Possibilities to Stabilize Organic Matter in Soil Using Various Biochars

Muhammad Farooq Qayyum

A thesis submitted for the requirement of the Doctoral Degree in Agriculture from the Faculty of Agricultural Sciences, Nutritional Sciences, and Environmental Management, Justus Liebig University Giessen

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2012

1st Edition 2012

Printed in Germany

édition scientifique
VVB LAUFERSWEILER VERLAG

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Institute of Plant Nutrition
Justus Liebig University Giessen
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M.Sc. (Hons.) Soil Sciences
Multan, Pakistan

Giessen 2012

Date of Defense: 23.05.2012

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Dedicated to

My family

CONTENTS

LIST OF ABBREVIATIONS

NCM	Net carbon mineralized
ANOVA	Analysis of variance
BC	Black carbon
BET	Brunauer-Emmett-Teller
C	Carbon
CAL	Calcium acetate lactate
CEC	Cation exchange capacity
CH_4	Methane
CO_2	Carbon dioxide
C_{org}	Soluble organic carbon
CRD	Complete randomized design
DTPA	Diethylene triamine pentaacetic acid
FF	Free fraction
FTIR	Fourier transform infrared
$g\ kg^{-1}$	Gram per kilogram
HF	Heavy fraction
HSD	Honestly significant difference
HTC	Hydrothermal carbonization coal
IAF	Intra-aggregate fraction
IPCC	Intergovernmental panel on climate change
LF	Light fraction
LOM	Labile organic matter
LSD	Least significant difference
LTC	Low temperature conversion coal
N_2O	Nitrous oxide
PE	Priming effect
Pg	Petagram ($1\ Pg = 10^{15} g$)
ppm / a	Parts per million / year
SOM	Soil organic matter
$t\ ha^{-1}$	Tons per hectare
TC	Total carbon
TN	Total nitrogen

INTRODUCTION

1.1. The need for soil organic matter stabilization

Soil organic matter (SOM) has an important role in soil fertility and agricultural productivity. In soils, organic matter includes all organic carbon either present free in the soils, bound in between soil stable aggregates, or chemically bound in the soil mineral matter. At any time, SOM may include a variety of compounds such as decomposing plant or microbial residues, root exudates etc. The SOM content in soils varies from traces to more than thirty percent of soil weight (Bot and Benites, 2005) and in most cases, is below the ecological potential of soil (Lal, 2006). Over the last century, many agricultural practices such as deforestation, biomass-burning, soil-cultivation and over-grazing, have resulted in decline of SOM. Soil degradation through accelerated erosion also decreases SOM (De Jong and Kachanoski, 1988). This decline in SOM leads to decreased soil fertility, increased mineral fertilization, and higher release of greenhouse gases into the atmosphere.

Practices used to increase the soil organic pool include optimum nitrogen fertilization (Rasmussen and Rohde, 1988), conservation agriculture (Ismail et al., 1994), and adding high amounts of manures to soil (Buyanovsky and Wagner, 1998; Fearnside, 2000). The contribution of these applications to SOM stabilization is small (Schlesinger, 1999) because most of the applied SOM is usually mineralized rapidly (Tiessen et al., 1994).

1.2. Factors affecting soil organic matter stabilization in soil

Many factors influence SOM stabilization including clay content of soil (Arrouays et al., 1995; Saggar et al., 1994), rainfall, and temperature regimes (Six et al., 2002). The mechanisms involved in the stabilization of SOM include formation of organo-mineral complexes (Gleixner et al., 2001; Tisdall and Oades. 1982), interactions with metal ions (von Lützow et al., 2006) and occlusion in micro pores, making SOM inaccessible to microorganisms (Guggenberger and Kaiser, 2003).

The stability of SOM in soils depends on the distribution of carbon (C) in various density fractions, which are differently protected against decomposition through distinct mechanisms (Bouajila and Gallali, 2008). The least stable SOM fraction in soils is the light fraction (LF)

or free fraction (FF) that may be plant-like and has higher C concentration (Golchin et al., 1994; Swanston et al., 2002). It has been suggested that the higher the FF in soil, the higher is the microbial activity (Alvarez and Alvarez, 2000; Janzen et al., 1992). The second fraction comprises the C protected in the soil stable aggregates and is called intra-aggregate fraction (IAF). The most stable fraction of C in soils is the heavy fraction (HF) which is protected by the soil minerals.

Incubation studies of density fractions separated from soils demonstrated higher respiration from LF and very small respiration in HF (Alvarez and Alvarez, 2000; Whalen et al., 2000). To study the stability of SOM density fractions, Swanston et al. (2002) isolated LF and HF from soils and incubated them both separate and combined for 300 d. They found higher respiration from LF than HF when expressed as per g of substrate but there was no difference between two fractions when expressed as per g of initial C. Their results suggested no significant difference between recalcitrance of LF and HF but the difference in stability was due to different mechanism. It was suggested that higher stability of C in the HF was due to less microbial accessibility.

1.3. Carbon sequestration into soils, a suitable tool to increase SOM

The increasing debate on global warming has augmented the emphasis on approaches to decrease emissions of greenhouse gases (carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)) in the atmosphere. According to an estimate, the atmospheric concentration of CO_2 has reached almost 400 ppm with an increasing rate of 2.2 ppm/a (IPCC, 2007). Carbon (C) sequestration into soils has been proved as an effective way of reducing greenhouse effect (Lal, 2004, 2009, and 2011).

Lal (2006) defined soil C sequestration as a process in which atmospheric CO_2 is fixed in soil either directly (conversion of CO_2 into soil inorganic carbon compounds such as calcium and magnesium carbonates because of chemical reactions) or indirectly (fixation of C in soil after decomposition of plant biomass). The global soil C pool is estimated to be 1550 Pg. (1Pg = 10^{15}g) of organic and 950 Pg. of inorganic C m^{-1} depth (Batjes, 1996) having a C sequestration potential of 1.2–3.1 billion tons C/a (Lal, 2011). The direct benefits of soil C sequestration are improvements of soil structure, nutrient use efficiency, and a decrease of

greenhouse gases (Lal, 2011). Thus, C sequestration in agricultural soils can be an important approach to restore the degraded organic soils.

1.4. Biochar for carbon sequestration

Recently, it has been suggested that C sequestration into soil by applying C-rich compounds (biochars) resulting from charcoal production from the above-ground biomass instead of converting it to CO_2 through burning can provide a significant C sink and can be an important step towards SOM stabilization and conservation in tropical agriculture (Glaser et al., 2002; Kamman et al., 2011). Applications of biochar to soils have resulted in decreased emissions of CH_4 (Priemé and Christensen, 1999), CO_2 (Spokas et al., 2009; van Zwieten et al., 2010), and N_2O (Augustenborg et al., 2011; Yanai et al., 2007). The net outcome of biochar applications is increased production of biomass that requires utilization of more atmospheric C in photosynthesis leading to a C-negative balance (Lehmann, 2006; Woolf, et al., 2010).

The idea of biochar as a soil amendment is not new. Pre-historic Amazonians had been practiced slash-and-burn to improve soil fertility and crop production (Glaser, et al., 2002; Lehmann et al., 2006). Highly fertile and organic-matter rich soils "*Terra Preta de Indigo"* in the Amazon basin are examples of anthropogenic additions of black carbon (BC) / charcoal (Glaser et al., 2000; Sombroek et al., 2003).

Using biochars as soil amendments enhance biological N_2 fixation (Rondon et al., 2007), decrease N leaching, and increase the efficiency of applied nutrients (Doydora et al., 2011; Glaser et al., 2001; Glaser et al., 2002; Major et al., 2011). Because of higher ash content, biochars increase soil pH and cation exchange capacity (CEC), ensuring plant-nutrient availability in acidic soils (Glaser et al., 2002; Streubel et al., 2011; Unger and Killorn, 2011). It has also been found that biochar applications in arid lands may reduce drought stress because of increasing water-holding capacity of soils (Karhu et al., 2011).

Steiner et al. (2007) applied charcoal (derived from secondary forest wood) to a Xanthic Ferralsol and they proposed that the charcoal application can improve soil chemical, biological, and physical properties but there is a need to optimize charcoal use for soil amelioration. Zhang et al. (2011) applied biochar from wheat straw in a calcareous soil and showed that in addition to higher maize yield, the release of greenhouse gases was

significantly decreased with higher application rates of biochars. The increase in crop yields with biochars as reported in many studies may be attributed to increased nutrient availability and improved soil structure (Asai et al., 2009; van Zwieten et al., 2010; Zhang et al., 2011).

1.5. Stability of biochars in soils

The combustion of organic materials while producing charcoal or biochars leads to a significant loss of hydrogen and oxygen, and some of the carbon present in organic materials (Antal and Gronli, 2003; Preston and Schmidt, 2006). Increasing pyrolysis temperature leads to more condensation of aromatic compounds in the remaining materials (Steinbeiss et al., 2009). These aromatic compounds in biochars are less prone to mineralization and very stable in soils.

Today, various biomass residues and waste materials are being converted into biochar by way of controlled pyrolysis (incomplete combustion under limited or no oxygen). Hydrothermal carbonization (HTC) of biomass and low-temperature conversion (LTC) of biomass into biochars are among such controlled carbonization practices. Hydrothermal carbonization is the conversion of biomass into biochar at low-temperature (200°C) in the presence of water (Titirici et al., 2007). The low-temperature conversion of sewage sludge is a thermo-catalytic conversion process that takes place anaerobically at (380-450°C) under normal pressure (Hossain et al., 2011). However, it has been proposed that all these carbonized products should be termed “biochars” when they are applied as a soil amendment (McLaughlin et al., 2009).

Overall, the stability of these biochars in soils is likely to vary significantly depending on the biomass source, properties, and the pyrolysis conditions such as temperature and catalyst application (Chan et al., 2007; Lehmann, 2007; Novak et al., 2010; Spokas, 2010). The mineralization of biochar C has been studied in soil and/or sand incubations with biochars produced by laboratory pyrolysis, and the loss of biochar C ranges from zero to > 50% in these studies (Baldock and Smernick, 2002; Hamer et al., 2004; Peng et al., 2011; Shindo, 1991; Steinbeiss et al., 2009; Wardle et al., 2008; Zimmerman, 2011). Steinbeis et al. (2009) studied C mineralization of glucose and yeast-derived biochars (produced by HTC) in soil, and they found C losses of 7 to 11% of initial biochar-C content. The production of biochars from sewage sludge, subsequent nutrient recovery, and plant availability of these nutrients

was described by Bridle and Pritchard (2004) and Hossain et al. (2011). However, there is a lack of information on the stability of the biochar C in temperate soils. There is a need to study the C mineralization kinetics of commercially available biochars when added to soils of differing properties.

Glaser et al. (2000) investigated the role of density fractions in black carbon (BC) stabilization in the *Terra Preta* compared to the adjacent soils and found highest C values in the light fraction (chemical inertness) followed by the heavy fraction (organo-mineral interactions). Kimetu and Lehmann (2010) also studied stabilization of biochar and green manure in SOM-poor soils and found higher stability of BC in the occluded fraction (aggregate fraction) in addition to the light fraction (recalcitrant BC). However, no information is available about the contribution of various biochars to SOM density fractions and changes occurring on biochar surfaces in various soils when applied in short-term or long-term laboratory incubation experiments.

1.6. Co-metabolization of biochar in soils

Although, most of the biochars are very recalcitrant in soil (Chen and Yuan, 2010; Major 2010), some studies have indicated increased mineralization of biochar when subjected to co-metabolization with some labile organic materials such as glucose (Hamer et al., 2004; Hamer and Marschner, 2002; Kuzyakov et al., 2009). This increased mineralization of biochar in soils containing high organic matter content or due to additions of labile organic matter is called priming effect (PE) that is considered as a short-term effect on the turnover of SOM (Kuzyakov et al., 2000).

The interactive priming effects between applied biochar and native soil C may result in either higher C loss from soils or improved C stabilization in soils. Wardle et al., (2008) were pioneers to report loss of soil organic C in boreal forests because of a charcoal-induced priming effect. Cross and Sohi (2011) investigated the effect of a biochar produced at high-temperature on stabilization of native organic matter of three soils. Their results showed no effect of biochar on mineralization of native SOM, rather it was concluded that biochar stabilizes native SOM.

The increased mineralization of biochars after glucose application has been reported in some studies. This may be due to enhanced microbial activity because glucose serves as readily available source of energy for microorganisms. However, the use of plant residues and manures may have a different effect because these are relatively more resistant as compared to the glucose. Little work has been done to study the effects of organic materials on biochars and vice versa. Cheng et al. (2006) observed changes in elemental compositions (increase in O and decrease in C) of a biochar when mixed with dairy manure and incubated for 4 months. Liang et al. (2010) applied organic-matter in BC-rich soil of Amazonian and adjacent BC-poor soil and studied the mineralization of applied C using changes of ^{13}C isotopic compositions. Their results suggested greater loss of applied C from BC-rich soil compared to BC-poor soil. It was also observed that applied organic-matter increased the loss of native soil C from BC-poor soil but interestingly, not in BC-rich soil. Similarly, Keith et al. (2011) studied interactive priming of biochars produced at various temperatures and sugarcane mulch as a labile organic matter (LOM). Their results indicated that LOM increased mineralization of biochars, and the biochars decreased mineralization of LOM. It was suggested by Keith et al. (2011) that the reactive surfaces of aged biochar particles in soils may protect LOM better as compared to the fresh biochar in soils.

Although, it is well known that conventional SOM is not stable in soils, co-metabolization of such SOM with biochars hints an idea that biochars, when present in soils for long time, may stabilize added SOM.

1.7. Objectives of the study

The stability of charcoal in Amazonian tropical soils (Oxisols, according to USDA soil classification) is well documented. In Germany, such soils are the remains of fossil Oxisols and they are present in the Rhenish Massif (Rhenish Slate Range), also spread in the Taunus (low mountain range in Hesse, Germany), in the Palatinate forest and in basalt of some parts of the Vogelsberg and the Rhoen. These soils are commonly known as Ferralsols (according to FAO soil classification system) or Ferrallites (Blume et al., 2010). In comparison to Ferralsols, Luvisols are widely spread soils in Western Europe used for crop production. There is a need to investigate the stabilization behavior of various biochars in these soils.

The main aims of our study were,

(i) To investigate the kinetics of carbon mineralization of various biochars compared to wheat straw in three soils.

(ii) To study the changes occurring on biochar surfaces in the SOM density fractions retrieved from three soils after 365 d of incubation.

(iii) To study the effect of ageing (time) of various biochars in soils on mineralization of wheat straw.

To achieve these objectives, the following approaches were made

(i) It was hypothesized that the charcoal, hydrothermal carbonization coal (HTC) and low temperature conversion coal (LTC) would be more stable in soils compared to wheat straw with stability dependent on soil properties.

In this study, incubation of various biochars (charcoal, hydrothermal carbonization coal, and low-temperature conversion coal) wheat straw and a control (non-amended) in three soils (Ferralsol, Luvisol topsoil, and Luvisol subsoil) was carried out over a period of 700 d.

(ii) In a second approach, it was hypothesized that the C in various biochars is stabilized in soils through distinct mechanisms.

In this line, soil samples were analyzed using the density fractionation technique after 365 d of incubation.

(iii) The third hypothesis was that biochar ageing in soil would stabilize the additional source of labile C.

In this study, various biochars were incubated for two residence times (fresh-applied and 365 d incubated), and wheat straw was applied in biochar-incubated soils. Carbon mineralization was studied over a period of 90 d. Kinetics of C mineralization and priming effects were calculated using CO_2-releasae data.

2. MATERIALS AND METHODS

2.1. Soils and the carbonized materials

The selected soils for the incubation experiment were sampled from various areas in Hesse, Germany. The Ferralsol was a mixture of various soil horizons from an area at Lich near Giessen, Germany; it is a relict tertiary Ferralsol from the Vogelsberg area in central Hesse, Germany (Blume et al., 2010). In comparison to the Ferralsol, Luvisols derived from loess were selected because these are the most important soils in Western Europe for crop production. The Luvisol topsoil (0-25 cm depth) was collected from a farmer's field in Hünfeld, Germany and the subsoil (80-120 cm depth) was sampled from Kleinlinden near Giessen, Germany, in spring 2009. Winter wheat and winter rape are the commonly grown crops of the sampled fields. Physical and chemical characteristics of the soils are documented in Table 1.

Wheat straw and the biochars used in the incubation experiment differed in biomass type and method of preparation. Charcoal (a wood coal that is used as a source of domestic fuel) was purchased from Sommer-hit Co. ® Germany, where it is produced by heating wood anaerobically at 550°C. Hydrothermal carbonization coal (HTC) was a product of hydrothermal carbonization of bark at low temperature (200°C) in the presence of water and citric acid as a catalyst (Titirici et al., 2007). The low-temperature conversion coal (LTC) was obtained from Loteco TEC Co. ® Germany, where it is produced by anaerobic thermo-catalytic conversion of sewage sludge at 400°C. The physicochemical properties of the wheat straw, charcoal, HTC, and LTC are given in Table 2.

Table 1. Physicochemical properties of the soils used in the experiment.

Parameter	**Ferralsol**	**Luvisol topsoil**	**Luvisol subsoil**
	(mix. of various soil horizons)	(0 – 25 cm)	(80 – 120 cm)
pH, 0.01 M $CaCl_2$	5.5	7.2	7.2
Total C, mg kg^{-1} soil	3300	17500	2800
Total N, mg kg^{-1} soil	200	1700	200
Total S, mg kg^{-1} soil	800	700	200
[1]CAL-P, mg kg^{-1} soil	0.32	39.53	5.94
[1]CAL-K, mg kg^{-1} soil	6.67	169.01	38.81
Exchangeable Mg, mg kg^{-1} soil	110.5	63.3	166.0
[2]DTPA-Cu, mg kg^{-1} soil	Not measureable	1.58	0.60
DTPA-Mn, mg kg^{-1} soil	11.10	20.20	11.03
DTPA-Fe, mg kg^{-1} soil	12.20	57.60	34.65
Oxalate-Fe, g kg^{-1} soil	2.20	1.90	1.42
Dithionite-Fe, g kg^{-1} soil	16.50	5.70	5.92
Oxalate-Al, g kg^{-1} soil	0.90	0.60	1.26
Dithionite-Al, g kg^{-1} soil	1.70	0.50	1.27
[3]CEC, cmol kg^{-1} soil	3.20	15.20	9.80
Sand, g kg^{-1} soil	304	88	479
Silt, g kg^{-1} soil	395	668	345
Clay, g kg^{-1} soil	301	245	176
Water holding capacity, %	31.6	33.3	30.0
Texture	Clay loam	Silt loam	Loam

[1] Available P and K in soil samples extracted using the CAL method (Schueller, 1969).

[2] DTPA, Diethylene triamine pentaacetic acid.

[3] Cation exchange capacity.

Table 2. Physicochemical characteristics of wheat straw, charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) used in the experiment.

Parameter		Straw	Charcoal	HTC	LTC
Ash content, mg g^{-1}		42.4	35.8	28.4	19.7
Mobile matter, mg g^{-1}		24.8	5.0	19.6	2.7
Brunauer-Emmett-Teller (BET) Surface area, m^2 g^{-1}		1.8	201.2	9.8	4.1
Carbon	**Total C, mg g^{-1}**	424.0	881.0	510.0	265.0
	Organic C, mg g^{-1}	424.0	881.0	510.0	261.0
Nitrogen	**Total N, mg g^{-1}**	9.4	3.1	5.9	34.9
	Heterocyclic N, mg g^{-1}	1.9	3.0	3.9	29.1
	Total hydrolyzable N, mg g^{-1}	6.8	0.1	1.5	4.3
	[4]Ammonium-N, amide-N, amino sugar-N, mg g^{-1}	3.4	0.7	0.5	2.5
	[4] α-amino acid N, mg g^{-1}	2.1	0.0	0.0	0.5
S, mg g^{-1}		3.4	4.1	2.1	6.5
K, mg g^{-1}		13.8	3.2	1.4	8.2
Na, mg g^{-1}		1.9	0.0	0.3	2.0
Ca, mg g^{-1}		5.3	2.4	9.8	70.8
Mg, mg g^{-1}		0.8	0.8	1.4	9.9
Cu, mg kg^{-1}		16.7	12.8	16.9	665.2
Mn, mg kg^{-1}		19.8	373.0	730.2	390.9
Fe, mg kg^{-1}		92.0	57.5	6954.6	33442.9
Zn, mg kg^{-1}		11.2	4.5	155.2	2149.3
P, mg g^{-1}		1.7	0.3	0.4	38.2

[4] the fractions of total hydrolyzable nitrogen.

2.2. Physicochemical analyses

Before the start of incubation experiment, soils and organic C compounds (wheat straw and biochars) were analyzed for physicochemical characteristics.

2.2.1. Pre-incubation soil analysis

Soil pH was measured in 0.01 M $CaCl_2$, total N and total C were measured using an elemental analyzer (Vario EL-III). Available P and K in soil samples were extracted using the CAL method (Schüller, 1969). Exchangeable cations and CEC were analyzed according to Mehlich (1948). Soil Fe and Al were determined according to Tamm (1932).

2.2.2. Biochars and wheat-straw characterization

2.2.2.1. Physical analyses

The volatile matter and ash content of straw and biochars were analyzed by determining weight loss of materials after drying at 450°C and 550°C for 0.5 h, respectively (McLaughlin, 2009). Specific surface area of the biochars and straw was determined using N_2 adsorption (at 77 K) isotherms (11 point, range p/p0 = 0.05-0.3) on a Quantachrome Autosorb-1. Samples were prepared by de-gassing until the rate of pressure increase by vapor evolution was < 1.3 Pa/min. Specific surface was calculated using the Brunauer-Emmett-Teller (BET) equation according to Gregg and Sing (1982).

2.2.2.2. Chemical analyses

Total C, N, and S were determined with an elemental analyzer (Vario EL- III).

Nitrogen fractions were quantified using a modified method described by Stevenson (1996). This method is used for the determination of organic N forms in soils and composts. Four fractions of N (heterocyclic N; total hydrolyzable N; sum of ammonium, amide, and hexose amine N; α-amino N) were determined with the Kjeldahl method after hydrolyzing the biochars and straw in 6 M HCl.

Briefly, 1 g material was weighed into Duran flasks of 100 mL capacity and 50 mL of 6 M HCl were added in each flask and heated in an oven at 160°C for 20 h. After hydrolysis, the

suspension was filtered through blue ribbon filter (589/3 ash-less, Schleicher and Schuell Microscience Co. Germany). The first fraction was the N present in the material and remained on the filter (non-hydrolysable called as heterocyclic N) and this was determined using Kjeldahl digestion followed by steam distillation with NaOH.

The second fraction (total hydrolyzable N) was analyzed using Kjeldahl digestion of filtered-hydrolysate followed by distillation with NaOH.

The third fraction (ammonium, amide, and hexose amine N) was obtained by steam distillation of hydrolysate with 6 M KOH (without Kjeldahl digestion).

The fourth fraction (α-amino N) was obtained from the hydrolysate material remaining after getting the third fraction. The hydrolysate material remaining after getting the third fraction was adjusted to pH 2.0 with HCl, 500 mg ninhydrin was added, and the suspension was boiled in a water bath for 30 min. After that, N was determined using steam distillation with 6 M NaOH.

For the analysis of other elements, biochars and straw were digested using a digestion mixture (HNO_3 65 %/ $HClO_4$ 70 %/ H_2SO_4 98 % at a ratio of 40 : 4 : 1) and filtrates were analyzed for P using the vanadate yellow method with a spectrophotometer. Potassium, Ca, Mg, Na, Fe, Cu, Mn, and Zn were measured with AAS (Rosopulo, 1985).

