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STABILITY OF BIOCHAR AND ITS INFLUENCE ON THE DYNAMICS OF SOIL PROPERTIES

**A thesis presented in partial fulfilment of the
requirements for the degree of**

**Doctor of Philosophy
(PhD)
in
Soil Science**



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*This Thesis is Dedicated to My
Father (Late Mr. H.M.S.P. Herath),
Mother (Mrs. W.M.P. Wijesinghe),
and All the Teachers Who Paved the
Way and Expected Me to Achieve
Such a Task One Day.....!!!*

ABSTRACT

The overall objective of this PhD was to investigate the stability of specific biochars – produced from corn stover (*Zea mays* L.) at 350 °C (CS-350) and 550 °C (CS-550) – and their roles on the dynamics of native organic matter (NOM) and physical properties of a Typic Fragiaqualf (Tokomaru soil; TK soil) and a Typic Hapludand (Egmont soil; EG soil). Except for the controls, all other treatments received a 7.18 t C ha⁻¹ application, either as fresh corn stover or as biochar. After 295 d, bulk density, saturated hydraulic conductivity (K_s), volumetric moisture content (θ_v), aggregate stability and soil water repellency were measured. At that sampling time, two undisturbed subsamples from each pot were taken: (i) in one subsample, lucerne (*Medicago sativa* L.) was seeded; (ii) in the other, the incubation was continued without plants. All pots were additionally incubated for 215 d. During the 510 d incubation, the CO₂-C efflux rate was determined for the selected 82 d, and samples for 19 d out of these 82 d were analysed for $\delta^{13}\text{C}\text{O}_2$. Soil samples at T0, T295 and T510 (with and without plants) were physically fractionated into coarse and fine free particulate organic matter (fPOM), silt+clay, and heavy fraction (HF), and analysed for $\delta^{13}\text{C}$ and total OC. Dichromate oxidation and acid hydrolysis were also conducted for the bulk soil and physical fractions.

Biochar application significantly increased ($P<0.05$) the aggregate stability of both soils (the effect of CS-550 biochar being more prominent in the TK soil than that in the EG soil, and the reverse pattern being observed for the CS-350 biochar), and the volumetric moisture content (θ_v). The latter effect was generally more evident in the TK soil than that in the EG soil, at both T0 and T295. Biochar addition significantly ($P<0.05$) increased the macroporosity in the TK soil and also the mesoporosity in the EG soil. Biochar also significantly increased ($P<0.05$) K_s of the TK soil but not that of the EG

soil. However, biochar was not found to increase water repellency of these soils. Overall, the results suggest that these biochars may facilitate drainage in the poorly drained TK soil and potentially reduce N₂O emissions.

Total accumulated CO₂-C evolved from the corn stover treatment was significantly higher ($P<0.05$) than that from rest of the treatments. No significant differences ($P<0.05$) were observed in the rate of CO₂-C evolution between the controls and biochar treatments. In both soils, fresh corn stover had a net positive priming effect on the NOM decomposition, while biochar had a net negative priming effect in the TK soil, but no effect in the EG soil. When a C balance was made considering the C lost during pyrolysis, the combination of CS-350 biochar and EG soil provided the greatest C saving of all treatments. When the different priming effects on NOM were also considered, differences among the two soils were balanced. The longer half-life (494 y) corresponded to the CS-550 biochar in the TK soil, while the half-lives of the other biochar-soil combinations were <200 y. It was estimated that 55 – 70 % of amended biochar-C would remain in soil after 100 y.

After 295 d, >78 % of biochar-C recovered in the TK soil and >64 % of biochar C in the EG soil ended in the coarse fPOM, >13 % (TK) and >21 % (EG) in the fine fPOM fraction, and the rest in the silt+clay fraction. The same pattern was observed after 510 d, both with and without plants. Most of the biochar particles thus concentrated into the “unprotected pool”. The use of dichromate oxidation to distinguish the recalcitrant fraction of C in the “unprotected pool” is suggested. Finally, the presence of both biochar and plants induced an additional accumulation of total organic carbon (OC) in the TK-350 and EG-550 soils ($P<0.05$), compared with the treatments with plants but no biochar.

The use of biochars in these OC-rich soils was proven to be adequate to promote C sequestration, especially when compared to the direct application of the fresh feedstock. This enhanced C sequestration is suggested to occur through (i) the addition of a stable C source (*e.g.*, condensed aromatic C in biochar), (ii) the protection of NOM (especially in the TK soil), and (iii) the interaction of biochar with new OC inputs to soil (*e.g.*, root exudates). The results from this study also indicated that long-term incubations in the absence of a continuous fresh input of plant material may create artefacts such as reduced aggregate protection and an apparent loss of aggregate protected OC. Future research should be directed to investigate (i) the influence of these physicochemical changes on microbial activity, population and diversity; and (ii) the evolution of these interactions under field conditions.

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Other contributions

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3. **Herath, H.M.S.K.**, Marta Camps-Arbestain, Mike Hedley, 2012. Effect of biochar on soil physical properties in two contrasting soils. New Zealand 2012 Biochar Workshop, Massey University, New Zealand.
http://www.biochar.co.nz/pdf/2012_Biochar_Final_programme_for%20web.pdf
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6. **Herath, H.M.S.K.**, Marta Camps-Arbestain, Mike Hedley, 2011. Mineralisation of carbon from fresh and carbonised corn stover in two New Zealand soils. New Zealand 2011 Biochar Workshop, 10 – 11 February 2011. Massey University, New Zealand.
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ACRONYMS

AWC	available water content
BET	Brunauer, Emmett and Teller surface area
BC	black carbon
C	carbon
C_{dichro}	dichromate oxidisable C
C_{Net}	net C
C_{org}	organic carbon (biochar/feedstock)
CP	cross polarisation
CS	corn stover feedstock
CS-350	corn stover biochar produced at 350 °C
CS-550	corn stover biochar produced at 550 °C
Ctr	control
d	day(s)
ECEC	effective cation exchange capacity
EG	Egmont soil
EG-350	CS-350 biochar amended Egmont soil
EG-550	CS-550 biochar amended Egmont soil
EG-CS	corn stover amended Egmont soil
f_{OA}	fraction of C from organic amendment
fPOM	free particulate organic matter
f_{SOC}	fraction of C from soil organic carbon
GC/MS	gas chromatography mass spectroscopy
GHG	greenhouse gas
h	hour(s)
HF	heavy fraction

IBI	international biochar initiative
IPCC	Intergovernmental panel on climate change
iPOM	intra particulate organic matter
K_s	saturated hydraulic conductivity
MED	molarity of ethanol droplet
MWD	mean weight diameter
NOM	native organic matter
NOM-C	C from native organic matter
OA	organic amendment
OA-C	C from organic amendment
OC	organic carbon (soil)
OM	organic matter
OC _{hl}	hydrolysable organic carbon
OC _{nhl}	non-hydrolysable organic carbon
OC _{nox}	non-oxidisable organic carbon
OC _{ox}	oxidisable organic carbon
RAWC	readily available water content
S	supportive information
SEM	scanning electron microscopy
SOM	soil organic matter
SSB	spinning side bands
T0	time zero
$t_{1/2}$	half life
T295 (R)	after 295 d of soil respiration
T510 (P)	after 510 d in the presence of plants
T510 (R)	after 510 d of soil respiration

TK	Tokomaru soil
TK-350	CS-350 biochar amended Tokomaru soil
TK-550	CS-550 biochar amended Tokomaru soil
TK-CS	corn stover amended Tokomaru soil
TPV	total soil pore volume
VM	volatile matter
VPDB	Vienna Pee Dee Belemnite
WDPT	water droplet penetration test
WHC	water holding capacity
y	year(s)
$\delta^{13}\text{C}$	stable C isotopic ratio
$\delta^{13}\text{C}_{\text{OA}}$	stable C isotopic ratio of organic amendment
$\delta^{13}\text{C}_{\text{SOC}}$	stable C isotopic ratio of soil organic carbon
ΔOC	difference of OC content between the amended and unamended treatments
$\Delta\text{OC}_{\text{hl}}$	difference of non-oxidisable OC content between the amended and unamended treatments
$\Delta\text{OC}_{\text{nhl}}$	difference of oxidisable OC content between the amended and unamended treatments
$\Delta\text{OC}_{\text{nox}}$	difference of non-oxidisable OC content between the amended and unamended treatments
$\Delta\text{OC}_{\text{ox}}$	difference of oxidisable OC content between the amended and unamended treatments
θ_v	volumetric water content

CHAPTER 1: Introduction

1.1 Overall importance of the research

Biochar is produced ideally from waste biomass under the process of thermal degradation with a limited presence of or absence of oxygen (Lehmann & Joseph, 2009; Shackley & Sohi, 2010). This conversion of biomass into biochar rapidly locks up a fraction of the carbon (C) present in the original feedstock into a much more durable form (Lehmann, 2007) that may remain for hundreds to thousands of years after application to soil (Lehmann *et al.*, 2008). Biochar has been proposed as a soil amendment (Lehmann *et al.*, 2006) to be used to mitigate greenhouse gas (GHG) emissions. For biochar technology to be accepted as a GHG mitigation strategy it must be proven that addition of biochar to soils results in net accretion of a permanent/stable form of soil C. To achieve this objective (i) biochar-C must be stable in soils within a centennial time frame, and (ii) the stable native organic matter (NOM) must not be significantly depleted when biochar is present.

The degree of biochar stability in soil – which not only depends on the intrinsic recalcitrance governed by the type of feedstock and process conditions but also on the pedoclimatic conditions of the area where it is deployed (Enders *et al.*, 2012) – determines how long biochar will contribute to the mitigation of GHG emissions. Robust evaluations thus need to be put in place to demonstrate the stability of biochar in soil over time. Unfortunately, the generally slow decay of biochar poses challenges to quantifying its longevity, as direct measures of turnover times may require centuries to millennia, which makes this approach unattainable. Therefore, predictions through modelling of observations from long-term studies may provide a potential way to achieve this task.

In the context of biochar storage as a GHG mitigation activity, any amount of C stored away from the atmosphere for at least 100 y can be computed as a GHG mitigation benefit (Pigneri & Anaya de la Rosa, 2009). The best estimate of the fraction of biochar C that will remain stable in soils for a minimum of 100 y is needed. To date, a number of studies have been conducted to investigate how biochar influences the cycling of NOM in soils (Cross & Sohi, 2011; Luo *et al.*, 2011; Zimmerman *et al.*, 2011; Singh *et al.*, 2012), although most of them have used soils with a low organic carbon (OC) content. The labile fraction of C in biochar provides C and energy to heterotrophic microorganisms, and the ash fraction may supply some of the mineral nutrient requirements (Chan & Xu, 2009; Bruun *et al.*, 2011). Therefore, biochar triggers microbial growth, although it may also include some compounds toxic to soil microorganisms (Fierer *et al.*, 2001; Fernandes & Brooks, 2003). Pores in biochar may also provide a suitable habitat for many micro-organisms by protecting them from predation and desiccation (Saito & Marumoto, 2002; Warnock *et al.*, 2007). Liang *et al.* (2010) observed that, in spite of the greater microbial biomass detected in charcoal-rich soils, these have a lower microbial metabolic quotient, which suggests the presence of a less active fraction of microorganisms within the biochar pore space compared to soils without charcoal. The extent to which this increase in microbial biomass affects NOM decomposition however needs a mechanistic understanding.

Surface interactions of biochar with soil particles will be influenced by the surface properties of the biochar. These properties will depend on the feedstock used and the temperature of pyrolysis, and will evolve with time as the surface of the charcoal oxidises (Joseph *et al.*, 2010). The addition of biochar to soil has been shown to induce a more rapid incorporation of fresh organic matter (OM) in to the aggregates (532 d) and association with the mineral fraction, compared to soils without charcoal (Liang *et*

al., 2010). The potential role of biochar surface properties in aggregation has been suggested by Joseph *et al.* (2010). Brodowski *et al.* (2005; 2006) also highlighted the role of charcoal-organo-mineral interactions in aggregate formation in soils in which charcoal has been present for at least 40 y. Biochar application to soil may thus improve physical properties of soils, and enhance NOM protection through aggregation. Nevertheless, some authors reported enhancement of NOM decomposition (positive priming effect), after adding carbonised material in to soil (Czimczik & Masiello, 2007; Luo *et al.*, 2011). Conversely, retardation of NOM decomposition (negative priming effect) was also found, after biochar application (Cross & Sohi, 2011; Zimmerman *et al.*, 2011; Singh *et al.*, 2012). Aggregation would not only protect biochar and other NOM constituents from on-going decomposition, but would also decrease the potential of biochar particles for clogging or cementing soil pores. The interactions between types of biochars and types of soils on biochar and NOM decomposition should therefore be carefully studied to enable the benefits of biochar as a C sequestration technology to be explored and demonstrated. More evidence is required on the potential biochar-induced enhancement of soil C on a wider range of soils, particularly those already rich in NOM.

The purpose of this PhD study is to develop a process-based understanding of three key areas: (i) the effect of biochar on aggregation and the implication of this process on other soil physical properties, (ii) the stability of biochar in NOM-rich soils, and (iii) the effect of biochar on the cycling of NOM.

1.2 Outline of the thesis with the hypotheses and objectives

This chapter (Chapter 1) provides the overall importance of the PhD research including the hypotheses and the objectives (Figure 1.1). The Chapter 2 composes a ‘Literature Review’ based on the previous related findings. Experimental works are discussed in Chapter 3 to 5. An overview about the details on the experimental works is presented in Figure 1.1.

Finally, Chapter 6 includes the overall conclusions and potentials for future research.

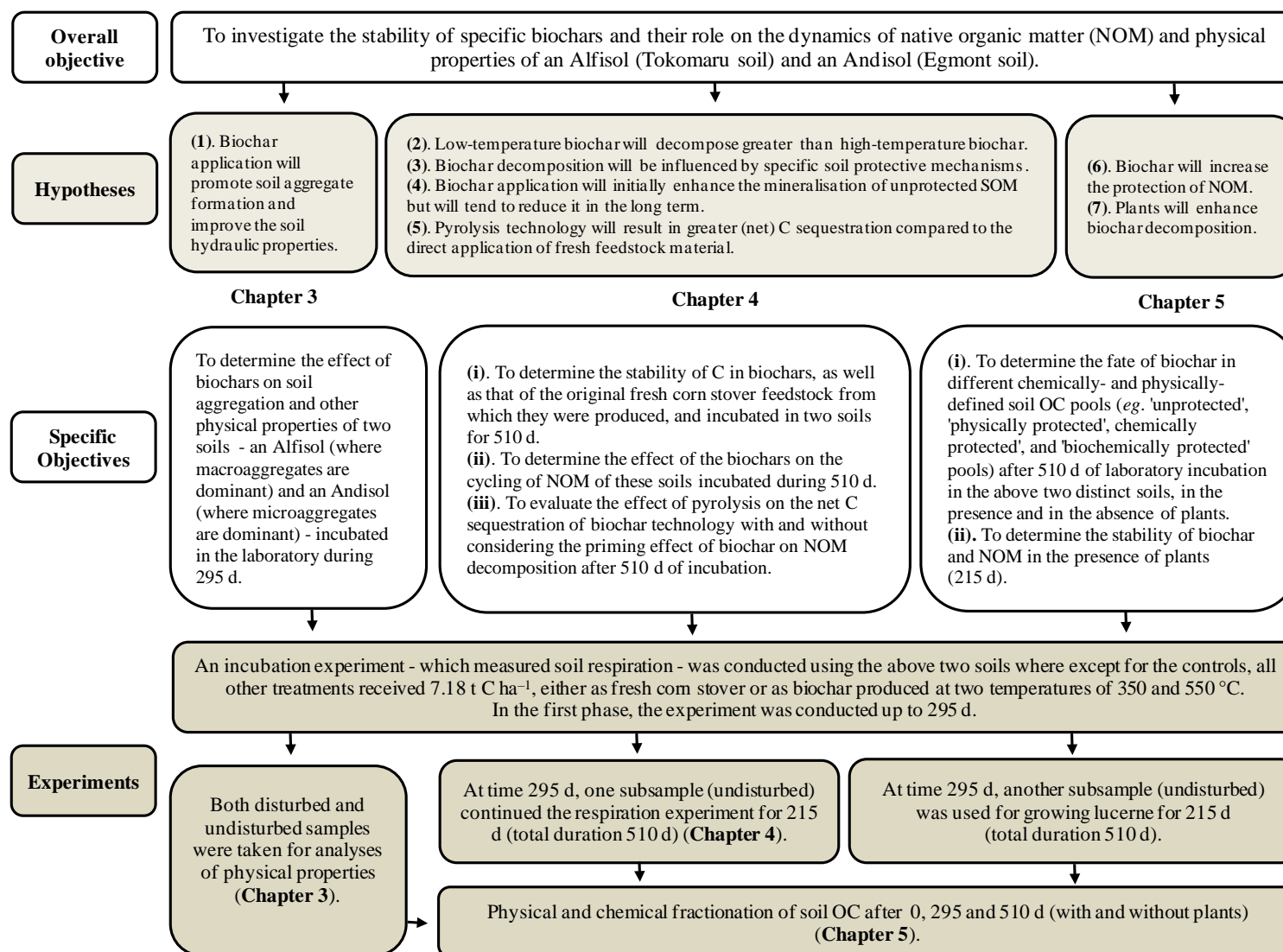


Figure 1.1: An overview of the three main research chapters comprising this thesis.

CHAPTER 2: Literature Review

The literature review of this thesis comprises several key areas, which target the use of biochar as a carbon sequestration strategy. Definitions for black carbon and biochar are provided. This is followed by a section on technology of pyrolysis and thereafter by another section that deals with the properties of biochar. The main agronomic and environmental uses of biochar are provided under the sections of “Agronomic significance of biochar” and “Stability of biochar and carbon sequestration potential”, respectively. The latter section is focussed in both the intrinsic stability of biochar – which is determined by the precursor materials and the applying conditions in pyrolysis – and the pedoclimatic conditions affecting biochar-C stability. A final section includes the conclusions drawn from this literature review and ‘research gaps’ identified in it.

2.1 What is black carbon?

Black carbon (BC) is considered as “a group of products, including partially charred carbonaceous material, char, charcoal, soot and graphite, formed under the thermal alteration processes” (Liang *et al.*, 2008; Liang *et al.*, 2010). Masiello (2004) defined BC as “a continuum of products resulted from combustion ranging from slightly charred biomass to highly condensed-refractory soot”. During thermal degradation, the structure of the original organic material becomes altered (Cheng & Lehmann, 2009); the final chemical composition and morphology of BC is mainly influenced by the type of fuel, temperature, and duration of thermal degradation (Hilscher *et al.*, 2009). As described in common char models, BC is made of clusters of fused rings, whose sizes vary widely (Knicker *et al.*, 2005; Preston & Schmidt, 2006). In a recent model presented by Knicker (2007), BC is described as a mixture of heat-altered biopolymers with domains of relatively small poly-aromatic clusters. The presence of BC can directly and

indirectly affect the C cycle and, to a certain extent, climatic conditions, through: (i) direct – the absorption of the solar radiation by the soot aerosols, (ii) indirect – the removal of CO₂ from the rapid C cycle, that is the biosphere-atmosphere gas exchange (Nguyen *et al.*, 2008). Once BC is formed, it can either remain *in situ* or be transported to other areas by means of wind or water (Kuhlbusch, 1998).

Quantification of soil BC is another important aspect that helps assess its real long-term effects. The longevity of BC is mainly attributed to its stability in the environment due to the resistance to biotic and abiotic decomposition (Liang *et al.*, 2010). The less-condensed BC types are more likely to react with oxygen (and other electron acceptors), and decompose through chemical oxidation and microbial activities than more-condensed BC types. The amount of BC present in soils has been estimated for different geographical locations with values ranging widely: 60 % of soil organic carbon (SOC) pools in Canadian Chernozems (Ponomarenko & Anderson, 2001); 45 % of SOC in German Chernozems (Schmidt *et al.*, 1999); 30 % Australian soils (Skjemstad *et al.*, 1996); 18 % in native US prairie (Glaser & Amelung, 2003); and 3.5 % in most US agricultural soils (Skjemstad *et al.*, 2002). Besides, it was noted that after a fire event only 3 % of the C in biomass remains as BC and, in global rates 50 – 270 Tg (Tg = 10¹² g) of BC is expected to be produced per year (Forbes *et al.*, 2006). In contrast, Kuhlbusch & Crutzen (1995) have estimated that this rate ranges between 50 and 600 Tg y⁻¹. The rate is still considered to be small compared with total C flows in the biosphere; 120,000 Tg y⁻¹ (IPCC, 2007). Nonetheless, total BC stocks in soils can be high as they tend to accumulate due to their recalcitrant nature (Schmidt & Noack, 2000).

2.2 What is biochar?

Biochar is basically defined as: “charcoal – biomass that has been pyrolysed in a zero or low oxygen environment – for which, owing to its inherent properties, scientific consensus exists that its application to soil at a specific site is expected to sustainably sequester C and concurrently improve soil functions (under current and future management), while avoiding short- and long-term detrimental effects to the wider environment as well as human and animal health” (Verheijen *et al.*, 2009). The definition given by Lehmann *et al.* (2009) is: “biochar is the C-rich product obtained when biomass, such as wood, manure or leaves, is heated in a closed container with little or no available air”. Shackley & Sohi (2010) provided an alternative definition: “biochar is the porous carbonaceous solid produced by thermo-chemical conversion of organic materials in an oxygen-depleted atmosphere which has physicochemical properties suitable for the safe and long-term storage of C in the environment and, potentially, soil improvement”. According to European Biochar Certificate (EBC) (2012), “biochar is defined as char produced by pyrolysis for use in agriculture (and other non-thermal applications) in an environmentally sustainable manner”. Further, “biochar is produced by biomass pyrolysis, a process whereby organic substances are broken down at temperatures ranging from 350 to 1000 °C in a low-oxygen (<2 %) thermal process”. The recent definition presented by the IBI (2012) describes biochar as “a solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment”.

The use of biochar as a soil additive has been proposed as a potential means to simultaneously “improve soil functions; and reduce emissions from biomass that would otherwise naturally degrade to GHG, by converting a portion of that biomass into a stable carbon fraction that has carbon sequestration value” (IBI, 2012). The particular

precursor type used and also the applying-pyrolysed conditions, such as temperature, time, heating rate, and level of oxygen, will decide the characteristics and usability of biochar (Antal & Grønli, 2003; Calvelo Pereira *et al.*, 2011). During pyrolysis, bio-oil and syngas are produced together with biochar, and these three different products are potentially valuable: the syngas and the bio-oil can be used as fuel and the biochar as a soil additive (Lehmann *et al.*, 2006) (Figure 2.1). However, the bio-oil needs upgrading as it contains a considerable amount of water (15 – 30 %), ash (<1 %), oxygen (35 – 40 %), and acids (with pH values of 2 – 3 due to the presence of some acids, such as acetic and formic acids) (Zhang *et al.*, 2007; Thangalazhy-Gopakumar *et al.*, 2010). The bio-oil could be upgraded through hydrodeoxygenation; catalytic cracking of pyrolysis vapours; emulsification; steam reforming; and the extraction of specific chemicals from the bio-oils (Zhang *et al.*, 2007). The syngas produced also need upgrading by removal of tars that may cause clogging and acid corrosion, and a final compression to increase its energy density (Jim Jones, personal communication). Additional details of the pyrolysis technology are discussed below under the section of ‘Technology of Pyrolysis’.

Biochar can be produced from a wide range of feedstocks (Van Zwieten *et al.*, 2009). Different biochars produced from different precursors have been tested: woody materials, *e.g.*, pine chips (Gaskin *et al.*, 2008; Hina *et al.*, 2010); agricultural wastes, *e.g.*, olive husk, corn cob, peanut hulls, and tea wastes (Gaskin *et al.*, 2008; Hilscher *et al.*, 2009; Fuertes *et al.*, 2010); green waste (Chan *et al.*, 2007); animal waste (Chan *et al.*, 2008; Gaskin *et al.*, 2008; Cantrell *et al.*, 2012; Wang *et al.*, 2012a); paper mill waste (Van Zwieten *et al.*, 2010b); and sewage sludge (Hossain *et al.*, 2010; Yao *et al.*, 2010). However, the stability of C in biochar is more related to the pyrolysed temperature, increasing its stability at increasing temperatures (Guo & Rockstraw,

2007), than with the type of feedstock (Calvelo Pereira *et al.*, 2011). The temperature effect on stability will be discussed under the “Stability of biochar and C sequestration potential” (Refer to Section 2.6).

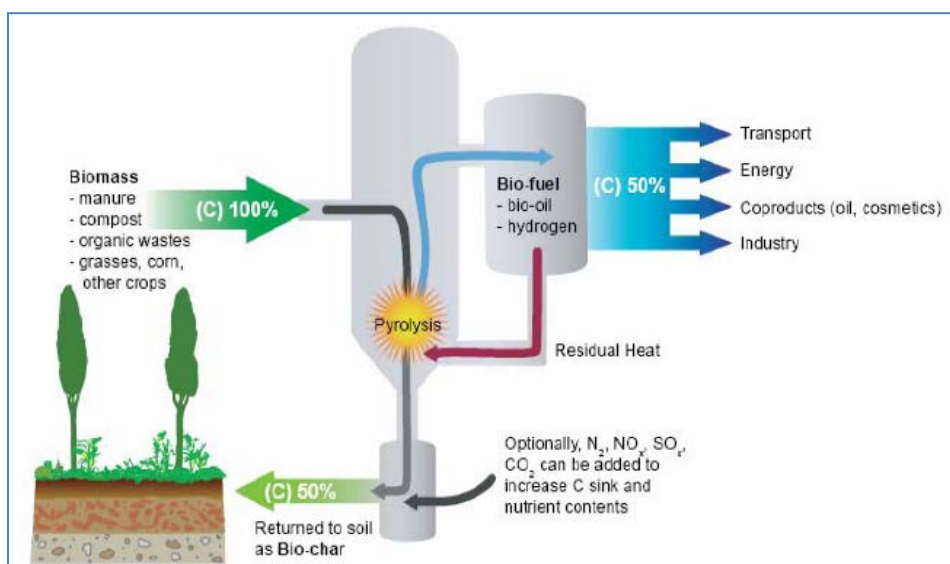
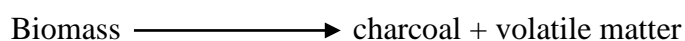


Figure 2.1: Schematic representation of biochar, bio-oil, and syngas production in pyrolysis and their use (source: www.csiro.au).

2.3 Technology of pyrolysis

Based on the conditions and also on the resulting products, different definitions are given to pyrolysis. Yaman (2004) considered pyrolysis as “the direct thermal decomposition of the organic matrix in the absence of oxygen to obtain an array of solid, liquid and gas products”. Ioannidou *et al.* (2009) have defined it as, “a thermochemical process that is used to transform low-density biomass ($\sim 1.5 \text{ GJ m}^{-3}$) and other organic materials into a high-energy density liquid, bio-oil ($\sim 22 \text{ GJ m}^{-3}$ or 17 MJ kg^{-1}), a high-energy-density solid, biochar ($\sim 18 \text{ MJ kg}^{-1}$), and a relatively low-density gas, syngas ($\sim 6 \text{ MJ kg}^{-1}$)”. Pyrolysis is also defined as “the thermal degradation of biomass by heat in the absence of oxygen, which results in the production of charcoal (solid), bio-oil (liquid), and fuel gas products” (Demirbas & Arin, 2002). The pyrolysis reaction is as follows.



During the pyrolysis, the major organic compounds such as cellulose ($C_6H_{10}O_5$)_n, hemicellulose ($C_5H_8O_4$)_n, and lignin ($C_{10}H_{12}O_3$) are expected to break down. The rates of thermal degradation process for these three different organic compounds are given as hemicellulose > cellulose > lignin (Demirbas & Arin, 2002), and therefore, the quality of the final product can be assumed to depend on the composition of the feedstock. Hemicellulose breaks down first (197 – 257 °C), followed by cellulose (237 – 347 °C), and lignin (277 – 497 °C) (Küçük & Demirbas, 1993). Nevertheless, irrespective of the type of compounds in feedstock, the whole process will affect the final yield in pyrolysis. For example, at high temperatures with fast heating rates, the biochar yield will be low. Conversely, a lower final temperature with smaller heating rates will yield higher biochar.

The main types of pyrolysis methods used – slow (conventional) pyrolysis, fast pyrolysis, and flash pyrolysis – are discussed elsewhere (Demirbas & Arin, 2002; Onay & Kockar, 2003; Laird *et al.*, 2009). The details are summarised in Table 2.1. The rate and extent of degradation in the above methods are governed by specific parameters, such as reactor type, temperature, particle size, heating rate, and pressure (Antal & Grønli, 2003).

Table 2.1: Range of the main operating parameters for pyrolysis process, as adapted from Demirbas & Arin (2002).

Condition	Slow pyrolysis	Fast pyrolysis	Flash pyrolysis
Pyrolysis temperature (°C)	277 – 677	577 – 977	777 – 1027
Heating rate (°C s ⁻¹)	0.1 – 1	10 – 200	>1000
Particle size (mm)	5 – 50	<1	<0.2
Solid residence time (s)	450 – 550	0.5 – 10	<0.5

As Demirbas & Arin (2002) identified, considering the temperature, the degree of thermal degradation in biomass may happen according to four different zones.

Zone A: The zone is also named as the ‘easily degradation zone’ and is extended up to 200 °C. The main changes observed in this zone are given by Demirbas & Arin (2002): “the surface of the biomass particle becomes dehydrated at this temperature, and water vapour, carbon dioxide, formic acid, acetic acid, and glyoxal are given off”.

Zone B: This zone includes the temperature range of 200 – 260 °C. The most obvious change occurring in this region is the evolving of water vapour, carbon dioxide, formic acid, acetic acid, glyoxal, and some carbon monoxide from the pyrolysing material. Endothermic reactions are expected to happen up to this level and non-condensable products are released. The other important activity limited to this area is the commencement of biomass conversion to charcoal.

Zone C: The temperature range of this zone extends from 262 to 502 °C. Actual pyrolysis commences at this particular zone. The zone is dominated by exothermic reactions. Accordingly, “combustible gases such as carbon monoxide from cleaving of carbonyl group, methane, formaldehyde, formic acid, acetic acid, methanol, and hydrogen are being liberated and charcoal is formed” (Demirbas & Arin, 2002).

Zone D: The most noticeable activity occurring in this zone is charcoal production. The zone is extended beyond 502 °C of temperature. The most desirable temperature range for carbonisation to occur has been identified as 402 – 602 °C (Chum, 1991).

2.4 Properties of biochar

2.4.1 Chemical structure and surface characteristics

The molecular structure of biochar primarily depends on the arrangement of ‘C’ in pyrogenic compounds formed during the thermal degradation process. It is noted that the core of biochar structure is highly condensed compared to the periphery (Liang *et al.*, 2008). Graetz and Skjemstad (2003) also noted that some biochars contained highly condensed graphite-like structures. But in some other biochars, they could see only a few clusters of aromatic rings. Considering the recalcitrant nature attributed by the condensed-chemical structure, biochar may have a less active role in the soil C and N cycles (Novak *et al.*, 2010). Nevertheless, biochar may also take part in some chemical reactions presumably due to the presence of some unstable C groups, mainly at the surface of chemical structure. An understanding of the dominant forms of C in biochar is crucial to know the chemical behaviour of biochars and, in turn the reactivity (Calvelo Pereira *et al.*, 2011). Dominant C forms found in biochar include aliphatic, aromatic, carboxylate, and carbonyl forms (Novak *et al.*, 2009a). Table 2.2 shows how the total C determined with a Bruker DSX-300 ¹³C NMR Spectrometer (Karlsruhe, Germany) is distributed among different structural groups (as a percentage) depending on the pyrolysis temperature for selected precursor materials.

Table 2.2: Percentage total C distribution among structural groups in biochars pyrolysed from different feedstocks and different temperatures as adapted from Novak *et al.* (2009a).

Feedstock	Pyrolysis Temperature (°C)	Total C (%)		Carboxylate	Carbonyl	Sum
		Aliphatic	Aromatic			
Peanut hull	400	35	57	5	3	100
	500	12	82	3	3	100
Pecan shell	350	49	42	4	5	100
	700	29	58	14	0	100
Poultry litter	350	36	57	4	3	100
	700	n.a. ^b	n.a.	n.a.	n.a.	n.a.
Switchgrass	250	63	29	5	3	100
	500	12	82	3	3	100

^bn.a. – not applicable

It is obvious that there is a strong relationship between the pyrolysis conditions, *i.e.* temperature, and the chemical structure of biochars. At high pyrolysis temperatures, for example 400 – 700 °C, feedstock is expected to be converted into poly-condensed-aromatic structures that contain high-recalcitrant C (Kloss *et al.*, 2011; Schimmelpfennig & Glaser, 2011). Carbon concentration increases as pyrolysis temperature increases, whereas H and O concentrations gradually decrease (Novak *et al.*, 2009a). Therefore, the relationship then between H and O with pyrolysis temperature is likely to be negative, as generally represented in the Van Krevelen diagram (Van Krevelen, 1950) (Figure 2.2). Conversely, the ash content will increase as the temperature of pyrolysis increases (Novak *et al.*, 2009a; Kloss *et al.*, 2011). When the pyrolysis happens comparatively at lower temperatures, *i.e.* 250 – 400 °C, a biochar rich in C=O and C-H functional groups is formed. The yield recovery is comparatively high under this lower temperature range, and the dominant organic compounds are aliphatic or less stable cellulose-like structures, which can easily be degraded by microbes.

As many of the studies concluded, a positive correlation between the pyrolysis temperature and the biochar stability is expected. Nguyen & Lehmann (2009) clearly observed this relationship, *e.g.*, the loss of C from biochar produced at 350 °C during an incubation study was greater than that at 600 °C. Chemically, higher charring temperatures are expected to increasingly change O-alkyl C to aryl and O-aryl furan-like structures (Baldock & Smernik, 2002), which would thus lead to enhance condensation of C structures (Nishimiya *et al.*, 1998; Kaal *et al.*, 2009a). More importantly, as a consequence of the peak temperature of pyrolysis, Nguyen & Lehmann (2009) observed higher aromaticity and lower H/C and O/C ratios of both corn-derived and oak-derived BC at 600 °C than at 350 °C (Table 2.3). However, irrespective of the pyrolysis temperature, it is obvious that surface C of biochars is

prone to conversion into nutrient exchange sites upon activation or weathering (Novak *et al.*, 2009a; Hina *et al.*, 2010; Joseph *et al.*, 2010).

Table 2.3: Properties of BC depending on the charring temperature as adapted from Nguyen & Lehmann (2009).

Biomass type	Charring temperature (°C)	C/N	O/C	H/C	Aromaticity ^a
Corn stover	350	73	0.37	0.07	77.6
	600	86	0.21	0.03	85.2
Oak wood	350	759	0.26	0.06	61.8
	600	737	0.10	0.02	68.4

^aQuantified via X-ray diffraction (XRD) analysis

The atomic ratios of C, H and O can be calculated to give an idea about the behaviour of BC in relation to its polarity and, thus, its potential interaction with water (Novak *et al.*, 2009a; Schimmelpfennig & Glaser, 2011). The Van Krevelen diagram (Van Krevelen, 1950) can be used to understand how the functionality of biochar changes over time in relation to the atomic ratios of H/C and O/C. Importantly, these ratios have been set by IBI (2012) to standardise biochars where <0.7 for H/C_{org} is taken into consideration, C_{org} being the C associated to the charred structure (that is, inorganic C, if present, is not considered). Materials ≥ 0.7 (H/C) are grouped as non-condensed aromatic structures like lignin (Schimmelpfennig & Glaser, 2011). From the Van Krevelen diagram (Van Krevelen, 1950), the dominant reactions occurring during pyrolysis (*e.g.*, dehydration, decarboxylation, and demethylation) can be inferred (Hammes *et al.*, 2006). Hammes & Schmidt (2009) further discussed these relationships in comparison to fresh biochar considering certain incubation studies undertaken (Shindo, 1991; Baldock & Smernik, 2002; Cheng *et al.*, 2006; Cheng *et al.*, 2008a) at different temperatures.

In Figure 2.2 a Van Krevelen diagram is represented based on the work of Hammes *et al.* (2006), as cited by Hammes & Schmidt (2009). The biochars represented in Figure 2.2 are termed as follows: ‘Fresh biochar’ for those produced at around 450 °C or higher; ‘oxidised biochar’ for biochars that have been subjected to oxidation conditions;

and ‘slightly charred biochar’ for biochars produced below 450 °C”. The arrows (lower right) indicate the processes involved in the change in elemental composition of biochar in the soil under different circumstances (Figure 2.2).

