The effects of biochar or activated carbon amendments on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil

Khaled Mohamed Mossa Bushnaf

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Environmental Engineering group

School of Civil Engineering and Geosciences

Abstract

The impact of biochar or activated carbon (AC) amendments on the biodegradation of a mixture of 12 volatile petroleum hydrocarbons (VPHs) in an aerobic sandy soil was investigated in batch microcosms and column experiments. The impacts of biochar amendments on nutrient availability and biogenic gases activity were also studied by batch microcosms. The maximum nutrient amount adsorbed by biochar (q_{max}) was very high. Therefore, biochar amendments decreased the readily available nitrogen with increasing biochar application rate and contact period. Biogenic gas activities in biochar amended soils had varied responses because these activities were dependent on soil properties. The effects of sorbent amendment significantly depended on the compound chemical structure and type of sorbent material. In the batch microcosms, the AC and biochar amendments resulted in a large increase in the K_{OC} values. The biodegradation of the water-dissolved fraction of most compounds was as fast or faster in the soil amended with activated carbon compared to the soil with or without biochar, but the strong sorption capacity of activated carbon, in particular, greatly reduced water-dissolved concentrations. The nutrient amendments accelerated the biodegradation of VPHs in the batch microcosms and nutrient availability was the main factor controlling the biodegradation rates of total petroleum hydrocarbons in sandy soil; whereas sorption was a secondary factor influencing the biodegradation of total petroleum hydrocarbons in biochar and activated carbon amended sandy soil. The biodegradation in sorbent amended columns was difficult to predict. The sorbent amendments decreased the availability of both VPHs and nutrients. Therefore, the biodegradation rate was reduced. However, the petroleum hydrocarbon vapour migration and volatilization was also reduced, which increased the residence time of contaminants in the sorbent amended column. This means there was more time available to degrade the pollutants before they emanated from soil, and therefore the sorbent amendments may result in a greater amount of biodegradation, if considered over a certain distance and over a long time period. It is concluded that biochar and activated carbon amendments are potentially a sustainable remediation strategy for dealing with volatile petroleum hydrocarbons pollution. These sorbents are able to reduce the risk of VPHs to biota and the also surrounding environments without using large scale, energy intensive and treatment processes.

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Chapter 1: Aims, objective and thesis outline

1.1 Overview

Over the last century, modern society's demands for petroleum hydrocarbons such as gasoline, kerosene, diesel and heating oil have increased. Petroleum hydrocarbons have been released in soils due to human errors, accidents or corrosion of storage tanks and pipes (Shabir *et al.*, 2008). Once the leaks are discovered and fixed, residual amounts of these compounds will still remain in soil pores and will spread by diffusion as hydrocarbon vapours in the surrounding soil matrix. Some of these vapours will dissolve in soil water and could reach ground water.

Contaminated land. The definition of contaminated land and contamination levels is not simple, due to different views on what should be considered as contaminated land by reference to original and/or background concentrations (Pollard *et al.*, 2001). However, the UK Environment Protection Agency provided the statutory definition of contaminated land in the UK by The Environmental Protection Act1990 Part IIA which defines contaminated land as:

" Contaminated Land is any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that:

(a) Significant harm is being caused or there is a significant possibility of such harm being caused; or

(b) Pollution of controlled waters is being, or is likely to be caused"

This statutory definition depends on a significant risk of harm which pollution may pose to the health of living organisms with current or intended land use. A site which is not suitable for use, is considered to be contaminated. For example, a certain contaminant level could pose risks to human health if this site were used for housing. However, there are no risks to human health if this site were used for parking. According to Pollard et *al.* (2001) there are approximately 100,000 land sites in England and Wales which are estimated to be contaminated sites by the Environment Agency. These areas range from 50,000 to 200,000 ha. Moreover, between 5,000 and

20,000 ha may require remediation, due to their high risk to human health or the environment (Pollard *et al.*, 2001).

Natural attenuation. The term "natural attenuation" is used to describe the fate and transport processes which reduce the concentration of contaminants in the environment (Wiedemeier *et al.*, 2007). These processes include dilution, dispersion, sorption, volatilization, hydrolysis and biodegradation. Processes such as dilution, dispersion, sorption, and volatilization reduce the aqueous phase pollutant concentration but not the total mass of pollutant. However, hydrolysis and biodegradation cause reduction in both the aqueous concentration and total mass of a contaminant (Wiedemeier *et al.*, 2007). The contribution of each process depends on contaminant properties and the chemical, physical and microbial nature of contaminated soil. Understanding the contribution of each process helps to predict the fate and transport of contaminants in different media and how long contaminants will persist and how far the contaminant will migrate from the source (Wiedemeier *et al.*, 2007).

Bioremediation. The term "bioremediation" is widely used to describe the biotransformation of environmental contaminants, due to biological processes. Biodegradation is used to describe the breakdown of organic molecular structures by microbial communities resulting in simplification of organic compounds into CO_2, CH_4, H_2O and mineral salts (Alvarez and Illman, 2006). Bioremediation includes two main techniques: biostimulation which provides the optimum environmental condition to indigenous pollutant degrading microbial communities by providing nutrients, electron acceptors and or adjusting the pH and redox potential. Bioaugmentation additionally comprises injection of specialized bacteria to enhance contaminant remediation. Biostimulation is a very common technique for the bioremediation of hydrocarbon contaminated soils because indigenous bacteria are typically able to degrade hydrocarbons. Moreover, the indigenous bacteria are more adapted to environmental conditions at contaminated sites in comparison with specialized bacteria. Bioremediation is amongst the most popular remediation approaches, due to low-cost, low risk and the ability to breakdown a wide variety of organics (Alvarez and Illman, 2006).

1.2 Risk assessment of contaminated land

Soil contamination may be a source of risk if toxic substances reach receptors by various pathways (Liptak and Lombardo, 1996). Risk assessment is a tool providing the necessary information that identifies risks of contaminated site to ensure that human health and the environmental are protected (Liptak and Lombardo, 1996; Nathanail and Bardose, 2004). The relevant legislation is Part IIA of the Environmental Protection Act 1990, that require risk assessment of potentially contaminated land. This requires local authorities to identify existing contaminated sites and to identify whether or not contamination poses an unacceptable risk to human health or the environment. After a site has been determined as contaminated land by the local authority, steps should be taken to reduce the risks to acceptable levels (Nathanail and Bardose, 2004). The risk assessment can help to make decisions for selecting an appropriate remediation approach and necessary extent of remediation based on scientific, social and economic cost to ensure that any unacceptable risk will be reduced to an acceptable level (Liptak and Lombardo, 1996; Nathanail and Bardose, 2004).

Risk analysis is preliminary based on health-based investigation levels that are the first considerations in assessing the potential for health effects from contaminated sites (Schmidt et al., 1998b). Official risk assessments guides which are followed today are provided by the Department for Environment, Food &Rural affairs (DEFRA) and Environment Agency, (2004). These guides are called CLR11 Model Procedures which provide the framework for conducting contaminated land risk assessment in the UK. Following the framework will help a local authority to satisfy that the conclusions of the risk assessment are valid. The CLR11 document recommends a three-tiered approach to simplify the risk-assessment process and focus the effort on areas where risks are potentially unacceptable:

Tier 1: Preliminary Risk Assessment;

Tier 2: Generic Quantitative Risk Assessment;

Tier 3: Detailed Quantitative Risk Assessment.

The preliminary risk assessment (tier 1) is the first stage aiming to develop a conceptual model of risk for the site. The conceptual model is a simplified description of the pathways between source and receptors and how exposure to any contamination at the site may occur (DEFRA and Environment Agency, 2004). The conceptual model aims to define how a contamination source may cause adverse effects, release mechanisms (migration in soil, leaching to water, emission to air), retention in the transport medium (soil, air, surface water, groundwater), exposure point and exposure route (DEFRA and Environment Agency, 2004). This model can be developed based on historical information, geology, hydrogeology, meteorological data, the source geometry (that may be present in surface soil, deep soil and/or groundwater, physical-chemical properties of the contaminant, sampling and chemical analysis. Fig 1.1 illustrates an example of a conceptual model describing the relationship between the source-pathway-receptor elements near a petroleum hydrocarbon contaminated site.

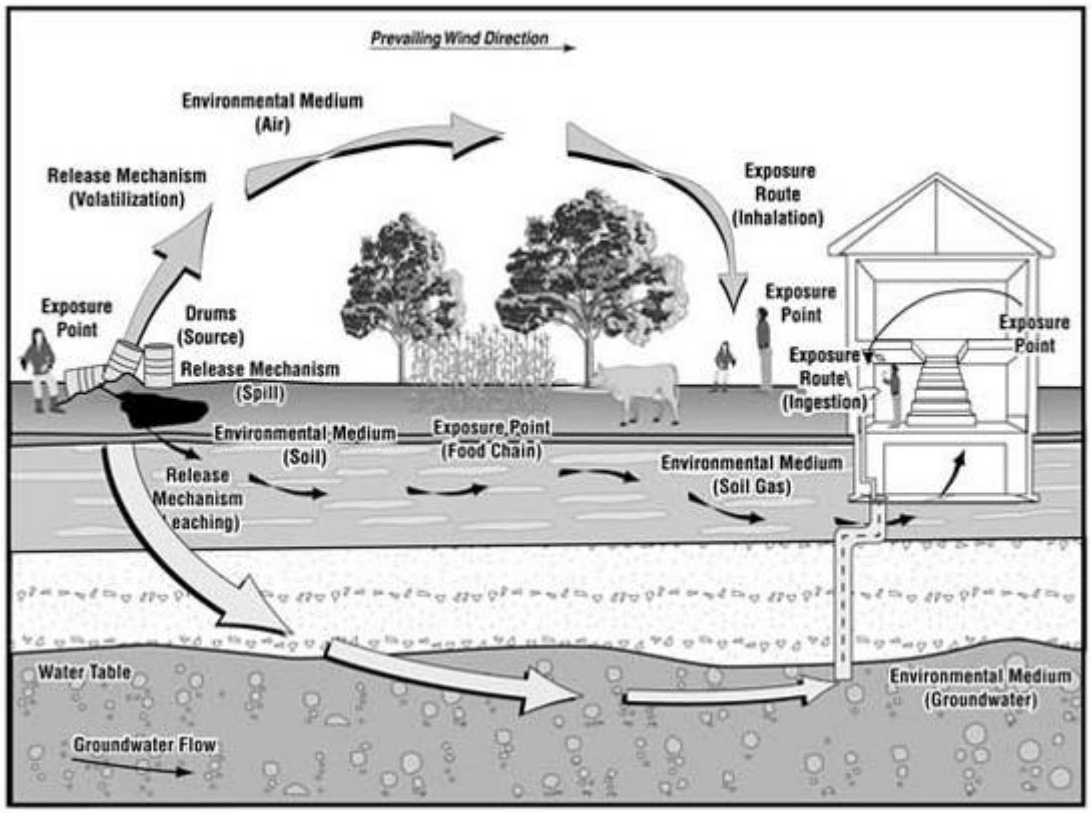

Fig 1. 1. The conceptual model of a site contaminated with petroleum hydrocarbons (ASTDR, 2005)

The second stage (tier 2) is generic quantitative risk assessment which aims to compare measured concentrations of contaminants in soil, water or soil gas at a site with the Soil Guideline Values (SGV), and identify if these concentration exceed the SGV and identify if the pathways between contaminant and receptors poses unacceptable risk. This stage also aims to propose next steps in relation to the site remediation (DEFRA and Environment Agency, 2004).

The third stage (tier 3) is the detailed risk assessment which may be carried out if required. This stage may involve toxicity assessment of particular contaminants and apply site-specific contaminated fate and transport models to measure the magnitude, frequency, and duration of exposure to contaminants which help to better understand the site. At the end of this stage, the risk assessment can identify unacceptable risks associated with these linkages and propose further action, if needed (DEFRA and Environment Agency, 2004).

The end stage is the risk management which is the process of evaluating alternative actions and to select a remediation strategy in response to unacceptable environmental risk to mitigate the potential risk (Provoost *et al.*, 2010). The three-tiered risk assessment approach is illustrated in Fig 1.2. This sketch is a useful guide which indicates the risk-assessment procedure and the basis of a decision and it underlines what is the next step of risk assessment and risk management.

Implementation of risk assessment can help local authorities to identify both the unacceptable risk of a contaminated site to human health or the environment and the exposure pathways. This is essential information which can help decision makers to select and design monitoring systems and appropriate remediation techniques and the necessary extent of remediation based on land use, scientific evidence, social aspects and economic cost.

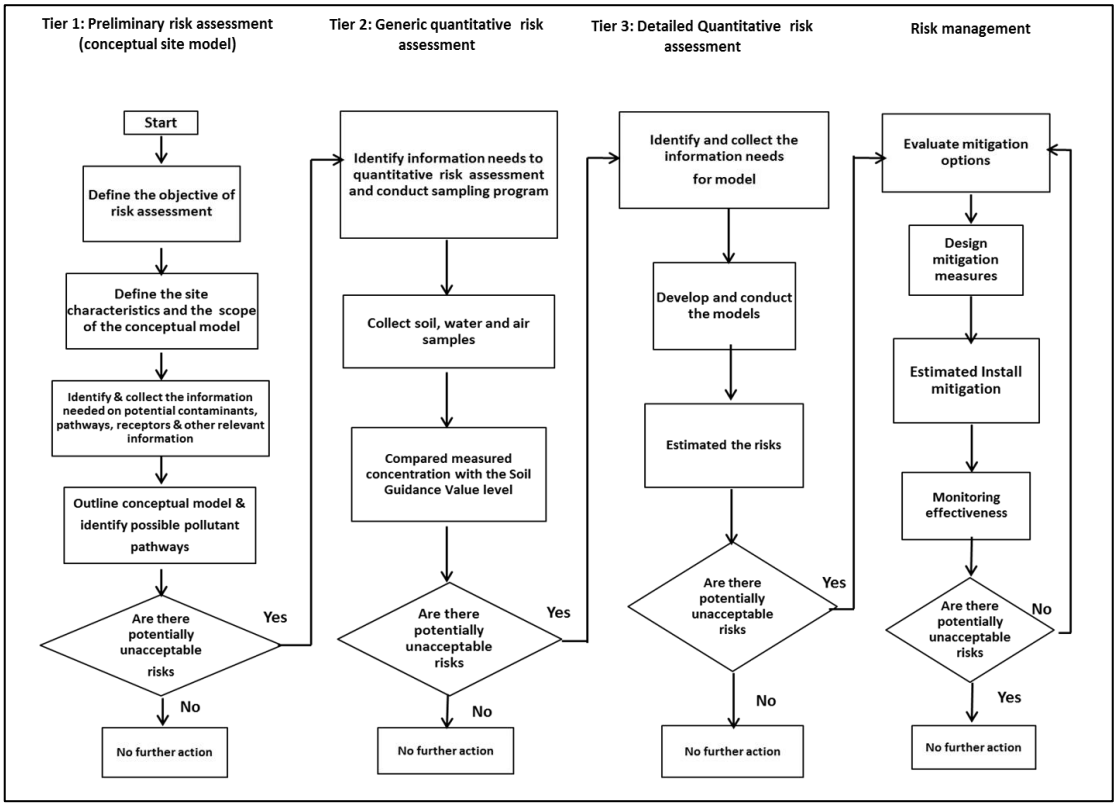

Fig 1. 2. General sketch of risk assessment (Provoost et al., 2010)

1.3 Aims and objectives

The overall aim of this project is to provide a comprehensive understanding of how strong carbonaceous sorbent addition to soil contaminated with volatile petroleum hydrocarbons affect pollutant sequestration and pollutant biodegradation and how biochar or activated carbon addition can impact soil inorganic nutrient content and biogenic gases activities in contaminated soils which are important considerations to develop more effective approaches to immobilize and reduce the toxicities of residual pollutants to levels that enable the re-use of contaminated areas. More specifically, and the research objectives are:

1) To compare the biodegradation of volatile petroleum hydrocarbon pollutants by indigenous microbial communities in the presence and absence of sequestering sorbents, such as biochar or activated carbon.

2) To examine the impact of sequestering sorbents such as biochar or activated carbon on the sorption of essential nutrients and their availability in soils.

3) To examine the impact of sequestering sorbents, such as biochar or activated carbon, on nitrification activity and methane oxidation in order to better understand secondary environmental impacts of remediation efforts.

4) To quantitatively assess the potential trade-offs between pollutant sequestration and pollutant biodegradation in biochar or activated carbon amended soils.

1.4 Research hypotheses and questions

The initial hypothesis of this research was that sorbent materials, such as biochar or activated carbons, can reduce the availability of residual pollutants after remediation, but also inhibit the intrinsic biodegradation. The research questions that arise from the objective are:

1) What are the impacts of biochar or activated carbon on biodegradation rates, apparent diffusion coefficients and reactive transport of the volatile petroleum hydrocarbons in soil?

2) What are the effects of biochar or activated carbon on the sorption of essential nutrients for bioremediation and their availability in soils?

3) What are the effects of biochar or activated carbon on biogenic gases in soil?

4) Is the risk reduction through binding and immobilization of volatile petroleum hydrocarbons with biochar or activated carbon sufficient to justify an eventual reduction in the intrinsic pollution removal rate thorough inhibition of the intrinsic pollutant biodegradation?

1.5 Outline of the thesis and acknowledgement of other contributors

This thesis is organised into seven chapters. An overview of the research motivation, aims, objectives, research hypotheses, research questions and an outline of thesis are introduced in **Chapter 1**.

Chapter 2 provides an introduction and a detailed literature review covering biochar definition, properties and its anticipated effects on nutrients content, availability and leaching. The relationship between availability, volatilization and sorption of volatile

petroleum hydrocarbons is also discussed. Also described are different petroleum hydrocarbon polluted soil remediation approaches and how sorbents materials effect the main processes leading to pollution attenuation.

Chapter 3 contains results of batch experiments which were performed to investigate the impacts of biochar and activated carbon on the sorption of essential nutrients and the effects of these sorbents on the availability of nutrients in soils. The chapter also investigates the effects of biochar on denitrification activity and methane oxidation. All the results reported in this chapter are the author's own work.

Chapter 4 presents data for the short-term impacts of biochar on the fate and transport of volatile petroleum hydrocarbons in column experiments for a one month experiment performed with different boundary conditions. Also presented are biochar effects on the solid–water partitioning coefficient and the first-order biodegradation constant of the pollutants investigated. The numerical pollutant fate model code in Section 4.36 was written by David Werner, and Sara Puricelli assisted with the one month column study during a research visit at Newcastle University. Otherwise, the results reported in this chapter are the author's own work. This chapter has been published in the Journal of Contaminant Hydrology: Bushnaf KM, Puricelli S, Saponaro S, Werner D. Effect of biochar on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil. Journal of Contaminant Hydrology 2011, 126(3-4), 208-215.

Chapter 5 presents data for the long-term impacts of biochar and activated carbon on the fate and transport of volatile petroleum hydrocarbons in column experiments for a one year experiment. Also presented in this chapter is a comparison between measured and modelled results. The numerical pollutant fate model code in Section 5.3.10 was written by David Werner, and the cell counts for the one year column study was provided by George Mangse. Otherwise, the results reported in this chapter are the author's own work.

Chapter 6 provides the results of laboratory batch experiments which were performed to investigate the effects of biochar and activated carbon with and without nutrient addition on the fate of volatile petroleum hydrocarbons in soil where nonaqueous phase liquid (NAPL) is present. All the results reported in this chapter are the author's own work.

Chapter 7 presents conclusions, answers for research questions and recommendations for further work.

Chapter 2: Literature review

2.1 Volatile petroleum hydrocarbon contamination and remediation approaches

Volatile petroleum hydrocarbons (VPHs) are very toxic at high concentration and some have mutagenic and carcinogenic effects (Breus and Mishchenko, 2006). Their negative effect is not only due to their direct adverse impact on humans and animals, but also they significantly deteriorate the properties of soil, and its cleaning is complicated (Breus and Mishchenko, 2006). Due to modern life style, society's energy demand has been increased. Therefore, large quantities of petroleum hydrocarbon products are extracted, refined and handled every year. There are many causes which contribute to releases of hydrocarbons such as blowouts, leakage from tanks, dumping of waste petroleum products and accidental release of hydrocarbon contaminants to the environment. Such incidents resulted in the formation of thousands of contaminated sites with a high level of environmental contamination (Shabir *et al.*, 2008; Tiehm *et al.*, 2010). VPHs are the most important contaminants which can be introduced in the soil as Non Aqueous Phase Liquids ($NAPL_S$). During the migration of NAPLs through the soil, capillary forces retain an amount of NAPLs which may occupy 2-20% of the available pore space (Falta *et al.*, 1989). These NAPLs emit vapour which moves away from the source and could contaminate clean soil, air or groundwater (Li and Voudrias, 1994). Contamination of unsaturated zone by VPHs and their ability to migration into groundwater have been identified as a serious threat for groundwater quality (English and Loehr, 1991; Pasteris *et al.*, 2002).

Various remediation approaches which depend on accelerating natural attenuation processes, such as addition of carbonaceous geosorbents and bioremediation have been introduced to mitigate environmental risks. Bioremediation is one of the most attractive approaches for the remediation of contaminated soil with petroleum hydrocarbons as it is a low cost technique, causes less environmental damage (Chaîneau *et al.*, 2005; Xu *et al.*, 2005; Lee *et al.*, 2008; Shabir *et al.*, 2008; Styriakova *et al.*, 2009; Tiehm *et al.*, 2010), is simple to maintain, applicable over large areas, and leads to complete destruction of the contaminants (Bolan and Baskaran, 1996; Jensen *et al.*, 2004; Lee *et al.*, 2008). It stimulates hydrocarbon

degrading microorganisms to clean up contaminated environments (Chaîneau *et al.*, 2005; Xu *et al.*, 2005; Lee *et al.*, 2008; Shabir *et al.*, 2008; Styriakova *et al.*, 2009). Biostimulation of microorganisms can be achieved by improving environmental conditions such as aeration, soil pH, water holding capacity (Lee *et al.*, 2008; Styriakova *et al.*, 2009; Jin *et al.*, 2010), and by providing nutrients (Xu *et al.*, 2005; Lee *et al.*, 2008). Indigenous microorganisms are well adapted to the presence of contaminants, but other conditions such as aeration, availability of nutrients and/or soil pH may be unfavourable. Therefore, contaminants persist in the environment (Lee *et al.*, 2008; Styriakova *et al.*, 2009). Another approach depending on enhanced natural attenuation is the addition of carbonaceous geosorbents, such as activated carbon or biochar which was suggested as a novel in-situ remediation approach to remediate sediments and soils contaminated with PAHs (Carmichael *et al.*, 1997; Carmichael and Pfaender, 1997; Cornelissen *et al.*, 2005; Rhodes *et al.*, 2008; Zhang *et al.*, 2010a), pesticides (Bolan and Baskaran, 1996; Guo *et al.*, 1999; Guo *et al.*, 2000; Tomaszewski *et al.*, 2007; Hale *et al.*, 2009), and PCBs (Werner *et al.*, 2005; Sun and Ghosh, 2007; Vasilyeva *et al.*, 2010; Werner *et al.*, 2010; Sopeña *et al.*, 2012). Addition of carbonaceous geosorbents could enhance sorption of petroleum hydrocarbons and sequestration of these compounds. As a result the organic pollutant bioaccessibility is reduced (Semple *et al.*, 2013; Werner *et al.*, 2010), and pollutants are released more slowly over time scales of weeks to years (Cornelissen *et al.*, 2005). Therefore, their toxicity, pore water concentrations and uptake may be decreased (Werner *et al.*, 2010).

These two approaches are the most attractive techniques to enhance natural attenuation of organic contaminants and they have been widely used in remediation efforts. However, the choice of the best remediation strategy depends on site-specific remediation goals and the remediation cost. Bioremediation is a low-cost technique for contaminated soil remediation when the main goal of the remediation strategy is a rapid reduction in the total pollution levels. This goal can be achieved by providing nutrients and or optimized soil conditions. Nutrient amendment cannot be applied to remedy contaminated marine sediments, due to disadvantages such as the high risk of eutrophication and the need for repeated applications of nutrients. Indeed, activated carbon could help to treat contaminated sediments, for instance marine and lake

sediments contaminated with PAHs and or PCBs which are highly toxic, less biodegradable and have a long life time in the environment. Furthermore, the biodegradation at these sites is very slow and tends to be anaerobic degradation. The remediation goals at these sites are to reduce the environmental risk of releasing the contaminants from sediments concentration to water and bioaccumulation of contaminants in benthic organisms can be achieved by activated carbon amendments.

2.2 Biodegradation of volatile petroleum hydrocarbons

Biodegradation refers to the use of biodegradative processes to remove contaminants which have found ways into the environment. Volatile petroleum hydrocarbons, including monoaromatic, straight, cyclic and branch alkanes are susceptible to enzymatic attack. Microorganisms are able to utilize organic contaminants as growth substrate and break down organic contaminants and transform them into less harmful compounds. The biodegradation process takes place in different environments including soil, sediment and groundwater. However, like other processes, biodegradation has its limitations. Many factors might inhibit biodegradation processes such as a limited presence of pollutant degraders in the indigenous microbial population, limited availability of organic pollutants to degrading microorganisms, limited availability of inorganic nutrients, the limited presence of oxygen and electron acceptors, low temperature and other soil chemical and physical properties (Vidali, 2001).

Pasteris *et al.* (2002) studied the natural attenuation of 12 VPHs in a large scale field lysimeter representing a 2.3 m thick sandy unsaturated zone over a gravel aquifer. The biodegradation of VPHs started on the first day after emplacing the fuel source in alluvial sand and the biodegradation removed about 235 g C of fuel mass added, while volatilization removed about 74 g C of fuel mass added (Pasteris *et al.*, 2002).

The biodegradation of toluene in pre-exposed and unexposed soil was also investigated by Jin *et al.* (1994). Toluene biodegradation was very rapid under both soil conditions and volatilization flux was very erratic. The toluene degradation rate

increased from 34.2 day^{-1} to 42.5 day^{-1} as a result of pre-exposing the soil to substrate prior to the experiment (Jin *et al.*, 1994). Diffusion of oxygen to contaminated zones is an important factor influencing aerobic biodegradation of VPHs. Zhou and Crawford (1995) reported that the microbial communities can adapt to contaminated soil through selective enrichment and degrade VPHs at faster rates when oxygen was supplied. Freijer (1996) results supported these findings. Freijer (1996) found that about 80% of the total hydrocarbon decrease could be attributed to mineralization, while the other 20% was assumed to be converted into biomass and metabolites. It is concluded that the concentration of oxygen in contaminated soil and VPHs content affected the mineralization rate of VPHs in soil (Freijer, 1996)

Many researchers have reported the relationship between the rate and speed of biodegradation and the chemical structure of contaminants. Pasteris *et al.* (2002) reported that aromatic compounds and long chain alkanes such as octane, decane, and dodecane have high biodegradation rates. These rates ranged from 2.5 to 8.7 d^{-1}. Slower rates were reported for the short chain alkanes, including pentane and hexane, to cyclic alkanes and isooctane, which ranged from 0.1-1.2 d^{-1}. Short chain alkanes are reported to inhibit microbial activity by virtue of their solvent effect (Wilson, 1997). Branched alkanes are known to be slowly biodegraded due to steric problems imposed by side chains (Atlas, 1981). Singh and Lin (2009) observations supported the results of Pasteris *et al.* (2002). It was found that the mineralization of hydrocarbon C>16 in crude oil was relatively faster than the shorter chain compounds such as C9 (Singh and Lin, 2009). Moreover, the presence of the methyl groups in aromatic compounds increased the availability of xylene and toluene to degrading microorganisms more than benzene (Kelly *et al.*, 1996; McBeath and Smernik, 2009). Therefore, the mineralization of xylene was more complete and faster than toluene, which was mineralized more completely and faster than benzene (Kelly *et al.*, 1996). Xylene was completely degraded after 95 h, while toluene and benzene degraded after 125 and 170 h respectively. Furthermore, the biodegradation rate of xylene was 0.383 mmol l^{-1} h^{-l}, whereas the biodegradation rate of toluene and benzene was 0.049 and 0.046 mmol l^{-1} h^{-l} respectively (Kelly *et al.*, 1996).

Due to increases in renewable fuel demand, to reduce oxygenate MTBE usage and to mitigate greenhouse emissions from road transport, fuel is blended by a low proportion of bioethanol (5-15%) with gasoline or biodiesel (up to 20%) with diesel (Kulczycki, 2006; Ryan *et al.*, 2006; Elazhari-Ali *et al.*, 2013). The biofuel addition of blended fuels is generally less toxic and more readily biodegraded than petroleum hydrocarbons, which may inhibit the microbial petroleum hydrocarbon degradation in the presence of ethanol (Da Silva and Alvarez, 2002) or biodiesel (Lapinskiene *et al.*, 2006). The effects of the biofuel addition on the biodegradation of gasoline or diesel in the unsaturated zone have been investigated. Additive bioethanol increased the solubility of BTEX in ground water and it inhibited the biodegradation of BTEX due to the consumption of electron acceptors and nutrients (Powers *et al.*, 2001). The effects of different ethanol additive on the migration of VPHs was investigated by Adam *et al.* (2002) in a soil column. It was shown that there was a greater movement of VPHs to ground water in ethanol additive diesel. For example, in only diesel treatment, negligible amount of diesel (0.64% of total diesel) was found fewer than 30 cm of column surface, whereas 24.1% of total diesel was found fewer than 40 cm in diesel blended with 5% ethanol (Adam *et al.*, 2002). Therefore, the environmental risk increased with the increase in the ethanol percentage in the fuel. The effect of the presence of ethanol on the biodegradation of BTEX in tropical soil has been studied by Osterreicher-Cunha *et al.*(2007). It was found that ethanol enhanced the enzymatic activity. However, the biodegradation of BTEX was delayed. For instance, after 20 day, the BTEX residual in soil amended with pure BTEX was 7.44 mg g^{-1} dry soil, while these residual was 9.25 mg g^{-1} dry soil in soil amended with BTEX and ethanol (Osterreicher-Cunha *et al.*, 2007). Owsianiak *et al.* (2009) investigated the influence of biodiesel (10%) on the biodegradation of petroleum diesel fuel. It was reported that the biodegradation at a low molecular weight fraction was enhanced, whereas the biodegradation at the highest molecular weight fraction was inhibited; the overall biodegradation of the mixture was 58.6% of total diesel amount compared to 76% of total diesel amount pure diesel. On the other hand, Prince *et al.* (2008) and Pasqualino *et al.* (2006) reported that the biodegradation of the biodiesel and VPHs mixture was enhanced and complete. The biodegradability of the mixture increased and reached 100% during the test period when biodiesel was added to petroleum diesel and petroleum gasoline, due to co-metabolism (Pasqualino *et al.*, 2006).

2.2.1 Effects of nutrient on biodegradation

The effects of nutrient amendments on the biodegradation of petroleum hydrocarbons have been extensively investigated in both lab and field experiments under different aeration conditions and in different media such as soil, sediment and ground water, as well as in different chemical forms (Chaîneau *et al.*, 2005; Styriakova *et al.*, 2009; Tiehm *et al.*, 2010). Enhancement of the biodegradation process of an oil spill under marine conditions by providing nutrients is an effective remediation strategy (Oh *et al.*, 2001). However, the effects of nutrients on the mineralization of petroleum hydrocarbons have not yet been completely assessed (Chaîneau *et al.*, 2005). Tiehm *et al.* (2010) and Styriakova *et al.* (2009) reported that the availability of electron acceptors and nutrients is a key factor in the enhancement of hydrocarbon degrading microbes at contaminated sites. Over six months, the total hydrocarbons in biopile soil was decreased from 100.7 mg C g^{-1} to 1.4 mg C g^{-1} after inorganic forms of nitrogen and phosphorus nutrients were added (see Table 2.1) (Styriakova *et al.*, 2009). Moreover, CO_2 production and the hydrocarbon degrading microorganism populations increased during the treatment (Styriakova *et al.*, 2009). Jin *et al.* (2010) investigated the effects nutrient forms and rate on the mechanism of biodegradation of diesel in ground water. It is found that under anaerobic conditions, providing only nitrate or nitrate and phosphate accelerate the biodegradation of diesel, 75% of the compounds in the ground water degraded within 152 days in microcosms amended with nitrate, while there was a 25% removal rate in the controls (Jin *et al.*, 2010), and denitrifying microbes were the main degrading microbes in the treatment receiving only nitrate. However, in the treatment receiving nitrate and phosphate, other heterotrophic bacteria played significant roles in the degradation of diesel (Jin *et al.*, 2010). The effects of nutrient application rate on the biodegradation of kerosene was studied by Shabir *et al.* (2008). It is found that 27% of total kerosene was biodegraded in low nutrient treatment (C:N:P ratio 500:10:1), while 65 % of total kerosene was biodegrade over 6 weeks in the higher nutrient treatment (C:N:P ratio 500:25:20)(Shabir *et al,*. 2008). On the other hand, Swindoll *et al*. (1988) investigated the effects of different phosphorus and nitrogen levels on the biodegradation of crude oil. It was found that additions of 100, 200, and 300 mg of N/kg stimulated biodegradation of crude oil in subarctic Taiga soils. However, although nitrogen was the major limiting nutrient in this biodegradation experiment, the biodegradation was maximally enhanced by

Table 2.1. List of published experiments results regarding the effect of nutrients application on the biodegradation of petroleum

Nutrient type and application rate	Environment	Impact upon biodegradation	Reason for responses	Impact mechanism	References
Inorganic nutrients **550 µg N g^{-1} dry soil and 33 µg P g^{-1} dry soil**	**Biopile soil**	Enhanced biodegradation (Total hydrocarbon decreased from 100700 to 1400 µg C g^{-1} dry soil over 180 days)	Improved nutrient availability (Nutrients were the limiting factor)	Increased number of hydrocarbon-degrading microbes under aerobic condition	**Styriakova *et al*., 2009**
Inorganic nutrients (nitrate) **C:N ratio 100:30**	**Groundwater**	Enhanced biodegradation of diesel over 156 days	Provide electron acceptors and nutrients	Increased number of denitrifying microbes under anaerobic condition	**Jin *et al*., 2010**
Inorganic nutrients **C:N:P ratio 100:30:3**	**Groundwater**	Enhanced biodegradation of diesel over 156 days	Provide electron acceptors and nutrients	Increased number of denitrifying and heterotrophic microbes under anaerobic condition	**Jin *et al*., 2010**
Inorganic nutrients **C:N:P ratio 500:10:1**	**Sandy soil**	27 % of total kerosene was degraded over 42 days	Improved nutrient availability (Nutrients were the limiting factor)	Increased number of hydrocarbon-degrading microbes under aerobic conditions and the numbers were higher for the high nutrient level	**Shabire *et al*., 2008**
C:N:P ratio 500:25:20		65 % of total kerosene was degraded over 42 days			
Inorganic nutrients **N:P ratio 100:45**	**Sandy soil**	Biodegradation of diesel was 3.86 µg C g^{-1} day^{-1}	Improved nutrient availability (Nutrients were the limiting factor)	Increased number of hydrocarbon-degrading microbes under aerobic condition	**Braddock *et al*., 1997**
Inorganic nutrients **N:P ratio 200:90**	**Sandy soil**	Biodegradation of diesel was 2.1 µg C g^{-1} day^{-1}	Increased soil salinity	Reduced water availability for degrading microbes	**Braddock *et al*., 1997**

Table 2.1. continued

Nutrient type and application rate	Environment	Impact upon biodegradation	Reason for responses	Impact mechanism	References
Inorganic nutrients N:P ratio 300:135	**Sandy soil**	Biodegradation of diesel was 1.0 µg C g^{-1} day^{-1}	Increased soil salinity	Reduced water availability for degrading microbes	**Braddock *et al.*, 1997**
Inorganic nutrients 3000 µg N g^{-1}, 450 µg P g^{-1} and 1500 µg K g^{-1}	**Clay soil**	Total petroleum hydrocarbons were decreased from 6000 µg C g^{-1} to 3600 µg C g^{-1}, while in controls it became 3480 µg C g^{-1}	Consumption of nutrients	Reduced nutrient concentration due to the acceleration of the biodegradation of other carbon sources in the soil or to non-stimulation of the saturated hydrocarbon-degrading microorganisms	**Chaîneau et al., 2005**
Inorganic nutrients 850 µg N g^{-1}, 85 µg P g^{-1} and 240 µg K g^{-1}	**Clay soil**	Total petroleum hydrocarbons were decreased from 6000 µg C g^{-1} to 2600 µg C g^{-1}	Improved nutrient availability (nutrients were the limiting factor)	Increased number of hydrocarbon-degrading microbes under aerobic condition	**Chaîneau et al., 2005**
Inorganic nutrients C:N:P ratio 100:10:1	**Beach sediments**	23.91% of total petroleum hydrocarbons were decreased, while 15.74% were degraded in controls	Availability of nutrients is very low after the first 20 days	Improved nutrient availability via leaching	**Xu and Obbard, 2003**
Organic nutrients (Osmocote) C:N:P ratio 100:10:1	**Beach sediments**	48.72% of total petroleum hydrocarbons were degraded, while 15.74% were degraded in controls	Maintained nutrient availability	Inorganic nutrient availability was higher due to slow release from osmocote over long periods	**Xu and Obbard, 2003**
Organic nutrients (Osmocote) C:N:P ratio 100:10:1	**Beach sand**	50.73% of total petroleum hydrocarbons were degraded	Maintained nutrient availability	Inorganic nutrient availability was higher due to slow release from osmocote over long periods	**Xu *et al.*, 2005**

providing both nitrogen and phosphorus (Swindoll *et al.*, 1988). It is concluded that the concept of a single limiting nutrient may not be applicable to ground water microorganisms or to soils, because there are many species of degrading microorganisms which have their own particular nutrient requirements. Therefore, the addition of a combination of nutrients is better than the use of a single nutrient (Swindoll *et al.*, 1988).

Many researchers reported that nutrients amendments did not accelerate the biodegradation of petroleum hydrocarbons (Rhykerd *et al.*, 1995; Barddock *et al.*, 1997; Chaîneau *et al.*, 2005; Singh and Lin, 2009). Braddock *et al.* (1997) investigated the effects of different phosphorus and nitrogen levels on the biodegradation of diesel in sandy soil (Table 2.1) and found that the biodegradation rate of diesel in the low nutrient treatment was 3.86 µg C g^{-1} day, due to improved nutrient availability in sandy soil. However, the biodegradation rate of diesel were 2.1 and 1.0 µg C g^{-1} day in the moderate and higher nutrient level treatment, due to increased soil salinity. Although the levels of nutrients added were modest, increased soluble fertilizer salt concentrations in pore water increased soil salinity, and this may have been high enough to be harmful, because of the reduction of water availability for microbial communities. Moreover, the soil water salinity has its main effects on biodegradation in nutrient-amended soil (Braddock *et al.*, 1997). These findings were supported by many other observations. For example, Singh and Lin (2009) investigated the effects of soil texture on the biodegradation of diesel. Inorganic nutrients enhanced the degradation of diesel in loam soil and sea sand, but not in clay soil, particularly during the first 60 days of the experiment. The effects of different nutrient levels on the biodegradation of crude oil was investigated by Chaîneau *et al.* (2005). The total petroleum hydrocarbons was decreased from 6.0 to 2.6 µg C g^{-1} in low nutrients treatment. In the higher nutrients treatment, it was decreased to 3.6 µg C g^{-1}, while in control, the total petroleum hydrocarbons was decreased to 3.5 µg C g^{-1}(Chaîneau *et al.*, 2005). The number of heterotrophic microorganisms and the respiration rate were higher with higher nutrients treatment. However, the biodegradation of hydrocarbons was inhibited. This may be the result of a reduction in the nutrient concentration due to the acceleration of the biodegradation of other carbon sources in the soil or to non-stimulation of the saturated hydrocarbon-degrading microorganisms (Chaîneau *et al.*,

2005). It is concluded that soil properties, including organic matter (Chaîneau et al., 2005) and soil salinity (Rhykerd *et al.*, 1995; Braddock *et al.*, 1997) could negatively influence the efficiency of nutrients amendment. Therefore, for successful bioremediation, it is necessary to understand the role of nutrients in enhanced microbial activity (Braddock *et al.*, 1997).

The biodegradation of crude oil in sand of the upper intertidal zone (Oh *et al.*, 2001) or oil-contaminated beach sediments amended with inorganic nutrients is inefficient, due to the loss of soluble inorganic nutrients via leaching by water (Oh *et al.*, 2001; Xu and Obbard, 2003). Xu and Obbard (2003) investigated the effect of organic and inorganic nutrients on the biodegradation of crude oil in beach sediments and the effect of leaching on the biodegradation efficiency. It is reported that inorganic nutrients enhanced the biodegradation of oil-contaminated beach sediments within the first two weeks. After this period, the difference between treated sediment and the control was no significant (Xu and Obbard, 2003) and 23.9% of total petroleum hydrocarbons were degraded in inorganic nutrients treatment but 15.7 % of total petroleum hydrocarbons were degraded in controls. In beach sediment amended with Osmocote, microbes degraded 47.7% of total petroleum hydrocarbons. It is concluded that organic nutrients enhanced the biodegradation by maintaining available nutrients at appropriate levels which make the remediation approach more effective (Xu and Obbard, 2003). In some environment, such as marine, lake and rivers sediments or sea beach where water movement could increase the losses of inorganic nutrients which reduces the beneficial effects of nutrients, the design of a nutrient supplement protocol to maintain adequate nutrient concentrations in pore water is necessary to maintain higher biodegradation rates (Oh *et al.*, 2001). To overcome the loss of soluble nutrients and to make the remediation approach more effective, a slow-release fertilizer, such as Osmocote, has been used (Xu and Obbard, 2003; Xu *et al.*, 2005; Nikolopoulou and Kalogerakis, 2008). The application of Osmocote maintained nutrient concentrations at high levels that enhanced bioremediation (Xu and Obbard, 2003; Xu *et al.*, 2005; Nikolopoulou and Kalogerakis, 2008). Slow-release fertilizer (Osmocote) enhanced biodegradation of total petroleum hydrocarbons in beach sand. It is reported that 50.7% of total petroleum hydrocarbons was biodegraded in beach sand over 6 months. Moreover, the biodegradation rates of the straight and branched

alkanes, 2-ring PAHs, aerobic respiration rates, and microbial biomass were significantly enhanced in contaminated beach sediments that were treated with slow-release fertilizer (Xu *et al.*, 2005). The mineralization rates of total PAHs in the sediments treated with slow-release fertilizer were significantly higher compared to the control. On day 45, approximately 90.7% of the total PAHs had degraded in the sediments amended with slow-release fertilizer alone, whereas 55.8 to 42.0% of the total PAHs had degraded in the control (Xu and Obbard, 2004). On the other hand, the Osmocote treatment has its one disadvantage, such as increasing the length of the lag period before biodegradation, because Osmocote is a carbon source (Xu and Obbard, 2003). Moreover, Swindoll *et al.* (1988), Lee *et al.* (2008) and Carmichael and Pfaender (1997) found two issues; one was that with the receipt of carbon source substrates, such as glucose or amino acids, biodegradation was inhibited, and the other was that the length of the lag period before biodegradation increased. These issues were attributed to the depletion of oxygen and/or the microbial preference for utilization of easily degradable carbon sources. Xu and Obbard (2003) and Nikolopoulou and Kalogerakis (2008) suggested the incubation of both inorganic and organic nutrients to overcome the loss of water-soluble nutrients; this enhanced microbial adaptation and reduced the lag phase, as well as accelerating the rate of biodegradation. In leached oil-contaminated sediments which had received organic and inorganic fertilizer, the aliphatic hydrocarbon content (n-C12 to n-C33, pristane, and phytane) was reduced by 95-97% over a 45-day period, while in the control the loss was 26%. A combination of two types of fertilizer maintained adequate nutrient concentrations during the experimental period (Xu and Obbard, 2003). Furthermore, biodegradation and stimulation of the indigenous microorganisms was very fast (Xu and Obbard, 2003).

2.2.2 Biodegradation kinetics

A prediction of the retention, mobility and degradation of VPHs in contaminated sites of requires understanding of the kinetics of these essential mechanisms, which can help to improve remediation approaches (English and Loehr, 1991; Unger *et al.*, 1996). The reactive transport model is the main mathematical model which have been used to perform risk assessment and interpret the behaviour of VPHs in contaminated sites

(Unger *et al.*, 1996). This model is derived from Fick's second law and refers to the many processes controlling the transport and concentration of contaminants in porous media, including the biodegradation rate kinetics, the solid water partitioning coefficient and the effective diffusion coefficient of contaminants through the pore space. The biodegradation kinetics are essential information for the prediction of the natural attenuation of volatile organic compounds in the vadose zone and to assess the risk of VPHs (Alvarez *et al.*, 1991; Hohener *et al.*, 2006). A variety of expressions, including zero-order, first-order and Monod kinetics have been used to describe biodegradation processes which have been reported in the literature. Moyer *et al.*(1996) used first-order biodegradation constants of major hydrocarbon compounds to interpret a concentration profile. It was concluded that the biodegradation kinetics in the unsaturated zone were approximately first-order. Pasteris *et al.* (2002) conducted a large scale field lysimeter to monitor the upward and downward transport of fuel vapours and their biodegradation by indigenous soil microorganisms. It was concluded that the first-order biodegradation rate for 11 VPHs could be derived from the measured data. Hohener *et al.* (2003) conducted field, lab column and batch experiments to derive first-order kinetic rates for the aerobic biodegradation of 13 VPHs, and compared them to the analytical solution of a reactive transport model. It was concluded that the first-order kinetic rate model was useful in describing most compound's behaviour in all the systems, and was therefore used in all the models. Lahvis *et al.* (1999) concluded that the first-order biodegradation rate near the water table was highest for cyclohexane and nearly equivalent for ethylbenzene. Zero-order or Monod kinetics has been used to interpret the biodegradation in many studies. Chan and You (2010) investigated the effect of a non-ionic surfactant on toluene biodegradation behaviour in a biofilter. It was concluded that a zero-order kinetic model was adequate to interpret the biodegradation of toluene. A zero-order kinetic model was fitted to the biodegradation of BTEX compounds in sandy soil (Mohammed and Allayla, 1997). The biotransformation rate of toluene in sandy soil under nitrogen-limited conditions was studied by Allen-King *et al.* (1996), who suggested that the biotransformation of toluene appeared to follow zero-order kinetics. On the other hand, many researchers used Monod kinetic to describe biodegradation of organic hydrocarbons. Hohener *et al.* (2003) determined the aerobic biodegradation kinetic rates for 12 VPHs and methyl tert-butyl ether in unsaturated alluvial sand using a column experiment. It was found that Monod kinetic parameters, such as half

saturation constants and maximum growth rates, could be derived from the concentration profiles of toluene, m-xylene, n-octane and n-hexane, because substrate saturation was approached. Alvarez *et al.*(1991) concluded that Monod kinetics are adequate for describing the biodegradation rate of benzene in a sandy aquifer at a concentration of less than 100 mg/l.

Differences in describing biodegradation rates of VPHs and their kinetics have been reported in the literature. These differences could be attributed to differences in the degrading microbial species, in the used expressions for describing biodegradation kinetics, availability of contaminants, soil properties and to the use of sole contaminants vs. mixtures (Kelly *et al.*,1996). The biodegradation of the mixed VPHs is more complex than that of a sole substrate. Therefore, biodegradation rates derived from experiments using a sole compound were unsuitable for modelling degradation in aquifers and soils contaminated with complex hydrocarbon mixtures, because the degrading microorganisms preferred using certain compounds as carbon source, rather than other compounds (Kelly *et al.*, 1996). Moreover, the biodegradation assumes that the test pollutant concentration is the single factor limiting the growth of degrading microbes. However, this assumption is unlikely to be true in the environment, due to many factors limiting microbial growth, such as the availability of nutrients and condition of the soil (Battersby, 1990). Kinetics expressions have different assumptions. For example, first-order kinetics assumes that biodegradation depends on the contaminant concentration, while zero-order kinetics assumes that biodegradation is independent of contaminant concentration and biodegradation depends on other factors. In the environment, it is unlikely to be true that the contaminant concentration is the single factor limiting the growth of degrading microbes, due to many factors limiting microbial growth, such as the availability of nutrients and condition of the soil (Battersby, 1990). Jin *et al.* (1994) attributed failure of first-order kinetics for describing the biodegradation of toluene in column experiments not only to slow diffusion, but also to possible competition between different microbial species. Moreover, degrading bacteria at different column depths did not follow the same kinetic reaction and they did not have to be the same species (Jin *et al.*, 1994). Therefore, the correct interpretation of kinetic biodegradation parameters in unsaturated soils remains a difficult task (Hohener *et al.*, 2003).