2.2.2.3. Fourier transform infrared spectroscopy (FTIR)

The chemical characterization of wheat straw and biochars was performed with Fourier transform infrared (FTIR) spectroscopy (Tatzber et al., 2007). For this, 1 mg of sample was mixed with 200 mg KBr and finely ground. The pellets were made by pressing a mixture of sample and KBr under a pressure of 10 t cm^{-2}. The spectra were recorded in the 4000 to 400 cm^{-1} range with a Brucker IFS 48 spectrometer.

2.3. Experiment 1. Long-term soil incubation of various C compounds

An incubation experiment was conducted to study the kinetics of C mineralization of straw, charcoal, HTC, and LTC along with a control (non-amended soil) in three soils.

2.3.1. Application rates of treatments and nutrient elements

Application of C in form of various C compounds was equivalent to the C content of 50 t charcoal ha^{-1} (Table 3). Detailed description of treatment rates is given in Table 3. Other nutrients (N, P, K, and Mg) were also maintained considering their concentration in applied C compounds.

2.3.2. Incubation conditions

Calculated amounts of C compounds (Table 3) were mixed with 900 g soil (air-dried ≤ 4 mm) and the mixture was filled into plastic pots. Moisture was adjusted to 60% water-holding capacity of the soils. Each treatment was replicated 16 times at the start of experiment. After 5 d and 365 d of the incubation period, three replicates of each treatment were taken for further soil analyses. Two pots of each treatment were placed in a closed bucket along with a beaker containing KOH to capture CO_2 released during the incubation (Figure 1).

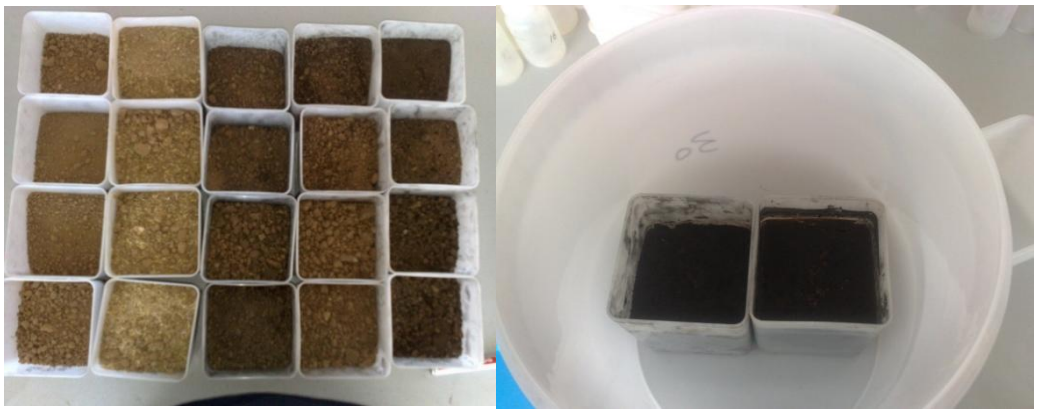

Figure 1. Incubation pots containing mixtures of soil and C compounds (left), and placed in a closed bucket (right).

Table 3. Experiment 1. Application rates of treatments and nutrients. The salts (NH_4NO_3, $Ca(H_2PO_4)_2$, KCl and $MgSO_4$) were used to maintain the differences of nitrogen, phosphorus, potassium and magnesium in treatments.

Treatments	Compound application rate	Applied C	Nitrogen		Phosphorus		Potassium		Magnesium	
			from treatment	from NH_4NO_3	from treatment	from $Ca(H_2PO_4)_2$	from treatment	from KCl	from treatment	from $MgSO_4$
	g kg^{-1} soil	g C kg^{-1} soil	g kg^{-1} soil		g kg^{-1} soil		g kg^{-1} soil		g kg^{-1} soil	
Control	0	0	0	1.49	0.00	1.63	0.00	0.37	0.00	0.42
Straw	26.66	11.29	0.25	1.24	0.04	1.58	0.37	0.00	0.02	0.40
Charcoal	12.82	11.29	0.04	1.45	0.01	1.62	0.04	0.33	0.01	0.41
HTC	22.15	11.29	0.14	1.35	0.01	1.62	0.03	0.34	0.03	0.39
LTC	42.57	11.29	1.49	0	1.63	0.00	0.35	0.02	0.42	0.00

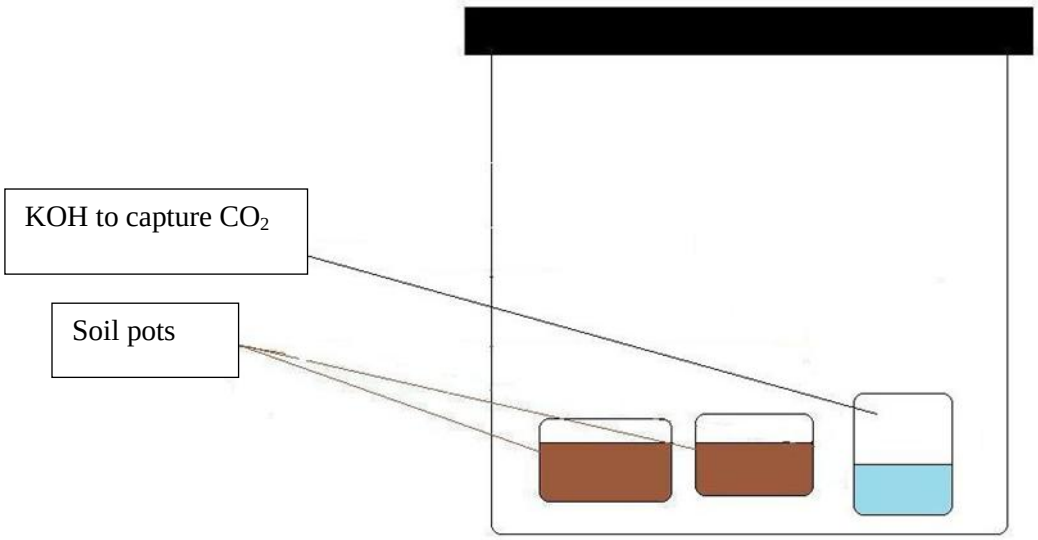

Figure 2. Model of the incubation chambers used in experiment.

For the first 5 d of incubation, 100 mL of 2 M KOH were placed in a beaker inside the bucket and then after each replacement, 100 mL of 1 M KOH was placed (Figure 2). The buckets were placed in a climate chamber at 26°C and KOH in the buckets was replaced after 5 d, 15 d, and 30 d and after each consecutive month.

2.3.3. Determination of CO_2 evolved

Sampled KOH was back-titrated against 0.1 M HCl using phenolphthalein as indicator in the presence of excess of $BaCl_2$ solution (Hopkins, 2008). The cumulative CO_2 release and C mineralization kinetics were calculated based on the amount of CO_2-C released during incubation.

2.3.3.1. Calculations

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O \qquad \text{(Equation 1)}$$

$$BaCl_2 + K_2CO_3 + KOH + HCl \rightarrow 3KCl + H_2O + BaCO_3 \qquad \text{(Equation 2)}$$

For each mole of CO_2 trapped in the KOH, two moles of KOH are converted to K_2CO_3. Therefore, the total CO_2 produced was twice the depletion of KOH in the trap. $BaCl_2$ was

added in titration flasks to precipitate the K_2CO_3. The KOH remained unused was determined through back-titration against acid.

$$CO_2 in\ trap = 0.5 \times (\frac{V_{KOH} \times C_{KOH}}{1000}) - \frac{V_{HCl} \times C_{HCl}}{1000} \times 100 \times 44 \times 1000 \qquad \text{(Equation 3)}$$

Where

- V_{KOH} = volume of KOH inside beaker
- C_{KOH} = concentration of KOH
- V_{HCl} = volume of acid used in titration
- C_{HCl} = concentration of acid used for titration
- 100 = dilution factor, 44 = molar mass of CO_2, 1000 = to convert into mg

Based on cumulative CO_2 production during incubation, the loss of C in the control treatment (un-amended) at day 365 was subtracted from the total C loss of the respective treatment (soil C loss + applied C loss). Net C mineralized (NCM) in biochars and straw was calculated according to Ribeiro al. (2010).

$$NCM, \% = \frac{CMC_{treatment(365d)} - CMC_{control(365d)}}{organic\,C_{applied}} \times 100 \qquad \text{(Equation 4)}$$

where CMC is the cumulative C mineralized in form of CO_2-C released during incubation. This approach assumes that there was no priming effect.

2.3.4. Carbon-mineralization kinetics

A single first-order equation and double-exponential equation were used to describe C mineralization kinetics. The single first-order kinetic equation is one of the most commonly used equations for organic matter mineralization in soils (Ajwa & Tabatabai, 1994; De Neve et al., 1996; Saviozzi et al., 1997; Sparks, 1989; Tian et al., 1992). In its simplest form, it is assumed that C mineralization is proportional to the amount of available C at any time (t).

$$\frac{dC}{dt} = -kC \qquad \text{(Equation 5)}$$

and in integrated form, it can be written as

$$C_t = C_o(1-e^{-kt}) \qquad \text{(Equation 6)}$$

or

$$\ln C_t = -kt + \ln C_o \qquad \text{(Equation 7)}$$

The half-life of C in soil can be calculated by

$$C_{\frac{1}{2}} = \frac{\ln(2)}{k} \qquad \text{(Equation 8)}$$

where C_t is the amount of C at time *t*, C_o the potential available C at time *t*, and *k* is the apparent rate constant.

The values of kinetic parameters of Equation 6 were determined using regression analysis of $\ln C_t$ against time t (Ajwa and Tabatabai, 1994) using SPSS 18.0.

The double-exponential equation was also used according to Liang et al. (2008).

In a general integrated form, the equation is written as

$$C_t = C_1[(1-e^{-k_1 t}) + C_2(1-e^{-k_2 t})] \qquad \text{(Equation 9)}$$

Non-linear regression using a double-exponential model (Sigma-plot 11.0, tolerance $1e^{-10}$, stepsize 100, and iterations 1200) was performed to mathematically define the size and turnover rate of C_1, which is conceptually corresponding to a smaller and easily mineralizable C pool of higher turnover rate (k_1); and C_2 which is a large stable pool with a slow turnover rate (k_2) comprising stable C.

The slow turnover rate value (k_2) was used in calculating the half-life ($t_{1/2}$) of the most stable C fraction by using Equation 8. Both of the kinetic equations (single and double-exponential equation) were compared based on the adjusted r^2 (coefficient of determination).

2.3.5. Soluble organic carbon and inorganic nitrogen

Soil samples taken after 5 d, 365 d, and 730 d of incubation were dried at 40°C, ground to pass a 2 mm sieve, and stored in plastic bottles for analyses. All replicates of each treatment were analyzed twice for 0.01 M $CaCl_2$-extractable C_{org}, NO_3-N, and NH_4-N using an autoanalyzer (Braan and Luebbe, Germany). Ten g soil were extracted with 100 mL of 0.01M $CaCl_2$ in 250 mL plastic bottles for 2 h (Houba et al., 1986). After shaking, the filtrates were analyzed with the autoanalyzer.

2.3.6. Soil organic matter density-fractionation

The soil samples taken after 365 d of incubation were dried at 40°C, ground to pass a 2 mm sieve, and analyzed for the SOM density fractions according to Sohi et al. (2001). Briefly, for one essay, six polycarbonate centrifuge bottles were prepared, each containing 15 g soil, and 90 mL NaI solution at a density of 1.80 g cm^{-3} (determined using hydrometer). To isolate the free fraction (FF), the bottles were swirled by hand for 30 s and centrifuged at 8000 g for 30 min. After centrifugation, the floating material was drawn from each bottle using a plastic pipette attached to a vacuum flask and pump. The material collected was filtered through a pre-weighed glass fiber filter (type GF/A, 47 mm, Whatman, Germany) connected with a Millipore vacuum filtration unit. The glass filter containing filtered material was rinsed with double-distilled water, separated from the filtration system carefully, and collected in glass crucibles. Before rinsing of glass filter, the flask containing the filtrate was separated from the filtration unit and an empty flask was connected to collect the washing material. The filtrates were returned into the respective centrifuge bottles. The fraction retrieved after this procedure was designated as free fraction (FF).

Figure 3. Process of soil organic matter density fractionation. From left to right: placing glass filter on vacuum filtration unit, filtration of material, collection of fraction.

After getting the FF, the bottles were placed in large beakers with crushed ice filled around the bottles. The lids of bottles were opened carefully to avoid ice drop into bottles. To release intra-aggregate fraction (IAA) from the soils the materials were sonicated at 58 W for 195 s using a Sonopuls GM 70 Sonicator (Bandeln, Germany) equipped with sonotrode SH 70 G. The required energy of sonication was achieved by using a frequency of the sonicator at 85 % for 195 s. After completion of sonication, the bottles were centrifuged at 8000 g for 30 min and filtration was done using a similar methodology as for FF.

The heavy fraction (HF) was the material that remained in bottles after removing IAF was washed with double-distilled water, and a small amount was taken on a glass petri dish.

All fractions (FF, IAF, and HF) were oven-dried at 40°C, weighed, finely ground using hard pestle and mortar, and kept for further analysis.

2.3.7. Elemental analyses and FTIR of the soil density-fractions

The total carbon (TC) and total nitrogen (TN) concentration in the bulk soil samples and soil density fractions were determined with an elemental analyzer (Vario EL-III, Germany). The TC contents in the SOM density fractions were calculated by multiplying their respective TC concentrations with the dry weights (Liang et al., 2010). The amounts of TC increased in the SOM density fractions relative to control were regarded as stabilized C. The proportion of stabilized C among various fractions was calculated as,

$$\%\,of\ total\ stabilized\ C\ in\ a\ fraction = \frac{(Fraction\,TC_{(C\,compound)} - Fraction\,TC_{(control)})}{(Bulk\ soil\,TC_{(C\,compound)} - Bulk\ soil\,TC_{(control)})} \times 100 \qquad \text{Equation 10}$$

The C compounds before incubation, soil density fractions, and bulk soil samples after incubation were scanned using Fourier Transform Infrared (FTIR) spectroscopy. For FTIR, a mixture comprising 1 mg sample and 200 mg KBr was finely ground and pellets were made under a pressure of 10 t cm^{-2} with an electrohydraulic press (Perkin-Elmer, Germany). The FTIR spectra were recorded with a Brucker IFS 48 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) in the range from 4000 to 400 cm^{-1} with 32 scans per spectra. Mean spectrum of three replicates were used for interpretation.

Figure 4. Process of Fourier transform infrared spectroscopy. From left to right: grinding of material, pellet formation, infrared spectrum on the computer screen.

The infrared peaks assignments were made according to Cheshire et al. (1993), Cox et al. (1999), Haberhauer and Gerzabek (1999), Madejova and Komadel, (2001), Terhoeven-Urselmans et al. (2010), and Steinbeiss et al. (2009).

2.4. Experiment 2. Effect of soil pH on C mineralization of straw and hydrothermal carbonization coal (HTC)

2.4.1. Treatments

To investigate the effect of soil pH on C mineralization, a low-pH soil (Ferralsol, pH = 5.5, was selected (Table 1). Wheat straw and HTC were used as source of C. the treatments included pH levels (5.5 and 7.2), and C compounds (control, straw, and HTC). The experiment was based on complete randomized design (CRD) with four replications. Detailed description of treatments and nutrients is given in Table 4.

Table 4. Experiment 2. Application rates of treatments and nutrients.

Treatments	Treatment Rates	Carbon	Nitrogen (NH_3NO_3)	Phosphorus ($Ca(H_2PO_4)_2$	Potassium (KCl)	Magnesium ($MgSO_4$)
	g 50 g^{-1} soil		mg 50 g^{-1} soil			
Control	0	0	212.2	330.2	35.1	214.5
Straw	1.33	0.56	176.6	321.3	0	204.3
HTC	1.11	0.56	193.5	328.3	32.2	198.4

2.4.2. Incubation conditions

For incubation, soil was air-dried and ground to pass through a 2 mm sieve. Fifty g soil were weighed in Petri dish and the treatments and nutrients (N, P, K, and Mg) were mixed with the soil. The calculation of nutrients was made in such a way that each treatment and soil had the same concentration of nutrients.

The treatments were calculated based on carbon content equivalent to 50 tons charcoal per hectare (Table 4). The Petri dish containing soil was placed in the glass jar containing 20 mL of 1 M KOH (Figure 5). Moisture was adjusted to 60 % water-holding capacity of the soil.

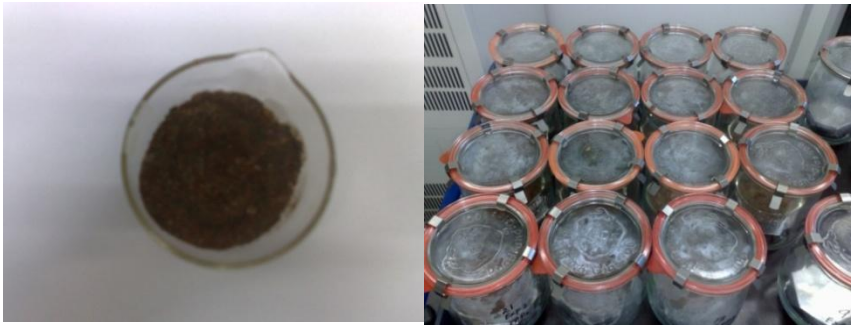

Figure 5. Petri-dish containing mixture of soil and treatment (left), incubation jars containing petri dishes and alkali for CO_2 absorption (right).

2.4.3. Determination of the soil pH-buffer curve for base requirement

The amount of NaOH required to increase pH of the Ferralsol was determined with a pH buffer curve drawn at various concentrations of NaOH added to soil (Figure 6).

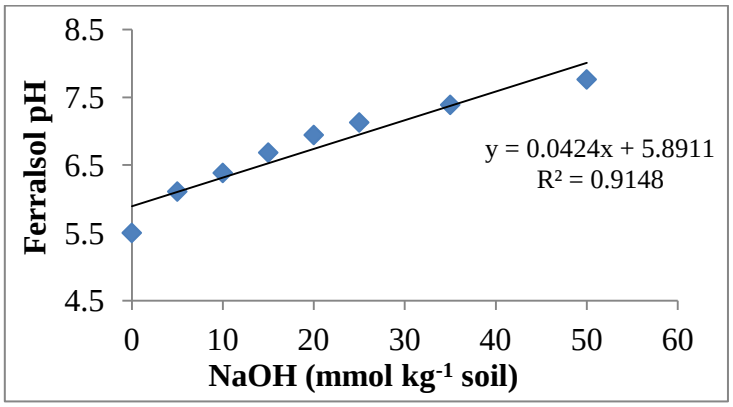

Figure 6. The pH buffer curve of the Ferralsol.

2.4.4. Determination of CO_2 evolved

After scheduled time intervals, KOH from glass jars was replaced with newly prepared solution and Petri dishes were weighed to check water loss. The glass jars were closed air tight and placed in a climate chamber. After scheduled period of incubation, the KOH samples were taken from the glass jars and titrated against 0.1 M HCl. The KOH samples were diluted ten times and 10 mL were taken for titration against 0.1 M HCl using phenolphthalein as indicator. The calculations were made according to equation 3.

2.5. Experiment 3. Biochar ageing in soils affects mineralization of added straw.

2.5.1. Description of treatments

1. **Treatments** = control, straw, straw + charcoal, straw + HTC, straw + LTC
2. **Residence times** =
    1. straw and biochars applied fresh to the soils
    2. straw applied to biochar-aged soils
3. Soils = Ferralsol, Luvisol topsoil, and Luvisol subsoil
4. Replications = three

To obtain the described experimental layout, the following steps were involved,

2.5.1.1. Residence time of 365 d (biochars aged in soils)

The detailed description of the treatments and their application rates is documented in Table 5. The calculated amounts of charcoal (50 t ha^{-1}), HTC, and LTC (equivalent to C of 50 t charcoal ha^{-1}) were mixed with 900 g soil, filled into pots and incubated for 365 d along with a control (soil without C additions).

All treatments were replicated nine times to have enough numbers of soil pots. In each pot, the soil moisture was adjusted to 60% water-holding capacity of the soils. After the residence time of 365 d, three replicates of each treatment were used for soil analysis. The remaining soil-incubation pots were regarded as 365 d-aged soils.

2.5.1.2. Residence time of 0 d (biochars applied fresh to soils)

After 365 d of first incubation, an experimental setup similar to the first incubation experiment was prepared using fresh biochars and soils, and the pots were regarded as fresh-amended soils.

Table 5. Experiment 3. Application rates of treatments and nutrients.

Treatments	**Carbon source**		**Nitrogen**		**Phosphorus**		**Potassium**		**Magnesium**	
	Wheat straw	biochar	From treatments	from NH_4NO_3	From treatments	from $CaH_2PO_4.H_2O$	From treatments	From KCl	From treatments	From $MgSO_4$
					g kg^{-1} soil					
Control	0.00	0.00	0.00	1.49	0.00	1.63	0.00	0.37	0.00	0.42
Straw	2.05	0.00	0.25	1.23	0.04	1.58	0.37	0.00	0.02	0.40
Straw + charcoal	2.05	12.82	0.04	1.45	0.00	1.62	0.04	0.33	0.01	0.41
Straw + HTC	2.05	22.15	0.13	1.36	0.01	1.62	0.03	0.34	0.03	0.39
Straw + LTC	2.05	42.57	1.49	0.00	1.63	0.00	0.35	0.02	0.42	0.00

2.5.1.3. Application of wheat straw

Wheat straw (8 t ha^{-1}) was mixed with the biochar-amended soils and the control soils (soils without biochars) after 12 months of incubation and filled into incubation pots. In addition, control (no straw, no biochar) was also kept for all soils and both incubation times.

Other nutrients (N, P, K, and Mg) were also applied along with treatments (Table 5). The pots were arranged in a randomized complete block design. Three replicates were used for the C-mineralization experiment.

2.5.2. Determination of CO_2 release during incubation

Soil-incubation pots along with a beaker containing 1 M KOH were placed inside closed buckets. Carbon dioxide released from soils during incubation was trapped in KOH and analyzed by back-titration of KOH with HCl using phenolphthalein as an indicator. Soil carbon mineralization was measured at different intervals of time (5, 5-15, 15-30, 30-45, 45-60, and 60-90 d of incubation).

2.5.3. Calculation of carbon-mineralization kinetics

First-order kinetics was employed to calculate the parameters of mineralization kinetics. The CO_2-C release data were used to calculate the kinetics equation 7.

2.5.4. Quantification of priming effect

The interactive priming effect (PE) of various biochars and straw on C mineralization was quantified according to Hamer et al. (2004). In our case, as an assumption, the values of CO_2-C release in the control treatment (non-amended) were subtracted from all other treatments to obtain CO_2-C release values for straw treatment and straw + biochar treatments. A significant difference between CO_2–$C_{straw\ +\ biochar}$ and CO_2–C_{straw} ($p \leq 0.05$, t-test) was considered a prerequisite to calculate the priming effect (Hamer et al., 2004). Therefore, prior to the calculation of priming effects, statistical analysis was performed to compare C mineralization in straw-amended soils with straw + biochar-amended soils. The priming effect (PE) was calculated according to the following equation:

$$PE,(\%) = 100 \times \frac{(CO_2 - C_{(straw+biochar)} - CO_2 - C_{(straw)}}{CO_2 - C_{(straw)}} \quad \text{(Equation 11)}$$

while, $CO_2 - C_{(straw)} = CO_2 - C_{(straw+soil)} - CO_2 - C_{(un-amended\,soil)}$ (Equation 12)

and $CO_2 - C_{(straw+biochar)} = CO_2 - C_{(straw+biochar+soil)} - CO_2 - C_{(un-amendedsoil)}$ (Equation 13)

2.6. Statistical analyses

Statistical analyses were performed using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL).