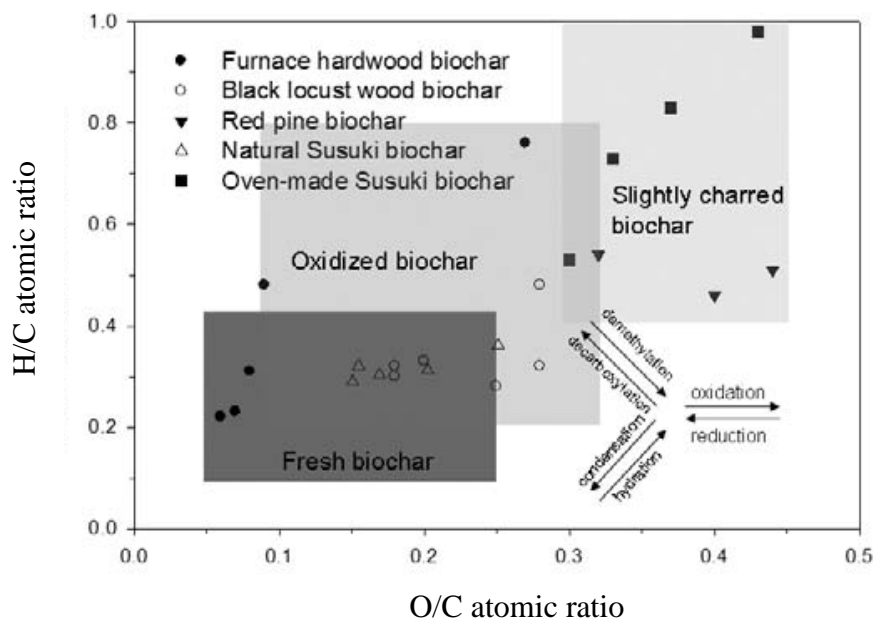


Figure 2.2: Van Krevelen plot of the elemental composition changes of five types of biochar with incubation and over time as adapted from Hemmes & Schmidt (2009).

2.4.2 Elemental composition

The elemental composition of the final pyrogenic material is basically dependent on the feedstock type and the pyrolysis temperature. In other words, these factors mostly determine the quantity and also the properties of biochar. Table 2.4 provides information about the elemental analysis of carbonised charcoals after pyrolysing certain feedstocks (Antal & Grønli, 2003).

In general, the amount of ash in the charcoal is highly influenced by the type of feedstock (Yaman, 2004; Kloss *et al.*, 2011; Spokas *et al.*, 2011). For example, the ash concentration in biochar from softwoods will have less than 1 %, whereas the ash content of charcoal produced from herbaceous plants and agricultural residues can reach values of 15 % (Yaman, 2004). Basically, ash contains Ca, Mg, Na, K, and other

anionic nutrients such as phosphates, sulphates, oxides and carbonates. However, as this depends on the feedstock types, the composition of ash is highly variable.

Table 2.4: Elemental analysis of carbonised charcoals, as adapted from Antal & Grønli (2003).

Feedstock	C (wt %)	H (wt %)	O (wt %)	N (wt %)	S (wt %)	Ash (wt %)
Coconut shell hull	92.28	1.09	3.08	0.47	0.04	2.78
Corn cob	86.38	1.20	5.34	0.56	0.05	4.31
Kukui nut shell	90.31	1.03	4.31	0.42	0.02	3.27
Leucaena wood	85.41	1.27	6.37	0.53	0.04	4.62
Macadamia nut shell	94.58	0.97	2.93	0.47	0.03	1.04
Oak board	91.50	1.22	3.55	0.18	0.01	1.04
Oak slabs	92.84	1.09	3.49	0.24	0.04	1.46
Pine wood	94.58	1.06	3.09	0.11	0.04	0.69
Rice hulls	52.61	0.82	3.87	0.57	0.06	41.34

wt – weight

As Laird *et al.* (2009) observed, some biomass sources such as corn stover, rice husks, and *Miscanthus* spp. are rich in silicon (Si). Thus, biochars obtained from these feedstocks will contain a high amount of Si. Examples for the ash composition of different charcoals produced from flash pyrolysis are given in Table 2.5.

Table 2.5: Ash composition of switch grass, corn stover, and hardwood chars by X-ray fluorescence spectroscopy prepared by the pressed pellet method as adapted from Brewer *et al.* (2009).

Element	Switchgrass char	Corn stover char	Hardwood char
Al ₂ O ₃	0.49	2.33	0.60
CaO	3.65	3.80	22.37
Cl	0.47	0.59	0.03
Fe ₂ O ₃	0.76	1.87	2.36
K ₂ O	6.00	4.03	1.35
MgO	1.55	2.02	0.48
MnO ₂	0.15	0.13	0.83
Na ₂ O	0.07	0.20	0.06
P ₂ O ₅	3.86	1.19	0.20
SiO ₂	43.62	29.98	5.67
SO ₃	0.99	0.28	0.27
Other	0.25	0.64	0.51
Total	61.86	47.06	34.73

All values are dry weight %. Elements are represented as their respective oxides.

The biochars produced from sewage sludge, especially those from highly industrialised areas, may contain a considerable amount of heavy metals. For example, Hossain *et al.* (2010) found that biochar produced from a wastewater sludge collected from a waste water treatment plant in Sydney (Australia) had substantial concentrations of heavy metals and trace elements (Table 2.6). However, this was not the case in biochars produced from sewage sludge of a non-industrialised area in New Zealand (Yao *et al.*, 2010).

Table 2.6: Concentration (mg kg⁻¹) of heavy metals and trace elements in biochar produced from waste water sludge as adapted from Hossain *et al.* (2010).

Element	Concentration in biochar (mg kg ⁻¹)
Arsenic (As)	8.8
Cadmium (Cd)	4.7
Chromium (Cr)	230
Copper (Cu)	2100
Lead (Pb)	160
Nickel (Ni)	740
Selenium (Se)	7
Zinc (Zn)	3300
Antimony (Sb)	8
Boron (B)	20
Silver (Ag)	29
Barium (Ba)	750
Beryllium (Be)	1
Cobalt (Co)	21
Tin (Sn)	310
Strontium (Sr)	390

2.4.3 Physical characteristics

Microscopic images, particularly those obtained using the scanning electron microscope (SEM), are important in analysing the physical structure of biochar. The physical structure of biochar is expected to be dependent on certain factors: temperature of pyrolysis, heating rates, and feedstock type (Downie *et al.*, 2009; Fuertes *et al.*, 2010). Brodowski *et al.* (2005) identified some structures of the parenchyma and also other primary tissues of higher plants and associated fungi were still distinguishable after

pyrolysing at 350 °C. Figure 2.3 shows the scanning electron micrographs of charcoal produced after charring maize straw at a temperature of 350 °C for 2 h (Brodowski *et al.*, 2005). Jones & Chaloner (1991) found that the structure of cell walls of *Pinus sylvestris* wood remained unchanged after exposure to heat at 340 °C. Simultaneously, they could observe some cracks along the side of the middle lamellae. The structural changes due to heat treatment are prominent when the thermal alteration of biomass happens at comparatively higher temperatures. Brodowski *et al.* (2005) observed no structure remains or complete destruction of the structure of the above feedstock material once the heating was undertaken at 600 °C. Therefore, it is clear that the structure of charcoal remaining after pyrolysis may give an idea about the level of heat applied during the carbonisation process.

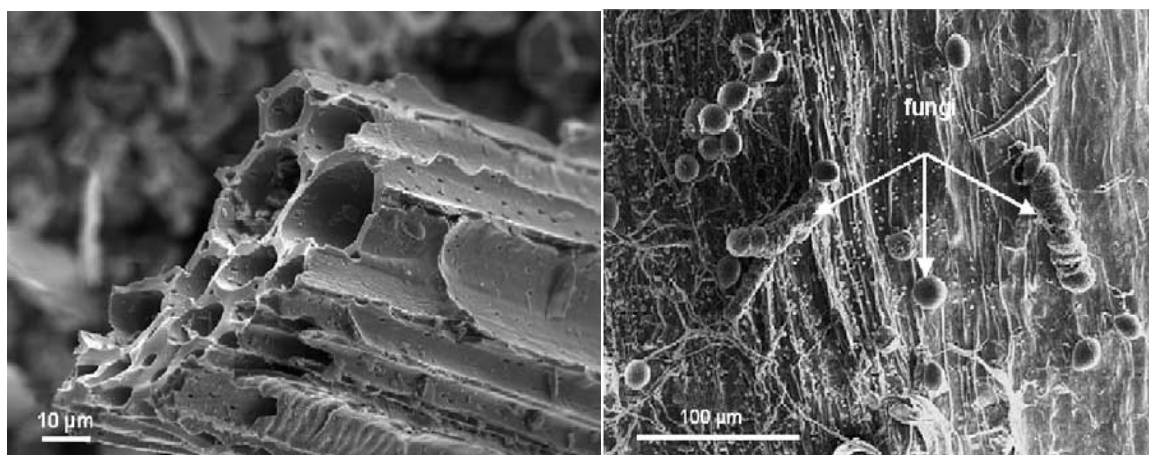


Figure 2.3: Scanning electron micrographs of BC prepared by charring maize straw at a temperature of 350 °C for 2 h, as adapted from Brodowski *et al.* (2005).

2.5 Agronomic significance of biochar

Application of biochar to soil was reported to give numerous benefits including improvements in soil quality – increase in cation exchange capacity (CEC), water retention, liming ability, microbial functions of soils – SOC content and plant growth (Glaser *et al.*, 2002; Liang *et al.*, 2006; Chan *et al.*, 2007; 2008; van Zwieten *et al.*, 2010a; Slavich *et al.*, 2012), especially in tropical areas. Other studies report similar

beneficial effects under temperate conditions (Streubel *et al.*, 2011). A recent study conducted by Wang *et al.* (2012a) under glasshouse condition showed that biochars – produced from dairy manure-wood and biosolid-wood mixtures at 250, 350, 450, and 550 °C – had similar effects to P fertilizers in increasing the shoot yield of rye grass. Thus, they concluded that the “high-ash biochars with high P concentrations are potential P sources with high-agronomic efficiency”. Use of biochar as a bulking agent has also been discussed elsewhere (Dias *et al.*, 2010; Steiner *et al.*, 2011). Recent studies using biochar in temperate areas may, however, give ambiguous results (Kolb *et al.*, 2009; Atkinson *et al.*, 2010; Hass *et al.*, 2012). And this has been mainly associated to the greater natural fertility of these soils compared to the highly weathered soils in tropical areas. Under specific conditions, biochar application has been reported to reduce the greenhouse gas (GHG) emissions such as non-CO₂ gases (*e.g.*, N₂O, CH₄) from soil (van Zwieten *et al.*, 2010a; Rogovska *et al.*, 2011; Taghizadeh-Toosi *et al.*, 2011; Augustenborg *et al.*, 2012; Zheng *et al.*, 2012) whereas Clough *et al.* (2010) did not observe any effect. Spokas & Reicosky (2009) attributed the reduction of N₂O emissions to either a direct effect of biochar preventing the formation of such gas or by inducing the oxidation of the produced gases (Van Zwieten *et al.*, 2010b).

The effect of biochar on water retention in soil cannot be disregarded (Laird *et al.*, 2010b). Biochar may affect soil moisture retention in both direct and indirect ways. The direct effect is caused by increasing the soil water holding capacity (WHC) due to the high internal surface area of some biochars, although this property depends greatly on the feedstock type and the charring temperature (Verheijen *et al.*, 2009; Mašek *et al.*, 2012). The indirect influence on soil water retention is related to the improvement of soil aggregation or the soil structure, through which the soil water retention ability may be subsequently affected. However, the percentage change in available moisture in soils

caused by the application of biochar will primarily depend on the texture of the soils (Tryon, 1948). This researcher observed an 18 % increase of available moisture after adding $\sim 72 \text{ t ha}^{-1}$ charcoal to sandy soils, but observed no considerable change for loamy and clayey soils. With regard to the nature of biochar, Verheijen *et al.* (2009) hypothesised that the typical water retention curve of a standard soil (Van Genuchten, 1980) will be modified by amending with biochar. However, water available to plants may not necessarily increase, as the biochar may store water in very small pores, retained at very low tension. Figure 2.4 shows the hypothesised effect of biochar on soil water retention compared with the typical water retention curve. Finally, it should be noted that the long-term effect of biochar on water retention in soils is highly determined by (i) mechanical stability, and (ii) the stability of biochar under the soil environment (Verheijen *et al.*, 2009).

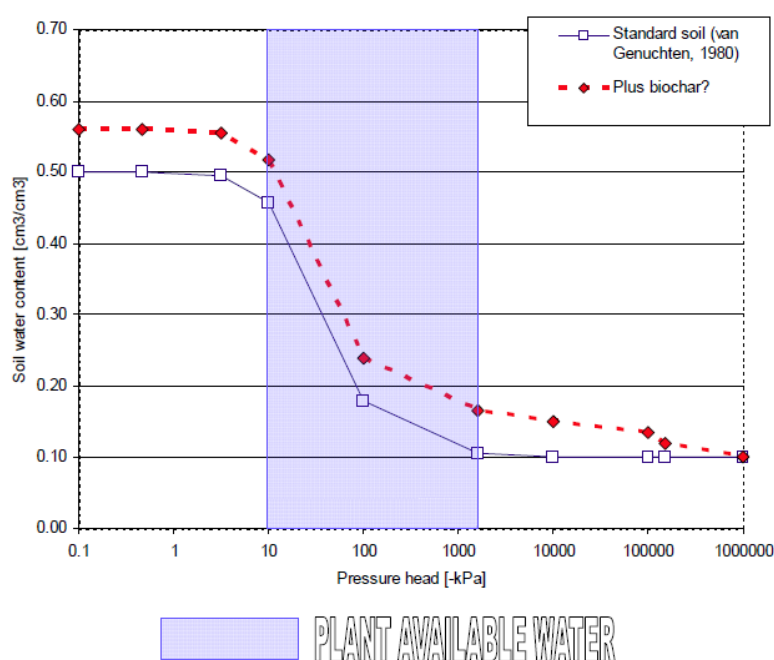


Figure 2.4: Typical representation of the soil water retention curve as provided by Van Genuchten (1980) and the hypothesised effect of the addition of biochar to this soil as adapted from Verheijen *et al.* (2009).

The role of biochar on the improvement of other soil physical properties is less well documented. There is a general reduction of bulk density (Agusalim *et al.*, 2010; Laird

et al., 2010a), as well as a decrease of soil strength and penetration resistance (Chan *et al.*, 2007; Agusalim *et al.*, 2010; Busscher *et al.*, 2010) and, an improvement of saturated hydraulic conductivity (Asai *et al.*, 2009) with the application of biochar to mineral soils.

2.6 Stability of biochar and C sequestration potential

2.6.1 Effect of the type of feedstock on the stability of biochar

Many studies have been undertaken to examine the behaviour of biochars that were produced from different precursory materials (Novak *et al.*, 2009b; Lee *et al.*, 2010; Bruun *et al.*, 2011). The physical structure of biochar, which primarily depends on the type of feedstock, is considered to be important. This is because the fate of biochars in the soil could be affected, to a certain extent, by its physical arrangement. Biochar products range from a powdery to a brittle appearance, depending on the microstructure of the material from which they are derived. Biochars produced from woody feedstock display a predominantly xylemic structure that is coarse and strong (Sohi *et al.*, 2009). These woody feedstocks tend to have high C contents, in excess of 70 % C, with a maximum up to 90 % C. However, these carbonised materials are low in nutrients. Conversely, biochars produced from rye grass and maize feedstocks are powdery, lower in C (~ 60 %), and enriched with a mineral fraction. The C in ash-rich biochars tends to be less stable as the ash limits the degree of aromatic condensation (Singh *et al.*, 2012). Hamer *et al.* (2004) observed that biochars derived from corn stover and rye grass mineralised more rapidly than those derived from oak wood, *i.e.* 0.8 and 0.3 %, respectively. This lower C stability in ash-rich biochars (corn stover) compared to ash-poor biochars (oak shavings) has also been observed by Nguyen & Lehmann (2009). A similar study was conducted with grass- and pine-derived chars (350 °C) by Hilscher *et al.* (2009). These researchers found the highest C mineralisation rates in the grass-

derived chars compared with wood-derived chars (Figure 2.5). Since no mineral nutrient solution was added, it was suggested that the lower N content of the pine-derived biochar (C/N ratio of 218 – 326) over the grass-derived biochar (C/N ratio of 13 – 14) might have limited microbial activity. However, a considerable fraction of N was probably forming part of the heterocyclic structures and thus are difficult to decompose by microbes, irrespective of the feedstock type as reported recently (Wang *et al.*, 2012b). Overall, these studies demonstrated that the different precursory materials attributed by different chemical compositions would affect the rate of microbial degradation, and therefore, the stability of pyrogenic organic material.

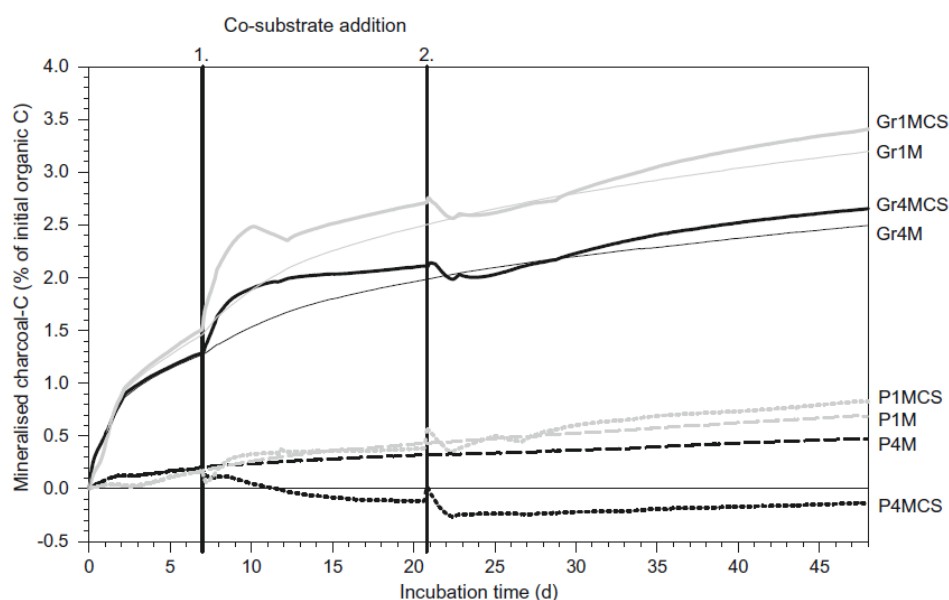


Figure 2.5: Cumulative CO₂-C release of pyrogenic OM produced from *Lolium perenne* and *Pinus sylvestris* at 350 °C with and without co-substrate addition. Values are corrected by subtraction of the CO₂-C emission of the blank values, as adapted from Hilscher *et al.* (2009).

Some authors considered that certain basic properties of the derived biochar are directly ascribed to the properties of feedstock type. For instance, Hamer *et al.* (2004) noticed that biochar from corn was soft, friable, and easily broken down. In contrast, biochar from oak was hard and more resistant to external pressure. As Byrne & Nagle (1997) concluded, a lower plant (wood/structure) density corresponds to a lower biochar density, thus reflecting the structures of the original plant tissue from which the biochar

materials were formed (Brodowski *et al.*, 2005). However, this has to be carefully evaluated as the density of biochar can be affected by the amount of ash from the particular feedstock type.

2.6.2 Pedoclimatic conditions and biochar-C stability

2.6.2.1 Effect of external temperature on environmental stability of biochar

Regardless of the type of feedstock and pyrolysis temperature, a considerable percentage of pyrogenic material will decompose, and this value may depend on pedoclimatic conditions of the area where it is deployed (Lehmann *et al.*, 2009). The stability of biochar in the soil environment is likely influenced by a group of external factors of which ambient temperature is important because the environmental temperature has a great influence on (i) abiotic surface oxidation, and (ii) biotic decomposition (Cheng *et al.*, 2006; Zimmerman, 2010).

It is noteworthy that the studies on BC decomposition have been conducted across a range of temperatures. However, a detectable degradation of BC has occurred even at the lowest temperatures at a relatively slow rate, for example, $-24\text{ }^{\circ}\text{C}$ (Raunemaa *et al.*, 1994). Conversely, while BC can degrade between $-22\text{ }^{\circ}\text{C}$ and $70\text{ }^{\circ}\text{C}$ (Cheng & Lehmann, 2009), the most favourable temperature range is between $20 - 40\text{ }^{\circ}\text{C}$; whereas microbial growth is favoured and optimal in the range between 25 and $30\text{ }^{\circ}\text{C}$ (Pietikäinen *et al.*, 2005). In addition, Cheng *et al.* (2008a) have reported a significant relationship between mean annual temperature and the role of BC on OC storing process in soil, concluding that soil temperature is one of the decisive factors that may govern BC decomposition.

The Q_{10} value – defined as “the increase in decomposition rate resulting from a $10\text{ }^{\circ}\text{C}$ rise in temperature” (Davidson & Janssens, 2006) as cited by Lehmann *et al.* (2009) –

may greatly increase depending on the chemical recalcitrance of a particular organic material and its own chemical structural complexity (Davidson & Janssens, 2006). However, it is noteworthy to mention that this temperature dependence of OM decomposition is likely to be greater at low (up to ~10 °C) than high (above ~20 °C) temperatures, particular for short-term responses (Kirschbaum, 1995; 2010). Kirschbaum (2010) further explained that therefore, the temperature dependence of OM decomposition has a potential to deviate strongly from the assumption of constant Q_{10} across temperature. Compared with other OM sources, biochar and other BC types are more recalcitrant, and hence Q_{10} may be relatively high (Lehmann *et al.*, 2009). This is assumed to be due to the complex structure owned by all BC types. The complex molecular attributes are characterised by “a low decomposition rates, high activation energies, and inherently high temperature sensitivity” (Davidson & Janssens, 2006). Considering the properties and also the performance in the soil, it is expected that biochar will also show high temperature sensitivity in decomposition. The environmental constraints that affect apparent temperature sensitivities of decomposition are given by physical protection, chemical protection, drought, flooding, and freezing (Davidson & Janssens, 2006; Conant *et al.*, 2011).

2.6.2.2 Stability of biochar in different soil types

The contribution of biochar to the total CO₂ efflux in amended soils is expected to be influenced by some of its unique properties: amount of labile carbon (Singh *et al.*, 2012), internal microporosity (Hammes *et al.*, 2008), carbonate content of ash (Bruun *et al.*, 2008), nutrient content of ash (Nguyen *et al.*, 2010), and susceptibility to abiotic oxidation (Cheng *et al.*, 2006; Zimmerman, 2010). The stability of biochar will also be influenced by the pedoclimatic conditions of the area of deployment. As studies reported so far are mostly from tropical soils (Liang *et al.*, 2008; Kimetu & Lehmann,

2010; Zimmerman, 2010; Singh *et al.*, 2012), with few being conducted in temperate regions (Hamer *et al.*, 2004; Hilscher *et al.*, 2009), there is presently insufficient data to build models, which predict the fate of BC in temperate soils (Atkinson *et al.*, 2010). Thus, more descriptive data need to be compiled for better predictions. Depending on the pedoclimatic conditions biochar (BC) may behave in different ways irrespective of their intrinsic lability (Cheng *et al.*, 2008b; Liang *et al.*, 2008; Liang *et al.*, 2010; Nocentini *et al.*, 2010; Novak *et al.*, 2010; Cross & Sohi, 2011; Jones *et al.*, 2011; Luo *et al.*, 2011). Thus, the influence of soil type and climate on the stability of biochar may be critical but is relatively unexplored, especially under temperate conditions.

For example, from Anthrosols in the central Amazon, Brazil, evolution of CO₂-C in 532 d was relatively 61 – 80 % lower than from the Oxisol (adjacent soils) (Liang *et al.*, 2008). The authors concluded that, regardless of the texture of the soils, the stability of BC was more prominent in Anthrosols than in adjacent soils where the quantity of BC was very low. These results agree with another study (Cheng *et al.*, 2008a), in which the OC present in BC-containing soil was more stable than in the adjacent soils. This is assumed to be due to the attributes of less labile OC in BC-containing soils. Kolb *et al.* (2009) studied soil amendment with charcoal under temperate conditions using four different soil types: a Mollisol, an Alfisol, an Entisol, and a Spodosol. Presence of biochar (0, 10, 25, 50, and 100 g biochar kg⁻¹ soil) had a significant influence on microbial biomass and activity, as initially hypothesised; however, the effect was dependent on the texture and fertility level of each soil. Kolb *et al.* (2009) further observed that charcoal application increased Bray P, but reduced extractable soil N content.

Stability of biochar in different soils can also be affected by the NOM and *vice versa*. This is explained by the priming effect where one source of C could either increase (positive priming effect) or retard (negative priming effect) (Woolf & Lehmann, 2012) the decomposition of the other. Some results suggest that biochar applications increase SOC content due to a dominant negative priming effect (Zimmerman *et al.*, 2011; Singh *et al.*, 2012) and this beneficial effect may persist for several hundred years (Woolf & Lehmann, 2012). However, other researchers have reported the opposite effect. Sohi *et al.* (2009) reported that, after adding biochar to soil, mineralisation of NOM may be stimulated by the presence of a labile C fraction in biochar and associated soluble nutrients. This is supported by certain findings related to charcoal in soil (Steiner *et al.*, 2008).

2.6.2.3 Environmental stability of biochar with soil water regimes

So far only a few studies have been undertaken to evaluate the effect of soil water content on the stability of biochar. The influence of the soil-water regime on the stability of biochar – weathering in soil and interactions with soil biota – in relation to different processes such as dissolution, hydrolysis, carbonation and decarbonation, hydration, and redox reactions, has been discussed elsewhere (Joseph *et al.*, 2010). Nguyen & Lehmann (2009) studied the loss of C from BC under different water regimes, and observed that it was significantly higher in unsaturated and alternating wet/dry conditions than under saturated conditions, as expected. For corn biochar, unsaturated conditions resulted in the highest C loss rate (16 % for the first year), whereas for oak biochar, the loss was highest when incubated under alternating saturated-unsaturated conditions (12 % for the first year). The lowest C loss (7 – 8 %) was recorded under saturated conditions for both oak and corn biochars.

2.6.2.4 Microbial activity and biochar decomposition

Although not fully identified, most of the micro-organisms able to oxidise the aromatic structure of biochars are assumed to be lignin degraders (Hilscher *et al.*, 2009) and importantly, these microbes prefer oxic conditions to activate their enzymatic systems. As discussed by Hilscher *et al.* (2009), the dominant micro-organisms able to decompose BC are wood-rotting and leaf-litter-decaying basidiomycetes. Under oxic conditions, basidiomycetes have an ability to cleave C-C linkages in aromatic structures with the aid of extracellular enzymes such as Mn-peroxidase, lignin-oxidase, and laccase in co-metabolic processes. Nevertheless, for the best functioning of these enzymes, the presence of H₂O₂ – which is normally generated by the oxidation of non-aromatic C sources (*i.e.*, glucose) – is required (Czimczik & Masiello, 2007). A close correlation between glucose mineralisation and additional BC mineralisation during biotic incubation has been observed and as a result the findings suggest that co-metabolic degradation of biochar is possible (Hamer *et al.*, 2004). Similarly, Hilscher *et al.* (2009) observed, after a co-substrate addition for the second time, a continuous increase in the microbial activity in pine-biochar amended soils over 21 – 48 d (Figure 2.5).

The physical structure of typical biochar may provide a secure environment for microbial colonies (Steiner *et al.*, 2008). As such, these authors observed that BC-containing soils had higher microbial reproduction rates and greater species (bacterial) richness, than soils without BC amendment. Biochar addition has been observed to produce a microbial biomass along with significant changes in their community composition and enzyme activities (Lehmann *et al.*, 2011). A study on Anthrosols also called Amazonian dark earths (*Terra Preta de Indio*) and adjacent soils showed a clear difference in bacterial community composition between the two soil types. Irrespective

of the similar climate and parent materials, they could observe significantly higher and more-diverse bacterial populations in Anthrosols compared with adjacent soils (O'Neill *et al.*, 2009). The bacterial groups found in the two types of soils also showed a clear difference in their activities. Indeed, the adjacent soils had mostly r-selected bacteria, which are responsible for a rapid C mineralisation, whereas the Anthrosols contained k-selected bacteria, characteristic of slower C turnover rates. More details on the r-selected and k-selected bacteria are given in (Fierer *et al.*, 2007).

In comparison, as a result of incubation experiments for 30 d at 30 and 70 °C temperatures, Cheng *et al.* (2006) observed that abiotic mechanisms were more important in the oxidation of the charcoals under study than biotic mechanisms. This is in agreement with the observations made by Brunn *et al.* (2008) who observed an abiotic oxidation, particularly during the initial period of their 113-d incubation. Moreover, the former authors (Cheng *et al.*, 2006) could not observe a significant effect of chemical oxidation for BC degradation when the incubation was extended up to 120 d. They suggested that microbial oxidation of BC could be assisted by this initially significant abiotic oxidation through which hydrophilicity increases.

Cheng *et al.* (2006) have discussed the effect of time on the process of degradation caused by either biotic or abiotic mechanisms. Accordingly, a certain time is expected for the expansion of the oxidation process into interior regions of BC particles and thereby for the development of surface functional groups, such as phenolic and carboxylic groups. These functional groups will be especially responsible for further chemical reactions with external compounds. However, Cheng & Lehmann (2009) observed that oxidation could be kinetically restricted and, subsequently, time could become the major factor affecting the on-going BC ageing process. Cheng & Lehmann (2009) discuss the ageing process in terms of changing elemental composition such as

increasing O and decreasing C concentrations; the development of carboxylic-functional groups resulting in lower pH, higher surface acidity, higher negative surface charge, less surface basicity, and a lower point of zero net charge (PZNC) than unweathered biochars.

2.6.2.5 Long-term environmental stability of biochar

The most labile fraction of biochar is susceptible for microbial degradation and is expected to decompose within a few weeks to few years, depending on the pedoclimatic conditions of the area of biochar deployment (Kuzyakov *et al.*, 2009; Whitman, 2011; Zimmerman *et al.*, 2011). The most stable fraction of C in biochar may persist up to 13,900 y in environments such as deep sea (Masiello & Druffel, 1998) and have a mean residence time (MRT) of 10,000 y in soils (Swift, 2001). Existence of anthropogenic-mediated soils in Amazon region for several hundreds to thousands of years support in long-term C sequestration potential of charred materials (Glaser *et al.*, 2001). Similar observations have been reported in several other parts of the world as well (Davidson *et al.*, 2006).

Recent findings working with biochar-amended soils (Bird *et al.*, 1999; Hilscher *et al.*, 2009; Zimmerman, 2010; Singh *et al.*, 2012) have proposed half-lives of biochar to be several hundred years. Cheng *et al.* (2008a) studied BC particles from historical charcoal furnaces across North America, and observed that these were considerably oxidised after 130 y of outdoor exposure. They also observed that the effect of temperature on the oxidation of BC was more significant than the exposure time in soil. Compared with short- and medium-term studies, long-term field experiments, *e.g.*, (i) Bird *et al.* (1999) in sandy savanna soil at a fire trial site; (ii) Czimczik *et al.* (2003) in fire-affected Siberian Scots pine forest soil; and (iii) Solomon *et al.* (2007) in Amazonian dark earth, suggested that BC may degrade faster than commonly assumed.

This unstable nature of BC over time is further documented by Hilscher *et al.* (2009). However, these contradictions are due to the loose definition of BC, which includes charred material of very different degrees of condensed aromatic structure. Fortunately, the current definition of biochar by the IBI (2012) restricts the use of this term to charcoal with a specific degree of condensation ($H/C_{org} < 0.7$). Thus, the BC produced by grassland fires or incomplete combustion of litter during some forest fires probably does not fulfil the definition of biochar and this may explain that their microbial degradation was equivalent to that for other SOM fractions.

2.7 Conclusions and the research gaps

From the above review it can be concluded that biochar comprises both labile and stable C fractions and the dominance of labile or stable C depends on the pyrolysis conditions applied (*e.g.*, the lower the temperature higher the labile fraction of C in biochar) and also the type of precursor material. The labile C fraction is more susceptible to microbial decomposition and abiotic oxidation whereas the stable C fraction may stay in soils for more than 100 y. The degradability of biochar C also depends on the type of soils where biochar is to be deployed. Most of the research carried out to present on biochar C decomposition in soils is related to soils with low OC contents. Biochar may affect the decomposition of NOM either positively or negatively, that is, having a positive or negative priming effect. Moreover, a mechanistic understanding on how biochar interacts with the soil matrix and the role of the latter in protecting NOM is not yet available. To present, knowledge on the influence of biochar on the dynamics of soil physical properties are also scarce.

Thus, there is a need to investigate the stability of biochar and its role on the dynamics of properties in soils with high OC content. In this thesis, the stability of specific biochars in two OC-rich soils and their role on the cycling of NOM have been studied

and reported in detail. The role of biochar in aggregate formation and hence on the improvement of soil physical properties is also carried out as this will affect most of the soil processes, either directly or indirectly.

CHAPTER 3: Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol

Abstract

Improving soil physical properties by means of biochar application has been hypothesised in recent publications. The objective of this study was to investigate to what extent the addition of corn stover and biochars produced from the pyrolysis of corn stover feedstock at 350 and 550 °C temperatures (CS-350, CS-550) affected aggregate stability, volumetric water content (θ_v), bulk density, saturated hydraulic conductivity (K_s) and soil water repellency of specific soils. Organic amendments were incorporated into a Typic Fragiaqualf (TK) and a Typic Hapludand (EG) soils at the rate of 7.18 t C ha⁻¹, which corresponded to 17.3, 11.3 and 10.0 t biochar ha⁻¹ for the corn stover, CS-350 and CS-550 treatments, respectively. After 295 d of incubation (T295), soils were sampled as (i) undisturbed samples for bulk density and K_s ; and (ii) mildly disturbed samples for θ_v (at -15, -1, -0.3, -0.1, -0.08, -0.06, -0.04, and -0.02 bar), aggregate stability and soil water repellency. The θ_v at time 0 (T0) was also determined at -15, -1 and -0.3 matric potentials for the different treatments. Biochar application significantly increased ($P < 0.05$) aggregate stability of both soils, the effect of CS-550 biochar being more prominent in the TK soil than that in the EG soil, and the reverse pattern being observed for the CS-350 biochar. Biochar application increased the θ_v at each matric potential although the effect was not always significant ($P < 0.05$) and was generally more evident in the TK soil than that in the EG soil, at both T0 and T295. Biochar addition significantly ($P < 0.05$) increased the macroporosity (*e.g.*, increase in θ_v at -0.08 to 0 bar) in the TK soil and also the mesoporosity in the EG soil (*e.g.*, increase in θ_v from -1 to -0.1 bar). Biochar significantly increased ($P < 0.05$) the K_s of the TK soil, but not that of the EG soil. Biochar was not found to increase the water repellency

of these soils. Overall results suggest that these biochars may facilitate drainage in the poorly drained TK soil, which has potential to reduce N₂O emissions from grazed pastures. However, the present results are biochar-, dose- and soil-specific. More research is needed to determine changes produced in other biochar, dose and soil combination, especially under field conditions.

Key words

Biochars, corn stover, soil physical properties, Andisol, Alfisol

3.1 Introduction

Production of biochar from the pyrolysis of forest and crop wastes has the potential to sequester atmospheric CO₂ into more stable soil C pools (Lehmann *et al.*, 2009; Liang *et al.*, 2010; Zimmerman, 2010). Agronomic benefits are mainly derived from the fertilizer value of biochar and its effects on the improvement of soil physical conditions, in particular, the soil water holding capacity (WHC) and soil drainage characteristics. There is however a number of logistic and financial constraints limiting the immediate adoption of biochar as a GHG mitigation strategy. Among these is the lack of sound economic evidence for its true agronomic value. When carbon dioxide credit values are low, a high agronomic value is important to offset the cost of biochar production. Obtaining an agronomic value is complicated because beneficial effects are dependent on the interaction between the different types of biochar and pedoclimatic conditions of the area where they are deployed. Therefore, a mechanistic understanding of these interactions is needed.

The use of biochar as a means to ameliorate soil physical properties and, particularly, the soil WHC, has emerged after identifying its general high porosity (Liang *et al.*, 2006; Hina *et al.*, 2010) and large inner surface area (Kishimoto & Sugiura, 1985; Van Zwieten *et al.*, 2009). The porosity of biochar depends on (i) the temperature of pyrolysis – increasing with increasing temperature up to ~ 750 °C (Schimmelpfennig & Glaser, 2011) – and (ii) the type of feedstock used (Hina *et al.*, 2010; Calvelo Pereira *et al.*, 2011). Pore sizes in biochar have been reported to range from <2 nm to >50 nm, with an increase in the small diameter pore fraction as temperature of pyrolysis increases (Downie *et al.*, 2009). However, a high porosity in charcoal particles does not necessarily increase the amount of plant-available water in soil, as pore sizes <200 nm tend to retain water at greater water potential than that generated by plants (Lal &

Shukla, 2004). Biochar-soil interactions through aggregation (Brodowski *et al.*, 2006) and soil texture (Tryon, 1948) may in turn affect the soil moisture retention pattern of the biochar-amended soil as well as soil drainage. While microporosity and mesoporosity are primarily important to retain both available water content (AWC) and readily available water content (RAWC) of a soil, macroporosity influences on the hydraulic conductivity and aeration of soil.