2.3 Biochar fundamentals

2.3.1 Biochar definition

Biochar is defined as a carbon-rich product produced by pyrolysis of organic material such as wood or manure with a limited supply of oxygen (Lehmann and Joseph, 2009). Most carbon atoms in biochar are organized in aromatic rings of six carbon atoms linked together by double bonds without oxygen or hydrogen. However, arrangements of carbon atoms in biochar are more irregular, complex and variable due to the effects of the mineral content of feedstock (Schmidt and Noack, 2000). There are many terms that have been used to describe carbon-rich material, such as black carbon, activated carbon and char. Black carbon is a term widely used to describe a spectrum of rich carbon materials from combustion processes, for example graphite, char, charcoal and biochar. The term includes the solid carbonaceous residue of combustion and heat, as well as the condensation products, known as soot (Schmidt and Noack, 2000). Activated carbon is a term used to define carbon-rich substances that are treated after pyrolysis by steam or CO_2 or chemicals for a partial re-oxidation of surfaces, often at high temperatures (>700°C) (Boehm, 1994). Char is a term is often used to refer to carbon material that is produced by fire (Schmidt and Noack, 2000).

2.3.2 Physical properties of biochar

Factors such as the structure of feedstock biomass (Wildman and Derbyshire, 1991) heating rate, final pyrolysis temperature, retention time and the flow rate of ancillary inputs (e.g. nitrogen, carbon dioxide, steam, etc.) are the main influences on biochar physical properties, including surface area, pore size distribution and density (Pandolfo *et al.*, 1994; Byrne and Nagle, 1997). Downie *et al.* (2009) reported that the final pyrolysis temperature is expected to be the main important factor. Heating rates are expected to have the second greatest effects (Lua *et al.*, 2004; Boateng, 2007). Lua *et al.* (2004) found that the pyrolysis temperature has a highly significant influence on surface area and pore size distribution, followed by the pyrolysis heating rate. Moreover, retention time and N_2 flow rate are the least significant effects. Wildman and Derbyshire (1991) found that the original cellular structures of biomass are present and identifiable in biochar, and have a massive effect on the porosity of the final biochar.

The total pore volumes of the sorbent materials were divided into three groups: micropores of an internal diameter of less than 2 nm; mesopores with an internal diameter of 2-50 nm; and macropores with an internal width greater than 50 nm (Rouquerol *et al.*, 1999). Micropores make the greatest contribution to the surface area of a sorbent material and are responsible for the sorption capacities of gases and solvents (Rouquerol *et al.*, 1999). Mesopores pores are considered important to liquid-solid adsorption processes (Lua *et al.*, 2004). Macropores are considered to be important for water, gases and roots movement in soil (Troeh and Thompson, 2005). The volume of macropores is larger than micropores and, therefore, it can provide a suitable habitat for microbial communities (Downie *et al.*, 2009). Biochar not only increases the surface area but also improves both the aeration condition and soil structure in fine-textured soils. Furthermore, the water holding capacity is increased (Kolb, 2007). The total pore volume of biochar is affected by the pyrolysis temperature and retention time, because they organize the biochar structure, resulting in larger surface areas per volume. Zhang *et al.* (2004) and Brown *et al.* (2006) reported that total pore volume of oak wood increased from 0.146 $cm^3\ g^{-1}$ (wood material), to 0.41 $cm^3\ g^{-1}$ (final pyrolysis temperature is 700°C), oak wood biochar made at pyrolysis 800°C had higher total pore volume which was approximately 0.62 $cm^3\ g^{-1}$. The total pore volume of biochars made from corn hulls or corn stover biochar had same behaviour (see Table 2.2). These results were supported by Pastro-villegas *et al.* (1997) who reported that the total pore volume of Rock rose stems biochar increased from 0.87 $cm^3\ g^{-1}$ (final pyrolysis temperature is 400°C) to 0.93 $cm^3\ g^{-1}$ (final temperature 800°C) (Table 2.2).

The final pyrolysis temperature has the main influences on the surface area of biochar. Brown *et al.* (2006) reported that the surface area of pine biochar increased from < 10 $m^2\ g^{-1}$ (final pyrolysis temperature was 450°C) to approximately 400 $m^2\ g^{-1}$ (final temperature was 750°C). Ding *et al.* (2010a) reported that the surface area of bamboo charcoal produced at 600°C was 330 $m^2\ g^{-1}$. While Yoshizawa et al., (2007) reported that the surface area of bamboo charcoal produced at 650°C was 420 $m^2\ g^{-1}$. A high temperature destroyed the walls between the pores, and as result the surface area decreased (Brown *et al.*, 2006). The surface area of pine wood biochar decreased to

between 75-330 m^2 g^{-1} with increased the pyrolysis temperature to 1000°C (Brown *et al.*, 2006) .

The solid density of biochar increases with greater pyrolysis temperatures and retention times (Pandolfo *et al.*, 1994). Brown *et al.* (2006) reported that the solid density of pine wood biochar increased from 1.37 g cm^{-3} (final pyrolysis temperature was 450°C) to 1.74 g cm^{-3} (the final pyrolysis temperature was 750°C). These results

Table 2.2. List of published experiments results regarding the effect of feedstock materials and pyrolysis temperature on some physical properties of biochars.

Feedstock material	Pyrolysis temperature	Surface area	Solid density	Total pore volume	References
		(m^2 g^{-1})	(g cm^{-3})	(cm^3 g^{-1})	
Pine wood	450 ℃	≤ 10	1.37	-	Brown *et al.*, 2006
Pine wood	600 ℃	370	1.46	-	Brown *et al.*, 2006
Pine wood	750 ℃	400	1.74	-	Brown *et al.*, 2006
Pine wood	1000 ℃	75-330	1.37	-	Brown *et al.*, 2006
Bamboo	600 ℃	330	-	-	Ding *et al.*, 2010a
Bamboo	650 ℃	420	-	-	Yoshizawa *et al.*, 2007
Oak wood	Raw material (wood)	92	-	0.146	Zhang *et al.*, 2004
Oak wood	700 ℃	643	-	0.41	Zhang *et al.*, 2004
Oak wood	800 ℃	850	-	0.62	Zhang *et al.*, 2004
Corn hulls	Raw material	48	-	0.058	Zhang *et al.*, 2004
Corn hulls	700 ℃	945	-	0.86	Zhang *et al.*, 2004
Corn hulls	800 ℃	995	-	0.75	Zhang *et al.*, 2004
Corn Stover	Raw material	38	-	0.054	Zhang *et al.*, 2004
Corn Stover	700 ℃	540	-	0.41	Zhang *et al.*, 2004
Corn Stover	800 ℃	670	-	0.49	Zhang *et al.*, 2004
Rock rose stems	400 ℃	270	0.87	0.474	Pastro-villegas *et al.*, 1997
Rock rose stems	600 ℃	350	0.89	0.568	Pastro-villegas *et al.*, 1997
Rock rose stems	800 ℃	85	0.93	0.560	Pastro-villegas *et al.*, 1997

are agreement with previous observations of Pastro-villegas *et al.* (1997) who found that increasing the pyrolysis temperature from 400°C to 800°C increased the solid density of Rock rose stems from 0.87 to 0.93 g cm^{-3} (Table 2.2). Bulk densities of biochars produced from different types of woods in different traditional kilns ranged from 0.30 g cm^{-3} to 0.43 g cm^{-3} (Rodríguez-Reinoso, 1997). In contrast, Yoshizawa *et al.* (2007) measured the bulk density of bamboo biochar at 0.17 g cm^{-3}. Byrne and Nagle (1997) found that feedstock density is the main influence on density of biochar. From their results, the density of biochar pyrolyzed at 900°C had 82 percent of the density of wood. Pan and van Staden (1998) studied the relationship between the percentages of pore types and the solid density of activated carbon. The bulk densities of activated carbon ranged from 0.40 g cm^{-3} to 0.50 g cm^{-3}, and activated carbons, which have a higher percentage micropores, have a solid density higher than those that have a high percentage of mesopores and macropores (Pan and van Staden, 1998).

2.3.3 Effects of biochar on chemical properties

A limited amount of literature has been published discussing the chemical properties of biochar and its effects on soil chemical properties. From these papers, the pyrolysis temperature is the main factor influencing biochar chemical properties, such as pH, electrical conductivity (EC) and cation exchange capacity. Biochars can be manufactured with any pH between 4 and 12 (Lehmann, 2007). High-temperature (800°C) pine wood biochar has a high pH and EC compared to low temperature (Gundale and DeLuca, 2006). This result is agreement with Hossain *et al.* (2011), who reported that the pH and EC of sewage sludge were 4.42 and 1.95 dS m^{-1} respectively, these values increased to 5.32 and 4.12 dS m^{-1} respectively, in sewage sludge biochar made at 300°C. Pyrolysis sewage sludge at 500 produced biochar had high pH and EC values by 7.27 and 4.7 dS m^{-1} respectively. Moreover, sewage sludge biochar made at 700°C had had higher pH and EC values (Table 2.3) (Hossain *et al.,* 2011). These findings are in agreement with previous results of Singh *et al.*, (2010a) who found increases in pH and EC of poultry litter biochar and wood biochar with increased pyrolysis temperature from 400°C to 550°C. Singh *et al.* (2010a) investigated the effect feedstock materials on the pH and EC of biochar made at the same pyrolysis temperature. It is found that biochars from different feedstock have different pH and EC values. The pH values of Wood, poultry litter and cow manure biochar which

Table 2.3. List of published experiments results regarding the effect of feedstock materials and pyrolysis temperature on some chemical properties of biochars.

Feedstock material	Pyrolysis Temperature	pH	EC	Nitrogen		Phosphorus		C/N ratio	References
			dS m^{-1}	Total	Available	Total	Available		
				g kg^{-1}	mg kg^{-1}	g kg^{-1}	mg kg^{-1}		
Sewage sludge	450 ℃	-	-	6.4	-	56	-	7	Bridle and Pritchard 2004
Broiler litter	700 ℃	-	-	7.5	-	48	-	34	Lima and marshal 2005
Poultry litter	450 ℃	9.9	-	20	2	25.2	11,600	19	Chang *et al*., 2007b
Green wastes	450 ℃	6.2	-	1.7	≤ 2	0.2	15	400	Chang *et al*., 2007b
Wood	Local farmer	-	-	11	-	6.7	-	65	Lehmann et al 2003
Sewage sludge	Raw material	7.08	-	18.2	-	31.7	-	-	Bagreev *et al*., 2001
Sewage sludge	400 ℃	7.7		0.038	-	-	-	-	Bagreev *et al*., 2001
Sewage sludge	600 ℃	11.5		0.032	-	-	-	-	Bagreev *et al*., 2001
Sewage sludge	800 ℃	11.3		0.016	-	-	-	-	Bagreev *et al*., 2001
Sewage sludge	250 ℃			-		56	9,800		Shinogi 2004
Sewage sludge	400 ℃			51		81	4,300		Shinogi 2004
Sewage sludge	600 ℃	-	-	40	-	12	1,200	-	Shinogi 2004
Sewage sludge	800 ℃	-	-	2.3	-	12.8	600	-	Shinogi 2004
Sewage sludge	Raw material	4.42	1.95	3.27	7300	-	-	9.9	Hossain *et al*., 2011

Table 3.2. continued

Feedstock's material	Pyrolysis Temperature	pH	EC	Nitrogen		Phosphorus		C/N ratio	References
			dS m^{-1}	Total	Available	Total	Available		
				g kg^{-1}	mg kg^{-1}	g kg^{-1}	mg kg^{-1}		
Sewage sludge	300 ℃	5.32	4.12	33.2	1175	-	-	7.8	Hossain *et al*., 2011
Sewage sludge	400 ℃	4.87	4.15	24	142.5	-	-	8.4	Hossain *et al*., 2011
Sewage sludge	500 ℃	7.27	4.7	21.3	25	-	-	9.5	Hossain *et al*., 2011
Sewage sludge	700 ℃	12.0	2.5	12	1.34	-	-	17	Hossain *et al*., 2011
E. saligna wood	400 ℃	6.93	0.27	2.1	-	0.13	7.8	332	Singh *et al*., 2010a
E. saligna wood	550 ℃	8.82	0.32	2.6	-	0.22	-	322	Singh *et al*., 2010a
Poultry litter	400 ℃	9.2	9.27	51.8	-	5.8	1,756	8.3	Singh *et al*., 2010a
Poultry litter	550 ℃	10.26	10.17	37.9	-	5.8	2,446	11	Singh *et al*., 2010a
Cow manure	400 ℃	9.03	12.28	13.5	-	4.4	-	13	Singh *et al*., 2010a
Cow manure	550 ℃	8.94	10.97	11.4	-	4.9	-	14.5	Singh *et al*., 2010a
Pine wood	350 ℃	5.2	0.15	-	5.7	-	7.1	-	Gundal and Deluca 2006
Pine wood	800 ℃	9.8	15.0	-	3.2	-	2.5	-	Gundal and Deluca 2006

made at 550℃ were 8.82, 10.26 and 8.94 and the EC value had the same behaviour (Table 3.2) (Singh *et al.*, 2010a). The cation content of the feedstock is an important factor affecting the liming potential of biochar. During the pyrolysis process, a small part of the feedstock becomes ash, which consists of cation oxides. These oxides have their main effect on the liming potential of biochar. Moreover, the percentage of carbonate ranges between less than 0.5 to 33 % for biochars made from different materials and under different conditions (Chan *et al.*, 2007b). Therefore, adding biochar containing carbonate might overcome issues of soil acidity (VanZwieten *et al.*, 2007).

The cation exchange capacity (CEC) of biochar is very low. For example, the CEC of wood biochar produced at 600℃ was 0.8 cmol g^{-1} which is lower compared to soil (Clough *et al.*, 2010). The CEC of green waste biochar was 9.0 (VanZwieten *et al.*, 2010b). Moreover, the CEC of biochar decreased with increased pyrolysis temperature (Gundale and Deluca, 2006). The CEC of pine wood biochar made at 350°C was 36.2 cmol g^{-1}. This value was decreased to 21.1cmol g^{-1} when pyrolysis temperature increased to 800°C (Gundale and Deluca, 2006). The CEC of freshly made biochars is lower than for aged biochar due to the oxidation being insufficient to create enough negative surface charge (Cheng *et al.*, 2008). The CEC of biochar increased with greater incubation time in soil, and this is probably due to the oxidation of the surface functional group of biochar producing carboxyl groups (Liang *et al.*, 2006; Preston and Schmidt, 2006). This increased the CEC of biochar (Liang *et al.*, 2006), and reduced the sorption of organic compounds. Moreover, aged biochar has a much greater negative surface charge which can explain a much greater cation exchange capacity (Cheng *et al.*, 2008). The ability of Amazonian Dark Earths to reduce leaching, combined with the availability of nutrients in them, is attributed to their having large amounts of aged biochar (Lehmann *et al.*, 2003). Overall, applying biochar to acid soil decreased soil exchangeable acidity and increased both soil exchangeable base cations and soil pH (Yuan *et al.*, 2011). The effects of applying biochar as a conditioner of soil properties are important (Glaser *et al.*, 2002; Lehmann *et al.*, 2003).

2.3.4 Biochar nutrient content

Despite the extensive study of biochar, research related to the contents and availability of nutrients in biochar is still limited. Moreover, information provided by these studies is thus far incomplete. The available data is equivocal, and this may be attributed to using different starting materials and manufacturer operation conditions. The nutrients content of biochar depends upon many factors such as the feedstock biomass type and the operating conditions of pyrolysis (Bridle and Pritchard, 2004; DeLuca *et al.*, 2009). Changing pyrolysis operation parameters can produce biochar with different nutrients content from the same feedstock (Chan *et al.*, 2007b).

2.3.4.1 Effect of feedstock material and pyrolysis temperature on the nutrient content of biochar

Despite the extensive study of biochar, research related to the contents and availability of nutrients in biochar is still limited. Moreover, information provided by these studies is thus far incomplete. The available data is equivocal, and this may be attributed to using different starting materials and manufacturer operation conditions. The nutrients content of biochar depends upon many factors such as the feedstock biomass type and the operating conditions of pyrolysis (Bridle and Pritchard, 2004; DeLuca *et al.*, 2009). Changing pyrolysis operation parameters can produce biochar with different nutrients content from the same feedstock (Chan *et al.*, 2007b). Many types of organic material can converted to biochar, such as poultry litter, sewage sludge, green waste and wood (Bridle and Pritchard, 2004). The type of feedstock biomass has a significant influence on biochar nutrients, in addition to the heavy metal content. For example, the phosphate and nitrogen content of biochar produced from sewage sludge and poultry litter feedstock are higher than those from plant biomass for the same pyrolysis conditions. For example, the total nitrogen content of biochar from sewage sludge at 450 ℃ was 6.4 g kg^{-1} (Bridle and Pritchard, 2004). Chan *et al.* (2007a) found that the total nitrogen content of biochar from poultry litter was 20 g kg^{-1}, yet 1.7 g kg^{-1} in biochar made from green waste at same pyrolysis temperature. Wood biochar had a low total phosphate content at 0.13 g kg^{-1}, whereas the total phosphate in poultry biochar was much higher at 5.8 g kg^{-1} (Singh *et al.*, 2010a). The lower concentration of phosphate and nitrogen in green waste and wood biochar is due to their low contents. The concentrations of heavy metals and their availability are the main concerns regarding the application of biochar to soils. Some feedstock, such as sewage

sludge, can have a high heavy metal content (Bridle and Pritchard, 2004; Hospido *et al.*, 2005). Muralidhara *et al.* (1982) reported high concentrations of copper and zinc and chromium in biochar produced from tannery wastes by 2% of total dry weight, due to higher heavy metals content of tannery wastes. Hossain *et al.* (2011) reported results about effects of pyrolysis temperatures on the heavy metal contents of biochar made from sewage sludge. The heavy metal contents increased with increased pyrolysis temperatures (Hossain *et al.*, 2011). For example, lead, cadmium and nickel contents of raw sewage sludge were 86.5, 2.07 and 70 mg kg^{-1}respectively. These contents were increased to 115, 2.62 and 182.5 mg kg^{-1} respectively, when sewage sludge biochar was made at 300°C. Moreover, the sewage sludge biochar made at 500°C have higher heavy metals contents which were 140, 3.17 and 292.5 mg kg^{-1} respectively.

Feedstock materials, under different pyrolysis operation conditions, give vast differences in the biochar properties in terms of nutrient contents and their availability. The most important operating parameters affecting biochar properties are higher temperature (Knoepp *et al.*, 2005; Lang *et al.*, 2005; DeLuca *et al.*, 2009; Singh *et al.*, 2010b), heating rate and retention time (Lang *et al.*, 2005). The total nitrogen content of high-temperature biochar is extremely low. Tryon (1948), and Lang *et al.* (2005) studied the effect of pyrolysis temperature on nutrient content of sewage sludge biochar. It is found that the total nitrogen content of biochar made from sewage sludge reduced from 3.8% at 400°C to 0.94 % at 950°C. These observations were supported by Singh *et al.* (2010a) who reported that increased pyrolysis temperature from 400 to 550°C decreased the total nitrogen content of poultry litter biochar from 51.8 g kg^{-1} to 37.9 g kg^{-1}. These finding are agreement with Shinogi *et al.* (2004) and Hossain *et al.* (2011) (Table 3.2). On the other hand, increased pyrolysis temperature from 400 to 550°C enhanced or did not have effect on the total phosphorus content of cow manure or poultry litter biochar. The total phosphorus content of cow manure increased from 4.4 g kg^{-1} to 4.9 g kg^{-1} which is agreement with Knoepp *et al.* (2005), results. This enhancement was attributed to phosphate becoming concentrated in biochar with increased pyrolysis temperature until 700°C. After this temperature, the phosphate content was lower, due to phosphate volatilization (Knoepp *et al,.* 2005). Yu *et al.* (2005) found about half of the total potassium and sodium content was lost when the

pyrolysis temperature increased from 473°C to 673°C, and the water-soluble form of potassium, which is bioavailable, decreased from 90% of the total potassium to 20%. However, less available potassium forms, such as, exchangeable and acid extractable forms were increased. On the other hand, Gundale and Deluca (2006) and Deluca *et al.* (2009) found that during the pyrolysis, some nutrients are reduced, while other nutrients become concentrated in the biochar. The extractable concentrations of ammonia and phosphate were increased with increases in temperature thermal conversion from 350°C to 800°C, whereas extractable NO_3^- was significantly increased (Gundale and DeLuca, 2006). These changes in nutrient content is attributed to volatilization of nutrients at higher temperature (Bagreev *et al.,* 2001; Knoepp *et al.,* 2005; Gundale and Deluca, 2006; Deluca *et al.,* 2009), and changed functional groups (Chan and Xu, 2009). During the thermal conversion, the chemical and structural properties of feedstock and functional groups will change depending on the operating conditions (Chan and Xu, 2009). Increasing the pyrolysis temperature from 150°C to 550°C decreased the amounts of -OH and $–CH_3$ groups and increased C=C double bonds. These changes can be attributed to the change from an aliphatic to an aromatic C structure of the biochar (Chan and Xu, 2009). As a consequence, the low temperature biochars are found to have higher amounts of acid–based functional surface groups. The changes from aliphatic groups to aromatic ones causes a reduction in the availability of nutrients bound in the organic structure, such as N, P and S (Chan and Xu, 2009).

2.3.4.2 Availability of nutrients in biochar-amended soil

Information about the effects of biochar on the available nutrient content of soil is limited. From the limited data, however, availability of nutrients in soil and black carbon amended soil is affected by many factors such as pH, ion exchange capacity, the availability of nutrients in biochar and microbial activity. The availability of nutrients is a small fraction of the total content of which organisms can uptake (Chan and Xu, 2009). For example, although the total nitrogen content in biochar produced from sewage sludge was 6.4 g kg^{-1}, the concentration of available forms of ammonium and nitrate was negligible in soil after 56 days of incubation (Bridle and Pritchard, 2004). Moreover, wood biochar had a low total phosphate content at 0.13 g kg^{-1} and

the available phosphate was less than 7.8 mg kg^{-1} (Singh *et al.*, 2010a). Moreover, the concentration of available nitrogen in green waste biochar was < 2 mg kg^{-1}. The activity of microbial communities in soil mainly influences nitrogen transformation processes, such as ammonification, nitrification, denitrification and nitrogen fixation, all of which affect the content and availability of nitrogen in soil. On the other hand, pH has a great influence on phosphate availability (DeLuca et al., 2009), and precipitation reactions of phosphate (Stevenson and Cole, 1999), due to its effects on the activities of calcium, aluminium and ferric ions (Stevenson and Cole, 1999). The reaction of phosphate with calcium ion and precipitation as apatite is a main pathway in alkaline soils. Furthermore, in acid soils, phosphate availability is regulated by its interaction with aluminium and ferric ions (DeLuca et al., 2009). VanZwieten *et al.* (2010b) found that available nitrogen forms and phosphate in green waste biochar were very low. The available ammonium, nitrate and phosphate in biochar were <0.3 mg NH_4-N kg^{-1}, 1.2 mg NO_3-N kg^{-1} and 6.3 mg P kg^{-1} respectively, and amended soil with biochar (11% w/w) reduced available ammonium and nitrate from 3.7 mg NH_4-N kg^{-1} and 180 mg NO_3-N kg^{-1} in control to 3.3 mg NH_4-N kg^{-1} and 130 mg NO_3-N kg^{-1} in biochar amended soil respectively. However, biochar amendment did not affect available phosphate concentration (VanZwieten *et al.*, 2010b). Moreover, applying biochar to soils reduced the concentration of NH_4^+ and NH_3 in soil solution due to the sorption to the biochar surface area (Lehmann *et al.*, 2006). Lehmann *et al.* (2003), Rondon *et al.* (2007) and Liang *et al.* (2006) studied the causes of the reduction of available nitrogen in soil treated with biochar. This reduction was attributed to the immobilization of mineral nitrogen because of its high C/N ratio. On the other hand, activated carbon-amended soil (Ward *et al.*, 1997; Paavolainen *et al.*, 1998) or biochar-amended soil (Zackrisson *et al.*, 1996), increased the nitrification rate, possibly because of the decreased availability of nitrogen in the soil. On the other hand, the application of biochar enhanced the availability of phosphate in acid soil (Lehmann *et al.*, 2003). The available phosphate was increased from 8.1 mg P kg^{-1} in ferralsol to 258.3 mg P kg^{-1} in same soil amended with biochar. This was attributed to alteration of soil pH to neutrality when the soil pH increase from 5.14 to 5.9 and or to increases in sorption of cations, such as calcium and available Al^{3+} which interact with phosphorus, due to the high ion exchange capacity of biochar (Lehmann *et al.*, 2003). Moreover, Steiner et al. (2007) attributed the increased bioavailability and plant uptake of phosphate in forest soils treated with biochar to the increased cation

exchange capacity which increased soluble exchangeable forms in soil treated with biochar.

2.3.5 Biochar effects on nutrient leaching

Leaching is an important aspect affecting fertilizer-use efficiency and the health of the soil environment. It occurs when nutrients migrate outside the rooting zone and plants cannot take them up. According to Lehmann *et al.* (2004), leaching contributed to a loss of up to 80% of applied nitrogen. In intensive agricultural areas of the United States, the nitrate concentration in 26% of groundwater wells exceeded the maximum contaminant level in 1990 (Mueller *et al.*, 1995). Several studies have been performed to investigate the effect of the addition of biochar on the leaching of nutrients. Total nitrogen and phosphate lost via leaching decreased by 11% and 69%, respectively, in soil columns treated with biochar and manure (Laird *et al.*, 2010a). In the greenhouse, lysimeters showed that a mixture of typical Hapludox soil with biochar made locally reduced the leaching of ammonium by up to 60% over 40 days of cropping rice, compared to treatments not receiving biochar (Lehmann *et al.*, 2003). In addition, in high rainfall climates, the addition of a mixture of biochar with mineral fertilizer improved the yield of plants more than the use of fertilizer alone, due to a reduction in nutrient leaching (Chan and Xu, 2009). Sorghum dry matter production increased by up to 266% in soil amended with both nitrogen fertilizer (by 100 kg N ha^{-1}), and biochar (by 11 t ha^{-1}), compared to the control that received the same amount of N but no biochar due to a reduction in the amount of nutrients lost via leaching (Chan and Xu, 2009). The reduction of nutrient loss in biochar-amended soil via leaching is the result of different mechanisms, such as decreased water mobility (Lehmann *et al.*, 2003), and the increased retention time of water (Major *et al.*, 2009). This increased retention is, due to increased pore-size distribution, surface area and water holding capacity (Lehmann *et al.*, 2003; Duenisch *et al.*, 2007; Major *et al.*, 2009), and/or to increased plant biomass and evaporative surfaces (Lehmann *et al.*, 2003) and/or to the increased sorption capacity of biochar-amended soil (Duenisch *et al.*, 2007). The ability of biochar to adsorb nutrients not only leads to a reduction in leaching and the entrance into local water sources, which causes an eutrophication problem, but also to a reduced need for fertilizer application and less pesticide contamination (Ding *et al.*, 2010a; Laird *et al.*, 2010b).

2.3.6 Effects of biochar on the biological properties of amended soil

The diversity, abundance and activity of soil microorganisms is greatly affected by the addition of biochar (Wardle *et al.*, 1998), due to the changes in the chemical and physical properties of the soil that alter biological activities and the composition of communities (Thies and Rillig, 2009). For example, soil pH influences the diversity, abundance and activity of soil microorganisms in different ecosystems (Wardle *et al.*, 1998). The predominant microorganism and the microorganisms diversity and activities are highest in neutral soils and lowest in both acidic and alkaline soils (Thies and Rillig, 2009). Under the extremes of pH, fungi will probably predominate due to their wide range of pH tolerance. Adding biochar to soil could lead to significant changes in the soil community composition, ratio of bacteria to fungi and the predominant microorganism (Thies and Rillig, 2009). The porous structure of biochar could provide a suitable habitat for soil microorganisms, increase their population, protect them from natural predators, and provide essential nutrients and carbon sources (Saito and Marumoto, 2002; Warnock *et al.*, 2007). Moreover, the water-holding capacity may be increased in treaded soil with biochar thus increasing its habitability. According to Wardle *et al.* (1998), plant growth and biomass increased in boreal forest soils treated with biochar. For example, birch shoot and root were increased from 50 and 20 mg plant^{-1} respectively, in humus without biochar treatment to 280 and 62 mg plant^{-1} respectively, in biochar amended humus. This increase was attributed to the adsorption of heavy metals, as well as toxic secondary metabolites and phenolics in the soil (Wardle *et al.*, 1998). The Saito and Marumoto (2002) results support these findings. It is found that the fresh roots weight of alfalfa increased from 35 mg plant^{-1} in soil without biochar to 55 mg plant^{-1} in biochar amended soil. According to Zackrisson *et al.* (1996), microbial biomass was enhanced in humus treated with biochar. This enhancement might be attributed to an increase in the interaction between microorganisms, nutrients and organic substrate when these sorb on the surface area of biochar (Ortega-Calvo and Saiz-Jimenez, 1998).

2.3.7 The effects of biochar on crops and the response of microorganisms

Crop and microbial response to the application of biochar is not only related to increases in nutrients (Lehmann *et al.*, 2003; Chan *et al.*, 2007b; VanZwieten *et al.*, 2007), but also improvement the chemical and physical characteristics of soil and

reduction some of the effects of toxic compounds (Lehmann *et al.*, 2003; Yamato *et al.*, 2006; Steiner *et al.*, 2007). The addition of biochar has a direct effect related to increased nutrient values, while indirect effects are attributed to improvements in the chemical and physical characteristics of soil and a reduction in toxicity. However, the addition of biochar to soil which has sufficient amounts of nutrients may not significantly increase plant production (Blackwell *et al.*, 2009). The indirect positive impacts of biochar benefit crop production more than direct ones (Chan and Xu, 2009). One of the positive indirect effects of adding biochar is the increase in the pH of acidic soils due to the alkaline nature of biochar. VanZwieten *et al.* (2007) reported that the height of wheat increased by 30-40% in acidic soil when biochar was applied at a rate of 10 t ha^{-1} to an acidic soil, but not when applied to a neutral soil. This increase in wheat yield was attributed to overcoming the toxic effects of the exchangeable aluminium (Al^{3+}) in the acidic soils (VanZwieten *et al.*, 2007). This observation was supported by Yamato *et al.* (2006), who reported that fresh biomass and roots weight of maize were increased from 9 and 0.75 t ha^{-1} respectively, in control soil to 11.5 and 1.4 t ha^{-1} respectively, in Bark charcoal amend soil (0.5 t ha^{-1}). This enhancement in crop production was attributed to the decrease in available Al^{3+} from 2.93 cmol kg^{-1} in control soil to 0.12 cmol kg^{-1} in the charcoal-amended (Yamato *et al.*, 2006). Combination of fertilization and biochar enhanced the crop production, while fertilizer application alone did not influence crop production (Van Zwieten *et al.*, 2010b). It is reported that dry weight of wheat and soybean were significantly increased from 0.5 and 1.2 g $plant^{-1}$ respectively, in nitrogen fertilizer amended Forrosol soil to 1.2 and 2.6 g $plant^{-1}$ respectively, in Forrosol soil amended with both biochar and nitrogen fertilizer (Van Zwieten *et al.*, 2010b). However, the alkaline nature of the biochar might be harmful to calcifuge plant species (Mikan and Abrams, 1995). For example, Kishimoto and Sugiura (1985) reported that the application of biochar to soil at rates of 5 t ha^{-1} and 15 t ha^{-1} reduced soybeans yields by 37% and 71% respectively, and they attributed the reduction to the reduced availability of micronutrients due to increased pH. The positive indirect effects, such as increased fertilizer-use efficiency in soil treated with biochar by reduction of nutrients losses via leaching (Lehmann, 2007; VanZwieten *et al.*, 2010a), improved physical properties of soil, such as increased water-holding capacity (Iswaran *et al.*, 1980) and reduced soil strength have also been documented (Chan *et al.*, 2007b).

2.3.8 Effects of biochar on the microbial process of amended soil

The effects of biochar on microbial processes, such as denitrification, methane oxidation, respiration, nitrification, nitrogen fixation and immobilization, have been investigated. Varied effects of biochar on microbial processes have been reported in the literature. Some researchers found a reduction and others showed increases in these processes. Lehmann *et al.* (2003) and Rondon *et al.* (2007) reported that there is no evidence for the effect of biochar on nitrification in grassland or agricultural soils. On the other hand, treating soil with activated carbon (Ward *et al.*, 1997) or biochar (Zackrisson *et al.*, 1996) increased the nitrification rate due to adsorption of organic compounds that inhibited nitrifying microorganisms. The nitrification activity in activated carbon amended soil was almost double (6 mg NO_3–N g^{-1} compared to mg NO_3–N g^{-1}) that in the control treatment (Berglund *et al.*, 2004). This was attributed to mitigation of soil acidity in activated carbon treatment which increased nitrifying bacteria activity, due to nitrifying bacteria's preference for neutral soil (Berglund *et al.*, 2004). The biological nitrogen fixation was increased by 49, 78 and 72% in biochar-amended soil with 30, 60 and 90 g biochar/kg respectively (Rondon *et al.*, 2007), and by 15% in another study when biochar was added to soil (Nishio and Okano, 1991). Rondon *et al.* (2007) attributed increases in nitrogen fixation to the increased availability of B, Mo, K, Ca, and P, increased soil pH , lower Al^{3+} saturation and higher C/N ratios (24-35) in the acid soil. Lehmann *et al.* (2006) attributed the reduction of ammonification in biochar-amended soil to increased sorption of NH_4^+ and NH_3 from the soil solution to the biochar surface area, while Liang *et al.* (2006) attributed this to the immobilization of inorganic forms of nitrogen in biochar-amended soil with a high C/N ratio. The respiratory rate of biochar-amended soil was reduced by 80% compared with unamended soil over a 532 day period (Liang, 2008); this was attributed to the higher metabolic efficiency of the microbial community in amended soil due to the increase in microbial biomass from 43 to 125%. However, Jin *et al.* (2008) attributed the reduction in CO_2 to the chemosorption of respired CO_2 on the biochar surface area. However, Liang (2008) suggested other reasons to explain the reduction in the respiratory rate in biochar-amended soil such as changing of bacterial to fungal ratios and increasing C-use efficiency, whereas (Steiner *et al.*, 2008) attributed this reduction to changes in substrate quality and/or the availability of organic compounds.

Over the last few years, the denitrification process and methane oxidation have received much attention due to their roles in global warming. To mitigate greenhouse gases emissions, the addition of biochar to soil has been suggested as a geoengineering approach (Lehmann, 2007; Clough *et al.*, 2010; Karhu *et al.*, 2011; Taghizadeh-Toosi *et al.*, 2011; Feng *et al.*, 2012; Zhang *et al.*, 2012a; Zhang *et al.*, 2012b). Zhang *et al.* (2012b) and Feng *et al.* (2012) both suggested that the production of biochar from crop residues in developing countries may provide many advantages for agriculture production, and avoid the burning of crop residues in fields, which has been reported as one of the greatest global CO_2 sources; biochar addition may also reduce soil greenhouse gas emissions. Varied differences in the flux rates of CO_2, N_2O and CH_4 from biochar-amended soils have been reported in the literature. Some researchers found a reduction in emissions and others showed no effects or increased N_2O and CH_4 emissions. Cumulative fluxes of CH_4 from rice paddy soil were significantly decreased from 390 kg CH_4-C h^{-1} in un amended soil to 160 390 kg CH_4-C h^{-1} in biochar amended soil (Feng *et al.*, 2012). In pasture soil amended with corn stalk biochar and bovine urine, N_2O flux decreased by 70% in comparison with urine only amended soil (Taghizadeh-Toosi *et al.*, 2011). Cumulative fluxes of N_2O in urine only amended soil were 140.6 kg N_2O–N h^{-1}. This value was increased to 212.8 kg N_2O–N h^{-1} in biochar and urine-amended soil (Table 2.4), this increases was attributed to poor ability of biochar to sorb nitrate and nitrite (Clough *et al,*. 2010). Some studies in the literature showed an increased emission rate for one of the greenhouse gases and decreased emissions for other gases. Zhang *et al.* (2012a) reported that the cumulative fluxes of N_2O was decreased from 1.99 kg N_2O–N h^{-1} to 0.98 in Chinese paddy soil amended with biochar (40 t h^{-1}), while CH_4 emissions were increased from 69.3 to 104.9 kg CH_4-C h^{-1}, and no significant difference was found in soil respiration between soil amended with biochar and the control. The reduction in cumulative fluxes of N_2O are agreement with previous result of Yanai *et al.* (2007) and Zhang *et al.* (2012c). Karhu *et al.* (2011) found that the cumulative CH_4 flux from boreal agricultural soil amended with biochar at a rate of 9 t ha^{-1} decreased by 96% (Table 2.4), while N_2O cumulative fluxes which were decreased from 360 kg N_2O–N h^{-1} in the control to 387 kg N_2O–N h^{-1} in birch biochar amended soil. The effect biochar on the N_2O cumulative fluxes differences was not statistically significant (Karhu *et al.*, 2011).

Table 2.4. Effect of biochar on methane oxidation and denitrification frame studies performed in different soils.

Production (+), consumption (-)

Soil type	Biochar type and nutrient source	Control		Biochar treatments		Statistical difference	References
		N_20	CH_4	N_20	CH_4		
Pasture soil	Wood biochar (20 t h^{-1}) & Bovine urine (760 kg h^{-1})	140.6 kg N_2O-N h^{-1}	-	212.8 kg N_2O-N h^{-1}	-	It is significant. Biochar did not decrease concentration of nitrate and nitrite	Clough *et al.*, 2010
Boreal agricultural soil	Birch biochar (9 t h^{-1})	360 kg N_2O-N h^{-1}	97.4 kg CH_4-C h^{-1}	387 kg N_2O-N h^{-1}	3.9 kg CH_4-C h^{-1}	The concentration of N_2O did not significantly increase The concentration of CH_4 was significantly decreased	Karhu *et al.*, 2011
Chinese paddy soil	Corn stalk biochar & urea 250 kg N h^{-1} & 75 kg P h^{-1}	-	390 kg CH_4-C h^{-1}	-	160 kg CH_4-C h^{-1}	The concentration of CH_4 was significantly decreased	Feng *et al.*, 2012
Chinese paddy soil	Unknown biochar (40 t h^{-1}) & urea 300 kg N h^{-1}	1.99 kg N_2O-N h^{-1}	69.3 kg CH_4-C h^{-1}	0.98 kg N_2O-N h^{-1}	104.9 kg CH_4-C h^{-1}	The concentration of N_2O was significantly decreased The concentration of CH_4 was significantly increased	Zhang *et al.*, 2012a
Semiarid soil	Wheat straw biochar (40 t h^{-1}) & urea 300 kg N h^{-1} & 75 kg P h^{-1}	1.22 kg N_2O-N h^{-1}	-3.9 kg CH_4-C h^{-1}	0.71 kg N_2O-N h^{-1}	-0.11 kg CH_4-C h^{-1}	The concentration of N_2O was significantly decreased The consumption of CH_4 was significantly decreased	Zhang *et al.*, 2012c
Japanese grassland	Sorghum biochar (10% w/w)	19.9 µg N_2O-N g^{-1} soil	-	0.5 µg N_2O-N g^{-1} soil	-	The concentration of N_2O was significantly decreased	Yanai *et al.*, 2007
	Corn stover biochar (10% w/w)			-0.8 µg N_2O-N g^{-1} biochar	0.3 µg CH_4-C g^{-1} biochar		
Minnesota agriculture soil	Wood biochar (10% w/w)	-0.5 µg N_2O-N g^{-1} biochar	-0.1 µg CH_4-C g^{-1} biochar	-2.8 µg N_2O-N g^{-1} biochar	3.2 µg CH_4-C g^{-1} biochar	Changes in N_2O or CH_4 were significant	Spokas and Reicosky 2009
	Biosource (10% w/w)			5.7 µg N_2O-N g^{-1} biochar	-4.1 µg CH_4-C g^{-1} biochar		

The effects of 16 different biochars (10% w/w) on greenhouse gas emissions from Minnesota agriculture soil over 100 days were investigated by Spokas and Reicosky (2009). It was concluded that the application of biochar appeared to elicit different responses. CO_2 production increased in five biochar-amended soils, decreased in three and had no significant effect in eight biochar-amended soils. CH_4 oxidation decreased or showed no significant effect. N_2O emissions decreased in biochar-amended soil (Table 2.4).

It appears that factors such as biochar properties, soil type, fertilization and water management regime influence greenhouse gases fluxes (VanZwieten *et al.*, 2009). One of the explanations for the mitigation of N_2O emissions from soil amended with biochar is the reduced availability of ammonium and nitrate ions via sorption (Karhu *et al.*, 2011). Biochar increases nitrogen utilization efficiency (Karhu *et al.*, 2011; Zhang *et al.*, 2012b) and facilitates liming, all of which can reduce the activity of denitrifying communities and/ or reduce rewetting of the soil to 73% of water-filled pore space (Yanai *et al.*, 2007). However, Clough *et al.* (2010) attributed no effects of biochar on N_2O emission to the poor ability of this particular biochar to sorb ammonium and nitrate ions, and this biochar did not reduce the available nitrogen to denitrifying microorganisms. The reductions in CO_2 and CH_4 emissions were attributed to the sorption of dissolved organic carbon by biochar surfaces (Thies and Rillig, 2009; Knoblauch *et al.*, 2011), or to improving the soil porosity and soil aeration by biochar which could facilitate CH_4 oxidation in soil (Van Zwieten *et al.*, 2010b) or to variable soil and biochar properties affecting N_2O, CH_4 and CO_2 emissions (Spokas and Reicosky, 2009). Microbial methane oxidation in soil is the main process contributing to a reduction of methane emissions to the atmosphere. The methane oxidation rate in semiarid soil was 3.9 kg CH_4-C h^{-1}. This rate was decreased to 0.11 kg CH_4-C h^{-1} in the same soil amended with wheat straw biochar (40 t h^{-1}) (Zhang *et al.*, 2012c). Spokas and Reicosky (2009) reported that The methane oxidation rate was increased from 0.1 kg CH_4-C h^{-1} in Minnesota agriculture soil to 4.1 kg CH_4-C h^{-1} after amended soil with biochar (10% w/w). Many factors affect the activity of methanotrophic microorganisms, such as soil pH (Hütsch *et al.*, 1994), the availability of nitrate and ammonium ions (Castro *et al.*, 1994; Hütsch *et al.*, 1994; Le Mer and Roger, 2001), and soil texture (Hütsch *et al.*, 1994). Methanotrophic

microbial communities are sensitive to the acidification condition. However, methane oxidation was observed in soil at a soil pH of about 3.2 (Steudler *et al.*, 1989) and at pH 4.1 (Nesbit and Breitenbeck, 1992). Hütsch *et al.* (1994) reported that the methane oxidation was higher in soil at pH 7.4 in comparison with soil at pH 4.1. Many articles have described the impacts of different nitrogen forms on methane oxidation. It is reported that there is an inverse relationship between the availability of nitrogen and methane oxidation (Steudler *et al.*, 1989). Nitrogen fertilization reduces the methane oxidation of soil (Castro *et al.*, 1994; Le Mer and Roger, 2001). Castro *et al.* (1994) found a reduction in methane oxidation rate of 5-20 times in fertilized soils. Moreover, methane oxidation decreased by 64% in soil which had received 150 kg NH_4NO_3-N ha^{-1} $year^{-1}$ (Le Mer and Roger, 2001). The extent of nitrogen effects on methane consumption depend on the chemical form of the nitrogen (Mochizuki *et al.*, 2012). Low concentrations of nitrate can suppress methane oxidation in forest soil to a great degree and, moreover, nitrate has greater inhibitory impacts on methane consumption than ammonium (Mochizuki *et al.*, 2012). The influence of ammonium on methane oxidation is attributed to competition between methane and ammonium at the methane-monooxygenase enzyme (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994; Sitaula *et al.*, 1995) and or a transfer of the CH_4 oxidizing activity towards nitrification (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994). Furthermore, soil texture plays a role in this inhibition, as it slowly releases ammonium ions from the clay minerals maintaining the inhibition of methane consumption (Hütsch *et al.*, 1994).

2.4 Sorption to biochar

Sorption is one of the most important processes which affect the availability, leaching and behaviour of organic or inorganic chemicals (Downie *et al.*, 2007), and controls the toxicity, fate and transport of organic pollutants in the environment (Smernik, 2009), especially in black carbon-amended soil or sediment. Sorption occurs when organic or inorganic chemicals become attached to the surface area of particles or collide with them. There are many factors affecting sorption, such as the surface area of sorption material, salinity (Rysgaard *et al.*, 1999; Hou *et al.*, 2003), and type of functional group (Hina *et al.*, 2010b). Many researchers have attempted to investigate the abilities of black carbon, such as biochar or activated carbon, for two purposes:

one is the ability of sorption of inorganic chemicals to remove nutrients from water, and reduce the leaching of nutrients from the soil. The second purpose is to increase sorption of organic contamination, such as pesticides and PAHs, for reducing their mobility and availability for bio-uptake.

2.4.1 Biochar sorption of inorganic compounds

Activated carbon is widely used for treating drinking and wastewater due to its high sorption capacity. Although the surface area of biochar is lower than activated carbon, the high cost of activated carbon encouraged researchers to use biochar as a soil and water amendment, because biochar is a low cost material (Iyobe *et al.*, 2004). It is found that the surface area of wood biochar was 330.2 m^2 g^{-1} which was lower compared to activated carbon (1256 m^2 g^{-1}). However, the efficiency of wood biochar for sorption ammonia was 90% compared to 17 % of activated carbon (Iyobe *et al.*, 2004). Mizuta *et al.* (2004), investigated the ability of bamboo biochar or activated carbon for the removal of nitrate from contaminant water in the range of 0-10 mg/l. It is found that the nitrate sorption capacity of bamboo powder biochar was 1.25 mg g^{-1}, whereas the activated carbon sorption value was 1.09 mg g^{-1} activated carbon. The bamboo biochar is an attractive option for ammonium due to high ammonium adsorption capacity, which was 0.852 mg g^{-1} at 25°C for bamboo produced at 600°C (Ding *et al.*, 2010a). Downie *et al.* (2007) found that biochar removed up to 52% of dairy farm effluent phosphorus when chicken litter biochar was added at a ratio of 100:1 effluent/biochar. That is due to precipitation of minerals, such as calcium phosphate.

The nutrient sorption efficiency of biochar and activated carbon depends on many factors, including functional groups on the surface area and competition with other compounds on the sorption position. Rysgaard *et al.* (1999) conducted experiments to investigate the effects of different salinity level on the ammonium sorption capacity of Danish estuarine sediment. It was found that the salinity has a great influence on the ammonium sorption capacity of estuarine sediments, and the sorption capacity of Danish estuarine sediment was significantly decreased from 400 µg g^{-1} to 300 µg g^{-1} when salinity was increased from 0‰ to 10‰, and increased salinity over 10‰ did

not have significant effects on ammonium sorption (Rysgaard *et al.*, 1999). This results was supported by Hou *et al.* (2003) observations who found that the desorption rate of estuarine sediment was increased from 6.9 µg NH_4-N g^{-1} to 14 µg NH_4-N g^{-1} when salinity was increased from 0‰ to 10‰. Asada *et al.* (2006) investigated the effect of pyrolysis temperature on the ability of bamboo biochar to remove ammonium from liquids. It is found that bamboo biochar generated at 400°C and treated with diluted sulphuric acid was most efficient for the removal of ammonia from liquid rather than biochar generated at 700°C and 1000°C or activated carbon. Kastner *et al.* (2009) results support this observation, as it is found that the sorption capacity of low pyrolysis temperature palm oil biochar generated at 500°C ranged between 0.70 to 0.95 mg $NH_3 g^{-1}$ which was higher than the sorption capacity of AC (by 0.45 to 0.60 mg $NH_3 g^{-1}$). Hina *et al.* (2010b) attributed the high efficiency of low pyrolysis biochar for sorption to the nature of the surface and dominating functional group, rather than the surface area. This result was supported by Iyobe *et al.* (2004) findings who investigated the effect surface area on sorption capacity of ammonium. Although the surface area of activated carbon is higher, the efficiency of biochar generated at 500°C for sorption of ammonia is higher in comparison with activated carbon, due to the intensity of the acidity function group on the biochar surface being higher than on the activated carbon surface (Iyobe *et al.*, 2004). Moreover, Hina *et al.* (2010b) produced results showing that despite the decreased total surface areas of biochar treated with alkaline slurry, the removal percentage of ammonium increased from 61% to 83% of the total amount.