2.6.1. Experiment 1

Analysis of variance (ANOVA) and Fisher's LSD test were performed to distinguish the treatments within a soil for

1. TC and TN in bulk soils and soil density fractions
2. CO_2-C mineralization and kinetic parameters

2.6.2. Experiment 2

Carbon mineralization within a treatment at the two-pH levels of Ferralsol was compared using standard t-test.

2.6.3. Experiment 3

Initially, for each soil, a two-way analysis of variance (ANOVA) was performed on the cumulative CO_2 release data and subsequently, the post-hoc test (Tukey HSD) was employed to differentiate between the treatments within each soil and residence time.

For all kinetic parameters, comparison between two residence times within a soil was performed using the t-test using Microsoft Excel 2010.

3. RESULTS

3.1. Biochars and wheat straw characterization

3.1.1. Physicochemical characteristics

The volatile matter and ash concentration were higher in straw as compared to biochars. The LTC biochar had the lowest volatile matter and ash concentration as compared to charcoal, and HTC (Table 2). Total C concentration was higher in charcoal as compared to straw, HTC and LTC. Total N concentration was higher in LTC followed by straw, HTC and charcoal. Interestingly, most of the LTC-N and charcoal-N consisted of heterocyclic N. The greatest surface area was detected in charcoal followed by HTC, LTC and straw. Except for K and C, the highest nutrient concentrations were detected in LTC.

3.1.2. FTIR of carbon compounds

Fourier transform infrared spectroscopy was employed to investigate the chemical composition of the C compounds. Figure 7 shows infrared spectra collected for wheat straw, charcoal, HTC, and LTC. The infrared peak assignments were made according to Haberhauer and Gerzabek (1999), Cheshire et al. (1993), and Steinbeiss et al. (2009). The IR spectrum of HTC was similar to that described by Steinbeiss et al., 2009. In brief, the bands at 3500 to 3300 cm^{-1} and 1650 to 1600 cm^{-1} were attributed to OH stretching and deformations of water. The peaks at 2920 cm^{-1} were interpreted as the CH stretching and at 1050 cm^{-1} - 1000 cm^{-1} as the stretching of C-O of polysaccharides. The bands at 1600 cm^{-1}, 1513 cm^{-1}, and 1420 cm^{-1} were due to the C=C stretching of aromatic rings. The peaks at 800 to 500 cm^{-1} may be attributed to inorganic substances, mainly clay, quartz, and SiO_2. The differences in the C compounds are apparent from the FTIR spectroscopy. In wheat straw and HTC, the bands arising from the major components lignin and carbohydrates were easily detected.

In the charcoal, there were no observable peaks due to the total absorption of infrared radiation. In LTC, there was an accumulation of water, carboxylic groups and many inorganic substances, especially an enhanced absorption of a silica-rich phase, probably quartz coming from sewage sludge. The differences in the spectra of C compounds suggest the removal of easily degradable substances (carbohydrates) in the coals during pyrolysis (Figure 7).

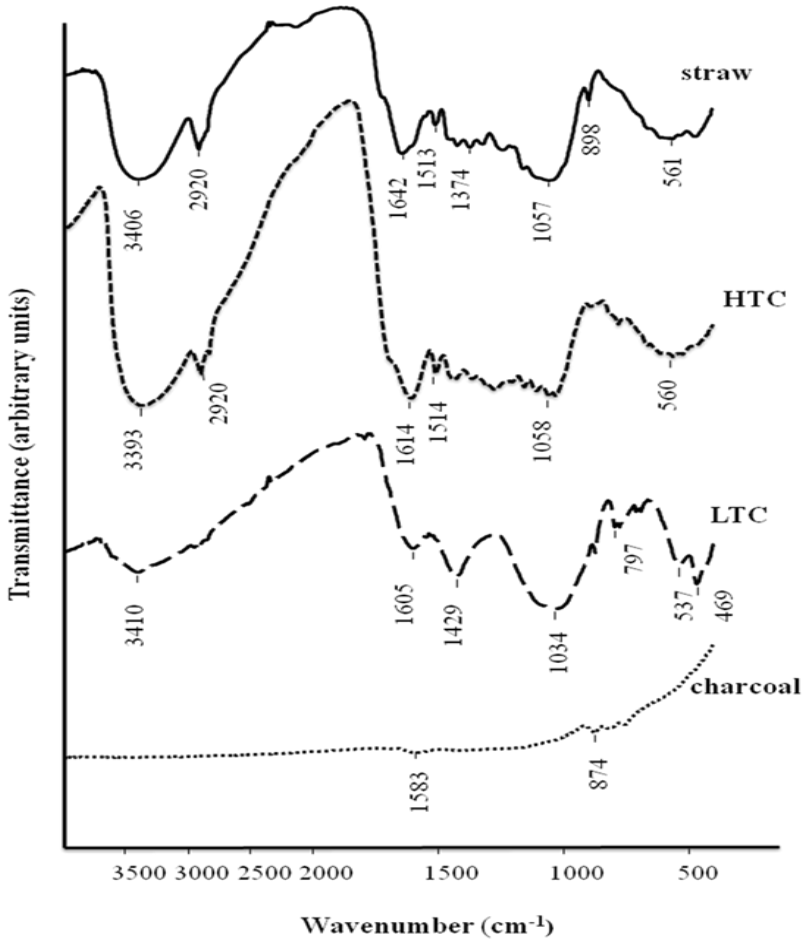

Figure 7. Fourier transform infrared (FTIR) spectra of wheat straw (straw), charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) used in the incubation experiment.

3.2. Experiment 1. Long-term soil incubation experiment

3.2.1. Carbon mineralization

Carbon mineralization determined as cumulative CO_2 release during the incubation period is presented in Figure 8. During the first 5 d of incubation, the CO_2 release was much higher than in the following periods. Charcoal was the most stable compound and did not show any significant C mineralization. In the Ferralsol, the C mineralization followed the sequence straw > HTC > LTC > charcoal = control (Figure 8). However, in the Luvisol topsoil, the LTC treatment showed less cumulative CO_2 after 270 d as compared with charcoal, control, and HTC treatments, and the sequence of C mineralization was straw > HTC > charcoal = control > LTC.

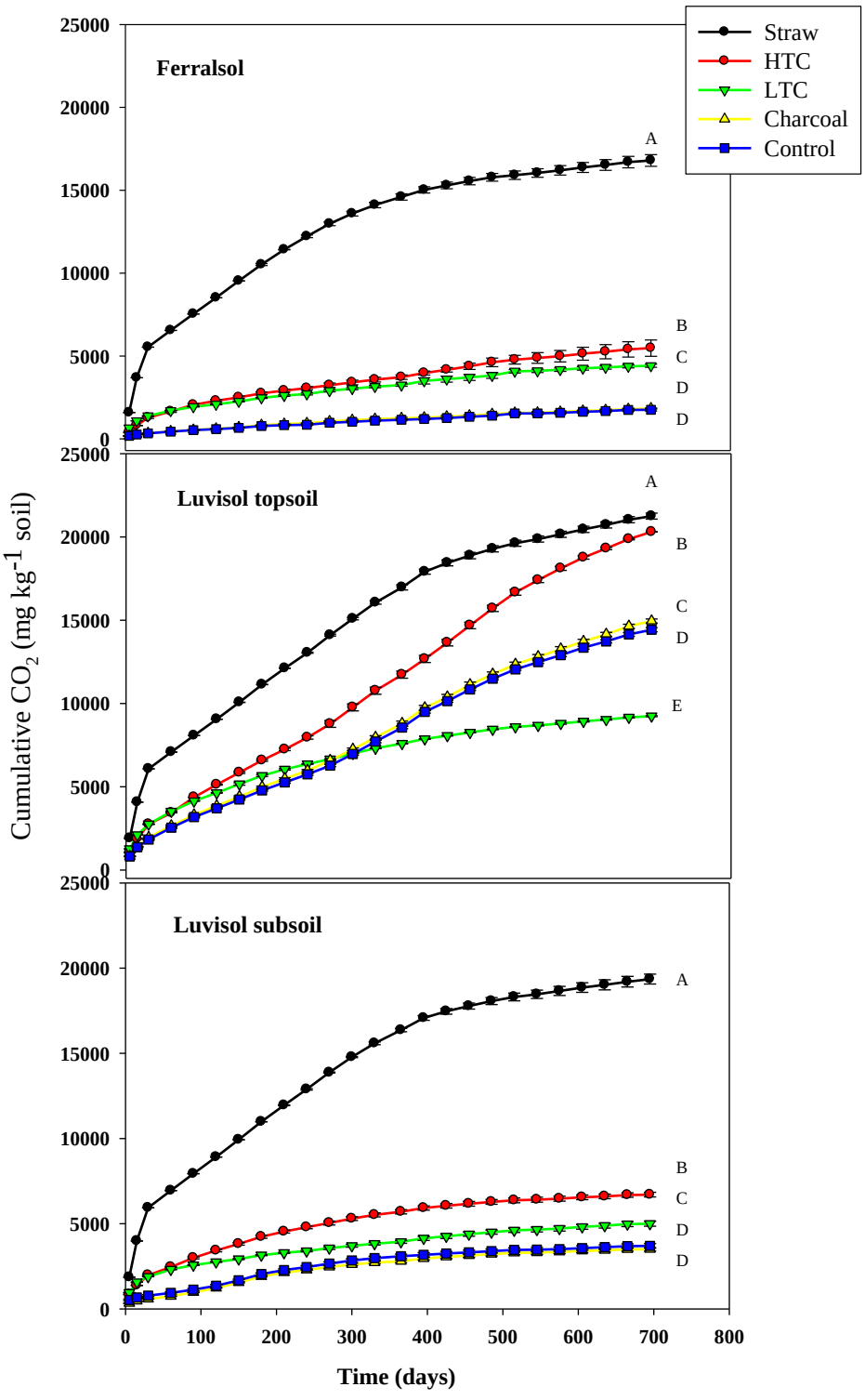

Figure 8. Experiment 1. Cumulative C release (as CO_2) from Ferralsol, Luvisol topsoil, and Luvisol subsoil amended with wheat straw (straw), hydrothermal carbonization coal (HTC), low-temperature conversion coal (LTC), charcoal, and non-amended soil (control) over a period of 730 d. The lines designated with similar letters are not significantly different at P = 0.05 using Fisher's LSD test. The values are means ± SE of three replicates.

Maximum loss of net C was in the straw treatment in all soils (Figure 9). There was no significant difference between HTC and LTC in the Ferralsol, but in the Luvisol topsoil and subsoil, a higher C loss was observed in the HTC treatment. The charcoal treatment showed the lowest C loss in the Ferralsol and a slightly negative loss in the Luvisol subsoil. Similarly, LTC showed a negative C loss in the Luvisol topsoil. The negative loss values in charcoal and LTC indicate that there was SOM stabilization in these treatments (Figure 9).

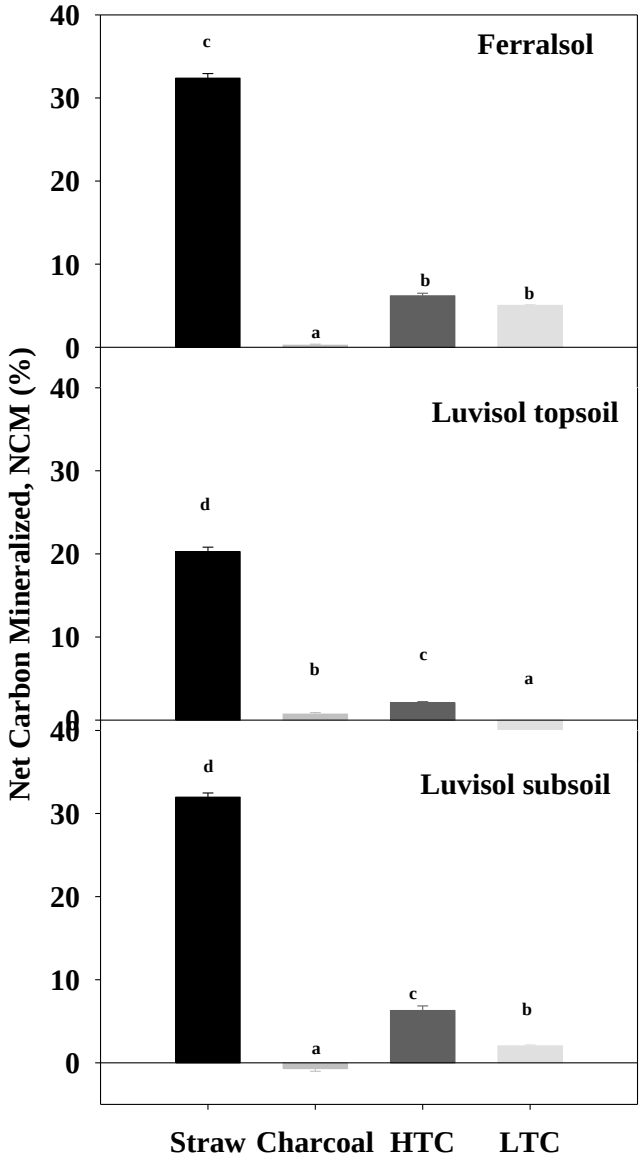

Figure 9. Net carbon mineralization (NCM) during the incubation of Ferralsol, Luvisol topsoil, and Luvisol subsoil amended with wheat straw (straw), charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) over a period of 365 d. The columns with different letters mean that the difference between treatments was significant at P = 0.05. The values are means ± SE of three replicates.

3.2.2. Kinetics of C mineralization

Single first-order and double-exponential equations were employed to describe C mineralization of straw, charcoal, HTC, and LTC in various soils. The degree of conformity between experimental data and the equation-predicted values is expressed by the coefficient of determination (r^2). The double-exponential equation describes the C mineralization better than the single first-order equation (Table 6). The kinetic parameters of C mineralization calculated with the double-exponential equation suggest a biphasic C-mineralization process. In the Ferralsol, for both the charcoal and the control the first phase was very short because of a relatively small mineralizeable C pool (C_1) and rate constants (k_1) were high when compared to other treatments (Table 6). Straw and HTC had higher amounts of C_1 and the first phase was longer with lower rate constants. For k_1, the LTC was not significantly different from other treatments. During the second phase of C mineralization, charcoal, HTC and LTC showed larger pools of relatively stable C (C_2) and the rate constants (k_2) were lower in these treatments as compared to straw and control.

The half-life of C in the Ferralsol calculated based on the slow reaction rate constant (k_2) followed the sequence charcoal > HTC = LTC > control > straw (Table 6). In the Luvisol topsoil during the first reaction phase, rate constants (k_1) of control, charcoal, and HTC were not significantly different and were higher as compared to straw, and LTC (Table 6). During the slow reaction phase, k_2 followed the sequence straw = control = HTC > charcoal > LTC. The half-life (slow reaction phase) of C in the Luvisol topsoil followed the sequence LTC > charcoal > HTC = control = straw. In the Luvisol subsoil during the first reaction phase, straw, charcoal, HTC, and LTC were not statistically different and showed lower rate constants as compared to the control treatment (Table 6). During the slow reaction phase, k_2 values for HTC and LTC were significantly different.

Half-lives of C in the Luvisol subsoil followed the sequence LTC > charcoal = HTC > straw = control. Overall, with the exception of straw the higher half-life of C occurred in the Ferralsol followed by the Luvisol subsoil and Luvisol topsoil.

Table 6. Experiment 1. Kinetic parameters of C mineralization. Parameters of the single first-order equation were calculated by plotting ln Ct against time (t) from data of cumulative CO_2 release over a period of 365 d. In the double-exponential equation, the parameters were obtained by non-linear regression using Sigma plot 11.0. The values within the same column in each soil followed by the same letter in parenthesis are not significantly different at the 5% probability level.

		Single first-order equation				**Double-exponential equation**					
		C_o (g kg^{-1} soil)	***k*** (day^{-1})	**Half-life of C** (years)	**r^2**	**C_1** (g kg^{-1} soil)	***K_1*** (day^{-1})	**C_2** (g kg^{-1} soil)	***K_2*** (day^{-1})	**Half-life of C** (years)	**r^2**
Ferralsol	Control	3.25	2.43E-04 (c)	7.8 (b)	0.963	0.08	0.139 (b)	3.23	2.00E-04 (b)	9.50 (b)	0.995
	Straw	13.75	7.88E-04 (d)	2.4 (a)	0.939	1.39	0.069 (a)	13.22	6.33E-04 (c)	3.01 (a)	0.997
	Charcoal	14.54	5.8E-05 (a)	32.6 (d)	0.977	0.08	0.132 (b)	14.52	5.24E-05 (a)	36.52 (d)	0.995
	HTC	14.40	1.76E-04 (b)	10.8 (c)	0.911	0.42	0.034 (a)	14.16	1.00E-04 (a)	18.99 (c)	0.997
	LTC	14.34	1.40E-04 (b)	13.6 (c)	0.879	0.38	0.079 (ab)	14.20	9.71E-05 (a)	19.59 (c)	0.995
Luvisol topsoil	Control	17.22	3.46E-04 (d)	5.5 (b)	0.986	0.33	0.154 (b)	17.11	3.00E-04 (c)	6.33 (a)	0.996
	Straw	27.85	4.18E-04 (e)	4.6 (a)	0.958	1.38	0.089 (a)	27.37	3.67E-04 (c)	5.26 (a)	0.999
	Charcoal	28.50	2.13E-04 (b)	9.0 (d)	0.983	0.36	0.137 (b)	28.37	2.00E-04 (b)	9.50 (b)	0.998
	HTC	28.20	2.88E-04 (c)	6.6 (c)	0.991	0.48	0.137 (b)	28.26	3.00E-04 (c)	6.33 (a)	0.997
	LTC	28.24	1.77E-04 (a)	10.8 (e)	0.913	0.78	0.056 (a)	27.91	1.00E-04 (a)	18.99 (c)	0.993
Luvisol subsoil	Control	2.63	9.59E-04 (c)	2.0 (a)	0.983	0.13	3.405 (b)	2.61	9.26E-04 (d)	2.05 (a)	0.993
	Straw	13.17	9.27E-04 (c)	2.0 (a)	0.959	1.33	0.090 (a)	12.72	8.00E-04 (c)	2.37 (a)	0.999
	Charcoal	13.95	1.53E-04 (a)	12.5 (d)	0.974	1.27	0.205 (a)	13.94	1.71E-04 (ab)	11.37 (b)	0.986
	HTC	13.69	2.88E-04 (b)	6.6 (b)	0.927	0.56	0.044 (a)	13.42	2.00E-04 (b)	9.50 (b)	0.992
	LTC	13.66	1.68E-04 (a)	11.3 (c)	0.828	0.54	0.086 (a)	13.47	1.00E-04 (a)	18.99 (c)	0.992

3.2.3. Soluble organic carbon and mineral nitrogen

For soluble organic carbon (C_{org}), a statistical comparison was made between incubation times (5 d, 365 d, and 735 d of incubation). In the Ferralsol, straw treatment resulted in a higher amount of C_{org} after 5 d of incubation that was significantly decreased after 365 d but remained non-significant with 735 d of incubation (Figure 10). In the control, and charcoal treatments, there were no significant differences in C_{org} from 5 d to 365 d of incubation but a significant decrease was recorded after 735 d of incubation. The HTC treatment showed no significant change at any stage of incubation. The LTC treatment showed significant decrease of C_{org} along with incubation time. In the Luvisol topsoil, C_{org} was significantly increased in the control, charcoal, and HTC treatments, but in the straw treatment, there was no significant change at any stage of incubation (Figure 10). The LTC treatment showed an increase in C_{org} after 365 d but it was significantly decreased after 735 d of incubation. In the Luvisol subsoil, there was a significant increase in C_{org} in the control, charcoal, and HTC after 365 d of incubation but after 735 d, C_{org} was decreased significantly. In straw and LTC treatments a trend similar to these treatments in the Ferralsol, was recorded.

After 365 d of incubation, in the Ferralsol, incubation of the straw resulted in a strong decrease in NO_3–N and NH_4–N (Figure 11 and Figure 12). In all other treatments, there was no significant change in NO_3–N and NH_4-N from 5 d to 365 d but after 735 d of incubation NO_3-N increased significantly in all treatments.

There was a significant increase in NH_4-N after 365 d in control and charcoal treatments but in HTC and LTC treatments, no significant change was recorded. In the Luvisol topsoil and Luvisol subsoil, there were significant increases in NO_3–N in all treatments except straw. The concentration of NH_4–N decreased significantly in all treatments after 365 d of incubation (Figure 12).

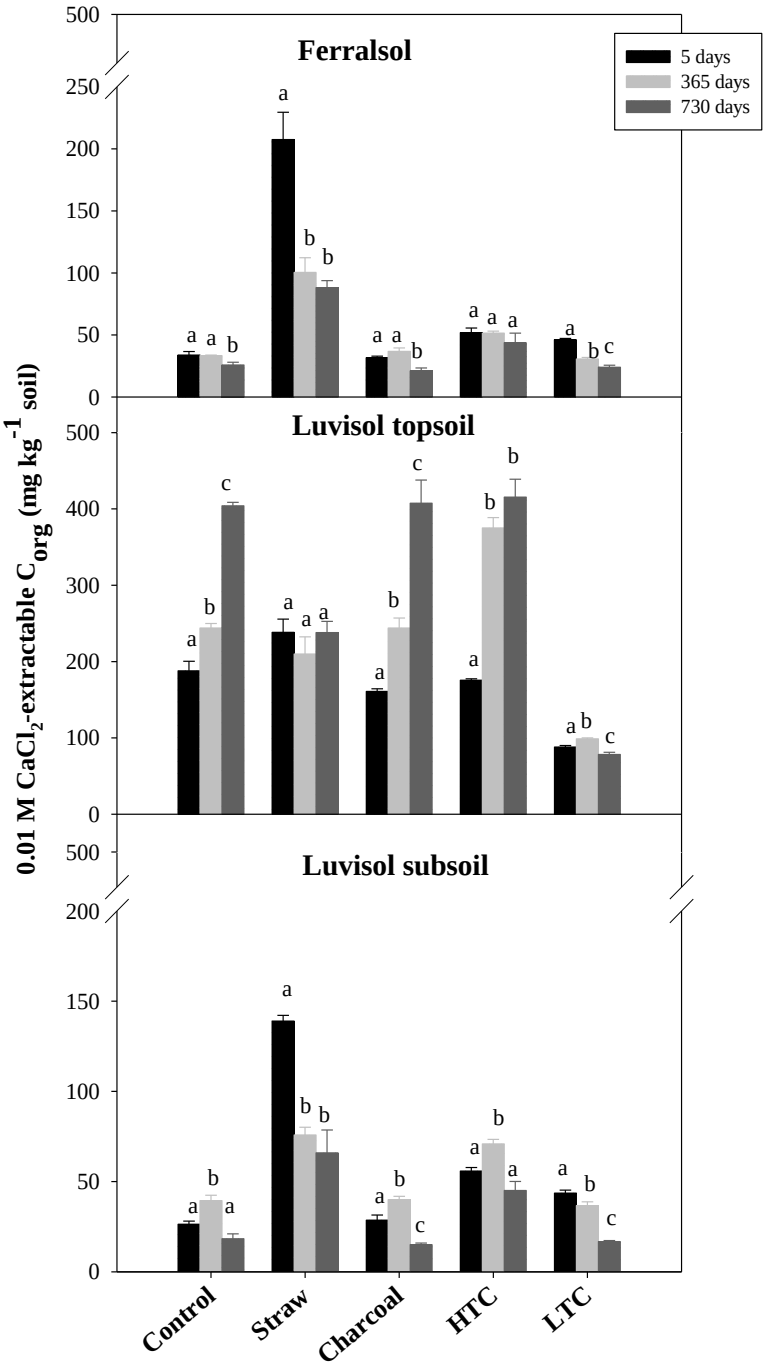

Figure 10. Soluble organic C (0.01 M $CaCl_2$-extractable-C_{org}) in the Ferralsol (mixture of various horizons), Luvisol topsoil, and Luvisol subsoil incubated as non-amended soil (control), with wheat straw (straw), charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) after 5 d, 365 d and 730 d of incubation. The columns with different letters mean that treatments significantly differed (P = 0.05) among three time intervals.