Application of charred material to soil has been shown to have a clear effect on AWC and/or WHC at field capacity (Tryon, 1948; Glaser *et al.*, 2002; Chan *et al.*, 2007; Kammann *et al.*, 2011), although most experiments carried out to date have used high rates of biochars – 100 and 200 t ha⁻¹ (Kammann *et al.*, 2011); 50 and 100 t ha⁻¹ (Chan *et al.*, 2007); ~ 70 t ha⁻¹ (Tryon, 1948), – which are not practically feasible at the farmer level. Studies using lower rates have only measured the WHC at specific soil water potential and/or shortly after application to soil (Agusalim *et al.*, 2010; Laird *et al.*, 2010b; Karhu *et al.*, 2011). Moreover, the question arises whether the same level of soil physical improvement can be achieved by incorporation of the feedstock given the cost of biochar manufacture. Waste biomass feedstocks such as manures and corn stover residues are prone to decompose rapidly (Torn *et al.*, 2005; Weerakkody & Parkinson, 2006) and need to be applied in large quantity (between 50 and 200 Mg ha⁻¹), which is not affordable at a farm scales (Piccolo *et al.*, 1996). Given the high stability of biochar in soils (Lehmann *et al.*, 2009; Liang *et al.*, 2010), long-term effects are expected in the context of soil WHC and other physical properties if these are proven to occur. The effects of biochar on other soil physical properties, such as penetration resistance, hydraulic conductivity, bulk density, and soil structure, have not been fully evaluated in field conditions (Glaser *et al.*, 2002; Chan *et al.*, 2007; Asai *et al.*, 2009; Agusalim *et al.*, 2010; Busscher *et al.*, 2010; Laird *et al.*, 2010b; Peng *et al.*, 2011).

Under this context, we hypothesised that soil application of biochar could improve the soil aggregation and thus the WHC (including AWC and RAWC) and drainage facility of soil. The objective of this study was to determine the effect of biochars – produced from the pyrolysis of corn stover at two temperatures (350 and 550 °C) – on soil aggregation and other main physical properties of two soils – an Alfisol (where macro-aggregates are dominant) and an Andisol (where micro-aggregates are dominant) – incubated in the laboratory during 295 d. These soils were chosen as they have distinct OC content, mineralogy and also soil physical conditions.

3.2 Materials and methods

3.2.1 Biomass used and carbonisation process

Corn stover (*Zea mays*), with a cellulose, hemicellulose and lignin content of 38.3, 35.7 and 9.6 %, respectively, was used as feedstock. The feedstock was first cut into pieces of 25-mm size with an electronic chipper, and thereafter cut to 5 mm using a cross-cutting mill. The material was dried for 24 h at 60 °C before pyrolysis. Two hundred grams of corn stover were pyrolysed at highest heating temperatures of 350 and 550 °C with an average heating rate of 36 and 51 °C min⁻¹, respectively, using a gas-fired, stainless steel, rotating drum kiln. When the desired temperature was reached, the kiln was allowed to cool to room temperature. The carbonised material was stored in sealed plastic bags until used. The two biochars produced were referred to as CS-350 and CS-550, respectively. The yield, biochar chemical composition, and recovery of C, N and S are reported in Table 3.1.

Table 3.1: Elemental analysis of feedstock and biochars and yield of biochar.

Sample	Chemical composition (%)						Biochar yield (%)	Atomic ratio (d.a.f.) ^b		Recovery (%)		
	C	N	H	O ^a	S	Ash		(H/C)	(O/C)	C	N	S
Feedstock	41.4	0.8	6.0	40.6	0.1	10.9	n.a.	1.98	0.74	n.a.	n.a.	n.a.
CS-350	63.5	0.7	3.7	21.6	0.4	9.8	35.0	0.64	0.26	67	29	100
CS-550	71.8	0.7	2.9	13.5	0.1	11.5	27.0	0.45	0.14	57	21	25

^a estimated by difference as $O = 100 - (C+H+N+S+Ash)$

^b dry ash free basis

n.a. – not analysed

3.2.2 Particle-size distribution of biochar

Particle-size distribution of biochars was determined by dry sieving the samples using a sieve shaker (Endecott Test Sieve Shaker, Watson Victor Ltd.). Seven different fractions were obtained using 2.00, 1.00, 0.50, 0.25, 0.15, and 0.05 mm sieves (Figure 3.1). Three consecutive shakings were conducted, as it was observed that the weight of different fractions remained unchanged thereafter. The first shaking was continued for 3 min; the other two shakings were only done for 2 min.

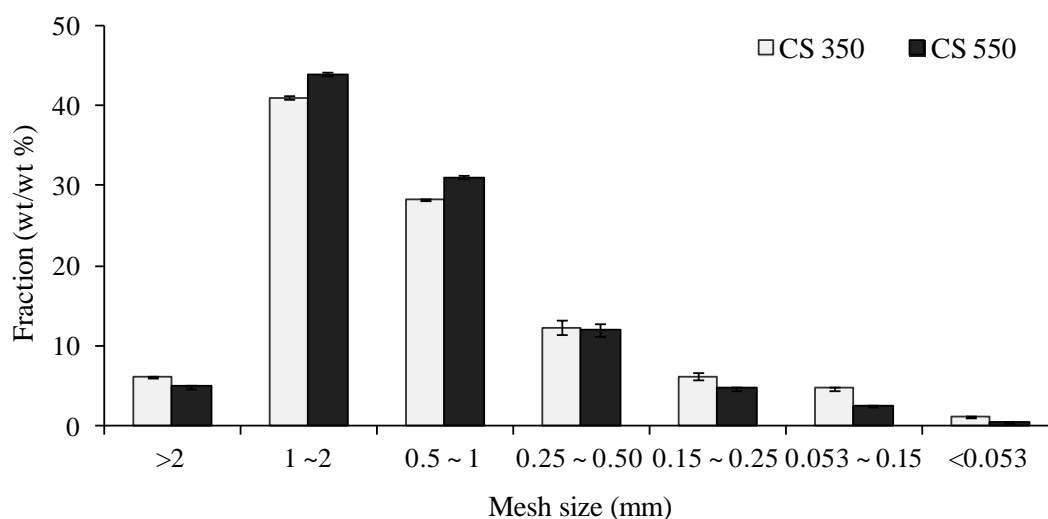


Figure 3.1: Particle size distribution of CS-350 and CS-550 biochars before adding in to soils.

3.2.3 BET surface area and scanning electron microscope (SEM)

Measurements of N_2 gas adsorption for BET surface area determination of biochars were undertaken with a Micromeritics ASAP 2020 volumetric adsorption system. The

surface physical morphology of the biochars at time 0 (T0) and after 295 d of incubation (T295, biochar particles separated from incubated soil) was examined by Quanta 200 equipment (FEI, Eindhoven, The Netherlands) after coating the particles with gold using a Bal Tec SCD 500 cool sputting device (Balzers Union, Wallruf, Germany).

3.2.4 Soil collection

Undisturbed soil cores were taken (0 – 100 mm depth, using 150 mm-diameter cylinders) from two different sites: Manawatu (Tokomaru Silt Loam; TK soil) (40°18' S, 175°23' E, 24 m above sea level), and Hawera (Egmont Silt Loam; EG soil) (39°37' S, 174°21' E, 66 m above sea level) in New Zealand. The two soils are classified as Typic Fragiaqualf and Typic Hapludand (Soil Survey Staff, 2006), respectively. Both sites have been under permanent pasture for at least 50 y (Parfitt *et al.*, 1984; Roberts & Thompson, 1984). The basic properties of these soils are given in Table 3.2. Soils were then thoroughly mixed, sieved to 5 mm, and stored in the cold room (temperature <4 °C) until used.

Table 3.2: Basic properties of the Tokomaru and Egmont soils.

Soil	Texture	Bulk density (Mg m ⁻³)	OC ^a (g C kg ⁻¹ soil)	Total N (%)	pH-H ₂ O
Alfisol	Silt Loam	1.00	41.7	0.32	5.7
Andisol	Loam	0.75	102.0	0.64	5.8

^aOC – organic carbon (total C content)

3.2.5 Sample preparation, incubation and sampling

Biochars and fresh corn stover were incorporated into soils at a rate of 7.18 t C ha⁻¹, which corresponds to the following application rates: 17.3, 11.3, and 10.0 t ha⁻¹ of corn stover, CS-350, and CS-550 amendments, respectively. This was achieved by adding 1.73, 1.13, and 1.00 g 100 g⁻¹, respectively, for the TK soil, and 2.30, 1.50, and 1.33 g 100 g⁻¹, respectively, for the EG soil (oven dry wt/wt basis), based on the initial bulk densities of the soils. A control, without organic amendment, was also prepared for each

soil. The amendments were evenly mixed with soils and the mixtures were packed into PVC columns (150 mm in diameter and 100 mm in height) based on pre-calculated bulk densities, which included the contribution of the amendments to the final bulk density. Treatments were run in triplicates and identified as follows: (i) control treatments (TK-Ctr, EG-Ctr), (ii) fresh corn stover (TK-CS, EG-CS), (iii) low-temperature biochar (TK-350, EG-350), and (iv) high-temperature biochar (TK-550, EG-550). Soil water content was returned to 70 % water holding capacity (w/w) every 2 d throughout the experiment. The soil columns were stored in a chamber provided with an open plastic container of 2 L of water to maintain the moisture of the atmosphere and thus minimising water evaporation from soils, while allowing air circulation. The average room temperature was ~20 °C (details are provided in Chapter 4). After 295 d of incubation, undisturbed ring samples (50 mm in height × 48 mm in diameter) were taken for the determination of K_s and bulk density. Samples also were taken for the determination of soil WHC, aggregate stability, and soil water repellency.

3.2.6 Determination of soil physical properties

3.2.6.1 Aggregate stability

Aggregate stability at T295 was determined according to Le Bissonnais's (1996) method. Air-dried samples were forced through 4.75 and 3 mm sieves, sequentially, and the 3 – 4.75 mm size aggregates were selected for the analysis. Three different procedures were applied by simulating conditions at the laboratory level following Le Bissonnais (1996) using five replicates in each: (i) procedure I (fast wetting) simulated a heavy rain storm in summer; (ii) procedure II (slow wetting) corresponded to a field condition of wetting under gentle rain; and (iii) procedure III, where samples were exposed to a mechanical breakdown by shaking after pre-wetting. At the end of all procedures, dry sieving was carried out using a column of six sieves: 2, 1, 0.5, 0.2, 0.1

and 0.05 mm. The mean weight diameter (MWD), which represents the aggregate stability, was calculated as follows:

$$MWD = \sum_{i=1}^n X_i W_i$$

where X_i is the mean diameter of each size fraction (mm) and W_i the proportion of the total sample mass in the corresponding size fraction.

3.2.6.2 Soil water holding capacity measurements

Moisture contents of the samples at T295 were measured at different matric potentials (–15, –1, –0.3, –0.1, –0.08, –0.06, –0.04, and –0.02 bar). At T0, they were measured at –15, –1, and –0.3 bar matric potentials. Haines' apparatus was used to determine the moisture contents from –0.08 to 0 bar pressures, while suction plate was used for –0.1 bar matric potential. Five- and 15-bar pressure plate apparatus were used to measure the water contents at –0.3 and –1 bar, and –15 bar, respectively (Dane & Hopmans, 2002). Gravimetric analysis was undertaken to determine the moisture contents and these were converted to volumetric basis using the corresponding bulk density values. Total porosity was estimated as (i) the volume equivalent to the moisture content at saturation using slightly disturbed samples, and (ii) as the volume equivalent calculated using a soil particle density of 2.65 Mg m^{–3}. Both estimations provided similar values and only the former are reported.

3.2.6.3 Saturated hydraulic conductivity and bulk density

Saturated hydraulic conductivity was measured using a constant head permeameter (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). The bulk density of each undisturbed sample (50 mm in height × 48 mm in diameter) was also measured.

3.2.6.4 Soil water repellency

The water droplet penetration test (WDPT) was conducted to quantify the persistence of soil water repellency and the molarity of ethanol droplet (MED) test to determine the degree of water repellency. For the potential WDPT, samples were prepared as described by Kawamoto *et al.* (2007). Thereafter, the time taken from the initial contact of the water droplet until complete penetration into the soil layer was recorded (Dekker & Jungerius, 1990; Täumer *et al.*, 2005). For the MED test, soil samples were prepared similarly to the WDPT. Once the suitable molarity – for which the ethanol drops did not penetrate within 10 s – was identified, the contact angle was calculated using the average value of the detected and the immediate high molarities (Deurer & Müller, 2010).

3.2.7 Statistical analysis

The statistical differences between the treatments under study were determined by analysis of variance (ANOVA) using SPSS software (General Linear Model, Multivariate) with version 16.0 (SPSS Inc., Chicago, USA). Post hoc analysis were computed using Duncan test at $P = 0.05$.

3.3 Results

3.3.1 Pyrolysis yield and biochar characteristics

The elemental analysis and yield of the biochars are provided in Table 3.1. Briefly, the biochar yield (*i.e.* the mass ratio of biochar recovered after pyrolysis and the initial

feedstock) of CS-350 biochar was 35.0 %; that of CS-550 biochar was 27.0 % (Table 3.1). As expected, biochar C content (635 and 718 g kg⁻¹ C for CS-350 and CS-550, respectively) was high compared with the original feedstock (413 g kg⁻¹). Recovered C, which is the proportion of the original C retained in the biochar sample, decreased with the increase of pyrolysis temperature (67.0 and 56.6 % for CS-350 and CS-550 biochars, respectively) (Table 3.1). As the amount of inorganic C was <0.9 % (data not shown), total C was considered organic C (C_{org}). The elemental concentrations of H and O were always higher in the CS-350 biochar than in the CS-550 biochar. As such, the H/C_{org} and O/C_{org} atomic ratios decreased as temperature of pyrolysis increased (Table 3.1), as did the volatile fraction (from 31.2 to 18.5 %). Conversely, the fixed C content increased with the increase of temperature from 57.2 to 67.4 % for CS-350 and CS-550 biochars, respectively, as did the pH and the ash content (Table 3.1).

The particle-size distribution of two biochars is reported in Figure 3.1. Most biochar particles were >0.25 mm, irrespective of pyrolysis temperature. This fraction corresponded to 88 and 92 % for the CS-350 and the CS-550 biochars, respectively.

3.3.2 Aggregate stability

Biochar application significantly ($P<0.05$) improved the aggregate stability of both soils, except in the EG-550 treatment for procedure II, where no effect was observed (Figure 3.2). The fresh corn stover amendment also improved significantly ($P<0.05$) the aggregate stability, except for the EG soil following procedure I. According to procedure I, CS-350 and CS-550 biochars improved the aggregate stability of the TK soil by 17 and 38 %, respectively, compared with the control. According to procedures II and III, this increase was 4 and 11 % for the CS-350 biochar and 9 and 16 % for CS-550 biochar, respectively, when added to the TK soil. The effect of fresh corn stover

application on aggregate stability of the TK soil was 14, 8 and 16 % for procedures I, II and III, respectively.

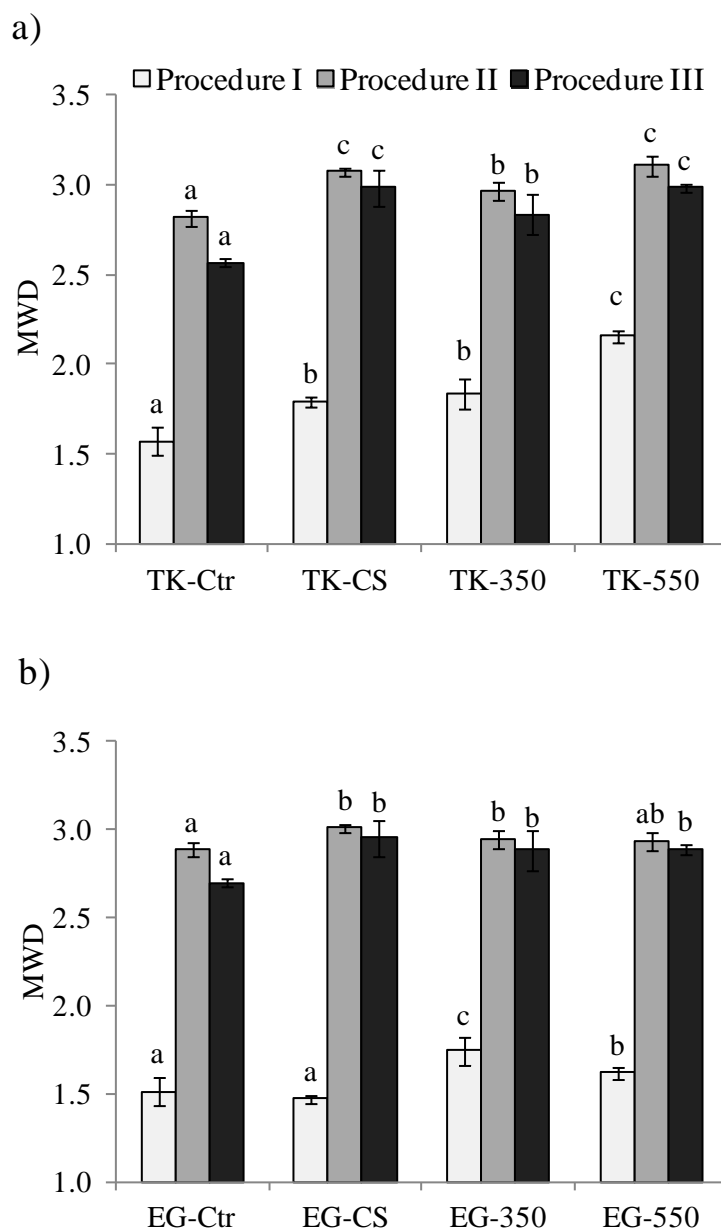


Figure 3.2: Aggregate stability as given by MWD determined using the Le Bissonnais (1996) method for the (a) TK soil, and (b) EG soil at T295. Least significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

In the EG soil, increased aggregate stability due to biochar addition was observed following all procedures, although this increase was not as prominent as in the TK soil: values for the CS-350 treatment were 15, 3 and 8 % in the procedure I, II and III,

respectively, compared with the control; values for the CS-550 treatment were 7 and 8 % only in procedures I and III respectively. Fresh corn stover only improved the aggregate stability of the EG soil in procedures II and III by 6 and 10 %, respectively.

3.3.3 Effect of biochar on soil porosity

Total soil pore volume (TPV) was estimated based on the water content of the soil samples at saturation (0 bar). After 295 d, TPV of the TK soil significantly increased ($P<0.05$) with the addition of organic amendments compared with the corresponding control soil. This increase was 13, 10, and 19 % for the fresh corn stover, CS-350, and CS-550, respectively. Smaller increases in TPV were observed in the EG soil (4 – 6 %) and these were not statistically significant at $P<0.05$. A general increment of macro-, meso- and micro- pore volumes – calculated based on θ_V data – was observed in both soils on the addition of biochars (except for the macropore volume in the EG-350). However, these differences were only significant ($P<0.05$) for the macro pore volume in the TK soil, with an increase of 13, 7, and 20 % for the TK-CS, TK-350 and TK-550, respectively.

3.3.4 Soil water holding capacity

3.3.4.1 Effect of biochar on soil water holding capacity at T0

Immediately after biochar application a significant increase ($P<0.05$) in θ_V at -0.3 , -1 and -15 bar was observed compared with the corresponding unamended controls (Table 3.3). Application of fresh corn stover showed a similar trend, although the effect was not always significant at $P<0.05$. The increase of θ_V at -0.3 bar was 6 % in the TK soil and 11 – 12 % in the EG soil. At -15 bar, the increase of θ_V in fresh corn stover, CS-350 and CS-550 treatments was 15, 13 and 10 % in the TK soil, and 2, 6 and 10 % in the EG soil. Temperature of pyrolysis did not affect the θ_V at the matric potential tested, except at -1 bar pressure in the EG soil, with the soil amended with CS-350 biochar

having a significantly ($P < 0.05$) greater WHC than that amended with CS-550 (Table 3.3).

Table 3.3: Mean ($n = 3$) volumetric soil moisture contents measured at different matric potentials, AWC and RAWC immediately after establishment (T0) of soil-feedstock and soil-char mixtures. Least significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

Treatment	-15 bar	-1 bar	-0.3 bar	^a AWC	^b RAWC
TK-Ctr	0.1300a	0.1767a	0.2200a	0.0867a	0.0433b
TK-CS	0.1500b	0.2100c	0.2200a	0.0700a	0.0100a
TK-350	0.1467b	0.2000b	0.2333b	0.0833a	0.0300b
TK-550	0.1433b	0.2033bc	0.2333b	0.0800a	0.0300b
EG-Ctr	0.2100a	0.2400a	0.3067a	0.0967a	0.0667b
EG-CS	0.2133a	0.2733b	0.3167a	0.1034a	0.0467a
EG-350	0.2233b	0.2900d	0.3400b	0.1167b	0.0500a
EG-550	0.2300b	0.2800c	0.3433b	0.1133b	0.0600b

^aAWC – available water content

^bRAWC – readily available water content

The AWC was calculated as the difference between θ_v at -0.3 and -15 bar; the biochar amendments significantly increased ($P < 0.05$) the AWC in the EG soil, with values of 21 and 17 % for the CS-350 and CS-550 biochar treatments, respectively. No significant effect ($P < 0.05$) of the organic amendments on AWC was observed in the TK soil. RAWC calculated as the difference in θ_v at -0.3 and -1 bar tended to decrease with the organic amendments, this decrease being significant ($P < 0.05$) in both soils for the fresh corn stover, and for CS-350 biochar in the EG soil.

3.3.4.2 Effect of biochar on soil water holding capacity after 295d

After 295 d, all amendments increased the θ_v of both soils compared with the controls; however, this increase was only significant ($P < 0.05$) at a particular range of matric potentials (0 to -0.08 and -0.3 bar in the TK soil, and -0.08 to -1 bar in the EG soil; Table 3.4). At low tensions (> -0.1 bar), high-temperature biochar had a greater effect on increasing the θ_v than the low-temperature biochar, but the effect was only

significant ($P<0.05$) at saturation in the TK soil, and at -0.1 bar in the EG soil (Table 3.4). Incubation for 295 d increased θ_v at the three common matric potentials investigated (-0.3 , -1 and -15 bar) for all the treatments with respect to T0 (Table 3.3 and 3.4). However, that increase was only significant ($P<0.05$) at -0.3 and -15 bar matric potentials.

At T295, biochar-amended soils tended to have greater WHC than those amended with fresh corn stover, although these differences were only significant at $P<0.05$ for the TK soil at saturation. The amendments had no significant effect ($P<0.05$) on the AWC of both soils, but a general increasing trend was observed compared with the controls, and this increase was always greater with the carbonised material than with the fresh corn stover. Increases were 14, 22 and 22 %, for the fresh corn stover, CS-350 and CS-550, respectively, in the TK soil, and 33 and 19 % for the CS-350 and CS-550, respectively, in the EG soil. Differences were more evident for the RAWC in the TK soil, as the organic amendments significantly increased ($P<0.05$) the RAWC by 133, 100, and 78 % for the fresh corn stover, CS-350 and CS-550, respectively (Table 3.4). Compared to T0, AWC and RAWC significantly increased ($P<0.05$) in the TK soil after 295 d, but not in the EG soil (Table 3.5). A significant ($P<0.01$) interactive effect of treatment x time was observed in the RAWC of the TK soil (Table 3.5).

Table 3.4: Mean ($n = 3$) volumetric soil moisture contents measured at different matric potentials, AWC and RAWC after 295 d of incubation of soil-feedstock and soil-char mixtures. Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

Treatment	-15 bar	-1 bar	-0.3 bar	-0.1 bar	-0.08 bar	-0.06 bar	-0.04 bar	-0.02 bar	0 bar	AWC ^a	RAWC ^b
TK-Ctr	0.1450a	0.1950a	0.2250a	0.3200a	0.3150a	0.3350a	0.3650a	0.4100a	0.5850a	0.0850a	0.0300a
TK-CS	0.1567a	0.1900a	0.2533b	0.3200a	0.3500b	0.3733ab	0.4267b	0.4833b	0.6600b	0.0967a	0.0700b
TK-350	0.1533a	0.2000a	0.2600b	0.3433a	0.3633b	0.3867b	0.4100b	0.4833b	0.6433b	0.1033a	0.0600b
TK-550	0.1567a	0.2067a	0.2600b	0.3533a	0.3733b	0.3967b	0.4200b	0.5000b	0.6933c	0.1033a	0.0533ab
EG-Ctr	0.2200a	0.2533a	0.3100a	0.3867ab	0.4267a	0.4800a	0.5033a	0.5833a	0.7133a	0.0900a	0.0600a
EG-CS	0.2333a	0.2667a	0.3200ab	0.3833a	0.4600b	0.5033a	0.5467a	0.6033a	0.7400a	0.0867a	0.0567a
EG-350	0.2400a	0.3000b	0.3600b	0.4133b	0.4533ab	0.4900a	0.5267a	0.5867a	0.7533a	0.1200a	0.0600a
EG-550	0.2433a	0.2767ab	0.3467ab	0.4167c	0.4567ab	0.4967a	0.5500a	0.6100a	0.7533a	0.1067a	0.0733a

^aAWC – available water content

^bRAWC – readily available water content,

Table 3.5: Analysis of variance (ANOVA) for the effect of treatment and time on AWC and RAWC of the TK and EG soils. The significance of the ANOVA is given: ** – significant at $P \leq 0.01$; * – significant at $P \leq 0.05$; n.s. – not significant.

	Source	^a DF	^b AWC	^c RAWC
Model	TK soil	7	*	**
	EG soil	7	n.s.	n.s.
Treatment	TK soil	3	n.s.	n.s.
	EG soil	3	*	n.s.
Time	TK soil	1	*	**
	EG soil	1	n.s.	n.s.
Treatment x time	TK soil	3	n.s.	**
	EG soil	3	n.s.	n.s.

^aDF – degree of freedom

^bAWC – available water content

^cRAWC – readily available water content

3.3.5 Hydraulic conductivity

The hydraulic conductivity values at saturation (K_s) for the different treatments after 295 d of incubation are shown in Figure 3.3. Application of amendments had a significant ($P < 0.05$) effect on the K_s in both soils. In the TK soil, the lowest K_s value was observed in the TK-Ctr ($2.8 \cdot 10^{-5} \text{ m s}^{-1}$). TK-CS, TK-350 and TK-550 treatments had K_s values of $4.2 \cdot 10^{-5}$, $3.7 \cdot 10^{-5}$, and $6.7 \cdot 10^{-5} \text{ m s}^{-1}$ respectively, which corresponded to a K_s increase of 50, 32 and 139 %, respectively. The effect of the amendments on the K_s of the EG soils did not always follow the same trend. The K_s values of CS-350 and CS-550 biochar-amended pots were $4.8 \cdot 10^{-5}$ and $3.4 \cdot 10^{-5} \text{ m s}^{-1}$ respectively, which corresponded to a K_s increase of 41 and 0.9 %, respectively, compared with the control. The EG-CS treatment, in contrast, showed a decrease in the K_s value compared with the control ($2.3 \cdot 10^{-5} \text{ m s}^{-1}$ vs. $3.4 \cdot 10^{-5} \text{ m s}^{-1}$).

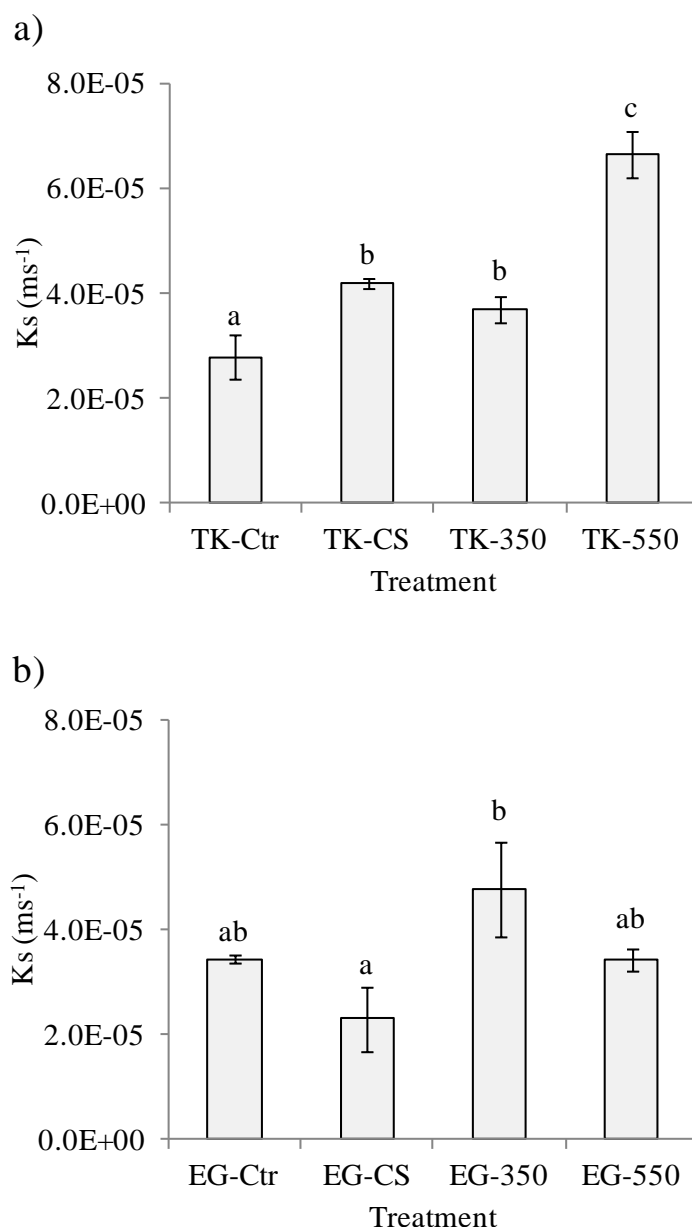


Figure 3.3: Saturated hydraulic conductivity (K_s) determined for the (a) TK soil, and (b) EG soil at T295. Least significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

3.3.6 Bulk density

Amended TK soils had a significantly smaller ($P < 0.05$) bulk densities (0.93 , 0.94 , and 0.91 Mg m^{-3} for the fresh corn stover, CS-350 and CS-550 amended soils) than the control (1.01 Mg m^{-3}) (Figure 3.4). Bulk densities of the EG soil treatments were not significantly different ($P < 0.05$) and values were always below 0.8 Mg m^{-3} , as expected for an Andisol (Soil Survey Staff, 2006).

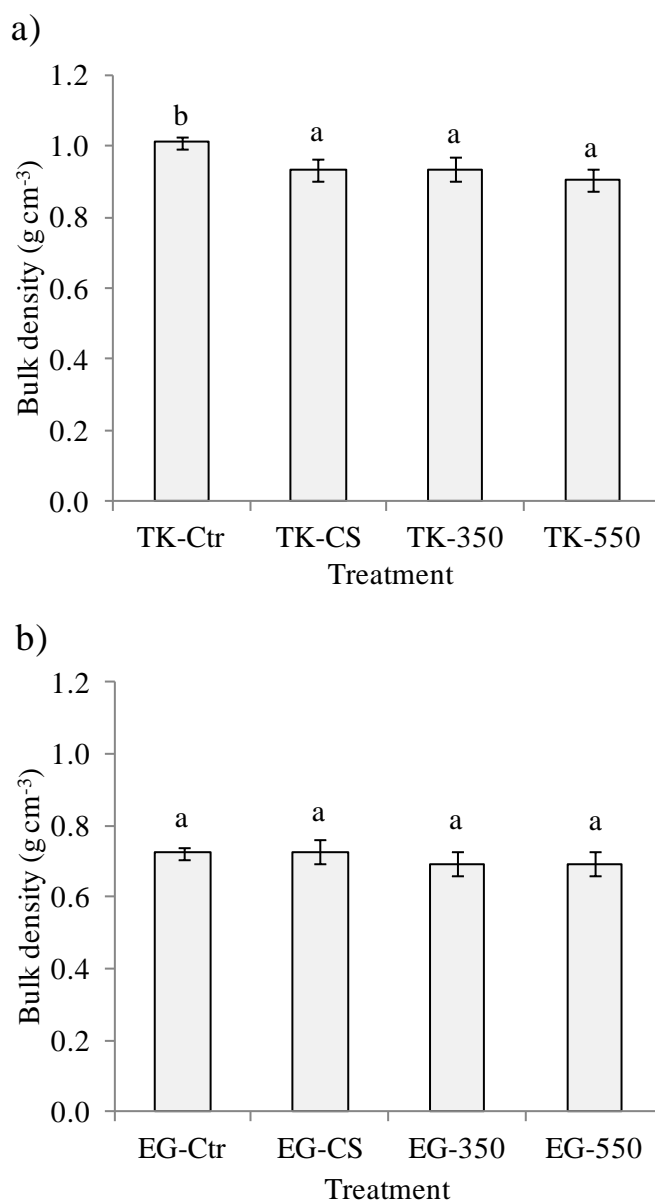


Figure 3.4: Bulk density determined for the (a) TK soil, and (b) EG soil at T295. Least significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

3.3.7 Soil water repellency

The determined WDPT classes and the calculated contact angles using the MED test are reported in Table 3.6. The two contrasting soils fall in either WDPT class 1 (TK soil) or 2 (EG soil) (Täumer *et al.*, 2005). The only exception was EG-350. Little change in WDPT class occurred as a result of biochar addition. The results obtained with the MED test showed a similar trend. The smallest contact angle was observed in the CS-

350 biochar-amended TK and EG soils (92.5 and 92.9°, respectively). Hydrophobicity determined in the EG-Ctr and EG-CS was significantly higher ($P < 0.05$) than that of the EG-350 (Table 3.6). For the remaining treatments, contact angles ranged between 93.4 and 95.5° (Table 3.6).

Table 3.6: The persistence and the degree of water repellence determined for the fresh and charred corn stover amended soils from the incubation experiment after 295 d. Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

Treatment	^a WDPT Class	Contact angle
TK-Ctr	1	93.7a
TK-CS	1	94.3a
TK-350	1	92.5a
TK-550	1	93.4a
EG-Ctr	2	95.5b
EG-CS	2	95.5b
EG-350	1	92.9a
EG-550	2	94.2ab

^aWDPT – water droplet penetration test (Täumer *et al.*, 2005)

3.4 Discussion

This research forms part of a long term study in which, the rates of decomposition of native soil organic matter as influenced by biochar and crop residue addition are being investigated (Chapter 4 and 5). This chapter reports the changes in soil physical properties that were observed on addition (T0) and 295 d (T295) after addition of the corn stover residue and biochars to two soils that have contrasting mineralogy. To place in context the degree of microbial activity that had occurred during the 295 d incubation the following brief description is provided. The initial soil OC content was 41.7 and 102.0 g C kg⁻¹ in the TK and EG soils, respectively (Table 3.2). The TK and EG soils received 5.6 and 6.3 g C kg⁻¹, respectively, as biochar or feedstock amendment. In both soils OC declined by 6.3 – 9.3 % through respiration over the 295 d (Chapter 4). Soil OC contents and pH values of the soils at T295 are reported in Table 3.7. Biochar

addition to soils immediately increased soil pore volume per unit mass and this is discussed below under the section ‘total soil porosity’.

Table 3.7: Total C and soil pH (H₂O) data determined after 295 d of the incubation study. Minimum significant (alpha = 0.05) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

Sample	Total C (g C kg ⁻¹ soil plus amendment)	pH (H ₂ O)
TK-Ctr	38.1a	4.82a
TK-CS	41.5b	5.12a
TK-350	43.5c	5.05a
TK-550	42.7c	5.06a
EG-Ctr	96.5a	5.04a
EG-CS	97.9a	5.14a
EG-350	101.1b	5.13a
EG-550	102.7c	5.21a

3.4.1 Soil aggregation

The creation of soil aggregates is unlikely to occur immediately upon biochar addition but will be a function of time and the interaction of chemical and physical properties of the biochar with those of the soil and its biological community. Therefore, the overall change in soil porosity observed after 295 d due to biochar application can be related to the induced soil aggregation, which will change the volume associated with micro- and macropores and, in turn, the hydraulic properties of the two soils. This time dependent effect is reflected in the increased aggregate stability (Figure 3.2). However, the enhanced formation of microaggregates – defined as those with a diameter <250 µm (Six *et al.*, 2000) – with biochar application after 295 d of incubation was not observed in this study (Chapter 5). Brodowski *et al.* (2006) suggested that charcoal in different land-use systems contributed to the formation of microaggregates under a long term field experiment (25 – 85 y) studying different land-use systems. Therefore, it may simply be that a much longer time than the 295 d of this investigation, is required to obtain significant formation of new microaggregates.

In the present study, a time dependent increase in aggregate stability was observed, particularly after adding biochar, and this was probably associated with the formation of water stable macroaggregates. These store more water than small aggregates (Liu *et al.*, 2011) by increasing the total pore volume (Aggelides & Londra, 2000). The enhanced formation of slaking-resistant macroaggregates with exogenous amendments has been observed to occur within a few weeks (Wortmann & Shapiro, 2007; Clark *et al.*, 2009) to many years (Liu *et al.*, 2011). Development of macroaggregates can also be expected over the time without any effect of external amendments, although the degree of formation tends to be lower than in the amended soils (Denef *et al.*, 2002). The increase of θ_V (discussed below), after 295 d, at the three common matric potentials investigated (-0.3, -1 and -15 bar) for all the treatments with respect to T0 (Tables 3.3 and 3.4) could thus be explained by 'time dependent macroaggregate formation' (Denef *et al.*, 2002). Macroaggregate formation in corn stover-amended soils was also higher than that of the control (except for the procedure I in the EG soil; Figure 3.2), as it would be expected after the addition of a high cellulose and hemicellulose source (Li *et al.*, 2007).