2.4.2 Biochar Sorption of Organic Compounds

Sorption is a predominant process in soils and sediments controlling the mineralization and environmental risk of hydrophobic organic contaminants (Carmichael and Pfaender, 1997; Guo *et al.*, 1999; Jensen *et al.*, 2004; Werner *et al.*, 2010; Sopeña *et al.*, 2012), their movement in the environment (Guo *et al.*, 1999) and bioavailability to degrading microorganisms (Carmichael *et al.*, 1997; Werner *et al.*, 2010), due to their ability to bind organic and inorganic chemicals to the surface area. The impacts of sorption on biodegradation of organic contaminants are very complicated and depend on many factors such as microbial species, the properties of compounds, and soil

conditions (Guo *et al.*, 1999). However, the sorption capacity of soils and sediments can be enhanced by adding strong sorption materials, such as biochar or activated carbon (Smernik, 2009). The understanding of the impacts of the sorption and desorption rate on the bioavailability and biodegradation of organic contaminants is essential for predicting their persistence in the environment and their health risk, and for improving remedial approaches (Guo *et al.*, 2000). The effects of activated carbon amendments on the sorption and degradation of pesticide 2,4-D was investigated by Guo *et al.* (2000), who reported that the adsorption coefficient in soil was increased from 0.81 ml g^{-1} in silty loam to 26.19 ml g^{-1} in activated carbon amended soil (2.5%). Moreover, the biodegradation rate in liquid phase was 0.15 day^{-1} while in sorbed phase it was 0.00246 day^{-1}. The black carbon's ability to absorb organic compounds is greater compared to the inorganic compounds, due to the hydrophobic nature of both carbon black and organic contaminants. The ability of organic contaminants to interact with the π–π electron force of the biochar surface area (Sander and Pignatello, 2005) and the ability to access small and narrow pores (Jonker and Koelmans, 2002; Van Noort *et al.*, 2004) are the main influences on the sorption of organic compounds. Sander and Pignatello (2005) pointed out that planar aromatic hydrocarbons are strongly sorbed to biochar surface areas. This is attributed to π–π interactions between the aromatic rings and those of the biochar and to better access to small and narrow micropores. The sorption capacity of organic contaminants in biochar-amended soil is higher than in non-amended soil (Smernik, 2009). Weak and linear sorption into amorphous organic matter, and strong and non-linear sorption onto the surfaces of biochar are the two sorption mechanisms controlling bioaccessibility, biodegradation and sequestration of organic contaminants in the environment (Cornelissen *et al.*, 2005; Koelmans *et al.*, 2006; Smernik, 2009). McGroddy and Farrington (1995) reported that a low concentration of PAH in the highly polluted water of Boston Harbour was attributable to the non-linear sorption to soot particles.

The bioavailability of organochlorine insecticides decreased in soil treated by activated carbon (Lichtenstein *et al.*, 1968) as well as PCB (Strek *et al.*, 1981), trinitrotoluene (Vasilyeva *et al.*, 2001), and phenanthrene (Rhodes *et al.*, 2008). It is found that there is a significant inverse relationship between sorption strength of MCPA (Jensen *et al.*, 2004), simazine (Jones *et al.*, 2011), phenanthrene (Rhodes *et al.*, 2008), or 2,4-dichlorophenoxyacetic acid (Bolan and Baskaran, 1996; Guo *et al.*,

2000), and their biodegradation. Jones *et al.* (2011) found that a small amount of simazine mineralization was observed just in the presence of biochar alone (0.34% of the total simazine added), however, the amount of simazine mineralization in the soil-only treatment over the same 21 day period was 34.1% of that mineralized. Increased sorption capacity in activated carbon amended soil decreased the biodegradation rate of MCPA. It is found that the first order biodegradation rate of MCPA was decreased from 0.05 day^{-1} in activated carbon amended soil (0.005%) to 0.004 day^{-1} in the same activated carbon amended soil at higher amendment dose (0.05%) (Jensen *et al.*, 2004). The mineralization of PAHs after 20 days decreased by up to 50% with increasing concentrations of black carbon, from 0 to 5%. For example, after 1 d soil-PAH contact time, mineralization declined from 70.8 to 19.7% in black carbon amended soil with 0 and 5% (Rhodes *et al.*, 2008). This is attributed to a reduction in water-dissolved concentration and decreases in bioaccessibility and bioavailability to degrading microorganisms (Bolan and Baskaran, 1996; Guo *et al.*, 2000; Jensen *et al.*, 2004; Rhodes *et al.*, 2008; Jones *et al.*, 2011). Werner *et al.* (2005) reported that PCB sequestration improved in Lake Hartwell sediment amended with activated carbon (2% as dry sediment weight), and total water concentrations of PCB decreased by more than 98% after 6 months. The concentration of these compounds remained below detection limits following the 18 months of contact. Moreover, just 7% of total sorbed PCB was desorbed after 6 months of treatment, while 74% of total sorbed PCB was desorbed in untreated sediment after the same contact period (Werner *et al.*, 2005). Moreover, the bioaccumulation of PCB in the benthic organisms (*Lumbriculus variegatus*) decreased by 70% to 92% in sediment treated with activated carbon (Sun and Ghosh, 2007) and decreased by 93% and 90% in *Neanthes arenaceodentata* and *Leptocheirus plumulosus*, respectively, in sediment amended with activated carbon (3.4%) (Zhang *et al.*, 2010b). This reduction was attributed to decreases in free water concentration (Sun and Ghosh, 2007; Zheng *et al.*, 2010) and to reduced desorption rates (Sun and Ghosh, 2007).

The sorption capacities of activated carbon or biochar do not only depend on sorbent properties and the type of organic pollutants, but also the interaction with soils or sediments. (Werner *et al.*, 2010) and (Cornelissen *et al.*, 2005) reported that the sorption strength capacity of PCBs or phenanthrene by activated carbon in clean water

were much higher compared to sorption capacities when these sorbents are mixed with sediments or soils in *in-situ* treatments. In addition, the sorption capacity of biochar-amended soils is not as high as expected based on the sorption capacity of pure biochar and soil without amendment (Cornelissen *et al.*, 2004; Ran *et al.*, 2007; Rhodes *et al.*, 2008; Rhodes *et al.*, 2010). This reduction is likely due to competition between native organic compounds and organic pollutants on sorption sites and/or blockage of black carbon sorption sites (Cornelissen *et al.*, 2004; Cornelissen *et al.*, 2005; Ran *et al.*, 2007; Rhodes *et al.*, 2008; Rhodes *et al.*, 2010; Werner *et al.*, 2010). This idea is supported by the findings of Pasqualino *et al.* (2006), who concluded that in *in-situ* indigenous organic substrates blocked the entry of organic contaminants, especially large molecules, into the pore network of the sorbent area. Moreover, indigenous organic substrate reduced the sorption of organic contaminants by competing on the external surface area. These observations were also agreed with the modelling results of Hale *et al.* (2009), who found decreases in DDTs sorption capacity with shorter contact time between sediment and activated carbon in sediments. This result was attributed to precipitate dissolved organic matter in the macropores and mesopores, which may slow mass transfer of DDTs to sorption sites in the micropores of activated carbon (Hale *et al.*, 2009). The effect of precipitation of dissolved organic matter on the mineralization of phenanthrene was investigated by Rhodes *et al.* (2010) who find that the total percentage of phenanthrene mineralization in higher organic content (19.5%) soil was 34% of added phenanthrene over 20 day, while this percentage was 21% in lower organic content (1.7%) soil. This phenomenon enhances the phenanthrene availability and ultimately accelerated the biodegradation rates (Rhodes *et al.*, 2010). Zheng *et al.* (2010) studied the effects of competion between atrazine and simazine on their sorption on biochar. It was found that the sorption capacities were reduced by 34% for atrazine and 59% for simian. The efficiency of biochar and activated carbon on the sorption of hydrophobic organic contaminants depended on contact time (Carmichael *et al.*, 1997; Werner *et al.*, 2005; Zimmerman *et al.*, 2005; Tomaszewski *et al.*, 2007), and sorbent size (Sun and Ghosh, 2007; Tomaszewski *et al.*, 2007; Jones *et al.*, 2011). Due to the enhancement of activated carbon sorption when the particle size of the activated carbon was decreased, the bioaccumulation of PCB in the benthic organisms and total water concentrations of PCB was decreased (Sun and Ghosh, 2007). Moreover, the reduction of total water concentrations of PCB in Lake Hartwell decreased from 170 ng l^{-1} in the first day

contact time to 7 ng l^{-1} after 1 month contact time and this value become < 3 ng l^{-1} after 6 months contact time (Werner *et al.*, 2005). Carmichael *et al.* (1997) found that freshly preloaded PAHs degraded while aged PAHs persisted in soil containing a large and active community of PAH-degrading microorganisms. On the other hand, (Vasilyeva *et al.*, 2010) found that adsorption of PCB in historically contaminated soil amended with granular and powdered activated carbons did not inhibit PCB degradation, because the total PCB mineralization in activated carbon-amended soil over three years was similar compared with the soil in the control. These results are supported by the findings of (Rhodes *et al.*, 2008) and (Rhodes *et al.*, 2012), who reported that the biodegradation of phenanthrene in the presence of activated carbon was faster than predicted by the rapid desorption rate which indicates that phenanthrene does remain to some extent bioaccessible. Therefore, it is hypothesized that degrading microbes may be able to overcome mass-transfer limitations and utilize the sorbed phenanthrene through direct contact by adhering to black carbon particles (Rhodes *et al.*, 2008; Rhodes *et al.*, 2012).

The mobility of VPHs in unsaturated soils is an important exposure pathways of these contaminants, which are significant sources of human health or environmental risk (Liptak and Lombardo, 1996; Environment agency, 2009b; Environment agency, 2009c). Therefore, effective attenuation of VPHs in contaminated site can considerably reduce exposure risks by reducing the volatilization and mobility of VPHs. However, soil types with low VPH sorption capacity, for example, those with low organic matter content and high contents of gravel and sand tend to attenuate VPH plumes poorly (Höhener *et al.*, 2006). Gravel and sand is widely used as a geotechnical construction material, including at sites with significant past or future VPH pollution risks. It is therefore desirable to identify practicable ways of enhancing the VPH attenuation capacity of gravel and sands, so they may for instance be used as VPH vapor barriers.

Traditionally, enhanced natural attenuation of VPHs relies on the optimization of growth conditions for pollutant degrading soil microorganisms through the provision of nutrients and electron acceptors. This strategy can be highly successful and low-cost. However, this strategy has its own disadvantages, such as, reliance on active on-

site remediation over extended time periods with diminishing returns, the need for repeated applications of nutrients and the risk of nutrient leaching into groundwater. Enhancing contaminant sorption in soil through the addition of activated carbon or biochar is a relatively new soil remediation strategy which also builds on the concept of enhanced natural attenuation (Meynet *et al.*, 2012; Hale *et al.*, 2012). Compared with other forms of organic matter, activated carbon and biochar is very stable in the soil environment and may therefore lastingly enhance a soils pollutant binding capacity and reduces the contaminant mobility in soil. Whether enhanced sorption will lastingly contribute to the attenuation of VPH vapors in biochar or activated carbon amended soils or whether it will just delay VPH breakthrough and hence exposure risks depends on the interaction between contaminant sorption and contaminant biodegradation, which is still poorly understood.

There is still a very limited understanding of the long-term effects of added sorbents on bioavailability, biodegradation and volatilization of different pollutants, for instance volatile petroleum hydrocarbons, which tend to be fairly biodegradable, but also quite mobile in the soil environment. Up until now, research on the relation between adding sorbent material such as activated carbon or biochar and availability of hydrocarbon pollutants has mainly focused on uptake by organisms, and much less on trade-offs between reduced contaminant mobility and effects on microbial pollutant biodegradation. To better understand potential trade-offs between pollutant binding, pollutant immobilization, pollutant biodegradation, pollutant flux from contaminated soil and risk of vapours inhalation is the primary motivation for this work. There is a clear need to improve our understanding of the relationships between adding different type of sorbent materials and pollutant biodegradation, pollutant flux from contaminated soil and the critical role inorganic nutrients may play in reduction of risk on indoor air quality. The outcomes of the project will contribute to the development of better in-situ remediation approaches that reduce risks to human health and ecosystems and enhance the long-term natural attenuation processes.

Chapter 3: Effects of biochar on soil nutrients and biogenic gases

3.1 Introduction

An increase of contaminated areas and global demand for agricultural food production requires new approaches to treat contaminated soils and sediments and to increase agriculture land health and productivity. Biochar is considered a valuable soil amendment due to its properties (Lehmann, 2007; Zhang *et al.*, 2012b). It mitigates global warming by sequestering atmospheric carbon dioxide in soils and may also reduce emission of biogenic gases such as methane and nitrous oxide (Clough *et al.*, 2010; Taghizadeh-Toosi *et al.*, 2011; Feng *et al.*, 2012; Zhang *et al.*, 2012b). In agriculture, reduced nutrient leaching and providing a source of nutrients are the main purposes of using biochar, and interactions of biochar with nutrients are also important for contaminated soil remediation applications. The fertility value of biochar is changeable and dependent upon the feedstock biomass type and the operating conditions of pyrolysis, but biochar may reduce the need for fertilizer in agriculture due to increased fertilizer utilization efficiency as well as reduced nutrients loss via leaching (Lehmann *et al.*, 2003; Chan and Xu, 2009; Ding *et al.*, 2010b; Laird *et al.*, 2010b). Furthermore, the ability of biochar to improve physical soil properties and increase acidic soil pH (VanZwieten *et al.*, 2007) can be a benefit of using biochar as soil amendment. Reduction of greenhouse gases emissions is one of the most important motivations for the use of biochar in agriculture. Producing biochar from crop residues and applying it in Paddy soils not only reduced CH_4 emission (Feng *et al.*, 2012) but also reduced CO_2 fluxes as result of avoiding the burning of crop residues in fields (Zhang *et al.*, 2012b). However, these effects depended on the type of biochar, soil chemical and physical properties and indigenous microbial communities.

3.2 Aims

The aim of this chapter is to investigate the effect of biochar on total and available contents of essential nutrients and to examine the influences of biochar on biogenic gases consumption or production in different soils. This will be achieved by setting up nitrate, ammonium and phosphate sorption isotherm batch experiments. Nitrous oxide

production and methane oxidation batch experiments will be carried out to measure biochar effects on biogenic gases. Soils with or without biochar will also be used to examine changes in nutrient contents.

3.3 Materials and methods

3.3.1 Soil sample

The sandy soil used in this study was obtained from the Newcastle Law School building construction site on the Newcastle University campus in the U.K. The moist soil was sieved (< 4.0 mm). The solid density of sandy soil was 2.5 g cm^{-3}, the total organic carbon (TOC) content was 1.6±0.1% and the total carbon (TC) content was 4.1±0.1%. The sandy soil had a pH of 7.43±0.04. It had following grain size composition: 89.5% sand, 8.4% silt and 2.1%. The sandy soil had an EC of 675±81 µS cm^{-1}. The clayey loam soil, loamy soil and sandy loam soil used in this study was obtained from the Cockle Park farm (Newcastle University farm) in Morpeth, Northumberland. The clayey loam soil had following grain size composition: 39.8% sand, 33.2% silt and 27% clay. The loamy soil had the following grain size composition: 39.4% sand, 48.5% silt and 12.1% clay. The sandy loam soil had the following grain size composition: 63.5% sand, 27.4% silt and 9.1% clay. Soil was stored at 3°C in the cold room until usage.

3.3.2 Biochar

The biochar obtained from Environmental Power International EPI (Wiltshire, UK), was produced from wood chips by fast pyrolysis at high temperature (800°C) in a fixed bed reactor. The biochar was ground to a particle size < 163 µm. The biochar solid density was 1.44±0.2 g cm^{-3}, and the biochar had a pH of 9.25±0.16. The biochar C: N: S ratio was 86.5:1.2:0.3, and a total surface area of 928 m^2 g^{-1}as determined from CO_2 adsorption isotherms. The TOC content was 83.9%. Ready available ammonium, nitrate and phosphate were1.3±0.1 µg NH_4^+ g^{-1}, 0.7±0.1 µg NO_3^- g^{-1} and 5.3±0.6 µg PO_4^{-3} g^{-1}, and respectively.

3.3.3 Activated carbon

A bitumen activated carbon (Chemviron Carbon Ltd, Lancashire, UK) was used in this study. It had a measured surface area of 1012 m^2 g^{-1}, a pore volume of 0.6 cm^3 g^{-1} and the following pore distribution: ultramicropores 44.6%, micro/supermicropores 23%

and meso/macropores 32.4%. The surface area and pore size disruption were measured by adsorption of nitrogen and carbon dioxide using an Intelligent Gravimetric Analyser (IGA) model 003 (Hiden Isochema Ltd., Warrington, U.K). The bitumen activated carbon was ground to a particle size < 163 µm. The solid density was 1.45±0.06 g cm^{-3}, and the TOC content was 72.7±0.3%. Activated carbon had a pH of 8.73±0.10.

3.3.4 Ammonium, nitrate and phosphate sorption isotherms

A batch method (three replicates) was used to obtain ammonium, nitrate and phosphate sorption isotherms for soil, soil & 2% activated carbon, soil & 2% biochar, activated carbon and biochar. Either 0.1 g of activated carbon or 0.1 g of biochar or 5 g of soil or 5 g of soil & 2% activated carbon or 5 g of soil & 2% biochar were added to 50 ml centrifuge tubes. Different ammonium chloride loadings (VWR, Leicestershire, UK) ranging from 0-400 mg l^{-1} or 6-7 different potassium nitrate loadings (VWR, Leicestershire, UK) ranging from 0-20 mg l^{-1} or Different potassium dihydrogen phosphate loadings (VWR, Leicestershire, UK) ranging from 0-20 mg l^{-1} were added to the centrifuge tubes. To assess the influence of competition of other cations on the sorption of ammonium, ammonium chloride was also dissolved in 0.02 M calcium chloride ($CaCl_2$) (VWR, Leicestershire, UK) and 6-7 different ammonium loadings ranging from 0-400 mg l^{-1} were used in batch experiments to obtain ammonium sorption isotherms for soil, soil & 2% activated carbon, soil & 2% biochar, activated carbon and biochar. In addition, centrifuge tubes without sorbent material or soil served as blanks. All tubes were shaken for 20 h at 120 rpm to establish sorption equilibrium. After equilibration the slurries were centrifuged at 4,000 rpm for 8 min and the supernatant was immediately filtered by using syringe filters (pore size 0.2 µm, diam. 25 mm, VWR, Leicestershire, UK). Changes in ammonium, nitrate or phosphate concentration from the initial concentration were used to calculate the sorbent of ammonium, nitrate or phosphate (µg g^{-1}). Adsorption isotherms were fitted with the Langmuir model according to equation 3.1,

$$q = q_{max} \frac{k C_e}{1 + k C_e} \qquad Equation\ 3.1$$

where q is amount adsorbed (µg g^{-1}), q_{max} is maximum amount adsorbed (µg g^{-1}), k is Langmuir equilibrium constant (l mg^{-1}) and C_e is equilibrium aqueous concentration (mg l^{-1}).

3.3.5 Ammonium quantification

Ammonium determination was carried out using a steam distillation method. Analysis was performed on the Vapodest 30s steam distillation system (Gerhardt, Northlands, UK). The sample of supernatant solution (50 ml) was steamed for 4 min, and ammonia was received in 50 ml of indicating boric acid solution (4% boric acid). The ammonia released in the distillation system was back-titrated with standard 0.02 M sulfuric acid (H_2SO_4). The concentration of ammonium was calculated according to equation 3.2.

$$NH_4^+ - \mathrm{N}\ (\mathrm{mg\ l^{-1}}) = \frac{(\mathrm{A} - \mathrm{B}) * 280 * 1000}{\mathrm{ml\ of\ sample}} \qquad Equation\ 3.2$$

where A is the volume of sulfuric acid used for the sample (ml) and B is the volume of sulfuric acid used for the blank (ml).

3.3.6 Nitrate, sulphate and phosphate quantification

Nitrate, sulphate and phosphate analysis was performed on a Dionex ICS 1000 Ion Chromatography system provided with a conductivity detector (Dionex, Sunnyvale, CA, USA). An aliquot of the sample (5 ml) was injected by a Dionex AS40 automated sampler. The separation was performed on a Ionic pac AS14A analytical column (250 mm × 4 mm i.d, Dionex, Sunnyvale, CA, USA). The ion chromatograph was held isothermally at 20°C with 8.0 mM Na_2CO_3/1.0 mM $NaHCO_3$ solution as the mobile phase (flow rate of 1 ml min^{-1}, initial pressure 1800 psi). Instrumental quantification was calibrated using standard nitrite, nitrate and phosphate solutions for a three point calibration.

3.3.7 Total nitrogen

Total nitrogen was carried out using the Kjeldahl method. Soil sample crushed to <0.5 mm and 2.0 g of soil sample was placed in 100 ml digestion tubes and 14 ml of concentrated H_2SO_4 (VWR, Leicestershire, UK) and 2 tablets of Kjeltabs CT catalyst tablets (Thompson & Capper, Cheshire, UK) were added before the samples were digested by heating in the Vapodest digestion unit (Gerhardt, Northlands, UK) for 40 min at 400°C. Digestion tubes were then moved to the Vapodest 30s steam distillation system (Gerhardt, Northlands, UK), and sample digestion was steamed for 4 min after adding 33 mls of 40% NaOH, and ammonia was received in 50 ml of indicating boric acid solution (4% boric acid). The ammonia released in the distillation system was back-titrated with standard 0.02 M sulphuric acid (H_2SO_4). The total nitrogen was calculated according to equation 3.3.

$$N_{\text{total}}\ (\%) = \frac{(\text{A} - \text{B}) * \text{N} * 1400}{\text{sample weight}} \qquad \text{Equation 3.3}$$

where A is the volume of sulfuric acid used for the sample (ml), B is the volume of sulfuric acid used for the blank (ml) and N is the normality of sulphuric acid used for titration.

3.3.8 Total phosphorus

Extraction of total phosphorus was carried out using wet mineralization method (Pansu and Gautheyrou, 2006) by placing 2.0 g of crushed soil sample in 100 ml digestion tubes and 30 ml of concentrated nitric acid (VWR, Leicestershire, UK) was added then samples were digested by heating in a Vapodest digestion unit (Gerhardt, Northlands, UK) for 3 h at 180°C, then filtered by using syringe filters (pore size 0.2µm, diam. 25mm, VWR, Leicestershire, UK), and evaporated to almost dry, then 1 ml concentrated sulphuric acid (VWR, Leicestershire, UK) was added and diluted to 100 ml with deionized water. Phosphorus in extracts was determined colorimetrically using the ascorbic acid methods.

3.3.9 Extraction of available ammonium, nitrate, sulphate and phosphate

Readily available nutrients were extracted using 1:1 (soil: $CaCl_2$ 0.02 M) suspensions for soils and 1:2 suspensions for biochar and activated carbon by mixing 25 g of soil and 25 ml of $CaCl_2$ 0.01 N (VWR, Leicestershire, UK) in centrifuge tubes then shaken for 20 h, and the slurries were centrifuged at 4,000 rpm for 10 min and the supernatant was immediately filtered by using syringe filters (pore size 0.2µm, diam. 25mm, VWR, Leicestershire, UK).

3.3.10 Available ammonium quantification

Available ammonium determination was carried out using an ammonium reagent kit (Merck no. 1.4752.002, Merck, Darmstadt, Germany) and a Spectroquant Nova 60 photometer (Merck, Germany). The sample of supernatant solution (5 ml) was put into test tube, and 0.6 ml of sodium hydroxide buffer solution and 0.5 g of EDTA was added. After 5 min of shaking 3 drops of sodium nitroprusside indicator was added. The concentration of ammonium was determined on a Spectroquant Nova 60 photometer.

3.3.11 Soil electrical conductivity (EC)

Soil electrical conductivity was measured using a 1:1 (mass: volume) suspension by mixing 20 g of soil and 20 ml deionized water in a centrifuge tube then shaken for 3 h, and the slurries were centrifuged at 3,000 rpm for 5 min, then the electrical conductivity was measured by a EcoSense EC300 conductivity meter (VWR, Leicestershire, UK).

3.3.12 Soil pH

Soil pH was carried out using a 1:1 (soil:$CaCl_2$ 0.02 M) suspension by mixing 20 g of soil and 20 ml of $CaCl_2$ 0.02 M (VWR, Leicestershire, UK) in a centrifuge tube then shaken for 3 h, and the slurries were centrifuged at 3,000 rpm for 5 min, then the pH was measured by a Jenway 3020 pH-meter (Jenway , Staffordshire, UK).

3.3.13 Nitrous oxide production experiments

Nitrous oxide production from denitrification was examined by monitoring N_2O concentrations in 10 ml crimp-top vials (H×Ø= 46×23), closed with grey butyl rubber stoppers, and capped with aluminium crimp caps (Sigma–Aldrich, Dorset, UK) containing 2 g (as dry weight) of sandy soil with and without biochar (2%, 10% as dry weight of soil), clayey loam soil with and without biochar (2%, 10% as dry weight of soil), loamy soil with and without biochar (2%, 10% as dry weight of soil) or sandy loam soil with and without biochar (2%, 10% as dry weight of soil). To avoid substrate limitation the following procedures described by Smith and Tiedje (1979) were used: Soils were saturated with a solution containing KNO_3 (200 µg NO_3^--N g^{-1} dry soil, VWR, Leicestershire, UK), glucose (0.5 mg C g^{-1} dry soil, VWR, Dorset, UK) and glutamic acid (0.5 mg C g^{-1} dry soil, Sigma-Aldrich, Gillingham, UK). To simulate a more natural situation, a second set of crimp-top vials were set up in which soils were saturated with deionised water, and no substrates were added. The vial's gas phase was exchanged by flushing with nitrogen gas (BOC, Guildford, UK), which was then replaced with a 1% acetylene in nitrogen blend (CK gases, Hampshire, UK) to inhibit N_2O reductase activity. The experiments were carried out for 14 days in duplicate. Head space gas samples (60 µl) were taken every day using a 100 µl Hamilton gastight syringe to inject samples into the GC-MS for N_2O quantification. To study the effects of the biochar-soil contact period on N_2O production, another set of experiments was set up using soils incubated 30 days at room temperature prior to the measurements. Denitrification enzyme activity (DEA) (µg N_2O h^{-1} g^{-1} dry soil) was then determined from the slope of the linear regression of plots of N_2O production (µg N_2O g^{-1} dry soil) against sampling times. DEA was calculated by using three linear time points (Smith and Tiedje, 1979).

3.3.14 Methane oxidation experiments

Batch microcosm experiments were set up by injection of 1 ml of 10% methane standard gas (Scientific and Technical Gases, Staffordshire, UK) (to get an initial concentration of 10,000 p.p.mv. in the headspace) in 10 ml crimp-top vials (H×Ø= 46×23), closed with grey butyl rubber stoppers, and capped with aluminium crimp caps (Sigma–Aldrich, Dorset, UK) containing 2 g (as dry weight) of sandy soil with

and without biochar (2%, 10% as dry weight of soil), clayey loam soil with and without biochar (2%, 10% as dry weight of soil), loamy soil with and without biochar (2%, 10% as dry weight of soil) or sandy loam soil with and without biochar (2%, 10% as dry weight of soil). The experiments were carried out for 10 days in triplicate. The head space of the vials was sampled every day with a 100 µl Hamilton gastight syringe to inject 60 µl into a GC for CH_4, CO_2 and O_2 quantification. To study the effects of the biochar-soil contact period on CH_4 oxidation, another set of experiment was set up using soils incubated 30 days at room temperature. CH_4 oxidation rates (µg CH_4 h^{-1} g^{-1} dry soil) were then determined from the slope of the linear regression of plots of CH_4 concentration (µg CH_4 g^{-1} dry soil) against sampling times. CH_4 oxidation rates were calculated by using three linear time points.

3.3.15 Methane quantification

GC-FID analysis was performed on a Carlo Erba HRGC 5160 mega series Gas Chromatography. The sample (60 µl) of headspace gas was injected with a 100 µl Hamilton gastight syringe. The separation was performed on a capillary HP-Plot-Q phase column (30 m x 0.320 mm i.d) coated with 20 µm film thickness (Agilent Technologies, Palo Alto, USA). The injection port used a split ratio of 10 and was heated to 200^{o}C. The GC was held isothermally at 36°C with hydrogen as the carrier gas (flow rate of 30 ml min^{-1}, initial pressure 55 kPa). Instrumental quantification was calibrated using standard methane gas (Scientific and Technical Gases, Staffordshire, UK) for a five point calibration.

3.3.16 CO_2, O_2, N_2O and SF_6 quantification

GC-MS analysis of CO_2, O_2, N_2O and SF_6 was performed on a Fisons 8060 Gas Chromatograph linked to a Fisons MD800 MS (electron voltage 70 eV, filament current 4A, source current 800 µA, source temperature 200°C, multiplier voltage 500V, interface temperature 150°C). The sample (60 µl) was injected in split mode with a 100 µl Hamilton gastight syringe. The separation was performed on a HP-PLOT-Q capillary column (30 m x 0.32 mm i.d) packed with 20 µm Q phase (Agilent Technologies, Palo Alto, USA). The GC was held isothermally at 35°C with helium

as the carrier gas (flow rate of 30 mlmin^{-1}, initial pressure 65 kPa, split at 100 ml min^{-1}). The instrument was calibrated using standard CO_2, O_2, N_2O (Scientific and Technical Gases, Staffordshire, UK) and SF_6 (Sigma –Aldrich, Dorset, UK) gases for a five point calibration.

3.3.17 Statistical analysis

The data were statistically analysed using Minitab for Windows (Version 16). Significant effects of biochar amendment rate, contact time, on the denitrification rate and methane oxidation rate were evaluated through the use of ANOVA using the Fisher's multiple-comparisons test for means ($P < 0.05$). Correlation between denitrification enzyme activity rates or methane oxidation rates and soil properties were analysed using SPSS for Windows (Version 19).

3.4 Results and discussion

3.4.1 Biochar and soil chemical and nutrients characterization

The biochar pH and EC were 9.25±0.16 and 23,133±904 µS cm^{-1} which is high due to high pyrolysis temperature (800°C). At higer pyrolysis temperature all cations in the feedstock become oxides which increases pH and EC when the sorbent is dissolved in water (Gundale and DeLuca, 2006). Adding biochar to soil increased pH and EC of soils as is shown in Table 3.1. For instance, the pH value of sandy soil increased from 7.43±0.04 in sandy soil without biochar to 7.83±0.03 and 7.99±0.02 in sandy soil with biochar (2% and 10%), respectively, and the pH value of loamy soil increased from 5.92±0.01 to 6.73±0.01 in loamy soil with biochar (10%). The effect of biochar on the level of pH and EC was depended on soil pH and EC properties before addition of the biochar. For example, biochar improved pH values of clayey loam soil, loamy soil and sandy loam soil because these soils were acid soils, while in the sandy soil which is alkaline applying biochar increased pH to 7.99 which may be non-ideal for microorganisms. The EC value of loamy soil was increased from 1577±40 µS cm^{-1} to 3290±620 µS cm^{-1} and 4313±655 µS cm^{-1} in soil with biochar (2% and 10%), respectively. The EC in clayey loam soil with biochar (10%) and sandy loam soil with biochar (10%) were higher which could have negative effects on microbial population biomass and microbial enzyme activity (Rietz and Haynes, 2003). The pH and EC results are consistent with Gundale and Deluca (2006) study which found that the values of pH and EC were high in the biochar made from Douglas-fire wood or ponderosa pine wood at 800 ℃. The high pH and EC has been attributed to presence of high amount of water-soluble salts in these biochar (Gundale and Deluca, 2006). The increases in pH and EC of soil amended with biochar (Table 3.1) are also consistent with the results of Van Zwieten *et al.* (2010) and Chan *et al.* (2007) who reported higher pH and EC increases in the biochar amended soils due to high liming value which can effectively ameliorate soil acidity. Total nitrogen and phosphate contents of biochar and its effects on total nitrogen and phosphate contents of soils with biochar are presented in Table 3.1. Total nitrogen and phosphate contents of biochar were 2.21±0.21% and 2300±110 µ PO_4^{-3} g^{-1}, respectively. Total nitrogen and phosphate contents of biochar are not very high due to the biochar being made from wood for which nitrogen and phosphate contents tend to be low (Singh et al., 2010) and the

biochar was produced at 800°C at which temperature nitrogen may be lost by volatilization (Knoepp et al., 2005; DeLuca et al., 2009). Adding biochar to soil increased total nitrogen from 0.45±0.07 % in sandy soil without biochar to 0.61±0.02 % and 1.25±0.19 % in sandy soil with biochar (2% and 10%), respectively, and total phosphate increased from 35.5±4.6 µg PO_4^{-3} g^{-1} in sandy soil without biochar to 40.3±8.2 µg PO_4^{-3} g^{-1} and 73.4±12.1 µg PO_4^{-3} g^{-1} in sandy soil with biochar (2% and 10%), respectively. Total nitrogen and phosphate contents of our biochar was higher compared to Singh *et al.* (2010) who reported that total nitrogen and phosphate contents of biochar made from *Eucalyptus saligna* wood at 550 ℃ were 0.26 % and 665 µg PO_4^{-3} g^{-1}. This is likely the result of differences in nitrogen and phosphate contents of feedstock material.

Table 3.1. Chemical properties of biochar and soils

	pH	EC	Total nitrogen	Total phosphate
	-	µS cm^{-1}	N % -N	µg PO_4^{-3} g^{-1}
Biochar	9.25±0.16	23,133±904	2.21±0.21	2300±110
Sandy soil (SS)	7.43±0.04	675 ± 81	0.45±0.07	35.5±4.6
SS & 2% biochar	7.83±0.03	1601±49	0.61±0.02	40.3±8.2
SS & 10% biochar	7.99±0.02	3503±110	1.25±0.19	73.4±12.1
Clayey loam soil (CL)	5.70±0.02	3424±1183	0.863	66.5
CL & 2% biochar	6.17±0.05	5585±300	n.m.	n.m.
CL & 10% biochar	6.62±0.05	7045±113	n.m.	n.m.
Loamy soil (LS)	5.92±0.01	1577±40	0.736	46.8
LS & 2% biochar	6.34±0.03	3290±620	n.m.	n.m.
LS & 10% biochar	6.73±0.01	4313±665	n.m.	n.m.
Sandy loam soil (SL)	5.58±0.02	2378±102	0.717	102.2
SL & 2% biochar	6.08±0.04	4679±32	n.m.	n.m.
SL & 10% biochar	6.51±0.04	8033±1150	n.m.	n.m.

n.m.: not measured

The effects of adding biochar and contact period on readily available nitrate, ammonium, phosphate and sulphate in amended soils are presented in Table 3.2. Available nitrate, ammonium, phosphate and sulphate contents of biochar are

Table 3.2. Availability of nitrate, ammonium, phosphate and sulphate in biochar and soils for contact period

	a 1 day contact period				a 30 day contact period			
	Available nitrate	Available ammonium	Available phosphate	Available sulphate	Available nitrate	Available ammonium	Available phosphate	Available sulphate
	µg NO_3^- g^{-1}	µg NH_4^+-N g^{-1}	µg PO_4^{-3} g^{-1}	µg SO_4^{-2} g^{-1}	µg NO_3^- g^{-1}	µg NH_4^+-N g^{-1}	µg PO_4^{-3} g^{-1}	µg SO_4^{-2} g^{-1}
Biochar	**0.65±0.09**	**1.3±0. 1**	**5.26±0.57**	**8320±25**	-	-	-	-
Sandy soil (SS)	**33±2**	**3.2±0.2**	**2.53±0.44**	**135±17**	**23±14**	**3.2±1.3**	**0.004±002**	**159±26**
SS & 2% biochar	**28±2**	**6.4±1.5**	**0.71±0.06**	**454±33**	**16±1**	**3.7±0.2**	**0**	**432±30**
SS & 10% biochar	**24±1**	**12.8±0.3**	**0.54±0.04**	**1116±118**	**10±0.1**	**2.8±0.00**	**0**	**1229±172**
Clayey loam soil (CL)	**666±13**	**6.4±0.6**	**7.1±0.5**	**89±12**	**612±13**	**3.0±0.8**	**9.70±0.56**	**66±1**
CL & 2% biochar	**632±16**	**8.8±1.3**	**4.4±0.2**	**540±37**	**572±17**	**5.9±0.1**	**4.1±0.43**	**432±9**
CL & 10% biochar	**392±130**	**21.1±5.2**	**4.0±0.5**	**1777±130**	**317±60**	**4.2±0.1**	**1.76±0.25**	**1266±14**
Loamy soil (LS)	**180±6**	**5.8±0.6**	**0.90±0.04**	**29±13**	**163±13**	**2.9±0.1**	**0.46±0.22**	**58±1**
LS & 2% biochar	**102±22**	**6.7±1.8**	**0.97±0.14**	**227±18**	**89±5**	**3.2±0.5**	**0.35±0.05**	**413±20**
LS & 10% biochar	**8±2**	**8.7±0.5**	**0.60±0.24**	**892±213**	**11±2**	**2.3±0.2**	**0**	**593±92**
Sandy loam soil (SL)	**961±54**	**3.5±0.2**	**9.4±0.4**	**80±4**	**918±8**	**2.3±0.4**	**5.6±0.4**	**39±1**
SL & 2% biochar	**756±210**	**14.1±0.4**	**6.1±0.4**	**455±97**	**667±6**	**5.9±0.9**	**3.9±0.1**	**336±15**
SL & 10% biochar	**569±34**	**18.2±0.8**	**5.5±0.1**	**1709±115**	**214±66**	**4.2±0.8**	**1.6±0.2**	**1368±165**

0.65±0.09 µg NO_3^- g^{-1}, 1.3±0.1 µg $NH_4^+ - N$ g^{-1}, 5.26±0.57 µg PO_4^{-3} g^{-1} and 8320±25 µg SO_4^{-2} g^{-1} respectively. The values of nitrate and phosphate are lower in comparison with total nitrogen and phosphate which agrees with Singh et al. (2010b) and Chan and Xu (2009) results. Adding biochar to soils actually reduced available forms of nitrate and phosphate. For example, available nitrate was decreased from 33±2 µg NO_3^- g^{-1} in sandy soil to 28±2 µg NO_3^- g^{-1} and 24±1 µ NO_3^- g^{-1} in sandy soil with biochar (2% and 10%), respectively, and available phosphate was decreased from 2.53±0.44 µg PO_4^{-3} g^{-1} in sandy soil to 0.71±0.06 µg PO_4^{-3} g^{-1} and 0.54±0.04 µg PO_4^{-3} g^{-1} in in sandy soil with biochar (2% and 10%) respectively. On the other hand, available ammonium and sulphate was increased. For example available sulphate increased from 135±0.17 µg SO_4^{-2} g^{-1} in sandy soil to 454±33 µg SO_4^{-2} g^{-1} and 1116±118 µg SO_4^{-2} g^{-1} in sandy soil with biochar (2% and 10%) respectively. Moreover, available ammonium was increased from 6.4±0.6 µg $NH_4^+ - N$ g^{-1} in clayey loam soil to 8.8±1.3 µg $NH_4^+ - N$ g^{-1} and 21.1±5.2 µg $NH_4^+ - N$ g^{-1} in clayey loam soil with biochar (2% and 10%). These results agree with observations of Lehmann *et al.*, (2003), Bridle and Pritchard (2004), Rondon *et al.* (2007) and van Zwieten *et al.* (2010a). These changes were variably attributed to immobilization of available nitrogen and phosphate because of its high C/N ratio in soil amended with biochar (Lehmann *et al.*, 2003; Rondon *et al.*, 2007) and or to increased nitrification rates (Zackrisson *et al.*, 1996; Ward *et al.*, 1997; Paavolainen *et al.*, 1998), and or to precipitation of phosphate as calcium phosphates as a main way to reduce the availability of phosphate in calcareous soils (Jalali and Ranjbar, 2010) and or to sorption of these nutrients to the biochar surface area (Berglund *et al.*, 2004)

Availability of nitrate, ammonium and phosphate in soil with or without biochar (2% and 10%) were decreased with increasing contact period between soil and biochar from one day to 30 days. For example, available nitrate was slightly decreased from 666±13 µg NO_3^- g^{-1}, 632±16 µg NO_3^- g^{-1} and 392±130 µg NO_3^- g^{-1} in clayey loam soil without or with biochar (2% and 10%) respectively to 612±13 µg NO_3^- g^{-1}, 572±17 µg NO_3^- g^{-1} and 317±60 µg NO_3^- g^{-1} respectively. Furthermore, availability of phosphate in sandy loam soil without or with biochar (2% and 10%) was decreased from 9.4±0.4 µg PO_4^{-3} g^{-1}, 6.1±0.4 µg PO_4^{-3} g^{-1} and 5.5±0.1 µg PO_4^{-3} g^{-1} respectively to 5.6±0.4 µg PO_4^{-3} g^{-1}, 3.9±0.1 µg PO_4^{-3} g^{-1} and 1.6±0.2 µg PO_4^{-3} g^{-1} respectively. The effect of

contact period on availability of phosphate was higher in sandy soil due to its pH being higher, this pH can accelerate precipitation of phosphate as calcium phosphate (Jalali and Ranjbar, 2010), while the effect of contact period was lower in clayey loam soil due to their lower pH of 5.7-6.6.

The effects of biochar application rates and contact period on shifts of readily available nitrogen forms from nitrate to ammonium in biochar amended soils are illustrated in Fig. 3.1. This figure shows the percentage of readily available nitrate and percentage of available ammonium (both as N) relative to total readily available nitrogen. From this figure it can be seen that the available form of nitrogen in biochar amended soils (2% and 10%) was shifted from nitrate towards ammonium, and higher shifts were observed in soils treated with 10% biochar. For example, the percentage of readily available nitrate in sandy soil was 70.0% of total readily available N, while in sandy soil with biochar (2% and10%) these percentages dropped to 49.7% and 29.8%, respectively (Fig. 3.1a). On the other hand, the percentage of readily available NH_4^+ increased from 30.0% in sandy soil to 50.3% and 70.2% in sandy soil with biochar (2% and 10%), respectively (Fig. 3.1c). Although, total readily available N had decreased after 30 day contact time, the readily available nitrogen transformation after 30 day contact time had the same trend. This shift may be attributed to strong sorption of NH_4^+ by biochar rather than NO_3^-.

3.4.2 Effects of material type on nutrient sorption

The effects of soil, biochar and activated carbon on the sorption of nitrate, phosphate and ammonium are shown in Fig 3.2. The ability of activated carbon and biochar to sorb ammonium ions was very high. For example, the maximum amount of ammonium adsorbed (q_{max}) by activated carbon was 90,100 µg g^{-1}, while q_{max} of biochar was 20,000 µg g^{-1} (Table 3.3) and the maximum amount adsorbed by soil was significantly lower at 272 µg g^{-1}. Moreover, the sorption capacity of activated carbon for phosphate was slightly higher than the sorption capacity of biochar. Soil had the lowest phosphate sorption capacity (Fig 3.2 b). The q_{max} values were 5740 µg g^{-1}, 3080 µg g^{-1} for activated carbon and biochar and 134 µg g^{-1} for sandy soil. Maximum

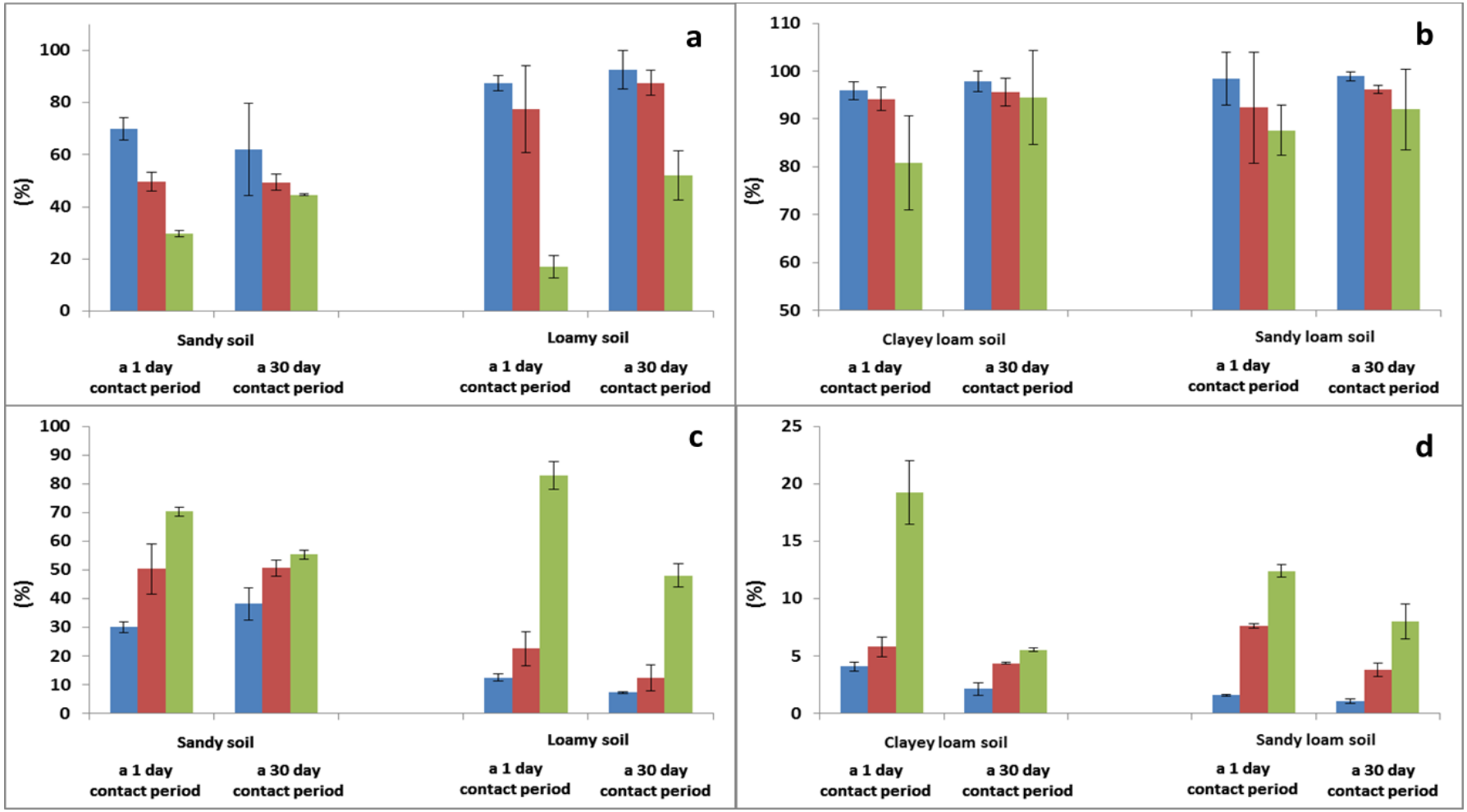

Fig. 3.1. Percentage of available form of nitrate and ammonium relative to total available nitrogen for nitrate (a) and (b), and ammonium (c) and (d); comparing soil (■), soil amended with 2% biochar (■) and soil amended with 10% biochar (■). Error bars: ± 1 standard deviation (SD, n=3).