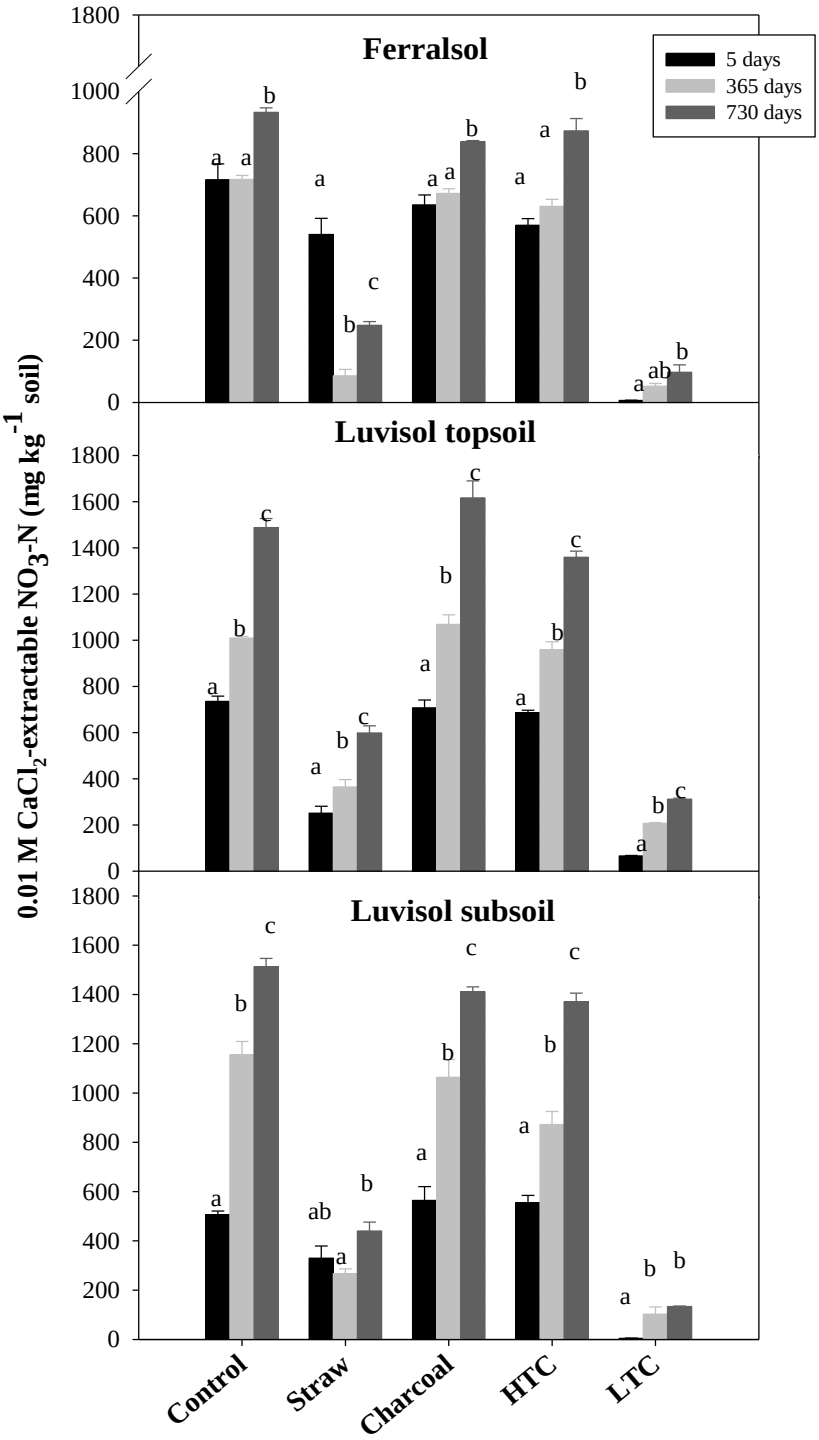

Figure 11. Effect of wheat straw (straw), charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) in comparison to non-amended soil (control), on 0.01 M $CaCl_2$-extractable NO_3-N in the Ferralsol (mixture of various horizons), Luvisol topsoil, and Luvisol subsoil after 5 d, 365 d, and 730 d of incubation. The columns with different letters mean that treatments significantly differed (P = 0.05) among three time intervals.

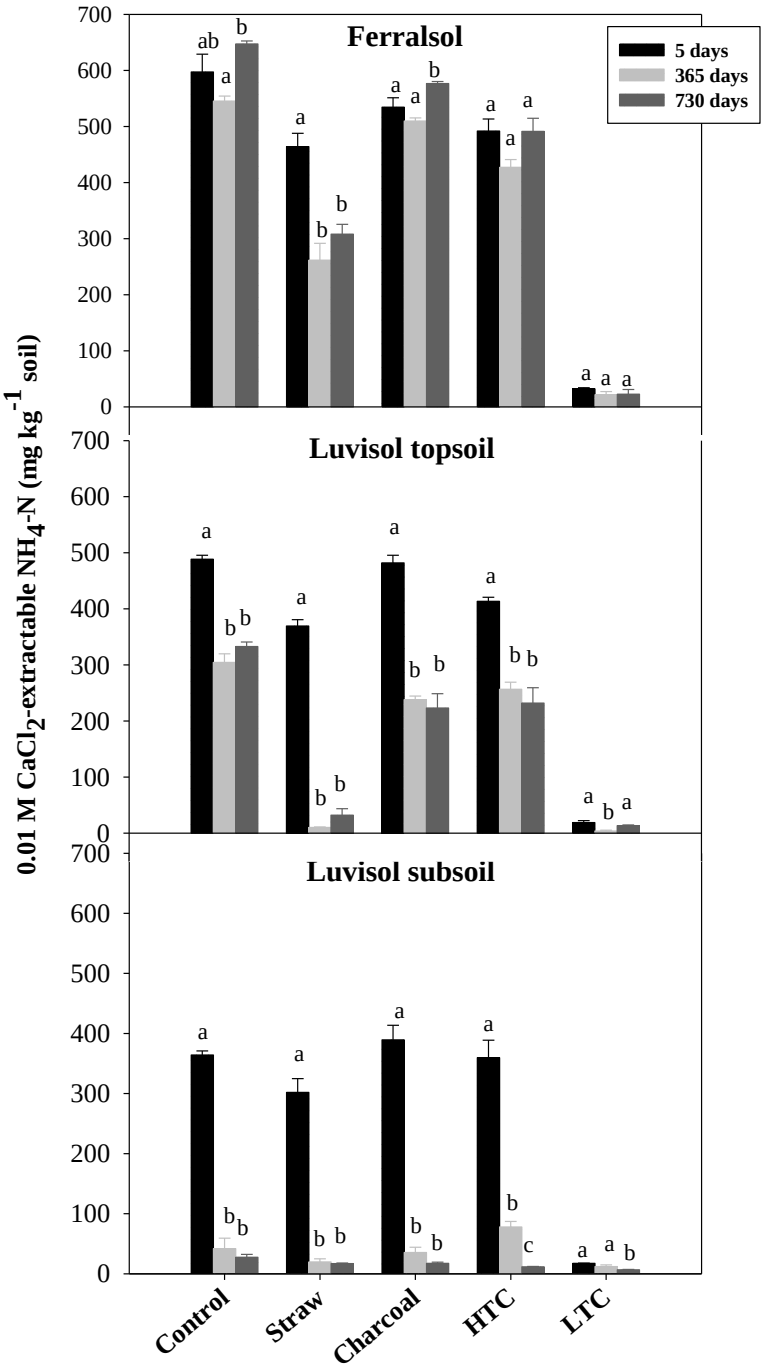

Figure 12. Effect of wheat straw (straw), charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) in comparison to non-amended soil (control) on 0.01 M $CaCl_2$-extractable NH_4-N in the Ferralsol (mixture of various horizons), Luvisol topsoil, and Luvisol subsoil after 5 d, 365 d, and 730 d of incubation. The columns with different letters mean that treatments significantly differed (P = 0.05) among three time intervals.

3.2.4. Total carbon content in soil density fractions

3.2.4.1. Bulk soil

The total carbon (TC) contents in three soils were significantly different, although at the start of experiment, application of C through various C compounds was equivalent in all treatments (11.29 g kg^{-1} soil) except control.

In the Ferralsol, the biochar treatments (charcoal, HTC, and LTC) showed the highest values of TC content (Table 7). The TC content in the straw treatment was similar to that with the HTC and LTC treatments but significantly smaller than charcoal. In the Luvisol topsoil and Luvisol subsoil, the LTC and charcoal treatments showed a maximum increase of TC content over the control (Table 7). The TC contents in the HTC treatment were statistically similar to those of the charcoal and straw treatments in the Luvisol topsoil, and to LTC in the Luvisol subsoil. Overall, the relative increase of TC content of the bulk soil was more pronounced in the Luvisol subsoil compared to the Ferralsol, and Luvisol topsoil.

3.2.4.2. Free and intra-aggregate fractions

In the Ferralsol, the highest TC contents of the FF and IAF were found in the charcoal treatment. In the LTC treatment, TC contents were statistically similar to those with HTC but significantly smaller than with charcoal (Table 7). Straw treatment showed smaller TC values of FF and IAF. In the FF and IAF of Luvisol topsoil, charcoal treatment showed highest values for TC content. The HTC was statistically similar to charcoal in the FF but smaller in IAF. The straw and LTC treatments increased TC content of FF and IAF when compared to the control treatment but less than charcoal and HTC treatments. In the Luvisol subsoil, all biochars showed significantly higher values of TC in the FF and IAF compared to the control. The straw application did not show a significant difference with the control in the FF, but in IAF, a significant increase over control was observed (Table 7).

3.2.4.3. Heavy fraction

In the heavy fraction (HF) of all soils, in LTC treatment the highest relative increase of TC content was found in comparison to the control (Table 7). In the Ferralsol, straw treatment also increased TC content of HF but the increase was smaller than with the LTC treatment.

Table 7. Effect of straw, charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) on the total carbon (TC) content and changes in TC relative to control treatment in the bulk soils and soil density fractions after 365 d of incubation. The values are means of three replicates, ± standard error. For each soil, values followed by different letters in columns are statistically different at $P \leq 0.005$.

Soils	Treatments	Bulk soil		Free fraction		Intra-aggregate fraction		Heavy fraction	
		g C kg^{-1} soil	[a] %	g C kg^{-1} soil	[a] %	g C kg^{-1} soil	[a] %	g C kg^{-1} soil	[a] %
Ferralsol	**Control**	3.3 ± 0.1 c		0.0 ± 0.0 c		0.0 ± 0.0 c		3.2 ± 0.1 c	
	Straw	8.5 ± 0.1 b	157.6	0.4 ± 0.1 c	40.0	1.1 ± 0.2 bc	110.0	4.3 ± 0.3 b	34.4
	Charcoal	14.3 ± 1.3 a	333.3	6.2 ± 1.1 a	620.0	3.7 ± 0.3 a	370.0	4.0 ± 0.4 bc	25.0
	HTC	11.3 ± 0.7 ab	242.4	4.6 ± 0.4 ab	460.0	2.0 ± 0.0 b	200.0	3.9 ± 0.3 bc	21.9
	LTC	11.9 ± 2.6 ab	260.6	3.5 ± 0.8 b	350.0	1.7 ± 1.0 b	170.0	6.5 ± 0.4 a	103.1
Luvisol topsoil	**Control**	14.1 ± 0.1 d		0.5 ± 0.1 c		0.6 ± 0.0 d		12.2 ± 0.4 d	
	Straw	19.9 ± 0.4 c	41.1	2.9 ± 0.3 b	480.0	1.3 ± 0.2 cd	116.7	13.6 ± 0.3 bc	11.5
	Charcoal	26.3 ± 2.5 ab	86.5	6.0 ± 0.3 a	1100.0	5.1 ± 0.6 a	750.0	14.3 ± 0.7 b	17.2
	HTC	21.8 ± 2.7 bc	54.6	5.4 ± 0.8 a	980.0	2.6 ± 0.1 b	333.3	12.8 ± 0.5 cd	4.9
	LTC	28.4 ± 1.6 a	101.4	3.7 ± 0.3 b	640.0	1.8 ± 0.1 bc	200.0	19.7 ± 0.1 a	61.5
Luvisol subsoil	**Control**	2.2 ± 0.1 d		0.0 ± 0.0 b		0.0 ± 0.0 b		1.8 ± 0.2 b	
	Straw	6.4 ± 0.3 c	190.9	1.9 ± 0.1 b	190.0	1.2 ± 0.2 a	120.0	2.2 ± 0.1 b	22.2
	Charcoal	13.6 ± 1.9 a	518.2	8.3 ± 1.8 a	830.0	2.0 ± 0.4 a	200.0	1.5 ± 0.3 b	-16.7
	HTC	9.5 ± 0.4 b	331.8	6.2 ± 0.3 a	620.0	1.5 ± 0.2 a	150.0	1.8 ± 0.1 b	0.0
	LTC	11.6 ± 0.4 ab	427.3	5.5 ± 1.1 a	550.0	1.7 ± 0.4 a	170.0	5.2 ± 1.6 a	188.9

[a] = percent increase or decrease of total carbon content relative to control treatment

3.2.5. Carbon distribution in soil density-fractions

The distribution of stabilized C (increased amount of TC over control treatment) among various soil density-fractions is provided in the Table 8. In all soils, biochars (charcoal, HTC and LTC) showed relatively higher proportions of stabilized TC in the FF (Table 8). The behavior of straw treatment was different in three soils. In the Ferralsol, straw addition resulted in higher proportions of stabilized C in the IAF and HF (21 % of increased TC). While in the Luvisol topsoil and Luvisol subsoil, straw treatment showed a higher proportion of stabilized TC in the FF (41 and 45 % of increased TC). In the Ferralsol and Luvisol topsoil, after stabilization in FF, higher proportions of TC were found in the IAF of all treatments except LTC, in which higher proportions of stabilized TC were found in the HF. In the Luvisol subsoil, only LTC and straw treatments showed positive values of stabilized TC in the IAF and HF. In the LTC and straw treatments, relatively higher proportions of TC were recorded in the HF (Table 8).

Table 8. Effect of straw, charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) on the distribution of stabilized total carbon (TC) content in the soil density fractions after 365 d of incubation. [a] = percent of increased total carbon (TC) content relative to control treatment.

Soils	Treatments	Free fraction	Intra-aggregate fraction	Heavy fraction
		[a] %	[a] %	[a] %
Ferralsol	**Straw**	7.7	21.2	21.2
	Charcoal	56.4	33.6	7.3
	HTC	57.7	25.0	8.8
	LTC	40.7	19.8	38.4
Luvisol topsoil	**Straw**	41.4	12.1	24.1
	Charcoal	45.1	36.9	17.2
	HTC	63.6	26.0	7.8
	LTC	22.4	8.4	52.4
Luvisol subsoil	**Straw**	45.2	28.6	9.5
	Charcoal	72.8	17.5	-2.6
	HTC	84.9	20.5	0.0
	LTC	58.5	18.1	36.2

3.2.6. Fourier transform infrared spectroscopy (FTIR)

3.2.6.1. Bulk soils

In the FTIR spectra of bulk-soil samples, there were no clear differences between control and other treatments. The possible reason for this may be the very low concentration of C in the

bulk soils or the low amount of material used to obtain spectra (1 mg). By increasing the sample amount, noise was increased and spectra became complex and difficult to interpret. Therefore, the FTIR spectra of only control treatments of the three soils are provided in Figure 13. In the Ferralsol, major infrared peaks were observed at wavenumbers 3694, 3620, and 3526 cm^{-1} (due to OH stretching associated with kaolinite clay minerals), 3444 cm^{-1} (characteristic for OH stretching vibrations of water), 1631 cm^{-1} (deformation vibrations of water), 1384 cm^{-1} (N-O stretching of nitrate), 1031 cm^{-1} (Si-O stretching), and 913 cm^{-1} (Al-Al-OH deformation). The FTIR spectra of bulk soils of the Luvisol topsoil and subsoil were almost similar with major infrared peaks at wavenumbers; 3431 cm^{-1} (OH stretching of water), 1636 cm^{-1} (deformation vibrations of water), 1384 cm^{-1} (nitrate), 1031 cm^{-1} (Si-O), and 778 cm^{-1} (quartz) (Figure 13).

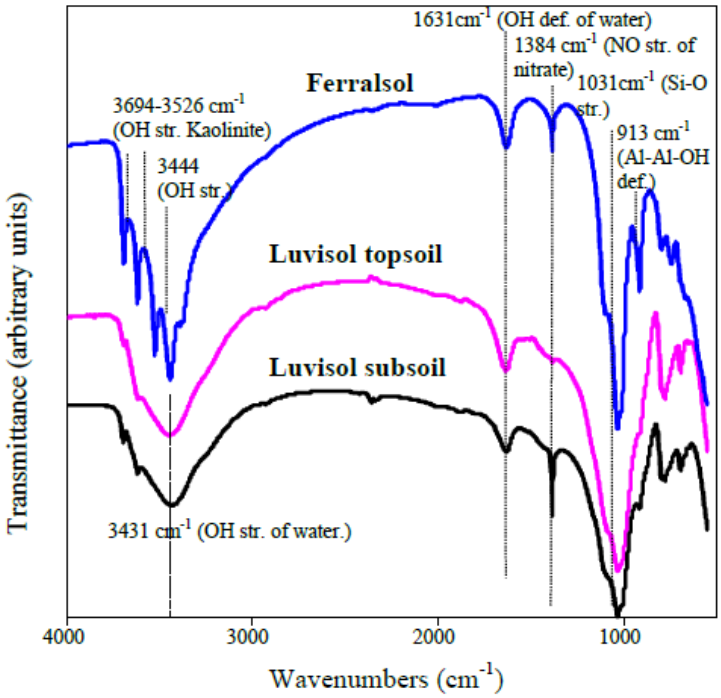

Figure 13. Fourier transform infrared spectra of the control treatments of Ferralsol, Luvisol topsoil, and Luvisol subsoil after 365 d of incubation. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

3.2.6.2. Free and intra-aggregate fractions

Straw

The FTIR spectra of straw without incubation, FF and IAF of straw treatment in three soils are presented in Figure 14. Straw FF derived from all soils showed a decrease in OH stretching vibrations (3410-3440 cm^{-1} in combination with 1642 cm^{-1}) when compared with straw without incubation (Figure 14). There was a decrease in intensity of infrared absorption at 1050 – 1000 cm^{-1} in the three soils indicating loss of polysaccharides in the straw treatment. The C-H stretching vibrations due to aliphatic methyl and methylene groups at wavenumber 2920 cm^{-1} in the straw without incubation were missing in straw FF of Ferralsol and Luvisol topsoil, but present in Luvisol subsoil with decreased intensity.

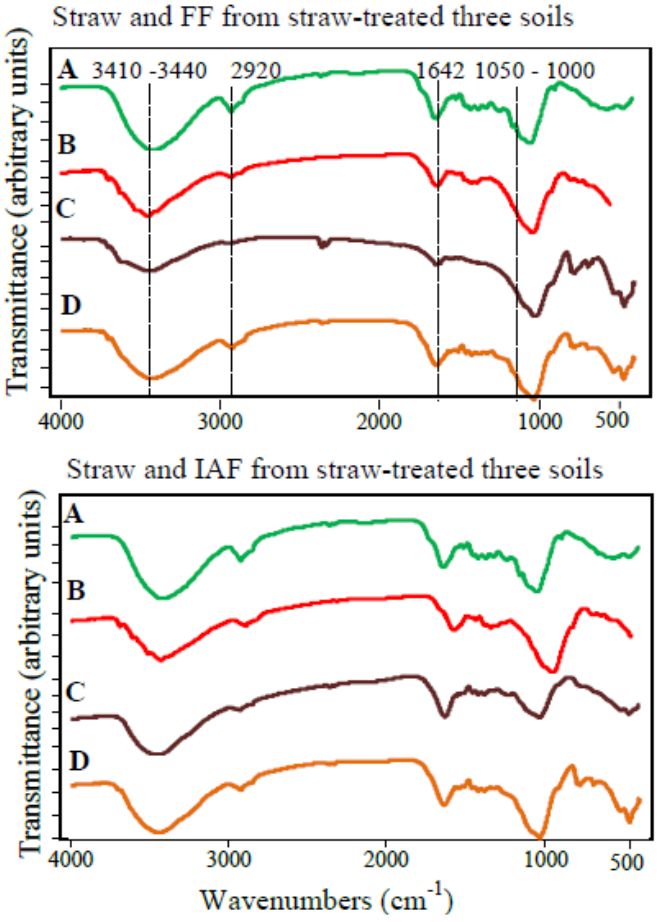

Figure 14. Fourier transform infrared spectra of straw without incubation (A), free fractions, and intra-aggregate fractions retrieved from Ferralsol (B), Luvisol topsoil (C), and Luvisol subsoil (D) after 365 d of incubation with straw. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

There were some new peaks in the straw FF at wavenumbers 2361, 778, 693, and 468 cm^{-1} in Luvisol topsoil and at 532, and 469 cm^{-1} in Luvisol subsoil. In IAF, no differences were observed in the infrared spectra of straw-treated soils (Figure 14).

Charcoal

The FTIR spectra of charcoal without incubation showed highly condensed C with no characteristic band (Figure 15). After 365 d of incubation in soils, the FTIR spectra of charcoal and of FF and IAF were similar in the infrared range 4000-1030 cm^{-1}. The infrared absorption was increased in the fingerprint region (1400 – 500 cm^{-1}) of FF retrieved from charcoal-treated soils. This was due to additions of inorganic components of soils on charcoal surfaces. The FTIR spectra of charcoal and of AIF of Ferralsol and Luvisol topsoil were similar (Figure 15). It seems that only in the Luvisol subsoil inorganic substances of soil were present in IAF of the charcoal treatment.

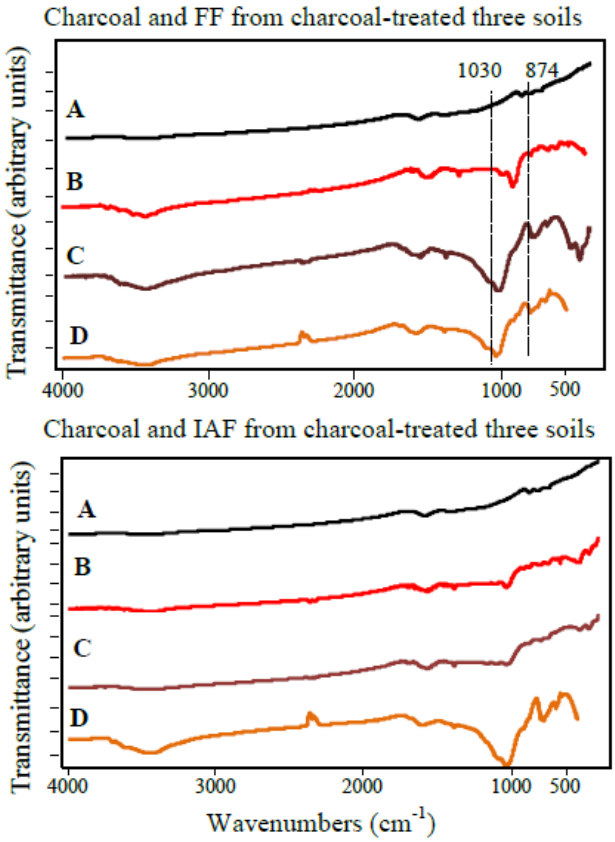

Figure 15. Fourier transform infrared spectra of the charcoal without incubation (A), free fractions, and intra-aggregate fractions retrieved from Ferralsol (B), Luvisol topsoil (C), and Luvisol subsoil (D) after 365 d of incubation with charcoal. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

HTC

The FTIR spectra of HTC without incubation, FF, and IAF are presented in Figure 16. The FTIR spectrum of HTC without incubation showed infrared peaks at 3392 cm^{-1} (OH stretching of water), 2920 cm^{-1} (CH stretching), 1614 cm^{-1} (deformations of water), 1058 cm^{-1} (polysaccharides), and 559 cm^{-1} (inorganic substances). The FTIR spectra of HTC retrieved from soils in the form of FF showed a decrease in absorbance intensity at wavenumber 2920 cm^{-1}, which indicates a decrease of aliphatic methyl and methylene functional groups (Figure 16). There were additional absorbance bands at 1032 cm^{-1} (smectite or Si-O) and 3527 – 3620 cm^{-1} (Si-O-H) in FF of the HTC treatments as compared to spectra of HTC without incubation.