The increased aggregate stability in corn stover-amended soils can be related to an increase in the carbohydrate-C content of the soil (Angers *et al.*, 1993), as well as to polysaccharides formed with the additional microbial growth, especially that of fungi (Tiessen & Stewart, 1988). Fungi can also have a role in biochar-amended soils, as biochars have been reported to enhance fungal growth (Ogawa, 1994). The SEM images taken after 295 d confirmed the presence of fungal hyphae within biochar pores (Figure 3.5).

The observed resistance of the two soils against fast wetting (procedure I) in the biochar-amended pots (Figure 3.2) denotes the higher aggregate stability provided by this amendment. In contrast to our findings, Peng *et al.* (2011) did not observe any

effect of biochar on soil aggregation using the Le Bissonnais method; however, they determined the aggregate stability only after 11 d of incubation study. The differences observed in this study for MWD between the three procedures is in accordance with other studies using organic amendments other than biochar (Leroy *et al.*, 2008a; Leroy *et al.*, 2008b). CS-350 biochar had a greater effect on aggregate stability in the EG soil than that in the TK soil, while the opposite was observed for CS-550 biochar. More research is needed to understand the mechanisms of these different responses.

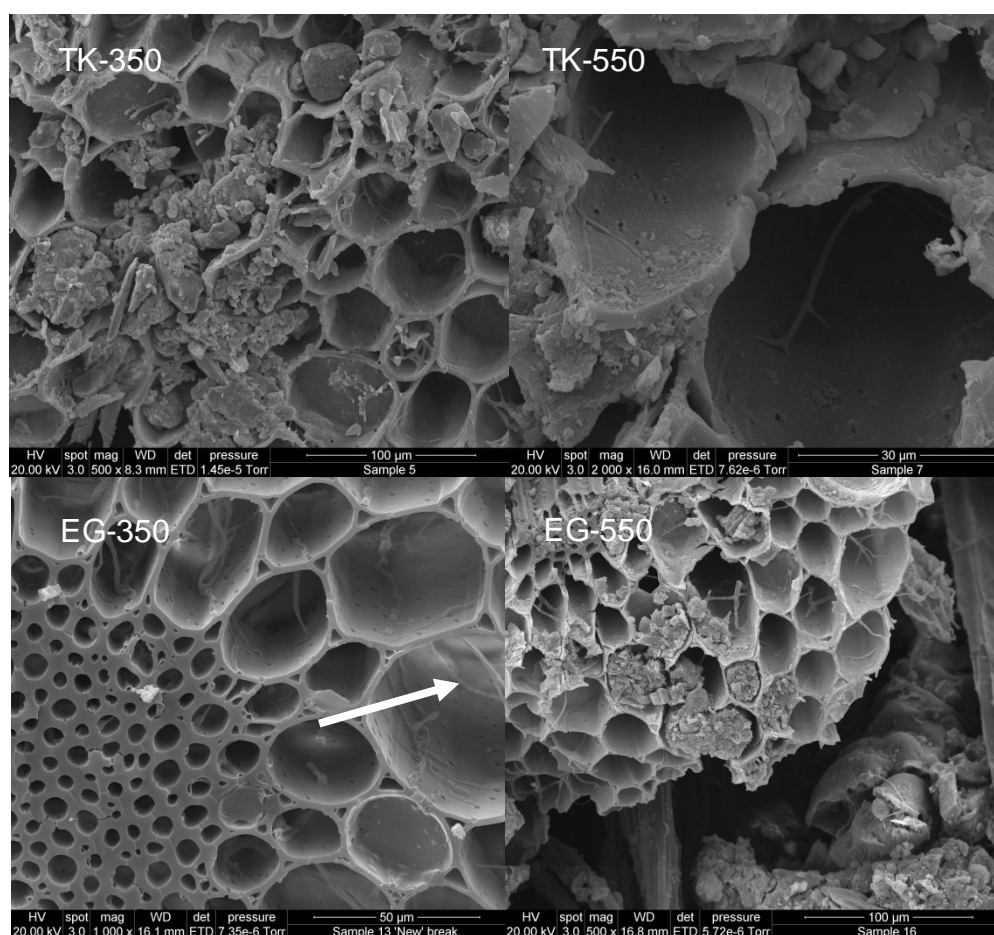


Figure 3.5: The SEM images of corn stover biochar particles picked out from the experimental pots after 295 d. Arrow points towards the fungal hyphae.

3.4.2 Total soil porosity

Total pore volume was considered equivalent to the space occupied by water at saturation (only measured at T295, Table 3.4). In addition to being influenced by the macroaggregate formation, the observed overall increase of soil pore volume caused by

the organic amendments is mainly attributed to the dilution effect of a low bulk density amendment to the soil (Soane, 1990; Hati *et al.*, 2007; Bhogal *et al.*, 2009). As differences between the bulk density of the organic amendments and the soil were greater in the TK soil (soil bulk density = 1.00 Mg m⁻³) than in the EG soil (soil bulk density = 0.75 Mg m⁻³), the effects in the final total porosity were also more evident in the TK soil (Table 3.3). Other studies have noted an increase in total pore volume of soils caused by the application of biochars (Teixeira & Martins, 2003; Oguntunde *et al.*, 2008; Jones *et al.*, 2010) and other organic amendments (Aggelides & Londra, 2000; Bhogal *et al.*, 2009; Du *et al.*, 2009) but have not reported whether change occurred in micro- or macropore volumes. The newly created pore volume in the TK soil was mostly associated with macroporosity. It should be noted that most of the biochar particles (~90 %) were found to be greater than 0.25 mm (Figure 3.1); these particles can create additional pore spaces by settling in between the particles of the matrix, without clogging pores, thus contributing to the generation of macroporosity (Steiner *et al.*, 2011). The effect of biochar on the porosity of the EG soil after 295 d of incubation was more apparent at high tensions, and was attributed to the contribution of biochar to microporosity (Tseng & Tseng, 2005), as suggested by Novak *et al.* (2009b). This was less evident in the TK soil, as this soil has a greater fraction of fine particles (silt and clay) than the EG soil.

3.4.3 Water holding capacity at different matric potentials

At a specific matric potential, the EG soil had always greater values of θ_V than the TK soil (Tables 3.3 and 3.4), which were attributed (i) to the greater native OC content of the former, 103 g C kg⁻¹ soil vs. 42 g C kg⁻¹ soil, and (ii) to the presence of short-range order inorganic compounds (*e.g.*, allophane) (Shoji *et al.*, 1996). The nature of these

two soils and specifically the different type of reactive surfaces of these soils have been discussed previously (Bolan & Baskaran, 1997).

However, the observed increase in θ_V at any matric potential in both TK and EG soils is related to the increases of micro-, meso-, and macroporosity caused by macroaggregate formation and the dilution effect. An increase in soil θ_V with the addition of biochar to soil was evident from T0, while the effect of the corn stover amendment at T0 was less apparent, especially in the EG soil. After 295 d of incubation, the effect of all organic amendments, including the fresh corn stover, on the θ_V of the TK soil was evident only at low tensions (≥ -0.3 bar), which corresponds to water mostly retained in macropores. In the EG soil, the effect of biochar was evident in a part of the meso- as well as macropore range (from -0.08 to -1 bar), but not in the -0.06 bar to saturation range. As observed at T0, fresh corn stover had barely any effect in the EG soil after 295 d incubation.

For both the Alfisol (TK soil) and the Andisol (EG soil), water retained at -0.3 bar was considered as field capacity based on their textural properties (Nachabe, 1998) (Table 3.2). After 295 d incubation, field capacity increased by 16 % in the TK soil and by 12 – 16 % in the EG soil. These proportional increases are above those value of that obtained by Zeelie (2012) who used similar application rates of biochar made from pine sawmill waste pyrolysed at 450 °C temperature to amend a sandy soil. An increase of field capacity by 18 % has also been reported for Anthrosols rich in charcoal (Glaser *et al.*, 2002), although the historical application rates were not identified. Conversely, Busscher *et al.* (2010; 2011) found no effect on the WHC at field capacity when biochar – produced at 700 °C from pecan shell – was applied to a Typic Kandudult (a loamy sand soil).

There was no sizeable difference in θ_V at T295 for TK and EG soils amended with the two biochars (CS-350 and CS-550) except under specific conditions. The θ_V at permanent wilting point of both soils was greater when CS-550 biochar was used compared with CS-350 biochar. This is attributed to the increased biochar microporosity (Liang *et al.*, 2006; Hina *et al.*, 2010) achieved with increasing the maximum pyrolysis temperature (Kishimoto & Sugiura, 1985; Van Zwieten *et al.*, 2009). However, BET surface analysis of the two charcoals at time zero did not reveal any measurable microporosity. It is possible that the inorganic ash fraction could initially clog micropores of the charcoal fraction, but dissolve thereafter during the experimental incubation. More research is needed to prove this.

3.4.3.1 Available water content

In a climate with variable periodic rainfall an increase in AWC can cause increased plant growth (Yamato *et al.*, 2006; Van Zwieten *et al.*, 2010b; Uzoma *et al.*, 2011). Therefore, the extra AWC generated due to biochar application may help to decrease the irrigation frequency during dry spell and also will allow the plants to survive longer time. The general increase in soil θ_V at T0 was not always paralleled by an increase in AWC. In fact, this was only observed in the EG soil amended with biochar. Moreover, the RAWC at T0 tended to decrease with organic amendment, although not always significantly at $P < 0.05$. Therefore, whereas the available water content of these two soils increased in the presence of organic amendments at T0, it is apparent the plant water potential may need to become more negative in order to absorb water from the amended soils compared with plants growing in the control soils. Nonetheless, this decrease in RAWC was only observed at the start of the experiment.

At the end of the incubation (T295), the increase in AWC in the biochar-amended soils compared with the mean value of the unamended control was 22 % in the TK soil and

19 – 33 % in the EG soil, and is in agreement with other studies where biochar was applied to soil (Tryon, 1948; Glaser *et al.*, 2002; Chan *et al.*, 2007). However, those experiments used high rates of biochars, 50 and 100 t ha⁻¹ (Chan *et al.*, 2007), and ~ 70 t ha⁻¹ (Tryon, 1948), which are not economically feasible at the farmer level. An increase of ~16 % in AWC after the application of rice husk biochar, has been observed by Agusalim *et al.* (2010) using similar rates (10 t ha⁻¹) to the present study. Importantly, an increase in RAWC was observed after biochar application, especially in the TK soil, for all amendments, and in the EG soil for the CS-550 amended soils (although the latter was not significant at $P < 0.05$). This increase would be important particularly during dry spells in cropping seasons to keep more water stored in biochar-amended soils that is available for the plants growth.

3.4.4 Hydraulic conductivity

The results on K_s followed a similar trend to that of aggregate stability in that the effect of CS-550 biochar was more prominent in the TK soil than that in the EG soil, whereas the reverse effect was observed for the CS-350 biochar. The increase in K_s agrees with the increase in the overall porosity of these soils. Development of macroporosity causes the hydraulic conductivity to increase, which reflects the drainage level of a given soil (Heard *et al.*, 1988; Logsdon *et al.*, 1990; Azooz & Arshad, 1996). As expected, the improvements observed in the K_s due to biochar application were corroborated by the increases of macropore volume, in particular in the poorly drained TK soil.

The effect of fresh corn stover on the K_s of these two soils was opposite, with an increase in the TK soil and a decrease in the EG soil. The overall effect of the organic amendments on the K_s of the TK soil can have positive implications if aeration is to be increased in this poorly drained soil. This may have direct environmental implications, such as the reduction of N₂O emissions. However, the extent of this benefit will always

depend on how the soil layer underneath the amended soil responds to drainage. Oguntunde *et al.* (2008) found comparable improvements of K_s from $7.5 \times 10^{-6} \text{ m s}^{-1}$ in unamended soil to a K_s of $1.3 \times 10^{-5} \text{ m s}^{-1}$ in charcoal amended soils under field conditions. Uzoma *et al.* (2011) reported K_s values several-folds higher than those of the present study using similar doses of biochar of 10 to 20 t ha⁻¹ but working with a sandy soil (sand 95 %, bulk density $\sim 1.5 \text{ Mg m}^{-3}$). Laird *et al.* (2010b), however, found no effect of biochar ($\sim 5 - 20 \text{ t ha}^{-1}$) made from mixed hardwood on the K_s of a Typic Hapludoll.

3.4.5 Hydrophobicity

The biochar-amended soils displayed less hydrophobicity than the corn stover-amended soils, specially the CS-350 biochar treatments (Table 3.6). Therefore, the observed improvements in soil physical conditions with less hydrophobicity in biochar-amended soils may give positive implications on the utility of biochar as a better soil amendment. Otherwise, it would create negative impacts on soil hydraulic properties (Clothier *et al.*, 2000; Blanco-Canqui & Lal, 2009). However, arguments are found that a moderate hydrophobicity could help improve soil aggregation (Blanco-Canqui *et al.*, 2007), but this needs to be further studied for stable C sources, such as biochars, and under varied conditions. The observed difference between biochar-amended and other treatments might be explained by the nature of C compounds (Capriel, 1997). Hydrophobicity of biochar is expected to increase with the temperature of pyrolysis as decarboxylation proceeds, although tars trapped within pores in the low temperature, poorly carbonised biochar (Antal & Grønli, 2003; Hina *et al.*, 2010; Calvelo Pereira *et al.*, 2011) may contribute to hydrophobicity. Once charcoal is produced and comes in contact with moist air, its surface will tend to oxidise. This ageing process will continue once biochar is in the soil, and the acidic functional groups created will contribute to soil

hydrophilicity (Zimmerman, 2010). The formation of these functional groups was confirmed by XPS spectroscopy (data not shown). It should be noted that the soil water repellency observed was clearly related to the type of soil, this being generally greater in the Andisol; this observation is at present only partially understood (Notario *et al.*, 2010; Deurer *et al.*, 2011). The higher soil water repellency observed in the corn stover-amended soil compared with those amended with biochar (Table 3.4) may be related with the greater content of aliphatic-C compounds in the former (Capriel, 1997).

3.5 Conclusions

After 295 d of incubation, biochars produced from corn stover at 350 and 550 °C and added at a dose of 7.18 t C ha⁻¹ had significant effects on the physical properties, partially influencing porosity of the two soils tested: an Alfisol (TK soil) and an Andisol (EG soil). Changes in porosity were reflected in changes in soil hydraulic properties. Biochar treatment led to increase soil WHC, particularly at lower tensions in the TK soil, suggesting that these biochars may facilitate drainage in the poorly drained soil (the TK soil). This has both agronomic and environmental implications as it may not only increase root growth but also decrease N₂O emissions from poorly drained soils. However, the extent of this benefit will always depend on how the soil layer underneath the amended soil responds to drainage. The mesoporosity of both soils increased, which corresponds to increased AWC, has key implications with regards to the resilience of the plant-soil system during a dry spell. This may be important to the enhancement of plant productivity as well as to the reduction of irrigation frequency. For example, if rye grass growing in these soils, has a rooting depth of 60 cm, and evapotranspiration was 5 mm d⁻¹, and the soil water depletion fraction was 60 %, it was estimated that in the presence of biochar, the irrigation interval could be extended for 1 d in the TK soil and up to 3 d in the EG soil. Biochar properties will evolve with time, as biochar will

continue oxidising and will likely keep contributing to aggregate formation. However, the present results are biochar-, dose- and soil-specific. More research is needed to determine changes produced in other biochar, dose, and soil combination, especially under field conditions.

CHAPTER 4: Experimental evidence for sequestering C by avoidance of CO₂ emission from feedstock and enhanced protection of soil organic matter with the use of biochar

Abstract

An incubation experiment was conducted (i) to investigate the evolution of CO₂-C from soils amended with fresh corn stover or with biochar produced from corn stover at either 350 or 550 °C (CS-350 and CS-550) and incubated for 510 d, and (ii) to evaluate the priming effect of these biochars on native organic matter (NOM) decomposition. Two soil types were studied: a Typic Fragiaqualf (Tokomaru soil, TK soil) and a Typic Hapludand (Egmont soil, EG soil). Except for the controls, all other treatments received a 7.18 t C ha⁻¹ (5.6 and 6.3 g kg⁻¹ for the TK and EG soils, respectively). No significant differences ($P < 0.05$) were observed in the rate of CO₂-C evolution between the controls and biochar treatments for a given soil, but total accumulated CO₂-C evolved from the corn stover treatment was significantly higher ($P < 0.05$) than that from the other treatments, with a loss of >65 % of the material added. In both soils, uncharred corn stover had a net positive priming effect on decomposition (enhanced the rate of decomposition) of NOM, whereas biochar had a net negative priming effect in the TK soil. No distinct net pattern on the priming of NOM by biochar was evident in the EG soil by the end of the incubation. A C balance indicates that, in less than 35 weeks, the CO₂ lost from both biochar production and decomposition “breaks even” with that lost from residue decomposition. The “break-even” point is reached earlier for the low-temperature biochar and in the allophanic soil. When the different priming effects on NOM were also considered in the C balance, the differences among the two soils disappeared. We estimated a half-life of 494 y for CS-550 biochar in the TK soil, while estimated half-lives of all other biochar-soil combinations were <200 y. It was estimated that 55 – 70 % of added biochar-C would remain in soil after 100 y in both soils. The

use of biochars in these organic C-rich soils was proven to be adequate for promoting C sequestration, especially when compared to the direct application of fresh feedstock.

Key words

Biochar, corn stover, Alfisol, Andisol, priming effect, stability, microbial respiration

4.1 Introduction

Biochar is produced by the thermal degradation of biomass and is intended to be applied to soils to achieve an agronomic and/or environmental benefit, while decreasing the greenhouse gas (GHG) emissions that would otherwise occur if the original biomass decomposed naturally (Lehmann & Joseph, 2009; Smith *et al.*, 2010; Zimmerman, 2010; Cross & Sohi, 2011). Biochar application to soil is proposed as a strategy to offset C emissions and combat global climate change (Lehmann *et al.*, 2006; Lehmann *et al.*, 2008; Kuzyakov *et al.*, 2009; Nguyen & Lehmann, 2009; Novak *et al.*, 2010). The stability of biochar in soils depends on (i) its inherent properties (intrinsic stability) (Calvelo Pereira *et al.*, 2011); and (ii) the pedo-climatic conditions to which biochar is exposed after its application to soils (Nguyen *et al.*, 2008; Hilscher *et al.*, 2009; Lehmann *et al.*, 2009; Nguyen & Lehmann, 2009). Not all biochars are the same, and their beneficial effects in a specific soil may not be observed in another soil (Kimetu & Lehmann, 2010; Luo *et al.*, 2011). Properties of biochars are primarily determined by (i) the feedstock used; and (ii) conditions under which it is produced (Antal & Grønli, 2003; Nguyen & Lehmann, 2009; Calvelo Pereira *et al.*, 2011). For biochar application to soils to become accepted as a C sequestration strategy, not only must the longevity of biochar C in soil be proven but, over the same period, the application must not lead to a reduction in soil native organic matter (NOM).

The influence of NOM on biochar decomposition has been mainly associated with co-metabolic reactions (Hamer *et al.*, 2004; Cheng *et al.*, 2006; Hilscher *et al.*, 2009; Kuzyakov *et al.*, 2009; Keith *et al.*, 2011; Zimmerman *et al.*, 2011). Conversely, an enhancement of NOM decomposition – a positive priming effect of biochar on NOM – after the addition of carbonised materials into soil has been attributed to enhanced microbial activity produced by (i) the chemical changes induced by the addition of

biochar (*e.g.*, addition of nutrients, liming effect), and/or (ii) the incorporation of a labile fraction of C in biochar (Czimczik & Masiello, 2007; Wardle *et al.*, 2008; Luo *et al.*, 2011). The latter work (Wardle *et al.*, 2008) was, however, controversial because the exact source from which the CO₂ evolved was not identified (Lehmann & Sohi, 2008).

Retardation of NOM decomposition after biochar application has also been reported (Jonker & Koelmans, 2002; Cross & Sohi, 2011; Jones *et al.*, 2011). This negative priming effect could be due to one or several of the following mechanisms: (i) sorption of NOM on to biochar surfaces (Kwon & Pignatello, 2005); (ii) the effect of biochar in promoting aggregation and the entrapment of NOM within those aggregates (Brodowski *et al.*, 2005); (iii) deactivation of microbial enzymes at the surface of biochar particles; and/or (iv) toxicity of biochar to microorganisms (Zimmerman *et al.*, 2011). Zimmerman *et al.* (2011) concluded – based on a 500 d-incubation experiment – that biochar could either have a positive or a negative priming effect on NOM decomposition depending on (i) the type of biochar; (ii) the type of soil; and (iii) the duration of incubation.

Research is required to obtain a mechanistic understanding of (i) the influence of soil types on the stability of a specific type of biochar; and (ii) the influence of the type of biochar on the cycling of NOM in a specific soil. This requires long-term incubations (>1 y) of different soils amended with biochars produced from various feedstocks under different pyrolysis conditions. In this study, a laboratory incubation was carried out for 510 d to determine (i) the stability of C in biochars produced from corn stover (*Zea mays* L.) at two different temperatures (350 and 550 °C) compared to the stability of the original fresh corn stover feedstock from which the biochar was produced, when

incubated in two distinct soils, and (ii) the effect of these biochars on the cycling of NOM of these soils.

4.2 Materials and methods

4.2.1 Biochar production

Corn stover was collected immediately after harvest from the Rangitikei region (40° 09′ 06.71″ S, 175° 16′ 51.45″ E, North Island, New Zealand). Two batches of 200 g of corn stover were pyrolysed in a gas-fired rotating drum kiln made of stainless steel (inner volume of 5 L), as described by Calvelo Pereira *et al.* (2011). One batch was pyrolysed at a final temperature of 350 °C (CS-350) and the other one at 550 °C (CS-550). Heating rates were 36 and 51 °C min⁻¹ respectively. Details on the carbonisation process are provided in Chapter 3.

4.2.2 Characterisation of feedstock and biochar

Corn stover and biochars were analysed for: total C, H, N, and S, ash content, C oxidisable with potassium dichromate, and C hydrolysable with HCl. Effective cation exchange capacity (ECEC), pH, particle-size distribution, N₂ adsorption analysis for specific surface area (BET), solid state CP-MAS ¹³C NMR spectroscopy and Py-GC-MS of biochar samples were also conducted. Methodologies are described in detail in the Supportive Information.

4.2.3 Soil material preparation

Details of the soil collection and sample preparation are provided in Chapter 3. A Typic Fragiaqualf (Tokomaru silt loam, TK) and a Typic Hapludand (Egmont black loam, EG) (Soil Survey Staff, 2006), both under a C₃ pasture ecosystem, were used for this study. The Alfisol (Rongotea, New Zealand) has developed on loess with a layer of Aokautere ash and is rich in silt and clay, the latter being dominated by 2:1 clay minerals. The

Andisol (Wanganui-Hawera, New Zealand) has developed on andesitic volcanic ashes of Oakura and Okatotephas and allophane is dominant in its mineralogy. The climatic conditions are similar for both sites, with a mean annual soil temperature of ~ 12 °C and a mean annual rainfall in the range between 1000 and 1300 mm y^{-1} (Baisden *et al.*, 2010). Some general chemical properties of the two soils are given in Table 4.1.

The PVC columns used in the incubation experiment were 100 mm in height and 150 mm in diameter. The treatments considered in this study were: (i) control treatments (TK-Ctr, EG-Ctr), (ii) fresh corn stover feedstock (TK-CS, EG-CS), (iii) low-temperature biochar (TK-350, EG-350), and (iv) high-temperature biochar (TK-550, EG-550). All treatments were run in triplicate.

Each organic amendment was added at a dose equivalent to 7.18 t C ha^{-1} and evenly mixed into the soil. Due to the differences in bulk density between these two soils, the amount added per unit of mass was 5.6 and 6.3 g C kg^{-1} soil for TK and EG soils, respectively. Moisture content was gravimetrically maintained at 70 % of water holding capacity, checked by weighing samples every two days and adding water to maintain samples at a constant weight. After 295 d, an undisturbed sub-sample (100 mm in height and 65 mm in diameter) was taken and incubated for an additional 215 d; while another subsample was used for a parallel study involving the use of plants (Chapter 5). Total duration of incubation was thus 510 d.

Table 4.1: Basic chemical properties of Tokomaru and Egmont soils.

Soil	pH (H ₂ O)	Total OC ^a	Dichro-C ^a	Hydro-C ^a	C _p ^a	Al _b	Fe _p ^b	(Al _p +Fe _p)/C _p	δ ¹³ C (‰)	Ava. N ^d	Olsen P ^e	Ex. K ^f	Ex. Ca ^f	Ex. Mg ^f	Ex. Na ^f	ECEC ^f	Main mineral types
Alfisol	5.7	41.7	34.0	22.3	19.9	1.1	2.3	0.05	-28.80	256	45	0.38	4.5	0.60	0.22	17	Micaceous clay minerals
Andisol	5.8	102.0	72.3	39.8	43.7	7.3	2.5	0.08	-27.60	380	34	0.48	6.5	0.65	0.15	29	Allophane, kaolinite, gibbsite

^a g C kg⁻¹ soil^b g Al or Fe kg⁻¹ soil^c molar ratio^d kg N ha⁻¹^e μg g⁻¹^f c mol (+) kg⁻¹

Ava. = Available

Ex. = Exchangeable

4.2.4 CO₂ evolution measurements

Each pot was placed in an air-tight, fixed-size 7 L PVC chamber as described by Saggari *et al.* (2008). The CO₂ evolved from the soil was trapped in 30 mL of 1 M NaOH solution for 3 h. From day 295 onwards, when the incubation soil was reduced to ~330 g, the trapping of CO₂ was carried out using 1.8 L Agee jars and 10 mL of 1 M NaOH. The amount of CO₂ trapped in the alkali solution was determined by titrating 5 mL aliquot with standardised 0.5 M HCl after the addition of 5 mL of 0.25 M BaCl₂ solution. The titrations were carried out with Titra Lab 865 Potentiometric Titration Workstation (Radiometer Analytical SAS, Lyon, France) in the presence of N₂ gas.

4.2.5 Determination of total soil C and $\delta^{13}\text{C}$

Total C content was determined using a CNS analyser (LECO FP- 2000 CNS Analyser; Corp. St. Joseph, MI) at 0, 295 and 510 d. The $\delta^{13}\text{C}$ in the two biochar types, the two soils, and the soil samples taken at 295 and 510 d were determined using a Carlo Erba NA1500 elemental analyser connected to a Thermo Delta Plus Advantage isotope ratio mass spectrometer at Isotrace Research (Dunedin, New Zealand). Samples were finely ground and loaded into tin capsules prior to isotopic determination. For all analyses, the recommended scale used was VPDB (Vienna Pee Dee Belemnite, absolute isotope ratio $(11237 \pm 60 \times 10^{-6})$) and the units in the following are given as per mil (‰). The $\delta^{13}\text{C}$ of caustic trapping solutions were determined at time intervals of 3, 7, 11, 14, 47, 49, 105, 112, 119, 197, 203, 275, 282, 289, 330, 365, 390, 450, and 510 d. Details of the sample treatments and analysis are provided in the Supportive Information.

The fraction of CO₂ evolved from the organic amendment (f_{OA}) and from native OC (f_{soc}) in the amended soils was calculated according to a two-component isotopic

mixing model as given below (Cheng *et al.*, 2008b; Liang *et al.*, 2008; Zimmerman *et al.*, 2011):

$$\delta^{13}\text{CO}_{2\text{-total}} = f_{\text{OA}}*\delta^{13}\text{CO}_{2\text{-OA}} + (1-f_{\text{OA}})*\delta^{13}\text{CO}_{2\text{-SOC}} \quad (1)$$

$$f_{\text{OA}}+f_{\text{SOC}} = 1 \quad (2)$$

There was a small effect of slow pyrolysis on $\delta^{13}\text{C}$, with the signature becoming more negative by ca. <1 ‰ after pyrolysis (Table 4.2).

The priming effect of biochar on NOM decomposition was calculated as follows:

$$\text{Priming effect (\%)} = \frac{(C_t - C_c)}{C_c} \times 100 \quad (3)$$

Where C_t is the CO_2 evolved from NOM in the treated soils and C_c that evolved from the control soils. C_t was calculated by the rate flux multiplied by a ratio determined from $\delta^{13}\text{CO}_2$.

4.2.6 Modelling and statistical analysis

4.2.6.1 Model 1

The rate of added C decomposed during the experiment – calculated using $\delta^{13}\text{CO}_2$ from 19 different time points – was used to estimate the percentage C lost (C_{lost}) after 100 y (Equation 4) and the half-life ($C_{t_{1/2}}$) (Equation 5) of amendments following the model suggested by Zimmerman (2010). The neperian logarithmic transformations of the degradation rate of added biochar-C were plotted against the corresponding transformations of time in order to determine m (slope) and b (intercept). Detailed equation derivation steps and other information can be found in Zimmerman (2010).

$$C_{lost} = \left(\frac{C_0 e^b}{m+1} \right) t^{m+1} \quad (4)$$

Table 4.2: Elemental analysis, proximate analysis, and other properties of corn stover feedstock and two biochar types.

Sample	Elemental composition (g kg ⁻¹ amendment)					Proximate analysis (g 100 g ⁻¹ amendment)				Atomic ratio (d.a.f.) ^b		Yield (%)	Recovery (%)			$\delta^{13}\text{C}$ (‰)	C _{dichro}	Hydro-C
	C	N	H	O ^a	S	Ash	Fixed	VM	VC	H/C _{org}	O/C _{org}		C	N	S			
Feedstock	413	8.3	60.8	406.6	1.3	10.9	14.0	71.1	27.3	1.88	0.79	n.a.	n.a.	n.a.	n.a.	-12.47	40.6	36.5
CS-350	635	7.1	37.7	216.2	4.4	9.8	57.2	31.2	6.3	0.68	0.25	35.0	67.0	28.6	100	-13.13	22.0	20.5
CS-550	717	7.6	29.2	135.5	1.2	11.5	67.4	18.5	4.3	0.47	0.14	27.0	56.6	20.9	25	-13.38	14.9	13.1

^aEstimated by difference as $\text{O} = 100 - (\text{C} + \text{H} + \text{N} + \text{S} + \text{Ash})$

^bDry ash free basis

VM – volatile matter

VC (volatile C) = total C – fixed C

n.a. – not analysed

C_{dichro} = oxidisable C by dichromate oxidation (g C 100 g⁻¹ amendment)

Hydro-C = hydrolysable C (g C 100 g⁻¹ amendment)

$$C_{t_{1/2}} = \left(\frac{m+1}{2e^b} \right)^{\left(\frac{1}{m+1} \right)} \quad (5)$$

where C_0 = initial C (mg C kg⁻¹ soil), t = time, $C_{t_{1/2}}$ = half-life.

4.2.6.2 Model 2

In this model, C in the soil is separated into any number of separate notional pools each of which decays exponentially (Bauer *et al.*, 2008), with the rate of decay of each pool determined by a pool-dependent rate constant modified by a temperature response function. The sum of C loss rate from all pools equates to the observable rate of CO₂ efflux. The amount of C remaining over time is modelled as the initial C content that gradually decreases over time at a rate that must correspond to the observed rate of CO₂ efflux. Performance of the model is thus constrained through the measured cumulative C loss and the amount of C added at the start of the incubation.

In order to calculate the half-life ($t_{1/2}$) of added-C compounds, the model was first used to describe the time course of CO₂-C evolution rate from the control treatments (NOM) with a sub-division into two pools. The sizes and decomposition rate constants of those two pools were then fixed, and two additional pools were added to describe the additional C loss attributable to further C amendments (Equation 6). These additional two pools represented the decomposition of added-C under the assumption of no priming effect. For both NOM and amendments, the system is thus described to consist of a fast and a slow soil OC pool.

$$C_{S(t)} = C_{1(t-\Delta t)} \cdot e^{-\Delta tr1} + C_{2(t-\Delta t)} \cdot e^{-\Delta tr2} + C_{3(t-\Delta t)} \cdot e^{-\Delta tr3} + C_{4(t-\Delta t)} \cdot e^{-\Delta tr4} \quad (6)$$

where $C_{S(t)}$ = total remaining C in the soil (mg C kg⁻¹soil) at time t (d), Δt = time difference (d) between two consecutive sampling days, C_1 and C_2 being the remaining

labile and stable C remaining in NOM, and C_3 and C_4 being the remaining labile and stable C in the added material. The terms $r_1..r_4$ represent the apparent first order mineralisation constants (d^{-1}) for the pools 1..4, respectively.

The CO₂ efflux rate at time t, E_t , was calculated as:

$$E_{(t)} = C_{1(t-\Delta t)} \cdot (1 - e^{-\Delta t r_1}) + C_{2(t-\Delta t)} \cdot (1 - e^{-\Delta t r_2}) + C_{3(t-\Delta t)} \cdot (1 - e^{-\Delta t r_3}) + C_{4(t-\Delta t)} \cdot (1 - e^{-\Delta t r_4}) \quad (7)$$

The apparent first-order decay constants, $r_1..r_4$, are calculated as:

$$r_i = k_i \cdot f(T) \quad (8)$$

where k_i is the inherent first-order decay constants of pool i (1..4) and $f(T)$ is a temperature response function given by an equation in the form given by Kirschbaum (2000):

$$f(T) = e^{[\beta(T-40)/(T+\gamma)]} \quad (9)$$

where T is the measured temperature (°C) at respective measurement dates, β and γ are empirical parameters '40' is an arbitrary normalisation parameter (Kirschbaum, 2000).

We first obtained the parameters of the temperature function by simultaneously considering the respiration rates observed in all different treatments (4 treatments x 2 soil types). Those parameters of the temperature function were then used for each individual data set of particular treatments. This assumed that the temperature response of decomposition was the same for NOM and all the different amendments.

Only the results for C_{lost} and $C_{t_{1/2}}$ are presented for fresh corn stover incubation. When simulating biochar decomposition, the extra C addition caused virtually no extra C efflux (data not shown).

4.2.6.3 Statistical analysis

The statistical differences between the applied treatments were determined by analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc., Chicago, USA). Post hoc analyses were computed using Duncan test at $P = 0.05$.

4.3 Results

4.3.1 Properties of the feedstock and biochars at time zero

Selected properties of the corn stover feedstock and biochars used in this study are reported in Table 4.2 and have been described in Chapter 3. As expected, with an increase in the pyrolysis temperature, the H/C_{org} and O/C_{org} atomic ratios of the organic material decreased (Table 4.2), as did the volatile matter fraction (from 31.2 to 18.5%) and the fraction of C oxidisable with potassium dichromate (from 22.0 to 14.9 %), as expected. Conversely, fixed C increased with the increase in the pyrolysis temperature (from 57.2 to 67.4 %), as did the pH (from 8.9 to 9.4). The increase in C stability as carbonisation intensity increased was also seen by solid state CP-MAS ^{13}C NMR spectroscopy (Figure 4.1A and 4.1B). Both biochars showed a large peak in the aromatic region at ca. 128 ppm, attributed to C- and H-substituted aromatic C. CS-350 biochar had also a broad band in the alkyl-C region (0 – 45 ppm); part of the alkyl intensity detected could be due to the presence of spinning side bands (SSBs), which were more evident in the CS-550 biochar. The peak observed in the CS-350 biochar at 56 ppm is attributed to methoxyl C, which is commonly associated with lignin structures.

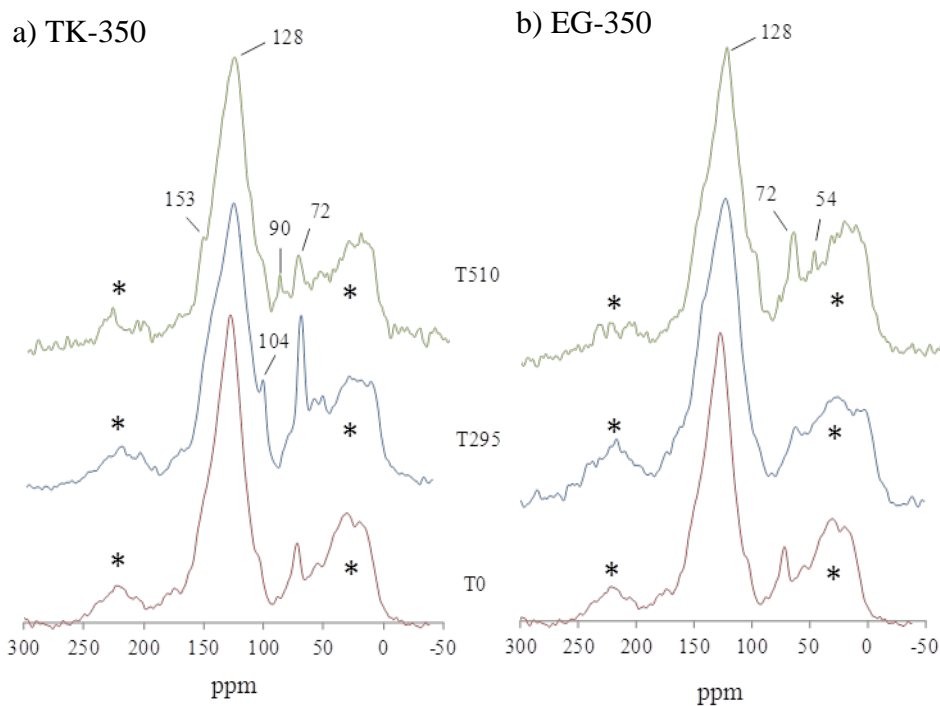


Figure 4.1A: Solid-state ^{13}C NMR spectra of CS-350 biochar obtained at T0 (before adding to the soils), T295, and T510 (handpicked particles from the incubation pots) for (a) the TK soil and (b) the EG soil. The SSBs are given with * symbol.