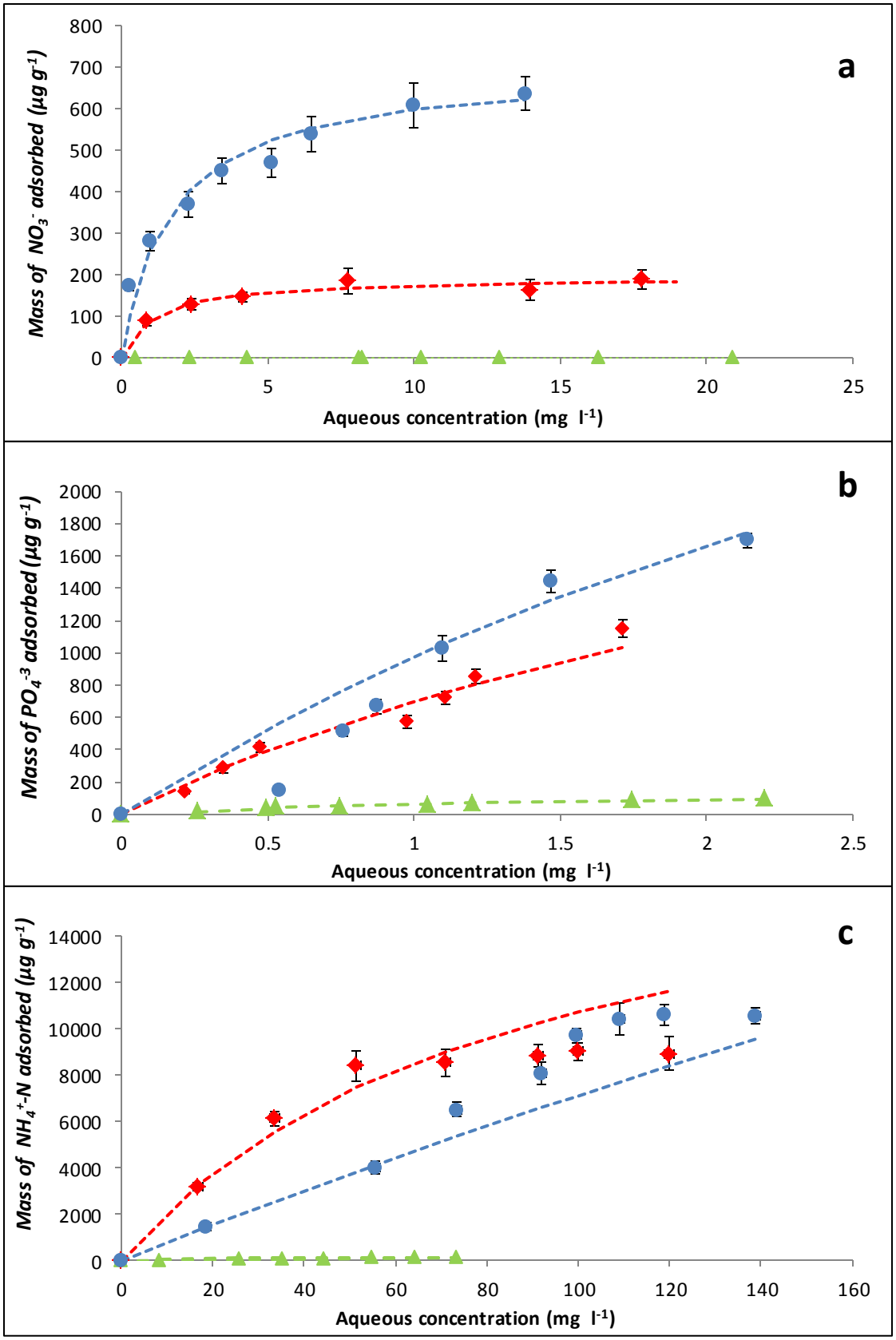

Fig. 3.2. Measured (symbols) and fitted (lines) sorption isotherms of soil (▲ …), biochar (♦ …) and activated carbon (● …) for (a) nitrate, (b) phosphate and (c) ammonium.

Table 3.3. Langmuir sorption isotherm parameters of sandy soil, biochar and activated carbon

Materials	*Nitrate*		*Phosphate*		*Ammonium*			
					Deionised water		*$CaCl_2$ Solution*	
	q_{max} ($\mu g\ g^{-1}$)	$K\ (\frac{l}{mg})$	q_{max} ($\mu g\ g^{-1}$)	$K\ (\frac{l}{mg})$	q_{max} ($\mu g\ g^{-1}$)	$K\ (\frac{l}{mg})$	q_{max} ($\mu g\ g^{-1}$)	$K\ (\frac{l}{mg})$
Sandy soil	**0**	**0**	**134**	**0.91**	**272**	**0.046**	**220**	**0.021**
Biochar	**193**	**0.91**	**3080**	**0.29**	**20000**	**0.012**	**4370**	**0.036**
Activated carbon	**700**	**0.59**	**5740**	**0.20**	**90100**	**0.001**	**7900**	**0.007**
Sandy soil & 2% biochar	**25**	**0.14**	**168**	**0.63**	**793**	**0.008**	**270**	**0.016**
Sandy soil & 2% A. C.	**31**	**0.18**	**192**	**0.37**	**370**	**0.033**	**170**	**0.028**

amount of nitrate adsorbed by activated carbon was 700 µg g^{-1} , whereas it was 193 µg g^{-1} for biochar. This value was 0.0 µg g^{-1} in soil. The high ability of activated carbon and biochar to adsorb ammonium ions may be attributed to high surface area and high pH value (Table 3.4) which increases negative charges on the surface area of particles and colloids. The addition biochar or activated carbon to sandy soil enhanced sorption of nitrate, phosphate and ammonium. Effects of biochar and activated carbon sorption results are consistent with those of Mizuta *et al.* (2004) and Iyobe *et al. (*2004). However, the maximum amount adsorbed was higher compared to these amounts from some published study (Ding *et al.*, 2010a, Kastner *et al.*, 2009). These differences can be explained in part by the differences in surface area of biochar used, type of feedstock material and pyrolysis temperature. The effects of biochar-amended soil and activated carbon-amended soil on the sorption of nitrate, phosphate and ammonium and the comparison between measured and calculated sorption isotherms of nitrate, phosphate and ammonia are shown in Fig 3.3. The maximum ammonium amount adsorbed (q_{max}) of biochar-amended soil was 793 µg g^{-1}, while q_{max} of activated carbon-amended soil was 370 µg g^{-1} (Table 3.3). Moreover, the sorption capacity of activated carbon-amended soil for phosphate was slightly higher than the sorption capacity of biochar-amended soil (Fig 3.3b). The q_{max} values were 192 µg g^{-1} and 168 µg g^{-1} for activated carbon-amended soil and biochar-amended soil

respectively. Maximum amount of nitrate adsorbed by activated carbon-amended soil was 31 µg g^{-1}, while q_{max} of biochar-amended soil was 25 µg g^{-1} (Table 3.3).

Measured and calculated sorption isotherms of nitrate, phosphate and ammonia in sandy soil & 2% biochar and sandy soil & 2% activated carbon are compared in Fig 3.3. The sorption isotherms calculated by using the mass weighted sorption isotherms of sandy soil and biochar or sandy soil and activated carbon was slightly higher than measured for ammonium and phosphate. Competition between ammonium ion and other cations in soil solution for negative charges on surface areas could cause this reduction in sorption capacity in measured isotherm compared to the calculated isotherm. The difference between measured and calculated sorption isotherms of phosphate is small (Fig 3.3b) , and may be attributed to the calcareous nature of sandy soil and its higher pH (Table 3.4) which increased activity of calcium ions and reaction with phosphate resulting in precipitation of phosphate as calcium phosphate. The precipitation reaction is probably the prevalent reaction not sorption. As shown in Fig 3.3a, measured nitrate isotherms were higher than the calculated isotherms. This result could be attributed to the effects of cation-bridging when cations, such as Ca^{+2}, adsorbed to the negatively charged surface area of biochar in the soil solution. This reduces the ammonium adsorption but facilitates the adsorption of nitrate.

Table 3.4. pH value in different sorption experiment solutions

Materials	***Nitrate***	***Phosphate***	***Ammonium***			
			Blank		***20 mg l^{-1}***	
	Deionised water	**Deionised water**	**Deionised water**	**$CaCL_2$ Solution**	**Deionised water**	**$CaCL_2$ Solution**
Sandy soil	**8.33**	**8.31**	**8.57**	**8.07**	**8.53**	**8.01**
Biochar	**8.90**	**8.72**	**9.34**	**8.57**	**8.70**	**8.28**
Activated carbon (AC)	**8.92**	**8.70**	**7.33**	**7.39**	**7.81**	**7.21**
Sandy soil & 2% biochar	**8.27**	**8.24**	**8.27**	**7.85**	**8.57**	**7.93**
Sandy soil & 2% AC	**8.49**	**8.48**	**9.01**	**8.10**	**8.71**	**8.04**

3.4.3 Effects of calcium chloride on ammonium adsorption

The effect of competition between ammonium and calcium ions on the sorption isotherm of ammonium is shown in Fig 3.4 and Fig. 3.5 by comparing the sorption isotherm of ammonium in calcium chloride solution (red quadrangle and red broken line) and ammonium in deionized water (blue circle and blue broken line). As is shown, the adsorbed mass of ammonium was significantly decreased in experiments that used calcium chloride solution for all types of materials. Therefore, q_{max} was reduced. For example, q_{max} of activated carbon was decreased by 91.2 % in calcium chloride solution treatment (Table 3.3). It was decreased from 90,100 ug g^{-1} in deionised water treatment to 7900 ug g^{-1} in calcium chloride solution. Biochar, sandy soil and sandy soil & 2% biochar or sandy soil & activated carbon had same trends with different percentages of change. Moreover the pH values in deionised water treatments are higher than those in calcium chloride treatments. For example, while the pH values in sandy soil treated with ammonium solution in deionised water was 8.53, it was 8.01 in soil treated with ammonium solution in calcium chloride solution. The ammonium sorption capacity reduction in calcium chloride is in agreement with results of Rysgaard *et al.* (1999) and Hou *et al.* (2003), who reported increased ammonium desorption with increasing salinity. This change of sorption capacity could not only attribute to a higher competition between ammonium and calcium cations for negatively charged binding sorption sites on the surface area but also to decreased pH which decreased negative charge on surface areas.

3.4.4 Effects of biochar on nitrous oxide production without nutrient supplements

The effect of biochar on nitrous oxide production under anaerobic conditions without substrate supplements is shown in Fig. 3.6, for sandy soil (Fig. 3.6a) and clayey loam soil (Fig. 3.6b). For better data visibility and comparison, the effect of biochar on nitrous oxide production rates under the same conditions in loamy and silty loam soil are shown in Fig. 3.7a and Fig. 3.7b, respectively. High variability was observed in the N_2O emitted from soils treated with different amounts of biochar. With only one day prior contact in the case of soil amendment with biochar, N_2O production in sandy soil

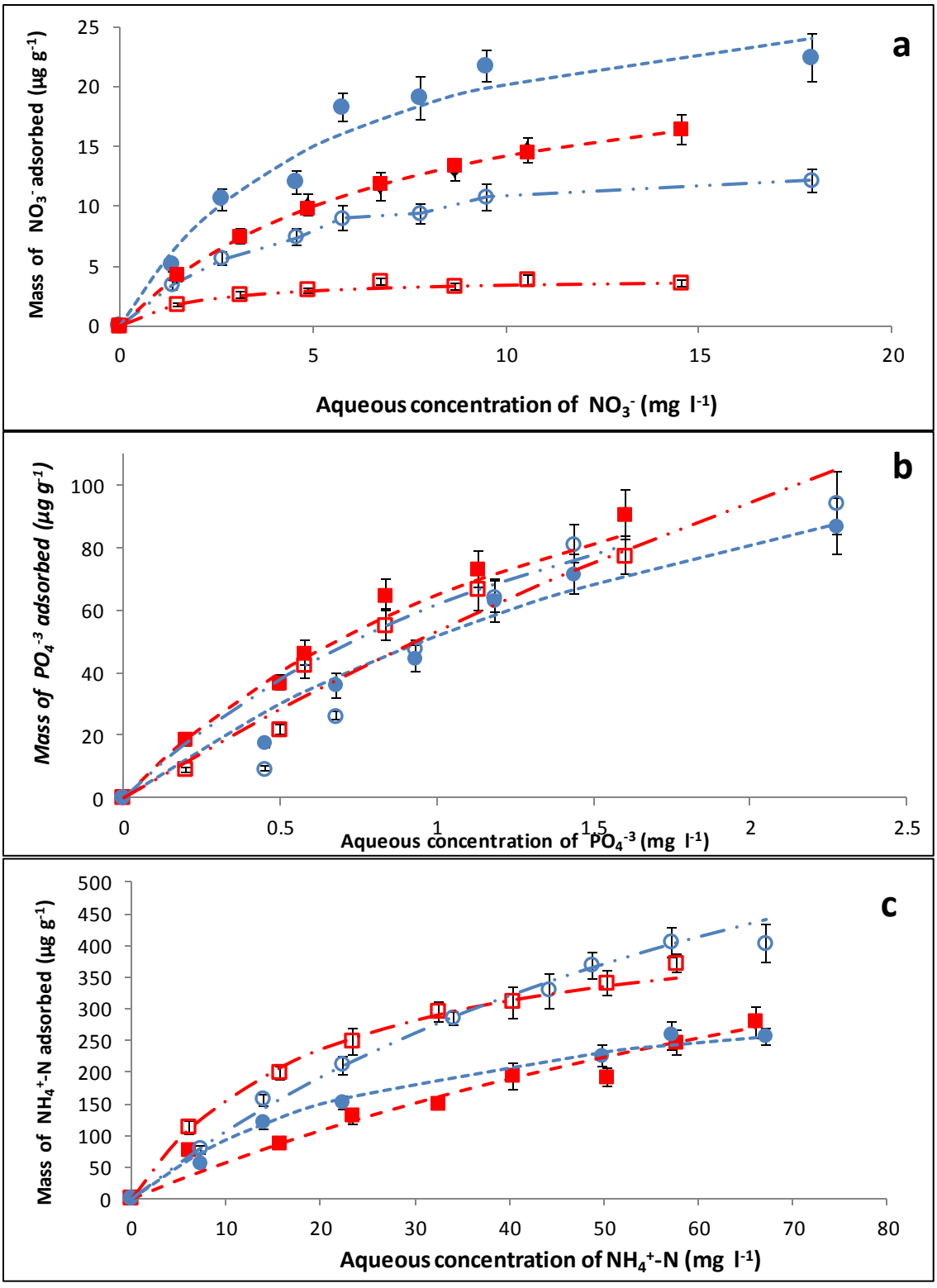

Fig. 3.3. Measured (symbols), and interpolated (lines) sorption isotherms of sandy soil & 2% biochar (measured) (■ ....), sandy soil & 2% biochar (calculated) (□ – ·· –), sandy soil & 2% activated carbon (measured) (● ....), and sandy soil & 2% activated carbon (calculated) (○ – ·· –), for (a) nitrate, (b) phosphate and (c) ammonium.

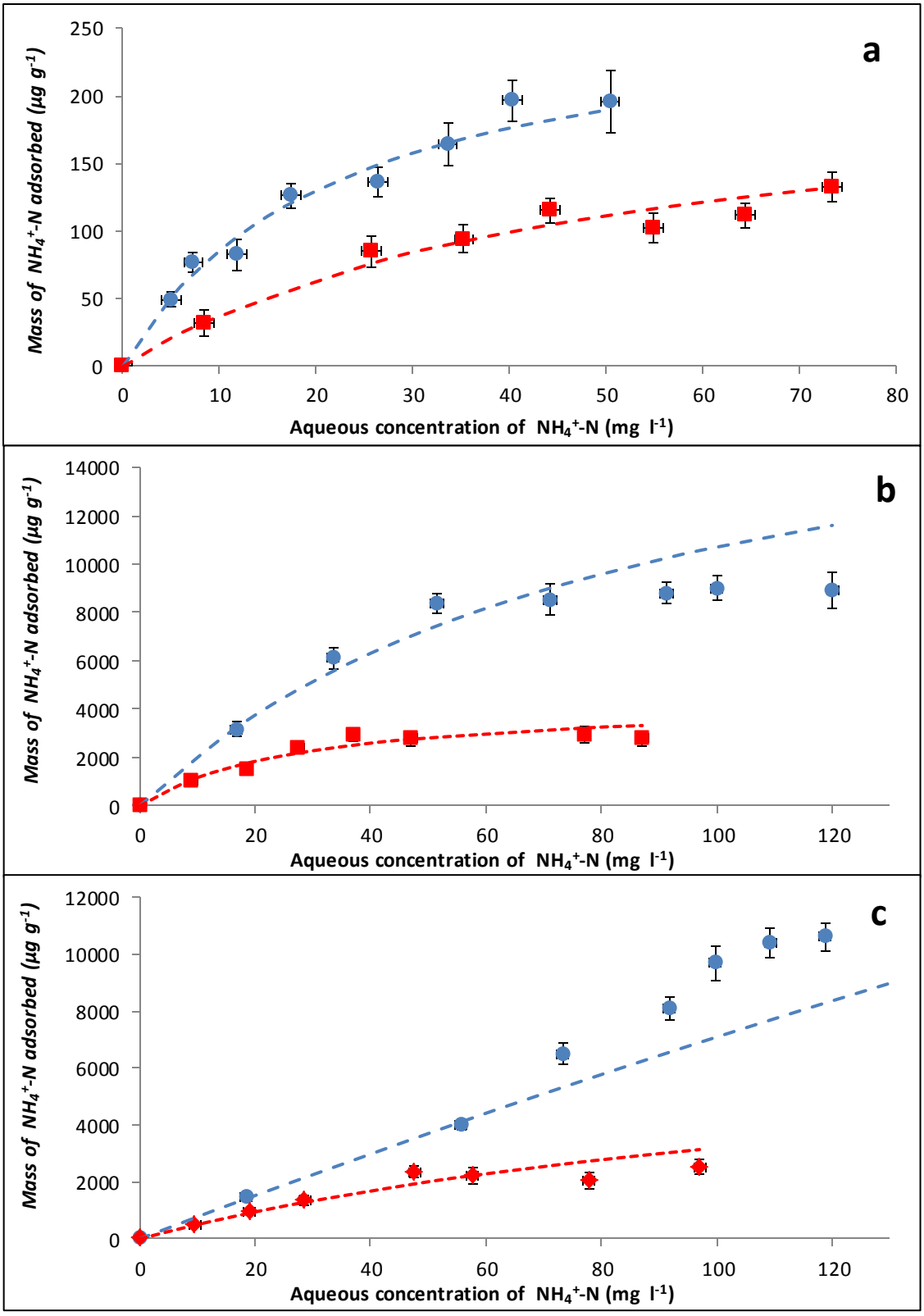

Fig. 3.4. Measured (symbols), and interpolated (lines), sorption isotherms of ammonium in deionised water (● ….), and ammonium in 0.01 N calcium chloride solution (■ ….), for sandy soil (a), biochar (b) and activated carbon (c).

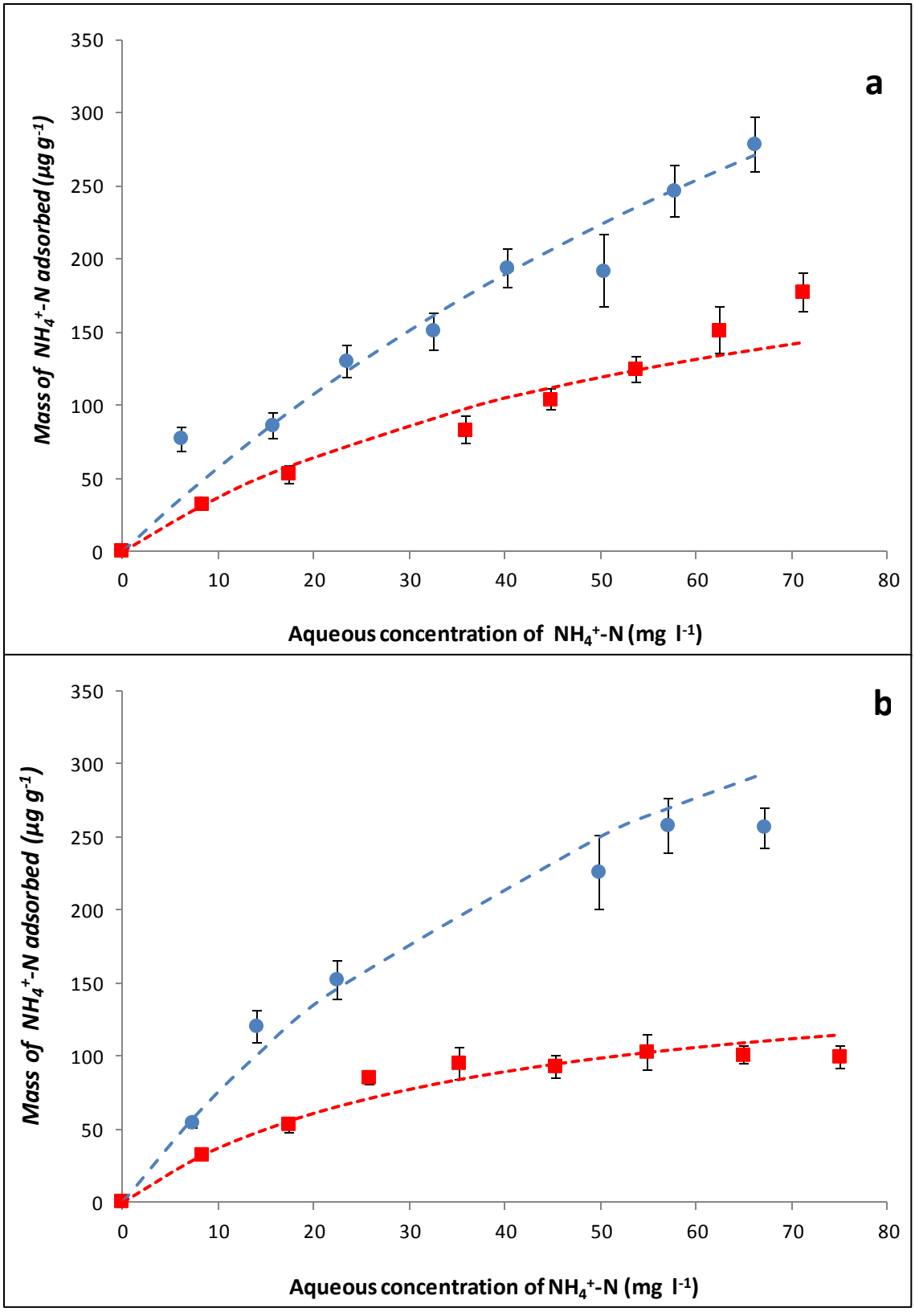

Fig. 3.5. Measured (symbols), and interpolated (lines), sorption isotherms of ammonium in deionized water (● ….), and ammonium in 0.01 N calcium chloride solution (■ ….), for sandy soil & 2% biochar (a), sandy soil & 2% activated carbon (b).

(Fig. 3.6a) started slowly without and with biochar addition (2%, 10%) then rose gradually to reach a peak of 2.3±0.3, 4.5±0.4 and 9.2±0.3 µg N_2O g^{-1} dry soil, respectively on the fourth day. The N_2O production was below the detection limit in the sandy soil with or without biochar after a 30 days prior contact period, which may be attributed to the decreased available concentration of nitrate and phosphate in 30 days contact period treatments. The effect of soil biochar contact time and biochar amended rate on the N_2O production was statistically significant for sandy soil $P<0.02$ and $P<0.000$, respectively (ANOVA-Fisher's test).

The N_2O productions in clayey loam soil without and with biochar (2%, 10%) rose sharply within two days and continued gradually to reach a peak on the sixth day, and the N_2O productions in clayey loam soil were higher in comparison with sandy soil for both contact periods. For example, the 1 day incubation treatment produced nearly double the production of N_2O in sandy soil. The difference between these two soils could be attributed to difference in availability of nitrate and phosphate and soil pH. For example, concentrations of available nitrate were 666±13 µg $NO_3^-g^{-1}$, 632±16 µg $NO_3^-g^{-1}$ and 392±13 µg $NO_3^-g^{-1}$ in clayey loam soil without and with biochar (2%, 10%) respectively, while these concentration were 33±2 µg $NO_3^-g^{-1}$, 28±2 µg $NO_3^-g^{-1}$ and 24±1 µg $NO_3^-g^{-1}$ in sandy soil without and with biochar (2%, 10%) respectively. Moreover, soil pH value in sandy soil without and with biochar (2%, 10%) were 7.43±0.04, 7.83±0.03 and 7.99±0.02 respectively. The sandy soil pH was higher in comparison with the optimum soil pH for denitrification which is between 5.5 - 6.0 (Dalal et al., 2003), while in clayey loam soil with and without biochar (2%, 10%) pH values ranged between 5.70 ±0. 02 to 6.62 ± 0.05.

The influence of biochar on the N_2O production in loamy and sandy loam soil is illustrated in Fig. 3.7a and Fig. 3.7b. From these figures, it can be seen that the concentration of N_2O increased gradually. However, the effect of soil biochar contact time on the N_2O production was not statistically significant for sandy loam soil $P<0.28$ (ANOVA-Fisher's test). This result may be attributed to the differences in availability of nitrate between the two incubation periods also not being significant in sandy loam soil. The N_2O production increased in loamy soil with biochar (2% and 10) compared

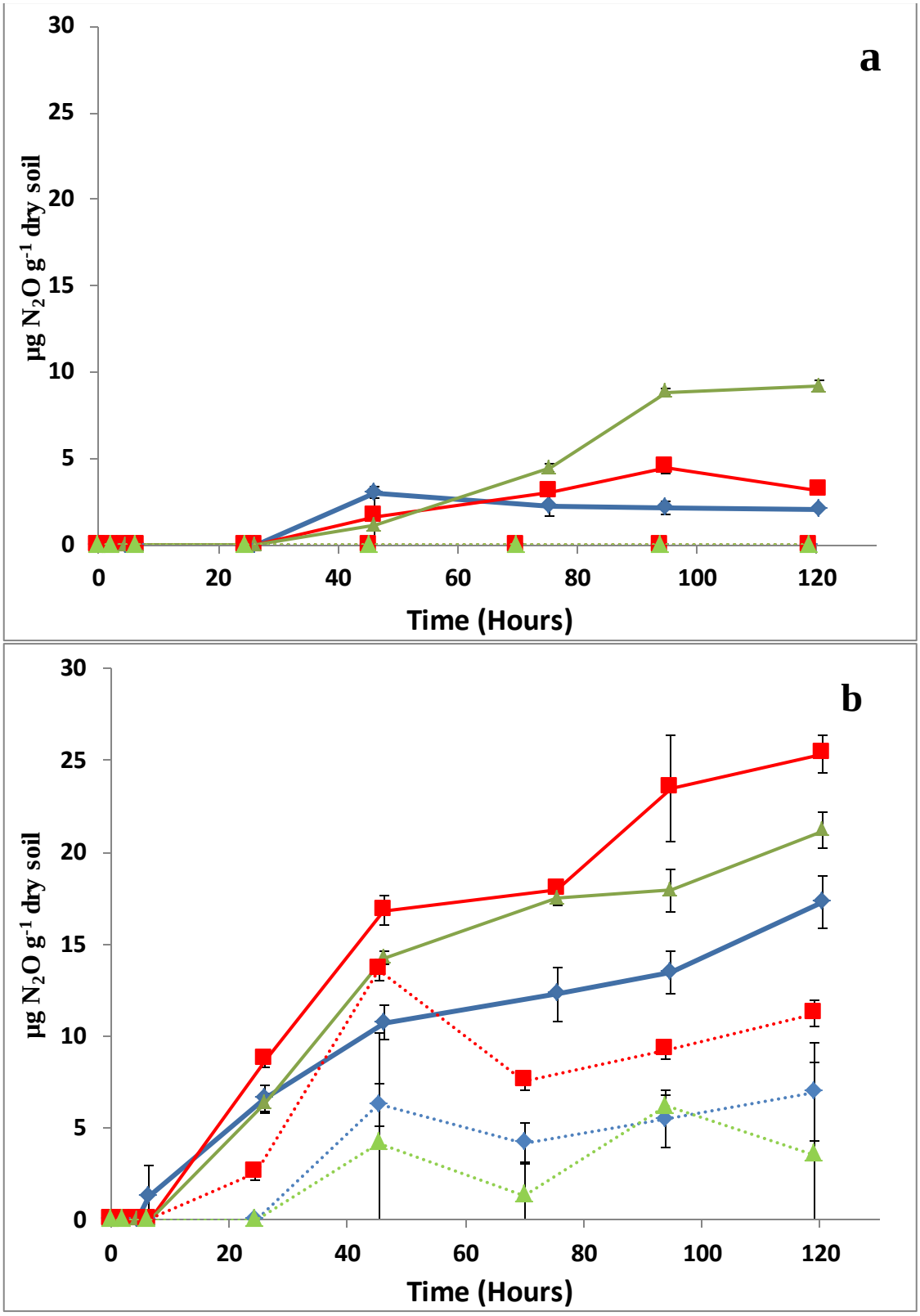

Fig. 3.6. Influence of biochar amendment and contact time on nitrous oxide production in soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines) and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

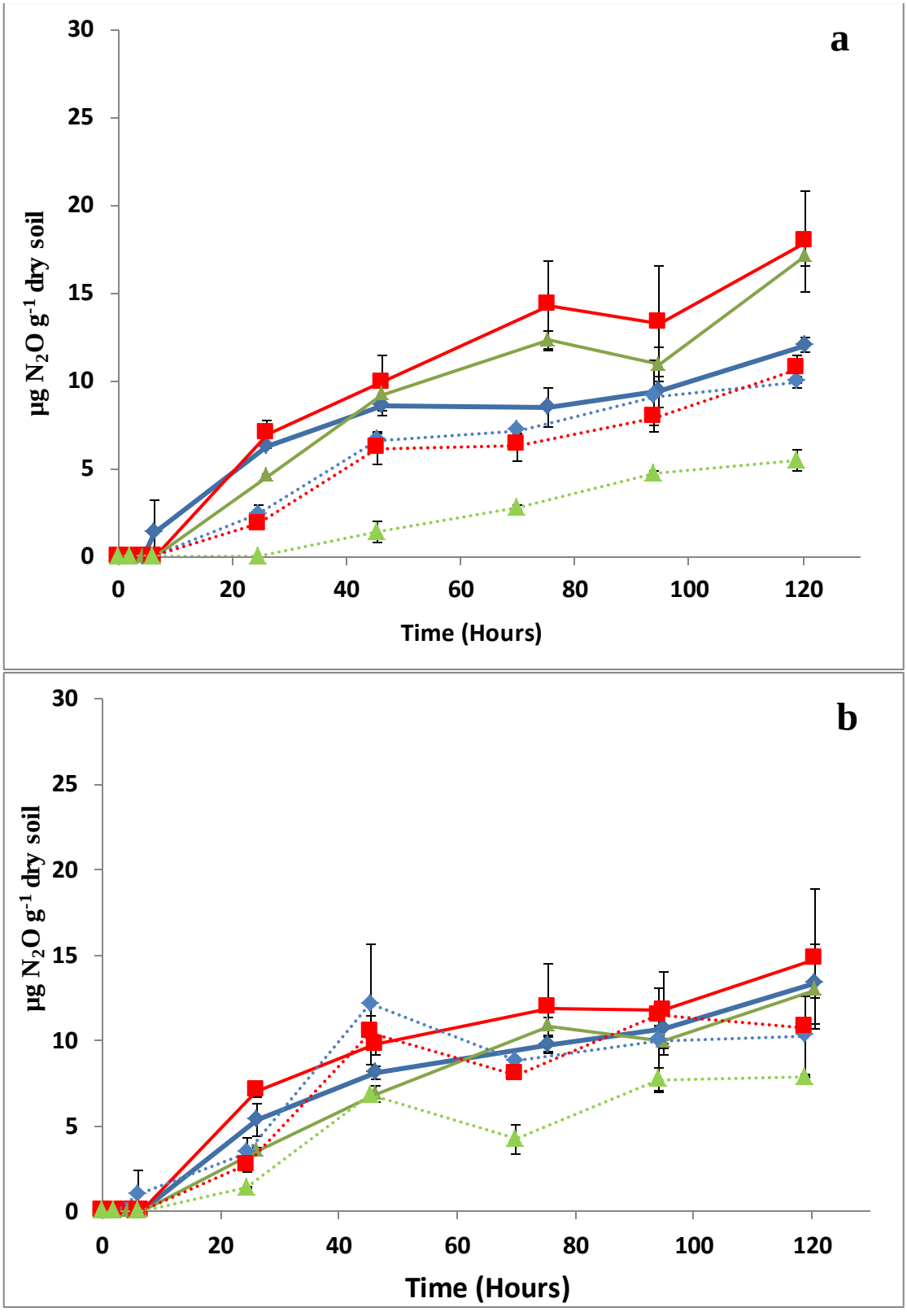

Fig. 3.7. Influence of biochar amendment and contact time on nitrous oxide production in soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines) and 30 days contact (broken lines), comparing (a), loamy soil and (b) sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).

to loamy soil without biochar (Fig 3.7a). This may be attributed to the increases in soil pH. However, the increase in contact time resulted in decreases in the N_2O production $P<0.011$ (ANOVA-Fisher's test).

3.4.5 Effects of biochar on nitrous oxide production with nutrient supplements

The influence of biochar amendments and contact period on nitrous oxide production when substrate provided is illustrated in Fig. 3.8 and Fig. 3.9 for sandy soil (Fig. 3.8a), clayey loam soil (Fig. 3.8b), loamy soil (Fig. 3.8a) and sandy loam soil (Fig. 3.8b). The N_2O production in soils supplied with nitrate, glucose and glutamic acid was ten times higher in comparison with those without substrate supplements. For example, the N_2O concentration in clayey loam soil provided with supplements reached a peak at 230±15 µg N_2O g^{-1}, while the peak for a clayey loam soil without substrate supplements was 17.3±1.5 µg N_2O g^{-1} dry soil. The higher N_2O production in substrate supplements treatments is due to provision of soluble organic carbon (glucose and glutamic acid) and nitrate which are the main factors affecting denitrification in soil (Dalal *et al.*, 2003). The batches with substrate supplements therefore measure potential denitrification enzyme activity when substrates are abundantly available. Adding 2% or 10% biochar to sandy soil had slight reducing effects on the N_2O production in comparison with sandy soil without biochar (Fig. 3.8a). However, after 30 day contact period, the N_2O production only increased in sandy soil without or with 2% biochar. The difference in the N_2O production from sandy soil with 10% biochar between two contact periods was not significant (Fig. 3.8a). The one day contact period results (Fig. 3.8b) show that, the N_2O produced in the clayey loam soil without biochar was higher in comparison to clayey loam soil with 2% biochar and the N_2O production was the lowest in clayey loam soil with 10% biochar. Increasing incubation period from one day to 30 days contact period enhanced the N_2O production in clayey loam soil with biochar (2%, 10%), while the N_2O production was reduced in clayey loam soil without biochar. These increases in the N_2O production may be attributed to alkalinity effects of biochar which enhanced pH in clayey loam soil. From Fig. 3.9a and Fig. 3.9b, it can be clearly seen that the differences in the N_2O emitted from loamy soil without or with 2% biochar and sandy loam soil without or with 2% biochar were

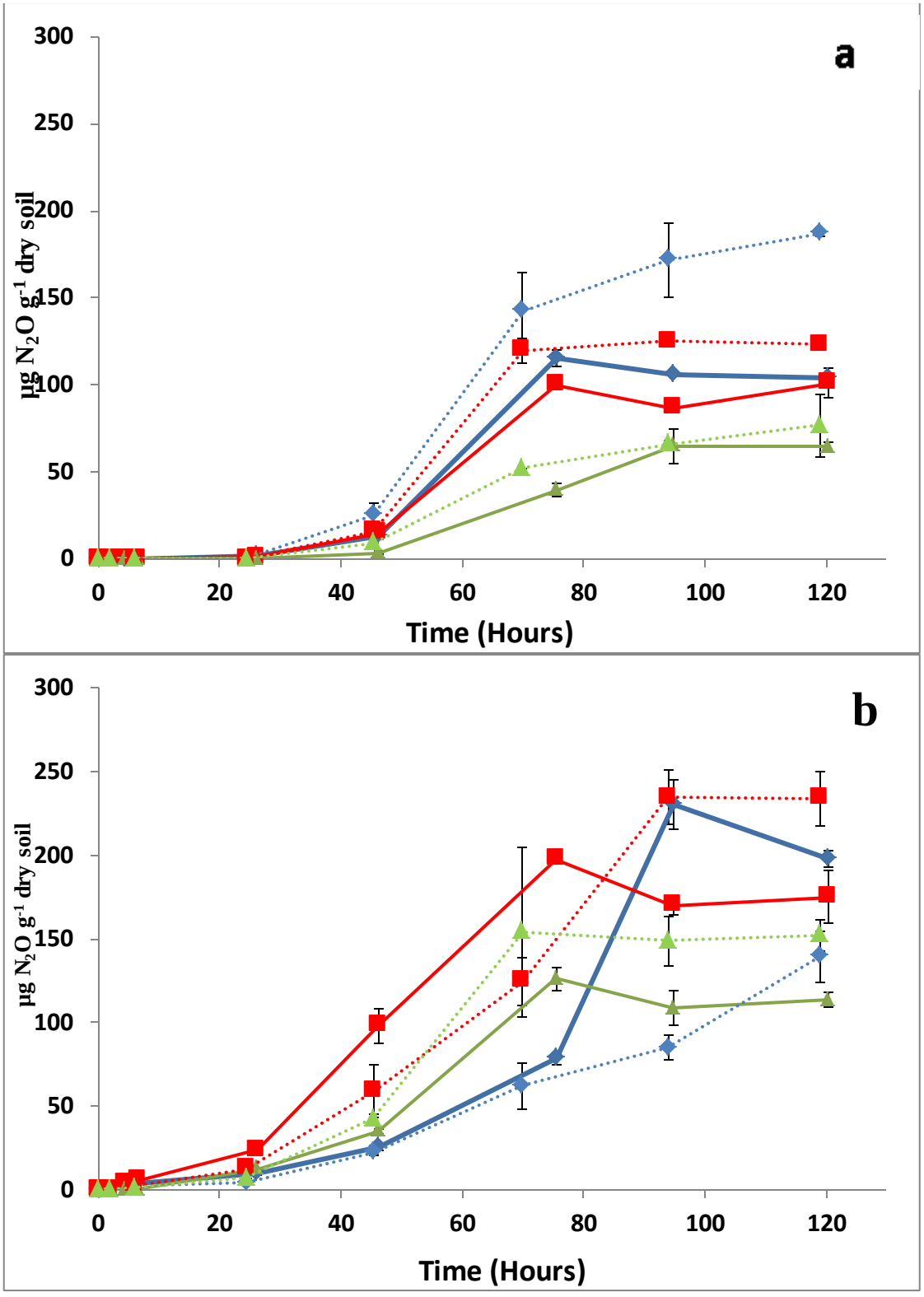

Fig. 3.8. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines) and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

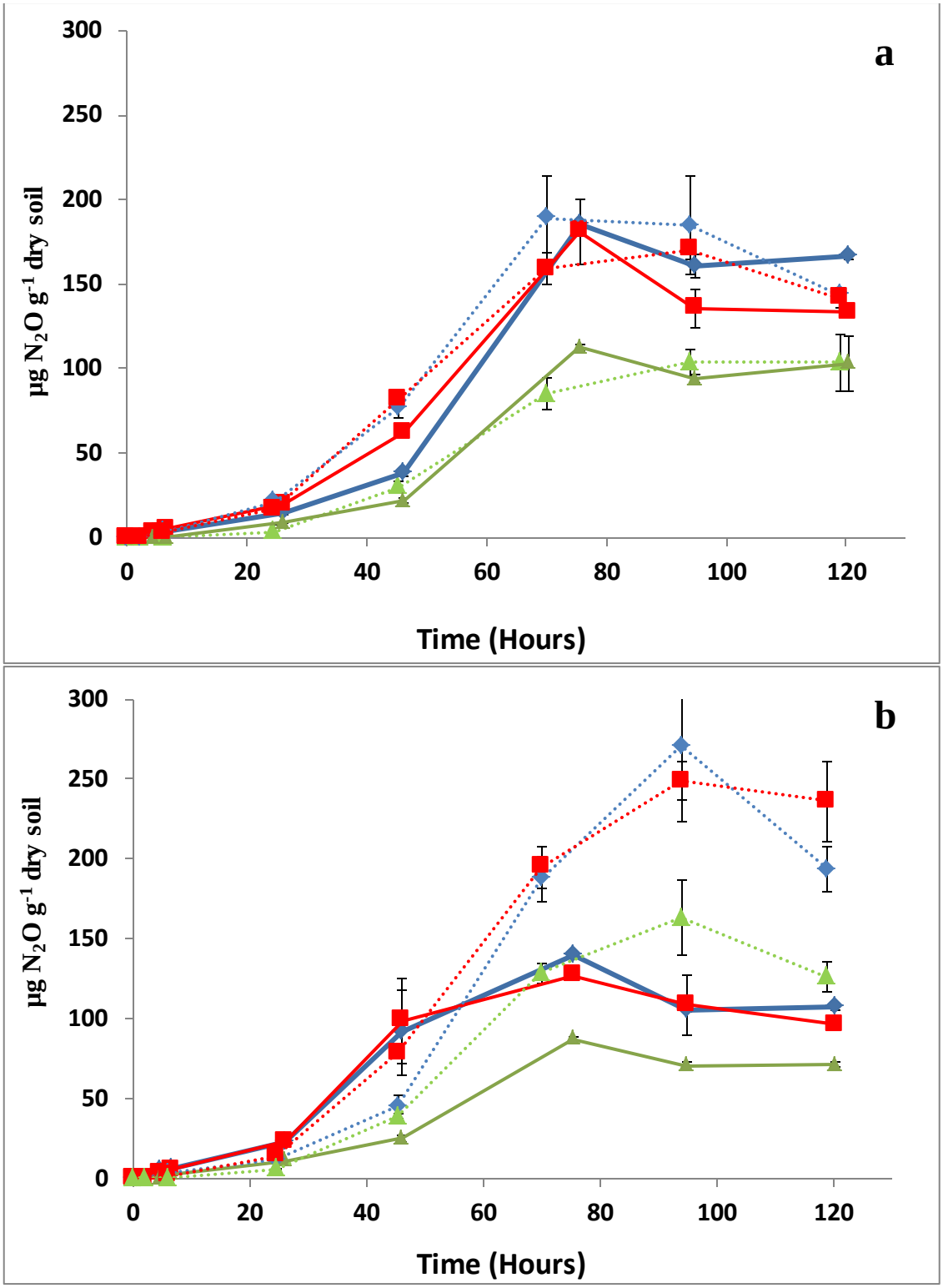

Fig. 3.9. Influence of biochar amendment and contact time on nitrous oxide production in nutrients supplemented soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines), and 30 days contact (broken lines), comparing (a), loamy soil and (b), sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).

not significant, whereas addition of 10% biochar significantly reduced the N_2O production from loamy soil and sandy loam soil. Increasing the soil biochar contact period from one day to thirty days resulted in increasing N_2O production in sandy loam soil without or with biochar (2%, 10%) $P<0.000$ (ANOVA-Fisher's test), while the effects of two contact period on the N_2O production in loamy soil was not significant $P<0.502$ (ANOVA-Fisher's test).

The effects of biochar amendment percentage on the N_2O production could possibly be attributed to the ability of biochar to sorb nitrate, glucose and glutamic acid and 10% of biochar could adsorb more of these compounds than 2% biochar amendment. For example, the maximum nitrate adsorbed (q_{max}) is 193 µg g^{-1} for biochar, 0 µg g^{-1} for sandy soil and 25 µg g^{-1} for sandy soil with (2%) biochar. For sandy soil with biochar (10%) the maximum amount of nitrate adsorbed is expected to be higher than 100 µg g^{-1} which may have reduced the availability of nitrate, since 200 µg g^{-1} was added to each treatment.

Denitrification rates in soils with or without biochar (2%, 10%) and with or without supplements are presented in Table 3.5. Denitrification rates should correspond to the denitrification enzyme activity rates for the treatments with substrate supplementation solution. For example, these rates were 1.3±0.03 and 2.2±0.04 µg N_2O h^{-1} g^{-1} dry soil for 1 and 30 days prior contact time in sandy soil respectively, while the rates were 0.01±0.003 and 0.00±0.00 µg N_2O h^{-1} g^{-1} dry soil in the same soil without supplements. The denitrification enzyme activity rates in sandy soil provided with supplement solution decreased with increasing biochar application rate $P<0.001$ (ANOVA-Fisher's test), but the difference between 0% and 2% was not statistically significant. That could be related to soil pH which increased from 7.48±0.4 in sandy soil to 7.99±0.02 in sandy soil with biochar (10%). Although the denitrification rates in sandy loam soil and loamy soil with biochar (10%) were lower in comparison with these soils without or with a lower amount of biochar (2%), the differences were not statistically significant $P<0.12$ and $P<0.36$ (ANOVA-Fisher's test) for sandy loam soil and loamy soil respectively. It would seem that in supplement solution

Table 3.5. Denitrification rates (μg N_2O h^{-1} g^{-1} dry soil). The error range is the standard deviation of duplicated bottles.

Soil	Without supplement solution		With supplement solution	
	1 day incubation	30 days incubation	1 day incubation	30 days incubation
Sandy soil	0.01 ±0.003	0.00±0.00	1.3±0.03	2.2±0.04
Sandy soil & 2% Biochar	0.04±0.004	0.00±0.00	1.2±0.05	1.5±0.02
Sandy soil & 10% Biochar	0.11±0.02	0.00±0.00	0.8±0.06	0.9±0.15
Clayey loam soil	0.10±0.01	0.05±0.02	2..4±0.2	1.2±0.2
Clayey loam soil & 2% Biochar	0.17±0.01	0.08±0.002	1.6±0.05	2.4±0.02
Clayey loam soil &10 % Biochar	0.14±0.01	0.05±0.00	1.2±0.07	1.7±0.12
Sandy loam soil	0.08±0.02	0.03±0.06	0.8±0.1	1.8±0.7
Sandy loam soil & 2% Biochar	0.07±0.04	0.07±0.006	0.7±0.2	2.7±0.2
Sandy loam soil & 10% Biochar	0.09±0.01	0.07±0.01	0.7±0.00	1.5±0.2
Loamy soil	0.05±0.003	0.05±0.004	1.8±0.00	1.5±0.2
Loamy soil & 2% Biochar	0.11±0.04	0.08±0.002	1.3±0.03	1.4±0.01
Loamy soil & 10% Biochar	0.11±0.003	0.06±0.002	1.1±0.1	1.2±0.1

experiments, increased biochar application reduced availability of nitrate and soluble organic carbon. However, the available nitrate and soluble organic carbon in these soils were still sufficient to reach high N_2O production. The reduced N_2O production observed for the DEA with supplements solution for the highest biochar dosage was not observed without supplement solution. This indicates that it might be a "method artifact", because biochar adsorbs supplements, as discussed here, but this biochar effect may not be so relevant in real soils.

3.4.6 Effects of biochar on methane oxidation

Result for the methane oxidation from sandy soil, clayey loam soil, silty loam soil and loamy soil without or with biochar (2%, 10%) are shown in Fig. 3.10 and Fig. 3.11, and the methane oxidation rates and lag phase period are presented in Table 3.6. The methane concentration decreased sharply after lag phase period then decreased sharply.

For example, in clayey loam soil, the methane concentrations were stable in the first 190 h of lag phase for clayey loamy soil without biochar and 120 h of lag phase for clayey loamy soil with biochar (2% and 10%) in a 1 day prior contact period experiment. In the experiments following a 1 day contact period, the methane oxidation rates were 0.01±0.003, 0.15±0.001 and 0.15±0.003 μg CH_4 h^{-1} g^{-1} dry soil in clayey loam soil without and with biochar (2%, 10%) respectively, whereas these rates were 0.036±0.01, 0.12±0.004 and 0.12±0.002 μg CH_4 h^{-1} g^{-1} dry soil in clayey loam soil without and with biochar (2%, 10%) following a 30 day contact period. Moreover, although the difference in the CH_4 oxidation rates, between the two contact periods were not statistically significant ($P< 0.397$, ANOVA-Fisher's test), the lag phase period was increased in clayey loamy soil without biochar (Table 3.6) while these lag phase periods decreased in clayey loamy soil with biochar (2% and 10%). The differences in lag phase period could be attributed to competition between methane and ammonium at the methane-monooxygenase (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994; Sitaula *et al.*, 1995) and a transfer of the CH_4 oxidising activity towards nitrification (Nesbit and Breitenbeck, 1992; Castro *et al.*, 1994; Sitaula *et al.*, 1995), because, although the ammonium concentration in clayey soil was decreased after a 30 day contact period, this concentrations were probably still high enough to inhibit methane oxidation in clayey loamy soil without biochar, while in clayey loamy soil without biochar could be regulated the ammonium.