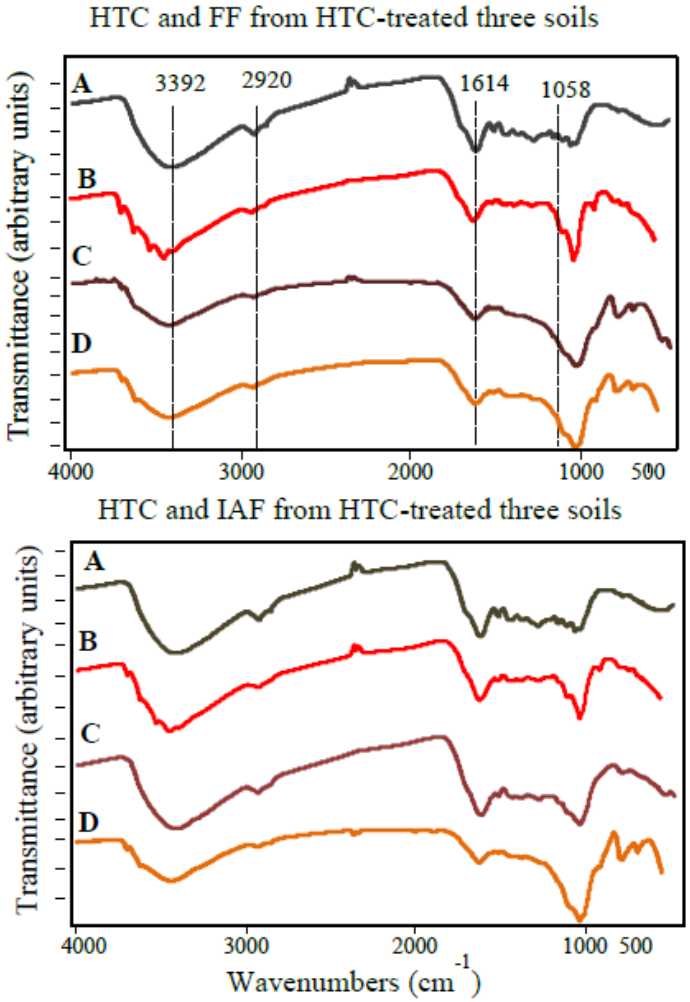

Figure 16. Fourier transform infrared spectra of the hydrothermal carbonization coal (HTC) without incubation (A), free fractions, and intra-aggregate fractions retrieved from Ferralsol (B), Luvisol topsoil (C), and Luvisol subsoil (D) after 365 d of incubation with HTC. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

The intensity of changes was higher in the Ferralsol followed by the Luvisol subsoil and Luvisol topsoil. Similar to the straw and charcoal treatments, no important differences were observed in the IAF collected from HTC treated soils (Figure 16).

LTC

The comparison of the FTIR spectrum of LTC without incubation with the spectra of SOM density fractions from LTC-treated soils is shown in Figure 17. The major infrared peaks of LTC without incubation were at 3428 cm^{-1} (OH str. of water), 1617 cm^{-1} (def. of water), 1435 cm^{-1} (C-O str. of carbonate), 1033 cm^{-1} (Si-O str.), 778 cm^{-1} (carbonate), and 469 cm^{-1} (Si-O-Si). The comparison of the LTC and SOM density fractions from three soils showed an absence of absorbance band at wavenumber 1435 cm^{-1} (carbonate) in the Luvisol topsoil (Figure 17). The intensity of this band at 1617 cm^{-1} was not changed in the FF and IAF of the three soils. The intensity of infrared peak at 1033 cm^{-1} (due to aluminosilicates) was increased in the Ferralsol but unchanged in the Luvisol topsoil and subsoil. Additional peaks in the FF of the Ferralsol at wavenumbers 3694, 3620, and 3525 cm^{-1} were due to kaolinte clay minerals, and in the FF of Luvisol topsoil and subsoil at wavenumbers 778, 693, and 468 cm^{-1} were due to smectite minerals (Madejova, 2003).

The FTIR spectra of the IAF were almost similar to those of FF (Figure 17).

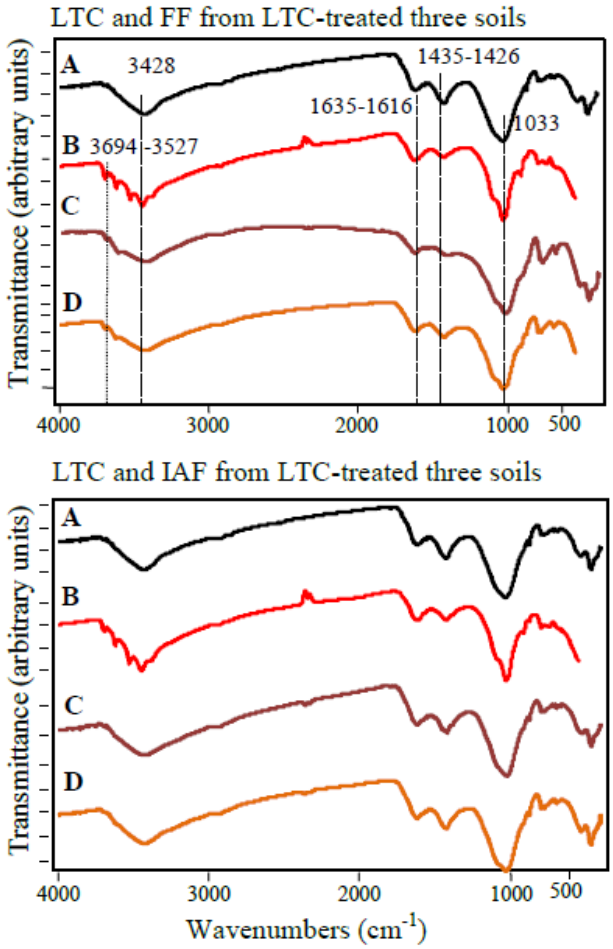

Figure 17. Fourier transform infrared spectra of the low-temperature conversion coal (LTC) without incubation (A), free fractions, and intra-aggregate fractions retrieved from Ferralsol (B), Luvisol topsoil (C), and Luvisol subsoil (D) after 365 d of incubation with LTC. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

3.2.6.3. Heavy fractions

The FTIR spectra of HF from the three soils are presented in Figure 18. The description of major infrared peaks is already provided in Figure 13. By comparing the infrared spectra of the HF, no differences between the treatments within soil were observed (Figure 18). These results suggest no change in HF of soils with the application of C compounds.

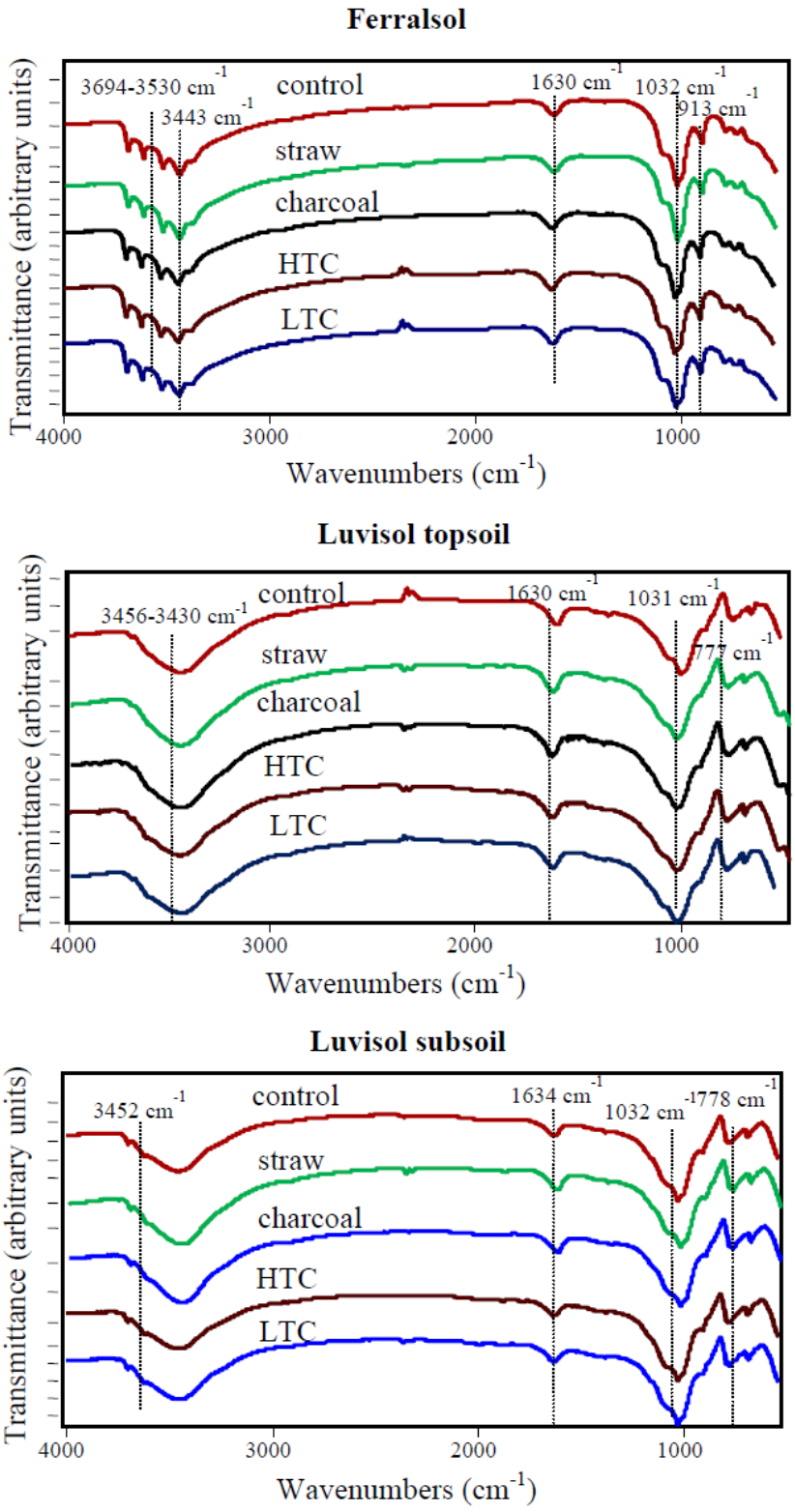

Figure 18. Fourier transform infrared spectra of the heavy fractions retrieved from three soils (Ferralsol, Luvisol topsoil, and Luvisol subsoil) after incubation with control, straw, charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) for 365 d. Each spectral line is the mean spectrum of three replicates. All spectra were recorded using KBr pellets (1 mg sample + 200 mg KBr).

3.3. Experiment 2. Carbon mineralization

In this experiment, data regarding cumulative CO_2 release during soil incubation showed no significant differences for two soil-pH levels. In the control treatment (no carbon compound), carbon mineralization was very low as compared to wheat straw and HTC coal (Figure 19). Comparing wheat straw and HTC coal, wheat straw was mineralized more than HTC coal. However, comparison of two pH levels showed no significant differences for control as well for wheat straw and HTC treatments (Figure 20 and Figure 21).

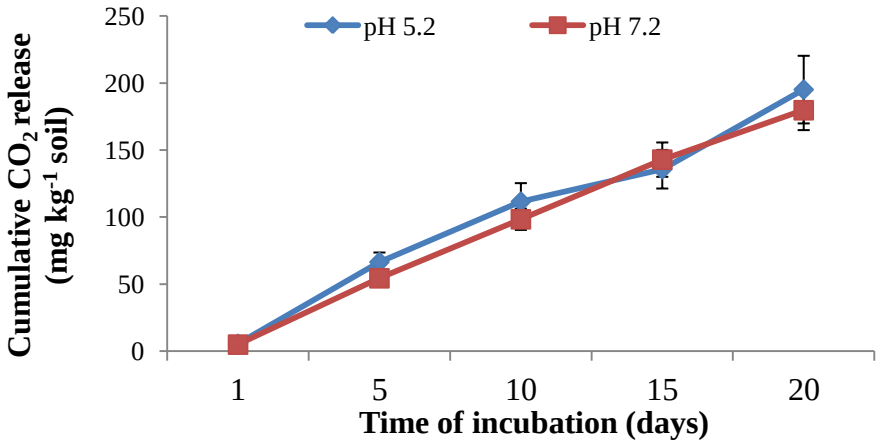

Figure 19. Cumulative CO_2 release from Ferralsol incubated without C amendment at two pH levels. To change the soil pH (from 5.5 to 7.2), 30 mmoles of OH kg^{-1} soil as NaOH were added.

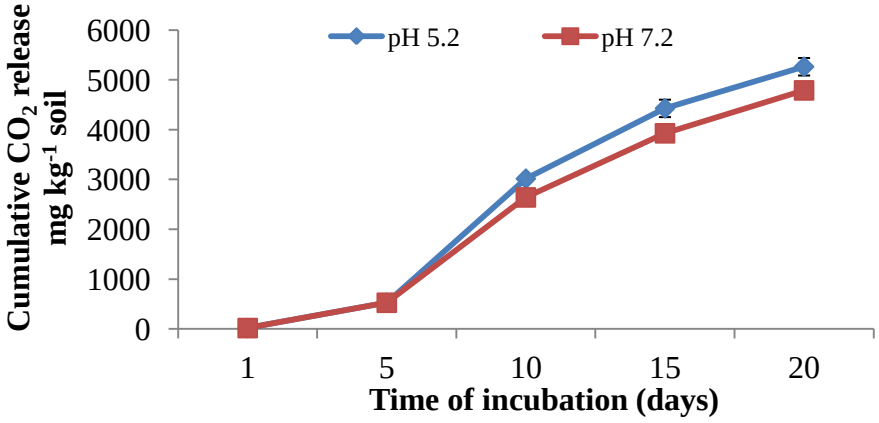

Figure 20. Cumulative CO_2 release from Ferralsol incubated with wheat straw at two pH levels. To change the soil pH (from 5.5 to 7.2), 30 mmoles of OH kg^{-1} soil as NaOH were added.

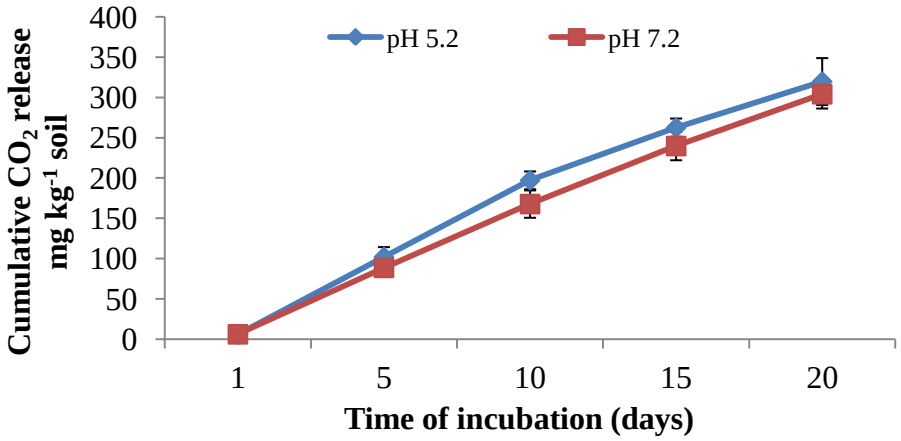

Figure 21. Cumulative CO_2 release from Ferralsol incubated with HTC at two pH levels. To change the soil pH (from 5.5 to 7.2), 30 mmoles of OH kg^{-1} soil as NaOH were added.

3.4. Experiment 3. Biochar ageing in soil improves soil organic matter stabilization

3.4.1. Carbon mineralization

In the Ferralsol, straw application in fresh soil resulted in a higher release of CO_2 from all treatments as compared to 365 d aged soil (Figure 22). In the Luvisol topsoil, there was no significant effect of ageing in the control treatment on CO_2 release. Application of straw in fresh soil resulted in higher CO_2 release as compared to 365 d aged soil (Figure 23). Similarly, straw application in biochar-aged soils also showed lower release of CO_2 as compared to fresh soil. In the Luvisol subsoil, CO_2 release was significantly higher from all treatments in the start of incubation but later on, the results showed no significant difference for charcoal + straw and LTC + straw (Figure 24). For biochar-amended soils, the difference between the two CO_2 release curves (fresh and 365 d aged) was higher as compared to differences in control and straw treatments.

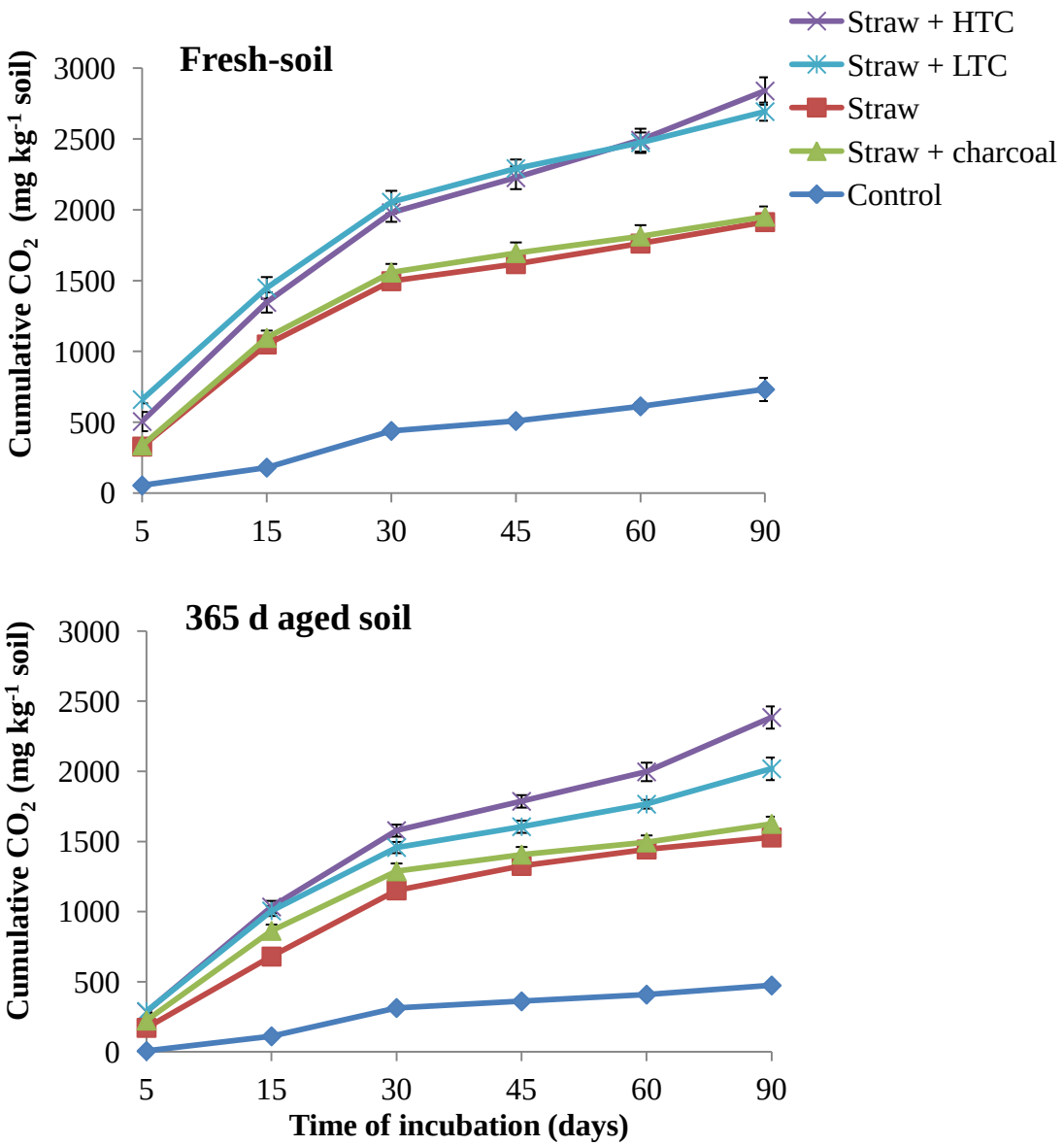

Figure 22. Cumulative release of CO_2-C during 90 d incubation from Ferralsol with straw, and straw mixed with charcoal, HTC or LTC applied to either fresh or aged soil for 365 d. The statistical comparison (t-test) is made between straw alone and straw mixed with biochars. Each data point represents mean ± standard error of three replicates.

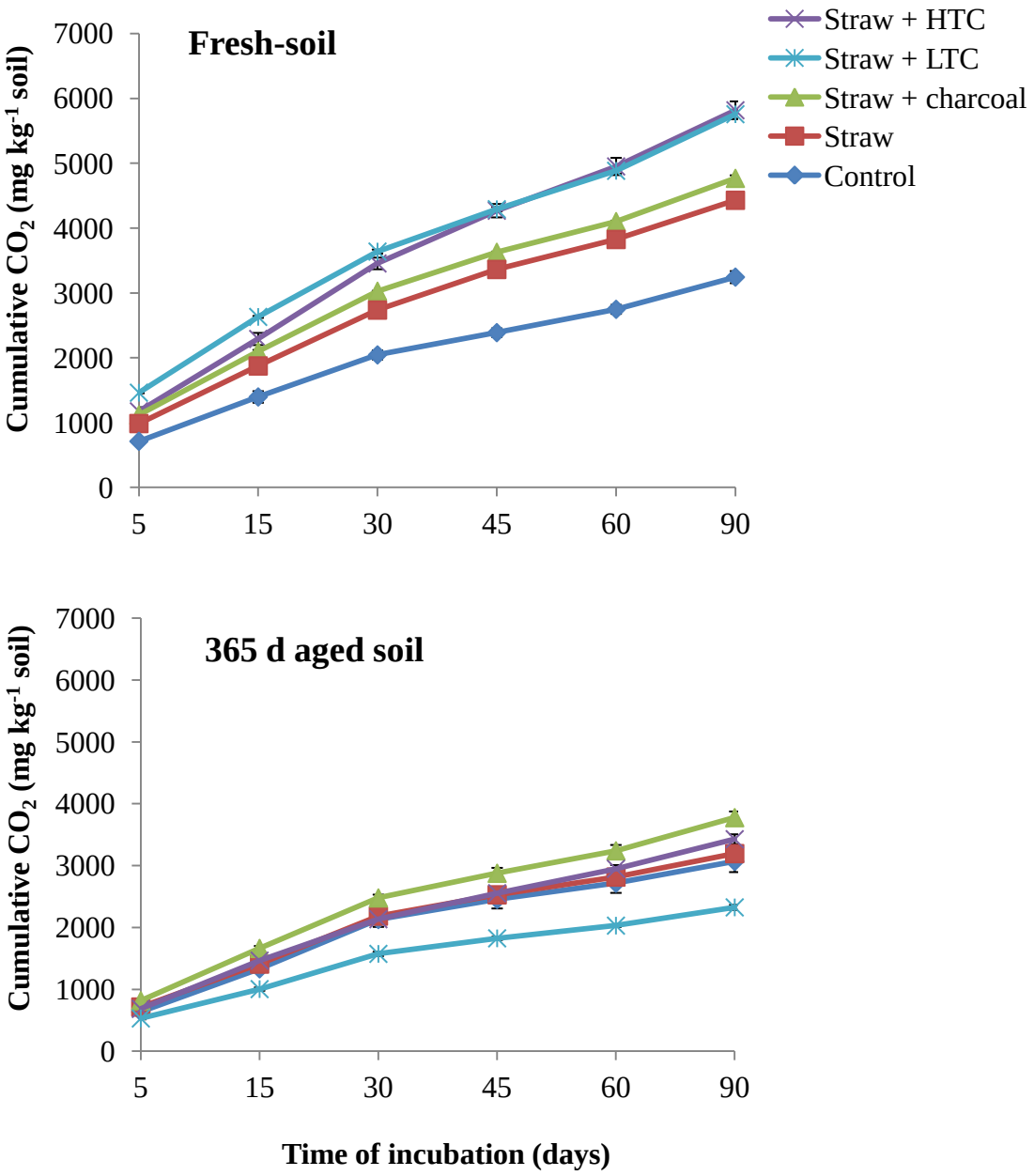

Figure 23. Cumulative release of CO_2-C during 90 d incubation from Luvisol topsoil with straw, and straw mixed with charcoal, HTC or LTC applied to either fresh or aged soil for 365 d. The statistical comparison (t-test) is made between straw alone and straw mixed with biochars. Each data point represents mean ± standard error of three replicates.