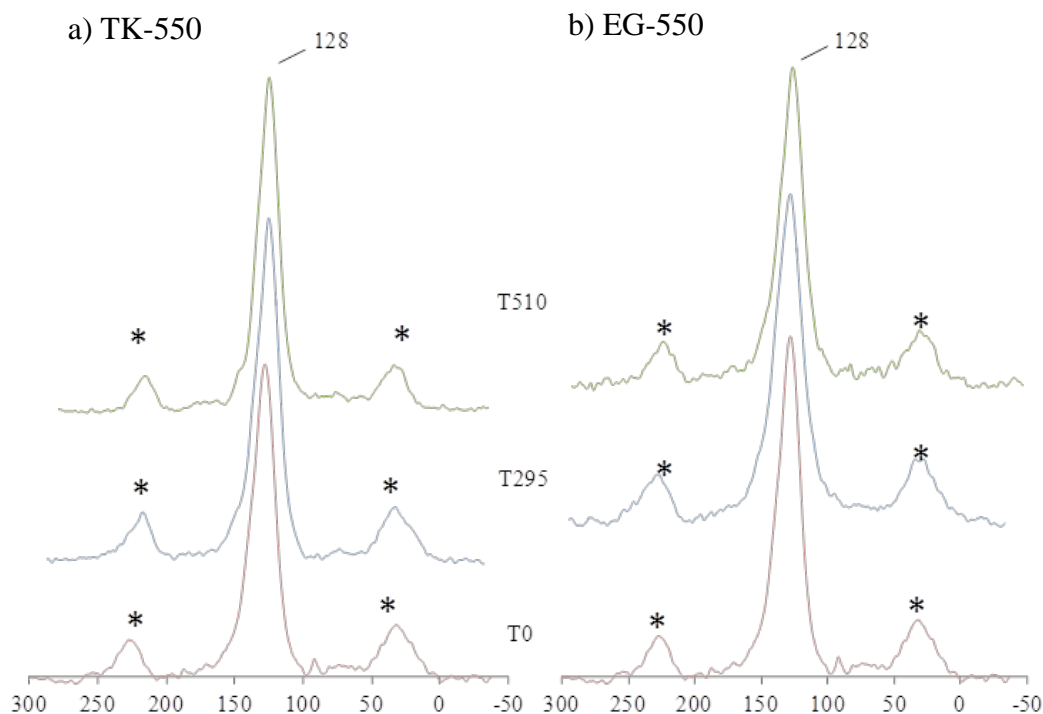


Figure 4.1B: Solid-state ^{13}C NMR spectra of CS-550 biochar obtained at T0 (before amending to the soils), T295, and T510 (handpicked particles from the incubation pots) for (a) the TK soil and (b) the EG soil. The spinning side bands (SSBs) are given with * symbol.

The peak detected at 30 ppm is attributed to C- and H- substituted alkyl C associated with the presence of long-chain polymethylene C, such as those found in lipids. The peak observed at 72 ppm is attributed to O-alkyl C associated with remaining cellulose.

4.3.2 Incubation experiment

4.3.2.1 Decomposition of charred and uncharred corn stover

The CO₂ evolution rates (mg CO₂-C kg⁻¹ soil h⁻¹) during the 510 d incubation are presented in Figure 4.2A and 4.2B. The EG-Ctr soil showed significantly greater CO₂-C evolution rates than the TK-Ctr soil (at $P < 0.05$ from day 103 onwards). The CO₂-C evolution from all treatments in both soils peaked at the start of the experiments (within the first 14 d) with values following the order TK-CS > TK-350 > TK-550 > TK-Ctr (3.93, 2.05, 1.82, and 1.66 mg CO₂-C kg⁻¹ soil h⁻¹, respectively), and EG-CS > EG-350 > EG-550 > EG-Ctr (4.09, 2.86, 1.99, and 1.28 mg CO₂-C kg⁻¹ soil h⁻¹, respectively). Thereafter, the mineralisation rate tended to decrease for all treatments. Both EG and TK soils amended with fresh corn stover exhibited significantly ($P < 0.05$) higher mineralisation rates than the rest of treatments up to day 204. From day 205 onwards, there were no significant differences ($P < 0.05$) in the CO₂-C evolution rates among TK treatments. The same pattern was observed in the EG soil from day 205 to 408; however, from day 408 to the end of the experiment, EG soils amended with both charred and uncharred materials had significantly higher ($P < 0.05$) CO₂-C evolution rates than the EG-Ctr treatment. No significant differences ($P < 0.05$) were detected between the two biochar treatments in the two soils under study at any time.

Cumulative CO₂-C evolved from the treatments under study after 510 d of incubation is shown in Figure 4.3a and 4.3b. A significantly greater ($P < 0.05$) cumulative CO₂-C evolution was detected in the EG-Ctr treatment than in the TK-Ctr treatment (6.3 and 5.3 g C kg⁻¹ soil). Cumulative CO₂-C evolved from the soils amended with fresh corn

stover was significantly greater ($P<0.05$) than those from the other treatments. At day 510, the additional amount of CO₂-C evolved from the pots amended with fresh corn stover compared with their respective control treatments was 4.3 and 4.9 g C kg⁻¹ soil for the TK and EG soil, respectively. No significant differences ($P<0.05$) were detected in the cumulative CO₂-C evolved over 510 d between biochar-amended soils and their respective control treatments.

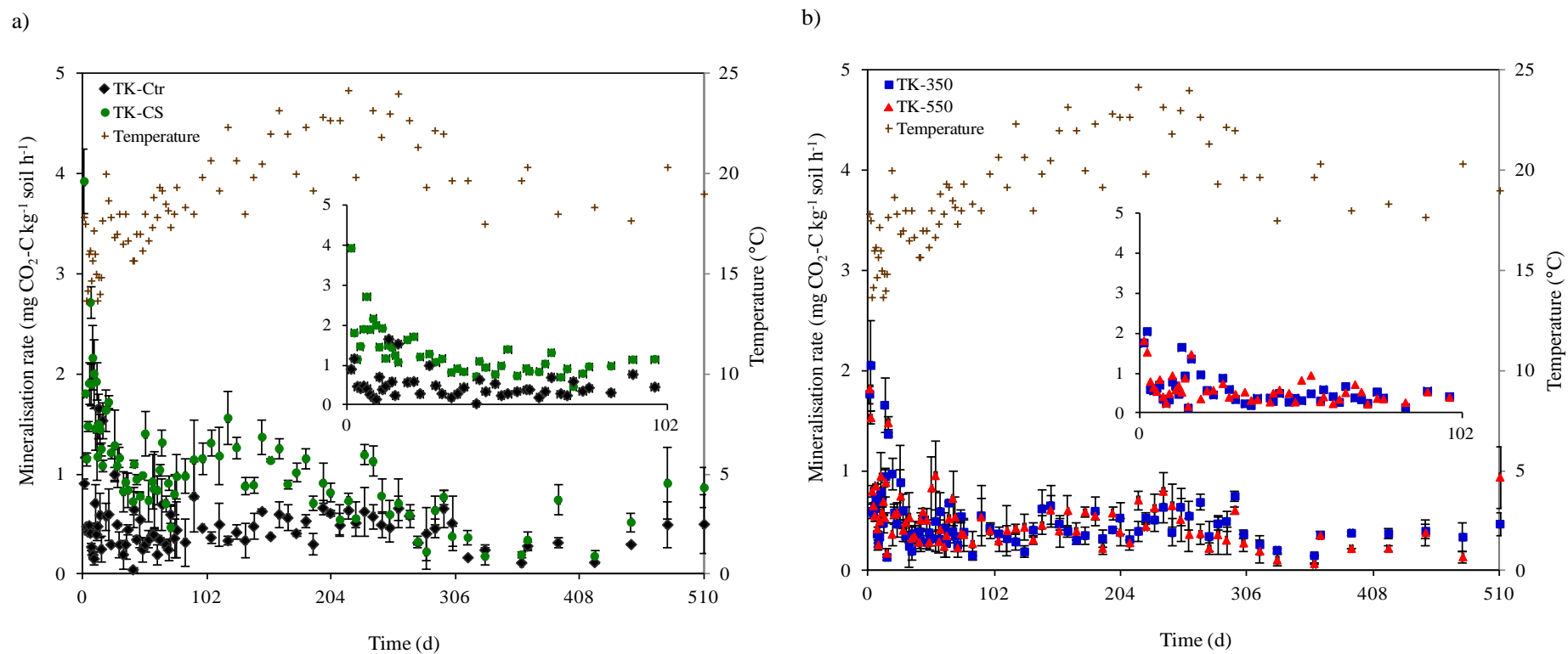


Figure 4.2A: The $\text{CO}_2\text{-C}$ evolution rates ($\text{mg CO}_2\text{-C kg}^{-1} \text{ soil h}^{-1}$) for the (a) corn stover amended TK soil with the control soil and (b) the two biochars amended TK soil during 510 d of the incubation experiment. Error bars represent the standard error ($n = 3$).

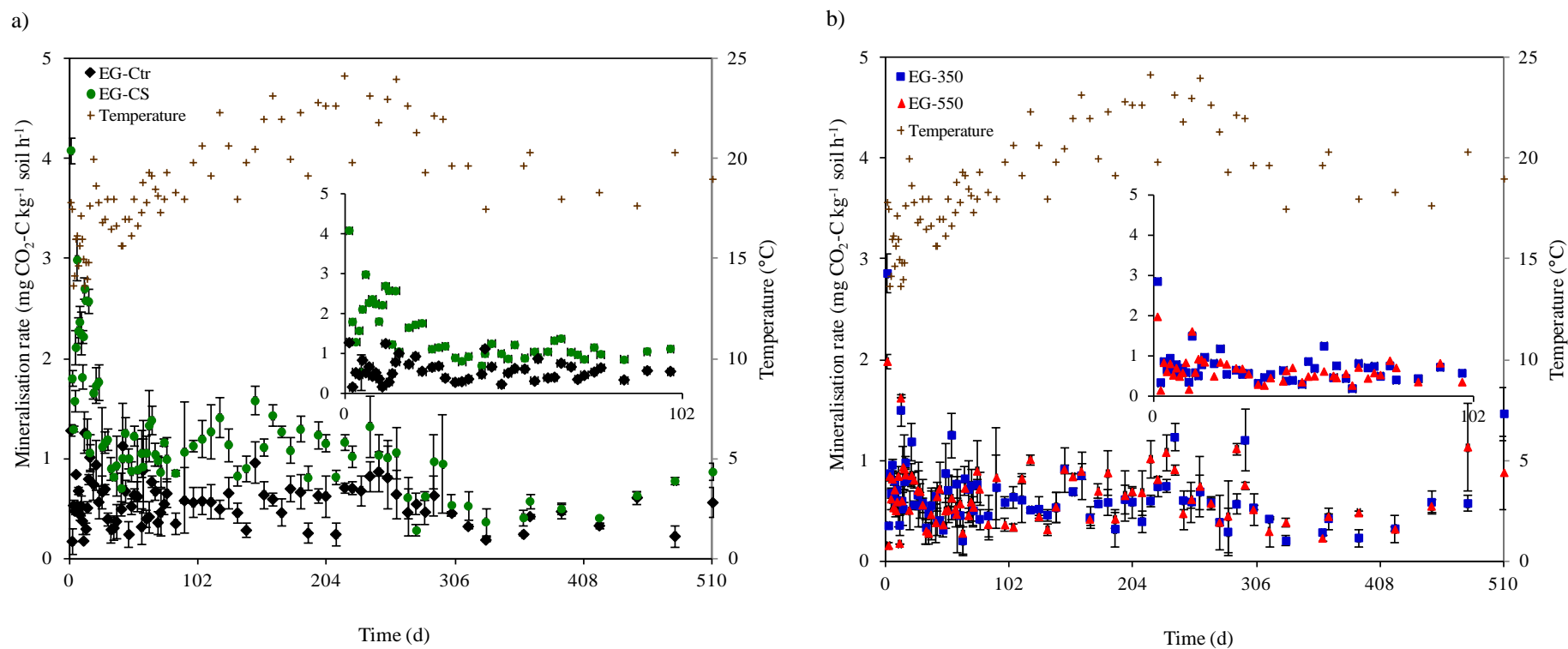


Figure 4.2B: The CO₂-C evolution rates (mg CO₂-C kg⁻¹ soil h⁻¹) for the (a) corn stover amended EG soil with the control soil and (b) the two biochars amended EG soil during 510 d of the incubation experiment. Error bars represent the standard error (n = 3).

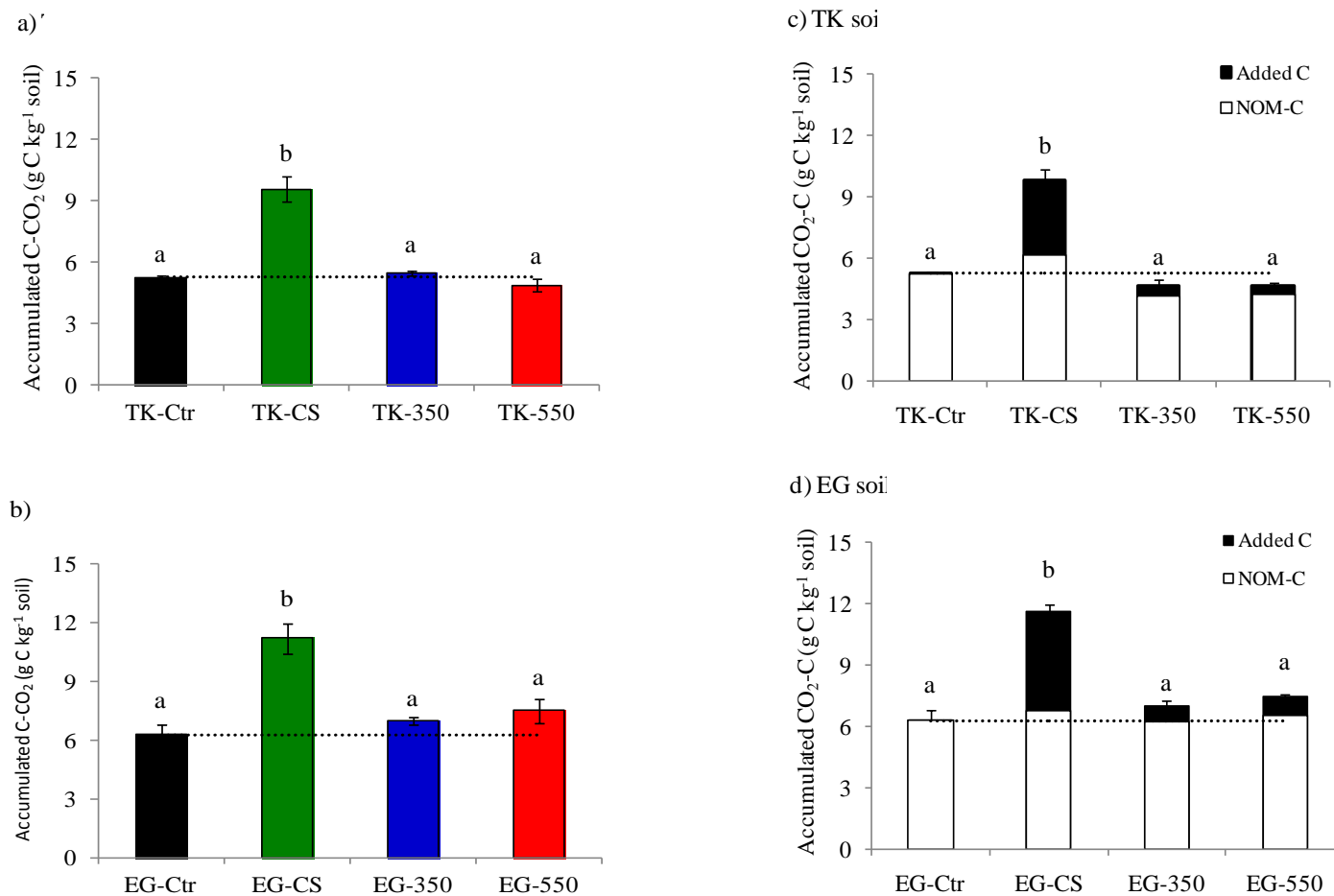


Figure 4.3: The total cumulative $\text{CO}_2\text{-C}$ mineralised from (a) the TK soil, (b) the EG soil, (c) the TK soil with the source of origin, and (d) the EG soil with the source of origin during 510 d of the soil incubation experiment. Error bars represent the standard error ($n = 3$).

4.3.2.2 $\delta^{13}\text{CO}_2$ and priming effect

Table 4.3 provides the $\delta^{13}\text{CO}_2$ data from specific dates of the experiment arranged in five time intervals of 102 d each. The $\delta^{13}\text{CO}_2$ of the TK-Ctr treatment ranged between –28.2 and –28.9 ‰ and that of the EG-Ctr between –27.6 and –29.4 ‰, which are values typical of C_3 ecosystems (Balesdent *et al.*, 1987; Cheng, 1996). The $\delta^{13}\text{CO}_2$ of the TK-CS and EG-CS treatments at each time interval was significantly higher ($P < 0.05$) than that of the corresponding biochar treatments (Table 4.3). A significantly higher ($P < 0.05$) $\delta^{13}\text{C}$ enrichment for $\text{CO}_2\text{-C}$ was always observed in biochar-amended soils compared with the respective controls, except in the TK soil within the 307 – 510 d time interval (Table 4.3). There were no significant differences ($P < 0.05$) between the $\delta^{13}\text{CO}_2$ evolved from soils amended with either CS-350 or CS-550 biochars at any time period considered.

The highest ^{13}C enrichment of $\text{CO}_2\text{-C}$ of the amended treatments was observed during the first two weeks of the incubation, this enrichment being 9.2, 7.2, and 7.5 ‰ in the TK-CS, TK-350, and TK-550 treatments, respectively, and 10.7, 7.3, and 6.7 ‰ in the EG-CS, EG-350, and EG-550 treatments, respectively. Accordingly, during this time period, fresh corn stover, CS-350 and CS-550 respectively contributed 60, 50, and 51 % of total $\text{CO}_2\text{-C}$ emission in the TK soil and 69, 46, and 43 % in the EG soil (Table 4.3). This ^{13}C enrichment tended to reduce over time in the biochar-amended soils, while there was no consistent trend in the soils amended with fresh corn stover (Table 4.3). During the second 102 d period (days 103 – 204), the same amendments contributed 36, 8 and 8 % of $\text{CO}_2\text{-C}$ in the TK soil and 33, 9, and 9 % of $\text{CO}_2\text{-C}$ in the EG soil. From day 204 onwards, the contribution of low- and high-temperature biochars to the $\text{CO}_2\text{-C}$ evolved from both soils was ≤ 7 %, while that of fresh corn stover was still high, ranging between 19 – 46 % in the TK soil and 18 – 49 %, in the EG soil (Table 4.3).

Figure 4.5 shows the evolution of the priming effect (%) caused by the amendment on NOM-C decomposition, as calculated from the stable C isotope ratio of evolved CO₂ and reported by averaging data at specific sampling points. In the TK soil, fresh corn stover amendment enhanced NOM decomposition during the first 100 d – that is, it had a positive priming effect – with an averaged maximum value of 128 %. The priming effect became negative for the following ~6 months and shifted back to positive in the final 2 months of the incubation (Figure 4.4a). A different pattern was observed when the TK soil was amended with biochar. With few exceptions, both biochars showed a predominant negative priming effect on NOM decomposition in the TK soil (Figure 4.4a). The cumulative priming effect on NOM decomposition in the TK soil after 510 d of incubation for corn stover, CS-350 biochar and CS-550 biochar treatments was 12, –5 and –15 %, respectively (Figure 4.3).

In the EG soil (Figure 4.4b), fresh corn stover amendment consistently enhanced NOM decomposition during the first 300 d of the incubation (averaged maximum of 160 %) with no evident effect thereafter. EG-350 and EG-550 treatments showed a brief initial negative priming effect, but this shifted to positive during the following 200 d of incubation. From then onwards, barely any priming effect was observed, except at the end of the experiment, when a positive priming effect was detected in all amended EG soils. The overall cumulative priming effect was 7, –1 and 4 % for the fresh corn stover, CS-350 biochar and CS-550 biochar, respectively (Figure 4.3b and 4.4b)

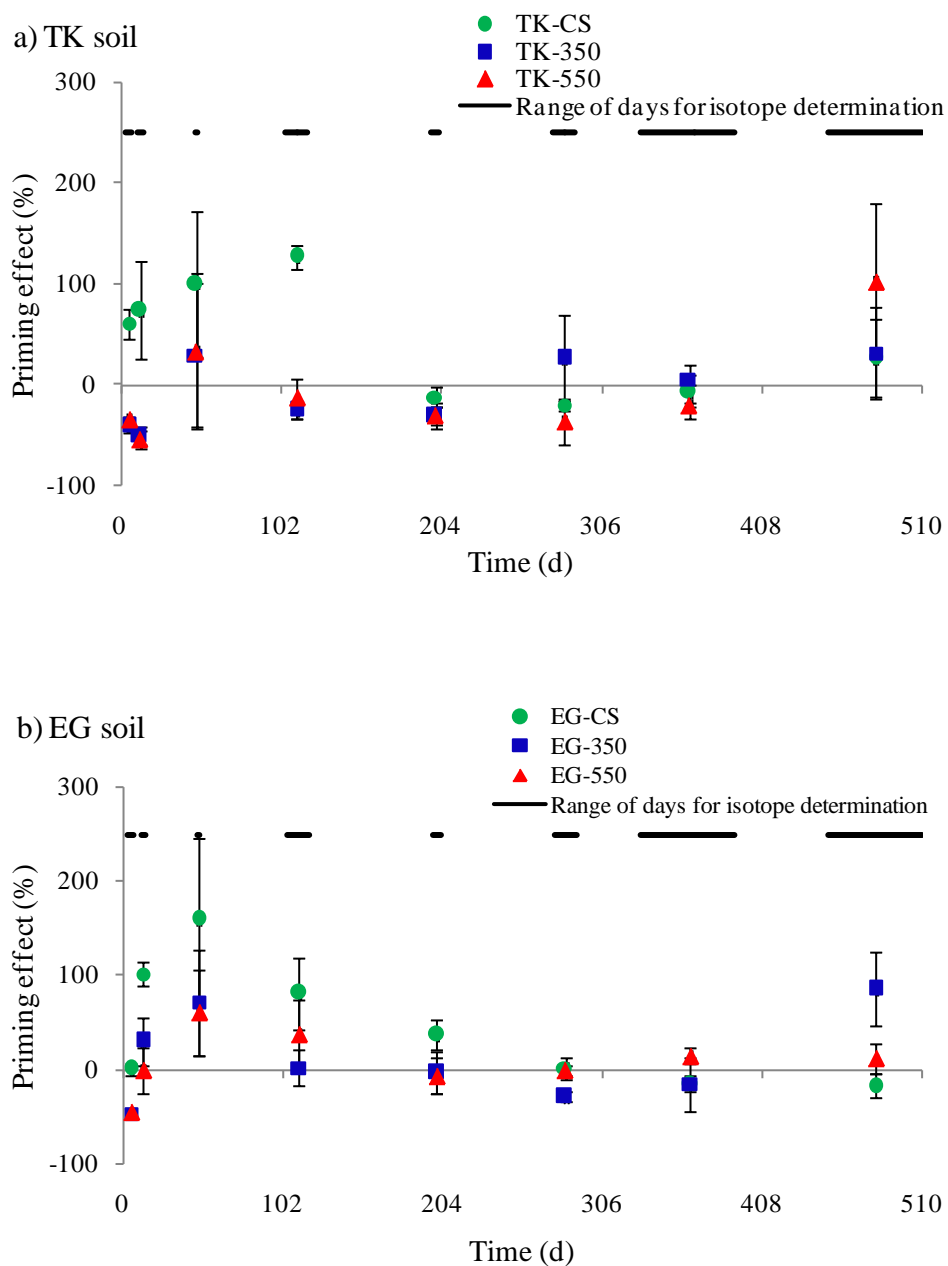


Figure 4.4: Priming effect (%) of amendment on NOM-C degradation as calculated using stable isotopic ratio of evolved CO₂ and reported by averaging data at specific sampling points. Error bars represent the standard error (n = 3).

Table 4.3: $\delta^{13}\text{C}$ and fraction of C coming from the organic amendments (f_{OA}) determined for the evolved $\text{CO}_2\text{-C}$ at different stages of soil incubation.

Treatment	1 – 102 d ^a		103 – 204 d ^b		205 – 306 d ^c		307 – 408 d ^d		409 – 510 d ^e	
	$\delta^{13}\text{C}$ (‰)	f_{OA}	$\delta^{13}\text{C}$ (‰)	f_{OA}	$\delta^{13}\text{C}$ (‰)	f_{OA}	$\delta^{13}\text{C}$ (‰)	f_{OA}	$\delta^{13}\text{C}$ (‰)	f_{OA}
TK-Ctr	-28.20a	–	-28.94a	–	-28.53a	–	-28.35a	–	-28.74a	–
TK-CS	-20.55c	0.49	-22.79c	0.36	-25.69c	0.19	-23.10b	0.35	-20.93b	0.46
TK-350	-23.79b	0.29	-27.38b	0.08	-27.88b	0.07	-27.58a	0.07	-27.78a	0.07
TK-550	-23.40b	0.30	-27.41b	0.08	-27.77b	0.07	-27.99a	0.05	-28.07a	0.05
EG-Ctr	-27.74a	–	-27.96a	–	-29.38a	–	-27.84a	–	-27.64a	–
EG-CS	-19.23c	0.52	-22.59c	0.33	-24.90c	0.18	-20.84c	0.49	-21.80c	0.43
EG-350	-22.81b	0.27	-26.16b	0.09	-26.66b	0.06	-26.60b	0.07	-26.68b	0.06
EG-550	-23.32b	0.25	-26.21b	0.09	-26.63b	0.07	-26.49b	0.07	-26.90ab	0.05

^a Measured on $\text{CO}_2\text{-C}$ evolved from incubations on 3, 7, 11, 14, 47, and 49 d

^b Measured on $\text{CO}_2\text{-C}$ evolved from incubations on 105, 112, 119, 197, and 203 d

^c Measured on $\text{CO}_2\text{-C}$ evolved from incubations on 275, 282, and 289 d

^d Measured on $\text{CO}_2\text{-C}$ evolved from incubations on 330, 365, and 390 d

^e Measured on $\text{CO}_2\text{-C}$ evolved from incubations on 450 and 510 d

4.3.2.3 Estimates of the added C remaining after 100 y and half-life

The evolution of CO₂-C from biochar and fresh corn stover was fitted to the model as described by Zimmerman (2010). Based on the fitted curves, the C in biochar and in fresh corn stover (in percentage) remaining in the two soils after 100 y was determined, as well as their corresponding half-lives (Table 4.4). Based on the modelled data, fresh corn stover is estimated to totally degrade in both soils in less than 4 y. It can also be inferred from the model that after 100 y of incubation under the conditions described here, 55 and 70 % of CS-350 and CS-550 biochar-C, respectively, would remain in the TK soil, and 59 % of CS-350 and CS-550 biochar-C would remain in the EG soil. Corn stover had an estimated half-life of 1.6 and 1.0 y, in the TK soil and in the EG soil, respectively (Table 4.4). Half-lives of CS-350 and CS-550 biochars incubated in the TK soil were 130 and 494 y, respectively. In the EG soil, these were 194 and 173 y, respectively.

Table 4.4: Percentage of remaining C after 100 y and half-lives of added C calculated using the power model. Standard deviations are given within parenthesis.

Soil	Amendment	C remaining %	t _{1/2} (y)
TK soil	Fresh corn stover	0.0 (±0.0)	1.6 (±0.1)
	CS-350	54.8 (±3.5)	129.9 (±23.3)
	CS-550	70.3 (±0.8)	493.7 (±61.2)
EG soil	Fresh corn stover	0.0 (±0.0)	1.0 (±0.1)
	CS-350	58.8 (±6.0)	193.6 (±86.3)
	CS-550	59.1 (±1.4)	173.0 (±9.6)

4.3.2.4 Changes in total soil organic C with time

The TK soil had an initial total OC content of 41.7 g kg⁻¹, while that of the EG was 102.0 g kg⁻¹ (Table 4.1). As the incubation proceeded, the total OC lost by the Ctr soils was 5.3 and 8.6 g kg⁻¹ over 510 d, respectively. At time 0, amended soils received 5.6 and 6.3 g C kg⁻¹ for the TK and EG soils, respectively. After 510 d of incubation, total OC content of TK and EG soils followed the order: biochar-350 ~ biochar-550 > fresh-

CS ~ Ctr soil (Table 4.5). At that sampling time, total OC contents of biochar-amended treatments surpassed those of the corresponding controls by 4.4 g C kg⁻¹ in the TK soil and by 5.5 g C kg⁻¹ soil in the EG soil.

A mass balance of C gains and losses was carried out. The relationship of estimates of total soil C changes at time 295 and 510 d in relation to time 0 and cumulative CO₂-C evolved after 295 and 510 d is shown in Figure 4.5. There is a good agreement between both groups of data for the TK soil (slope 1.02; R² = 0.82). There was a good fitting with EG soil data (R²= 0.93), but gas sampling tended to underestimate losses of soil C (slope 0.76).

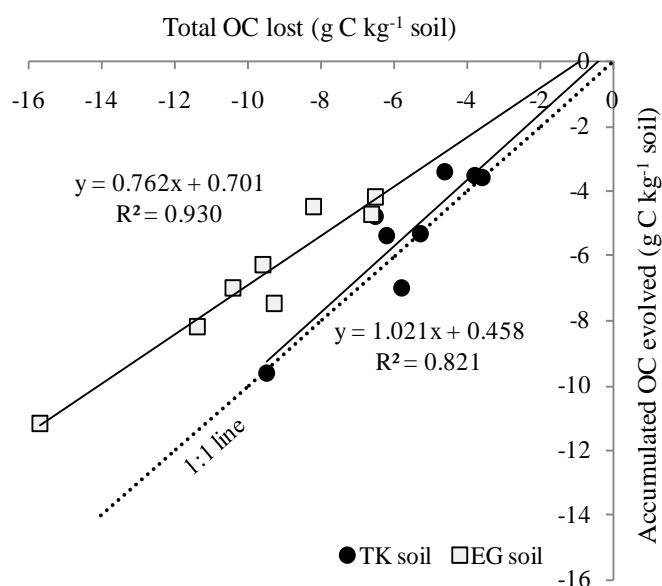


Figure 4.5: The relationship of estimates of total soil C changes at time 295 and 510 d in relation to time 0 and cumulative CO₂-C evolved after 295 and 510 d.

Table 4.5: Total C, non-oxidisable C, non-hydrolysable C, $\delta^{13}\text{C}$ (only at T510) and pH (H_2O) data determined after 295 and 510 d of the incubation study. Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test. Different letters denote significant difference between treatments in each soil.

Sample	Total C ^a		Non-oxidisable C ^a		Non-hydrolysable C ^a		Stable C isotope ratio (T510)		pH (H_2O)	
	T295	T510	T295	T510	T295	T510	$\delta^{13}\text{C}(\text{‰})$	f_{OA}	T295	T510
TK-Ctr	38.1a	36.4a	6.0a	7.0a	17.8a	19.3a	-29.00a	0	4.82a	4.72a
TK-CS	41.5b	37.8a	6.4a	7.2a	20.4b	21.4a	-28.32a	0.03	5.12a	4.84a
TK-350	43.5c	41.1b	11.7b	13.3b	25.4c	27.8b	-26.19b	0.14	5.05a	4.78a
TK-550	42.7c	40.8b	12.7b	13.2.b	24.7c	25.5b	-26.67b	0.14	5.06a	4.81a
EG-Ctr	96.5a	93.4a	25.8a	25.7a	62.3a	66.9a	-27.43a	0	5.04a	4.78a
EG-CS	97.9a	93.6a	26.8a	26.5a	63.1a	69.1ab	-26.98b	0.04	5.14a	4.94ab
EG-350	101.1b	98.9b	29.3b	31.0b	74.3b	81.3b	-26.29b	0.09	5.13a	5.00b
EG-550	102.7c	100.0b	31.7c	31.7b	75.4b	76.6ab	-26.26b	0.09	5.21a	5.10b

^ag C kg⁻¹ soil

4.3.2.5 CPMAS ^{13}C NMR spectra of biochar

The solid-state CPMAS ^{13}C NMR spectral data obtained for the handpicked biochar particles from the incubation experiment after 295 and 510 d are depicted in Figure 4.1A and 4.1B. The low-temperature biochar was the one that showed the greatest changes after 295 and 510 d of incubation. This was especially evident in the EG-350 biochar, with a decrease in the intensity of the peak at 128 ppm and the broad band at 45 – 0 ppm, which are associated with aromatic and aliphatic C groups, respectively. After 295 d, a new peak (ca.104 ppm) was observed in the TK-350 biochar, which was attributed to acetyl groups (di-O-alkyl C) such as anomeric C-1 of cellulose structures. The peak found at 72 ppm was also more prominent in this biochar compared with the corresponding fresh biochar. After 510 d, new peaks within the O-alkyl region (110 – 45 ppm) were found in the TK-350 and EG-350 biochars: ca. 90 ppm and 153 ppm in the former and ca. 54 ppm in the latter. These peaks are attributed to carbohydrate-derived structures (Kögel-Knabner, 1997) O-Aryl C associated with lignin (Hatcher, 1987), and methoxyl groups associated with lignin (Hatcher, 1987; Hatfield *et al.*, 1987), respectively. Barely any differences were observed in the high-temperature biochar after 295 and 510 d of incubation.

4.4 Discussion

4.4.1 Changes in soil OC stocks and the effect of different amendments on NOM decomposition

4.4.1.1 Control soils

Despite being under the same land use and similar climatic conditions, and having similar pH values, the EG soil has an OC content 2.5-fold that of the TK soil. This difference is mainly attributed to the different parent material from which they were formed, leading to differences in the mechanisms of SOM stabilisation. The TK soil has been developed mainly from loess and has a predominance of 2:1 clay minerals, the EG

soil has been formed on andesitic volcanic ashes and is dominated by allophane, a short-range alumina-silicate clay (Theng *et al.*, 1982; Parfitt, 2009). The presence of short-range ordered compounds and organo-Al complexes in Andisols has, for a long time, been considered to protect SOM from microbial attack (Jacquin *et al.*, 1978; Baldock & Skjemstad, 2000; Parfitt, 2009). In fact, the EG soil had an important fraction of OC (42 % out of total OC) extractable with sodium pyrophosphate and thus presumably associated with Al and Fe. Recent publications (Buurman *et al.*, 2007; Scheel *et al.*, 2008) further ascribe the stability of this SOM to entrapment within the micro-aggregates typical of these soils.

Mineralisation caused a greater absolute decrease in soil OC in the EG-Ctr soil (8.6 g kg⁻¹) than in the TK-Ctr soil (5.3 g kg⁻¹) by the end of the incubation, although the relative decrease was similar in both soils (~10 %). Greater mineralisation of OC was in agreement with the presence of more unprotected SOM in the EG soil, as determined by the Six *et al.* (2000) fractionation method (Chapter 5). This fractionation identified unprotected and protected OC of 3.5 and 25.6 g C kg⁻¹ in the TK soil and 5.9 and 74.8 g C kg⁻¹ in the EG soil (Chapter 5). The larger total protected OC pool in the EG soil, compared to the TK soil, concurs with the larger SOM protection capacity attributed to allophane soils. Presence of a greater unprotected amount of SOM in the EG soil could be related to the proximity of this soil to its organic C saturation limit, based on the concept developed by Six *et al.* (2002) and Stewart *et al.* (2008). However, the amount of mineralised OC lost from both soils after 510 d of incubation was surpassed by approximately 150 % of the size of their corresponding unprotected fraction, which brings into question the meaningfulness of this type of fractionation when carrying out long-term incubations and in the absence of a continuous fresh input of detritus, opposite to what occurs in field conditions. As already noted by Hirsch *et al.* (2009), if

the supply of OC to the soil is restricted, the contribution of soil OC to aggregation diminishes.

4.4.1.2 Fresh corn stover-amended soils

After 510 d, the amount of fresh corn stover decomposed in the TK and EG soils as estimated based on the isotopic ^{13}C composition of the CO_2 efflux, was 65.5 and 77.3 % respectively, while those from biochar amendments were <14 %. This illustrates the well-known concept that the uncharred precursor material is much less stable than the corresponding carbonised product. Moreover, it provides evidence of a greater decomposition of fresh corn stover in the EG soil than in the TK soil. The $\delta^{13}\text{C}$ obtained from evolved $\text{CO}_2\text{-C}$ at specific time periods suggests the existence of a dominant positive priming effect of fresh corn stover amendment on the decomposition of NOM in the TK soil, where the final cumulative positive priming was 12 %, whereas in the EG soil it was ~7 % (Figure 4.3c).