The influence of biochar amendments on methane oxidation may also depend on soil properties. For example, the methane oxidation rates in sandy loam soil were 0.02±0.006, 0.025±0.008 and 0.015±0.002 μg CH_4 h^{-1} g^{-1} dry soil in sandy loam without or with biochar (2%, 10%) respectively and there was no statistically significant difference ($P<0.842$ ANOVA-Fisher's test). 2% biochar added to sandy soil and sandy loam soil did not affect the methane oxidation rate (Fig. 3.8 and Fig. 3.8). However, the methane oxidation rate in sandy soil and loamy soil with biochar (10%) was lower in comparison with soil without and with biochar (2%). Adding biochar (2%) to loamy soil did have effects on the CH_4 concentration. While the lag phase period before methane oxidation was decreased in loamy soil without or with biochar (2%) with increased contact period from 1day to 30 days. Moreover, the

lag phase period was increased in loamy soil with biochar (10%) from 190 h to 300 when contact period increased from a 1day to 30 day.

Table 3.6. The methane oxidation rates (µg CH_4 $h^{-1}g^{-1}$ dry soil) and the lag phase periods before methane oxidation. The error range is the standard deviation of duplicated bottles

Soil	The methane oxidation rates		The lag phase period (hours)	
	1 day	30 days	1 day	30 days
Sandy Soil	0.11±0.005	0.15±0.01	120	95
Sandy Soil & 2% Biochar	0.12±0.004	0.15±0.008	120	95
Sandy Soil & 10% Biochar	0.02±0.004	0.018±0.01	300	300
Clayey loam soil	0.010±003	0.036±0.01	190	300
Clayey loam soil & 2% Biochar	0.15±0.001	0.12±0.004	120	95
Clayey loam soil &10 % Biochar	0.15±0.003	0.12±0.002	120	95
Loamy soil	0.11±0.004	0.12±0.01	170	90
Loamy soil & 2% Biochar	0.15±0.003	0.11±0.008	170	70
Loamy soil & 10% Biochar	0.02±0.004	0.041±0.01	190	300
Sandy loam soil	0.02±0.006	0.09±0.03	190	300
Sandy loam soil & 2% Biochar	0.025±0.008	0.08±0.01	190	300
Sandy loam soil & 10% Biochar	0.015±0.002	0.009±0.009	300	300

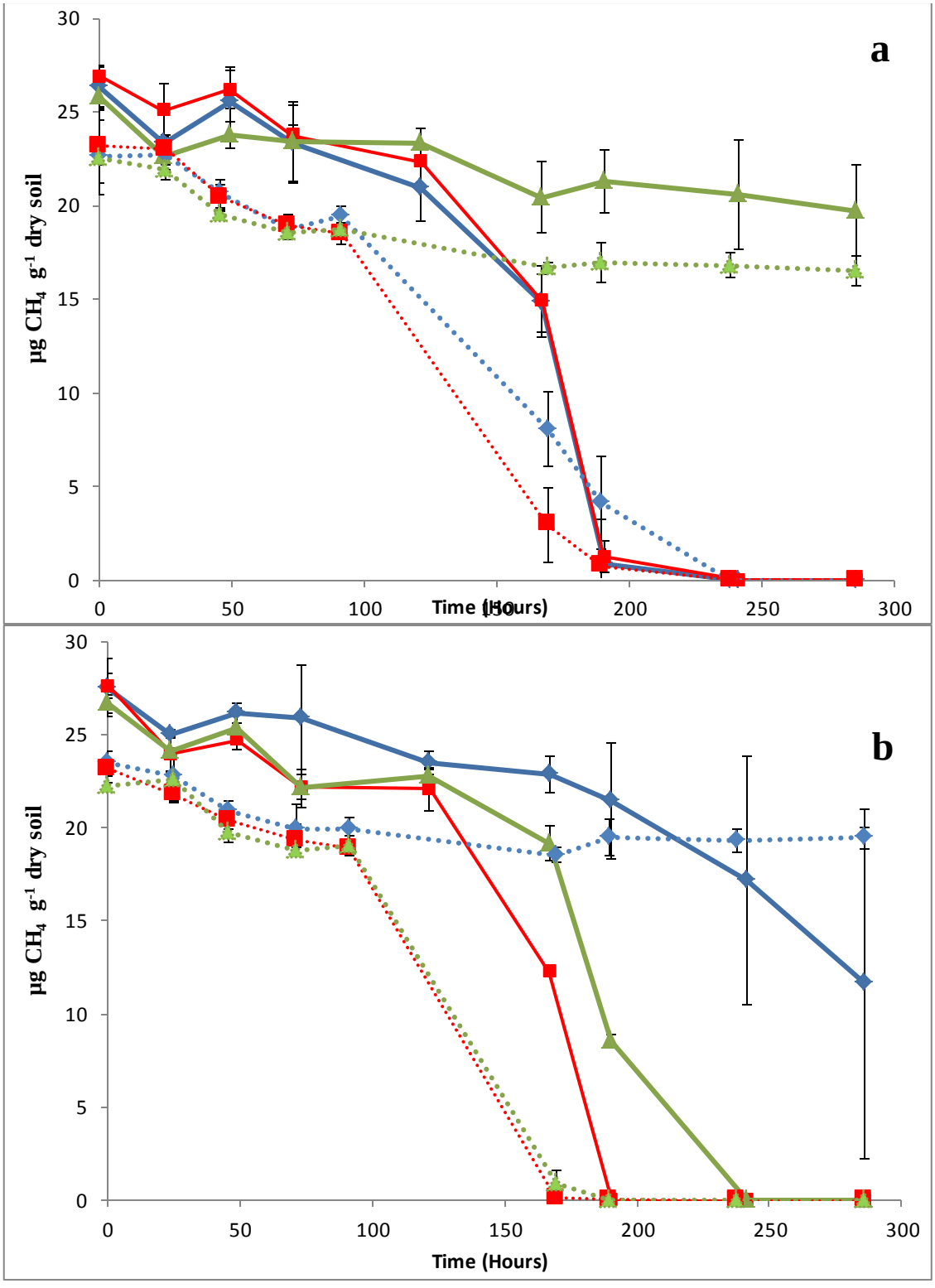

Fig. 3.10. Influence of biochar amendment and contact time on methane oxidation in soil (—◆—, ···◆···), soil & 2% biochar (—■—, ···■···), and soil & 10% biochar (—▲—, ···▲···), for one day contact (lines), and 30 days contact (broken lines), comparing (a) sandy soil and (b) clayey loam soil. Error bars: ± 1 standard deviation (SD, n=3).

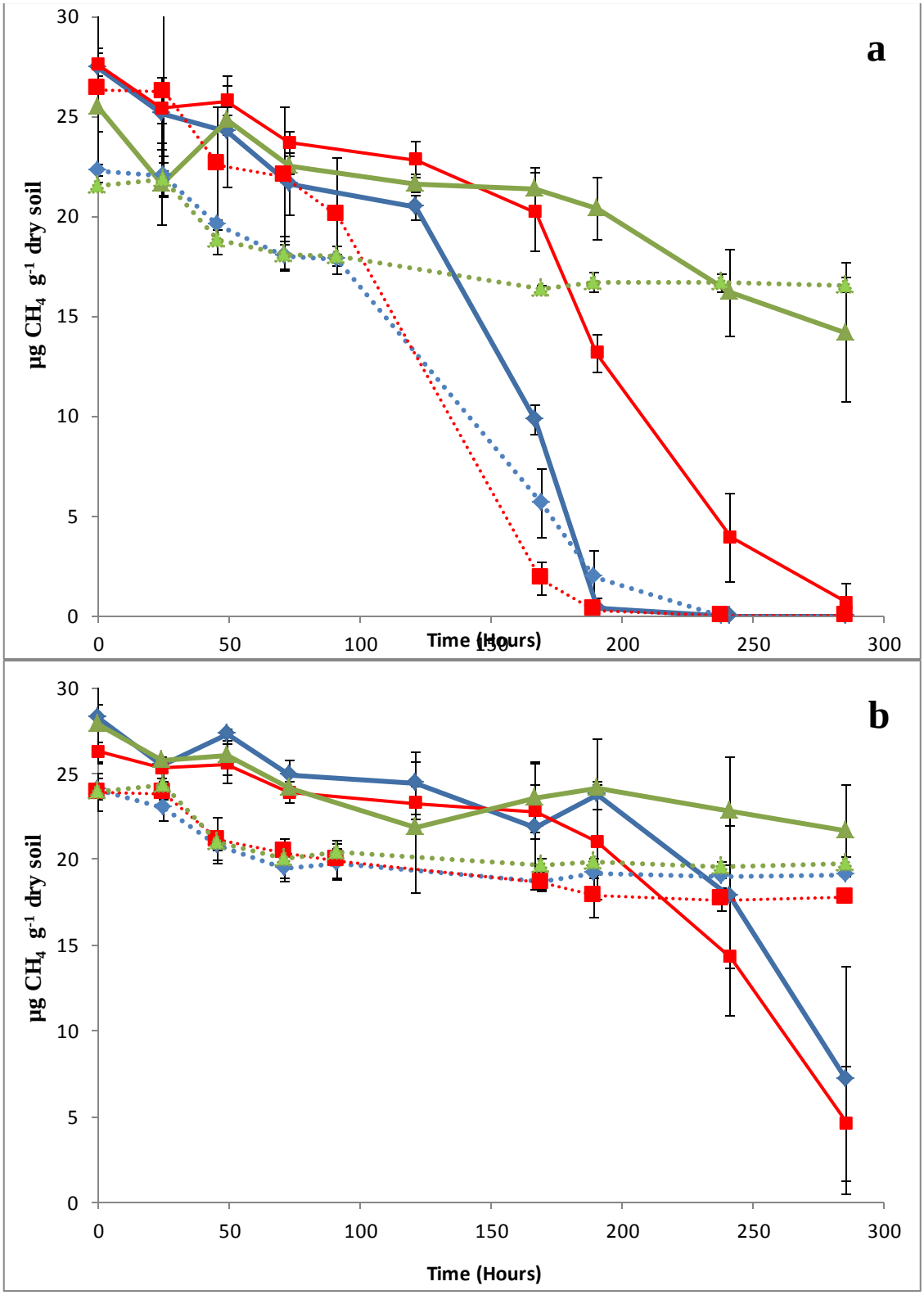

Fig. 3.11. Influence of biochar amendment and contact time on methane oxidation in soil (,), soil & 2% biochar (,), and soil & 10% biochar (,), for one day contact (lines), and 30 days contact (broken lines), comparing (a) loamy soil and (b) sandy loam soil. Error bars: ± 1 standard deviation (SD, n=3).

Correlations between the denitrification enzyme activity rate or the methane oxidation rate and soil pH, electrical conductivity, availability of nitrate, ammonium and phosphate are illustrated in Table 3.7. Without regards to type of soil, all soil properties have significant effects on the denitrification enzyme activity. The availability of phosphate and soil electrical conductivity have moderate negative effects on the methane oxidation rate. Correlation coefficients between soil properties and denitrification enzyme activity rate or the methane oxidation rate were different in different soil types. In sandy soil, correlation coefficients between available ammonium and soil electrical conductivity were significantly positive on denitrification enzyme activity rate, whereas in clayey soil and loamy soil only available ammonium has significant positive correlation coefficients, and in sandy loam soil the soil pH and soil electrical conductivity have significant positive effects on denitrification enzyme activity rate (Table 3.7).

From Table 3.7, it can seen that the significant negative correlation coefficient indicate that the methane oxidation rate was sensitive to increase in the soil pH and soil electrical conductivity in sandy soil, sandy loam soil and loamy soil. However, the soil pH and soil electrical conductivity in clayey soil seemingly facilitated the methane oxidation, although this may actually be due to better soil aeration following biochar amendment, while available nitrate and phosphate inhibited the methane oxidation. These results show that variable soil properties may be the main effects on the biogenic gases activities and biochar amendment effects must be interpreted in the context of biochar impacts on these variable soil properties.

This study has shown that biogenic gases activities depended on soil properties and biochar application rate. The some of study findings are consistent with those of Feng *et al.*(2012), Taghizadeh-Toosi *et al.(* (2011), Zhang *et al.(* (2012a) and Karhu *et al.(*2011) who found reduction in biogenic gases activities or the reduction is not significant. However, the other study findings do not support the previous research which could explain the different responses to biochar. However, high biochar application rate (10%) reduced biogenic gases activities except clayey loam soil amended with biochar (10%) where methane oxidation was

Table 3.7. Correlation coefficients and P value of relationships between soils properties and the denitrification rates or the methane oxidation rate.

		Global		Sandy soil		Clayey soil		Sandy loam soil		Loamy soil	
		Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate	Denitrification rate	CH_4 oxidation rate
Available Nitrate	**Correlation**	**0.38****	**-0.24**	**0.25**	**0.26**	**0.17**	**-0.51***	**-0.46**	**0.8****	**0.03**	**0.40**
	P value	**(0.001)**	**(0.051)**	**(0.3)**	**(0.3)**	**(0.5)**	**(0.03)**	**(0.06)**	**(0.000)**	**(0.9)**	**(0.1)**
Available ammonium	**Correlation**	**0.68****	**-0.081**	**0.96****	**-0.47***	**0.65***	**0.50***	**0.52**	**-0.16**	**0.80****	**-0.42**
	P value	**(0.000)**	**(0.5)**	**(0.000)**	**(0.048)**	**(0.006)**	**(0.04)**	**(0.03)**	**(0.55)**	**(0.04)**	**(0.084)**
Phosphate	**Correlation**	**0.34****	**-0.36** **	**0.08**	**0.08**	**-0.14**	**-0.79****	**0.28**	**0.5** *	**0.15**	**-0.058**
	P value	**(0.004)**	**(0.002)**	**(0.8)**	**(0.78)**	**(0.6)**	**(0.000)**	**(0.26)**	**(0.035)**	**(0.55)**	**(0.82)**
pH	**Correlation**	**-0.37****	**0.13**	**0.45**	**-0.65****	**0.16**	**0.85****	**0.5***	**-0.75****	**0.3**	**-0.51***
	P value	**(0.002)**	**(0.3)**	**(0.06)**	**(0.003)**	**(0.5)**	**(0.000)**	**(0.04)**	**(0.000)**	**(0.22)**	**(0.032)**
EC	**Correlation**	**0.50***	**-0.24** *	**0.51***	**-0.89****	**0.17**	**0.86****	**0.53***	**-0.64****	**0.29**	**-0.44***
	P value	**(0.000)**	**(0.04)**	**(0.03)**	**(0.000)**	**(0.5)**	**(0.000)**	**(0.03)**	**(0. 004)**	**(0.24)**	**(0.07)**

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level

increased. Biochar properties, soil properties, fertilization and water management regime influence greenhouse gases fluxes (VanZwieten *et al.*, 2009). the reduction in N_2O emissions from soil amended with biochar is could be explain by reduction of availability of ammonium and nitrate ions via sorption (Karhu *et al.*, 2011), and increases nitrogen utilization efficiency (Karhu *et al.*, 2011; Zhang *et al.*, 2012b) and facilitates the liming. The reductions in CH_4 emissions in biochar amended soil (10%) were attributed to the sorption of dissolved organic carbon by biochar surfaces (Thies and Rillig, 2009; Knoblauch *et al.*, 2011).

3.5 Conclusion

This chapter investigated the impact of biochar (2% and 10% on dry weight basis) or activated carbon amendment (2% on dry weight basis) on the sorption and availability of nitrate, ammonium and phosphate and the effects of these amendments on denitrification activity and methane oxidation in different type of soils. Biochar amendments increased the total nitrogen, total phosphorus, soil salinity and pH depend on the biochar application rate and soil properties. However, the availability of nitrate, ammonium and phosphate in soil with or without biochar (2% and 10%) were decreased with increasing prior contact period between soil and biochar and with an increasing biochar application rate, due to sorption of ammonium and nitrate by biochar. Furthermore, the available nitrogen form shifted towards ammonium in biochar amended soils, although nitrate mostly remained predominant. Soil properties have the most significant influence on the N_2O production and methane oxidation and the differences in soil properties could explain different soil responses to biochar amendments (Spokas and Reicosky, 2009, VanZwieten *et al.*, 2009). Furthermore, the N_2O production was lower without supplement treatments, and patterwere different from those observed with supplement treatments. However, contrary to other reports (Spokas and Reicosky, 2009; Taghizadeh-Toosi *et al.*, 2011; Zhang *et al.*, 2012a), biochar does not seem to have a strong and consistent impact on denitrification. Increased soil salinity or soil pH may have negatively affected microorganisms in sandy soil. Furthermore, since there were no differences between two soil-biochar contact periods, the results show that this variable did not influence methane oxidation rates.

Chapter 4: The fate and transport of volatile petroleum hydrocarbons in short term soil column studies with and without biochar

4.1 Introduction

Soil and sediment amendment with strong sorbent materials, such as activated carbon or biochar, is currently being evaluated as a low-cost in situ remediation approach for persistent pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Cornelissen *et al.*, 2006; Brandli *et al.*, 2008; Hale and Werner, 2010), polychlorinated biphenyls (Zimmerman *et al.*, 2004; Cho *et al.*, 2007; Sun *et al.*, 2009; Vasilyeva *et al.*, 2010), pesticides (Tomaszewski *et al.*, 2007; Hilber *et al.*, 2009; Yu *et al.*, 2010) and metals (Beesley *et al.*, 2010). Biochar is produced from biomass and may sequester atmospheric CO_2 in soil or sediments for many thousand years (Zimmerman, 2010) and thereby reduce the carbon footprint of sorbent-based soil remediation in comparison with the use of coal-derived activated carbons.

A potential concern about the application of strong sorbents at contaminated sites is their impact on the intrinsic biodegradation of organic pollutants (Rhodes *et al.*, 2008). Many sites are impacted by a range of pollutants with different physicochemical characteristics (Beesley *et al.*, 2010), and the impact of strong sorbents on the biodegradation of more readily available and more readily biodegradable organic pollutants such as volatile petroleum hydrocarbons has not yet been investigated. Volatile petroleum hydrocarbons (VPHs) are among the most common environmental pollutants that may enter the soil, usually as a separate phase (non-aqueous phase liquid-NAPL), due to accidental spills, chemical waste burials or leakages from storage tanks. They are present at many sites impacted by the more persistent PAHs, for which sorbent-based remediation has been shown to be effective (Hale *et al.*, 2010). Pollutants trapped in the micropores of carbonaceous sorbents become less bioaccessible for uptake by soil organisms, bacteria or plants, and the addition of activated carbons or biochars thereby reduces the transfer of these pollutants from soil into the terrestrial food-chain. This occlusion may, however, also reduce the pollutant's

bioaccessibility and increase persistence (Rhodes *et al.*, 2008). On the other hand, biochar addition to degraded soil has been shown to improve the soil structure and fertility and microbial cell numbers at loading rates between 0.5 and 5 kg m^{-2} (Glaser *et al.*, 2002; Major, 2010). Because of these antagonistic effects, the effect of biochar on the microbial degradation of soil pollutants cannot be readily anticipated.

4.2 Aims

This work aims to study the effect of biochar on the fate of VPHs emanating from a NAPL source. This aim will be achieved by performing batch tests with soil and soil amended with biochar to estimate the solid–water distribution coefficient and the first-order biodegradation rate for the pollutants investigated. The mechanisms affecting the pollutant transport through the soil and the soil amended with biochar will then be investigated in column experiments, with different boundary conditions at the top; a numerical pollutant fate model coded in Matlab will be calibrated with the batch data and its predictions compared to the data from the column studies.

4.3 Materials and methods

4.3.1 Volatile petroleum hydrocarbon compounds mixture

A mixture of 12 major constituents of gasoline or kerosene was prepared from high purity chemicals obtained from Sigma-Aldrich (Dorset, UK). Their weight percentage was chosen according to typical fuel compositions (Pasteris *et al.*, 2002) (Table 4.1). Sulfur hexafluoride (SF_6) (Sigma-Aldrich, Steinheim, Germany) was used as a volatile tracer recalcitrant to biodegradation under aerobic conditions.

Table 4.1.Composition of the petroleum hydrocarbon mixture and pollutant physico-chemical properties.

Compound	Formula	Initial NAPL mole fraction (-)	Initial vapor conc. (20 °C) (g cm^{-3})	Molecular diffusion coefficient D ($cm^2 s^{-1}$)	Henry's law constant (-)
n-pentane	C_5H_{12}	**0.068**	**56,5495**[e]	**0.082**[a]	**50.65**[b]
n-hexane	C_6H_{14}	**0.113**	**17,255.7**[e]	**0.074**[a]	**68.36**[b]
methylcyclopentane	C_6H_{12}	**0.116**	**18,583**[e]	**0.079**[a]	**14.65**[b]
cyclohexane	C_6H_{12}	**0.066**	**12,609.9**[e]	**0.079**[a]	**7.33**[b]
Isooctane	C_8H_{18}	**0.171**	**5442.2**[e]	**0.064**[a]	**132.35**[b]
methylcyclohexane	C_7H_{14}	**0.130**	**4911.2**[e]	**0.073**[a]	**17.6**[c]
Toluene	C_7H_8	**0.035**	**2920.2**[e]	**0.078**[a]	**0.26**[b]
n-octane	C_8H_{18}	**0.062**	**1460.1**[e]	**0.064**[a]	**120.67**[b]
m-xylene	C_8H_{10}	**0.046**	**1194.6**[e]	**0.072**[a]	**0.26**[b]
1,2,4-trimethylbenzene	C_9H_{12}	**0.048**	**192.5**[e]	**0.066**[a]	**0.27**[b]
n-decane	$C_{10}H_{22}$	**0.099**	**159.3**[e]	**0.057**[a]	**198**[c]
n-dodecane	$C_{12}H_{26}$	**0.046**	**39.8**[e]	**0.051**[a]	**293.05**[b]
CO_2	CO_2	-	**Not used**	**0.17**[d]	**0.75**[e]
Sulfur hexafluoride	SF_6	-	**Not used**	**0.093**[a]	**125.82**[e]

[a] (Schwarzenbach *et al.*, 1993)
[c] (Yaws and Yang, 1992)
[d] (Sander, 2011).
[e] (USEPA, 2007)(experimental data).

4.3.2 Soil

The sandy soil was obtained from King's Gate building construction site on Newcastle University campus (UK). It had the following grain size composition: 87.25% sand, 11.33% silt and 1.42% clay, with about 35% w./w. in the particle size range of 600–2000 μm. The total phosphorus 490±24 μg P g^{-1} d.w., total nitrogen 0.0007%, nitrate 3.9±0.6 μg NO_3^- g^{-1} d.w., nitrite <1.0 μg NO_2^- g^{-1} d.w. and ammonia nitrogen 6.7±0.3 μg $NH_4^+ - N$ g^{-1} d.w. The solid density (ρ_s) was 2.62±0.04 g cm^{-3}. The soil pH was 7.96±0.04. Soil pH after addition of 2% biochar was 8.1±0.1 and therefore not significantly different from the original soil pH.

4.3.3 Biochar

The biochar obtained from Environmental Power International EPI (Wiltshire, UK) described in Chapter 3, Section 3.3.2 was also used in this Chapter.

4.3.4 Laboratory batch microcosm experiments

Batch experiments were performed at room temperature (20±2°C) for 10 days, in bottles (63 ml) closed with Teflon Mininert valves (Supelco, Bellefonte, USA). Three replicates were prepared with soil (30 g) or soil amended with biochar (15 g, 2% biochar on soil d.w.) and injected with 2 μl of the pollutant mixture (Breus and Mishchenko, 2006). The amount of biochar was chosen based on the loading rates used to remediate contaminated soil and also used to improve soil fertility. Abiotic tests (three replicates) were prepared by autoclaving bottles with soil or soil amended with biochar at 126°C for 30 min in order to assess the pollutant distribution coefficient, K_d (cm^3 g^{-1}), at equilibrium conditions. The water content in the bottles was 0.12±0.01 g g^{-1} d.w.. Sorption on glass and lids was checked by injecting 2 μl of the pollutant mixture into free soil bottles (two replicates). Headspace samples (10 – 60μl) were daily collected with a Hamilton gastight syringe to measure VPHs in air, C_a (g cm^{-3}); O_2 was also measured during the biodegradation tests.

The soil and the soil amended with 2% biochar were considered as a three-phase system, with the solid surface wetted based on relative saturation to water in the system (Unger *et al.*, 1996). The VPH partitioning among the phases was assumed to be linear at equilibrium ((Lin *et al.*, 1996; Unger *et al.*, 1996). The VPH concentration in water C_w (g cm^{-3}) was estimated by the dimensionless Henry's constant, H (−) (Table 4.1). The adsorbed mass was calculated at stationary conditions by mass balance. Fate models typically assume that the microbial breakdown of pollutants occurs in the soil porewater (Steffan *et al.*, 2002), and the first-order biodegradation rate in the soil porewater, k_w (s^{-1}), was calculated according to:

$$k_w = \left(\frac{\theta_a}{\theta_w}H + 1 + \frac{\rho_s\theta_s}{\theta_w}K_d\right)k_a \qquad Equation\ 4.1$$

where θ_a (−), θ_w (−) and θ_s (−) were respectively the air-filled, the water-filled and the solid-filled porosity, and k_a (s^{-1}) was the apparent first-order biodegradation rate in the gas phase (Pasteris *et al.*, 2002; Hohener *et al.*, 2003), which was estimated by linear regression of ln(C_a) vs. time t (s), in the part of the experimental data that showed a clear concentration decrease (e.g. after an eventual lag phase).

4.3.5 Laboratory column experiments

Vertical columns (Fig. 4.1) made of glass were homogeneously packed with soil or soil amended with biochar (2% based on soil d.w.) to a bulk density of 1.48±0.01 g cm^{-3} and total porosity of 0.43±0.02 cm^3 cm^{-3}. θ_w at the beginning of the tests was 0.10±0.01 cm^3 cm^{-3}. The columns were placed in the fume cupboard at 20±2°C. After packing, the columns were left undisturbed for 5 days to monitor the background respiration. After that period, on day 0, a vial containing 10 ml of the pollutant mixture was tightly connected to the bottom of the funnel-shaped end of the column using a Teflon-lined rubber seal. The columns, open on the top, were run for 13 days. Then, the source was removed and the monitoring carried out for 2 days. After that period, the source was replaced and an inverted glass beaker was placed over the top (Fig. 4.1) from day 15 to 30, in order to compare fluxes of volatile petroleum hydrocarbons and CO_2 at the top of the columns. A known amount of SF_6 was injected through port 5

into the headspace of the columns covered by the beakers to estimate the air-phase tortuosity factor (τ_a) (−) (Moldrup *et al.*, 2000; Werner *et al.*, 2004) and the gap area A between the column wall and the beaker (Fig. 4.1). The sampling ports along the column length and on the glass beaker were sealed with GC septa (injection rubber plugs, Thermogreen LB-2, Supelco, Bellefonte, USA). Gas phase samples (10–60 µL) were daily collected by a Hamilton gastight syringe to quantify VPH, O_2, CO_2 or SF_6 concentrations.

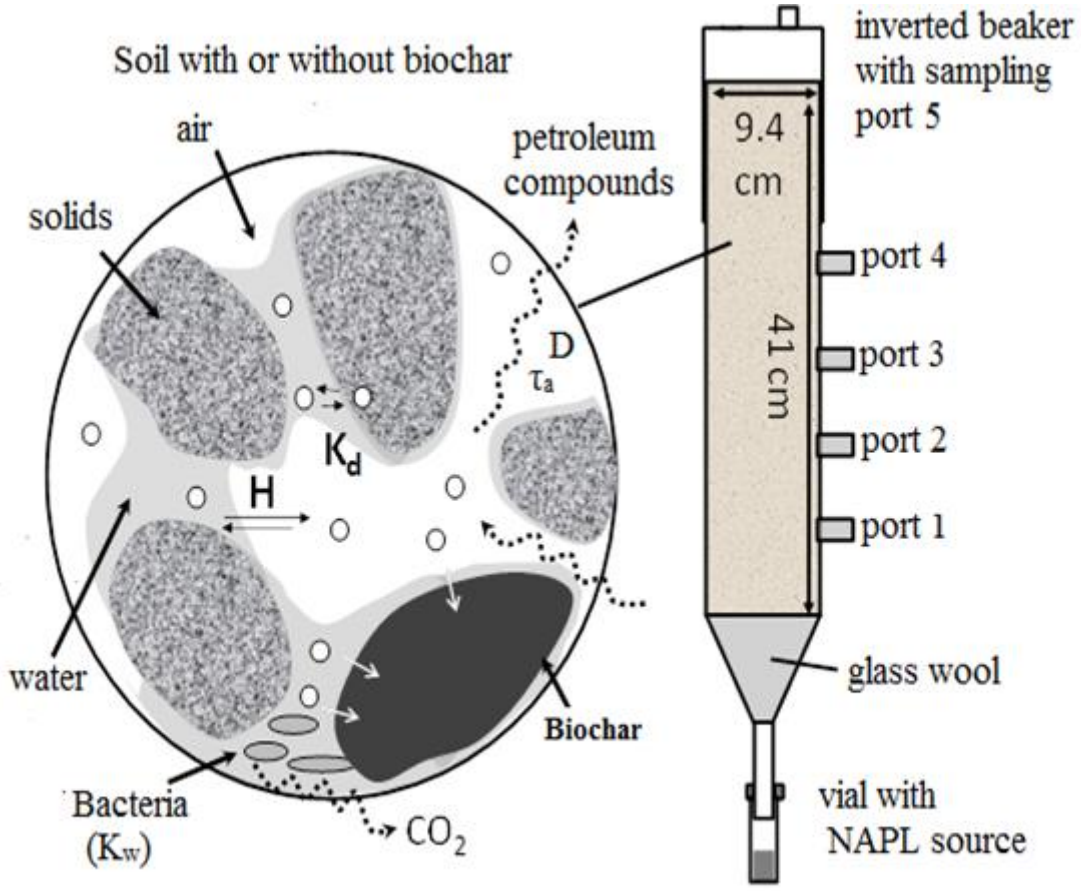

Fig. 4.1. Schematic drawing of the column and conceptual representation of the porous medium constituents and processes affecting the fate of petroleum hydrocarbons.

4.3.6 VPHs concentration quantification:

GC-FID analysis was performed on an Agilent HP-7890 Gas Chromatograph (Agilent Technologies, Palo Alto, USA). The gas sample (60 µl) of headspace gas was injected by a 100 µl Hamilton gastight syringe. The separation was performed on a HP-5 capillary column (30 m × 0.249 mm i.d) with 0.25 µm film thickness (Agilent Technologies, Palo Alto, USA). The injection port used a split ratio of 10 and was heated to 200^{o}C. The gas chromatography column temperature was held at 30^{o}C for 5 minutes, increased to 120^{o}C at a rate of 10^{o}C min^{-1}, and then held constant for 6 minutes with hydrogen as carrier gas (flow rate 2 ml min^{-1}, initial pressure 50 kPa). The instrumental was calibrated using dilutions of headspace concentrations sampled from closed batches containing the VPH mixture liquid for a five point calibration.

4.3.7 CO_2, O_2 and SF_6 quantification:

CO_2, O_2 and SF_6 in the gas samples of the headspace were quantified by gas chromatograph mass spectrometry according to Chapter 3, Section 3.316.

4.3.8 Physical and chemical analyses

Biochar was degassed under vacuum (180°C, 24 h) before measuring CO_2 adsorption at 273K to derive the biochar surface area. The partial pressure P/P0 ranged from 0.001 to 0.15. Grand canonical Monte Carlo simulations were used to interpreted CO_2 adsorption isotherms. The organic carbon content was measured by muffle combustion according to (Wiedemeier *et al.*, 2007). The moisture was determined by drying a soil sample at 105°C. The moisture content was expressed as a percent of the oven dry soil mass. pH of soil in a calcium chloride solution suspensions (1:1) was measured by using a Jenway 3020 pH-meter (Jenway, Staffordshire, UK). The solid density was measured by a pycnometer method according to ASTM method (F1815-97). Total phosphorus was measured by Derwentside Environmental Testing Services geology laboratory (Co Durham, UK) by extraction with Aqua Regia and analyzed using inductively coupled plasma atomic emission spectroscopy, ICP-OES. Total nitrogen was measured by Derwentside Environmental Testing Services geology laboratory by using the Kjeldahl method. Soil particle size distribution was measured by

Derwentside Environmental Testing Services geology laboratory. Water soluble nitrite, nitrate and ammonium were measured by extracting the sample in water and analyzing by Ion Chromatography (Dionex, Sunnyvale, CA, USA). The C:N:S ratio was measured with a Vario MAX CNS elemental analyser (Elementar Analysen systeme GmbH, Hanau, Germany).

4.3.9 Column modelling

A finite difference model based on Euler's method was developed to simulate VPH and SF_6 concentrations in the columns as a function of time, using central differencing for the first and second derivative terms. The column was assumed constant in diameter over the whole column length and filled with a porous medium described as a three-phase system (Fig. 4.1). The "extra-volume" outside the funnel-shaped section was filled with a non-sorbing solid medium, resulting in different values for θ_a (from 0.0191 to 1), θ_w (0), θ_s (0) and τ_a (1) in the lowest section of the system. The grid resolution along the z-axis was 1 cm.

The VPH partitioning among the phases was assumed as instantaneous, reversible and linear equilibrium (Werner and Hohener, 2002; Werner and Hohener, 2003). Sorption isotherms on chars tend to be non-linear if measured over a wide concentration range, and we therefore determined K_d values at concentrations similar to those observed in the column studies to minimize errors. Sorption on the solid phase and biodegradation in the water phase were accounted for by measured, compound-specific K_d and k_w parameter values obtained from the batch experiments. Diffusion in the gas phase was described by the molecular diffusion coefficient in air D ($cm^2\ s^{-1}$) (Table 4.1). The governing equation for the VPHs in the medium was described as a function of C_a according to (Massmann, 1989):

$$\left(\theta_a(z) + \frac{\theta_w(z)}{H} + \frac{\rho_s \theta_s(z) K_d}{H}\right) {}^{d}/_{dt}\, C_a(z,t) = {}^{\partial}/_{\partial z}\left(\theta_a(z)\tau_a(z) D\, {}^{\partial}/_{\partial z}\, C_a(z,t)\right) - k_w \theta_w(z) C_w(z,t) \qquad \text{Equation 4.2}$$

where z (cm) was the upward distance from the bottom of the column.

The experimental duration was divided in two simulation periods, according to the different initial and boundary conditions. The initial conditions for C_a were based on the experimental measurements in the gas phase. The lower boundary condition was determined by the VPH gas-phase concentration in the headspace of the vial attached to the bottom of the column, which was calculated according to Raoult's law. For each time step, the amount of pollutants in the pollutant mixture was recalculated based on the volatilization flux at the bottom of the column. The upper boundary condition was determined by assuming a constant zero VPH concentration at the top of the column. The time step was set to 1 s. The model mass balance closed within ± 5%.

4.4 Results and discussion

4.4.1 The laboratory batch experiments

Petroleum hydrocarbon concentrations in the sterilized batches were stable within the analytical coefficient of variance, while concentrations decreased to levels below the detection limit in the non-sterilized batches. The compound behaviour was broadly consistent amongst the group of the straight-chain alkanes, the group of the cyclic and/or branched alkanes, and the group of the monoaromatics and is illustrated by n-octane, methylcyclohexane, and toluene respectively in Fig. 4.2. The values for K_d derived from the sterile batch data and k_w derived from the live batch data are reported in Table 4.2. The addition of 2% biochar increased the K_d values of the straight-chain, cyclic and branched alkanes approximately by a factor of 1.1 to 4.2, which is consistent with the increase by a factor 2.4 in the TOC (from 1.2 % to 2.9%) due to the biochar amendment. Their organic carbon normalized partitioning coefficients (K_{OC}) were therefore mostly comparable (Fig. 4.3a). On the other hand, the K_d value of toluene (Table 4.2), increased 36 times and the K_{OC} value of toluene increased 15 times (Fig. 4.3a), because of the ability of this compound to interact via π–π electron forces with the aromatic surface of the biochar (McBeath and Smernik, 2009). Additional methyl-groups on the aromatic ring appear to interfere with these interatomic interactions, since m-xylene and 1,2,4-TMB did not show a comparable enhancement in their K_{OC} value in the biochar-amended soil. Similar observations were made in the comparison of sorption to biochar vs. un-amended soil (Fig. 4.3b). In the live soil without biochar, straight-chain alkanes and aromatic hydrocarbons were rapidly biodegraded, whereas the biodegradation of cyclic and branched alkanes (methyl-cyclohexane in Fig. 4.2b) had a lag phase before the onset of biodegradation roughly consistent with the time needed to degrade the straight-chain alkanes (n-octane in Fig. 4.2a), and aromatic hydrocarbons (toluene in Fig. 4.2 c). The apparent degradation rates in the gas-phase concentration, k_a, were fairly consistent with values reported by Pasteris *et al.* (2002), with differences within one order of magnitude. The estimated k_w values (Table 4.2), suggested that the biodegradation of the water-dissolved, available compounds was as fast or faster in the soil amended with biochar compared to the soil without biochar. Furthermore, the degradation of branched and

Table 4.2. Pollutant properties determined experimentally.

Compound	Solid-water distribution Coefficient K_d ($cm^3\ g^{-1}$)		First-order degradation rate k_w (s^{-1})		Volatilization through the beaker gab from day 15 to day 30(g)	
	Soil	Soil & 2% biochar	Soil	Soil & 2% biochar	Soil	Soil & 2% biochar
n-pentane	20±7	43±16	$(3.7\pm1.2)\cdot10^{-3}$	$(2.1\pm0.4)\cdot10^{-2}$	0.105	0.050
n-hexane	47±17	90±36	$(2.9\pm0.3)\cdot10^{-2}$	$(5.7\pm2.6)\cdot10^{-2}$	0.106	0.033
methylcyclopentane	9±4	10±7	$(1.2\pm1.1)\cdot10^{-3}$	$(5.6\pm0.9)\cdot10^{-3}$	0.111	0.070
cyclohexane	4±2	5±4	$(6.4\pm0.6)\cdot10^{-4}$	$(1.1\pm0.2)\cdot10^{-3}$	0.075	0.046
isooctane	44±16	73±70	$(1.6\pm0.2)\cdot10^{-2}$	$(1.8\pm0.7)\cdot10^{-2}$	0.057	0.035
methylcyclohexane	2.3±0.7	4±3	$(4.1\pm4.0)\cdot10^{-4}$	$(1.6\pm0.3)\cdot10^{-3}$	0.061	0.029
toluene	1.9±0.1	69±16	$(8.9\pm3.1)\cdot10^{-4}$	$(1.5\pm0.1)\cdot10^{-2}$	0.004	0.000
n-octane	104±27	314±69	$(1.7\pm0.5)\cdot10^{-1}$	$(2.1\pm0.1)\cdot10^{-1}$	0.002	0.000
m-xylene	1.3±0.2	14±3	$(7.3\pm3.0)\cdot10^{-4}$	$(5.1\pm0.4)\cdot10^{-3}$	0.001	0.000
1,2,4-trimethylbenzene	1.6±0.2	13±1	$(1.8\pm0.4)\cdot10^{-3}$	$(5.1\pm0.4)\cdot10^{-3}$	0.000	0.000
n-decane	123±27	520±190	$(1.7\pm1.0)\cdot10^{-1}$	$(3.7\pm0.4)\cdot10^{-1}$	0.000	0.000
n-dodecane	774±211	1630±350	$(1.6\pm0.4)\cdot10^{-1}$	$(3.1\pm0.3)\cdot10^{-1}$	0.000	0.000
CO_2	-	-	-	-	0.158	0.211

The error rang e for the distribution coefficient K_d is the standard deviation of triplicate bottles and the error of the first-order biodegradation rate k_w is based on the propagation of the errors (Taylor, 1997) in Eq. (4.1). The mass volatilized through the gap between the column and the beaker in the column experiments was calculated by integrating the flux calculated according to Eq. (4.3). The TOC content was 1.2% d.w. for the soil and 2.9% d.w. for soil + 2% biochar.

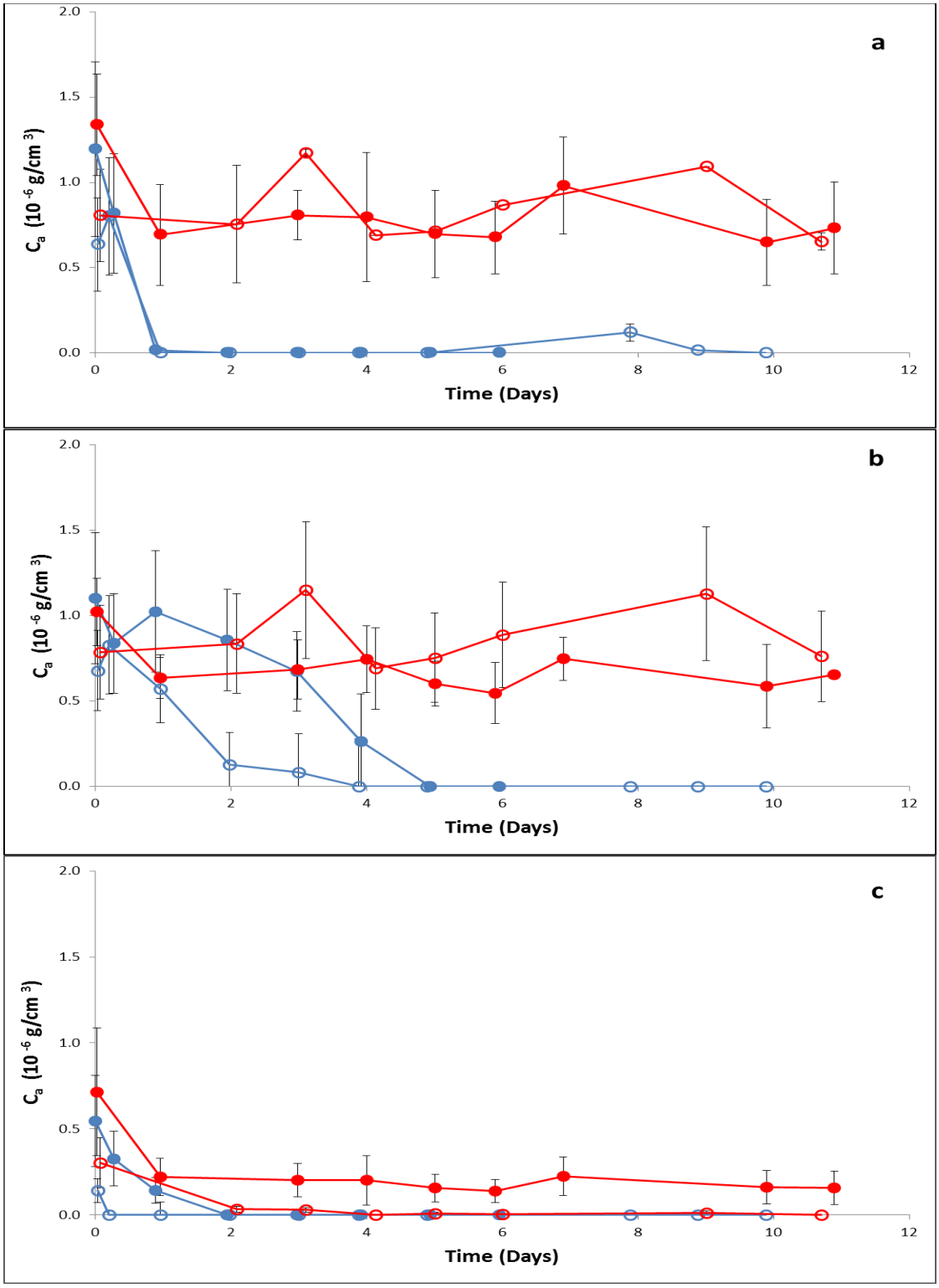

Fig. 4.2. Petroleum hydrocarbon gas concentration (C_a) in batches containing soil (●●) or soil amended with biochar (○○) for sterilized (●○) or live batches (●○). Typical data for straight chain alkanes are illustrated by n-octane (a), for cyclic or branched alkanes by methylcyclohexane (b), and for aromatics by toluene (c). Error bars are calculated as the standard deviation in triplicate batches.

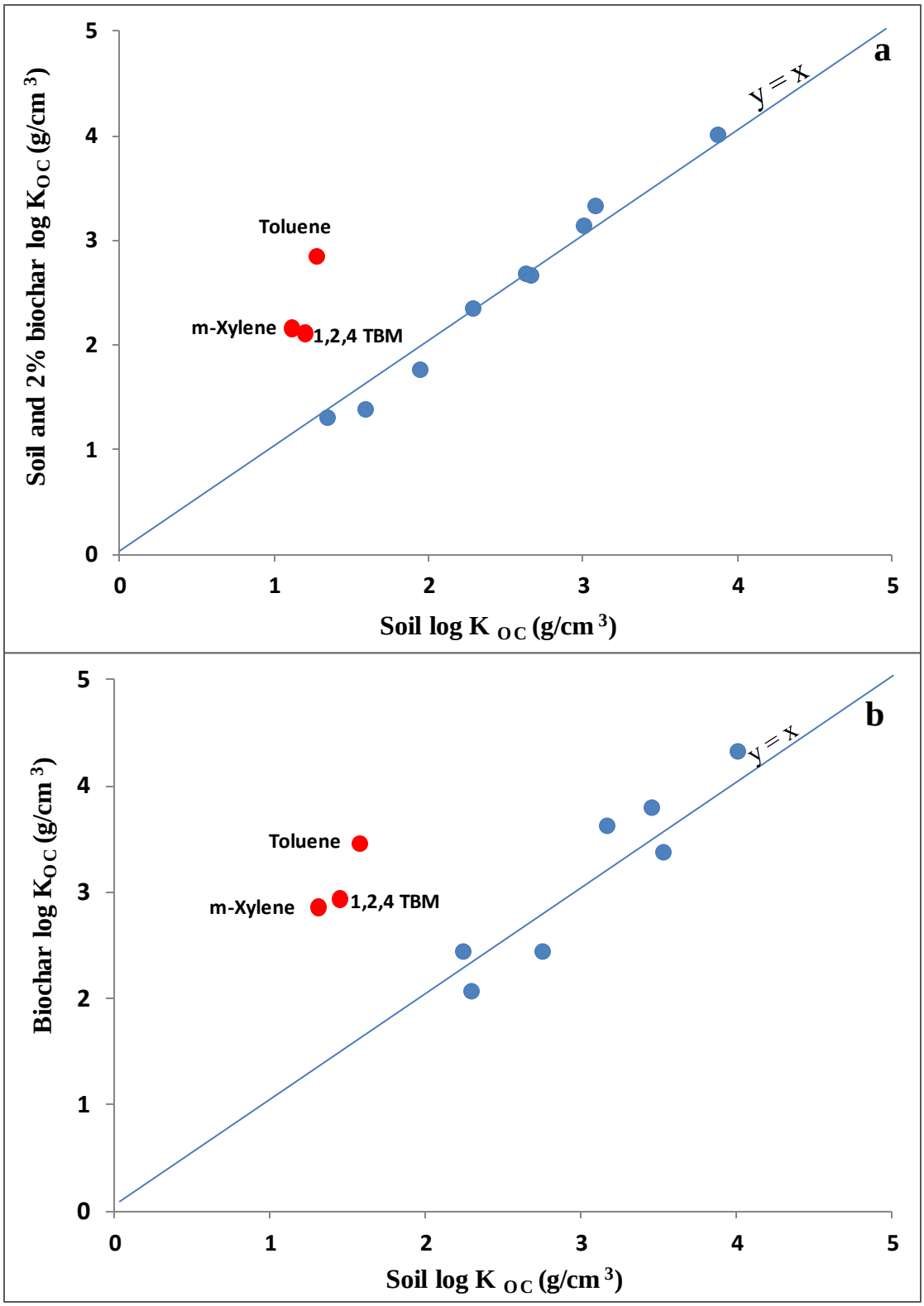

Fig. 4.3. Comparison between log K_{OC} (cm g^{-1}) in soil and (a) soil amended with 2% biochar and (b) pure biochar for straight-chain, branched or cyclic alkanes (●) and aromatic hydrocarbons (●).

cyclic alkanes was initiated without lag phase in the biochar amended soil resulting in an earlier onset of the concentration decrease (Fig. 4.2 b).