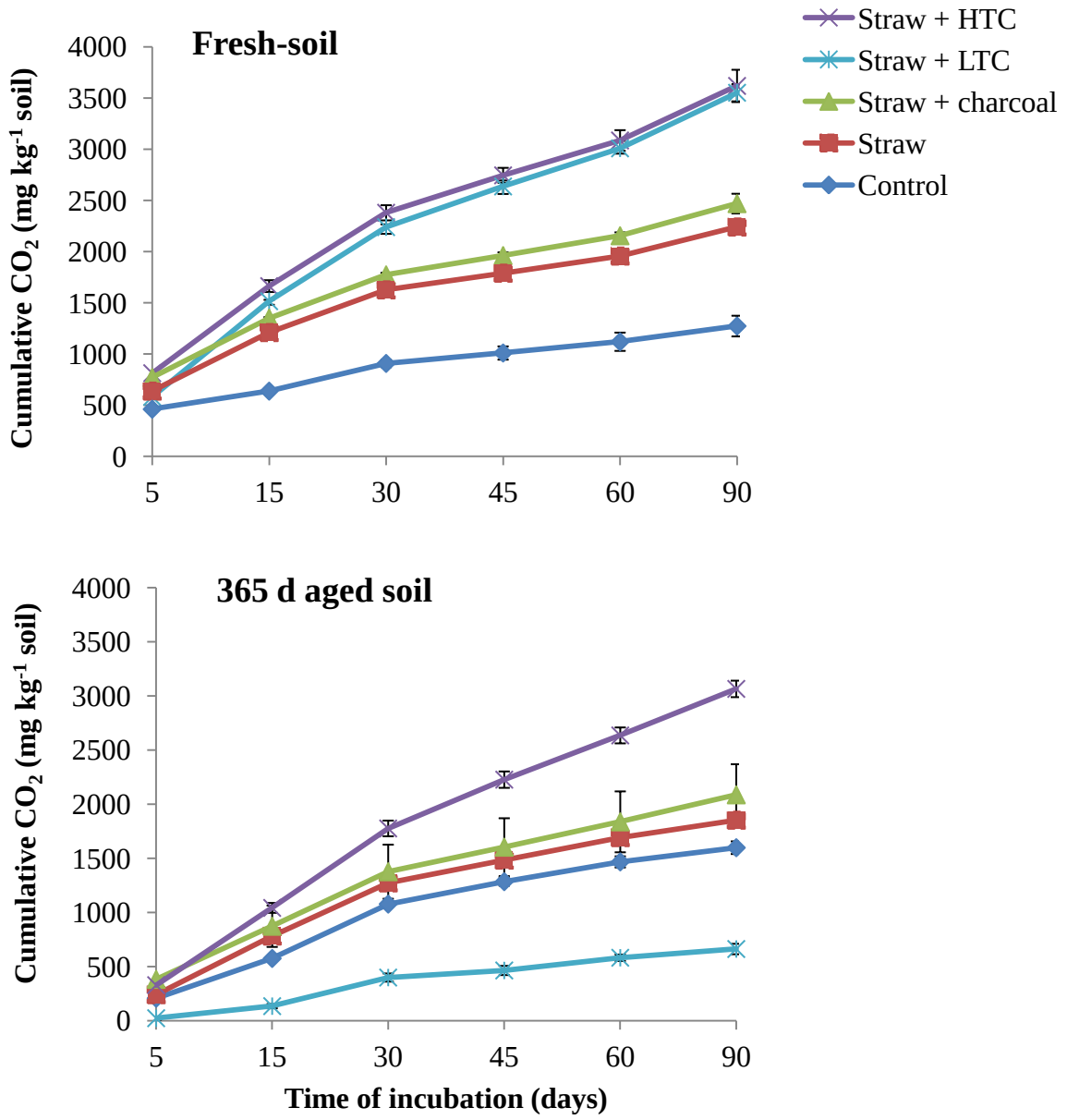

Figure 24. Cumulative release of CO_2-C during 90 d incubation from Luvisol subsoil with straw, and straw mixed with charcoal, HTC or LTC applied to either fresh or aged soil for 365 d. The statistical comparison (t-test) is made between straw alone and straw mixed with biochars. Each data point represents mean ± standard error of three replicates.

3.4.2. Kinetics of carbon mineralization

The data regarding parameters of C mineralization kinetics are presented in Table 9. A first-order exponential equation was used to describe the C mineralization of control, straw, straw + charcoal, straw + HTC, and straw + LTC in various soils. The degree of conformity between experimental data and the equation-predicted values is expressed by the r^2 value (ranging from 0.77 to 0.99). The results show a significant decrease of potential mineralizeable C in the 365 d aged soils when compared to the fresh soils (Table 9). In the Ferralsol, except in the control and straw treatments, mineralization rate constants (*k*) were significantly lower in the 365 d aged soils (Table 9). The half-lives of C in the control and straw + biochars treatments were higher in the 365 d aged soils as compared to the fresh soils. In the Luvisol topsoil, 365 d ageing significantly decreased the rate constants in all treatments and consequently the half-lives of C were higher in the 365 d aged soils. In the Luvisol subsoil, only straw + charcoal treatment resulted in a decreased rate constant and significantly higher half-life of C in the 365 d aged soil as compared to the fresh soil.

Table 9. Experiment 3, Kinetic parameters of the first order kinetics. Parameters of the single first-order equation were calculated by plotting ln Ct against time (t) from data of cumulative CO_2 release over a period of 90 d. (C_o = potential mineralizeable C, *k* = rate constant).

Soils	**Treatments**	**C_o (mg kg^{-1} soil)**		**k (day^{-1})**		**Half-life of C (years)**		**r^2**	
		Fresh soil	365 d aged	Fresh soil	365 d aged	Fresh soil	365 d aged	Fresh soil	365 d aged
Ferralsol	**Control**	3293	2984 *	0.00072	0.00053	2.7	3.6 *	0.99	0.88
	Straw	4043	3778 *	0.00145	0.00129	1.3	1.5	0.79	0.81
	Charcoal	15327	15018 *	0.00037	0.00032 *	5.1	5.9 *	0.77	0.78
	HTC	15302	14330 *	0.00054	0.00048 *	3.5	4.0 *	0.85	0.85
	LTC	15270	14462 *	0.0005	0.00041 *	3.8	4.6 *	0.79	0.82
Luvisol topsoil	**Control**	17282	15108 *	0.00054	0.00041 *	3.5	4.7	0.89	0.89
	Straw	18083	15902 *	0.00072	0.00057 *	2.7	3.3 *	0.9	0.86 *
	Charcoal	29341	27136 *	0.00046	0.00040 *	4.1	5.6 *	0.88	0.86 *
	HTC	29334	26274 *	0.00058	0.00041 *	3.2	4.6 *	0.91	0.88 *
	LTC	29273	27447 *	0.00055	0.00036 *	3.4	5.2 *	0.88	0.93
Luvisol subsoil	**Control**	2645	1724 *	0.00131	0.00133	1.4	1.5	0.83	0.92 *
	Straw	3314	2540 *	0.00183	0.00205	1.0	1.5	0.89	0.89
	Charcoal	14709	13878 *	0.00045	0.00054	4.2	4.8	0.81	0.82
	HTC	14687	13166 *	0.00071	0.00047 *	2.7	4.0 *	0.87	0.88
	LTC	14743	13605 *	0.00068	0.00071	2.8	2.7	0.91	0.92

* represents a significant difference ($P \leq 0.05$, using t-test) between fresh soil and 365 d aged soil for a parameter within each soil

3.4.3. Priming effect of biochars on straw mineralization

The priming effects (PE) of biochars on straw mineralization are presented in the Figure 25. For the calculation of PE, the amount of C mineralized in the control (non-amended) was subtracted from all other treatments. As a pre-requisite of PE, the C mineralized in the straw-alone treatment was statistically compared with C mineralized in straw + biochar treatments at both residence times (Figure 22, Figure 23, and Figure 24).

In the Ferralsol, straw + HTC and straw + LTC treatments showed significantly higher ($p < 0:05$, t-test) C mineralization as compared to the straw alone (Figure 22). This shows the presence of PE in the straw + HTC, and straw + LTC treatments. The cumulative PE of straw + HTC and straw + LTC in the Ferralsol is shown in Figure 25. Results show strong positive PE in these treatments throughout the incubation duration. The PE was stronger on 5th day of incubation (75 to 125 %), decreased to some extent after day 15 (25 to 75%) and again increased at the end of incubation (Figure 25). It is clear that there was a positive PE at both residence times (fresh soil and 365 d aged soils) and the statistical comparison between residence times showed no significant effect of biochar aging on PE.

In the Luvisol topsoil and subsoil, fresh applications of straw + charcoal, straw + HTC, and straw + LTC showed positive PE (Figure 25). However, in the 365 d aged soils only HTC and LTC showed PE. In the Luvisol topsoil, fresh application of straw + LTC showed significantly higher PE from 5 d to 60 d. However, in the Luvisol subsoil, HTC + straw showed very strong PE in the 365 d aged soil at the start of incubation, but after 30 d of incubation, the reverse occurred (PE was higher in the fresh-soil as compared to the 365 d aged-soil). In the straw + LTC treatment of Luvisol subsoil, 365 d aged soil showed significantly higher PE than the fresh soil (Figure 25).

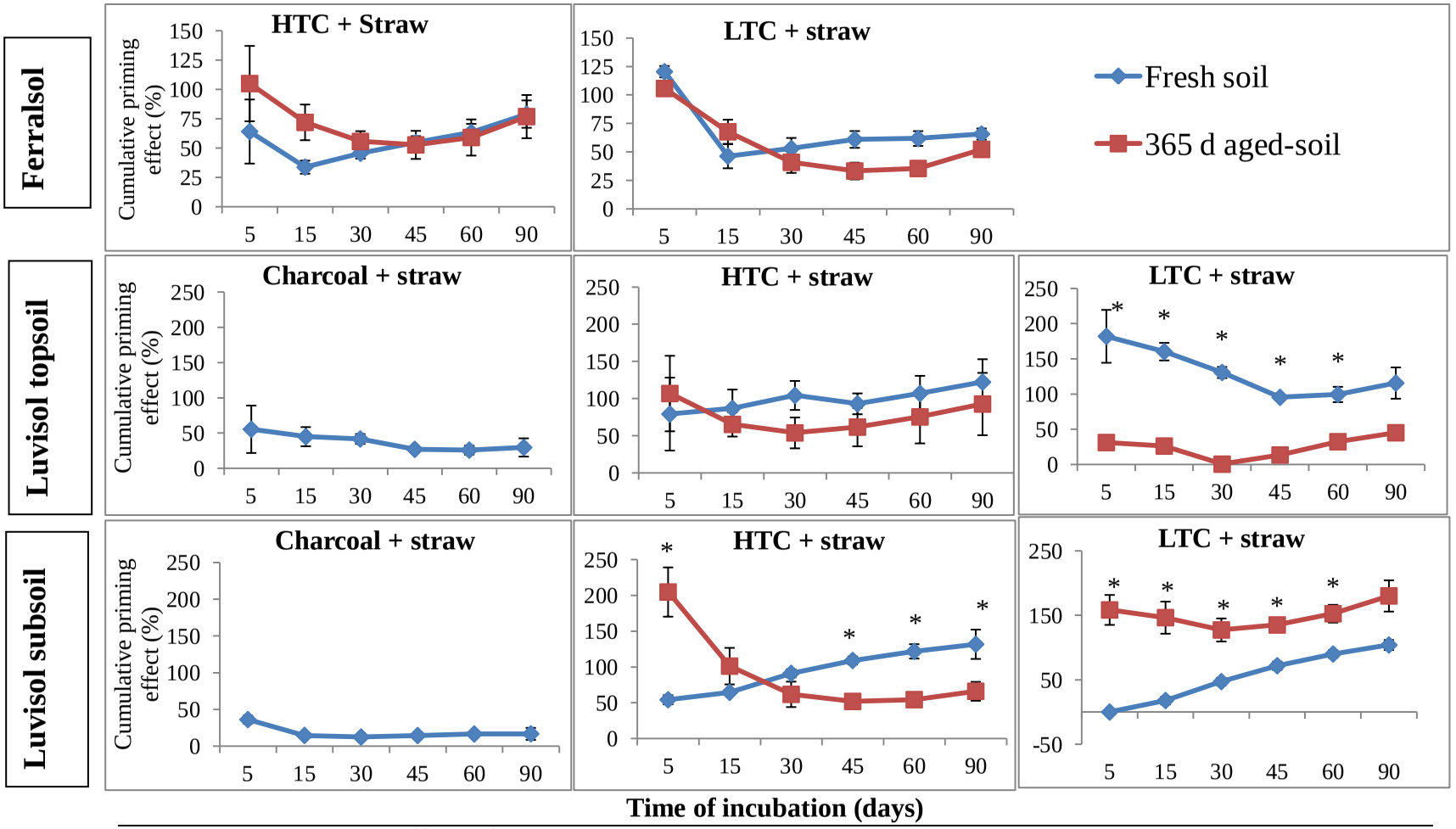

Figure 25. Cumulative priming effect of charcoal, hydrothermal carbonization coal (HTC), and low-temperature conversion coal (LTC) on mineralization of straw in the Ferralsol, Luvisol topsoil, and Luvisol subsoil. The priming effect was calculated by using the equation, $PE,(\%) = 100 \times \frac{(CO_2 - C_{(straw+biochar)} - CO_2 - C_{(straw)}}{CO_2 - C_{(straw)}}$ The statistical comparison (t-test) is made for residence times (fresh soil and 365 d aged soil).

4. DISCUSSION

4.1. Stability of biochars in soils as compared to wheat straw

It was hypothesized that charcoal, hydrothermal carbonization coal (HTC), and low temperature conversion coal (LTC) would be more stable in soils compared to wheat straw, with stability dependent on soil properties. The charcoal used in the present study was a high-temperature pyrolysis product of wood with an accumulation of black C (Figure 7). This property makes it very inert and recalcitrant to microbial degradation (Spokas, 2010). The charcoal and control treatments were not significantly different for C mineralization. In contrast, the C mineralization was higher for the HTC and LTC than the charcoal and control. This may be explained by the presence of mineralizeable C compounds in HTC and LTC as shown in the FTIR spectra i.e. CH-str. at 2920 cm^{-1}, and C-O str. of polysaccharides at 1050 cm^{-1} to 1000 cm^{-1} (Figure 7). Similar findings for low-temperature biochars have been reported (16% to 51% loss of biochar made from maize and rye residues at 350°C during the first 2 years) by Brodowski (2004). Steinbeiss et al. (2009) also compared C mineralization of high condensation-grade and low condensation-grade biochars and found higher C losses (10% and 11%) from low condensation-grade biochars in forest and arable soils. They concluded that the chemical structure of low condensation-grade biochars was the main factor affecting the stability of biochar in soil. Contrary to the biochars, application of wheat straw to the soils resulted in the higher loss of C. The significant decreases in inorganic N with the application of straw suggested the microbial immobilization of N (Figure 11 and Figure 12). Higher release of CO_2 from the straw treatment was due to the high amount of easily mineralizeable C compounds in straw as indicated by the FTIR spectrum of straw (Figure 7).

The stability of biochar depends on the production conditions and type of organic material used during pyrolysis (Novak et al., 2010; Spokas, 2010). The data show that C mineralization was also affected by soil properties. Charcoal was much more stable and had a higher half-life in the Ferralsol compared to the Luvisol topsoil & subsoil (Table 6). There may be two possible reasons for the higher stability of charcoal in the Ferralsol. Firstly, the presence of Fe and Al oxides in the Ferralsol may have enhanced the adsorption of charcoal C (Kaiser et al., 1996; Kalbitz and Kaiser, 2008; Joseph et al., 2010; Yao et al., 2011). The second reason may be the lower SOM content and microbial activity in the Ferralsol, since it

has been suggested that the soils with low SOM have higher carbon stabilization efficiency (Six, et al., 2002; Stewart, et al., 2007). Similar to the Ferralsol, Luvisol subsoil had also low organic matter and showed smaller carbon mineralization compared with Luvisol topsoil. The acceleration of microbial degradation of biochar in the presence of labile SOM was reported by Hamer et al. (2004). Thus, it could be suggested that higher C mineralization in the Luvisol topsoil may be due to less Fe and Al oxides (Table 1). In addition, high microbial activity and the presence of easily degradable SOM might have caused a positive priming effect and enhanced the C mineralization of HTC as stated by Kuzyakov et al. (2000).

Relatively low C mineralization in the Ferralsol might have been due to the more acidic soil pH. However, in second experiment it was found that low pH of the Ferralsol did not affect mineralization of HTC and straw (Figure 19, Figure 20, and Figure 21). In contrast, with application of wheat straw, carbon mineralization was increased at both pH values because wheat straw is easily degradable by the soil microorganisms. This shows that low pH did not limit carbon mineralization in the Ferralsol. It was also confirmed by Hassink, (1994) that low soil pH does not necessarily contribute to low mineralization of carbon compounds.

Incomplete combustion of organic substances leads to changes in chemical structure of biomass and more resistant-compounds are formed. This may be the reason for less mineralization of HTC coal in soil.

4.2. Carbon mineralization kinetics

Overall, the estimation of kinetics parameters with combined first-order equation using non-linear regression provided higher determinant of coefficient (r^2) values for all treatments in three soils as compared to the single first-order equation. Our results showing better performance of combined first-order equation are in accordance with the findings of Smith et al. (1980), and Talpaz et al. (1981) in N mineralization studies and those of Saviozzi et al. (1993), and Sleutel et al. (2005) for the decomposition of various organic materials in soils. The kinetic parameters of C mineralization showed a very low rate constant and a longer half-life for the resistant C pool in the charcoal treatment in the Ferralsol relative to the Luvisol topsoil and subsoil (Table 6). The reduced CO_2 release from the LTC treatment after 270 d of incubation (Figure 8) resulted in a lower rate constant (k_s) and ultimately higher half-life of LTC-C in the Luvisol topsoil and subsoil (Table 6). The possible reason for reduction of C

mineralization in LTC treatment after 270 d may be due to low availability of N in this treatment (Figure 11, and Figure 12). Although LTC had a higher total N concentration, much of this N was in the form of heterocyclic compounds and was not mineralized during the incubation period (Table 2). Although in the charcoal and HTC treatments N was not limiting higher half-lives were recorded in these treatments as compared to straw and control.

The calculated half-life of charcoal-C in the present study is much lower compared to previous studies where the half-lives of C in charcoal were estimated to be in a range of hundreds to thousands of years (Hammes et al., 2008; Harden et al., 2002; Liang et al., 2008; Middelburg et al., 1999). According to Cheng et al. (2008), the decomposition of charcoal in the natural environment is slower than under laboratory conditions. Lehmann (2007) also stated that the true half-life of C is always higher than calculated due to systematic overestimation of long-term black C decay in short-term incubations. Nevertheless, the standardized conditions in the incubation experiments are useful and provide meaningful information in comparing mineralization potentials of various organic C compounds, which can be used as soil amendments.

4.3. Nitrogen dynamics

In the Ferralsol, the significant decrease of mineral N in the straw treatment was due to the higher C mineralization resulting in immobilization of N (Figure 11 and Figure 12). However, in other treatments, there was no significant change in mineral N after 365 d of incubation. This could be due to less C mineralization in these treatments. Similar results were recorded by van Zwieten et al. (2010) who found a decrease of NH_4-N and increase of NO_3-N in a Ferralsol with the application of biochar. In the Luvisol (topsoil and subsoil), higher C mineralization resulted in strong immobilization of NH_4-N in all treatments, whereas a significant increase of NO_3-N in control and biochar treatments after 365 d of incubation was observed. In the straw treatment, there was no change in NO_3-N due to N immobilization after depletion of NH_4-N. It has been previously confirmed that in the presence of both available forms of N (NO_3-N and NH_4-N), the soil microorganisms immobilize NO_3-N only after NH_4-N is nearly exhausted (Recous et al., 1995).

The results of the first experiment showed an accumulation of NO_3-N after the application of charcoal (Figure 11) which are consistent to the findings of DeLuca et al. (2006) and Kolb et

al. (2009) who stated that biochar was capable of stimulating nitrifier activity. The LTC treatment in all soils showed very low 0.01 M $CaCl_2$-extractable NO_3-N and NH_4-N. The reason for this was the high concentration of heterocyclic N in LTC and this N was not mineralized (Table 2). The heterocyclic compounds are formed during the carbonization process in which amide-N is converted to stable pyrrolic N (Knicker et al., 1996). Bridle and Pritchard (2004) also indicated that the carbonization of sewage sludge resulted in the recovery of almost all N but this was not bio-available and was in a form that was resistant to decomposition and mineralization. However, C and N bound in heterocyclic forms have a great significance for improving soil structure.

4.4. Distribution of carbon in SOM density-fractions

With SOM density fractionation, the effect of various C compounds on C distribution in SOM density-fractions was studied. The sums of TC content of three fractions (FF, IAF, and HF) were almost equal to the TC content of the bulk soils (Table 7). Due to low C concentration of soils, no FF or IAF could be extracted from the control treatments of the Ferralsol and the Luvisol subsoil. Based on this observation it was argued that all C in the FF and IAF of the Ferralsol and Luvisol subsoil belonged to the added C compounds and not from the soils. In the straw treatments, the sums of TC in SOM density fractions were lower than TC of bulk soils. It is well known that straw mineralization in soils results in higher C losses as well as in an increase of soluble organic C. From these results, it can be speculated that soluble organic carbon could not be retrieved in SOM density fractionation due to losses during repeated centrifugation and sonication. In the control treatments of the Ferralsol and Luvisol subsoil, almost all C was present in the HF (Table 7) describing higher proportions of stabilized C in the HF (Gruenewald et al., 2006).