These results evidence that, in spite of the greater positive priming on NOM observed in the TK soil, the final gain of soil C in the TK soil was still greater than that in the EG soil, as the latter had a more active decomposition of the fresh corn stover. The different composition of NOM in these two soils may explain the different response observed (Figure 4.S1). The TK soil had an identifiable presence of ligno-cellulosic material (in the coarse free particulate material), as concluded from the dominance of methoxyphenols (guaiacols and syringols) and levoglucosan in the pyrolysates obtained by pyrolysis-GC/MS (Galletti & Reeves Iii, 1991). On the other hand, the pyrolysis fingerprints of the EG soil (in the same fraction above mentioned) were predominantly composed of markers of a recalcitrant aliphatic fraction (n-alkanes, n-alkenes and n-fatty acids) and secondary organic matter of microbial origin (furans, pyrroles and chitin markers) (Artur Stankiewicz *et al.*, 1998; Nierop, 1998). As such, the TK soil contains

largely primary, and theoretically easily degraded, organic matter while the EG soil contains larger amounts of degraded and recalcitrant components enriched through microbial activity. This is supported by the basal respiration of the Ctr soils which showed the presence of a greater microbial population in the EG soil than the TK soil, even though the former was less active on OC basis (6 and 13 g evolved C 100 g⁻¹ soil C, respectively). When a fresh C source was added, microbes that were inactive but “metabolically alert” (Dungait *et al.*, 2012), and probably present in greater numbers in the EG soil, were able to respond rapidly to fresh substrate as it became available, thus decomposing more added fresh C than the TK soil.

4.4.1.3 Soils amended with biochar

Total CO₂-C evolved from these carbonised materials were 8.1 and 7.5 % of original added OC biochar-C for the CS-350 and CS-550 incubated in the TK soil, and 13.0 and 13.8 % for the corresponding biochars in the EG soil. These values are similar to those reported by Whitman (2011) and Nguyen & Lehmann (2009) for biochars produced from corn stover, which lost 9.7 and 8.5 %, after an incubation of 3 y in sand (pyrolysed at 350 and 550 °C, respectively); and 21.0 and 11.0 % after an incubation of 1 y in sand (pyrolysed at 350 and 600 °C, respectively). Higher loss of biochar C from the EG soil than the TK soil can be explained by the (i) higher microbial population in the EG soil becoming metabolically active with the addition of the amendment (see previous section), and (ii) a greater pH buffering capacity in the EG soil (*e.g.*, with time the pH tended to decrease less in the EG soil than in the TK soil).

The lack of significant differences ($P < 0.05$) in the cumulative CO₂-C evolved among the two biochar types (Figure 4.3) – in spite of the different temperatures at which they were produced and their different lability as evidenced by the corresponding NMR spectra (Figure 4.1) and their H/C_{org} ratios (Table 4.2) – was unexpected. It should be

noted though that the estimated total amount of biochar-C evolved in biochar-amended soils was always below their labile C value, as estimated by dichromate oxidation (Table 4.2 and 4.6).

Total CO₂-C evolved from NOM at the end of the experiment, estimated from the isotope ratio of trapped carbonates, was 5.0 and 4.5 g C kg⁻¹ for both the TK-350 and TK-550 treatments, respectively, and 6.2 and 6.6 g C kg⁻¹ for the EG-350 and EG-550 treatments, respectively. Considering that the total CO₂-C evolved by the corresponding Ctr treatments was 5.3 and 6.3 g C kg⁻¹, the results suggest the clear existence of a negative priming effect of biochar on NOM decomposition of the TK soil (Figure 4.3c and d). This is in agreement with the findings of Zimmerman *et al.* (2011) and Cross and Sohi (2011) who found evidence for negative priming effect especially in soils with low SOM content. The negative priming effect of biochar on NOM decomposition was attributed to the sorption of NOM in pores and/or on to external surfaces of biochars (Kaiser & Guggenberger, 2000; Zimmerman *et al.*, 2011). The fact that biochars promoted the formation of (macro) aggregates in the TK soil further supports these results (Chapter 5).

Table 4.6: Amount of added C_{dichro} degraded and remaining after 510 d of soil incubation. The percentage values out of the total C content are given within parenthesis.

Treatment	Amended total C (g C kg ⁻¹ soil)	Amended C _{dichro} (g C kg ⁻¹ soil)	Degraded C _{dichro} (g C kg ⁻¹ soil)	Remaining C _{dichro} (g C kg ⁻¹ soil)
TK-CS	5.60	5.51 (98.4)	3.67 (65.5)	1.84 (32.9)
TK-350	5.60	1.94 (34.6)	0.45 (8.1)	1.49 (26.5)
TK-550	5.60	1.16 (20.7)	0.42 (7.5)	0.74 (13.2)
EG-CS	6.30	6.19 (98.3)	4.87 (77.3)	1.32 (21.0)
EG-350	6.30	2.18 (34.6)	0.82 (13.0)	1.36 (21.6)
EG-550	6.30	1.31 (20.8)	0.87 (13.8)	0.44 (7.0)

C_{dichro} = oxidisable C with dichromate solution

Degraded C_{dichro} = amount of C degraded based on the stable C isotope calculations of evolved CO₂-C

4.4.2 CO₂ evolution pattern

Carbon isotopic data evidenced a contribution of the CO₂-C evolved from amendments to the peaks observed in the first two weeks of 52 – 63 % for the corn stover and of 32 – 44 % for the biochars (Table 4.3). Other researchers have observed a peak in CO₂-C evolution within the first 1 – 3 wk of the experiment, and that was attributed to a stimulation of the microbial activity with the application of exogenous OM, including biochar, into soils (Hamer *et al.*, 2004; Zimmerman, 2010). Moreover, inorganic C present in minor quantities in the biochars (8.2 – 9.0 g CO₃-C kg⁻¹ biochar) could have contributed to this initial CO₂ evolution once added to these acidic soils. After 102 d – at the latter part of the spring and the beginning of summer in 2010 – the respiration rate showed an increase, irrespective of the treatment and the type of soil, and this increase was associated to a ~1.7 °C increase of temperature above the mean (~20 °C) (Figure 4.2). Additionally, an increasing pattern of CO₂-C evolution was also observed, towards the beginning of summer 2011, in the EG-350 and EG-550 treatments from the day 330 onwards and this change could be partly attributed to a temperature increase.

4.4.3 Predicted residence time

The predicted CO₂-C efflux rates from soils amended with fresh corn stover using Model 2 are represented in Figure 4.6 ($R^2 = 0.71$ and 0.57 , for the TK and EG soils, respectively). Variance in the observations was believed to be due to temperature fluctuations but could not be predicted more accurately by including a temperature correcting factor in the model (Figure 4.6). Thus, it is more likely that the variance in the observations is caused by opportunistic growth of soil microbes. The amount of C from fresh corn stover remaining at the end of the incubation as calculated using this model slightly overestimates the data obtained from isotopic analysis (or the latter underestimates it), as it predicts an ~89 % decomposition of added C after 510 d in both

soils and a half-life of 80 d. Model 1 estimated a half-life of fresh corn stover of 1.6 and 1.0 y for the TK and EG soil, respectively.

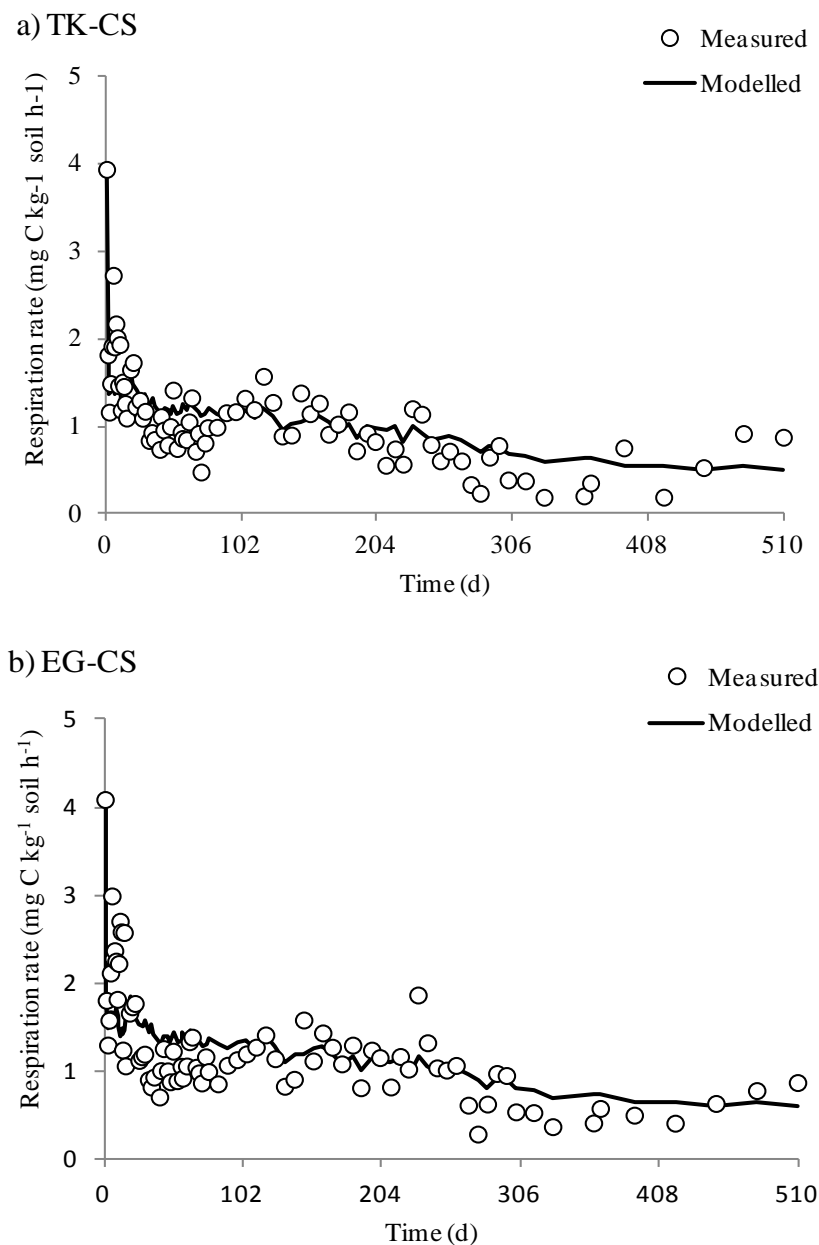


Figure 4.6: Measured and modeled respiration rates of corn stover amended (a) TK soil and (b) EG soil over 510 d.

Based on the Model 1, 55 – 59 % and 59 – 70 % of CS-350 and CS-550 biochar-C, respectively, would be remaining in these soils after 100 y under the present incubation conditions (Table 4.4). The power model considers that biochar loss rates are not

biphasic (Lehmann *et al.*, 2009) but vary along a continuum from more labile to extremely refractory carbon (Zimmerman, 2010). Estimated half-lives were 494 y for the CS-550 biochar incubated in the TK soil, and <200 y for the other biochar-soil combinations. Therefore, it is noteworthy that the residence time of biochar in soils thus depends not only on the inherent recalcitrance but also on the type of soil to which they are actually exposed.

4.4.4 C debt or credit ratio

When evaluating the net C sequestration of producing and adding biochar to soils, the C lost during pyrolysis should also be considered. This approach is essential if biochar is to be used as a C management strategy for climate change mitigation, since this baseline will be needed to quantify the impact of the production of biochar. In the present study, the C loss during the production of CS-350 and CS-550 biochars was 33 and 43 % of initial C, respectively, *i.e.* $(X - X_1)/X \cdot 100$ where X and X₁ depend on the pyrolysis temperature. When fresh corn stover was added to soil it mineralised 33 % of initial C by 157 and 101 d, and 43 % by 245 and 167 d in the TK and EG soils, respectively. The results obtained in this study indicate that in less than 35 weeks the CO₂ lost from biochar production “breaks even” with CO₂ lost from residue decomposition in these soils. From this point onwards the biochar system sequesters greater net C. The “break-even” point is reached earlier in the allophanic soil, in which the corn stover mineralises faster.

The proportion of feedstock C remaining in soil after 510 d was 34.5, 61.2, and 52.9 % for the fresh corn stover, CS-350 and CS-550 in the TK soil, respectively, and 22.7, 58.3 and 48.9 % in the EG soil, respectively (Figure 4.7). These percentages were used to calculate the “C debt or credit ratio” (Equation 9, Figure 4.7, based on Whitman *et al.*

(2012). Values were 1.8 and 1.5 for the TK-350 and TK-550 combinations, respectively, and 2.6 and 2.2 for the EG-350 and EG-550 combinations, respectively.

$$\text{C debt or credit ratio} = \text{Remaining biochar-C (\%)/Remaining feedstock-C (\%)} \quad (9)$$

When the same calculation was performed considering the net C gained, C_{Net} debt or credit ratio (Equation 10, Figure 4.7) – based on negative or positive priming effect of biochar on NOM-C decomposition during the incubation without plants – the new “C debt or credit ratio” values were 3.9 and 3.3 for the TK-350 and TK-550 combinations, respectively, and 3.8 and 3.0 for the EG-350 and EG-550 combinations, respectively.

$$C_{\text{Net}} \text{ debt or credit ratio} = \text{Remaining Net-C}_{\text{biochar}} (\%)/\text{Remaining Net-C}_{\text{feedstock}} (\%) \quad (10)$$

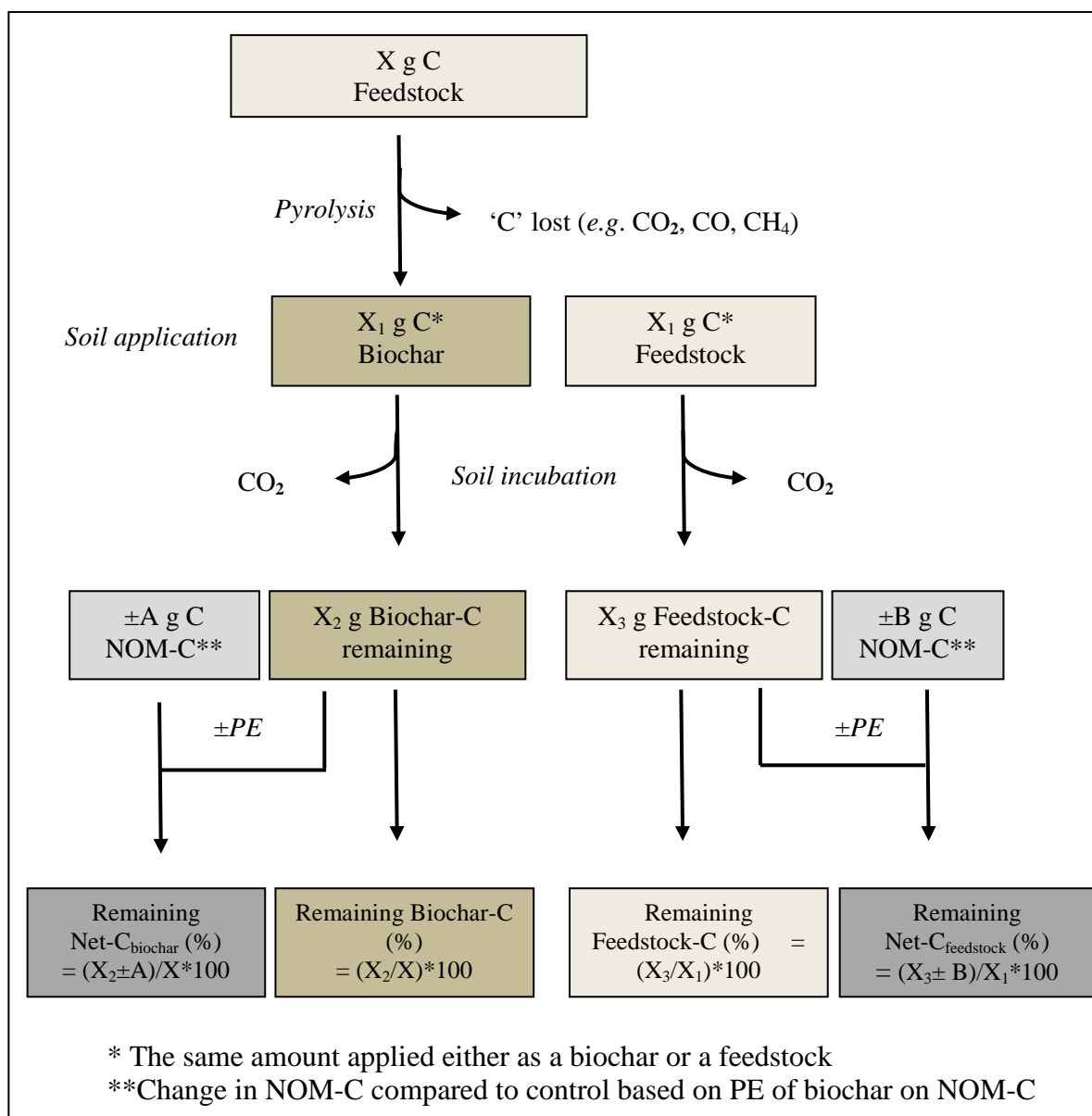


Figure 4.7: Procedure of calculation of “C debt or credit ratio” based on Whitman *et al.*, (2012) and C_{Net} debt or credit ratio”. PE stands for priming effect.

The “debt or credit” calculation for this 510 d incubation experiment shows that (i) the low temperature biochars saved more C, (ii) when only the decomposition of the amended residues was accounted for, the combination of low-temperature biochar and EG soil provided the greatest C saving of all treatments, and (iii) when the different priming effects on NOM were also considered, differences among the two soils were balanced.

4.5 Conclusions

After 510 d of incubation of corn stover feedstock and biochar in soils the following conclusions can be drawn about the decomposition rate of added material and their effect on NOM mineralisation. Corn stover biochar had a half-life in soils ranging from 130 – 494 y depending on pyrolysis temperature and soil type, compared to <2 y for the fresh corn stover. The added C, either fresh or carbonised, always had a slower decomposition in the Alfisol than in the Andisol. Only in the Alfisol, biochars caused a net negative priming effect on NOM decomposition, whereas fresh corn stover caused a net positive priming effect in both soils. In the Andisol, the priming effect on NOM caused by amended biochar C was more buffered, at least during the first 510 d of incubation. The different responses of these two soils were attributed to the different composition of NOM. Despite the fact that higher final pyrolysis temperature (550 vs 350 °C) produced more aromatic C, no differences were detected in the CO₂-C evolved from the two types of biochar after 510 d of incubation in either of the two soils studied. The effect of temperature of pyrolysis on priming NOM decomposition was soil-dependent. Overall pyrolysis temperature had only a small effect on net C sequestered when considering similar amounts of C added to soil, at least, during the 510 d-incubation. However differences became evident when the C lost during pyrolysis was taken into account, low temperature biochar being the one that sequestered more C. These results highlight the importance of (i) understanding biochar-soil interactions, and (ii) knowing the breakeven point of the C lost during pyrolysis with that of C decomposed when the fresh feedstock is added to the soil when establishing the C sequestration potential of the biochar technology.

4.6 Supportive information

4.6.1 Characterisation of feedstock and biochar

Total C, H, N and S of biochars and feedstock were determined using a TruSpec CHNS analyser (LECO Corp. St. Joseph, MI). The ash content was determined by the thermo gravimetric analysis (TGA) using a TA instrument (Alphatech, SDT Q600, TA Instruments, Melbourne, Australia) as described by Calvelo-Pereira *et al.* (2011). Oxygen content was estimated by $O = 100 - (C + H + N + S + \text{ash})$ (all wt %).

The effective cation exchange capacity (ECEC) of biochars at T0 was determined following the methodology of Matsue & Wada (1985). One gram of finely ground biochar was dissolved in 30 mL of 0.01M SrCl₂, and five washings were done with the same SrCl₂ solution, following consecutive centrifugations and shakings. After the last washing, 30 mL of 0.5M HCl was added, and the Sr concentration of supernatant was then determined with atomic absorption spectroscopy (AAS) after 24 h shaking followed by centrifugation.

The pH values of the biochars were determined in a suspension of a 1 % (wt/wt basis) biochar in deionised water heated in a water bath to 80 °C under stirring and cooled down before pH measurement (Ahmedna *et al.*, 1998). Biochar particle size distribution and BET surface area of these biochars was also determined, this information being provided in Chapter 3.

4.6.2 Chemical and spectroscopic characterisation of OC in the amendments

The fraction of OC in the amendments oxidisable by dichromate oxidation (C_{dichro}) was determined following the Walkley-Black method as modified by Calvelo Pereira *et al.* (2011) after Wolbach and Anders (1989) and Knicker *et al.* (2007). The organic

hydrolysable C fraction (hydro-C, non-hydro-C) was determined using 6M HCl acid following Silveira *et al.* (2008).

Solid state ^{13}C MAS NMR with cross polarisation (CP) was used to characterise the initial (T0) biochars (produced at 350 and 550 °C) and the biochars manually collected after 295 d (T295) and 510 d (T510) of incubation. The NMR analysis was conducted in a Bruker (Rheinstetten, Germany) DRX 200MHz horizontal bore magnet. Finely ground samples were packed into a 7 mm diameter cylindrical zirconia rotor with Kel-F end caps and spun at speeds of 5.0 ± 0.2 kHz in a dual resonance magnetic angle spinning (MAS) probe from Doty Scientific. During acquisition the sample temperature was maintained at 20 °C. Solid-state ^{13}C MAS NMR spectra were obtained at a ^{13}C frequency of 50.3 MHz on a Bruker (Rheinstetten, Germany) DRX 200 MHz spectrometer. Free induction decays FIDs were acquired with a sweep width of 30 KHz; 960 data points were collected over an acquisition time of 30 ms. The CP-MAS spectra were acquired with a ^1H 90° pulse for 5.5 μs , a cross-polarisation contact time of 1000 μs , an acquisition time of 30 ms, relaxation time of 2 s and 4 k scans. All spectra were zero filled to 4 k data points and processed with a 0.005 s Gaussian broadening. Chemical shifts were externally referenced with glycine.

Pt filament coil probe Py-GC-MS was performed with a Pyroprobe 5000 (Chemical Data Systems, Oxford, USA) coupled to a 6890N GC and 5975B MSD GC-MS system (Agilent Technologies, Palo Alto, USA). Finely ground samples (ca. 1 mg) of coarse fPOM at time zero (Chapter 5, Figure 5.1) were embedded in fire-polished quartz tubes that contained glass wool. The samples were pyrolysed at 750 °C for 10 s (10°C ms^{-1}). The interface and the GC inlet (splitless mode) were at 325 °C. The GC oven was heated from 50 to 320 °C (held 5 min) at $20^\circ\text{C min}^{-1}$. The GC-MS transfer line was at 325 °C, the ion source (electron ionisation mode, 70 eV) at 230 °C and the quadrupole

detector at 150 °C; scan range was m/z 45 – 500. The GC instrument was equipped with a (non-polar) HP-5MS 5 % phenyl, 95 % dimethylpolysiloxane column (ca. 30 m x 0.25 mm i.d.; film thickness 0.25 μm). He was the carrier gas (1 mL min^{-1}). Compounds were assigned using the NIST '05 library and Py–GC–MS literature (Galletti & Reeves *et al.*, 1991; Artur Stankiewicz *et al.*, 1998; Nierop, 1998).

4.6.3 Determination of $\delta^{13}\text{CO}_2$

Carbon isotope ratios of the trapped $\text{CO}_2\text{-C}$ were measured by reaction with phosphoric acid to form CO_2 gas, which was introduced into a stable isotope ratio mass spectrometer (IRMS). 100 μL of 1M NaOH trap solution was reacted with 200 μL of acid for 18 h at 25 ± 0.1 °C before sampling of the headspace in a 12 mL exetainer. Eight replicate 100 μL aliquots of CO_2 +helium headspace were injected into a Thermo Advantage IRMS (Thermo, Bremen) bracketed by pulses of CO_2 reference gas. Three standard carbonate materials were analysed at the beginning and end of each batch of 96 samples (LSVEC, lithium carbonate: -46.50 ‰; IRU-Bicarb, sodium bicarbonate: -5.03 ‰; IRU-Marble, calcium carbonate: 2.47 ‰); control materials were analysed at every 12th position. The results of the standard materials were used to correct the raw delta values (*vs.* ref gas) to the international scale (*vs.* VPDB) by 3-point calibration.

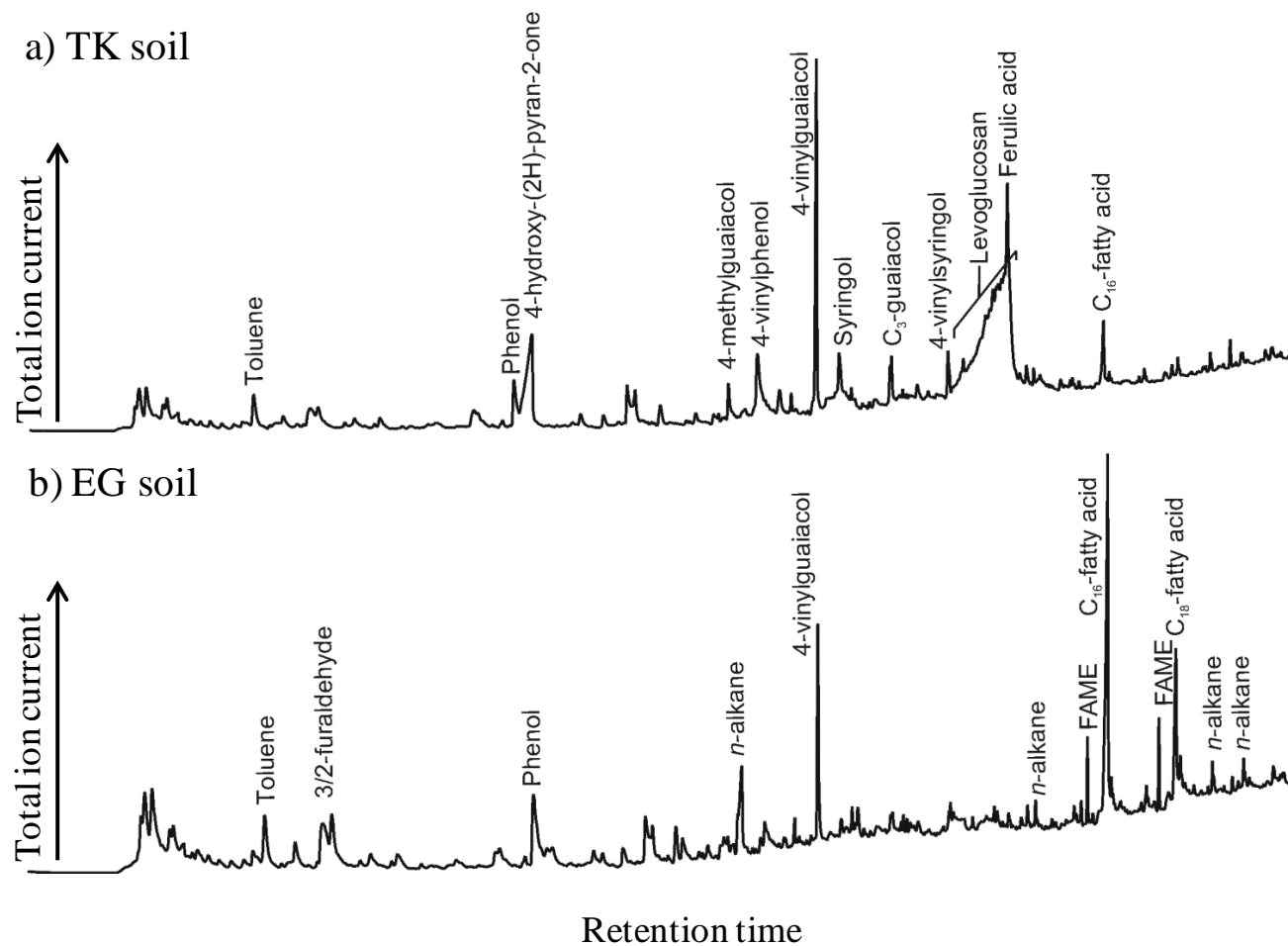


Figure 4.S1: Total ion current pyrograms of coarse free particulate organic matter (fPOM) samples (a) TK soil and (b) EG soil. FAME stands for fatty acid methyl ester.

CHAPTER 5: Fate of biochar in chemically- and physically-defined soil organic carbon pools

Abstract

The objective of this study was to elucidate the fate of biochar in physically- and chemically-defined fractions of organic carbon (OC) of two distinct soils after 510 d of laboratory incubation. Fresh corn stover and biochars produced from corn stover at 350 and 550 °C (CS-350 and CS-550) were incorporated in a Typic Fragiaqualf (Tokomaru soil; TK) and a Typic Hapludand (Egmont soil; EG) at a dose equivalent to 7.18 t C ha⁻¹. After 295 d, two undisturbed subsamples from each pot were taken: (i) in one subsample, lucerne (*Medicago sativa* L.) was seeded; (ii) in the other, the incubation was continued without plants. Soil samples at 0 (T0), 295 (T295) and 510 d (T510) were physically fractionated into coarse free particulate organic matter (fPOM), fine fPOM, silt+clay and heavy fractions, and analysed for $\delta^{13}\text{C}$ and total OC. Furthermore, the bulk soil and soil physical fractions were chemically fractionated into oxidisable and hydrolysable fractions using 0.17 M K₂Cr₂O₇ and 6 M HCl, respectively. After 295 d, $\delta^{13}\text{C}$ fractionation revealed that >74 % (TK soil) and >64 % (EG soil) of the biochar-C was recovered in the coarse fPOM fraction; 13 – 18 % (TK soil) and 21 – 30 % (EG soil) in the fine fPOM fraction, and minor amounts in the silt+clay fraction. The same pattern was observed after 510 d, both with and without plants, although a significant increase ($P < 0.05$) in the silt+clay fraction recovery of biochar C was observed, especially in the presence of plants. Most of the biochar particles thus concentrated into the so-called “unprotected pool”, which is generally considered as “labile C”. Treatments including the presence of both biochar and plants induced an additional accumulation of OC in the TK-350 and EG-550 soils ($P < 0.05$), compared with the treatments with plants but no biochar. A method to improve the physico-chemical fractionation of biochar-amended

soils is proposed in this study. The use of long-term incubations in studies of soil OM turnover (including biochar) where no inputs of fresh detritus are made is discouraged.

Key words

Biochar, corn stover, Alfisol, Andisol, physical fractionation, chemical fractionation, particulate organic matter (POM), microaggregates, silt and clay

5.1 Introduction

Soil organic carbon (OC) dynamics have implications at a global scale as changes in this pool influence the concentration of atmospheric CO₂. It has been estimated that, worldwide, the top 3 m of soil harbour 2,344 Pg OC (1 Pg = 10¹⁵ g) (Jiménez & Lal, 2006), the majority of which (~1,550 Pg) concentrated in the top 1 m (Batjes, 1996). The natural OC fluxes in terrestrial ecosystems involve a continuous input of OC to soils through the deposition of plant and animal residues of ~57 Pg C y⁻¹ and an output through the biological and abiotic decomposition of soil organic matter (OM) of ~55 Pg C y⁻¹ (Novotny *et al.*, 2009). Soil OM is defined as a complex mixture of fresh and partially decomposed plant and animal residues, including root exudates and soil biota (Totsche *et al.*, 2010; Stockmann *et al.*, 2013).

Biochar is charcoal aimed for soil amendment in order to improve soil functions and to reduce emissions from organic material that would otherwise rapidly degrade to greenhouse gases (GHG) (IBI, 2012). The C sequestration potential of biochar is mainly attributed to its recalcitrant nature, which is associated with the condensed aromatic structure of charcoal (Graetz & Skjemstad, 2003; Liang *et al.*, 2008). The intrinsic stability of biochar C is influenced by the type of feedstock used and pyrolysis conditions (Calvelo Pereira *et al.*, 2011). However, the efficiency of biochar as a C sequestration strategy also depends on its behaviour under the specific pedoclimatic conditions of the area in which it is deployed (Masiello, 2004), including soil climate and biochar-soil interactions. These interactions may involve (i) physical protection of biochar within soil aggregates (Brodowski *et al.*, 2005), (ii) chemical protection of biochar mostly through surface reactions (Joseph *et al.*, 2010), and (iii) the influence of native OM characteristics on biochar decomposition *i.e.* co-metabolic reactions (Hamer *et al.*, 2004; Hilscher *et al.*, 2009; Keith *et al.*, 2011; Zimmerman *et al.*, 2011).

On the other hand, the presence of biochar may affect the decomposition of native OM as well. The chemical changes that the addition of biochar causes in soil (*e.g.*, addition of nutrients, liming effect) may enhance microbial activity (Lehmann *et al.*, 2011) thus causing a positive priming effect, although a recent review where a modelling approach was used to quantify this priming effect suggests that enhanced decomposition of native OM is not a significant drawback in biochar's potential for C sequestration (Woolf & Lehmann, 2012). In fact, this effect is probably negligible in comparison with its negative priming effect on native OM decomposition (Woolf & Lehmann, 2012), which has been attributed to (i) the sorption of native OM on biochar surfaces (Kwon & Pignatello, 2005); (ii) improved aggregation and resultant entrapment of native OM (Brodowski *et al.*, 2005); (iii) deactivation of microbial enzymes at the surface of biochar particles; and (iv) toxicity of biochar to soil microorganisms (Zimmerman *et al.*, 2011).

The proposed mechanisms behind biochar-mineral-OM interactions and how they affect soil C dynamics in biochar-amended soils have not yet been fully experimentally validated. One approach to their study is through physical and chemical fractionation of biochar-amended soils before and after a long-term incubation (>1 y). Ideally, this incubation would involve a system including living roots, since aggregation is dependent on the supply and turnover of soil OM by microorganisms (Watts *et al.*, 2001; Hirsch *et al.*, 2009; Dungait *et al.*, 2012) and root exudates may create rhizosphere-associated microbial hotspots for a positive priming effect (Kuzyakov, 2010), although it may also induce non-specific hydrophobic aggregation (Buurman *et al.*, 2002; Bachmann *et al.*, 2008).

The main objective of the present study was to determine the fate of biochar in different physically- and chemically-defined soil OC pools after a long-term (>1 y) laboratory

incubation in two soil types (Typic Fragiaqualf/Alfisol and Typic Hapludand/Andisol) in the presence or absence of plants. Four physico-chemically defined OC pools, aimed to reflect different conditions with respect to the protective mechanisms against microbial decomposition, were targeted: (i) non-protected pool (Six *et al.*, 2002); (ii) micro-aggregate protected pool (Six *et al.*, 2002); (iii) silt+clay protected pool (Hassink, 1996; Hassink *et al.*, 1997); and (iv) biochemically protected pool (Baldock & Skjemstad, 2000) (by means of HCl hydrolysis). An additional chemical fractionation to the physically-defined OC fractions and the bulk soils was obtained on the basis of oxidisability (in K-dichromate solution).

5.2 Materials and methods

5.2.1 Biochar production and characteristics

Biochar from corn stover (*Zea mays* L.) was produced at the highest heating temperature of 350 °C (CS-350) or 550 °C (CS-550) (pyrolysis conditions described in Chapter 3). Selected properties of the feedstock and biochars (Chapter 3) are given in Table 1. The biochar particles were mostly >0.25 mm in size (88 % of CS-350 and 91 % of CS-550) (Table 5.S1).

Pyrolysis-GC/MS was applied to the CS-350 and CS-550 biochars in order to obtain information on their molecular properties, using a CDS Pyroprobe 5000, connected to a Agilent 6890 GC and 5975B MSD. Pyrolysis was performed at 750 °C for 10 s, which is a method considered suitable for black C species including biochar (Kaal *et al.*, 2009b). For details on GC/MS conditions please refer to Calvelo-Pereira *et al.* (2011). The pyrolysis fingerprint of the CS-350 biochar was dominated by phenol and alkylphenols (methyl-, dimethyl- and ethylphenols), in addition to traces of incompletely demethoxylated lignin moieties (reflected by guaiacols and syringol) and markers of strongly charred material such as naphthalene (data not shown). Also, traces

of charred polysaccharides were detected (furans). The CS-550 biochar produced mainly benzene, toluene and polycyclic aromatic hydrocarbons (PAHs) upon pyrolysis, from strongly charred moieties. Also, some short-chain aliphatic structures (C₁₀-C₁₆ alkanes) were detected. From comparison with previously published studies on the relation between charring temperature and molecular composition (Keiluweit *et al.*, 2010; Kaal *et al.*, 2012), it is concluded that the CS-350 is a moderately charred biochar (“transition char”) while the CS-550 biochar is strongly charred material (“amorphous char” and higher). Considering the production temperatures, these results are not considered surprising.

5.2.2 Soil material preparation and incubation experiment

A Typic Fragiaqualf (Tokomaru silt loam, TK soil) and a Typic Hapludand (Egmont black loam, EG soil) (Soil Survey Staff, 2006) were used in this study. The Alfisol (TK soil) is developed on loess (Rongotea, New Zealand) (40°18' S, 175°23' E, 24 m above sea level), and is rich in silt and clay, the latter being dominated by 2:1 clay minerals. The Andisol (EG soil) is developed on andesitic volcanic ashes of Oakura and Okato tephra (Wanganui-Hawera, New Zealand) (39°37' S, 174°21' E, 66 m above sea level), and the clay fraction is dominated by allophane. Both soils have been under pasture production for at least 50 y (Roberts & Thompson, 1984). The climate is similar at both sites with a mean annual soil temperature of 12 °C and a mean annual rainfall of 1000 to 1300 mm (Baisden *et al.*, 2010). Chemical properties of the two soils are given in Table 5.2. Undisturbed soils were sampled to 100 mm depth using cores of 150 mm in diameter. The soils were passed through a 5 mm sieve and stored in plastic bags under cold conditions (4 °C) prior to incubation.

