batch	Continuous
Feedstock	Sitka spruce	Sitka spruce
HTT (^{o}C)	400, 600, 800	400, 600, 800
Particle size of Feedstock (mm)	2	10
Residence time at HTT (minutes)	30	30
Heating rate (^{o}C/min)	10	8, 12, 16
Kiln/Oven atmosphere (Purge gas)	Nitrogen	Nitrogen
Flow rate of purge gas (L/min)	No information	0.9

The mostly similar production parameters between the two processes may likely explain the many similarities in properties of the biochars from the two production streams. In terms of trends of properties with pyrolysis temperature, the biochars do not appear different from one another as is evident from Figures 7.1 and 7.2. Even when actual values are considered, differences exist only between the 400^{o}C biochars for only two parameters: oxygen content (Figure 7.3b) and O:C ratio (Figure 7.3e). The difference in O:C ratio is directly related to oxygen contents of the biochar which may be the result of differences in post-pyrolysis handling of the product. Information supplied from the producers of Edinburgh biochar show that in addition to purging the

kiln with nitrogen gas throughout the process, at the end, the warm biochar fresh from the pyrolysis unit was purged with nitrogen and sealed to avoid oxidation. This may explain the lower oxygen content and subsequent O:C ratio for the ess400 biochar which possibly was not the case for the batch process.

Chapter 8 General conclusions and recommended further work

8.1 Conclusions

The results considered in Chapter 4 go a long way in addressing our first hypothesis:

- Biological, chemical and physical properties of the biochar such as fixed carbon, pH, functional group chemistry, water holding and cation exchange capacities, are altered with increasing pyrolysis temperature.

With the feedstock source fixed, the results of this experimental study clearly show that temperature of production is the most important factor that alters both physical and chemical properties of biochars (Wang *et al.*, 2013b), a position supported in the case of C content by the report of Enders *et al.* (2012). The 400^{o}C biochars from both production streams stand well apart from the higher temperature (600 and 800^{o}C) biochars which on their part show only minor differences between themselves in properties such as all proximate analysis except ash content, pH, TC, TOC, thermal behaviour, elemental ratios and CEC. Thus, producing and using the 600^{o}C biochar in place of the 800^{o}C biochar could be more cost effective in terms of energy input. The Sitka Spruce biochar possesses high fuel quality potentials as evidenced by its satisfactory fuel ratio.

Measurement of microbial mediated soil processes and microbial community diversity in the amended soils addressed the third hypothesis of this study:

- Increasing pyrolysis temperature progressively alters biochar's ability to influence the selection of resultant microbial communities and microbial mediated processes e.g. respiration, and nitrogen cycling in soil environments.

The addition of Sitka spruce biochar to the two test soils did influence the two microbial mediated soil processes measured (BR and DEA), enhancing basal respiration in the low pH sandy soil and suppressing it in the near neutral soil. The amendment suppressed DEA in terms of reduced N_2O emissions in the near neutral soil and no measurable effect in the acid soil. However, the reduced N_2O emissions may not be due to decreased enzyme activity as other physicochemical reasons such as nutrient immobilisation may explain the reductions. Moreover, there seems to be no change in

the microbial community structure in the test soils as a result of biochar addition. But increasing the pyrolysis temperature of the biochars used in this study did change how the biochar influenced the processes measured but not in a progressive way. Taken together therefore the third hypothesis put forward is partly accepted (HTT alters biochars influence on BR and DEA) and partly rejected (HTT alters biochars influence on microbial community selection).

Soil amendment using Sitka spruce biochar brought about significant soil property changes compared to unamended controls which included raising the TOC and lowering BD in both the acid and near- neutral soils ($p < 0.05$); increasing the pH in the acid soil ($p < 0.05$) but not in the near neutral soil ($p > 0.05$); and increasing the CEC and WHC ($p < 0.05$) although these last two properties were only determined in the acid soil. The significant alteration of these soil properties due to biochar addition was also significantly influenced by changing the pyrolysis temperatures of the biochars (HTT) used for amendment which makes the fourth hypothesis put forward in this study acceptable. The fourth hypothesis was:

- Different biochar pyrolysis temperatures and their application rates will significantly alter the pH, total organic carbon (TOC) contents, bulk density (BD) water holding (WHC), and cation exchange capacities (CEC) of soils to which the biochar was added.