4.4.2 The laboratory column experiments

For both soil and soil amended with biochar, the back-ground respiration was minimal, as the O_2 and CO_2 concentrations remained near to atmospheric levels over the five-day monitoring period prior to the introduction of petroleum hydrocarbons. Once NAPL was added the vial at the bottom of the column, petroleum hydrocarbon vapours and CO_2 levels increased at all sampling ports. Petroleum hydrocarbon gas-phase concentrations measured at the lowest port1 (as an example) during the initial 15-day period when the columns had open top are illustrated in Fig. 4.4 by n-octane (Fig. 4.4a), methylcyclohexane (Fig. 4.4b), and toluene (Fig. 4.4c). The VPH concentrations predicted by the numerical model at port 1 by assuming either no biodegradation (k_w= 0, marked no deg) or using the k_w data (marked deg) from Table 4.2 are also illustrated with solid and broken lines (for soil and soil with 2% biochar respectively) in Fig. 4.4. Microbial degradation in the columns was not always following first order rate laws, since measurements do not always correspond to the model predictions. In soil without biochar, gas-phase concentrations of VPHs at first generally increased in line with the model predictions assuming no biodegradation (blue solid line marked no deg), and then fell towards or even below those predicted based on the first-order biodegradation rates determined in the batch studies (blue broken line marked deg). For some compounds (n-octane and toluene in Fig. 4.4, n-hexane, n-decane and m-xylene, for which data are not shown) gas-phase concentrations towards the end of the experiment rose again somewhat above the predictions based on the first-order biodegradation rate determined in the batch studies.

In soil with biochar, the experimental observations were broadly in line with the predictions based on the batch data, assuming biodegradation (red broken line marked deg). After the initial monitoring period, the source vial at the bottom of the columns was replaced with a vial containing fresh NAPL, and an inverted beaker was placed over the top of the columns as illustrated in Fig. 4.4. A narrow gap between the beaker

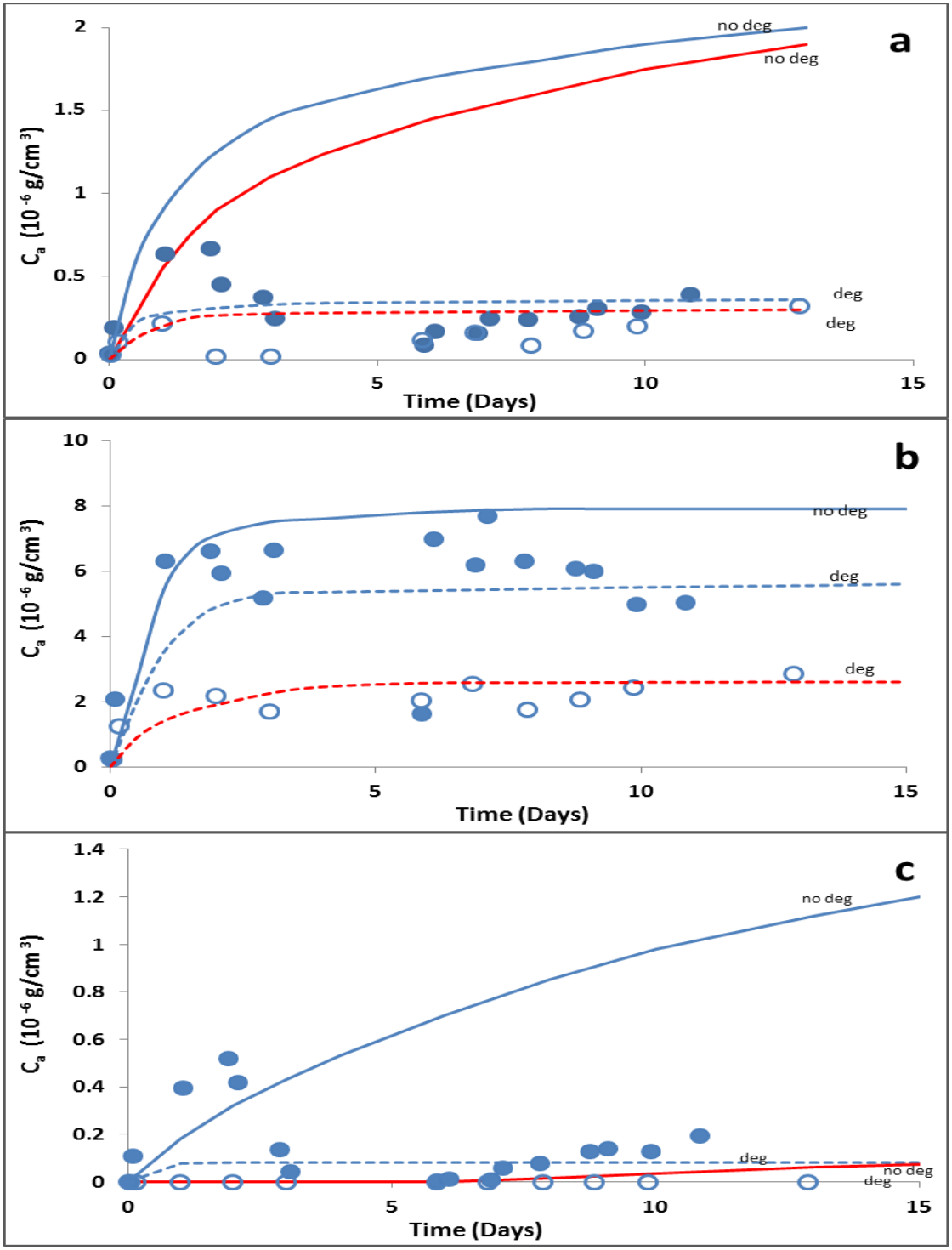

Fig. 4.4. Measured (symbols) and modeled (lines) petroleum hydrocarbon concentrations (C_a) at port 1 in the open-top columns: soil (blue lines and solid symbols, (– ●) or soil amended with biochar (red lines, open symbols, (– ○). Typical data for straight chain alkanes are illustrated by n-octane (a), for cyclic or branched alkanes by methylcyclohexane (b), and for aromatics by toluene (c). Simulations assuming no biodegradation (k_w= 0) are indicated by "no deg" (solid lines) and those using the k_w data from Table 4.2 are indicated by "deg" (broken lines).

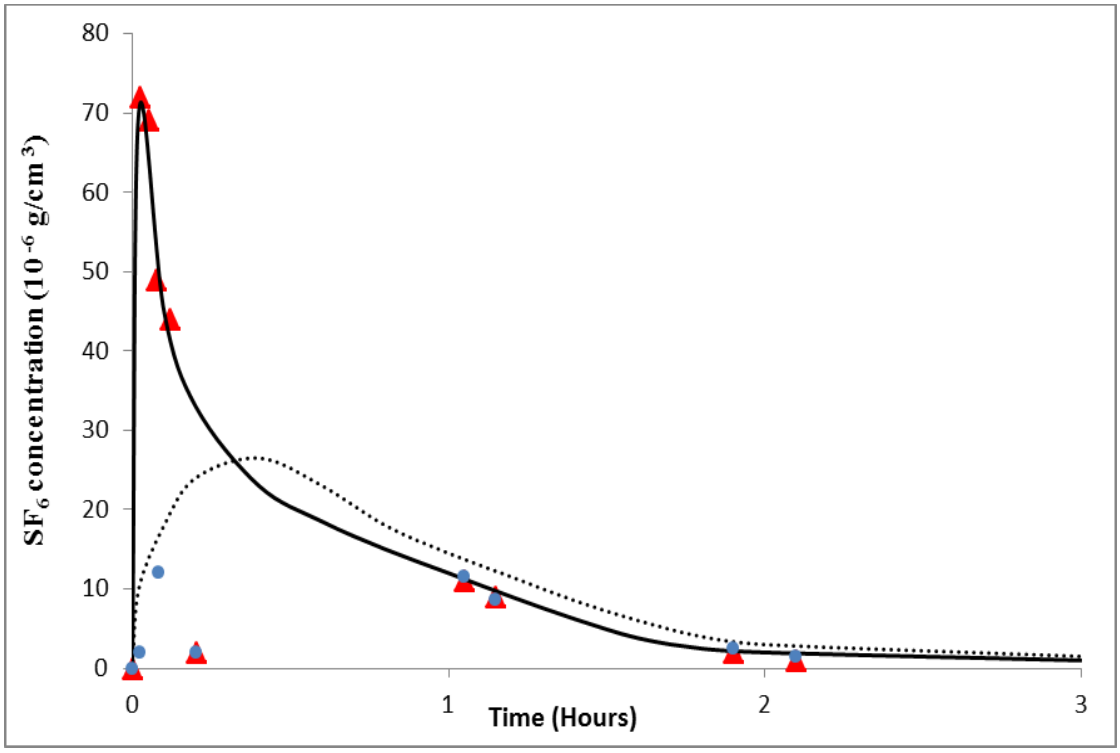

Fig. 4.5. Measured (lines) and modeled (symbols) SF_6 concentrations during tracer tests used to determine the size of the gap area. Data referred to port 5 (— and ▲) and port 1 (…and ●).

and the outside of the columns allowed for a diffusive oxygen flux into the column and a counter-flux of diffusive VPH and CO_2 out of the system. The size of the gap area between the inverted beaker and the wall of the columns was estimated from the tracer tests with SF_6 injected into the column headspace through port 5. Fig. 4.5 shows the agreement between experimental data and the simulated SF_6 concentration at ports 5 and 1 for the fitted gap area of 1.2 cm^2 which was used in subsequent simulations. The measurements and the simulations (k_w= 0 or k_w from Table 4.2) of VPH gas-phase concentrations at the sampling port 5 in the headspace of the column are illustrated in Fig. 4.6. In the soil without biochar, concentrations in the headspace of the column for most of the linear alkanes (n-octane in Fig. 4.6 a) fell in between the model predictions assuming no biodegradation or biodegradation with the rates k_w determined in the batch studies. Cyclic and branched alkanes concentrations (methylcyclohexane in Fig. 4.6 b) rose broadly in line with the predictions assuming no biodegradation. The concentrations of monoaromatics rose initially in line with the predictions assuming

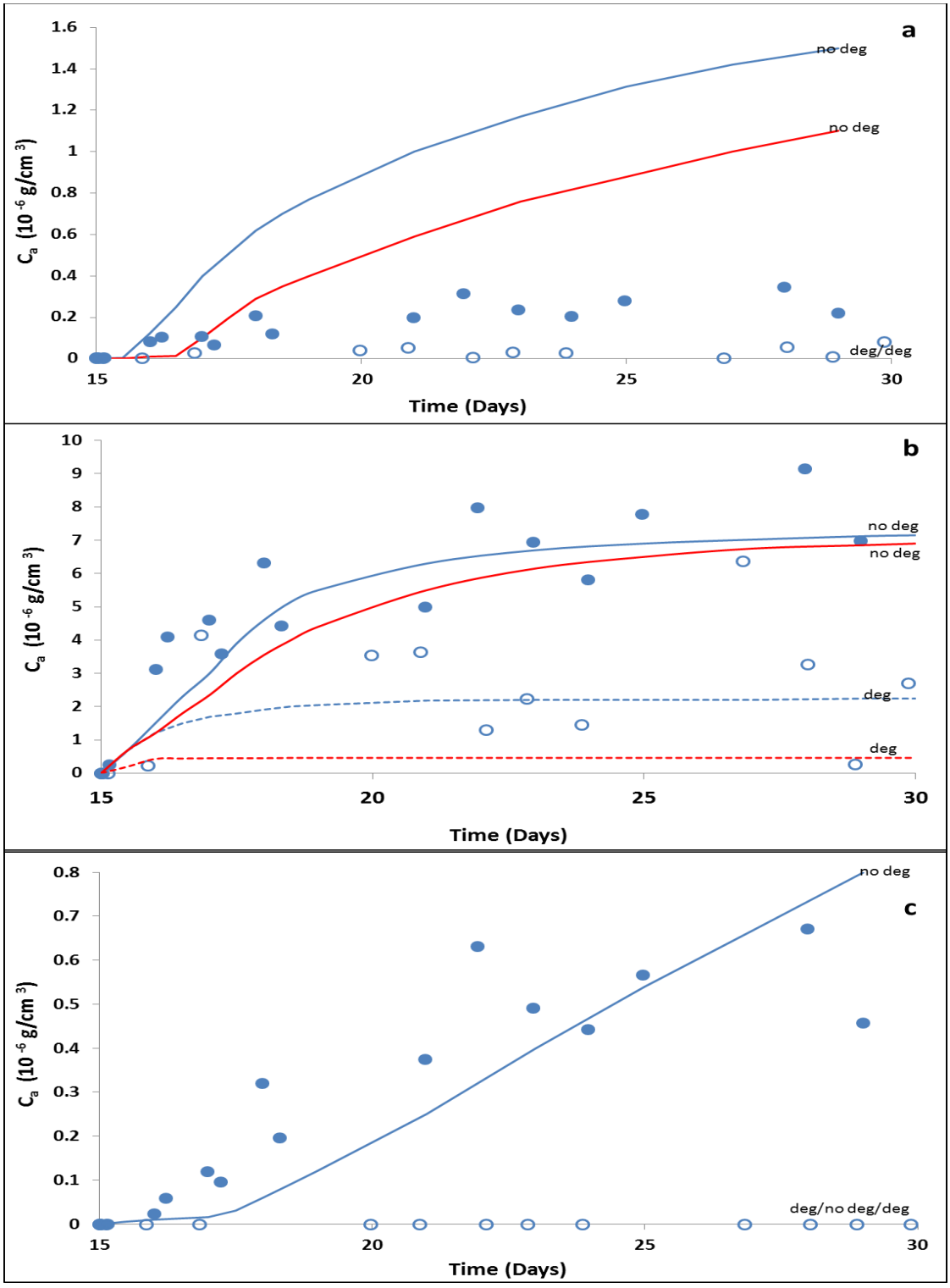

Fig. 4.6 Measured (symbols) and modeled (lines) petroleum hydrocarbon concentrations (C_a) at sampling port 5 in the headspace of the beaker-covered columns: soil (blue lines and solid symbols, (– ●) or soil amended with biochar (red lines, open symbols, (– ○). Typical data for straight chain alkanes are illustrated by n-octane (a), for cyclic or branched alkanes by methylcyclohexane (b), and for aromatics by toluene (c). Simulations assuming no biodegradation (k_w= 0) are indicated by "no deg" (solid lines) and those using the k_w data from Table 4.2 are indicated by "deg" (broken lines).

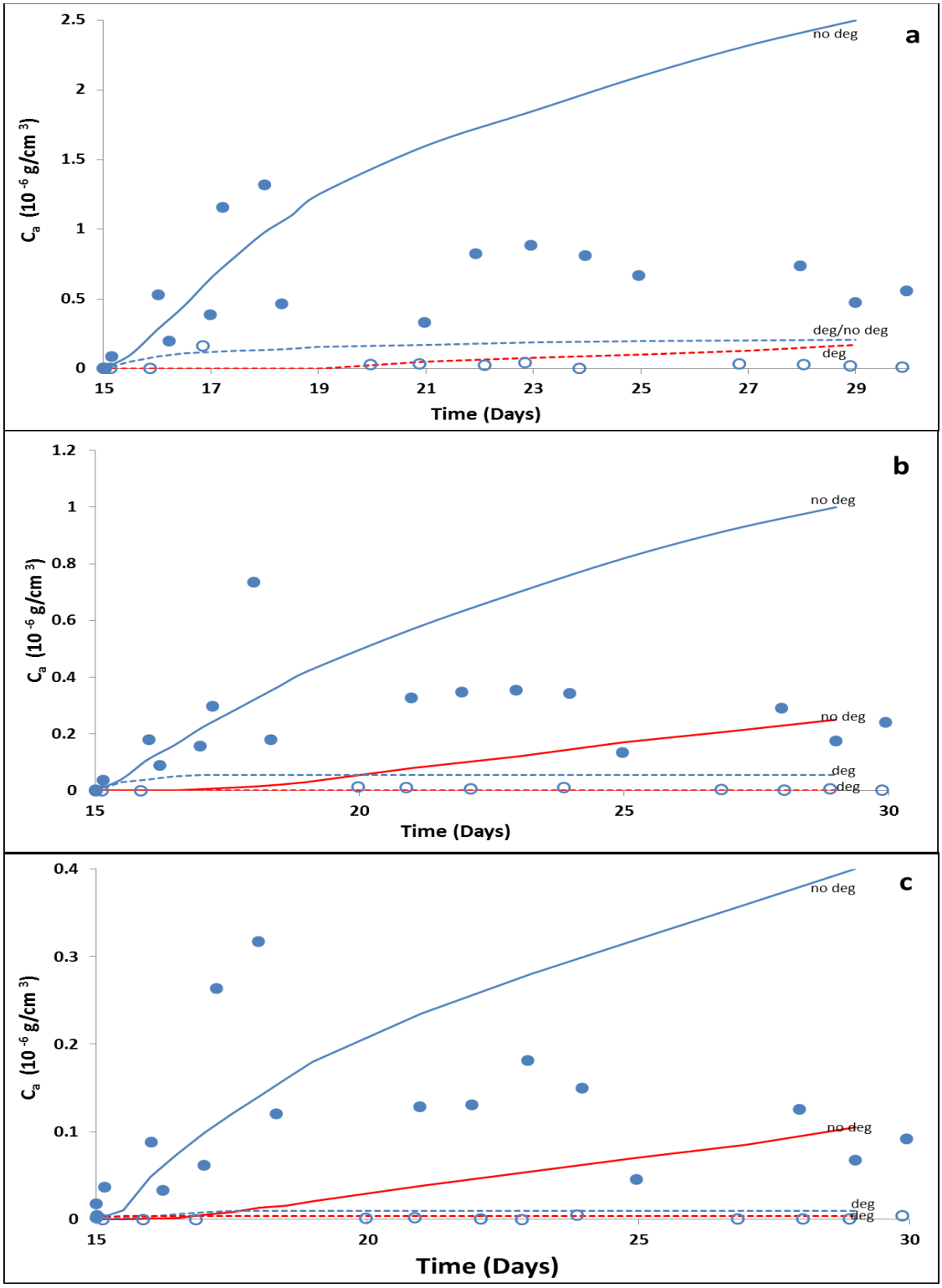

Fig. 4.7. Measured (symbols) and modeled (lines) petroleum hydrocarbon concentrations (C_a) at the lowest sampling port 1 of the beaker-covered columns: soil (blue lines and solid symbols, (– ●) or soil amended with biochar (red lines, open symbols, (– ○). The behavior of the monoaromatic compounds is illustrated for toluene (a), m-xylene (b), and 1,2,4 TMB (c). Simulations assuming no biodegradation (k_w= 0) are indicated by "no deg" (solid lines) and those using the k_w data from Table 4.2 are indicated by "deg" (broken lines).

no biodegradation and then stabilized at a level in between the concentrations of the two predictions (k_w= 0 or k_w from Table 4.2) indicating biodegradation at a rate below the one measured in the batch study. For toluene (Fig. 4.6c), this stabilization trend was only observed for the very last data point and therefore not ascertained. For soil with biochar, straight-chain, cyclic and branched alkanes concentrations fell in between the concentrations of the two predictions (k_w = 0 or k_w from Table 4.2) and were consistently lower than those observed in soil without the biochar. Monoaromatics did not break-through into the headspace of the column with biochar amended soil over the duration of the experiment. Monoaromatics concentrations at the lowest port 1 during the monitoring period with the beaker-covered columns are therefore compared in Fig. 4.7. Toluene, m-xylene and 1,2,4-TMB concentrations in soil with biochar stayed well below the predictions assuming no biodegradation (k_w= 0) towards the end of the experiment, indicating that biodegradation of these compounds occurred in the lowest part of the column filled with soil and biochar.

At the end of the experiments, the columns were still under aerobic conditions, with O_2 concentrations at about atmospheric conditions and CO_2 values below 0.8% v/v at all sampling ports. CO_2 concentrations in the headspace of the beaker-covered columns are compared in Fig. 4.8. They rose and then stabilized at broadly similar levels for soil with and without biochar towards the end of the column experiments. A stable concentration in the headspace of the columns indicates that the net flux emanating from the soil is equal to the diffusive flux leaving the headspace through the gap in between the inverted beaker and the column (Fig. 4.1). The mass flux MF (g/s) leaving the system through the gap can be quantified by as follows:

$$MF = -DA\frac{(C_{fc} - C_{P5})}{l} \approx= -DA\frac{C_{P5}}{l} \qquad Equation\ 4.3$$

Where l is the length of the gap (12 cm), C_{fc} (g/cm^3) is the concentration in the fume cupboard and C_{P5} (g/cm^3) is the concentration at port 5 and A (cm^2) is the gap area. Eq. (4.3) assumes that the concentration in the column headspace is much higher than in the fume cupboard. The mass of VPH and CO_2 volatilized through the gap over the 15-day monitoring period with the inverted beaker placed on top of the column was thus calculated and compared for the soil with and without biochar in Table 4.2.

Petroleum hydrocarbon emissions (Table 4.2) were lower for the system with the biochar amended soil, and CO_2 emissions were somewhat higher, but of the same order of magnitude as CO_2 emissions from soil without biochar. The biodegradation rates of VPH in the column experiments and the batch experiments were not always flow first-order rate kinetics. These results do not support the previous research (Moyer *et al.*, 1996; Pasteris *et al.*, 2002; Hohener *et al.*, 2003) who concluded that the first-order kinetic rate was useful in describing most compound's biodegradation. These differences could be explained by differences in soil properties, such as, nutrient contents, microbial communities. Moreover, the result of column experiments suggested that the overall biodegradation rate of total petroleum hydrocarbons in soil and soil with biochar was limited by many factors other than substrate availability, since CO_2 levels in the headspace of the capped columns were broadly comparable. This was observed despite of the greater sorption, slower migration and hence lower availability in particular of the monoaromatic compounds in the biochar amended soil.

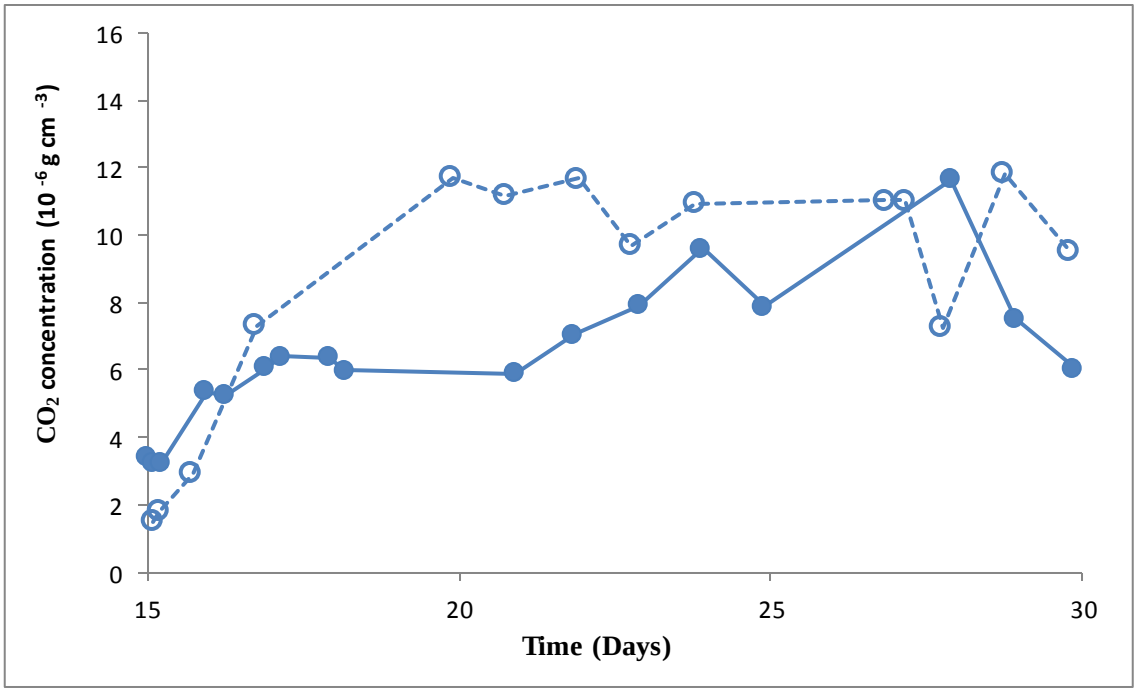

Fig. 4.8 Measured CO_2 concentrations at port 5 in the headspace of the beaker-covered columns: soil (solid, ●) or soil amended with 2% biochar (open symbols, ○).

In fact, the adsorption of monoaromatic compounds on biochar apparently accelerated the biodegradation of cyclic and branched alkanes, which were only poorly biodegraded in soil without biochar. These results are most consistent with the idea of a controlling factor such as the inorganic nutrient availability limiting the amount of total petroleum hydrocarbons which may be degraded within a given timeframe and volume of soil. In this scenario, the reduced availability of a strongly sorbing compounds in the biochar amended soil will allow for greater degradation of less strongly sorbing compounds, which is consistent for instance with the observation of greater methylcyclohexane degradation in the biochar amended soil in both batch and column studies (Fig. 4.2b, 4.4 b and 4.6 b). There was no evidence that the strong binding of monoaromatic compounds by biochar would have inhibited the biodegradation of residual fractions of monoaromatic compounds dissolved in soil porewater. On the contrary, the concentrations of the monoaromatic compounds at the lowest port 1 in the column containing soil with biochar stayed well below the levels predicted by the simulations assuming no biodegradation (Fig. 4.7). Therefore, at least over the duration of the current experiments and for the sandy soil investigated no detrimental effect of biochar was observed on the natural attenuation of the more readily biodegradable and volatile petroleum hydrocarbons. However the impact of biochar on the fate of VPHs needs to be investigated further for different soils, over longer periods, and also under field conditions.

4.5 Conclusions

Biochar addition to soil is currently being investigated as a novel technology to remediate polluted sites. A critical consideration is the impact of biochar on the intrinsic microbial pollutant degradation, in particular at sites polluted with a mixture of readily biodegradable and more persistent organic pollutants. We therefore studied the impact of biochar (2% on dry weight basis) on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil with batch and column studies. The soil-water partitioning coefficient, K_d, was enhanced in the biochar-amended soil up to a factor 36, and petroleum hydrocarbon vapor migration was retarded accordingly. Despite increased sorption, in particular of monoaromatic hydrocarbons, the overall microbial respiration was comparable in the biochar-amended and un-amended soil. This was

due to more rapid biodegradation of linear, cyclic and branched alkanes in the biochar amended soil. We concluded that the total petroleum hydrocarbon degradation rate was controlled by a factor other than substrate availability and the reduced availability of monoaromatic hydrocarbons in the biochar amended soil led to greater biodegradation of the other petroleum compounds.

Chapter 5: The fate and transport of volatile petroleum hydrocarbons in long term soil column studies

5.1 Introduction

The biodegradation and transportation of petroleum hydrocarbons in the vadose zone have been extensively studied. However, the process is complicated and much remains to be understood (Lin *et al.*, 1996). The study of *in-situ* biodegradation is a challenge because the vadose zone is difficult to characterize and the introduction of chemicals and the sampling of microorganisms is complicated. Moreover, field experiments are the most expensive and complex experiments. Therefore, many alternative different methods have been used to investigate the biodegradation of VPHs in the vadose zone. These methods include laboratory column or microcosm experiments, and large scale field lysimeter experiments.

Microcosm laboratory experiments have been used by many researchers (Kelly *et al.*, 1996; Hohener *et al.*, 2003; Hohener *et al.*, 2006) to investigate the biodegradation and sorption of VPHs. However, microcosm laboratory experiments have many drawbacks including the disruption of soil aggregates, changing concentration conditions and often a much higher contaminant to soil ratio than is observed in natural soil (Hohener *et al.*, 2003). On the other hand, microcosm laboratory experiments avoid mass-transport limitations (Kelly *et al.*, 1996).

Laboratory column experiments have been widely used to simulate the mobility and natural attenuation of VPHs. Laboratory column experiments are a more realistic simulation of nature than microcosms (Kelly *et al.*, 1996). Therefore, the biodegradation rate parameters which are determined by column experiments are preferable for the risk assessment of volatile organic hydrocarbons (Hohener *et al.*, 2003), and they can avoid some of the drawbacks of microcosms (Hohener *et al.*, 2003). However, the migration of VPHs and oxygen diffusion through the columns is quasi one-dimensional, whereas in the field it is three-dimensional.

Large scale field lysimeter experiments are even more complex then column experiments but also more representative of field conditions. Lysimeter experiments have been used to study the migration and biodegradation of petroleum hydrocarbons (Pasteris *et al.*, 2002) and to investigate the biodegradation and infiltration of medium molecular weight hydrocarbons to ground water and the impact of the addition of MTBE and ethanol on ground water quality (Dakhel *et al.*, 2003). While they are more realistic than microcosms and column experiments, lysimeter experiments still have their own drawbacks, such as the disturbance of the sand during filling. The migration of VPHs and oxygen diffusion through lysimeters is still quasi one-dimensional, whereas in the field it is truly three-dimensional.

Hohener *et al.* (2006) compared for the first time the biodegradation of volatile petroleum hydrocarbons using three different methods: laboratory microcosm, laboratory column and field experiments. The study illustrated that petroleum hydrocarbon biodegradation in the microcosms was underestimated by a factor of up to 5 due to the lack of sensitivity in the measurement of biodegradation rates for slowly degrading compounds. Moreover, the field data interpreted with different models suggested a significantly higher degradation rate for benzene than the rates measured in the laboratory.

Although laboratory column and field lysimeter experiments have many drawbacks, many researchers recommended this method for estimating risk. Hohener *et al.* (2003) concluded that the column approach is preferable to batch studies for measuring biodegradation rate parameters to be used in risk assessment models. The usage of biodegradation rates measured in the laboratory to predict biodegradation rates under field conditions is sound, when differences in environmental conditions have been taken into account (Freijer, 1996).

5.2 Aims

This work aims to study the long-term effects of biochar and activated carbon on the fate and volatilization of volatile petroleum hydrocarbons emanating from a NAPL

source and passing through soil over a period of 14 months. This aim will be achieved by first performing laboratory microcosms with soil, soil amended with biochar and soil amended with activated carbon to estimate the solid-water distribution coefficient and the first-order biodegradation constant for the pollutants investigated. The mechanisms affecting the pollutant transport through the soil, the soil amended with biochar and the soil amended with activated carbon were then be investigated with long-term column experiments. The data from the column studies were be compared to the predictions of a numerical pollutant fate model coded in Matlab.

5.3 Materials and methods

5.3.1 Volatile petroleum hydrocarbon compounds mixture

A mixture of 12 major constituents of gasoline or kerosene and sulphur hexafluoride (SF_6) described in the Shapter 4, Section 4.3.1 (table 4.1), was used in this Chapter.

5.3.2 Soil, biochar and activated carbon

The sandy soil was obtained from the Newcastle Law School building construction site on the Newcastle University campus in the U.K. which is described in Chapter 3, Section 3.3.1 was also used in this chapter. The biochar obtained from Environmental Power International EPI (Wiltshire, UK) and bitumen activated carbon (Chemviron Carbon limited, Lancashire, UK) which are described in the Chapter 3, Section 3.3.2 and Section 3.3.3 were also used in this Chapter.

5.3.3 Laboratory batch microcosm experiments

To measure soil solid-water distribution coefficients and biodegradation rates, batch experiments were performed at room temperature (20±2°C) for 14 days, in bottles (63 ml) closed with Teflon mininert valves (Supelco, Bellefonte, USA) contain soil, soil with biochar or activated carbon (2%), or pure biochar and activated carbon was used following the methods outlined in Chapter 4, Section 4.3.4.

The adsorbed mass was calculated at stationary conditions for sterilized batches by using a mass balance. The first-order biodegradation rate in the gas phase of the batch experiments (k_a) was estimated by linear regression of ln ($C_a/C_{a\,0}$) vs. time (t). Fate models typically assume that the microbial breakdown of pollutants occurs in the soil pore water (Steffan *et al.*, 2002), and the first-order biodegradation rate in the soil pore water, k_w (s^{-1}), was therefore calculated based on k_a according to equation 4.1.

5.3.4 Laboratory column experiments

Three horizontal columns (Fig. 5.1) of 120 cm length and 7.8 cm internal diameter made of glass were homogeneously packed with sandy soil or sandy soil amended

with biochar or with activated carbon (2% amendments on soil d.w. basis) to a bulk density of 1.12, 1.01 and 1.03 g cm^{-3} for the soil column, soil with 2% biochar column and soil with 2% activated carbon column respectively. The corresponding total porosities were 0.55±0.02, 0.60±0.03 and 0.59±0.02 cm^3/cm^3 for the soil column, soil with 2% biochar column and soil with 2% activated carbon column respectively. Volumetric water content at the beginning of the tests was 0.11±0.01, 0.12±0.02 and 0.11±0.01 cm^3 cm^{-3} for the soil column, soil with 2% biochar column and soil with 2% activated carbon column respectively. The columns were placed on a laboratory bench at 20±2°C. After packing, the columns were left undisturbed for 5 days to monitor the background respiration. After that period, on day 0, a vial containing 20 ml of the VPHs mixture was tightly connected to one end of the columns using a Teflon-lined rubber seal (Fig. 5.1). The void space on the other end of the column was purged into the fume cupboard with a water-saturated airflow at a rate of 5±1 ml min^{-1} in order to strip the fuel vapour and simulate an atmospheric boundary condition without drying out the sand (Fig. 5.1). Each column was equipped with 7 sampling ports positioned at every 15 cm distance and the sampling ports along the length of column were sealed

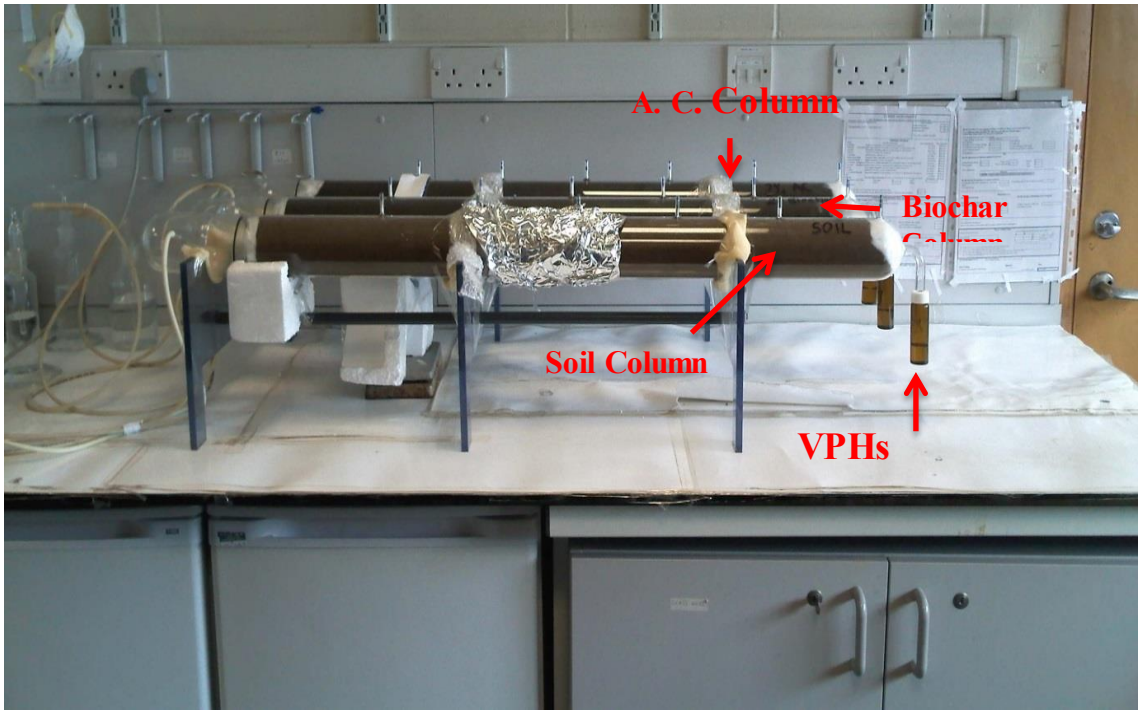

Fig. 5.1. Illustration of the columns experimental set up.

with GC septa (injection rubber plugs, Thermogreen LB-2, Supelco, Bellefonte, USA). Two sampling ports allowed measurements of gas concentrations in the air at the beginning and end of the sand and the others were for sampling of the gas phase in the sand. A known amount of SF_6 was injected through the columns to estimate the air-phase tortuosity factor (τ_a) (−) (Moldrup *et al.*, 2000) according to a tracer method described by Werner et al. (2004).

5.3.5 Tracer test for the measurement of column tortuosity

Due to differences in the empirical equations used for deriving the tortuosity factor based on soil texture and soil water content, there is uncertainty about the true values of effective diffusion coefficients. Therefore, a measurement of diffusion coefficients is desirable to eliminate the uncertainty associated with the use of theoretically derived effective diffusion coefficients. To examine the tortuosity factor of the soil column, soil with biochar column and soil with activated carbon column, 10 mL of SF_6 gas were injected into centre of each column. The soil air was sampled through the injection point and at a sampling port at 15 and 30 cm distance on either side, after 20, 40, 60, 80, 100, 130 and 160 min. Headspace purging was stopped for the duration of the tracer experiment. To avoid artefacts due to the spatial limitation of the experimental system, the duration of the experiments was no longer than 160 min. Samples of 60 µL of soil gas were taken with a gas-tight syringe and injected directly into the gas chromatograph for SF_6 analysis by mass spectrometry.

The measured concentration of SF_6 was compared with the modelled concentration which was obtained by interpreting the diffusive transport with an equation for an instantaneous plane source of SF_6 in a porous medium as described by Werner and Hohener (2002).

$$C_{a\,(x,t)} = \frac{m_0 f_a}{2A\theta_a\sqrt{f_a D_a \tau_a \pi t}} exp\left[x^2 / 4 f_a D_a \tau_a \pi t\right] \qquad \text{Equation 5.1}$$

C_a (gcm^{-3}) is the SF_6 concentration in soil air, m_0 (g) is the tracer mass injected into the column, f_a is the mass fraction of the gaseous tracer in the soil air (f_a = 1), x (cm)

is the lateral distance from tracer injection port, θ_a (cm^3 water cm^{-3} total) is the volumetric soil air content, τ_a (-) is a tortuosity factor for the air-filled soil pore space, D_a (cm^2 s^{-1}) is a molecular diffusion coefficient in air, t (s) is the time and A (cm^2) is the cross-sectional area.

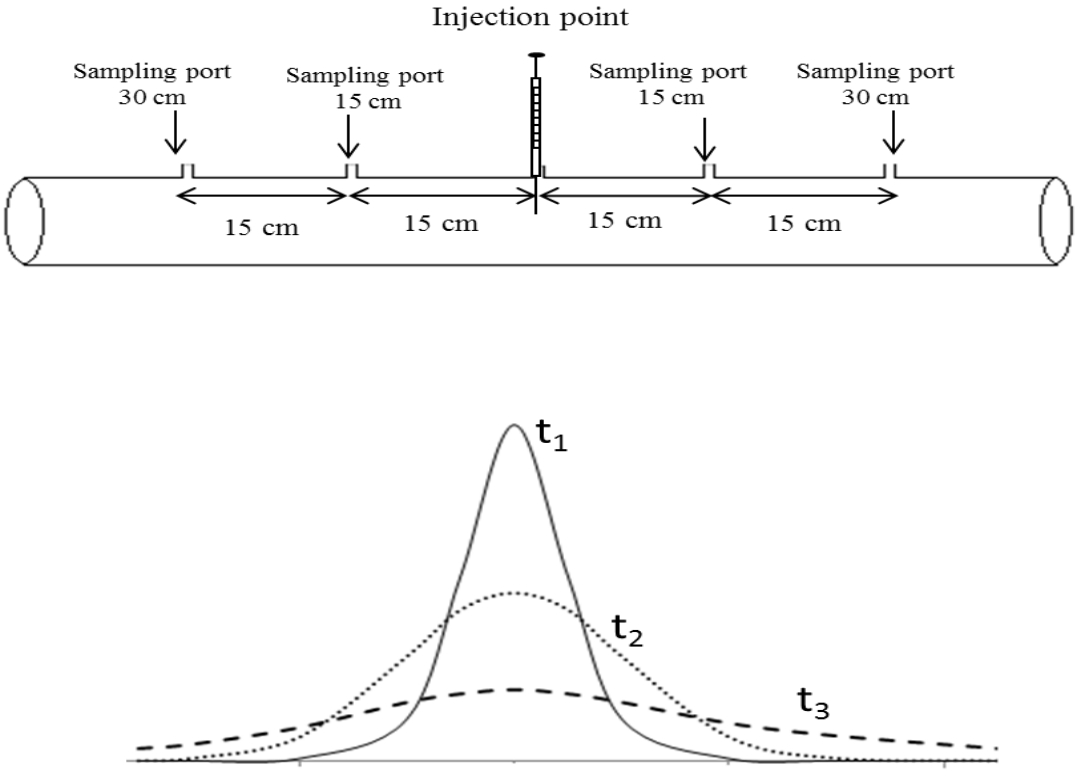

Fig. 5.2. The laboratory column experiment and schematic representation of a tracer distribution in one dimension at different times (t).

5.3.6 VPHs headspace concentration quantification:

VPHs in the soil air samples from the columns were quantified by an Agilent HP-7890 Gas Chromatograph with an FID detector according to Chapter 4 Section 4.3.6.

5.3.7 Volatile petroleum hydrocarbon residual quantification

To examine VPHs residual in columns at the end of the column experiments, 15 g of column sandy soil with or without biochar (2% as dry weight of soil) or with activated carbon (2% as dry weight of soil) were taken from the atmospheric boundary side, the middle and the NAPL source side of each column, extracted twice for 24 h in 10 ml dichloromethane/pentane mixture (40:60 by volume). The combined extracts were

passed through a glass chromatographic column (45 cm×10 mm id) pre-packed with 3 g activated silica gel topped by 1.5 g sodium sulphate (Sigma–Aldrich, Dorset, UK). Columns were rinsed with an additional 20 ml of the dichloromethane/pentane solvent and all solvent was collected in 40 ml amber vials from which 1 ml was transferred into a 1 ml GC-vial for analysis. Pentane could not be quantified by this method because of incomplete separation from the solvent peak. Compounds in cleaned-up solvent extracts were identified and quantified by GC-FID analysis on a Hewlett Packared 5890 series II in split less mode, injector temperature at 280°C. The separation was performed on an Agilent fused silica capillary column (30 m × 0.25 mm i.d) coated with 0.25 µm diethyl poly-siloxane (HP-5) phase (Agilent Technologies, Palo Alto, USA). The GC temperature was programmed from 50-310°C at 5°C min^{-1} and held at the final temperature for 20 min with hydrogen as the carrier gas (flow rate of 30 mLs min-1, initial pressure 50 kPa).

5.3.8 Total carbon and total organic carbon

Total carbon (TC) and total organic carbon (TOC) of soil, biochar, activated carbon and soil with 2% biochar or activated carbon were measured using a Leco CS230 carbon-sulphur analyser (LECO Corporation, Michigan, USA). To compare the change in TOC and TC in soil, biochar and activated carbon columns at the end of the column experiments, soil samples from the NAPL source side, the middle of columns and their atmospheric boundary side were sampled at the end of the column experiment and also analyzed. Samples for TOC were treated with 50% hydrochloric acid to remove carbonate before measuring TC. Total inorganic carbon is calculated as:

$$Total\ inorganic\ carbon\ (carbonate) = TC - TOC$$

5.3.9 CO_2, O_2 and SF_6 quantification:

CO_2, O_2 and SF_6 in the gas samples were quantified by gas chromatograph mass spectrometry according to chapter 3, section 3.3.16.

5.3.10 Modelling

To estimate the biodegradation and diffusive VPH transport in column experiments, a mathematical pollutant fate model is used in this research which is modified from a diffusive reactive transport model used by Jin *et al.* (1994). The model is based on the following assumptions: (1) diffusion is the dominant transport process and Fick's law applies, (2) the sorption of VPHs is linear and reversible and all solid surfaces are wetted, (3) Henry's law governs the dissolution of VPHs vapour in soil pore water, (4) volatilization from the NAPL obeys Raoult's law, (5) the biodegradation reactions occur in the soil pore water and obey first-order kinetics, and uptake and release of VPHs by biochar or activated carbon particles is described by intraparticle diffusion kinetics (Wu and Gschwend, 1988).

Based on these assumptions, the VPHs mass equation in soil with or without a carbonaceous sorbent (biochar or activated carbon) was described as:

$$\left(\theta_a + \frac{\theta_w}{H} + \frac{\rho_s \theta_s K_d}{H}\right) \cdot {}^{d}/_{dt}\, C_a(z,t) = \theta_a \tau_a D_a \cdot {}^{\partial^2}/_{\partial z^2}\, C_a(z,t)$$

$$-\theta_c \cdot \frac{d}{dt}\left[\frac{3}{R_c{}^3}\int_0^{Rc} r^2 S_c(r)\,dr\right] - \theta_w \cdot R_w \qquad \text{Equation 5.2}$$

Where C_a (g per cm^{-3}) is the VPH concentration in soil air, θ_a (cm^3 water cm^{-3} total) is the volumetric soil air content, θ_w (cm^3 water cm^{-3} total) is the volumetric soil water content, H (g per cm^3 air cm^{-3} water) is the Henry's law constant, θ_s (cm^3 solids cm^{-3} total) is the volumetric soil solid content, ρ_s (g cm^{-3}) is the soil solid density, K_d (cm^3 g^{-1}) is the partitioning coefficient between the water and solid phase, τ_a (-) is the tortuosity factor for the air-filled soil pore space, D_a (cm^2 s^{-1}) is the molecular diffusion coefficient in air, z (cm) is the distance from the source, t (s) is the time, R_c (cm) is the particle radius of the carbonaceous sorbent, S_c (g cm^{-3}) is the volumetric concentration of the VPH compound in the carbonaceous sorbent, r (cm) is the radial distance from the centre of the carbonaceous particle, θ_c (cm^3 carbonaceous sorbent per cm^3 total) is the carbonaceous sorbent-filled soil volume fraction, and the biodegradation removal rate of compound R_w (g cm^{-3} s^{-1}) in the aqueous phase is defined as:

$$R_w = k_w \cdot \frac{C_a(z,t)}{H} \qquad \text{Equation 5.3}$$

Where k_w (s^{-1}) is the first-order biodegradation coefficient and z (cm) is the distance from source and t (s) is the time.

The carbonaceous sorbent intraparticle diffusion equation is defined as:

$$\partial/\partial t\, S_c(r,t) = \frac{D_c}{r^2} \cdot \partial/\partial r \left(r^2\, \partial/\partial r\, S_c(r,t) \right) \qquad \text{Equation 5.4}$$

Where D_c ($cm^2\ s^{-1}$) is the intraparticle diffusion coefficient:

$$D_c = \frac{\theta_{w,c}}{\theta_{w,c} + (1-\theta_{w,c}) d_c K_c} \cdot \tau_{w,c} \cdot D_{aq} \qquad \text{Equation 5.5}$$

Where d_c is the carbonaceous sorbent solid density, D_{aq} ($cm^2\ s^{-1}$) is the molecular diffusion coefficient in the water, K_c ($cm^3\ g^{-1}$) is the carbonaceous sorbent-water partitioning coefficient, $\theta_{w,c}$ (cm^3 water cm^{-3} total) is the volumetric water content of the carbonaceous sorbent, $\tau_{w,c}$ (-) is the tortuosity factor for the water-filled sorbent pore space.

The mass flux over a time period dt, M (g) for each VPH compound passing through a cross-sectional area of the column in between two sampling ports was calculated using Fick's first law:

$$M = -A\tau_a\theta_a D_a dt(C_{a\,2} - C_{a\,1})/(z_2 - z_1) \qquad \text{Equation 5.6}$$

Where $C_{a\,2} - C_{a\,1}$ (g cm^3) is the difference in the soil air concentration of VPHs between two sampling points, A (cm^2) is cross sectional area of column, θ_a (cm^3 air cm^{-3} total) is the volumetric soil air content, τ_a (-) is the tortuosity factor for the air-filled soil pore space, D_a ($cm^2\ s^{-1}$) is the molecular diffusion coefficient in the soil air, t (s) is the time and $z_2 - z_1$(cm) is the distance between two sampling ports.