The treatments considered in this study were: (i) control treatments (TK-Ctr, EG-Ctr), (ii) fresh corn stover (TK-CS, EG-CS), (iii) low-temperature biochar (TK-350, EG-350), and (iv) high-temperature biochar (TK-550, EG-550).

Each organic amendment was added at a dose equivalent to 7.18 t C ha^{-1} down to 100 mm depth. Due to the differences in bulk density of these two soils, the amount added per unit of mass was different: 5.6 and 6.3 g C kg^{-1} soil for TK and EG soils, respectively. The experiment was conducted at 70 % water holding capacity and the moisture content was maintained by weighing the pots once in every two days. More details are provided in Chapter 4. The incubation experiment was set up inside the laboratory where the average daily room temperature was recorded at $\sim 20 \text{ }^{\circ}\text{C}$ ($\pm 6 \text{ }^{\circ}\text{C}$).

After 295 d, two undisturbed subsamples from each pot were taken: (i) in one subsample, lucerne (*Medicago sativa* L.) was seeded; (ii) in the other, the incubation continued without plants to study bacterial respiration alone. Eight seeds were planted and, after 2 weeks, thinned to leave 4 seedlings per pot, at which stage the pots were transferred to a glasshouse. Plants were provided with a standard nutrient solution without N during the first three months. Plants were watered to maintain the moisture level similar to the $\sim 70 \text{ } \%$ water holding capacity, as above. The experiment lasted 510 d in which plants were grown for 215 d. The respiratory and plant studies after 510 d are denoted as T510 (R) and T510 (P), respectively.

Table 5.1: Elemental analysis of feedstock and biochars.

Sample	Chemical composition (%)						Atomic ratio (d.a.f.) ^b		Recovery (%)			Recover rate (%)	$\delta^{13}\text{C}$
	C	N	H	O ^a	S	Ash	(H _{org} /C _{org})	(O/C _{org})	C	N	S		
Fresh corn stover	41.4	0.83	6.08	40.66	0.13	10.9	1.98	0.74	n.a.	n.a.	n.a.	n.a.	-12.47
CS-350	63.5	0.71	3.77	21.62	0.44	9.8	0.64	0.26	67.0	28.6	120.9	35.0	-13.13
CS-550	71.8	0.76	2.92	13.55	0.12	11.5	0.45	0.14	56.6	21.0	25.0	27.0	-13.38

Table 5.2: Basic chemical properties of Tokomaru and Egmont soils.

Soil	pH (H ₂ O)	OC (g kg ⁻¹)	Dichro-C (g kg ⁻¹)	Hydro-C (g kg ⁻¹)	Ava. N (mg kg ⁻¹)	Olsen P (µg g ⁻¹)	Ext. Al (CaCl ₂) (µg g ⁻¹)	ECEC (meq 100 g ⁻¹)	δ ¹³ C (‰)	Main mineral types
Alfisol	5.7	41.7	34.0	22.3	256	45	2.6	17	-28.80	Micaceous clay minerals
Andisol	5.8	102.0	72.3	39.8	380	34	2.7	29	-27.60	Allophane, kaolinite, gibbsite

Ava. = available, Ext. = extractable (with CaCl₂)

5.2.3 Soil organic matter chemical fractionation

5.2.3.1 Chemical fractionation with 0.17 M $K_2Cr_2O_7$

The OC of the bulk soil and of the free particulate organic matter (fPOM) – both coarse fPOM and fine fPOM – and silt+clay fractions as depicted in Figure 5.1 was chemically oxidised with dichromate oxidation (OC_{ox}) following the Walkley-Black method as modified by Calvelo-Pereira *et al.* (2011), after Wolbach and Anders (1989) and Knicker *et al.* (2007). Briefly, a 250 mg of finely ground soils (<500 μm) was used; 10 mL of 0.17 M $K_2Cr_2O_7$ and 20 mL of concentrated H_2SO_4 were added as the oxidising agents and the system was then placed in a digestion unit at 60 °C for 6 h allowing oxidation. After oxidation, 200 mL of water followed by 10 mL of phosphoric acid were added to the flask. Titration was subsequently continued with 0.5 M ferrous ammonium sulphate.

5.2.3.2 Chemical fractionation with 6 M HCl

The OC of the bulk soil and the silt+clay fractions, as depicted in Figure 5.1, were also further fractionated to quantify the non-hydrolysable C fraction (OC_{nhl}) and the hydrolysable fraction (OC_{hl}). Acid hydrolysis was undertaken with 6 M HCl according to Silveira *et al.* (2008). In brief, 1 g of each sample was digested for 24 h in a digestion unit at 105 °C after adding 25 mL 6 M HCl. The system was continuously refluxed, covering the tubes with glass funnels. The samples were finally filtered and dried at 60 °C.

5.2.4 Soil organic matter physical fractionation

Subsamples from each pot, taken at T0, T295 and T510 were used for physical fractionation following the scheme presented in Figure 5.1. Physical fractionation was initiated by wet sieving using a microaggregate isolator unit, which helped separate the coarse free POM (fPOM), microaggregates and the silt+clay sized (<53 μm) fractions

from air-dried 2 mm-sieved soil (Six *et al.*, 2000). This procedure breaks macroaggregates while preserving the microaggregates, which were subsequently separated (Warkentin & Maeda, 1980). After wet sieving, the light fraction, also known as fine fPOM, was isolated from microaggregates by density flotation using 1.85 Mg m⁻³ sodium polytungstate solution (Sometu Europe, Germany) (Six *et al.*, 1998). The final step of the physical fractionation involved the dispersion of the heavy fraction (HF) by shaking for 18 h on a reciprocal shaker in 0.5 % Na-hexametaphosphate (SHMP) solution.

The dispersed material was then passed through a 53 µm sieve to isolate microaggregate protected (intra-aggregate) POM (iPOM) from silt+clay associated OC. In the present study, only the fractions marked in grey in Figure 5.1 are described here. Most of the biochar was recovered in the free POM fractions (fine fPOM + coarse fPOM) and the free silt+clay associated fraction taken after wet sieving. For this reason and to facilitate the flow of the text, the sum of iPOM and silt+clay associated OC is referred together and that corresponds to the above-mentioned HF. The fraction denoted in the text as silt+clay refers solely to the fraction of silt+clay (grey box) obtained after wet sieving (Figure 5.1). The resultant fractions corresponding to T0 (*e.g.*, prior to the addition of the organic amendment) were, following pre-treatment by mild aqueous hydrofluoric acid (2 %) to concentrate organic materials (Zegouagh *et al.*, 2004), analysed by pyrolysis-GC/MS in order to understand differences in molecular composition and potentially the divergent behaviour of these fractions during incubation, with pyrolysis at 750 °C for 10 s, as described above.

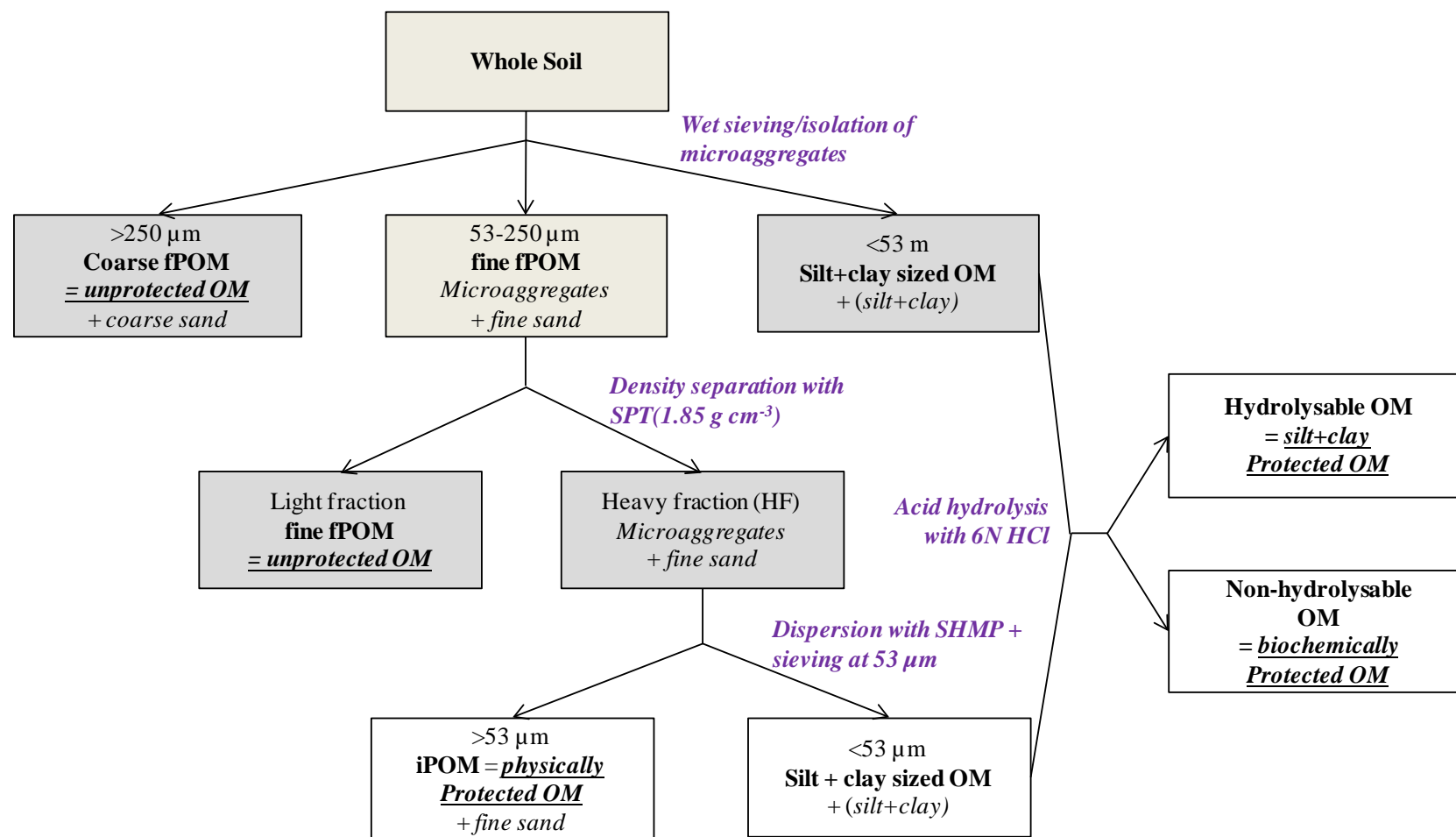


Figure 5.1: Schematic representation of physical fractionation procedure used to separate free POM, iPOM, and silt+clay sized OM according to the four isolated conceptual SOM pools as given by Six *et al.* (2002). In this study, only the fractions marked in grey are discussed. SPT and SHMP stand for sodium polytungstate and sodium hexameta phosphare, respectively.

5.2.4.1 Concentration and isotope ratio of soil organic carbon

Total C was equivalent to the total OC content as no inorganic C was present in these soils. Total OC was determined in the bulk soil and each of the fractions using a CNS analyser (LECO FP- 2000 CNS Analyser; Corp. St. Joseph, MI). The $\delta^{13}\text{C}$ of the fresh corn stover and the two biochar types at T0, as well as those of the bulk soil and the different fractions at T0, T295 and T510 were measured using a Carlo Erba NA1500 elemental analyser connected to a Thermo Delta Plus Advantage isotope ratio mass spectrometer at Iso-trace Research (University of Otago, New Zealand). Before measurements, the samples were finely ground and loaded into tin capsules. For all isotopic analyses, the recommended scale used was VPDB (Vienna Pee Dee Belemnite, absolute isotope ratio $(11237 \pm 60 \cdot 10^{-6})$) and the units are given as per mill (‰).

The fraction of OC in the bulk soil and in the different physical fractions corresponding to either the organic amendment (f_{OA}) or the native soil OC (f_{SOC}) was calculated according to a two-component isotopic mixing model as given below:

$$\delta^{13}\text{C}_{\text{measured}} = f_{\text{OA}} * \delta^{13}\text{C}_{\text{OA}} + (1 - f_{\text{OA}}) * \delta^{13}\text{C}_{\text{SOC}} \quad (1)$$

$$f_{\text{OA}} + f_{\text{SOC}} = 1 \quad (2)$$

There was a small effect of slow pyrolysis on $\delta^{13}\text{C}$, with the $\delta^{13}\text{C}$ becoming more negative by ca. 1 ‰ after pyrolysis (Table 5.1).

5.2.5 Statistical analysis

Results are expressed as an average of three replicates with standard deviation if not otherwise stated. The statistical differences between the applied treatments were determined by analysis of variance (ANOVA) using SPSS software with version 16.0 (SPSS Inc., Chicago, USA). Post hoc analyses were computed using Duncan test at $P = 0.05$.

5.3 Results

5.3.1 Total soil OC content

Without organic amendments, the Alfisol and Andisol studied had OC contents of 42 and 102 g kg⁻¹ soil, respectively (Table 5.2). After 510 d of incubation without plants, the OC contents of TK-Ctr, TK-CS, TK-350 and TK-550 were 36, 38, 41, and 41 g C kg⁻¹ soil, respectively, and the corresponding OC contents of the EG soil 93, 94, 99, and 100 g C kg⁻¹ soil (Table 5.3). At T510, there were no significant differences ($P < 0.05$, Duncan test) between the uncharred CS-amended treatment and the control of both soils. Both low- and high-temperature biochar-amended soils had significantly ($P < 0.05$) higher total OC content than the corresponding control soils (Table 5.3), but no differences were observed between the two biochars.

In the presence of plants, after 510 d total OC content of all treatments were larger ($P < 0.05$) than those of the corresponding treatments without plants, except for TK-Ctr. For a given soil, differences among treatments in the presence of plants followed a similar trend to those observed in their absence, except for the TK soil amended with uncharred CS; this treatment did not show significant differences ($P < 0.05$) with the control and biochar-amended soils (Table 5.3). It should be noted that, for a specific soil type, no significant differences ($P < 0.05$) in above-ground biomass and root biomass were detected among treatments (Table 5.S2).

Table 5.3: Total OC (g C kg⁻¹ soil) and the $\delta^{13}\text{C}$ of whole soil determined after 295 and 510 d (with and without plants). Minimum significant (alpha = 0.05) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments in each soil.

Treatment	T295 (R)		T510 (R)		T510 (P)	
	Total OC (g C kg ⁻¹ soil)	$\delta^{13}\text{C}$ (‰)	Total OC (g C kg ⁻¹ soil)	$\delta^{13}\text{C}$ (‰)	Total OC (g C kg ⁻¹ soil)	$\delta^{13}\text{C}$ (‰)
TK-Ctr	38.1a	-28.87a	36.3a	-29.00a	36.9a	-28.76a
TK-CS	41.5b	-27.72a	37.8a	-28.32a	41.3ab	-27.94b
TK-350	43.5c	-26.39b	41.1b	-26.19b	45.4b	-26.32c
TK-550	42.7c	-26.50b	40.8b	-26.67b	42.5b	-26.26c
EG-Ctr	96.5a	-27.57a	93.4a	-27.43a	98.3a	-27.46a
EG-CS	97.5a	-26.83b	93.6a	-26.98b	96.7a	-26.95b
EG-350	101.1b	-26.33b	98.9b	-26.29b	105.2b	-26.44c
EG-550	102.7c	-26.36b	100.0b	-26.26b	107.0b	-26.21c

5.3.2 Soil OC distribution in the oxidisable vs. non-oxidisable OC fractions

Total soil OC gain/loss balances (ΔOC) and differences in oxidisable OC content between treatments and corresponding controls ($\Delta\text{OC}_{\text{ox}}$), without physical fractionation, are presented in Figure 5.2. Values at T0 correspond to the amendments, *i.e.* 5.6 g kg⁻¹ soil by addition of uncharred CS or biochars to soil TK and 6.3 g kg⁻¹ in soil EG. Uncharred CS at T0 was almost completely oxidised by dichromate (*e.g.*, 5.5 g kg⁻¹ out of 5.6 g kg⁻¹ in respect to the TK soil; Figure 5.2). In the absence of plants, both ΔOC and $\Delta\text{OC}_{\text{ox}}$ in soils amended with uncharred CS sharply decreased during incubation, particularly in the EG soil.

Thirty four percent (34 %) of the CS-350 biochar-C was dichromate-oxidisable (Figure 5.2). In the TK soil, the $\Delta\text{OC}_{\text{ox}}$ of the TK-350 treatment became negative after 295 d of incubation and followed this negative trend as incubation time increased. The presence of lucerne plant produced an overall increase in total ΔOC and $\Delta\text{OC}_{\text{ox}}$ in the TK-350 treatment as compared with the parallel treatment in the absence of plants [T510 (P) – T510 (R)] of 3.8 and 5.6 g kg⁻¹, respectively. The CS-550 biochar-C – which had an

original C_{ox} value of 21 % (Figure 5.2e) – created a similar pattern of OC changes as when CS-350 biochar was added to the TK soil. In the EG soil amended with either CS-350 or CS-550 biochar, the ΔOC and ΔOC_{ox} decreased with time, although less accentuated than in the TK soil with values remaining positive (Figure 5.2b). Again, as in the TK soil, the presence of plants in biochar-treated soils caused an increase in both total OC (4.3 and 1.7 g kg⁻¹ for the CS-350 and CS-550 amendment, respectively) and ΔOC_{ox} (1.8 and 2.7 g kg⁻¹ for the CS-350 and CS-550 amendment, respectively).

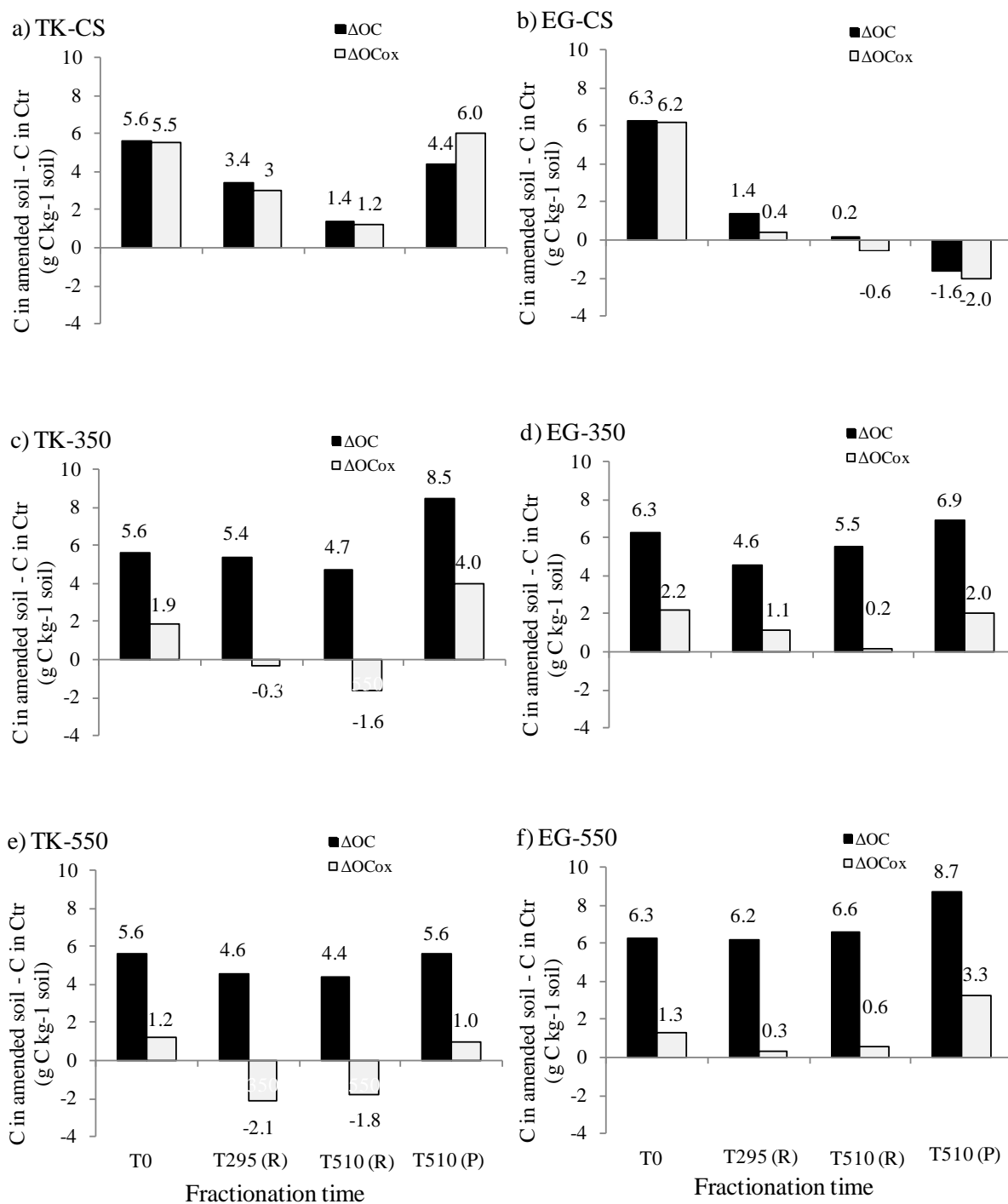


Figure 5.2: Change in soil OC and oxidisable OC contents calculated at different sampling times by subtracting the respective soil OC and oxidisable OC contents of the corresponding Ctr treatment at each sampling time, for (a) the TK-CS treatment, (b) EG-CS treatment, (c) TK-350 treatment, (d) EG-350 treatment, (e) TK-550 treatment, and (f) EG-550 treatment.

5.3.3 Soil OC distribution in physical fractions

The distribution of OC in different physical fractions of the control TK soil at time 0 followed the order: HF (53 %) >> silt+clay (23 %) ~ coarse fPOM (20 %) > fine fPOM (4 %). The HF fraction consisted of roughly 2/3 silt+clay obtained by dispersion and 1/3 % iPOM. The pyrolysis fingerprint (Figure 5.3) of HF (silt+clay from dispersion) was dominated by *n*-alkanes and *n*-alkenes, accompanied by 4-methylguaiacol, 4-vinylphenol, some polysaccharide products (but not levoglucosan), toluene, alkylphenols and pyrrols (including diketodipyrrole), which is indicative of a mixture of recalcitrant and/or root-derived aliphatics (waxes, cutin, suberin, *etc.*) and degraded lignin and non-lignin phenolics, with little intact or fresh OM. The HF (iPOM) fraction, on the other hand, gave a pyrolysis fingerprint typical of less degraded OM, with major peaks of markers of polysaccharides (*e.g.* levoglucosenone, 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one and levoglucosan) and non-lignin phenolics (4-vinylphenol and 4-vinylguaiacol), with in addition a substantial aliphatic fraction (*n*-alkane/*n*-alkene pairs and C₁₆- and C₁₈-fatty acid), which indicates that this material is a mixture of relatively intact biomass and more recalcitrant aliphatic material, the latter being dominant. The silt+clay fraction (after sieving) was similar to that of HF (silt+clay). Coarse fPOM, representing ca. 20 % of TOC, consisted of relatively intact lignocellulosic material (abundant C₃-guaiacols and -syringols from lignin and levoglucosan from cellulose), while the negligible fine fPOM fraction seems slightly more decomposed (toluene, phenol, alkylphenols) and has a higher aliphatic content. Chlorophyll can be detected in this sample as well (phytadienes). In summary, the more intact OM components, reflected by high polysaccharide content, are concentrated in the fPOM fractions while aliphatic and degraded/microbial material is enriched in the silt+clay and especially HF fractions. This can be explained by a combination of (i) selective depletion of labile OM

recovered in the coarse fPOM fraction, with minor amounts in the silt+clay and fine fPOM fractions (estimates based on isotope analysis; Table 5.S3). The native OM present in the coarse fPOM fraction of the TK-CS treatment – estimated based on the $\delta^{13}\text{C}$ – decreased with time and to a greater extent than that of the TK-Ctr treatment (77 vs. 64 % decrease after 510 d, respectively). In fact, at the end of the incubation without plants, the coarse fPOM fraction in the TK-CS treatment was as low as in the TK-Ctr treatment (Figure 5.4a), in spite of having added uncharred CS at T0. The silt+clay-associated OC content also significantly decreased ($P<0.05$) with time. No significant changes ($P<0.05$) were detected either in the fine fPOM or the HF fractions during the incubation period studied. In the presence of plants, only the silt+clay fraction increased significantly ($P<0.05$), while no changes were observed in the rest of fractions.

When sampling the TK soil amended with the CS-350 biochar after 295 d of incubation, the largest amount of biochar-C was recovered in the coarse fPOM, followed by the fine fPOM, with minor amounts detected in the silt+clay fraction (Figure 5.4c). Again, native OC in the coarse fPOM fraction decreased with time and to a greater extent than in the TK-Ctr treatment (Figure 5.4a) (76 vs. 64 % decrease, respectively, after 510 d). The native OC of the rest of fractions (fPOM, silt+clay, HF) followed a similar trend to that of the TK-Ctr treatment (Figure 5.4a). In the presence of plants, all other fractions increased significantly ($P<0.05$), except for fine fPOM. Isotope ratios suggested that, at the end of the experiment (in the absence and presence of plants), the distribution of biochar-C within the different particle-size fractions of the TK soil was as follows: 67 – 80 % $>250\ \mu\text{m}$, 13 – 24 % between $53 - 250\ \mu\text{m}$ and 6 – 11 % $<53\ \mu\text{m}$ (Table 5.S1), which is indicative of physical disintegration of biochar particles. There were hardly any differences in the fractionation of OM among the two types of biochars (CS-350 and CS-550) (Figure 5.4d), although in the presence of plants the increase in OC was

more accentuated in the CS-350, in agreement with the greater overall increase in ΔOC detected in this soil (Figure 5.2).

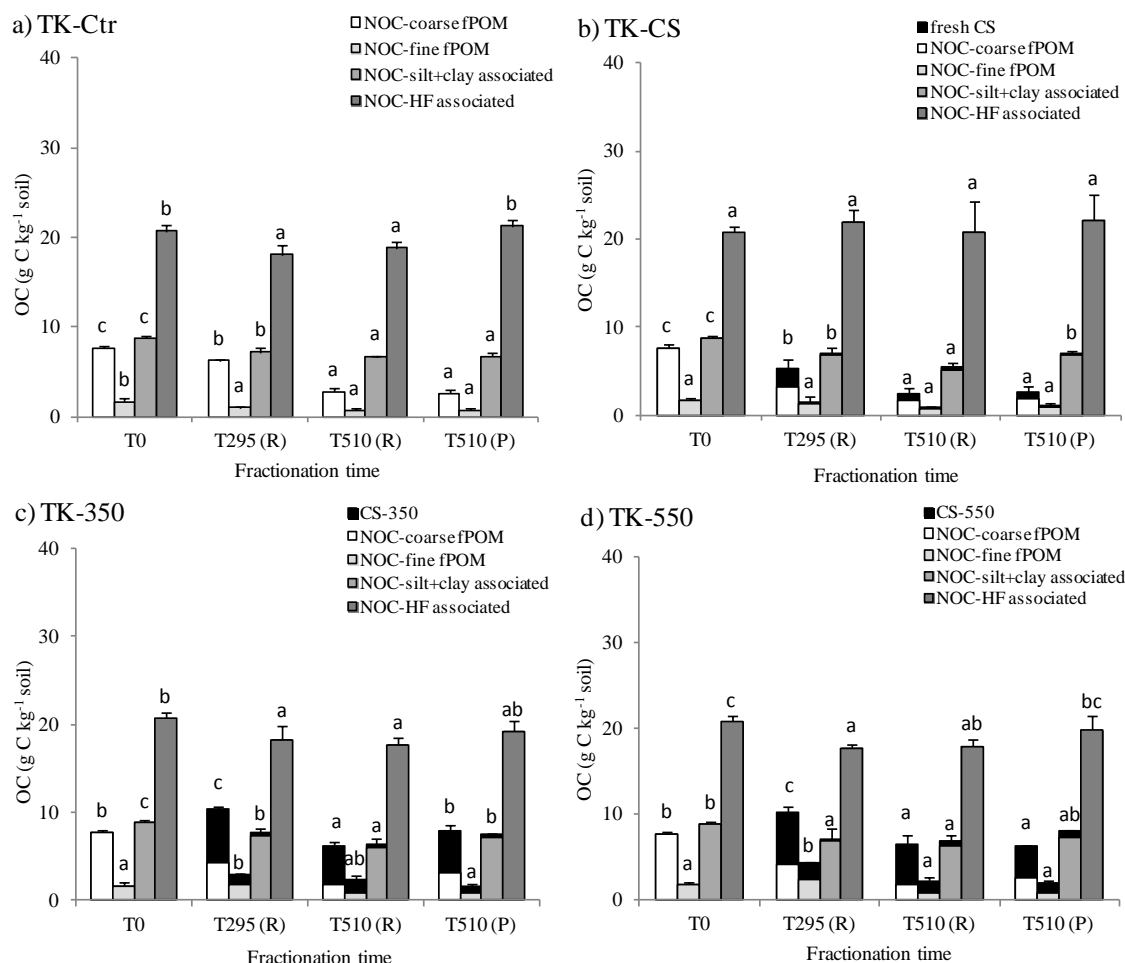


Figure 5.4: Distribution of native organic carbon (NOC) (g C kg⁻¹ soil) and added C in coarse fPOM, fine fPOM, silt+clay sized fraction and HF obtained from (a) control (b) corn stover amended (c) CS-350 amended and (d) CS-550 amended TK soils at T0, T295 (R), T510 (R), and T510(P). Minimum significant (alpha = 0.05) differences between any two means are based on the Duncan post hoc test and different letters denote the significant differences between treatments for the same fraction in each soil.

The distribution of OC in the different fractions of the EG soil at T0 followed the order HF (ca. 55 %) >> fine fPOM (ca. 21 %) ~silt+clay (ca. 20 %) > coarse fPOM (ca. 5 %) (Figure 5.5a). The pyrolysis fingerprint of the coarse fPOM fraction shows evidence of a probably grass-derived intact (poly) phenolic fraction (hydroxybenzoic acid, 4-vinylguaiacol) and a pyrolysis product of an intact aliphatic constituent (m/z 57, 69, 70), in addition to phenols, polysaccharide products (furaldehydes), fatty acids and

analogous fatty acid methyl and propyl esters. The fine fPOM is characterised by more degraded OM, both in comparison with coarse fPOM in soil EG and with the fine fPOM in soil TK, with major contributions of microbial biomass (including chitin markers) and *n*-alkanes/*n*-alkenes from selectively preserved aliphatics. The silt+clay fraction produced a distorted pyrogram dominated by microbial and aliphatic constituents. The pyrolysis fingerprints of both components of the HF fraction (silt+clay after dispersion, 69 % of HF, and the iPOM fraction) were strongly dominated by *n*-alkanes/*n*-alkenes, in combination with markers of degraded and microbial OM. In conclusion, analogous to soil TK, the fPOM fractions contain less degraded OM than the other fractions. The OM in soil EG was generally more degraded than that of soil TK.

After incubating the control EG soil for 295 d, the only significant decrease ($P < 0.05$) detected was in the coarse fPOM fraction which is the sample with the largest proportion of intact OM. When the incubation was extended to 510 d, a sharp OC content drop was observed in the fine fPOM ($\sim 13.9 \text{ g C kg}^{-1}$ soil) while a concomitant increase was observed in the HF fractions ($\sim 11.5 \text{ g C kg}^{-1}$ soil). In the presence of plants, this pattern was less accentuated. The silt+clay-associated OC content did not significantly change ($P < 0.05$) over time either in the presence or the absence of plants (Figure 5.5).

When fresh CS was added to the EG soil and incubated for 295 d without plants (Figure 5.5b), most of the amended OC was recovered in the coarse fPOM fraction, followed by the fine fPOM fraction, with minor amounts in the silt+clay fraction (Table 5.S1). Patterns of the different fractions during the incubation with either plants or without plants were similar to those observed for the EG-Ctr treatment (Figure 5.5a).

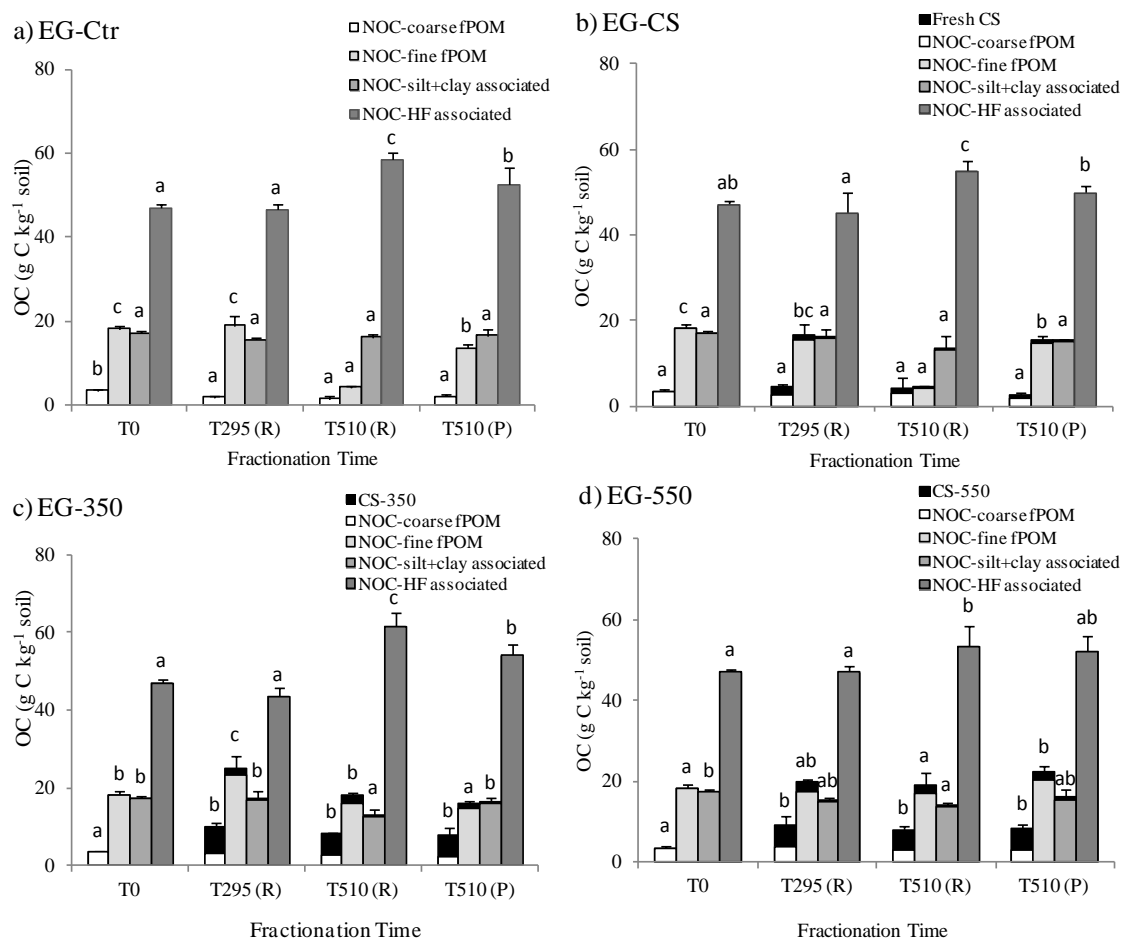


Figure 5.5: Distribution of native organic carbon (NOC) (g C kg^{-1} soil) and added C in coarse fPOM, fine fPOM, silt+clay sized fraction and HF obtained from (a) control (b) corn stover amended (c) CS-350 amended and (d) CS-550 amended EG soils at T0, T295 (R), T510 (R), and T510(P). Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments for the same fraction in each soil.

When sampling the EG-350 treatment after 295 d of incubation, most of the biochar fraction was recovered in the coarse fPOM, followed by the fine fPOM, while minor amounts were detected in the silt+clay fraction (Figure 5.5c). Moreover, there was a significant increase ($P < 0.05$) in the native OC recovered in the fine fPOM fraction compared to T0 and a concomitant decrease in that recovered in the HF fraction, although this was not significant at $P < 0.05$ (Figure 5.5c). It should be noted that the recovery of the different fractions of the EG soil was the lowest at T295 (Table 5.S4) and this could have influenced the differences in the HF fraction among sampling times.

As the incubation proceeded without plants for 510 d, there was a significant decrease ($P < 0.05$) in the native OC fraction of the fine fPOM ($\sim 2.1 \text{ g C kg}^{-1}$ soil) and silt+clay (4.5 g C kg^{-1} soil), whereas there was a parallel increase in the HF fraction ($\sim 14.4 \text{ g C kg}^{-1}$ soil), although the above-mentioned problems in the mass recovery at T295 could have contributed to these differences. In the presence of plants, the fine fPOM and HF fractions significantly decreased ($P < 0.05$) (Figure 5.5c). When amending the EG soil with CS-550, the same patterns as with the CS-350 biochar were observed (Figure 5.5d), except for the fact that (i) at T295 there was no significant increase ($P < 0.05$) in the native OC of the fine fPOM fraction, and (ii) at T510 and in the presence of plants, an increase in the native OC of the fine fPOM fraction was observed. The latter is in agreement with the greater overall increase in ΔOC detected in this soil (Figure 5.2). Isotope ratios suggested that, at the end of the incubation (in the absence and presence of plants), the distribution of biochar-C within the different particle-size fractions was as follows: 67 – 72 % $>250 \mu\text{m}$, 18 – 28 % between 53 – 250 μm and 4 – 10 % $<53 \mu\text{m}$ (Table 5.S1).