From the ANOVA results in Tables 5.1 and 5.2, a suggested suitable dosage of Sitka spruce biochar could be 5% of 600^{o}C biochars for enhanced pH in sandy acid soil; 1% of 400^{o}C biochars for enhanced TOC in both acidic sandy soil and near neutral loamy clayey soil; 5% of 600^{o}C biochars for enhanced CEC in acid soil; and 1% amendment level for enhanced WHC in the acid soil. Similarly from the ANOVA results in Tables 5.10 and 5.11, a dose of 5% 400^{o}C biochars could be suggested for enhanced leek growth in the acid soil only.

In answer to our fifth hypothesis:

- Different biochar pyrolysis temperatures and their application rates influence biochar's ability to impact on the growth rate of leek plant in amended soils compared to control soils.

The results in Chapter 5 and statistical treatment of the data considered showed that addition of Sitka spruce biochar to the test soils significantly influenced leek growth compared to the controls only in the acid soil (at 5 and 10% amendment rates) and not in the near neutral soil (Table 5.10). Impact of biochar addition on the growth of leek was achieved by altering some of the physico-chemical properties of the soil environment in which the plant was grown. However, altering the biochar HTT had no significant effect on leek growth in both soils (Table 5.11) and hence the fifth hypothesis is partly accepted (different biochar application rates influence biochar's ability to impact on the growth rate of leek plant in amended soils compared to control soils) and partly rejected (different biochar pyrolysis temperatures influence biochar's ability to impact on the growth rate of leek plant in amended soils compared to control soils).

The totality of our results (see Chapters 4, 5 & 6) and the discussion in section 7.5 of Chapter 7, go to answer the second hypothesis put forward:

- The biological and physico-chemical properties of biochars are altered differently based on the production process used.

There seems to be very little differences between the properties (see Chapter 4, Table 4.4) of the biochars from the batch and continuous production processes. In a similar way production process did not for example significantly influence the impact of these biochars on leek growth (see Chapter 5, Figure 5.13 and Tables 5.8 & 5.9). Hence, our second hypothesis is rejected.

On the whole and from the perspective of biochar's applications in agriculture and the environment, the result in this experimental study showed Sitka spruce biochar as possessing high percentage of recalcitrant (satisfactory recalcitrant index) organic carbon and hence, has strong potential worth pursuing as a tool for carbon sequestration. The suppression of N_2O emission from amended soil at higher biochar application rate (50-100 t/ha) may offer double advantages in using the Sitka spruce biochar as a climate change mitigation tool, namely; long term solid carbon sequestration and reduction in atmospheric N_2O concentrations especially as there is some evidence of unaltered microbial community structure in the soils. Additionally, the hypothesized mechanism for the observed reduction in N_2O emission (sorption

and immobilisation of NH_4^+ and NO_3^-) could be advantageous in nutrient availability and hence improved soil fertility in biochar amended soils.

8.2 Recommended further work

Consequent upon the strong need for a library or database of properties for individual biochars (Enders *et al.*, 2012; Kloss *et al.*, 2012; Schimmelpfennig and Glaser, 2012) resulting from systematic studies, a further analysis on the Sitka spruce biochars investigated in this study for areas that could not be covered in this thesis is desirable. These include a fuller proximate and ultimate analysis of the feedstock, PAH contents of the biochars, in addition to nutrient sorption, retention and leaching. Molecular analysis could be carried further to the point of identifying the identities of microorganisms introduced and/or removed by the biochar amendment.

The preliminary molecular study done can be improved further by for example, looking at other functional genes such as nitrate reductase and ammonia mono-oxygenase and how their functions are affected by biochar addition. Next generation DNA sequencing technologies could also be used to sequence the soil DNA extracts from the different experiments which will provide thousands of sequences for comparison.

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