The relative uncertainty for the mass flux was estimated depending on the relative uncertainty of the tortuosity factor ($Error\ \%\ of\ \tau_a$), the relative uncertainty of the volumetric soil air content ($Error\ \%\ of \theta_a$) and the relative uncertainty of the difference between soil air concentrations at two sampling ports ($Error\%\ of\ (C_{a2} - C_{a2})$) as:

$$\text{Uncertainty } \% = \sqrt[2]{(Error\ \%\ of\ \tau_a)^2 + (Error\ \%\ of \theta_a)^2 + (Error\ \%\ of\ (C_{a2} - C_{a1}))^2}$$

For cumulative mass fluxes it was assumed that the error contribution of the measured concentration differences is non-systematic and becomes negligible for a high number of added terms in the cumulative mass flux calculation.

5.4 Results and discussion

5.4.1 Batch experiments

Profiles of natural logarithm C_t/C_0 vs. time of selected VPHs in free soil, abiotic soil and live soil filled batch microcosms are shown together in Fig 5.3 and 5.4 for soil (Fig 5.3a and 5.4a) soil amended with 2% biochar (Fig 5.3b and 5.4b), and soil amended with 2% activated carbon (Fig 5.3c and 5.4c). The behaviour of VPHs was broadly consistent amongst the group of the monoaromatics, the group of the straight-chain alkanes, the group of the cyclic alkanes and the group of the branched alkanes, and is illustrated by toluene, n-pentane, methylcyclohexane and isooctane in Fig 5.3 and 5.4. In live, free soil and abiotic batches, the tracer SF_6 stayed within 90% of the initial concentration during the 12 day period. For soil and soil amended with 2% biochar batches, the differences in concentrations of n-pentane, toluene, isooctane and methylcyclopentane between live, free soil and abiotic microcosms were very significant, whereas differences between live and abiotic microcosms were small for soil amended with 2% activated carbon. The differences in the concentration of toluene in live and abiotic soil amended with 2% activated carbon microcosms was not significant. The batch results reflected the influence of the sorption capacity on the biodegradation of VPHs. For example, the differences between live and abiotic soil were not significant for toluene over the first 5 days. After this delay, microbes started the degradation of toluene and toluene in the headspace was decreased to levels below the detection limit on day 9. In soil amended with biochar microcosms, degradation of toluene started after a lag phase of 6 days and it required one day for decreasing the headspace toluene concentration to levels below the detection limit. This difference in the biodegradation of toluene in soil and soil with amended 2% biochar batches may be attributed to the increased sorption capacity of soil amended with 2% biochar. Both, sorption and biodegradation reduce toluene concentration in live batches. Due to the high toluene sorption capacity in soil amended with 2% activated carbon, there was no measurable biodegradation.

In the activated carbon amended batches, the biodegradation was lowest compared with soil or soil with biochar batches and isooctane for instance was decreased to levels below the detection limit within 8 days. The biodegradation behaviour of methylcyclopentane was the same as the isooctane behaviour, while different behaviour can be seen in the biodegradation of pentane. The degradation of pentane

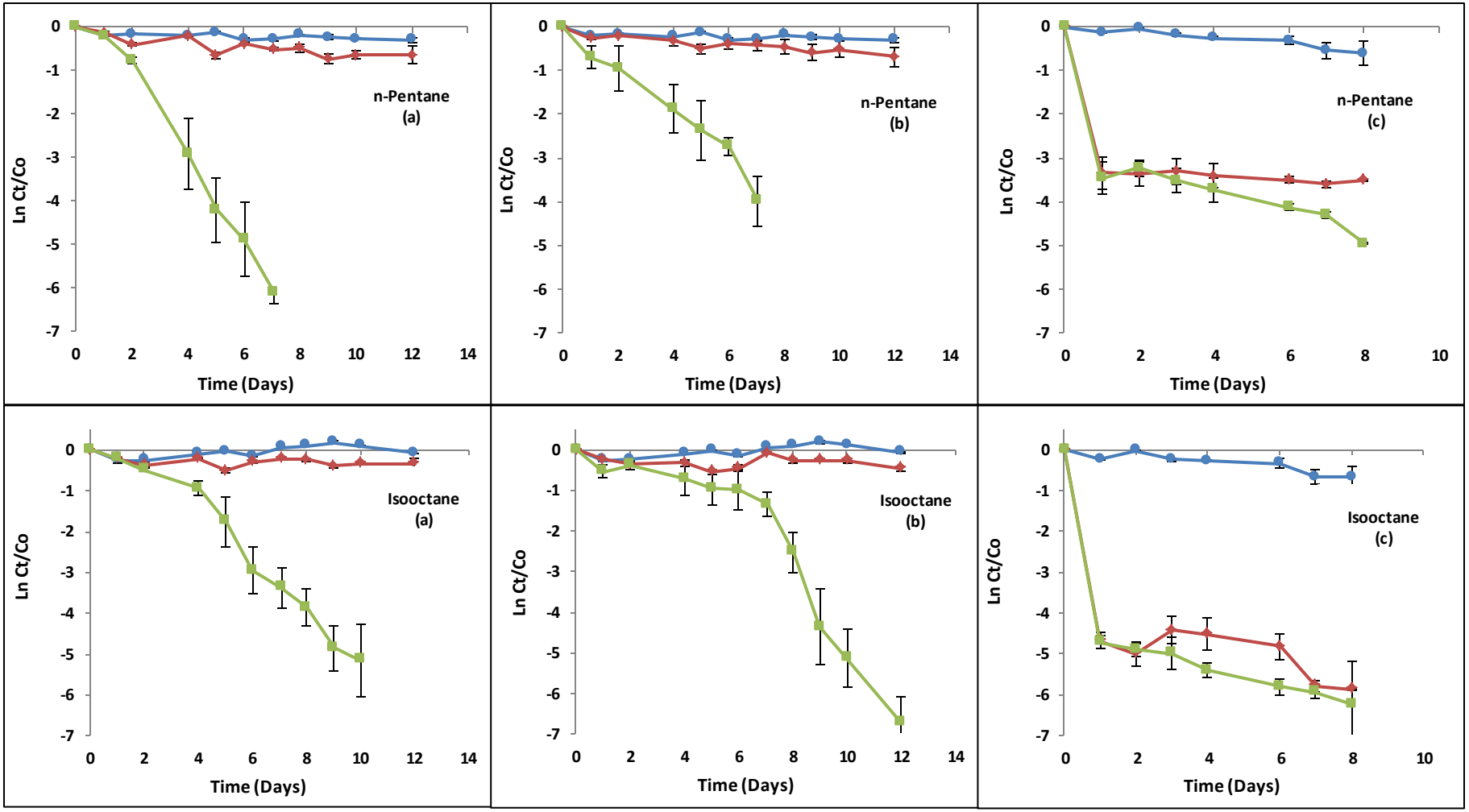

Fig. 5.3. Profile Ln C_t/C_0 of pentane and isooctane in the batch headspace for the soil (a), soil amended with 2% biochar (b) and soil amended with 2% activated carbon (c); comparing free soil batch (—●—), abiotic batch (—◆—) and live batch (—■—).

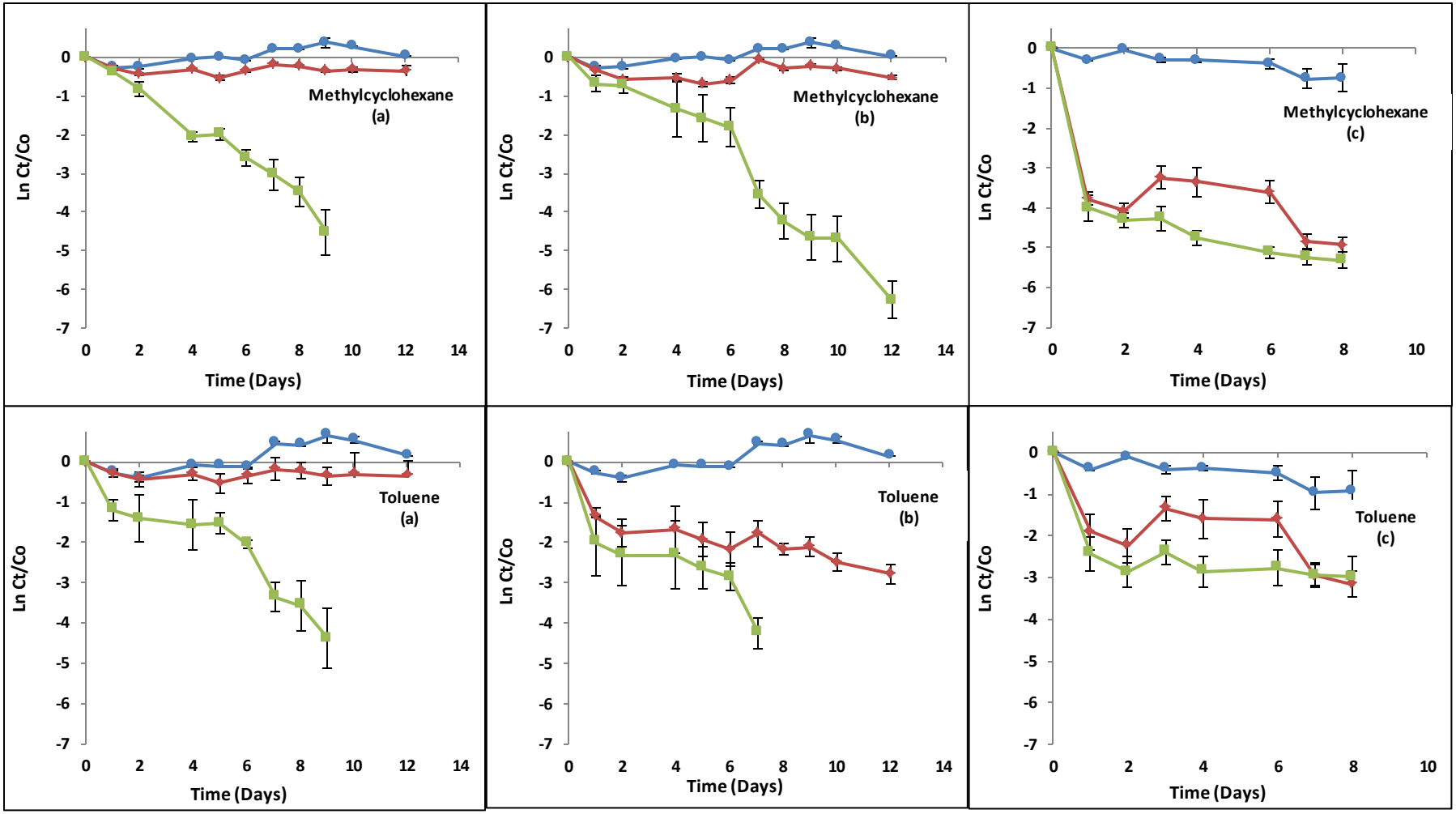

Fig. 5.4. Profile Ln C_t/C_0 of methylcyclohexane and toluene in the batch headspace for the soil (a), soil amended with 2% biochar (b) and soil amended with 2% activated carbon (c); comparing free soil batch (—●—), abiotic batch (—▫—) and live batch (—■—).

started after a lag phase of 1 day in soil batches, while in soil amended with 2% biochar, pentane rapidly biodegraded without a lag phase (Fig 5.3 a and 5.3b). There was a lag phase of 3 days in soil amended with 2% activated carbon batches before pentane started to decrease to a level below the detection limit (Fig 5.3c). Furthermore, the pentane biodegradation was slower in soil with activated carbon in comparison with soil or soil with biochar.

The estimated first-order biodegradation rate in the soil pore water (k_w) values (Table 5.1) suggested that the biodegradation of the water-dissolved portion of most compounds was as fast or faster in the soil amended with activated carbon compared to the soil with or without biochar. However, the strong sorption of by activated carbon inhibited the dissolution of VPH compounds in pore water, resulting in very slow or negligible overall biodegradation. This is consistent with the inverse relationship observed between sorption strength of petroleum hydrocarbons and their biodegradation, bioavailability and bioaccessibility in our previous work (Bushnaf *et al.*, 2011) and it is consistent with several earlier d studies microorganisms (Bolan and Baskaran, 1996; Guo *et al.*, 2000; Jensen *et al.*, 2004; Werner *et al.*, 2005; Rhodes *et al.*, 2008; Jones *et al.*, 2011).

The values for K_d derived from the sterile batch data and k_w derived from the live batch data are reported in the Table 5.1. A comparison between the log organic carbon normalized partitioning coefficients (K_{OC}) of VPHs with and without the sorbent amendment is shown in Fig. 5.5a for soil amended with 2% biochar and Fig. 5.5b for soil amended with 2% activated carbon. The addition of 2% biochar increased the K_{OC} values of the straight-chain, cyclic and branched alkanes approximately by a factor of 1.3 to 2.0, while the K_{OC} values of toluene, m-xylene and 1,2,4-trimethylbenzene approximately increased by a factor of 9.0, 3.3 and 2.3 respectively, due to the ability of these compounds to interact via π–π electron forces with the aromatic surface of the biochar (McBeath and Smernik, 2009), as previously discussed in Section 4.4.1. From Fig. 5.5b, it can be seen that the addition of 2% activated carbon resulted in a very large increase the K_{OC} values of the straight-chain, cyclic and branched alkanes by a factor of 50 to 3000, while the increases of K_{OC} values was a factor 139, 33 and 13 for toluene, m-xylene and 1,2,4-trimethylbenzene, respectively. Such results comply with many previous study where it has been found that the contribution of the micropores in

Table 5.1. VPH properties determined in the batch study.

Compound	First-order biodegradation rate k_w (s^{-1})			Distribution coefficient K_d (cm^3/g)		
	Soil	Soil with 2% biochar	Soil 2% with activated carbon	Soil	Soil with 2% biochar	Soil 2% with activated carbon
n-Pentane	$(6.5\pm0.9).10^{-3}$	$(6.0\pm0.5).10^{-3}$	0.66±0.1	53 ±16	86±19	23200±3000
n-Hexane	$(5.3\pm0.2).10^{-3}$	$(1.2\pm0.1).10^{-2}$	2.00±0.34	68±6	112±24	133000±27000
Methylcyclopentane	$(1.2\pm0.07).10^{-3}$	$(6.9\pm0.2).10^{-3}$	0.53±0.04	13±2	18±1	10600±700
Cyclohexane	$(6.0\pm0.3).10^{-4}$	$(3.2\pm0.1).10^{-3}$	0.18±0.01	6±0.8	8±1	3600±260
Isooctane	$(1.1\pm0.06).10^{-2}$	$(3.0\pm0.2).10^{-2}$	2.05±0.6	83 ±14	124 ±32	249000±76000
Methylcyclohexane	$(5.5\pm0.6).10^{-4}$	$(1.5\pm0.4).10^{-3}$	0.15±0.05	15 ±2	26±9	28800±8800
Toluene	$(6.9\pm0.9).10^{-5}$	$(8.8\pm3.2).10^{-4}$	0.0002±0.00004	1.3±0.2	12±5	170±59
n-Octane	$(8.1\pm1.9).10^{-3}$	$(1.4\pm0.4).10^{-2}$	Not detected	167±77	334±190	53300±19900
m-Xylene	$(4.8\pm1.2).10^{-5}$	$(1.2\pm0.3).10^{-4}$	$(8.0\pm3.0).10^{-5}$	1.8±0.6	6±2	59±20
1,2,4 TBM	$(1.6\pm0.7).10^{-4}$	$(1.4\pm0.4).10^{-4}$	$(8.4\pm1.4).10^{-4}$	4.4±2.0	10±3	59±10
n-Decane	$(8.9\pm2.9).10^{-3}$	$(1.1\pm0.3).10^{-2}$	0.73±0.13	805±355	1091±437	39800±6900
n-Dodecane	$(2.5\pm0.6).10^{-2}$	$(1.3\pm0.1).10^{-2}$	1.45±0.41	1714±490	2151±295	206900±5800

The error range for the distribution coefficient K_d is the standard deviation of triplicate bottles and the error of the first-order biodegradation rate (k_w) is based on the propagation of the errors (Taylor, 1997) in Eq. (4.1).

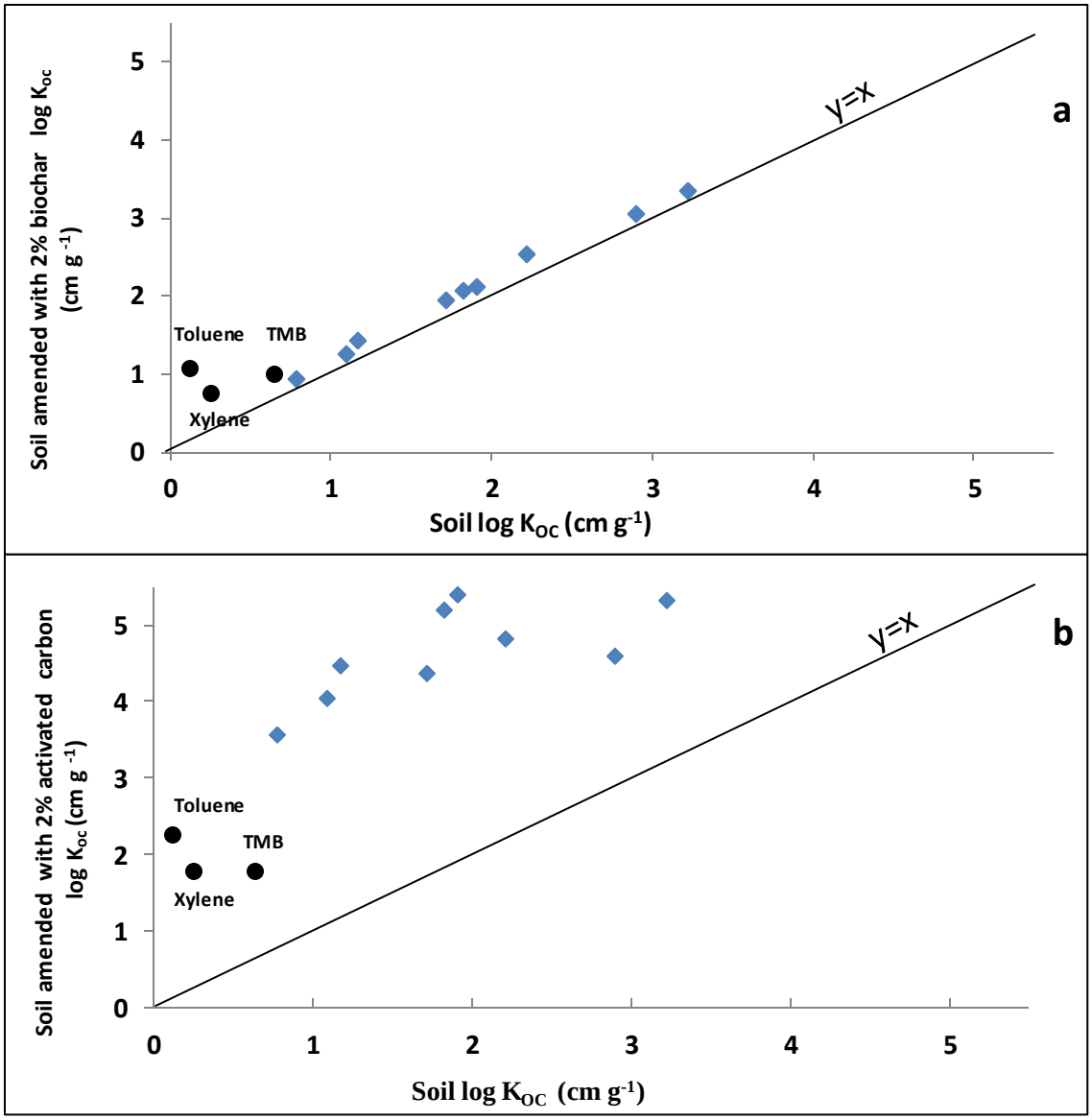

Fig. 5.5. Comparison between log K_{OC} (cm^3 g^{-1}) in soil and soil amended with 2% biochar (a), soil and soil amended with 2% activated carbon (b), for straight-chain, branched or cyclic alkanes (♦) and monoaromatic hydrocarbons (●).

the surface area of activated carbon may greater compared to biochar, making activated carbon more effective sorbent, because the sorption in micropores is likely to be the most important sorption mechanism in activated carbon amended soil (Jonker and Koelmans, 2002)

The K_d values, the first-order biodegradation rates and lag phase periods in this chapter were fairly different from values reported in Chapter 4, Section 4.4.1. For example, the K_d values of pentane, isooctane, toluene and methylcyclohexane in the Newcastle

University, Law School building construction site soil were 53±16, 83±14, 1.3±0.2 and 15±2 cm^3 g^{-1} respectively, while these value for the King's Gate building construction site soil were 20±7, 44±16, 1.9±0.1 and 2.3± 0.7 cm^3 g^{-1} respectively. Moreover, the lag phase period before degradation of toluene and n-octane (as examples) in batches contained the Newcastle University, Law School building construction site soil were longer compared to the lag phase periods of these compounds in the batches contained the King's Gate building construction site soil. For example, there was no lag phase before degradation of toluene and n-octane in the King's Gate building construction site batches, while in the Newcastle University, Law School building construction site soil batches the lag phases were 3 days and 4 day respectively. On the other hand, the lag phase periods before degradation of methylcyclohexane had the opposite behaviour. This period was higher in the Newcastle University, Law School building construction site soil batches compared to the King's Gate building construction site soil batches. Jin *et al.* (1994) suggested that different soils have different biodegradation rates, because degrading bacteria at different soil did not follow the same kinetic reaction and they did not have to be the same species. This may provide an explanation for the differences in the first-order biodegradation rates and lag phase periods between two soils.

5.4.2 Column experiments

5.4.2.1 Estimation of tortuosity

Predicted tortuosities by theoretical relationships based on the measured soil air filled porosity and total porosity yielded very different values. For example, the tortuosity of the soil, soil with biochar and soil with activated carbon columns was estimated to be 0.19, 0.25 and 0.24, respectively, using the Moldrup relationship, (Moldrup *et al.*, 2000) or were 0.41, 0.47 and 0.47, respectively, using the Millington-Quirk relationship, (Millington and Quirk, 1961). Due to these differences in theoretical estimated tortuosity values, column tortuosity was determined by fitting SF_6 measured tracer gas concentrations to the modelled concentrations, which yielded a more reliable tortuosity value. Fig. 5.6 shows the agreement between SF_6 experimental concentrations and the simulated concentrations at a 15 cm and 30 cm distance from the injection port using the diffusion transport equation for an instantaneous plane

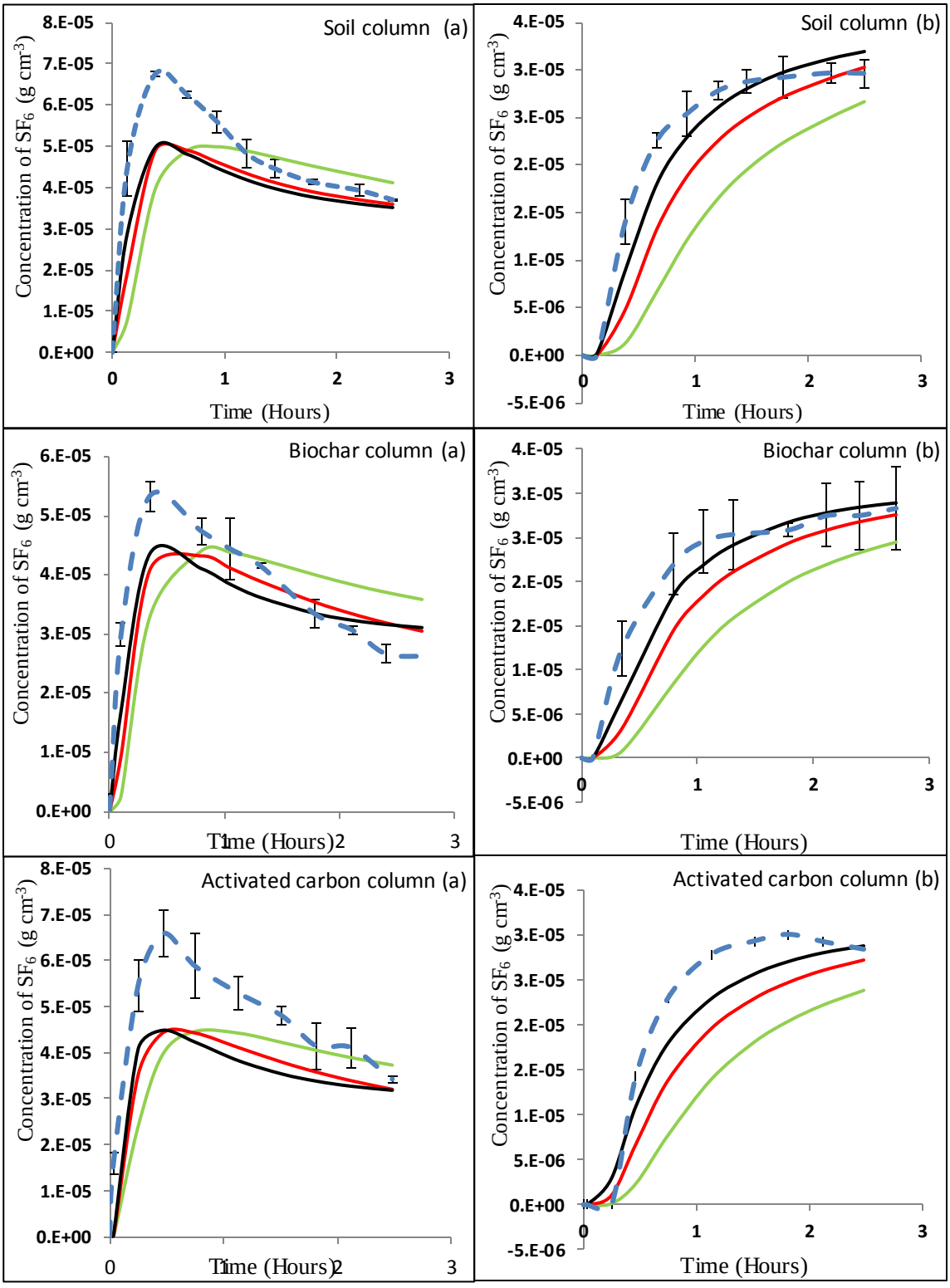

Fig. 5.6. SF_6 breakthrough curves in the soil, biochar and activated carbon column at the sampling port 15 cm aside from the injection port (a) and the sampling port 30 cm aside from the injection port (b); comparing measured (- - -) with modelled concentrations (—— τ_a= 0.4, —— τ_a= 0.6, —— τ_a= 0.8). Error bars are calculated as the standard deviation of duplicate measurements.

source (Equation 5.1) with different tortuosity values. From this figure, it can be seen that the simulated SF_6 concentration when using $\tau = 0.8$ yield more accurate concentration predictions in comparison with simulations using $\tau = 0.6$ or less. It can be concluded that 0.8 should be used as the tortuosity value to simulate the volatilization and migration of VPHs or CO_2. Such a high value of the tortuosity may be due to the fact that the columns were relatively loosely packed in comparison with natural soils.

5.4.2.2 VPHs concentration in soil air

The soil air concentrations of n-hexane, isooctane, methylcyclohexane and toluene measured at sampling port 2 and sampling port 6 (as examples) over a period of 420 days are illustrated in Fig. 5.7. The soil air concentrations of these compounds predicted by the numerical model at the same sampling ports by assuming no biodegradation (k_w = 0) are also illustrated in the same figure. The maximum VPH concentrations were found at sampling point 1 and these concentrations decreased towards the simulated atmospheric boundary at the other end of the column. The figure shows that there are clear differences in the measured soil air concentration profiles for different soil types (i.e. with or without biochar or activated carbon) and chemical groups of VPHs. From this figure, it can be seen that branched and cyclic alkanes soil air concentrations only decreased significantly below those predicted by assuming no biodegradation when concentrations of straight-chain alkanes and monoaromatics were low. This could be attributed to the preferred use of straight-chain alkanes and monoaromatics as a carbon source by the VPH degrading microbes. The sorbent amendments had a strong influence on VPH vapour migration and concentration. For instance, the measured gas-phase concentration of n-hexane and isooctane in the activated carbon column was 10 and 20 times lower compared to their concentration in the soil column, respectively. Moreover, during the first 18 days, the VPH concentrations at the sampling port 1 of the activated carbon column were low. After day 20, the gas phase concentrations of VPHs increased and reached a peak at day 50. The effect of biochar amendment on the measured soil air concentration of VPHs was less remarkable compared to the activated carbon effect. This difference is explained by the differences in K_d values (Table 5.1).

For better visibility and comparison, the measured and the modelled gas-phase concentrations of n-hexane, isooctane, methylcyclohexane and toluene measured at the sampling port 2 and the sampling port 6 during the first 80 days are illustrated in Fig. 5.8 together with the modelled concentrations (assuming no degradation). The measured headspace concentration (n-hexane as example) shows three distinct temporary phenomena which will be interpreted as "lag phase (no biodegradation)", "growth phase (biodegradation with growing bacterial populations)" and "stationary phase (biodegradation with a stable bacterial population equal to the soil's biomass carrying capacity)". These distinct phases were clearly observed in both the soil and biochar columns, but was less notable in the activated carbon amended column. Once the NAPL source was added at the inlet of the soil column or biochar column, VPH vapour concentrations increased at the first three sampling ports. There appears to be no biodegradation in this early period due to the fact that VPH degrading microbes need time to adapt to the use of the pollutant as a carbon source. This period will be called the lag phase. After the three day lag phase period, which is broadly consistent with the lag phase duration observed in batch experiments, the VPH degrading microbes consumed for instance n-hexane as the carbon source and their population probably increased. In this assumed growth phase, the n-hexane gas-phase concentration decreased very markedly. Probably due to the consumption of the available inorganic nutrients, the n-hexane soil-air concentration increased again when this assumed growth phase comes to an end and reached a peak after the 25^{th} day of the experiment. From this day onwards, the scarce availability of nutrients or another soil biomass limiting factor apparently restricted the VPH biodegradation. For instance, the inorganic nutrients may need to be recycled between decaying biomass and growing biomass. The total petroleum hydrocarbon degradation would then become a function of the biomass decay rate. This third period with slower VPH biodegradation will be called the stationary phase. This phenomenon was very clear when n-hexane and toluene were monitored. However, all the VPHs followed this general temporal trend (result not shown), but the extent of the growth phase was variable. These differences may be attributed to substrate-substrate inhibition which refers to the preference of microorganisms for certain substrates when several are available. Although the temporary concentration decrease during the assumed growth phases were not observed for isooctane and methylcyclohexane soil air concentrations at

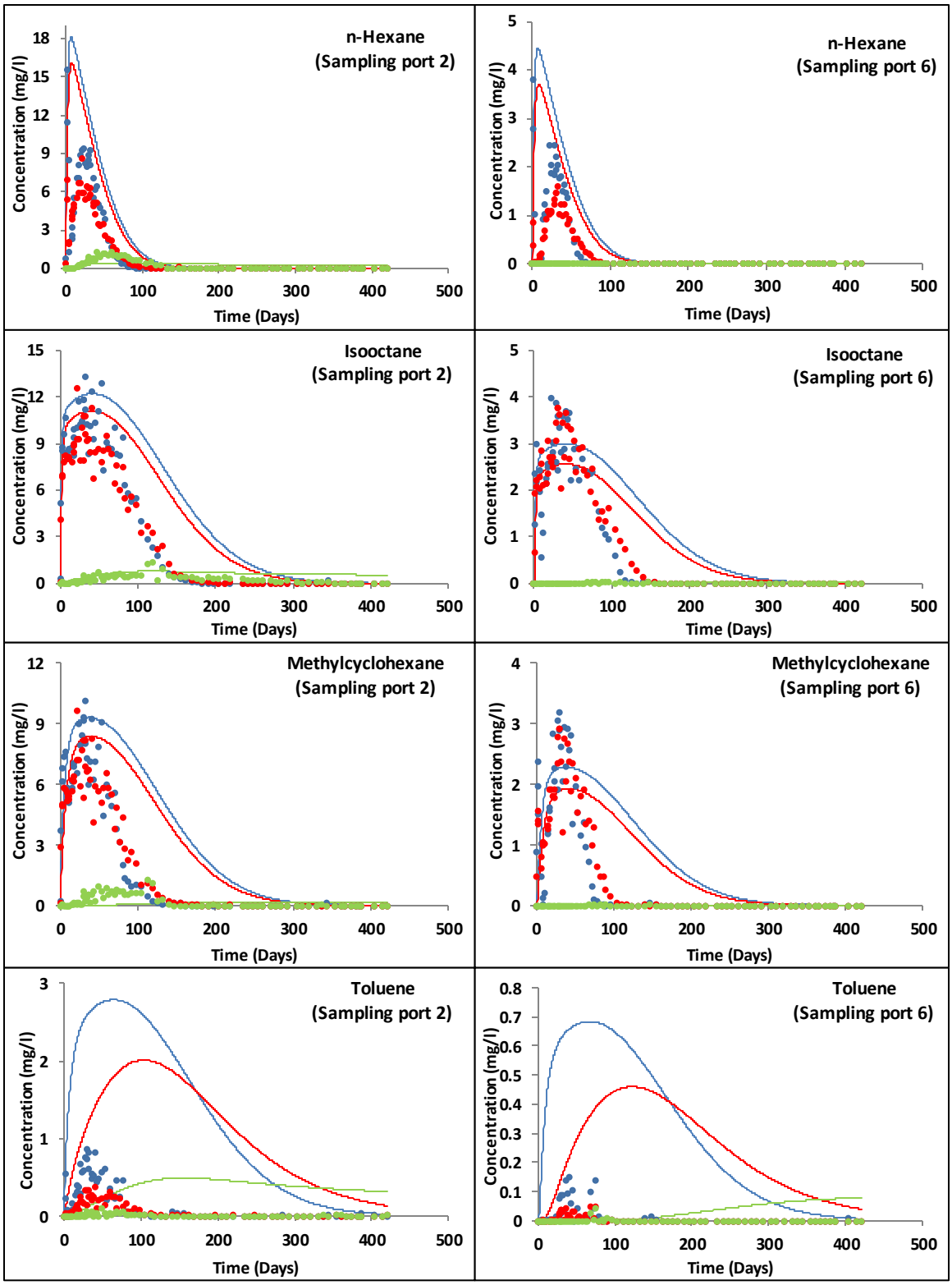

Fig. 5.7. Measured (symbols) and modelled (lines) petroleum hydrocarbon concentrations (C_a) at port 2 and 6 in the soil columns (– ●), the soil amended with biochar column (– ●), and the soil amended with activated carbon column (– ●). Simulations assume no biodegradation (k_w=0).

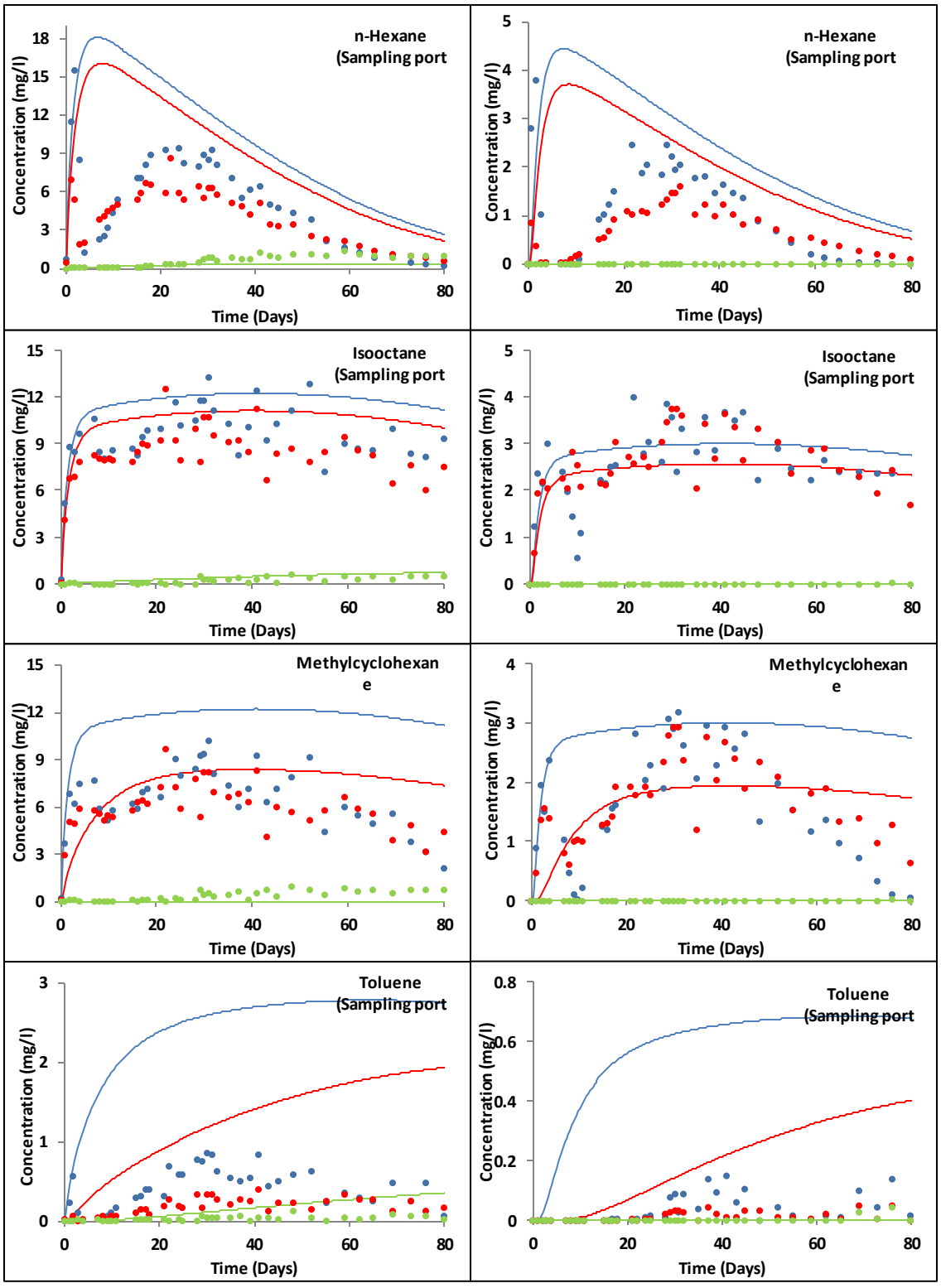

Fig. 5.8. Measured (symbols) and modelled (lines) petroleum hydrocarbon concentrations (C_a) at port 2 and 6 in the soil columns (– ●), the soil amended with biochar column (– ●), and the soil amended with activated carbon column (– ●). Simulations assume no biodegradation (k_w=0).

sampling port 2 in either the biochar or soil column; such a temporary concentration decrease was very clearly visible at sampling port 6 in the same columns, where the other VPH concentrations were much lower. The reduction in availability of other pollutants probably decreased the inhibition effects of monoaromatic and straight-chain alkanes on the biodegradation of branched and cyclic alkanes, which could encourage degrading microbes to consume branched and cyclic alkanes as an alternative carbon source. The lag phase and growth phase were not so clear in the activated carbon amended soil column, which may be attributed to the low availability of VPHs which may have limited biomass growth instead of for instance the inorganic nutrient availability.

The comparison between measurements and simulations assuming first-order rate biodegradation of VPHs in soil pore water (using k_w from Table 5.1) at sampling port 2 and 6 in the soil air of the three experimental columns is illustrated in Fig. 5.9. The measured soil- air concentration of n-hexane and toluene at sampling port 2 and 6 of the soil column show that concentrations initially rise above those predicted by the first-order rate model, which is consistent with an assumed lag-phase before the on-set of biodegradation. The measured gas-phase concentrations of hexane and toluene then fell below those of the model predictions based on the first-order biodegradation rates determined in the batch studies. These observations are consistent with an apparent growth phase during which an increasing abundance of hexane and toluene degrading microorganisms would accelerate the biodegradation of these compounds beyond the measurements of the batch study. Toluene and hexane concentrations rose again above those predicted by the first-order rate biodegradation model following this assumed growth phase, presumably when their transformation was limited by scarce inorganic nutrient availability when the readily available nutrients have been used up. For isooctane, on the other hand, measured concentrations were always higher than predicted, presumably because of substrate-substrate inhibition prevents the degradation of this least preferred substrate in the column study. In short, the microbial degradation in the columns cannot be accurately described by a simple first-order rate kinetics model, and the measurements therefore do not correspond well to the model prediction. This is probably a consequence of the complex dependence of the biodegradation on many factors such as substrate-substrate inhibition, the

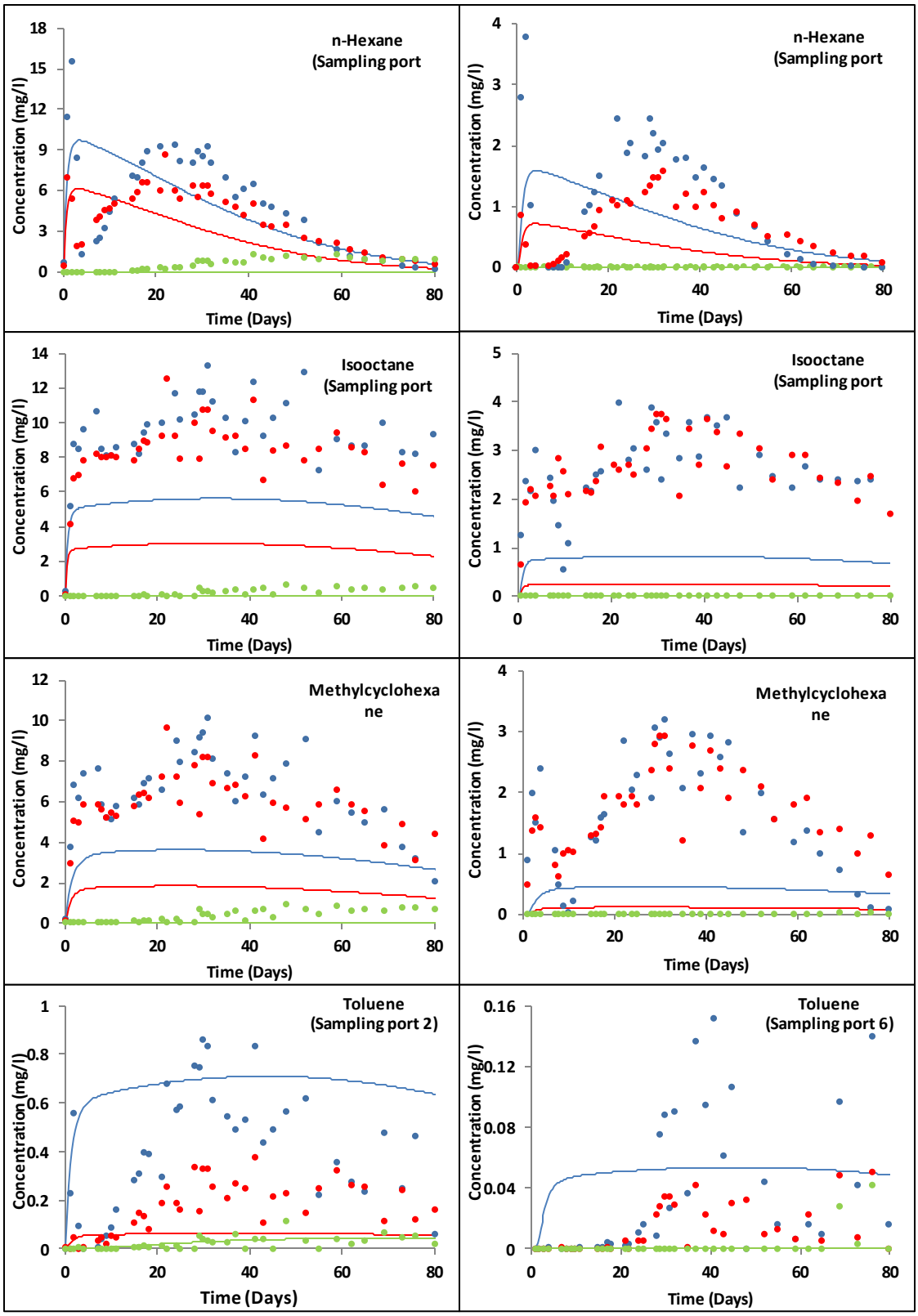

Fig. 5.9. Measured (symbols) and modelled (lines) petroleum hydrocarbon concentrations (C_a) at port 2 and 6 in the soil columns (– ●), the soil amended with biochar column (– ●), and the soil amended with activated carbon column (– ●). Simulations assuming biodegradation are using the k_w data from Table 5.1.

bioavailability of pollutants and nutrients, biomass growth and decay, nutrient recycling, and the variable composition of soil microbial communities. Moreover, the model could not describe the biodegradation in either the growth or stationary phase period, because the changes in nutrient availability and nutrient recycling were not represented in the model. Because of the large number of variables potentially involved in controlling VPH biodegradation, it is difficult to determine the contribution of a change in an individual factor. Therefore, the correct prediction of the biodegradation kinetics remains a difficult task. A more complicated model including substrate-substrate inhibition, the availability of nutrients, biomass decay, nutrient recycling, and microbial population dynamics is needed to describe the biodegradation process more accurately.

The column experiments have shown that the strongly inference of activated carbon amendment on the soil air concentration and migration of VPHs compared to biochar amendment. These results are consistent with many previous studies (Lichtenstein *et al.,* 1968; Strek *et al.,* 1981; Vasilyeve *et al.,* 2001, Rhodes *et al.,* 2008), which found that the availability of petroleum hydrocarbons decreased in soil treated by activated carbon and the great surface area of activated carbon makes activated carbon more effective sorbent (Jonker and Koelmans, 2002). This column experiments show the relationship between the biodegradation and chemical groups of VPHs which was cold substrate-substrate inhibition. The branched and cyclic alkanes soil air concentrations only decreased significantly when concentrations of straight-chain alkanes and monoaromatics were low which reflect preference of the VPH degrading microbes to consumption of straight-chain alkanes and monoaromatics as a carbon source. These results are consistent with those of Atlas (1981) who reported that the branched alkanes are known to be slowly biodegraded due to steric problems imposed by side chains and are consistent with those of Pasteris *et al.* (1997) who reported that aromatic compounds and long chain alkanes have high biodegradation rates compared to the branched and cyclic alkanes. Furthermore, the soil and biochar column results show that the scarce availability of nutrients in the stationary phase apparently restricted the VPH biodegradation and VPHs degradation become a function of the biomass decay rate which support the earlier studies (Styriakova *et al.,* 2009; Tiehm *et al.,* 2010)) who reported that the availability of nutrients is a key factor in the

enhancement of hydrocarbon degrading microbes at contaminated sites. The activated carbon amendment slowed both the biodegradation and migration of VPHs which increased their retention time in the column. This benefitted the VPHs attenuation in the activated carbon amended columns, because increased retention time means more time is available to degrade the pollutants and recycle nutrients which increased the cumulative amount VPHs vapours degraded as observed previous studies (Rhodes *et al.*, 2008; Vasilyeve *et al.*, 2010; Rhodes *et al.*, 2012). As demonstrated by Hohener *et al.* (2003), the interpretation of kinetic biodegradation parameters could be challenged, due to many factors limiting microbial growth (Battersby, 1990).