In all soils, despite of equivalent C application, the relative increases in TC content of bulk soil, FF and IAF were higher with application of biochars than straw treatment (Table 7). The significant differences among C compounds in increasing TC content were due to higher C losses in the straw and HTC treatments during 365 d incubation (Qayyum et al., 2011). After incubation for 365 d, the increased portion of C in treatments with C compounds can be regarded as stabilized C. The results show that in biochar treatments, higher proportions of this stabilized C were found in the FF (Table 7 and Table 8) which suggests the chemical

recalcitrance of biochars. Among biochars, charcoal and HTC treatments showed maximum increase of TC in the FF in the three soils. The charcoal used in the present experiment had the highest C concentration (Table 2) and most of this was black C as was confirmed by the FTIR spectrum of charcoal without incubation (Figure 7). In the *Terra Preta* soils, which contain huge amounts of BC, the higher stability of SOM is suggested due to chemical inertness (Schneour, 1966; Seiler and Crutzen, 1980; Glaser et al., 2002). Moreover, the results of the FTIR spectra (Figure 15) show strong indications of charcoal surface-depositions of the alumino-silicate minerals (an addition of infrared band at 1030 cm^{-1} (smectite) with higher intensity in the Luvisol topsoil and Luvisol subsoil and less in the Ferralsol). These surface interactions might have increased the stability of charcoal-C in FF through enhancing inertness. Similar findings have been reported by Joseph et al. (2010), who stated that charcoal surface-depositions of Al, Ca, and Si may enhance the inertness of the C in the soils. Similar to the charcoal, the FF from the HTC treatment also indicated some surface depositions of soil inorganic components especially clay minerals (Figure 16). This suggests that despite of higher mineralization (Qayyum et al., 2011) a part of C in the HTC is also stable in soils in form of recalcitrant BC.

Followed by the stabilization of C in FF, higher proportions of stabilized C were found in the IAF retrieved from the three soils (Table 7). The FTIR spectra of IAF from all treatments in the Ferralsol and Luvisol topsoil were not different from those of the C compounds. This suggests lack of surface reactions of the straw and biochars when present in micro-aggregates. However, the FTIR spectra of IAF from the Luvisol subsoil showed contamination of soil materials that was due to less stable aggregation in this soil. These observations suggest that the stabilization of C in IAF was only due to occlusion into micro-aggregates (physical protection). Similar findings have been reported by Mutuo et al. (2006) who incubated two soils and SOM fractions separately and found higher C concentrations in micro-aggregates as compared to macro-aggregates. Increased formation and stabilization of SOM with biochar application was also reported by Brodowski et al. (2006) who were able to identify larger portions of BC particles in micro-aggregates and suggested BC as a binding agent for soil aggregates.

The third mechanism of SOM stabilization (protection of C in the HF) is suggested due to interaction of C compounds with clay minerals (Dalal and Mayer, 1986; Golchin et al., 1995).

The results show that the LTC treatment in all three soils and straw treatment in the Ferralsol and Luvisol topsoil significantly increased TC in the HF (Table 7). This indicates a possibility of C stabilization in LTC-treated soils due to organo-mineral interactions (Hammes and Schmidt, 2009). The other possible reason may be that the LTC biochar was produced from sewage sludge that might contain C compounds with higher density, which were not retrieved in the FF and IAF. From the FTIR spectra of LTC-treated soils and SOM fractions in this treatment no information could be drawn to support any mechanism of stabilization. The exact mechanisms for C stabilization in this treatment need further research.

For straw, it can be speculated that higher mineralization in 365 d of incubation must have resulted in some heterocyclic C compounds, which might have interacted with soil HF. The charcoal treatment did not increase TC in the HF from Ferralsol and Luvisol subsoil. This indicates no stabilization of charcoal-C in the HF in these soils.

4.5. Effect of biochar ageing in soil on straw mineralization

In the third experiment, it was hypothesized that biochar ageing in soil would stabilize added wheat straw. The results show significantly higher values for C half-lives in biochar-aged soils as compared to the fresh amended-soils (Table 9). This suggests the possibility that ageing of biochars in soils may have lowered the rate constants of C mineralization. Possibly, the C mineralization in biochar-aged soils was in the second phase of mineralization after loss of the soil's easily degradable organic compounds during residence time of 365 d (Qayyum et al., 2011).

4.5.1. Stabilization of added carbon

As hypothesized, the results show C stabilization but also increased mineralization (priming) of applied wheat straw in some combinations. The increased stabilization of C is identified in charcoal-aged soils. In the Ferralsol, combination of straw and charcoal did not influence straw mineralization at any residence time, but in the Luvisol topsoil and Luvisol subsoil, this combination when applied fresh in the soils, resulted in a positive priming effect (Figure 25). The absence of PE in straw + charcoal treatment suggests stabilization of straw C in the charcoal-amended Ferralsol as compared to the Luvisol topsoil and Luvisol soil. This different behavior of charcoal in various soils might be due to different type of soil minerals.

However, it was observed that in the Ferralsol, the stabilization process might be fast as fresh combined application of straw and charcoal was not different from straw application in charcoal-aged soil.

It is possible that the recalcitrant nature of charcoal had been enhanced in the Ferralsol, due to relatively higher concentration of soil Fe and Al oxides (Table 1). In the first experiment, the FTIR of density fractions retrieved from charcoal treatment of three soils after 365 d incubation showed charcoal surface-depositions of aluminosilicate minerals (Figure 15). In the Luvisol topsoil and Luvisol subsoil, types of clay minerals were different and there was less intensity of charcoal surface reactions than in the Ferralsol. This might be reason that fresh application of charcoal + straw in these two soils resulted in higher CO_2 release as compared to the aged soil. These findings indicate that changes occurred on charcoal surfaces during 365 d of incubation (surface depositions of aluminosilicate minerals) might have resisted mineralization in the presence of easily degradable straw. These results confirm the findings of Liang et al., (2010) who documented increased stabilization (3-8 %) of added organic matter in biochar-rich Amazonian soil. They argued that the high surface area of black carbon may have stabilized added organic matter. It is also supposed that in the *Terra Preta* soils, the total carbon content is increasing rather than decreasing with the passage of time (Glaser et al., 2001; Grady and Rush, 2007). Our results of less mineralization in the straw treatment when combined with charcoal confirm the increase in soil organic matter in previously biochar amended soils.

The charcoal used in the present study was a high-temperature pyrolysis product (> 500°C), and it is reported that biochars produced at high temperature contain more condensed C that is recalcitrant in soils (Nguyen et al., 2010). Such biochars have relatively higher interaction affinity for organic and inorganic compounds in soils (Zimmermann et al., 2011). It is also argued that slow oxidation of biochars in soils results in production of carboxylic groups and increase in CEC (Glaser et al., 2002; Streubel et al., 2011). These formations may have affected soil-minerals interactions with C, making biochars more stable in soils.

4.5.2. Priming effects

In all soils, the combination of straw + HTC and straw + LTC at both residence times caused positive priming-effects (Figure 25). From the data of HTC mineralization in the 365 d

(Figure 8), it was suggested that HTC was not as stable in soil as charcoal, and the present results show that combination of straw + HTC may have enhanced mineralization of each other. The reason for lower stability of HTC may be the low temperature during production (180-200°C) that results in higher amount of labile organic compounds as compared to charcoal and LTC.

In the first experiment, the results show that stability of LTC biochar was not different to that of charcoal (Qayyum et al., 2011). It was argued that nitrogen may be a limiting factor in reduced mineralization of LTC during the first year of incubation. However, in the present study, results show that from the combination of straw and LTC, the release of CO_2 from soil was higher as compared to straw alone. This increased release of CO_2 from the described treatment suggests that mineralization of LTC is possible in soils in the presence of a labile source of C. These results confirm the findings of (Keith et al., 2011) who reported positive interactive PE between straw and low-temperature biochar as compared to high-temperature biochar. Hamer et al. (2004) also observed an increase of biochar mineralization with labile organic matter additions in soils, but in their study, glucose was used as a source of readily available C.

5. CONCLUSIONS

The incubation studies of various biochars in comparison to wheat straw in three soils provided a coherent view of the C stabilization in soils and considerably improved the understandings of mechanisms of organic matter (SOM) stabilization. Based on the results it is concluded that for long-term C sequestration and SOM stabilization, low temperature conversion coal (LTC), and charcoal are suitable options. The results show a higher half-life of hydrothermal carbonization coal (HTC) than of wheat straw. However, HTC is mineralized very fast in the soils as compared to charcoal and LTC. Therefore, HTC is not a suitable option for C sequestration in soils.

The selection of biochar as a soil amendment must be based on the intention of the amendment. If long-term soil C-sequestration is an aim then charcoal or the material produced at high temperature is the most suitable option. Based on the results of SOM density-fractionation, it is concluded that different mechanisms of biochar-C stabilization occur in various soils. Among these mechanisms, the recalcitrant nature of applied black-carbon (BC) and the occlusion into soil micro-aggregates are responsible for stabilization of charcoal and HTC in the soils.

Moreover, the Fourier transform infrared spectroscopy (FTIR) of the SOM density-fractions showed aluminosilicate mineral associations on the surfaces of charcoal and HTC present in the form of free fraction (FF). These surface interactions may enhance the stabilization potential of these biochars. However, for the LTC biochar produced from sewage sludge, the major mechanism of C stabilization is binding in the heavy fraction (HF) of SOM.

Charcoal application in soils may stabilize added organic matter such as wheat straw, while the low-temperature biochars (HTC and LTC) have no influence on stabilization; rather they increase the C mineralization of added materials. However, ageing of charcoal, HTC, and LTC biochars in soils and subsequent addition of labile organic materials such as wheat straw stabilize organic carbon in the soils.

Based on the present studies, it is suggested that further research is needed to understand detailed mechanism of stabilization of the LTC biochar. Although stabilization as well as interactive priming effect by the biochars on straw C is documented here, the results possess

lack of clear C source identification. The C mineralized in biochars could not be differentiated from that in the straw. There must be further research using C-labeled biochars and organic materials to study the detailed mechanisms of stabilization or priming.

6. SUMMARY

Soil organic matter (SOM) has an important role in soil fertility and agricultural productivity. Carbon (C) sequestration in soil can be an important step towards soil organic matter (SOM) stabilization and conservation. The traditional practices used to increase SOM do not sequester significant quantities of C into soil because most of the organic matter is not stable and is mineralized very fast. With the discovery of *Terra Preta*, it is evident that charcoal/biochar can be used to increase SOM. The potential positive effects of biochars on soils and plant production have been well elaborated. With increasing interest of biochar production using various biomass materials, it is necessary to study their mineralization and stabilization potential in various soils.

In the present study, three biochars (a charcoal produced by burning of wood at 550°C, a hydrothermal carbonization coal (HTC) of bark, and a low-temperature conversion coal (LTC) of sewage sludge) in comparison to wheat straw were selected with the objectives to investigate the C mineralization kinetics. Prior to the incubation experiment, Fourier transform infrared spectroscopy (FTIR) and N-fractionation were performed to characterize the C and N bonds in the biochars and straw. Charcoal was highly carbonized compared with the HTC and LTC. The LTC biochar contained more N in the heterocyclic-bound N fraction as compared with the charcoal, HTC and straw. The selected soils for the incubation experiment were sampled from various areas in Hesse, Germany. The Ferralsol (a mixture of various soil horizons) was a relict tertiary Ferralsol. In comparison, the Luvisol derived from loess (topsoil and subsoil) was selected because this is the most important soil type in Western Europe for crop production.

In the first experiment, the above-described soils were incubated at 25°C with the biochars, straw, and without amendment (control) over a period of 730 d. Carbon mineralization was analyzed as alkali absorption of CO_2 released at regular intervals. Soil samples taken after 5 d, 365 d, and 735 d of incubation were analyzed for soluble organic C and mineral N. To examine the reactions occurring on biochar surfaces during the first year of incubation, SOM density fractionation was performed. Total C and N in the density fractions were determined using an elemental analyzer. The bulk soil samples and SOM density fractions were scanned using FTIR to observe chemical changes on the surfaces of density fractions.

In a second experiment, the effect of soil pH on C mineralization of straw and HTC was investigated. The Ferralsol was selected as test soil in this experiment. In the third experiment, wheat straw (8 t ha^{-1}) was mixed with the biochar-amended (365 d and 0 d aged-soils) and control soils. Carbon mineralization was measured at different time intervals for a time scale of 90 d. Carbon mineralization kinetics was calculated using cumulative CO_2 release data.

The results show that wheat straw was mineralized very fast and had a lower half-life of C compared with the biochars. Among the biochars, HTC showed some C mineralization when compared with charcoal and LTC but less than straw. The kinetics show that half-lives of C were higher in the charcoal and LTC treatments in the Ferralsol than in the Luvisol topsoil and subsoil, possibly due to high concentration of Fe-oxide in the Ferralsol. The LTC-C had a higher half-life, possibly due to N-binding in a heterocyclic form. The results of density fractionation showed a higher proportion of applied C in the free fraction (FF). The FTIR spectra of the FF indicated surface depositions of alumino-silicate minerals on biochars, which might have increased the stabilization of biochars in the soils. Followed by the FF, higher amounts of C were found in intra-aggregate fraction (IAF). No surface depositions occurred in IAF. Except LTC, no treatment increased the C content of the heavy fraction (HF). The mechanisms involved in the increase of TC in the HF with LTC are unclear and need further investigations.

In the second experiment, it was shown that soil pH did not affect mineralization of HTC and straw. The results of the third experiment show that biochar ageing in soils influenced C mineralization of added straw. The half-lives of C in various treatments were significantly higher in 365 d biochar-aged soils. Stabilization of straw C was observed in the charcoal added-soils, while increased mineralization or priming was documented in HTC and LTC added-soils. The results suggest an interaction between biochar aged in soil and applied straw. However, to investigate the detailed mechanisms of C-stabilization and C priming, further research is needed using C-labeled biochars and straw.

7. ZUSAMMENFASSUNG

Die organische Substanz spielt eine wichtige Rolle für die Bodenfruchtbarkeit und für die landwirtschaftlich Produktivität. Die Kohlenstoff-Sequestrierung in Böden könnte ein wichtiger Schritt für die Stabilisierung der organischen Substanz im Boden sein. Traditionelle Praktiken zur Steigerung der organischen Substanz erfordern große Kohlenstoffmengen, da der eingebrachte Kohlenstoff relativ instabil ist und somit rasch mineralisiert wird. Mit der Entdeckung der *Terra Preta* ist es offensichtlich, dass Holzkohle / Biokohle verwendet werden könnte, um die organische Substanz nachhaltig zu erhöhen.

Für die vorliegende Studie wurden drei Biokohlen ausgesucht: eine Holzkohle produziert aus Holz bei 550°C, eine hydrothermale Kohle (HTC) aus Rinden und eine niedrigtemperatur Konvertierungskohle aus Klärschlamm (LTC). Das Ziel dieser Untersuchungen war es, die Kohlenstoffmineralisation dieser drei Biokohlen im Vergleich zu Weizenstroh zu analysieren. Vor den Inkubationsversuchen wurde die Fourier-transform-Infrarotspekroskopie (FTIR) sowie die Stickstofffraktionierung zur Charakterisierung der Kohlenstoff- und Stickstoffverbindungen in den verschiedenen Biokohlen angewendet. Holzkohle war deutlich stärker karbonisiert als die HTC- und die LTC-Kohle. Die LTC-Kohle enthielt im Vergleich zu Holzkohle, HTC-Kohle und Weizenstroh deutlich mehr heterocyclisch gebundenen Stickstoff. Die für die Inkubationsexperimente ausgewählten Böden stammten aus verschiedenen Regionen Hessens, wobei der Ferralsol (Gemisch aus verschiedenen Horizonten) von der Eisernen Hose in Lich geholt wurde. Im Vergleich dazu wurden ein Ober- und eine Unterboden von Lössböden ausgesucht, da die aus Löss entstandenen Böden eine große Bedeutung für die landwirtschaftliche Produktion in Deutschland aufweisen.

Im ersten Experiment wurden die drei Biokohlen und das Weizenstroh in die oben beschriebenen Böden eingearbeitet und im Vergleich zu einer Variante ohne organische Düngung bei 25°C über einen Zeitraum von 730 Tagen inkubiert. Mineralisiertes CO_2 wurde in Kalilauge aufgefangen und in regelmäßigen Abständen analysiert. Bodenproben wurden nach 5, 365, und 735 Tagen der Inkubation entnommen, um löslichen organischen Kohlenstoff und mineralischen Stickstoff zu analysieren.

Nach einer einjährigen Inkubationsphase der Biokohlen in den verschiedenen Böden wurde eine Dichtefraktionierung durchgeführt, um mögliche Reaktionen an den Oberflächen der Biokohlen zu untersuchen. In den Dichtefraktionen wurden die Gesamtkonzentrationen von Kohlenstoff und Stickstoff mit einem Elementaranalysator bestimmt. Ferner wurden die Ausgangsböden und die Dichtefraktionen einer Fourier-Transforms-Infrarotspekroskopie (FTIR) unterzogen, um Oberflächenänderungen an den Biokohlen zu messen..

Im zweiten Versuch wurde der Einfluss vom Boden pH-Wert auf die Kohlenstoffmineralisierung von Stroh und HTC-Kohle im Ferralsol untersucht.

In einem dritten Versuch wurde der Einfluss einer einjährigen Verweildauer (Alterung) von Biokohle im Boden im Vergleich zu einer frischen Applikation von Biokohle (keine Alterung) auf die Kohlenstoffmineralsierung von Weizenstroh analysiert. Dazu wurden die für 365 Tage und 0 Tage mit verschiedenen Biokohlen inkubierten Böden mit Weizenstroh (8 t ha^{-1}) gedüngt. Die Kohlenstoffmineralisierung wurde in verschiedenen Zeitabständen bis 90 Tage gemessen.

Die Ergebnisse zeigen, dass Weizenstroh im Vergleich zu den Biokohlen sehr rasch mineralisiert wurde, und dass die Halbwertszeit von Weizenstrohkohlenstoff niedriger war als von den Biokohlen. Von den Biokohlen wurde HTC-Kohle stärker mineralisiert als Holzkohle und LTC-Kohle. Die Ergebnisse der Kinetik-Berechnungen aus den Kohlenstoffmineralisationskurven ergaben für Holzkohle und NTK-Kohle eine längere Halbwertszeit von Kohlenstoff im Ferrasol als im Ober- und Unterboden der Lössböden, möglicherweise aufgrund der hohen Fe-Oxid-Konzentration im Ferralsol. Der in der LTC-Kohle gebundene Kohlenstoff hatte eine höhere Halbwertszeit, möglicherweise aufgrund von heterocyclisch gebundenem Stickstoff. Die Ergebnisse der Dichtefraktionierung zeigen einen hohen Anteil des zugeführten Kohlenstoffs in der freien Fraktion (FF). Die FTIR-Spektren der FF ergaben Oberflächenablagerungen an Aluminium-Silikat-Mineralen auf den Biokohlen, die die Stabilisierung von Biokohlen in den Böden gefördert haben könnten. Gefolgt von der FF, wurden höhere Kohlenstoffmengen in der Intra-Aggregat-Fraktion gefunden. In der Intra-Aggregat-Fraktion traten keine Oberflächenablagerungen auf. Mit Ausnahme der LTC-Kohle hatte keine Biokohle einen Einfluss auf den Kohlenstoffgehalt in der schweren Fraktion (HF). Die Mechanismen für die Erhöhung des Kohlenstoffgehalts in der schweren Fraktion (HF) infolge von LTC-Düngung sind unklar und sollten weiter untersucht werden.

Im zweiten Experiment wurde gezeigt, dass der Boden pH-Wert nicht die Mineralisierung von HTC-Kohle und Stroh beeinflusste. Die Ergebnisse des dritten Experiments dokumentieren, dass die Alterung von Biokohle im Boden die Kohlenstoffmineralisierung von Stroh beeinflusste. Die Halbwertszeiten von Kohlenstoff waren signifikant in den Varianten höher, in denen die Biokohle einer 365-tägigen Verweildauer (Alterung) in den Böden unterlag. In den Varianten mit Holzkohle wurde eine Stabilisierung von Strohkohlenstoff beobachtet. Im Vergleich dazu wurde eine höhere Kohlenstoffmineralisation oder ein priming Effekt in den Varianten mit HTC- und NTK-Kohle dokumentiert. Die Ergebnisse deuten auf Interaktionen zwischen den im Boden gealterten Biokohlen sowie gedüngtem Stroh. Es sind weitere Forschungen mit markiertem Kohlenstoff erforderlich, um die Mechanismen von Biokohlen auf Stabilisierung und Mineralisation von Kohlenstoff in unseren Böden besser zu verstehen.

8. REFERENCES

Ajwa, H.A., and M.A. Tabatabai. 1994. Decomposition of different organic materials in soils. Biol. Fertil. Soils 18:175–182.

Alvarez, R., and C.R. Alvarez. 2000. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Sci. Soc. Am. J. 64:84–189.

Antal, M.J., and M. Gronli. 2003. The art, science, and technology of charcoal production. Ind. Eng. Chem. Res. 42:1619–1640.

Arrouays, D., I.Vion, and J.L. Kicin. 1995. Spatial analysis and modelling of topsoil carbon storage in temperate forest humic loamy soils of France. Soil Sci. 159:191–198.

Asai, H., B. Samson, H. Stephan, K. Songyikhangsuthor, K. Homma, Y. Kiyono, Y. Inoue, T. Shiraiwa, and T. Horie. 2009. Biochar amendment techniques for upland rice production in Northern Laos1. Soil physical properties, leaf SPAD and grain yield. Field Crops Res. 111:81-84.

Augustenborg, C.A., S. Hepp, C. Kammann, D. Hagan, O. Schmidt, and C. Mueller. 2011. Biochar and earthworm effects on soil nitrous oxide and carbon dioxide emissions. J. Environ. Qual. doi:10.2134/jeq2011.0119.

Baldock, J.A., and R.J. Smernik. 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (red pine) wood. Org. Geochem. 33:1093–1109.

Batjes, N.H. 1996. Total carbon and nitrogen in soils of the world. Eur. J. Soil Sci. 47:151-163.

Blume, H.P., G.W. Brummer, R. Horn, E. Kandeler, I. Kogel-Knabner, R. Kretzschmar, K. Stahr, and B. M. Wilke. 2010. Scheffer/Schachtschabel: Lehrbuch der Bodenkunde (Text book of soil science). 16th ed. Ferdinand Enke Verlag Stuttgart, Germany. pp.351-352.

Bot, A., and J. Benites. 2005. Importance of Soil Organic Matter, Key to Drought-Resistant Soil and Sustained Food and Production. FAO. Rome, Italy.

Bouajila, A., and T. Gallali. 2008. Soil organic carbon fractions and aggregate stability in carbonated and no carbonated soils in Tunisia. J. Agron. 2:127-137.

Bridle, T.R., and D. Pritchard. 2004. Energy and nutrient recovery from sewage sludge via pyrolysis. Water Sci. Technol. 50:169–75.

Brodowski, S., B. John, H. Flessa, and W. Amelung. 2006. Aggregate-occluded black carbon in soil. Eur. J. Soil Sci. 574:539–546.

Brodowski, S.B. 2004. Origin, Function, and Reactivity of Black Carbon in the Arable Soil Environment. PhD thesis. University of Bayreuth, Germany.

Buyanovsky, G.A., and G.H. Wagner. 1998. Changing role of cultivated land in the global carbon cycle. Biol. Fertil. Soils 27:242–245.

Chan, K.Y., L.Van Zwieten, I. Meszaros, A. Downie, and S. Joseph. 2007. Agronomic values of green waste biochar as a soil amendment. Aust. J. Soil Res. 45:629–634.

Chen, B., and M. Yuan. 2010. Enhanced sorption of polycyclic aromatic hydrocarbons by soil amended with biochar. J. Soils Sediments 11:62–71.

Cheng, C., J. Lehmann, J. Thies, S. Burton, and M. Engelhard. 2006. Oxidation of black carbon by biotic and abiotic processes. Org. Geochem. 37:1477–1488.

Cheng, C.H., J. Lehmann, and M.H. Engelhard. 2008. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72:1598–1610.

Cheshire, M.V., J.D. Russell, and A.R. Fraser. 1993. The decomposition of straw in soil: Use of multiple internal reflectance infrared spectroscopy. J. Soil Sci. 44:693–702.

Cross, A., and S.P. Sohi. 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. Soil Biol. Biochem. 43:2127-2134.