5.3.4 Distribution of OC pools based on the Six's fractionation method

The percentage distribution of the OC fractions of the treatments studied here at the different sampling times, based on Six's fractionation method, are presented in Figure 5.6. These data include not only physical fractionation but also chemical fractionation using 6 M HCl, so that all fractions presented in Figure 5.1 are reported. Results showed an increase in the “unprotected OC” fraction of the soil – usually considered as “labile OC” – with the addition of biochar of 12 – 15 and 6 – 21 %, in the TK soil and EG soil, respectively, compared to the corresponding controls with the addition of biochar, and a decrease of “chemically + biochemically protected” fraction of 9 – 13 and 6 – 17 % in the TK soil and EG soil, respectively.

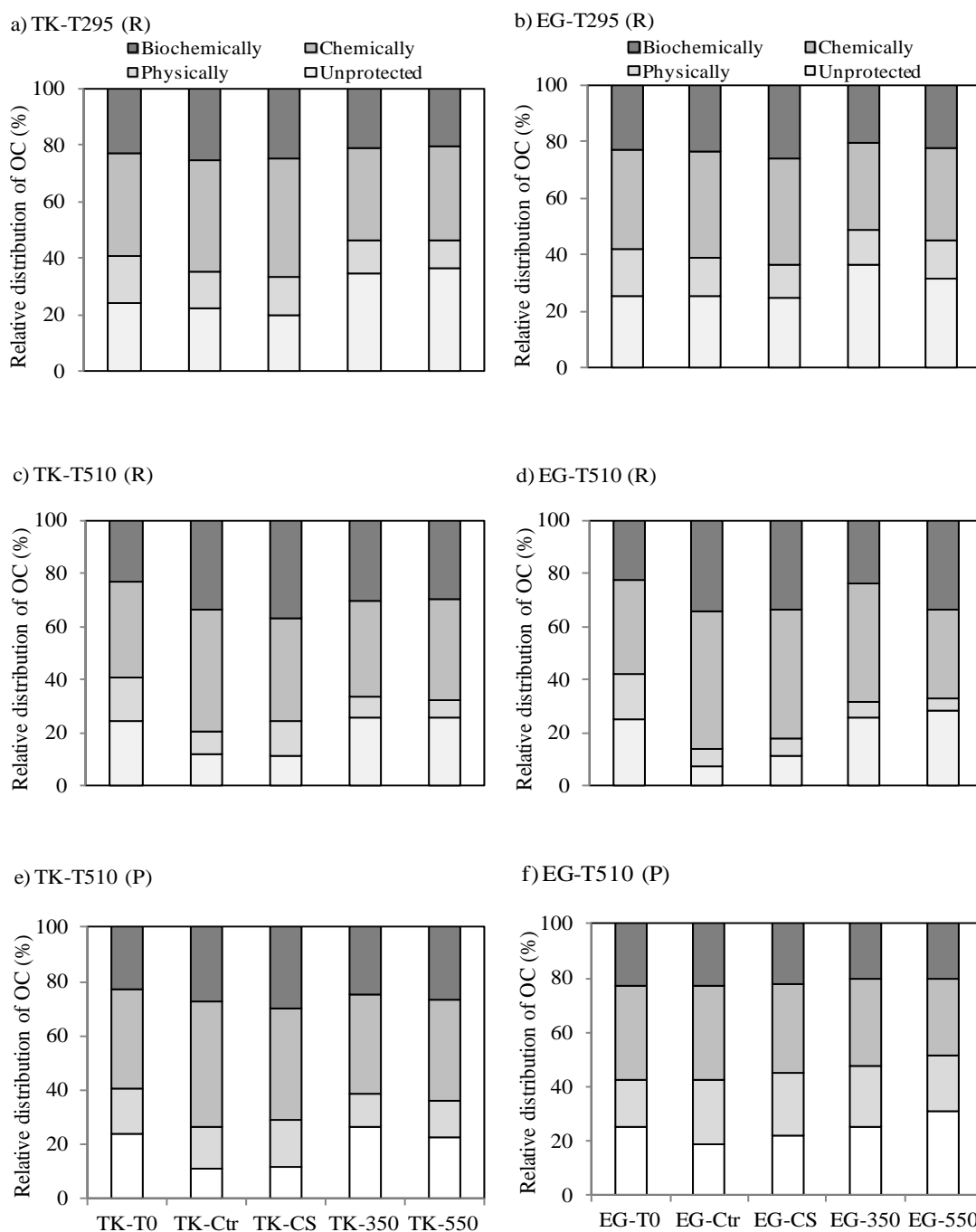


Figure 5.6: Relative distribution of OC in different SOC pools as given in Six *et al.* (2002): for (a) the TK soil at T295 (R), (b) the EG soil at T295 (R), (c) the TK soil at T510 (R), (d) the EG soil at 510 (R), (e) the TK soil at 510 (P), and the EG soil at T510 (P). Corresponding T0 data are included in each graph.

5.4 Discussion

5.4.1 Soil properties (TK-Ctr and EG-Ctr at T0)

The soils under study differed widely in OM content and composition. This was reflected by the total OC content, pyrolysis fingerprints and their physical and chemical fractionation. More specifically, the TK soil had an OC content of 42 g kg^{-1} of which 47 % was non-hydrolysable and 19 % was non-oxidisable (Table 5.2), while the EG soil had 102 g kg^{-1} OC of which 61 % was non-hydrolysable and 29 % was non-oxidisable (Table 5.2). Baisden *et al.* (2010), working with the same soils (but different samples), estimated a passive C pool of 15 % in the TK soil and of 27 % in the EG soil, which in rough agreement with the percentages reported here. These differences can, at least in part, be explained by the different mineralogical composition of these soils, with the TK soil developed in loess containing mostly 2:1 clay minerals (vermiculite and illite) which probably has a smaller negative effect on OM decomposition than the allophane in the andesitic EG soil (Jacquin *et al.*, 1978; Theng *et al.*, 1982; Baldock & Skjemstad, 2000; Parfitt, 2009), perhaps in combination with differences in OM composition.

The differing composition of native OM in these two soils was also reflected in their physical fractionation behaviour, with the EG soil having (i) a higher percentage of fine fPOM and silt+clay, and (ii) a lower percentage of coarse fPOM (Figure 5.4 and 5.5). The abundance of microaggregates is common in Andisols and has been attributed to the specific type of interaction existing between soil OM, allophane and Al cations (Parfitt *et al.*, 1997; Scheel *et al.*, 2008). The pyrolysis-GC/MS results indicate that soil TK contains an important fraction of primary OM in the fPOM fractions, which is theoretically easily degraded, while soil EG contains larger amounts of degraded and recalcitrant components that had accumulated through microbial activity – a feature often observed for OM in soils developed in Andisols (Verde *et al.*, 2010; Abelenda *et*

al., 2011) – and selective preservation. The abundance of aliphatic compounds in the EG soil may explain the low oxidisable and hydrolysable OC contents of this soil (Table 5.2), which could be justified by the low solubility of these compounds in aqueous reagents or by relative chemical stability against them (Abelenda *et al.*, 2012). Overall, the presence of a more stable OC fraction in the EG soil is consistent with its greater soil residence time (*e.g.*, 2-fold) compared with that of the TK soil, as reported by Baisden *et al.* (2010).

5.4.2 Changes in chemical and physical fractionation with time

5.4.2.1 Control treatments

At T510, in the absence of plants, mineralisation caused a greater absolute decrease in soil OC in the EG-Ctr soil (8.6 g kg⁻¹) than in the TK-Ctr (5.4 g kg⁻¹), although in relative terms the decrease was smaller in the EG-Ctr soil than in the TK-Ctr soil (~8 and ~12 %, respectively). The amount of OC lost from both soils exceeded by approximately 150 % the size of their corresponding so-called “unprotected fraction”, represented by fPOM (9.3 and 21.6 g C kg⁻¹ soil) in the TK-Ctr and EG-Ctr, respectively).

Decomposition of OC in the TK-Ctr treatment was most apparent in the coarse fPOM fraction, although fine fPOM also decreased with time. The decrease in coarse fPOM was expected, given the presence of fresh organic detritus, and is consistent with the conceptual model of Six *et al.* (2002). Decomposition of OC in the EG-Ctr treatment was most apparent in the fine fPOM fraction. However, a shift of OC from fine fPOM to HF partly contributed to these changes. Both fractions were dominated by microbial tissue and recalcitrant aliphatic OM. We hypothesise that, as the decomposition of native OM in the fine fPOM fraction preceded, its density increased so that it was

recovered in the HF fraction (it should be noted that these two fractions are separated by density fractionation). All soils were air-dried before fractionation; this may have affected to some extent aggregation in the allophanic soil (Warkentin & Maeda, 1980); however, this effect, if any, was assumed to be the identical for all treatments.

5.4.2.2 Fresh corn stover treatments

Uncharred CS was mostly recovered in the fPOM fractions of both soils, predominantly in the coarse fPOM (Figure 5.4b). Based on the isotopic data from evolved CO₂ (Chapter 4), 66 % of this amendment was decomposed after 510 d of incubation in the TK soil and 77 % in the EG soil. In that study, a positive priming effect of uncharred CS on native OM decomposition (in the absence of plants) was observed, this being especially evident in the TK soil (12 % in the TK soil vs. 7 % in the EG soil) (Chapter 4). The present study showed a greater decrease of native OM in the coarse fPOM fraction of the TK-CS treatment than the TK-Ctr soil; this would support the above-mentioned positive priming effect of uncharred CS on NOM decomposition.

The co-presence of plants and uncharred CS amendment had a positive effect on OC accumulation in the TK soil ($\Delta\text{OC} = 4.4 \text{ g kg}^{-1}$; Figure 5.2a). This increase was accompanied by an increase in $\Delta\text{OC}_{\text{ox}}$ of 6.0 g kg^{-1} . Therefore, a shift from non-oxidisable OC to oxidisable OC in the co-presence of plants and fresh CS occurs, as compared with the treatment with only plants, which might be explained by changes in the conformational structure of OM (Buurman *et al.*, 2002), although more research is needed in this regard. By contrast, the co-presence of plants and uncharred CS had a negative effect on the accumulation of OC in the EG soil ($\Delta\text{OC} = -1.6 \text{ g kg}^{-1}$; Figure 5.2b), which we cannot explain with the available data. The presence of plants in the EG soil with or without uncharred CS mitigated the decrease in the fine fPOM fraction

(and concomitant increase in the HF fraction) observed in the absence of plants (Figure 5.5a-b). More research is needed to explain this.

5.4.2.3 Biochar-amended treatments

When biochar was added to the soil, most of the biochar OC was recovered in the coarse fPOM, as expected given that the dominant particle size of the biochar used in this study was $>250 \mu\text{m}$ ($\sim 90 \%$; Table 5.S1).

The proportion of OC not oxidisable in dichromate solution, has traditionally been used as a broad estimate of the OC associated with charred material in soils (Bremner & Jenkinson, 1960b; Bremner & Jenkinson, 1960a; Kalisz & Sainju, 1991; Skjemstad & Taylor, 1999; Rodríguez Rodríguez *et al.*, 2004), even though it is known that some pyrogenic OC is susceptible to oxidation and some non-pyrogenic OC may actually survive the oxidation, *e.g.* hydrophobic aliphatic compounds (Knicker, 2007; Abelenda *et al.*, 2012). In this study, the non-oxidisable fraction, which can be inferred from Figure 5.2 by the difference between ΔOC and $\Delta\text{OC}_{\text{ox}}$, in soils incubated without plants for 510 d (T510 (R)) was greater than at T0. However, no explanation was found for this.

The co-presence of plants and biochar induced an additional accumulation of OC in the soil compared with the treatments with only plants (Table 5.3; Figure 5.4 and 5.5). This effect was greater in the TK-350 treatment than in the TK-550 treatment, while the opposite pattern was observed in the EG soil. It should be noted that pots were irrigated with a nutrient solution (without N) to avoid potential nutritional differences among biochar and non-biochar treatments; no differences in root and biomass growth were detected (Table 5.S2). Therefore, the observed enhanced C stocks in the co-presence of plants and biochar compared with only plant treatments point towards a potential role of

biochar in the stabilisation of fresh OC. This effect was soil and biochar type-specific, and more information is needed for a mechanistic understanding of the described observations. Finally, it should be noted that the proportion of non-oxidisable OC decreased when plants and biochar were both present (Figure 5.2). However, final values of $\Delta\text{OC}_{\text{nox}}$ were always above of those in the initial biochar added to soil. It is thus not possible to confirm whether (i) biochar decomposition was enhanced in the presence of plants, or (ii) the new inputs of plants favoured an increase in oxidability of native OM.

5.4.3 Implications of biochar on aggregate formation

No significant effects of biochar on microaggregate formation were observed in this study, although biochar-native OM interactions may in the long term influence the formation of microaggregates. The physical properties of these biochar-amended soils have been described in Chapter 3. In that study an increase in macroaggregate stability in the presence of biochar was reported; this may have had a role on OC protection in this short-term experiment. Studies on charcoal particles under field conditions for 44 to 85 yr suggests a role of charcoal on the formation of microaggregates (Brodowski *et al.*, 2006). Similarly, Kimetu and Lehmann (2010) observed an increase in the intra-aggregate light fraction of a Humic Nitosol 730 d after amending the soil with biochar made from *Eucalyptus saligna* Sm. (produced at 400 – 500 °C).

5.4.4 Implications of the presence of biochar in soils following Six's fractionation methodology

The results obtained in this study show that after 510 d, biochar was mostly recovered in the so-called “unprotected OC” pool (Figure 5.6), which corresponded to the coarse and fine fPOM. These fractions are considered “labile OC” (Six *et al.*, 2000; Six *et al.*, 2002). The increase in charred OC in this pool caused a percentage decrease in the OC

fraction considered as “biochemically protected”. This is inconsistent with the OC forms present in these physically-defined fractions. In fact, it is the particle size of the amended biochar what controls the fate of biochar C in these fractions, at least on the short- and mid-term. When oxidising the free fPOM and silt+clay fractions with dichromate, a 1:1 relationship ($R^2 = 0.88$) was found between the fraction of biochar-C and that of OC_{nox} (after normalising for initial OC_{nox}) (Figure 5.7). Therefore, we propose that, when analysing soils amended with biochar (or those that undergo recurrent fire events) following the Six’s fractionation method, a dichromate oxidation is carried out on these fractions to ensure the biochemically protected fraction (including biochar) is correctly identified.

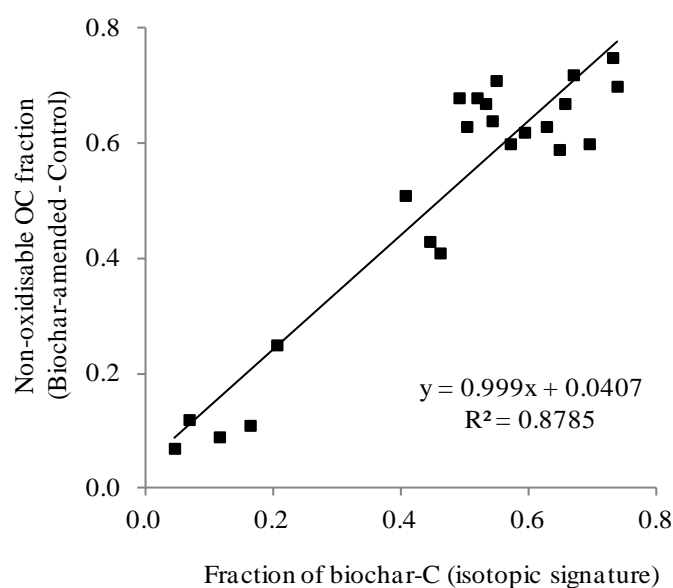


Figure 5.7: Relationship between the non-oxidisable OC fraction (biochar-amended – control) vs. fraction of biochar-C calculated using stable C isotope ratio of the free POM fractions.

5.5 Conclusions

After a 510 d incubation of biochar in an Alfisol and an Andisol, an increase in the proportion of biochar recovered in the silt+clay fraction is indicative of some biochar disintegration, even though most biochar particles were still recovered from the fPOM

fractions. No effect of biochar on microaggregate formation (and therefore physical protection of the iPOM fraction) was observed. The pyrolysis temperature used to create the biochars (350 or 550 °C) did not influence the fate of biochar C in the different physically- and chemically-defined fractions. The fact that the estimated total amount of biochar-C evolved in biochar-amended soils was always below their labile C value (Chapter 4), as estimated by dichromate oxidation, may partly explain the observed pattern. The fate of biochar-C in the physically-defined fractions studied was not influenced by the presence of plants but biochar did cause more total OC accumulation in their presence. This could not be attributed to differences in plant/root growth, as these were not detected. According to the conceptual soil OC pools (Six *et al.*, 2002), fPOM is considered as a labile SOC pool, which is inconsistent with the occurrence of stable, dichromate resistant biochar in this fraction. Acid hydrolysis was not able to identify the presence of biochar in the fPOM fraction; instead, dichromate oxidation can be used to detect this biochemically protected soil OC pool in biochar-amended soils and, in general, in soils with considerable amounts of charcoal (*e.g.*, areas with recurrent fire events).

5.6 Supportive information

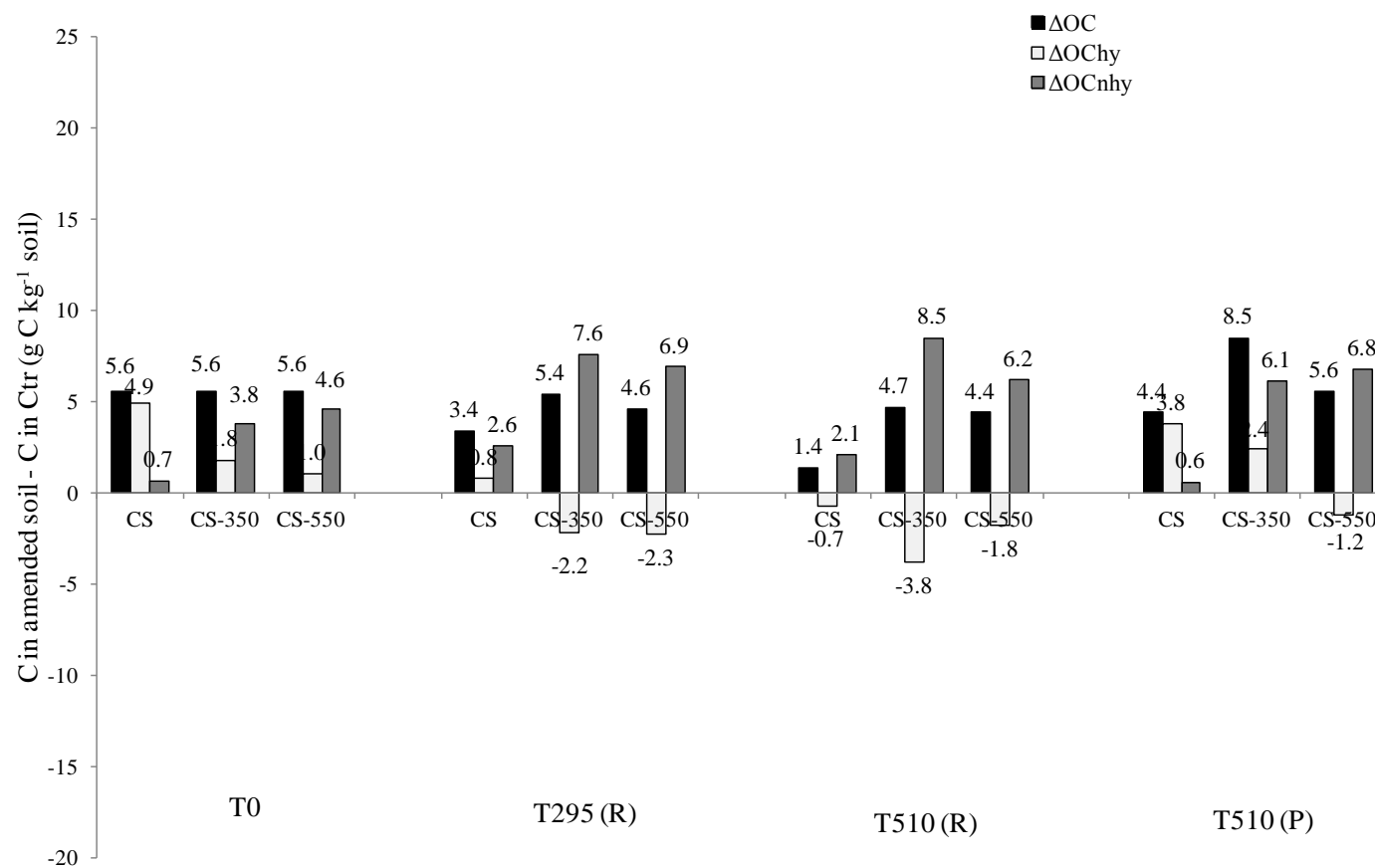


Figure 5.S1A: Soil OC, hydrolysable OC, and non-hydrolysable OC contents calculated at different sampling times after subtracting the respective soil OC, hydrolysable OC, and non-hydrolysable OC contents of the corresponding control treatment at that sampling time, for the TK soil.

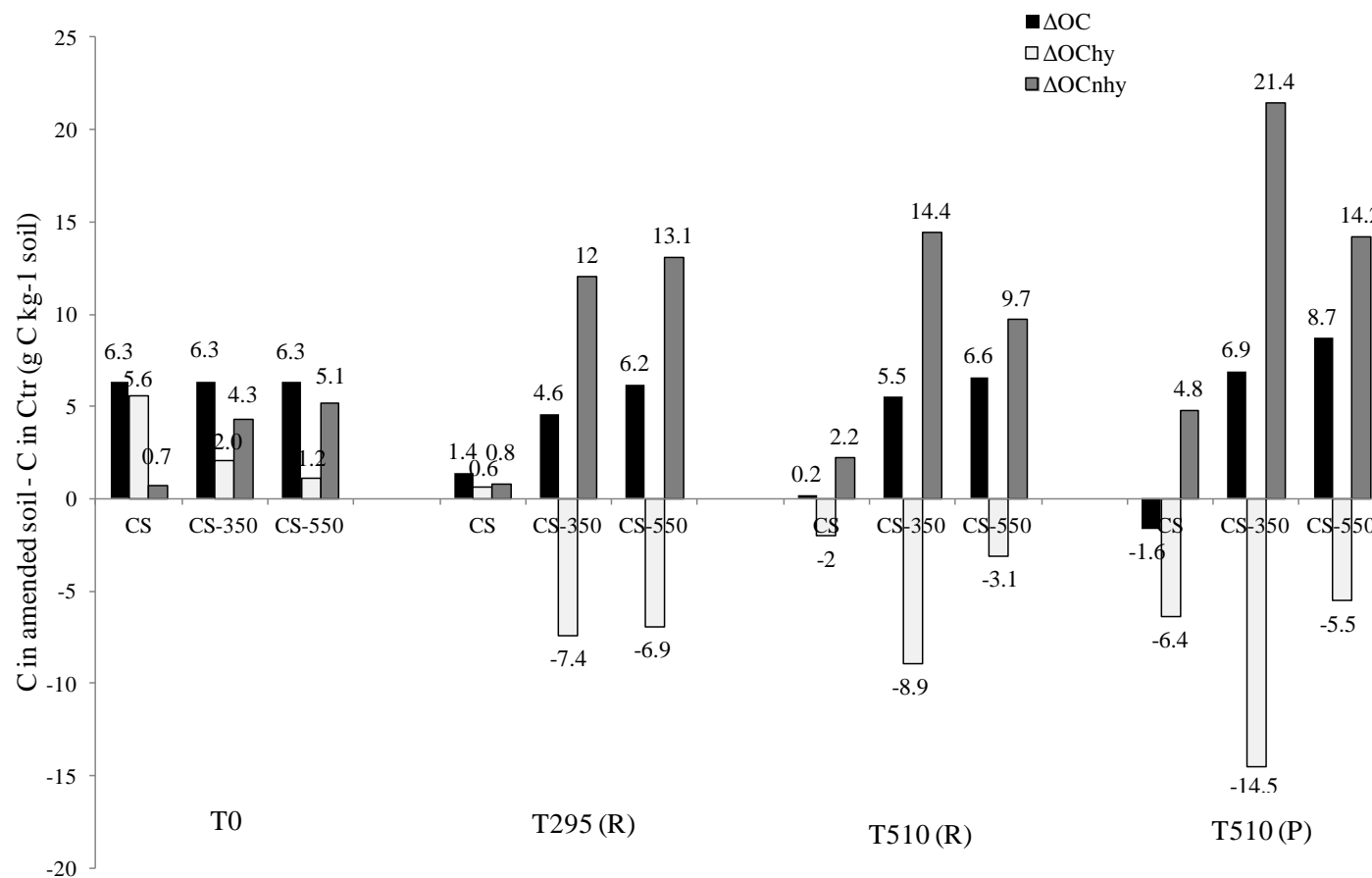


Figure 5.S1B: Soil OC, hydrolysable OC, and non-hydrolysable OC contents calculated at different sampling times after subtracting the respective soil OC, hydrolysable OC, and non-hydrolysable OC contents of the corresponding Ctr treatment at that sampling time, for the EG soil.

Table 5.S1: Distribution of biochar at T0 (expected %) and the recovered after 295 and 510 d (recovered %). Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between the fractionation times.

Soil	Physical fraction	CS-350				CS-550			
		Expected %	Recovered %			Expected %	Recovered %		
			T295 (R)	T510 (R)	T510 (P)		T295 (R)	T510 (R)	T510 (P)
TK soil	Coarse fPOM	87.8d	83.1b	70.5a	79.9b	92.1c	78.0b	71.6a	67.0a
	Fine fPOM	11.0a	13.4a	23.8b	12.7a	7.4a	18.4b	22.1b	21.6b
	Silt+clay sized	1.2a	3.6b	5.7c	7.4d	0.5a	3.6b	6.3c	11.4d
EG soil	Coarse fPOM	87.8b	74.3a	70.7a	71.8a	92.1b	64.3a	66.5a	67.0a
	Fine fPOM	11.0a	21.1bc	25.0c	18.8b	7.4a	29.7b	27.5b	23.6b
	Silt+clay sized	1.2a	4.6a	4.3a	9.5b	0.5a	6.0b	6.0b	9.5c

Table 5.S2: Dry weight of lucerne plants (total, shoots and roots) (g) determined after 215 d of growth in 510 d study (standard deviations are given within parenthesis).

Treatment	Total	Shoots	Roots
TK-Ctr	4.86 (± 0.11)	3.01 (± 0.33)	1.86 (± 0.22)
TK-CS	5.13 (± 0.38)	3.07 (± 0.24)	2.06 (± 0.16)
TK-350	4.97 (± 0.44)	2.76 (± 0.06)	2.21 (± 0.40)
TK-550	4.62 (± 0.13)	2.82 (± 0.03)	1.80 (± 0.14)
EG-Ctr	3.81 (± 0.57)	2.19 (± 0.36)	1.62 (± 0.22)
EG-CS	4.13 (± 0.21)	2.40 (± 0.15)	1.73 (± 0.06)
EG-350	4.09 (± 0.15)	2.36 (± 0.10)	1.73 (± 0.04)
EG-550	4.27 (± 0.09)	2.42 (± 0.16)	1.84 (± 0.14)

Table 5.S3: $\delta^{13}\text{C}$ and the fraction of C originated from the amendment in the coarse fPOM, fine fPOM and silt+clay sized fractions taken after 295 and 510 d of respiration study and 510 of the plant study. Minimum significant ($\alpha = 0.05$) differences between any two means are based on the Duncan post hoc test, and different letters denote the significant differences between treatments for the same fraction in each soil.

Fraction	Treatment	T295 (R)		T510 (R)		T510 (P)	
		$\delta^{13}\text{C}$	Amendment fraction	$\delta^{13}\text{C}$	Amendment fraction	$\delta^{13}\text{C}$	Amendment fraction
coarse fPOM	TK-Ctr	-29.54a	0.00	-29.39a	0.00	-28.92a	0.00
	TK-CS	-22.90b	0.39	-24.66b	0.28	-24.68b	0.26
	TK-350	-19.38c	0.59	-17.83c	0.70	-19.31c	0.62
	TK-550	-19.17c	0.59	-17.45c	0.72	-19.72c	0.59
	EG-Ctr	-28.56a	0.00	-28.77a	0.00	-28.70a	0.00
	EG-CS	-21.81b	0.42	-23.47b	0.33	-23.04b	0.35
	EG-350	-18.34c	0.67	-17.90d	0.68	-17.83c	0.71
	EG-550	-19.40d	0.61	-19.07c	0.63	-18.41c	0.67
fine fPOM	TK-Ctr	-29.54a	0.00	-28.08a	0.00	-28.18a	0.00
	TK-CS	-27.78b	0.10	-25.81b	0.15	-24.43b	0.24
	TK-350	-22.95c	0.41	-18.09c	0.68	-20.65c	0.51
	TK-550	-22.62d	0.43	-18.15c	0.68	-19.25d	0.60
	EG-Ctr	-28.07a	0.00	-27.32a	0.00	-27.04a	0.00
	EG-CS	-27.34b	0.05	-26.65b	0.05	-26.27b	0.05
	EG-350	-26.98c	0.07	-25.76c	0.11	-25.86c	0.09
	EG-550	-26.27d	0.12	-25.81c	0.11	-25.84c	0.09
silt+clay sized	TK-Ctr	-28.15a	0.00	-28.80a	0.00	-28.53a	0.00
	TK-CS	-27.86a	0.02	-28.15b	0.04	-28.14b	0.02
	TK-350	-27.61b	0.04	-27.91c	0.06	-27.61c	0.06
	TK-550	-27.50b	0.04	-27.85c	0.06	-27.34d	0.08
	EG-Ctr	-27.37a	0.00	-27.31a	0.00	-27.32a	0.00
	EG-CS	-27.04b	0.02	-27.04b	0.02	-27.01b	0.02
	EG-350	-27.04b	0.02	-26.94c	0.03	-26.75c	0.04
	EG-550	-26.91c	0.03	-26.88c	0.03	-26.63d	0.05

Table 5.S4: Amount of soil dry matter present in the size and density fractions (g 100 g⁻¹ soil) obtained by the physical fractionation (average \pm standard deviation) and the mass recovery (%) obtained at T0, T295 (R), T510 (R) and T510 (P).

Sampling time	Treatment	Coarse sand + coarse fPOM (>250 μ m)	Microaggregates + fine free POM + fine sand (53-250 μ m)		Silt + clay (<53 μ m)	Mass recovery (%)
			Light fraction (fine fPOM)	Heavy fraction (HF)		
T0	TK	2.7 \pm 0.2	0.9 \pm 0.2	58.2 \pm 1.8	36.4 \pm 1.7	98.2
	EG	8.0 \pm 0.1	11.1 \pm 0.7	50.8 \pm 1.6	21.8 \pm 1.4	91.7
T295 (R)	TK-Ctr	8.4 \pm 0.4	0.9 \pm 0.2	52.9 \pm 1.6	34.1 \pm 1.6	95.4
	TK-CS	5.6 \pm 3.4	0.8 \pm 0.4	59.3 \pm 5.8	32.3 \pm 0.7	95.1
	TK-350	8.2 \pm 0.4	1.0 \pm 0.3	50.4 \pm 3.3	36.5 \pm 1.8	96.6
	TK-550	10.5 \pm 0.3	1.7 \pm 0.6	56.3 \pm 1.6	31.4 \pm 0.5	97.2
	EG-Ctr	8.7 \pm 0.5	12.1 \pm 0.2	58.3 \pm 1.4	17.6 \pm 0.6	84.6
	EG-CS	9.8 \pm 0.2	10.6 \pm 2.5	56.0 \pm 6.2	18.2 \pm 1.4	82.4
	EG-350	10.2 \pm 1.2	13.3 \pm 2.0	53.0 \pm 4.6	19.2 \pm 1.1	85.8
	EG-550	11.4 \pm 0.7	10.4 \pm 0.2	57.9 \pm 2.5	16.5 \pm 1.1	83.9
T510 (R)	TK-Ctr	3.6 \pm 0.0	0.3 \pm 0.1	60.2 \pm 1.5	32.9 \pm 0.5	97.0
	TK-CS	2.6 \pm 1.1	0.4 \pm 0.1	68.5 \pm 3.3	26.7 \pm 2.0	98.2
	TK-350	3.3 \pm 0.2	0.6 \pm 0.2	63.2 \pm 2.6	30.5 \pm 2.2	97.6
	TK-550	3.2 \pm 0.3	0.6 \pm 0.1	63.0 \pm 3.3	31.4 \pm 2.7	98.3
	EG-Ctr	8.2 \pm 0.5	2.1 \pm 0.1	67.0 \pm 0.7	17.6 \pm 0.8	94.8
	EG-CS	10.8 \pm 0.1	2.8 \pm 0.3	68.0 \pm 2.2	14.9 \pm 2.3	96.5
	EG-350	9.3 \pm 1.6	7.1 \pm 0.4	63.2 \pm 0.8	14.9 \pm 0.6	94.5
	EG-550	10.9 \pm 1.3	8.3 \pm 0.6	60.3 \pm 1.2	15.4 \pm 0.3	94.9
T510 (P)	TK-Ctr	3.5 \pm 1.4	0.4 \pm 0.1	62.1 \pm 3.3	32.1 \pm 2.0	98.2
	TK-CS	2.9 \pm 0.9	0.5 \pm 0.1	63.2 \pm 2.4	31.5 \pm 1.7	98.2
	TK-350	5.0 \pm 0.5	0.5 \pm 0.1	59.6 \pm 0.3	33.2 \pm 0.1	98.3
	TK-550	4.2 \pm 0.6	0.6 \pm 0.1	58.7 \pm 1.1	34.9 \pm 0.3	98.4
	EG-Ctr	8.8 \pm 0.1	6.7 \pm 0.4	61.5 \pm 1.2	18.5 \pm 0.8	95.5
	EG-CS	9.0 \pm 0.1	6.8 \pm 0.4	62.4 \pm 0.5	17.2 \pm 0.7	95.5
	EG-350	9.5 \pm 0.8	7.8 \pm 0.2	60.3 \pm 0.8	17.9 \pm 0.7	95.4
	EG-550	9.0 \pm 0.7	8.7 \pm 1.8	60.2 \pm 2.9	17.5 \pm 1.4	95.5

CHAPTER 6: Overall conclusions and future research works

6.1 Conclusions

The addition of corn stover biochar produced at 350 and 550 °C in to the Tokomaru and the Egmont soil did not enhance the formation of soil microaggregates, which disagrees with what was initially hypothesised. The mesoporosity of both soils increased; this corresponds to an increase in available water content, and thus may have key implications with regards to (i) the resilience of the plant-soil system during a dry spell, and (ii) the reduction of irrigation frequency. Macroporosity of the poorly drained Alfisol increased with the biochar amendment. This can have crucial implications with regards to N₂O emissions reductions.

Stability of biochar in soil was more soil-dependent than pyrolysis temperature-dependent and no differences in CO₂ evolved were observed among the biochars tested in a specific soil. In the Alfisol, biochars retarded the NOM decomposition whereas the fresh corn stover showed the reverse effect. No clear effects of the amendments on NOM decomposition were observed in the Andisol. Results stressed the importance of pyrolysis as an effective method to sequester atmospheric CO₂ into the soil. The amount of C lost during pyrolysis was compensated for within a short time period (<245 d, in all biochar x soil combinations) after soil application. Although, a higher fraction of biochar-C degraded during +500 d compared to the reported literature, current results provide a solid base regarding the longevity of biochar-C in the soils, and this is further supported by the longer half lives (at least centennial) determined over the corn stover C (<2 y).

The mechanisms involved in stabilisation of biochar-C in different soil organic C pools will have direct implications in the length of time of the biochar benefits to soil. Biochar was mostly recovered from the unprotected C pool, defined as that not included in microaggregates and/or associated with silt+clay, which is generally considered as “labile” C. Biochar however, was shown to encourage macroaggregate formation. The present study proposed a further chemical fractionation of the unprotected C pool with dichromate solution to distinguish the biochemically protected fraction (recalcitrant) from the true labile C. Acid hydrolysis was not able to identify the presence of biochar in the unprotected C fraction as the acid hydrolysable NOM fraction was shown not to be stable with time. In fact, it is hypothesised that conformational changes occur during long-term incubations in the absence of a fresh organic matter input increasing the hydrophobicity of NOM and thus changing its recovery by acid hydrolysis. The presence of plants tended to increase accumulation of total C in the biochar-added soils compared with the plant only treatments. A slow shift of biochar-C from the unprotected pool into the silt+clay associations observed, particularly in the presence of plants, suggests the potential of biochar to have a greater role in long-term NOM stabilisation, although more studies on this are needed.

6.2 Implications for future research

The experimental works conducted in this thesis were constrained by the time period of study for a PhD thesis. There are a number of areas of research on the role of biochar in these OC-rich soils that still require investigation and thus any future research should be focussed on the following topics:

- Changes in chemical and physical properties of the biochar and biochar-amended soils over longer time intervals;

- The influence of the physicochemical changes observed in the present study on microbial activity, population and diversity;
- Developing the mechanistic understanding of the changes observed in the present study further so that there is less need to test a combination of a range of biochars and soil types; and
- Conducting an investigation into the soil-plant-biochar interactions considered in this PhD study under field conditions, in order to understand more realistic situations, which are far more complex than in incubation studies.

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