Cox, R.J., H.L. Peterson, J. Young, C. Cusik, and E.O. Espinoza. 1999. The Forensic Analysis of Soil Organic by FTIR. Forensic Sci. Int. 108:107-116.

Dalal, R.C., and R.J. Mayor. 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. IV. Loss of organic carbon from different density fractions. Aust. J. Soil. Res. 24:301–309.

De Jong, E., and R.G. Kachanoski. 1988. The importance of erosion in the carbon balance of prairie soils. Can. J. Soil Sci. 68:111–119.

De Neve, S., J. Pannier, and G. Hofman. 1996. Temperature effects on C- and N-mineralization from vegetable crop residues. Plant Soil 181:25–30.

DeLuca, T.H., M.D.M. Kenzie, M.J. Gundale, and W.E. Holben. 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Sci. Soc. Am. J. 70:448–453.

Doydora, S., M.L. Cabrera, K.C. Das, J.W. Gaskin, L.S. Sonon, and W.P. Miller. 2011. Release of nitrogen and phosphorus from poultry litter amended with acidified biochar. Int. J. Environ. Res. Public Health 8:1491-502.

Fearnside, P.M. 2000. Greenhouse gas emissions from land-use change in Brazil's Amazon Region. In: Lal, R., Kimble, J.M., Stewart, B.A. (Eds.), Global Climate Change and Tropical Ecosystems. CRC Press, Boca Raton, FL, pp. 438.

Galinato, G.I., A. Olanie, S. Uchida, and J.K. Yoder. 2011. Long-term versus temporary certified emission reductions in forest carbon sequestration programs. Aust. J. Agr. Res. Econom. 55:537–559.

Glaser, B., E. Balashov, L. Haumaier, G. Guggenberger, and W. Zech. 2000. Black carbon in the density fractions of anthropogenic soils of the Brazilian Amazon Region. Org. Geochem. 31:669–678.

Glaser, B., J. Lehmann, and W. Zech. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – A review. Biol. Fertil. Soils 35:219–230.

Glaser, B., L. Haumaier, G. Guggenberger, and W. Zech. 2001. The 'Terra Preta' phenomenon: A model for sustainable agriculture in the humid tropics. Naturwissenschaften 88:37–41.

Gleixner, G., D.J. Czimczik, C. Kramer, B. Luhker, and M.W.I. Schmidt. 2001. Plant compounds and their turnover and stability as soil organic matter. pp. 201–215. In: E.D. Schulze et al (Ed.) Global Biogeochemical Cycles in the Climate System. Academic Press, SD.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Study of free and occluded particulate organic matter in soils by solid-state 13C NMR spectroscopy and scanning electron microscopy. Aus. J. Soil Res. 32:285–309.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1995. Structural and dynamic properties of soil organic matter as reflected by 13C natural abundance, pyrolysis mass spectrometry and solid-state 13C NMR spectroscopy in density fractions of a Ferralsol under forest and pasture. Aust. J. Soil Sci. 33:59–76.

Grady, R.O., and R. Rush. 2007. The Terra Preta phenomenon. *In.* Managing the Carbon Cycle' Katanning Workshop 21-22 March. pp. 11-15.

Gregg, S.J., and S.W. Sing. 1982. Adsorption, surface area and porosity. Academic Press, NY.

Gruenewald, G., K. Kaiser, R. Jahn, and G. Guggenberger. 2006. Organic matter stabilization in young calcareous soils as revealed by density fractionation and analysis of lignin-derived constituents. Org. Geochem. 37:1573–1589.

Guggenberger, G., and K. Kaiser. 2003. Dissolved organic matter in soil. Challenging the paradigm of sorptive preservation. Geoderma 113:293–310.

Haberhauer. G., and M.H. Gerzabek. 1999. Drift and transmission FT-IR spectroscopy of forest soils: an approach to determine decomposition processes of forest litter. Vib. Spec. 19:413–417.

Hamer, U., B. Marschner, S. Brodowski, and W. Amelung. 2004. Interactive priming of black carbon and glucose mineralisation. Organic Geochem. 35:823–830.

Hammes, K., and W.I. Schmidt. 2009. Changes of biochar in soil. In: Lehmann, J., Joseph, S., (Eds.), Biochar for Environmental Management Science and Technology. Earthscan, London, pp. 169–182.

Hammes, K., M.S. Torn, A.G. Lapenas, and M.W.I. Schmidt. 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeoscience 5:1339–1350.

Harden, J.W., S.E. Trumbore, B.J. Stocks, A. Hirsch, S.T. Gower, K.P. O'neill, and E.S. Kasischke. 2002. The role of fire in the boreal carbon budget. Global Chang. Biol. 6:174–184.

Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralizaion. Soil Biol. Biochem. 26:1221–1231.

Hopkins, D.W. 2008. Carbon mineralization. In: M.R. Carter and E.G. Gregorich (eds.) Soil Sampling and Methods of Analysis. 2nd ed. CRC Press. Boca Raton, FL. pp. 589-599.

Hossain, M. K., V. Strezov, K.Y. Chan, A. Ziolkowski, and P.F. Nelson. 2011. Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. J. Environ. Manag. 92:223-228. doi: 10.1016/j.jenvman.2010.09.008.

Houba, V.J.G., I. Novozamsky, A.W.M. Huijbregts, and J.J. van der Lee. 1986. Comparison of soil extractions by 0.01 M $CaCl_2$, by UAF and some conventional extraction procedures. Plant Soil 96:433–437.

IPCC, 2007. Climate Change 2007: Impacts, Adaptation and Vulnerability. Working Group II Contribution to the 4th Assessment Report. Cambridge Univ. Press, Cambridge, UK.

Ismail, I., R.L. Blevins, and W.W. Frye. 1994. Long-term no-tillage effects on soil properties and continuous corn yields. Soil Sci. Soc. Am. J. 58:193-198.

Janzen, H.H., C.A. Campbell, S.A. Brandt, G.P. Lafond, and L. Townley-Smith. 1992. Light fraction organic matter in soils from long-term crop rotations. Soil Sci. Soc. Am. J. 56:1799–1806.

Joseph, S.D., M. Camps-Arbestain, Y. Lin, P. Munroe, C.H. Chia, J. Hook, L. van Zweiten, S. Kimber, B.P. Singh, J. Lehmann, N. Foidl, R.J. Smernik, and J.E. Amonette. 2010. An investigation into the reactions of biochar in soil. Aust. J. Soil Res. 48:501–515.

Kaiser, K., G. Guggenberger, and W. Zech. 1996. Sorption of DOM and DOM fractions to forest soils. Geoderma 74:281–303.

Kalbitz, K., and K. Kaiser. 2008. Contribution of dissolved organic matter to carbon storage in forest mineral soils. J. Plant Nutr. Soil Sci. 171:52–60.

Kammann, C.I., S. Linsel, J.W. Groesling, and H. Koyro. 2011. Influence of biochar on drought tolerance of *Chenopodium quinoa* and on soil-plant relations. Plant Soil. 345:195–210.

Karhu, K., T. Mattilab, I. Bergströma, and K. Reginac. 2011. Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity- Results from a short-term pilot field study. Agric. Ecosyst. Environ. 140:309–313.

Keith, A., B. Singh, and B.P. Singh. 2011. Interactive Priming of Biochar and Labile Organic Matter Mineralization in a Smectite-Rich Soil. Environ. Sci. Technol. 45:9611–618.

Kimetu, J.M., and J. Lehmann. 2010. Stability and stabilisation of biochar and green manure in soil with different organic carbon contents. Aus. J. Soil Res. 48:577–585.

Knicker, H., P.G. Hatcher, and A.W. Scaroni. 1996. A solid-state 15N NMR spectroscopic investigation of the origin of nitrogen structures in coal. International J. Coal Geo. 32:255–278. doi: 10.1016/S0166-5162(96)00040-7.

Kolb, S.E., K.J. Fermanich, and M.E. Dornbush. 2009. Effect of charcoal quantity on microbial biomass and activity in temperate soils. Soil Sci. Soc. Am. J. 73:1173–1181.

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32:1485–1498.

Laird, D.A. 2008. The charcoal vision: A win–win–win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. Agron. J. 100:178–181.

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123:1-22. doi:10.1016/j.geoderma.2004.01.032.

Lal, R. 2006. Carbon Management in Agricultural Soils. Mitigation and Adaptation Strategies for Global Change 12:303-322. doi:10.1007/s11027-006-9036-7

Lal, R. 2009. Sequestering atmospheric carbon dioxide. Critical Rev. Plant Sci. 28:90-96. doi:10.1080/07352680902782711.

Lal, R. 2011. Sequestering carbon in soils of agro-ecosystems. Food Policy. 36:33-39.

Lehmann, J. 2006. Black is the new green. Nature 442:624-626.

Lehmann, J. 2007. Bio-energy in the black. Front. Ecol. Environ. 57:381–387.

Lehmann, J., J. Gaunt, and M. Rondon. 2006. Bio-char sequestration in terrestrial ecosystems — A review. Mitig. Adapt. Strateg. Glob. Change 11:403–427.

Liang, B., J. Lehmann, D. Solomon, J. Kinyangi, J. Grossman, B. O'Neill, J.O. Skjemstad, J. Thies, F.J. Luizao, J. Peterson, and E.G. Neves. 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70:1719–1730.

Liang, B., J. Lehmann, D. Solomon, S. Sohi, J.E. Thies, J.O. Skjemstad, F.J. Luizão, M.H. Engelhard, E.G. Neves, and S. Wirick. 2008. Stability of biomass-derived black carbon in soils. Geochim. Cosmochim. Acta. 72:6069-6078. doi: 10.1016/j.gca.

Madejova, J., 2003. FTIR techniques in clay mineral studies. Vib. Spectrosc. 31:1–10.

Madejova, J., and P. Komadel. 2001. Baseline studies of the clay minerals society source clays: infrared methods. Clays Clay Miner. 49:410–432.

Major J., M. Rondon, D. Molina, S.J. Riha, and J. Lehmann. 2011. Nutrient leaching in a Colombian savanna Oxisol amended with Biochar. J. Environ. Qual. DOI: 10.2134/jeq2011.0128.

Major, J., M. Rondon, D. Molina, S.J. Riha, and J. Lehmann. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil 333:117-128.

McLaughlin, H., P.S. Anderson, F.E. Shields, and T.B. Reed. 2009. All biochars are not created equal, and how to tell them apart. Proceedings, North American Biochar Conference, Boulder, Colorado, August 2009. www.biochar-international.org/sites/default/files/All- Biochars--Version2--Oct2009.pdf

Mehlich, A. 1948. Determination of cation and anion exchange properties of soils. Soil. Sci. 66:429–445.

Middelburg, J.J., J. Nieuwenhuize, and P. van Breugel. 1999. Black carbon in marine sediments. Marine Chem. 65:245–252.

Mutuo, P.K., K.D. Shepherd, A. Albrecht, and G. Cadisch. 2006. Prediction of carbon mineralization rates from different soil physical fractions using diffuse reflectance spectroscopy. Soil Biol. Biochem. 38:1658–1664.

Nguyen, B.T., J. Lehmann, W.C. Hockaday, S. Joseph, and C.A. Masiello. 2010. Temperature sensitivity of black carbon decomposition and oxidation. Environ. Sci. Technol. 44:3324–3331.

Novak, J. M., W.J. Busscher, D.W. Watts, D.A. Laird, M.A. Ahmedna, and M.A.S. Niandou. 2010. Short-term CO_2 mineralization after additions of biochar and switchgrass to a Typic Kandiudult. Geoderma 154:281–288.

Peng, X., L.L, Ye, C.H. Wang, H. Zhou, and B. Sun. 2011. Temperature- and duration-dependent rice straw-derived biochar: Characteristics and its effects on soil properties of an Ultisol in southern China. Soil Tillage Res. 112:159-166.

Preston, C.M., and M.G. Schmidt. 2006. Black (pyrogenic) carbon in boreal forests: A synthesis of current knowledge and uncertainties. Biogeoscience 3:397–420.

Priemé, A., and S. Christensen. 1999. Methane uptake by a selection of soils in Ghana with different land use. J. Geophys. Res. 104:23617–23622.

Qayyum, M.F., D. Steffens, H.P. Reisenauer, and S. Schubert. 2011. Kinetics of carbon mineralization of biochars compared with wheat straw in three soils. J. Environ. Qual. doi:10.2134/jeq2011.0058.

Rasmussen, P.E., and C.R. Rohde. 1988. Long-term tillage and nitrogen fertilization effects on organic nitrogen and carbon in a semiarid soil. Soil Sci. Soc. Am. J. 52:1114–1117.

Recous, S., D. Robin, D. Darwis, and B. Mary. 1995. Soil inorganic N availability: Effect on maize residue decomposition. Soil Biol. Biochem. 27:1529–1538.

Ribeiro, H.M., D. Fangueiro, F. Alves, E. Vasconcelos, J. Coutinho, and R. Bol. 2010. Carbon-mineralization kinetics in an organically managed Cambic Arenosol amended with organic fertilizers. J. Plant Nutr. Soil Sci. 173:39–45.

Rondon, M.A., J. Lehmann, J. Ramirez, and M. Hurtado. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with biochar additions. Biol. Fertil. Soils 43:699–708.

Rosopulo, A. 1985. Schwermetallbestimmung direkt aus dem Feststoff und nach chemischem Aufschluss - ein Methodenvergleich. (In German, with English Abstract.) Fresenius. Z. Anal. Chem. 322:669-672.

Saggar, S., K.R. Tate, C.W. Feltham, C.W. Childs, and A. Parshotam. 1994. Carbon turn-over in a range of allophanic soils amended with 14C-labelled glucose. Soil Biol. Biochem. 26:1263–1271.

Saviozzi, A., R. Levi-Minzi, and R. Riffaldi. 1993. Mineralization parameters from organic materials added to soil as a function of their chemical composition. Biores. Technol. 45:131-135.

Saviozzi, A., R. Levi-Minzi, R. Riffaldi, and G. Vanni. 1997. Role of chemical constituents of wheat straw and pig slurry on their decomposition in soil. Biol. Fertil. Soils 25: 401–406.

Schlesinger, W.H. 1999. Carbon sequestration in soils. Science. 284:5423–2095.

Schneour, E.A. 1966. Oxidation of graphitic carbon in certain soils. Science 151:991–992.

Schueller, H. 1969. Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphors in Böden. (In German, with English Abstract) Z. Pflanzenernähr. Bodenkd. 123:48–63.

Seiler, W., and P.J. Crutzen. 1980. Estimates of gross and net fluxes of carbon between the biosphere and the atmosphere from biomass burning. Clim. Change 2:207–247.

Shindo, H. 1991. Elementary composition, humus composition, and decomposition in soil of charred grassland plants. Soil Sci. Plant Nutr. 37:651–657.

Six J., C. Feller, K. Denef, S.M. Ogle, J.C.M. Sa, and A. Albrecht. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils - effects of no-tillage. Agronomie. 22: 755–775.

Sleutel, S., S. De Neve, M.R. Prat Roibas, and G. Hofman. 2005. The influence of model type and incubation time on the estimation of stable organic carbon in organic materials. Eur. J. Soil Sci. 56:505–514.

Smith, J.L., R.R. Schnabel, B.L. Mc Neal, and G.S. Campbell. 1980. Potential errors in the first-order model for estimating soil nitrogen mineralization potentials. Soil Sci. Soc. Am. J. 44:996-1000.

Sohi, S.P., N. Mahieu, J.R.M. Arah, D.S. Powlson, B. Madari, and J.L.Gaunt. 2001. A procedure for isolating soil organic matter fractions suitable for modeling. Soil Sci. Soc. Am. J. 1128: 1121-1128.

Sombroek, W., M. de Lourdes Ruivo, P.M. Fearnside, B. Glaser, and J. Lehmann. 2003. Amazonian Dark Earth as carbon stores and sinks. In: Lehmann, J., D.C. Kern., B. Glaser, and W.I. Woods, W.I. (Eds.), Amazonian Dark Earths: Origin, Properties, Management. Kluwer Academic Publishers, Dordrecht. pp. 125-140.

Sparks, D.L. 1989. Kinetics of soil chemical processes. Academic Press. San Diego. CA. USA.

Spokas, K. A. 2010. Review of the stability of biochar in soils: Predictability of O : C molar ratios. Carbon Managem. 1: 289–303.

Steinbeiss, S., G. Gleixner, and M. Antonietti. 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41:1301–1310.

Steiner, C., W.G. Teixeira, J. Lehmann, T. Nehls, J.L.V. de Macedo, W.E.H. Blum, and W. Zech. 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291:275–290.

Stevenson, F.J. 1996. Nitrogen – Organic forms. In: Sparks, D.L., (Ed.), Methods of Soil Analysis. Part. 3. Chemical Methods. SSSA Book Set. 5. Madison, WI. pp. 1185-1200.

Stewart, C.E., K. Paustian, R.T. Conant, A.F. Plante, and J. Six. 2007. Soil C saturation: concept, evidence, and evaluation. Biogeochemistry 86:19–31.

Streubel, J.D., H.P. Collins, M. Garcia-Perez, J. Tarara, D. Granatstein, and C. E. Kruger. 2011. Influence of contrasting Biochar types on five soils at increasing rates of application. Soil Sci. Soc. Am. J. DOI: 10.2136/sssaj2010.0325.

Swanston, C.W., B.A. Caldwell, P.S. Homann, L. Ganio, and P. Sollins. 2002. Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. Soil Biol. Biochem. 34:1121–1130.

Talpaz, H., P. Fine, and B. Bar-Yosef. 1981. On the estimation of N-mineralization parameters from incubation experiments. Soil Sci. Soc. Am. J. 45:993-996.

Tamm, O. 1932. Über die Oxalat-Methode in der chemischen Bodenanalyse. Medd. Stat. Skogsforsoks. Stockholm. 27:1-20.

Tatzber, M., M. Stemmer, H. Spiegel, C. Katzlberger, G. Haberhauer, A. Mentler, and M.H. Gerzabek. 2007. FTIR-spectroscopic characterization of humic acids and humin fractions obtained by advanced NaOH, $Na_4P_2O_7$, and Na_2CO_3 extraction procedures. J. Plant. Nutr. Soil Sci. 170:522–529.

Tian, G., B.T. Kang, and L. Brussaard. 1992. Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions – decomposition and nutrient release. Soil Biol. Biochem. 24:1051–1060.

Tiessen, H., E. Cuevas, and P. Chacon. 1994. The role of soil organic matter in sustaining soil fertility. Nature 371:783–785.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. J. Soil Sci. 33:141–161.

Titirici, M.M., A. Thomas, and M. Antonietti. 2007. Back in the black: hydrothermal carbonization of plant material as an efficient chemical process to treat the CO_2 problem? New J. Chem. 31:787–789.

Terhoeven-Urselmans, T., T. Vagen, O. Spaargaren, and K.D. Shepher. 2010. Prediction of Soil Fertility Properties from a Globally Distributed Soil Mid-Infrared Spectral Library. Soil Sci. Soc. Am. J. 74:1792–1799.

Unger, R., and R. Killorn. 2011. Effect of three different qualities of Biochar on selected soil properties. Commun. Soil Sci. Plant Anal. 42:2274-2283.

van Zwieten, L., S. Kimber, S. Morris, A. Downie, E. Berger, J. Rust, and C. Scheer. 2010. Influence of biochars on flux of N_2O and CO_2 from Ferrosol. Aust. J. Soil Res. 48:555–568.

von Lützow, M., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions — a review. Eur. J. Soil Sci. 57:426–445.

Wardle, D., M. Nilsson, and O. Zackrisson. 2008. Fire-derived charcoal causes loss of forest humus. Science 320: 629-629

Whalen, J.K., P.J. Bottomley, and D.D. Myrold. 2000. Carbon and nitrogen mineralization from light and heavy fraction additions to soil. Soil Biol. Biochem. 32:1345–1352.

Woolf, D., J.E. Amonette, F.A. Street-Perrott, J. Lehmann, and S. Joseph. 2010. Sustainable biochar to mitigate global climate change. Nat. Commun. Doi: 10.1038/ncomms1053.

Yanai, Y., K. Toyota, and M. Okazaki. 2007. Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Sci. Plant Nutr. 53:181-188.

Yao, Y., B. Gao, M. Inyang, A.R. Zimmerman, X. Cao, P. Pullammanappallil, and L. Yang. 2011. Removal of phosphate from aqueous solution by biochar derived from anaerobically digested sugar beet tailings. J. Hazard. Mater. doi: 10.1016/j.jhazmat.2011.03.083.

Zhang, A., Y. Liu, G. Pan, Q. Hussain, L. Li, J. Zheng, and X. Zhang. 2011. Effect of biochar amendment on maize yield and greenhouse gas EMISSIONS from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant Soil. DOI: 10.1007/s11104-011-0957-x.

Zimmerman, A.R., B. Gao, and M. Ahn. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 43:1169–1179.

DECLARATION / ERKLÄRUNG

"I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice."

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Muhammad Farooq Qayyum

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ACKNOWLEDGMENTS

I would like to start with my praises and thanks to almighty Allah, the compassionate, the merciful, and the source of all knowledge and wisdom. I also offer my humble thanks to the Holy Prophet Muhammad (peace be upon him), whose moral and spiritual teachings enlightened our hearts and minds towards achieving high ideas of life.

I wish to express my sincere respect to Prof. Dr. Sven Schubert who not only served as my supervisor but also encouraged and challenged me throughout my doctoral program. I truly appreciate all the time and advice you gave me throughout my time in Giessen, Germany. Besides, I would like to thank my co-supervisor Prof. Dr. Sylvia Schnell for providing valuable suggestions during my research and reviewing of the present dissertation.

Thanks to the entire Institute of Plant Nutrition. Special credit and appreciation to Prof. Dr. Diedrich Steffens whose help, motivating suggestions and encouragement helped me in all time of research and for writing manuscripts and the present thesis.

All of the faculty and staff at the Institute were wonderful people and it was a real pleasure to work with them. Special thanks to Roland Pfanschilling, Ms. Anne Weber, Ms. Christina Plachta, Ms. Christa Lein, Ms. Rödiger, and Ms. Tripp for their cooperation and technical support during laboratory work. I am also thankful to Dr. Hans P. Reisenauer, who allowed me to complete spectroscopic analysis in his laboratory.

For my all happy moments in the laboratories and in the social-room of the Institute, I would like to thank all colleagues and friends in the Institute especially, Dr. Ahmad Naeem, Dr. Faiq, Imran Ashraf, Dr. Stefan Hanstein, Nesar Uddin, Dr. Muhammad Farooq, Phillip Eitenmüller, Franziska Faust, Alexandra Wening, Alexandra Riffel, Dr. Abdul Wakeel Dr. Abdel Kareem, and Stefan Jung.

I want to thank all of my friends particularly Imran Mehmood, Muqarrab, Sinan, Sarfraz, Shaghef, Hafiz Arshad, Dr. Jam Niaz, and Hammad who always offered me support and best wishes during my study. The moral support and care by the friends here in Giessen especially, Dr. Ijaz, Dr. Habib Ullah, Dr. Athar, Dr. Sajid Ali, Dr. Shafaqat, Ghulam Haider and Dr. Amir Rafiq helped me overcome setbacks and stay focused on studies. I greatly value their friendship.

I offer my grateful recognition to my parents, my brothers and sister, whose selfless devotion and prayers were always with me. Most importantly, my doctoral studies would have not been possible without the love and patience of wife Iram and my son Asad. My words cannot express thanks for their understanding, patience and love during the past few years. The love and affection with Muhammad Anas Wakeel will always remain alive in my recalls.

Finally, I sincerely acknowledge Higher Education Commission Pakistan (HEC) and German Academic Exchange Service (DAAD) for financial support and arrangements made during my stay in Germany.

Muhammad Farooq Qayyum

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.

The curriculum vitae was removed from the electronic version of the paper.

édition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN: 978-3-8359-5895-1

Cover photo: © matamu - Fotolia.com