In this context, the effect of sorbent amendments on the pollutant fate is even more difficult to predict, but some qualitative observations can be made. The activated carbon amendment apparently resulted in greater pollution attenuation, if one considers the pollution attenuation over the entire length of the soil column and the duration of the experiment. The activated carbon amendment slowed the petroleum hydrocarbon vapour migration and also slowed the biodegradation by decreasing the availability of both VPHs and possibly also nutrients. Biochar and activated carbon amendments reduced the early contaminant break-through in the lag phase, because the sorption-retarded vapour migration and chromatographic separation of different VPH compounds according to their solid-air partitioning coefficients gave soil microorganisms more time to adapt to the presence of VPHs. The higher sorption capacity and correspondingly lower VPH concentrations in soil air make the growth phase period less obvious in the activated carbon amended soil column. Low VPH availability means the activated carbon reduced the cumulative amount of petroleum hydrocarbon vapours degraded over a certain column length for the duration of the growth phase period. In the stationary phase; however, nutrient recycling or another factor seems to limit the biodegradation of petroleum hydrocarbons in un-amended soil and soil with biochar, and only a fixed amount of petroleum hydrocarbons can be degraded per volume of soil and time under such circumstances, equivalent to the amount of biomass which can for instance grow on the recycled nutrients. This "quasi-zero order" VPH degradation likely benefitted the VPHs attenuation in the activated carbon amended columns, because slower migration and chromatographic separation of different VPH compounds means more time is available to degrade the pollutants

and more time means more biodegradation, if the amount of pollutants degraded per time is constant ("quasi-zero order" kinetics). Furthermore, activated carbon amendment also benefitted the degradation of cyclic and branch alkanes by chromatographically separating them from other VPH compounds and by generally reducing bioavailable VPH concentrations, thus decreasing the impacts of substrate-substrate inhibition. The low availability of all VPHs in the activated carbon amended columns facilitates the biodegradation of less preferred carbon substrates such as the cyclic and branched alkanes.

5.4.2.3 Carbon dioxide concentration

Measured profiles of CO_2 concentrations in the soil air at two column sampling ports are illustrated in Fig. 5.10 for soil (Fig. 5.10a), soil amended with 2% biochar (Fig. 5.10b) and soil amended with 2% activated carbon (Fig. 5.10c). On day zero, the background CO_2 concentrations were 0.0022, 0.0029 and 0.0014 g l^{-1} for the soil, biochar and activated carbon columns respectively. Once the NAPL source was added at the bottom of the column, CO_2 levels slightly increased at the first three sampling ports of the soil and biochar amended soil columns. However, the CO_2 levels in the activated carbon amended soil column started to increase only after 8 days. The small CO_2 production in this early period was due to the fact that the degrading microbes need time to adapt and use the pollutant as a carbon source, which known as the 'lag phase'. Within the first 10 days, the CO_2 levels at sampling port 2 rose sharply to reach a peak of 0.068 and 0.031 g l^{-1} for the soil and biochar amended soil column, respectively, and then there was a steady drop of the CO_2 concentration until day 17. Again the CO_2 concentration increased to reach its highest level at day 21. The reduction followed by an increase in CO_2 production may be attributed to the reduction of the nutrients availability followed by nutrients recycling after a collapse of the newly formed microbial population which may be the reason why the CO_2 production increased again after day 17 to reach another peak. This indicates that microbial populations may be unstable during the apparent "growth phase", with rapid growth and decay and re-growth of VPH degrading biomass. The CO_2 levels at sampling port two after day 24 decreased and fluctuated between 0.025

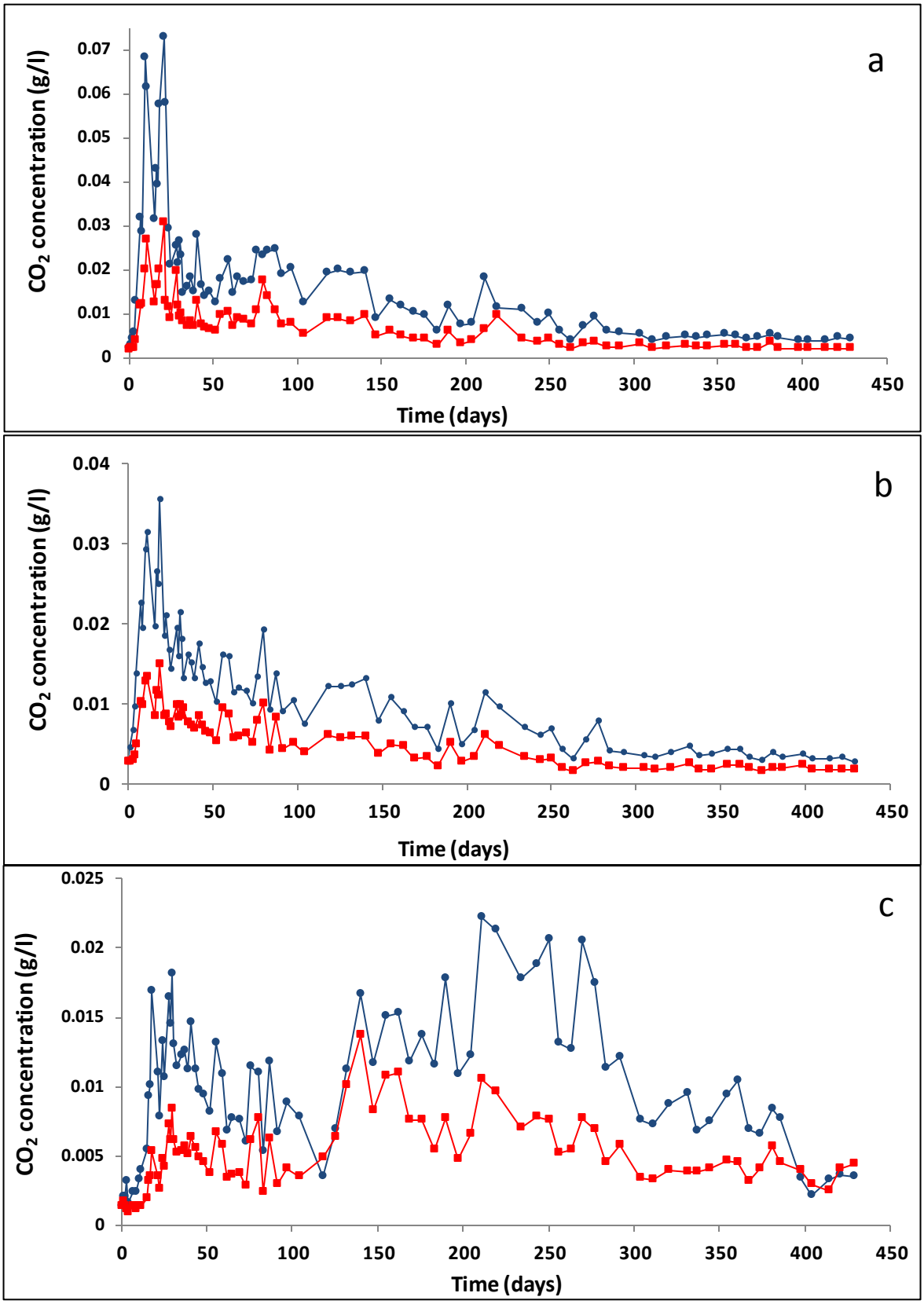

Fig. 5.10. CO_2 concentration profiles at sampling port 2 (—●—) and sampling port 6 (—■—), for soil column (a), biochar column (b) and activated carbon column (c).

to 0.017 g l^{-1} for the soil column and between 0.017 to 0.011 g l^{-1} for biochar amended soil column. After day 280, the CO_2 concentration remained fairly constant around 0.0045 g l^{-1} for soil column and 0.0035 g l^{-1} for the biochar amended soil column. In the activated carbon amended soil column, after an 8 day lag phase, the CO_2 concentration at sampling port 2 rose sharply to reach a peak of 0.017 g l^{-1}, and then the CO_2 concentration steadily dropped until day 24; when the CO_2 concentration increased again to reach its highest level on day 30. Between day 31 and 100, the CO_2 concentration decreased and fluctuated between 0.014 to 0.008 g l^{-1}. Between day 132 and 292, further changes in the CO_2 concentration were observed. The CO_2 concentration rose sharply to reach a peak of 0.017 g l^{-1} on day 140, and then fluctuated between 0.021 and 0.012 g l^{-1} during the next 5 months. Again, the CO_2 levels decreased to 0.008 g l^{-1} on day 304, and then fluctuated between 0.01 and 0.008 g l^{-1}; after day 400, the CO_2 levels decreased to around 0.003 g l^{-1}. During the experiments, all the columns were still under aerobic conditions, with O_2 concentrations at about atmospheric concentrations and CO_2 values at all sampling ports were always below 4.0, 1.9 and 1.3 % (v/v) for the soil, biochar and activated carbon amended soil columns, respectively. The CO_2 concentration was higher near the VPH source and decreased toward the end of column. This result is consistent with Hohener *et al.* (2003) and Pasteris *et al.* (2002) results. However, the CO_2 concentrations were fairly different with values reported by Hohener *et al.* (2003) and Pasteris *et al.* (2002).

5.4.3 Cumulative flux

5.4.3.1 VPHs cumulative flux

The cumulative flux of VPHs leaving the activated carbon amended soil column during 420 days was very low compared to the soil or biochar amended soil column, and these fluxes are reported in Table 5.2. The activated carbon column released 0.032 g of total VPHs (0.22 % of total VPHs added to column), while soil column and biochar column released 2.96 g and 3.28 g of total VPHs respectively, which represent 20.26 % and 22.45 % of total VPHs added for the soil column and the biochar column respectively. These mass fluxes have an estimated relative uncertainty of 15.0%, 10.6% and 14.9% for soil, biochar and activated carbon column because the

flux dependents on several measured parameters such as the effective diffusion coefficient and uncertainty in the measurement of these parameters will affect the mass flux. The differences in the total cumulative VPH mass flux and each compound mass flux between the activated carbon amended soil column and either, the soil or biochar amended soil columns, were greater than the estimated uncertainty, while the difference in total cumulative VPH mass flux and cumulative flux of n-pentane, n-hexane, methylcyclopentane, cyclohexane, methylcyclohexane, n-octane and m-xylene between the soil and biochar amended soil column was within the range of the estimated uncertainty. However, the difference in cumulative flux of toluene, 1,2,4 trimethylbenzene, n-decane and n-dodecane were greater than the estimated uncertainty for these two soil columns. The cumulative flux of n-pentane, isooctane, methylcyclohexane, and toluene respectively are shown in Fig. 5.11. From this figure, it can be seen that the cumulative isooctane mass flux from both, the soil and biochar amended soil columns, was higher in comparison with n-pentane or methylcyclohexane or toluene. For example, 0.78 g of isooctane was released from the soil column, while 0.27 g of pentane, 0.44 g of methylcyclohexane and 0.006 g of toluene were released from the same column (Table 5.1). The greatest isooctane flux was in the beginning of the soil column experiment when other VPHs were preferred by degrading bacteria. These results are consistent with Hohener *et al.* (2003) study who found that the fluxed amounts of branched and cyclic alkanes from alluvial sand column were great compared to monoaromatic or straight-chain alkanes. Cumulative fluxes from the activated carbon amended soil column were clearly the lowest. For instance, 0.005 g of n-pentane, 0.0022 g of isooctane, 0.0033 g of methylcyclohexane and 0.0014 g of toluene were released from the activated carbon amended soil column.

The effects of sorbent material type on cumulative fluxes are illustrated in Fig. 5.12 for pentane (Fig. 5.12a), isooctane (Fig. 5.12b), methylcyclohexane (Fig. 5.12c) and toluene (Fig. 5.12d). From this graph and Table 5.2, it can be seen that activated carbon amendment largely reduced the cumulative fluxes out of the column for all VPHs. Although biochar amendment slightly reduced fluxes of pentane, hexane and octane, as well as most monoaromatic compounds, the accumulative fluxes of straight-chain and cyclic alkenes increased in comparison with the unamended soil column. For example, the cumulative flux of hexane from the biochar column was 0.23 g,

which was somewhat lower in comparison with cumulative flux from the soil column (0.29 g). The cumulative fluxes of isooctane and cyclohexane from the biochar column were 0.88 g and 0.58 g respectively, which were somewhat higher compared to accumulative fluxes from soil column (0.78 g and 0.52 g respectively).

Table 5.2. Cumulative flux of VPHs and CO_2 from different columns over 420 days expressed as mass and % of the total mass initially in the NAPL source.

Compound	Added	Soil column		Biochar column		Activated carbon column	
	(g)	(g)	(%)	(g)	(%)	(g)	(%)
n-Pentane	0.62	0.27	43.55	0.25	40.32	0.005	0.81
n-Hexane	1.13	0.29	25.66	0.23	20.35	0.0004	0.04
Methylcyclopentane	1.11	0.63	56.76	0.74	66.67	0.0015	0.14
Cyclohexane	1.24	0.52	41.94	0.58	46.77	0.012	0.97
Isooctane	2.07	0.78	37.68	0.88	42.51	0.0023	0.11
Methylcyclohexane	1.83	0.44	24.04	0.57	31.15	0.0033	0.18
Toluene	0.69	0.006	0.87	0.002	0.29	0.0014	0.20
n-Octane	1.11	0.014	1.26	0.014	1.26	0.0025	0.23
m-Xylene	0.85	0.0056	0.66	0.005	0.59	0.0018	0.21
1,2,4 Trimethylbenzene	1.05	0.001	0.1	0.004	0.38	0.001	0.10
n-Decane	2.02	0.001	0.05	0.002	0.10	0.001	0.05
n-Dodecane	0.89	0	0.00	0.0001	0.01	0	0.00
Total VPHs	14.61	2.96	20.26	3.28	22.45	0.032	0.22
CO_2		12.77		10.73		13.55	

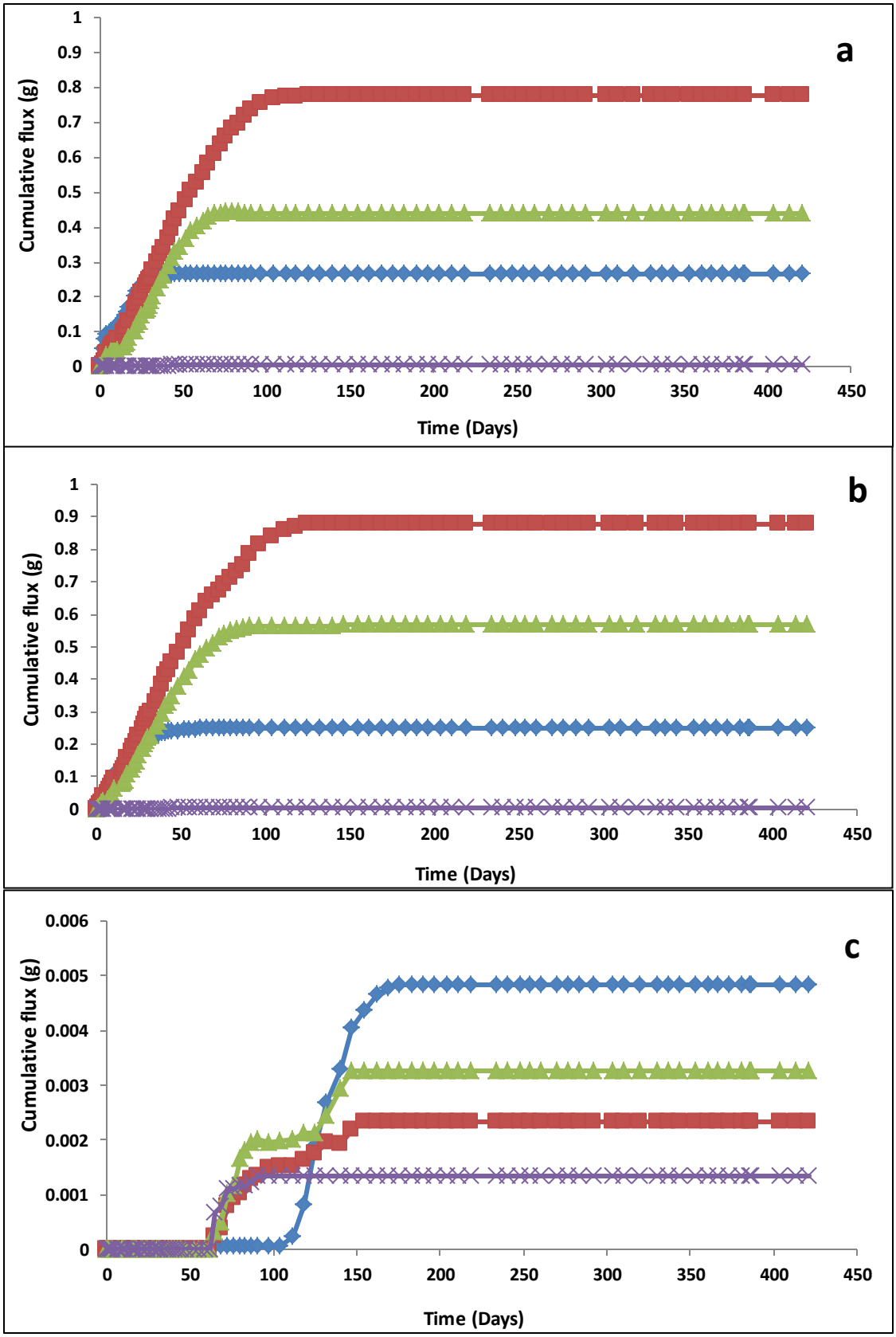

Fig. 5.11. Cumulative flux of n-pentane (), isooctane (), methylcyclohexane () and toluene () for the soil column (a), the biochar amended soil column (b) and the activated carbon amended soil column (c).

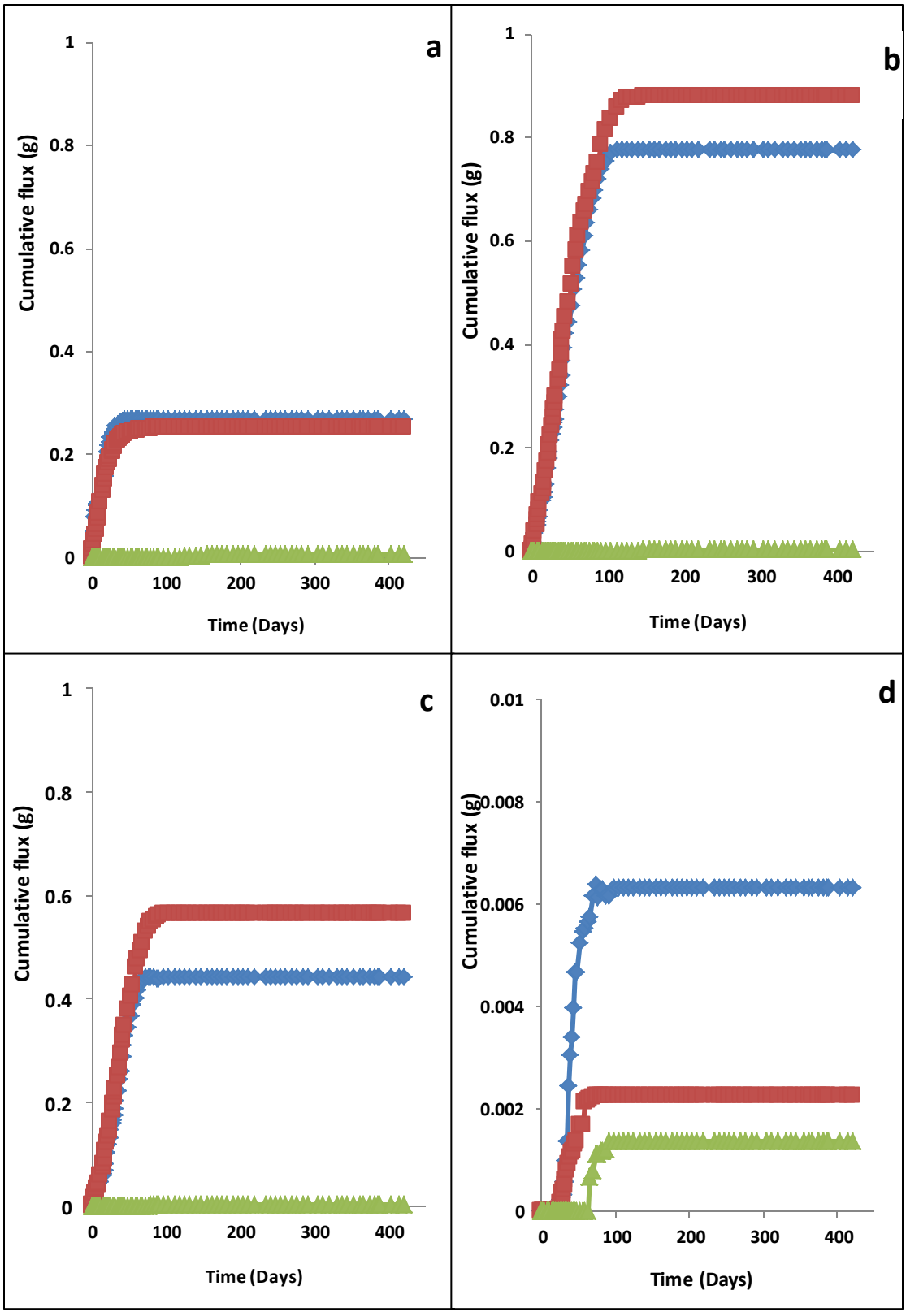

Fig. 5.12. Cumulative flux of n-pentane (a), isooctane (b), methylcyclohexane (c) and toluene (d), comparing ( ) soil column, ( ) biochar column and ( ) activated carbon column.

5.4.3.2 Cumulative flux of CO_2

Cumulative fluxes of CO_2 released from the soil, biochar and activated carbon columns are illustrated in Fig. 5.13. It can be seen that the flux of CO_2 from the activated carbon column was slightly higher compared to the soil column, and the biochar column released the lowest CO_2 flux. For example, 13.55 g of CO_2 was released from the activated carbon column, while the soil and biochar columns released 12.77 g and 10.73 g of CO_2, respectively (Table 5.2). However, these differences were within the range of the estimated uncertainty of the CO_2 mass flux. However, the trend in the CO_2 flux was very distinct for the soil with activated carbon. The cumulative CO_2 fluxes from the soil and biochar amended soil column significantly increased over the first 4 months, and then the flux became much slower, whereas the cumulative CO_2 flux from the activated carbon amended soil column rose slower, but more steadily. After 4 months, the cumulative CO_2 flux from the activated carbon amended soil column continued to increase steadily, and after 6 months the cumulative flux surpassed those in the other columns slightly to reach the highest overall cumulative flux (13.55g). This suggests that the VPHs retained in the activated carbon amended soil column continue to be biodegraded long after they have broken through and emanated from the other columns. The contribution of different parts of the column in the cumulative flux of CO_2 is illustrated in Fig. 5.13 for the NAPL source side of the column (Fig. 5.13a), the middle of column (Fig. 5.13b), and the atmospheric boundary side of column (Fig. 5.13c). Although the contribution of the NAPL source side column part in the cumulated flux of CO_2 was generally higher and the contribution reduced with increasing distance from the polluted source and decreasing VPH concentrations, as would be expected, there was a notable difference between the three columns. For instance, the NAPL source side of the soil column contributed 7.85 g of the cumulative CO_2 flux (representing 61.5% of total flux), while the middle and atmospheric boundary side of the soil column contributed 3.42 g and 1.5 g, respectively (representing 26.8% and 11.7% of total flux respectively). The NAPL source side of the activated carbon amended soil column contributed 5.3 g of the cumulative CO_2 flux (representing 39.1% of total flux), and the middle and atmospheric boundary side of the soil column contributed 4.3 g and 3.95 g, respectively (representing 31.7% and 29.2% of total flux respectively).

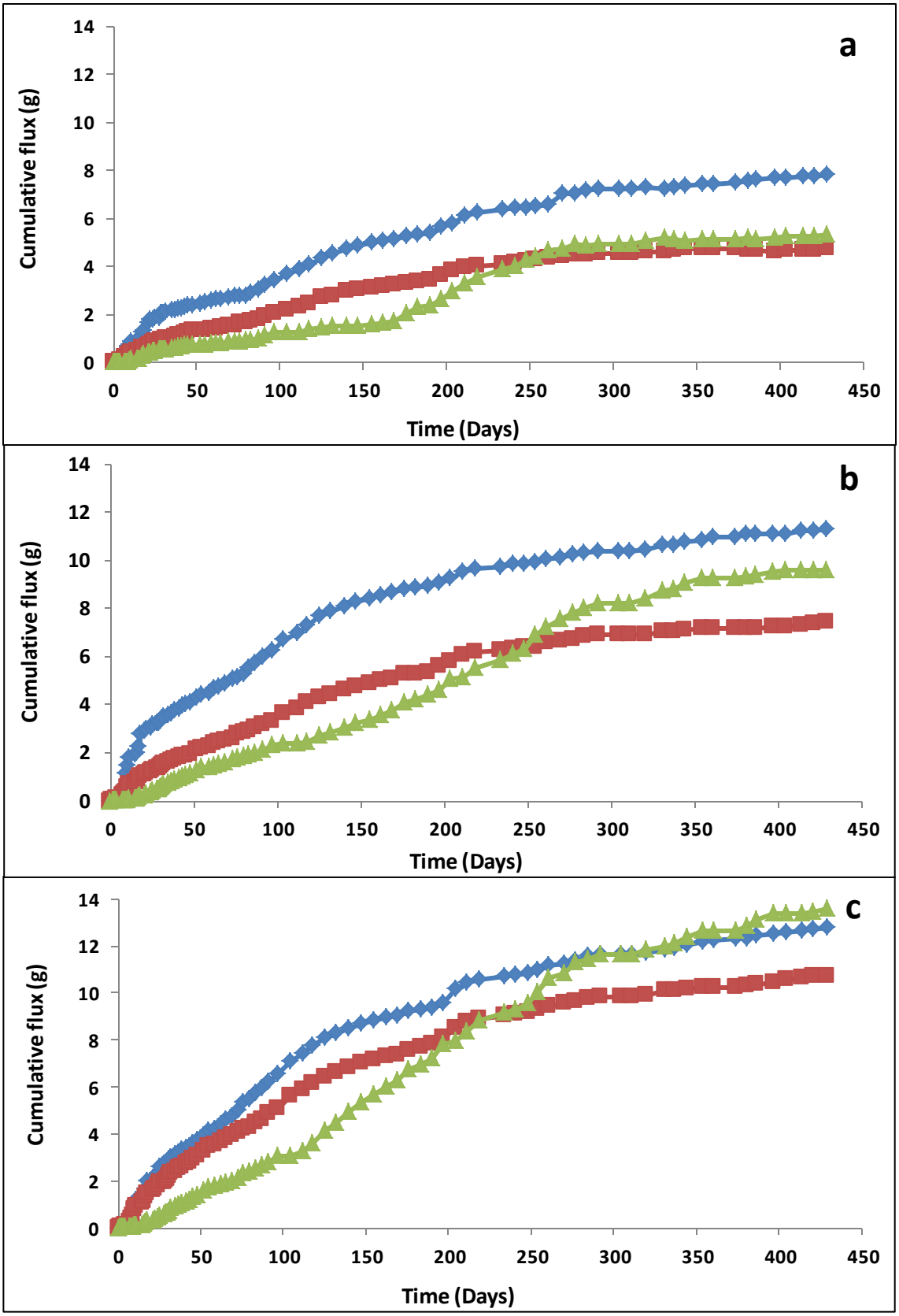

Fig. 5.13. Cumulative flux of CO_2 from the soil column (), biochar amended soil column () and activated carbon amended soil column () for the NAPL source side of the column (a), the middle of the column (b), and the atmospheric boundary side of the column (c).

5.4.4 Mass balance

The initial NAPL source was introduced to one side of each column by connecting a vial containing a mixture of 20 mls of VPHs, which represents 12.5 g of carbon weight per 20 mls. After 420 days, the NAPL sources still contained 1.15±0.09, 1.36±0.1 and 0.65±0.03 g VPH carbon for the soil, biochar amended soil and activated carbon amended soil columns, respectively. Interestingly, the activated carbon amended column had the lowest residual NAPL amount in the source, presumably because the sorption of the less biodegradable VPHs assisted with their volatilisation. The residuals of each VPH as the carbon weight, the estimated volatilized mass and VPH residuals in the soil, biochar amended soil and activated carbon amended soil columns are shown in Table 5.3. After 420 days, the concentrations of the most common compounds were very low in the NAPL sources, except for decane and dodecane, which were enriched in the NAPL residual. For example, there were 0.736±0.02, 0.766±0.06 and 0.58±0.03 g of dodecane in the soil column NAPL source, the biochar column NAPL source and the activated carbon column NAPL source, respectively. This phenomenon may be attributed to the very low emission of these compounds from the NAPL sources because these compounds are semi-volatile and their vapour pressure in comparison with other compounds in the VPHs mixture was very low. The VPH residuals in the soil column were under the detection limit, while the biochar amended soil column had 0.005±0.002 g of cyclohexane, which was sorbed. Moreover, the activated carbon amended soil contained 0.001±0.0005g of cyclohexane, 0.34±0.04 g of isooctane, 0.01±0.001 g of methylcyclohexane and 0.049±0.003 g of n-octane as carbon, which reflects the effect of the high VPH sorption capacity of activated carbon, and also the comparatively poor biodegradability of branched and cyclic alkanes as previously demonstrated (Bolan and Baskaran, 1996; Guo *et al.*, 2000; Jonker and Koelmans, 2002; Jensen *et al.*, 2004; Werner *et al.*, 2005; Rhodes *et al.*, 2008; Jones *et al.*, 2011).

The contributions of different factors in the total VPH mass balance for different columns are illustrated in Table 5.4. The residuals of VPHs in the NAPL source and soil were small. The contributions of VPH volatilization from the soil and biochar amended soil columns were high, whereas the contribution of VPH volatilization from activated carbon amended soil column was negligible. Carbon mass retained in the

Table 5.3. Mass balance of each VPH as the carbon weight for column experiments.

Compound	Added (g)	Soil column			Biochar column			Activated carbon column		
		Source (g)	Soil residual (g)	Volatilized from column (g)	Source (g)	Soil (g)	Volatilized from column (g)	Source (g)	Soil (g)	Volatilized from column (g)
n-Pentane	0.52	1.2E-04±6E-06	*	0.225	4.2E-04±1E-04	*	0.208	2E-04±2E-05	*	0.004
n-Hexane	0.94	9.8E-05±5E-05	0.0	0.243	5.2E-05±3E-05	0.0	0.192	3E-05±3E-05	0.0	0.000
Methylcyclopentane	0.95	1.4E-04±7E-06	0.0	0.539	8.3E-05± 2E-06	0.0	0.634	3E-05±1E-06	0.0	0.001
Cyclohexane	1.06	1.9E-04±9E-06	0.0	0.445	1.3E-04±1E-05	0.005±0.002	0.497	5-05±1E-06	0.001±0.0005	0.010
Isooctane	1.73	3.5E-04±2E-05	0.0	0.650	2.4E-04±3E-05	0.0	0.734	8 E-05±1E-06	0.344±0.04	0.002
Methylcyclohexane	1.57	3E-04±2E-05	0.0	0.377	2.0E-04±3E-05	0.0	0.488	6.7E-051E-06	0.010±0.001	0.003
Toluene	0.63	0	0.0	0.005	0	0.0	0.002	0	0.0	0.001
n-Octane	0.94	0	0.0	0.012	0	0.0	0.012	0	0.049±0.003	0.002
m-Xylene	0.77	0	0.0	0.005	0	0.0	0.005	0	0.0	0.002
1,2,4 Trimethylbenzene	0.94	0.056±0.01	0.0	0.001	0.071±0.01	0.0	0.004	0.0013±3E-04	0.0	0.001
n-Decane	1.71	0.36±0.06	0.0	0.001	0.419±0.06	0.0	0.002	0.07±6E-03	0.0	0.001
n-Dodecane	0.75	0.736±0.02	0.0	0.000	0.766±0.06	0.0	0.000	0.58±0.03	0.0	0.000
Total	12.50	1.15 ±0.09	0.0	2.5	1.36±0.1	0.005±0.002	2.78	0.65±0.03	0.40±0.05	0.028

* Pentane did not quantify due to the dichloromethane/pentane extraction method.

Table 5.4. Mass balance of total VPH as the carbon weight for column experiments. Unaccounted mass may be explained by experimental error or poor recovery of volatile petroleum hydrocarbon.

Column	Total added mass	Source	Soil residual	Volatilized from column	Volatilized as CO_2	Biomass [a] as C	Total	Retained carbon as carbonate	Retained carbon as organic matter [c]
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
Soil column	**12.50**	**1.15±0.09**	**0.0**	**2.5**	**3.49**	**0.080±0.026**	**7.22**	**2.0±0.03**	**5.4±0.1**
Biochar column	**12.50**	**1.36±0.1**	**0.005±0.002**	**2.78**	**2.93**	**0.037±0.023**	**7.11**	**14.1±0.1**	**1.6±0.01**
Activated carbon column	**12.50**	**0.65±0.03**	**0.40±0.05**	**0.028**	**3.70**	**0.063±0.019**	**4.84**	**5.73±0.1**	**16±2.7**

a Calculated based on difference between initial and final microbial cell numbers and the dry mass carbon of one microbial cell is equal to 100 femto-gram (Whitman *et al*., 1998).

b Calculated based on difference between total carbon and total organic carbon in the beginning and the end of experiments.

c Calculated based on difference between total organic carbon in the beginning and the end of experiments.

Table 5.5. Available ammonium, nitrate and phosphate, total carbon, total organic carbon and total cell number in column before and after the column experiments.

Material	Initial (before set up the experiment)					
	Ammonium	Nitrate	Phosphate	TOC	TC	Total cell number *
	$\mu g\ NH_4^+ - N\ g^{-1}$	$\mu g\ NO_3^- - N\ g^{-1}$	$\mu g\ PO_4^{-3} - P\ g^{-1}$	(% as dry weight)	(% as dry weight)	cells g^{-1} dry soil
Sandy soil	2.9 ±0.2	8.2±2.0	0.8±0.07	1.6±0.12	4.06±0.24	$2.74\times10^7\pm1.5\times10^6$
Soil with biochar (2%)	5.8 ±0.5	5.5±0.57	0.2±0.02	3.0±0.1	5.08±0.23	$7.75\times10^7\pm1.6\times10^6$
Soil with activated carbon (2%)	6.1 ±1.2	6.5±1.0	0.3±0.03	2.7±0.3	5.02±0.27	$3.87\times10^7\pm7.4\times10^6$
	Final (after the end of experiment)					
Soil column (the NAPL source side)	0.18±0.04	4.0±0.4	0.2±0.02	1.98±0.36	4.13±0.11	$2.14\times10^8\pm1.3\times10^7$
Soil column (the middle of column)	0.18±0.05	6.8±0.3	0.4±0.03	1.59±0.06	4.10±0.19	$1.15\times10^8\pm6.6\times10^6$
Soil column (the atmosphere boundary side)	0.24±0.03	6.5±0.5	0.7±0.03	1.86±0.02	4.27±0.02	$1.86\times10^8\pm4.1\times10^6$
Biochar column (the NAPL source side)	0.06±0.003	1.1±0.5	0.07±0.004	2.75±0.08	5.11±0.25	$2.02\times10^8\pm1.6\times10^7$
Biochar column (the middle of column)	0.05±0.01	2.1±0.1	0.12±0.02	3.16±0.06	5.10±0.02	$1.39\times10^8\pm1.1\times10^7$
Biochar column (the atmosphere boundary side)	0.04±0.01	2.2±0.1	0.13±0.03	3.0±0.26	5.86±0.02	$1.12\times10^8\pm4.6\times10^6$
Activated carbon column (the NAPL source side)	0.04±0.01	0.0±0.0	0.17±0.02	4.10±0.07	5.68±0.09	$1.8\times10^8\pm4.6\times10^7$
Activated carbon column (the middle of column)	0.05±0.01	0.18±0.01	0.16±0.01	3.84±0.17	5.7±0.28	$1.25\times10^8\pm1.1\times10^7$
Activated carbon (the atmosphere boundary side)	0.05±0.01	2.4±0.02	0.12±0.02	4.85±0.18	6.86±0.16	$1.79\times10^8\pm9.1\times10^6$

* The total microbial number is provides courtesy of George Mangse.

microbial cells was very low. However, the emanating carbon mass leaving the columns as CO_2 had large contributions in the total carbon mass balance of all the three columns. The retained carbon mass as carbonate was calculated based on the difference between total carbon and total organic carbon. The retained soil carbon mass either as carbonate or organic matter also had a potentially high contribution in the mass balance, but with great uncertainty due to the high initial carbonate and organic content of the soil columns relative to the VPH carbon added. The contributions of biomass were small (Table 5.4), due to scarce availability of nutrients probably restricting the soil biomass carrying capacity. At the beginning of the column experiment, when the inorganic nutrients were available, the bacteria grew and increased their population until the available inorganic nutrients were consumed. From then onwards, biomass decay is necessary to recycle inorganic nutrients and the biomass weight in the columns is limited. For example, initial microbial numbers in soil were $2.74\times10^{7}\pm1.5\times10^{6}$ cells g^{-1} of dry soil and after 420 days they were $2.14\times10^{8}\pm1.3\times10^{7}$, $1.15\times10^{8}\pm6.6\times10^{6}$ and $1.86\times10^{8}\pm4.1\times10^{6}$ cells g^{-1} of dry soil for the NAPL source side, the middle and atmospheric boundary side of the soil column respectively, about a tenfold increase. Biochar and activated carbon amended soil columns have the same behaviour (Table 5.5). The increases in the total microbial numbers added 0.08±0.026, 0.037±0.023 and 0.063±0.019 g of carbon to soil, biochar and activated carbon column, respectively. The increases in the total microbial numbers in sandy soil after exposure to VPH vapour are consistent with Hohener *et al.* (2003) study who found same behaviour but different microbial numbers. This could be explained by differences in soil, properties, nutrient content and the duration of experiment.

The differences in available ammonium, nitrate and phosphate concentrations between the beginning and end of the column experiments are presented in Table 5.5. It is clear that the reduction in the availability of ammonium and phosphate in soil, biochar and activated amended soil columns were greater compared to the reduction in nitrate availability after 420 days of the columns experiment. For example, available nitrate was decreased from 8.2±2 μg $NO_3^- - N\ g^{-1}$, 5.5±0.57 μg $NO_3^- - N\ g^{-1}$ and 6.5±0.1 μg $NO_3^- - N\ g^{-1}$ in soil, biochar and activated carbon amended soil columns respectively to the range between 4.0±0.4 μg $NO_3^- - N\ g^{-1}$ to 6.8±0.3 μg $NO_3^- - N\ g^{-1}$ in soil

column, to the range between 1.1±0.5 μg $NO_3^- - N\ g^{-1}$ to 2.2±0.1 μg $NO_3^- - N\ g^{-1}$ in biochar column and to the range between 0.0±0.0 μg $NO_3^- - N\ g^{-1}$ to 2.4±0.02 μg $NO_3^- - N\ g^{1}$ in activated carbon amended soil column.

Microorganisms may assimilate ammonia for their growth, whereas nitrate is less commonly assimilated, which can explain the particular reduction in ammonia availability. The availability of nutrients was very low in the columns. Therefore it appears that the recycling of nutrients from decaying microbial biomass to newly formed biomass had to maintain VPH biodegradation for a long period. The initial TOC contents were 1.6±0.12%, 3.0±0.1% and 2.7±0.3% for soil, soil with biochar (2%) or with activated carbon (2%) respectively. The TOC contents of soil with biochar (2%) or activated carbon (2%) were higher compared to soil TOC contents, because biochar and activated carbon had high TOC contents (83.9±0.15% and 72.7±0.32% for biochar and activated carbon respectively). The TC contents had the same trend (Table 5.5). The TC and TOC contents in the columns at the end of column experiment were significantly higher compared to the beginning of the column experiments, indicating that some of the added VPH carbon is retained in the form of inorganic or organic soil carbon. Why this increase is especially notable at the atmospheric boundary side of columns (Table 5.5), whereas CO_2 production was most notable on the NAPL source side of the columns needs more investigation. It is possible that the less preferred carbon substrates such as the cyclic and branched alkanes which migrated further due to substrate-substrate inhibition were only partially degraded leading to soil humus formation.

5.5 Conclusion

This chapter investigated the soil solid-water distribution coefficient (K_d) and the first-order biodegradation rate in porewater (k_w) of VPHs in sorbent amended soils and how sorbent amendment impacted the biodegradation, vapour migration, and volatilization of VPHs. Sorbent amendments greatly increased the values of K_d and much more so in activated carbon amended soil as compared to biochar amended soil. The estimated biodegradation rates in soil pore water of most VPHs were faster in the soil amended with activated carbon compared to the soil with or without biochar,

although VPHs sorption by activated carbon greatly reduced the water dissolved fraction of VPHs and hence slowed overall VPH biodegradation rates. The sorbent amendments slowed the vapour migration and thereby early volatilization of VPHs in the first few days of the column experiments, especially in the activated carbon column. The impacts of substrate-substrate inhibition were reduced in activated carbon column and this facilitated the biodegradation of less preferred carbon substrates, such as the cyclic and branched alkanes over the length of the soil column. Consequently, the ratio of VPH mass biodegraded relative to VPH mass emanating from activated carbon column was greatly improved. The biodegradation of VPHs in the columns does not correspond to the first-order kinetics model prediction, due to a complex dependence of the biodegradation on many factors which are not easily represented in numerical models. Consequently, it is difficult to simulate the biodegradation kinetics.

Chapter 6: Comparing the effects of biochar, activated carbon and nutrient amendments on the biodegradation of volatile petroleum hydrocarbons in an aerobic soil

6.1 Introduction

The problem of persistent hydrocarbon contamination in soils or sediments is one of the most pressing environmental pollution challenges in many countries (Xu and Obbard, 2004). The application of carbonaceous sorbents, particularly activated carbon, is a novel *in situ* remediation approach (Jensen *et al.*, 2004; Tomaszewski *et al.*, 2007). This approach has been widely used to reduce the bioaccessibility and the toxicity of organic pollutants in soils and sediments (Rhodes *et al.*, 2008; Hilber *et al.*, 2009; Werner *et al.*, 2010). However, the decrease in the biodegradation of organic pollutants at contaminated sites treated with activated carbon is an important concern needing further investigation (Rhodes *et al.*, 2008). Over the last decade, biochar has received more attention because it can be produced from a variety of biomass materials, whilst also being cost-effective compared to activated carbon. These advantages have encouraged many researchers to investigate the usage of biochar rather than activated carbon as a soil remediation amendment (Iyobe *et al.*, 2004). The surface area and micropore volume of activated carbon is bigger compared to biochar, because activation increases micro-porosity (Zhang *et al.*, 2004), and the ability of chemical molecules to access the micropores via macropore networks makes activated carbon a more efficient sorbent material than biochar. This ability may explain why the bioaccessibility of organic pollutants in soil or sediments treated with activated carbon tends to be lower in comparison with biochar amended soil. Unlike activated carbon, biochar may contain available nutrients such as nitrogen, phosphate and potassium due to its ash content which can facilitate the activity of pollutant degrading microorganisms. However, due to high nutrient sorption capacity, the addition of nutrients may not always greatly increase nutrient availability in biochar or activated carbon amended soils. Therefore, the effects of biochar and activated carbon on the biodegradation of petroleum hydrocarbons in soil with and without nutrient addition need to be investigated.

6.2 Aims:

The study was aim to investigate and compare the effect of biochar and activated carbon, with and without nutrient addition, on the fate of volatile petroleum hydrocarbons in soil to which VPHs were initially added as nonaqueous phase liquid (NAPL). This was achieved by monitoring the VPH vapour-phase concentrations, oxygen, carbon dioxide and VPH residuals in batch experiments after a certain time period.

6.3 Material and methods

6.3.1 Fuel compound mixture

A mixture of 12 major constituents of gasoline or kerosene and SF_6 (Sigma–Aldrich, Steinheim, Germany) described in Chapter 4, Section 4.31 were used in this chapter.

6.3.2 Soil, biochar and activated carbon

The sandy soil was obtained from the Newcastle Law School building construction site on the Newcastle University campus in the U.K., the biochar used in this study was obtained from Environmental Power International EPI (Wiltshire, UK) and bitumen activated carbon (Chemviron carbon limited, Lancashire, UK), which were described in the Chapter 3, Sections 3.3.1, 3.3.2 and 3.3.3 was used in this study.

6.3.3 Batch experiments

Batch microcosm experiments were performed in 65 ml amber vials **(**Jencons, a VWR Division, Leicestershire, UK) closed with Teflon Mininert valves (Supelco, Bellefonte, USA) containing 15.0 g of wet sandy soil (water content 10% wet weight of soil) with and without biochar (2% as dry weight of soil) or activated carbon (2% as dry weight of soil). VPH headspace concentrations, CO_2 and O_2 were monitored after injected 0.03 ml of VPH mixture as liquid. Sterilized controls were prepared by autoclaving the soils at 126 ^oC for 30 min. Further live controls were set up to account for CO_2 production from organic matter and biochar without injection of the VPH mixture. To study the effect of inorganic nutrients, sets of batch experiments were performed by adding 1.8 mg of nitrogen in the form of NH_4Cl and 0. 18 mg of phosphorus in the form of KH_2PO_4 or 1.8 mg of nitrogen in the form of NH_4Cl or 1.8 mg of phosphorus in the form of KH_2PO_4 were added separately to each nutrient amended batch, only nitrogen amended batch or only phosphate amended batch respectively. On day 4 and day 6, 10 ml of the headspace air in the nutrient amended batches containing soil with or and without biochar were replaced with pure air to keep the batch aerobic which was done using gas-tight syringes. The amount of VPHs and CO_2 vapour removed with the syringe was measured and considered in the mass balance.

6.3.4 VPHs residual quantification

To measure the VPHs residual in the batch microcosm, 15 g of sandy soil with or without biochar (2% as dry weight of soil) or with activated carbon (2% as dry weight of soil) were extracted, cleaned up and analysed according to the method outlined in Chapter 5, Section 5.3.7.

6.3.5 VPHs headspace concentration quantification:

GC-FID analysis of VPHs in the headspace was performed on an Agilent HP-7890 Gas Chromatograph with a split ratio of 10 (200 °C) according to Chapter 4, Section 4.3.6.

6.3.6 CO_2, O_2, N_2O and SF_6 quantification:

GC-MS analysis of CO_2, O_2, N_2O and tracer (SF_6) in the soil air samples were performed on a Fisons 8060 Gas Chromatograph linked to a Fisons MD800 MS according to Chapter 3, Section 3.3.16.

6.3.7 Statistical analysis

The data were statistically analysed using Minitab for Windows (Version 16). Significant effects of sorbent amendments, nutrients, on the total residuals of VPH were evaluated through the use of ANOVA using the Fisher's multiple-comparisons test for means ($P < 0.05$).

6.4 Results and discussion

6.4.1 Vapour phase concentration of VPHs

Profiles of natural logarithm C_t/C_0 of VPH vs. time in non-nutrient amendment batches are shown in Fig. 6.1 for hexane (Fig. 6.1a), methylcyclohexane (Fig. 6.1b) and toluene (Fig. 6.1c), respectively. For better visibility and comparison, the natural logarithm of the concentration ratio C_t /C_0 of VPH in nutrients amendment batches vs. time are illustrated in Fig. 6.2 for hexane (Fig. 6.2a), methylcyclohexane (Fig. 6.2b) and toluene (Fig. 6.2c), respectively. The behaviour of VPHs was broadly consistent amongst the group of the monoaromatics, the group of the straight-chain alkanes and the group of the cyclic and/or branched alkanes and is illustrated by toluene, n-hexane and methylcyclohexane, respectively in Fig. 6.1 and Fig. 6.2. It can be seen from Fig. 6.1a, that the hexane concentration in the batches without nutrients amendment of two sets of sterilized and live batches was stable within the analytical coefficient of variance, while the concentration decreased in live batches containing the nutrients, see Fig. 6.2a. The lack of difference between sterilized and live batches is likely due to the equilibrium between hexane in headspace and hexane in VPH liquid droplets (NAPL), which may mask the effect of biodegradation. The lack of difference between sterilized and live batches observed for some batches without nutrient amendment therefore does not necessarily exclude partial biodegradation of a compound. The fact that VPH headspace concentrations in batches containing activated carbon amended sterile soil were substantially below those in un-amended sterile soil suggests that the NAPL phase has completely volatilized and sorbed to the activated carbon, which is a much stronger VHPs sorbent than biochar, especially for the saturated hydrocarbons (see Chapter 5, Table 5.1) . The methylcyclohexane concentration in the two sets of batches, non-nutrients amendment and nutrients amendment of sterilized and live batches were also broadly constant within the analytical coefficient of variance, see Fig. 6.2b and Fig. 6.2b. The concentration of toluene in the headspace of sterilised soil with or without biochar is broadly stable, while toluene concentration in soil with activated carbon decreased within the first two days then became stable. A slight difference is observed between live and sterilised batches in the two activated carbon amended soil sets with and without nutrients amendment (Fig. 6.2c and Fig. 6.2c).

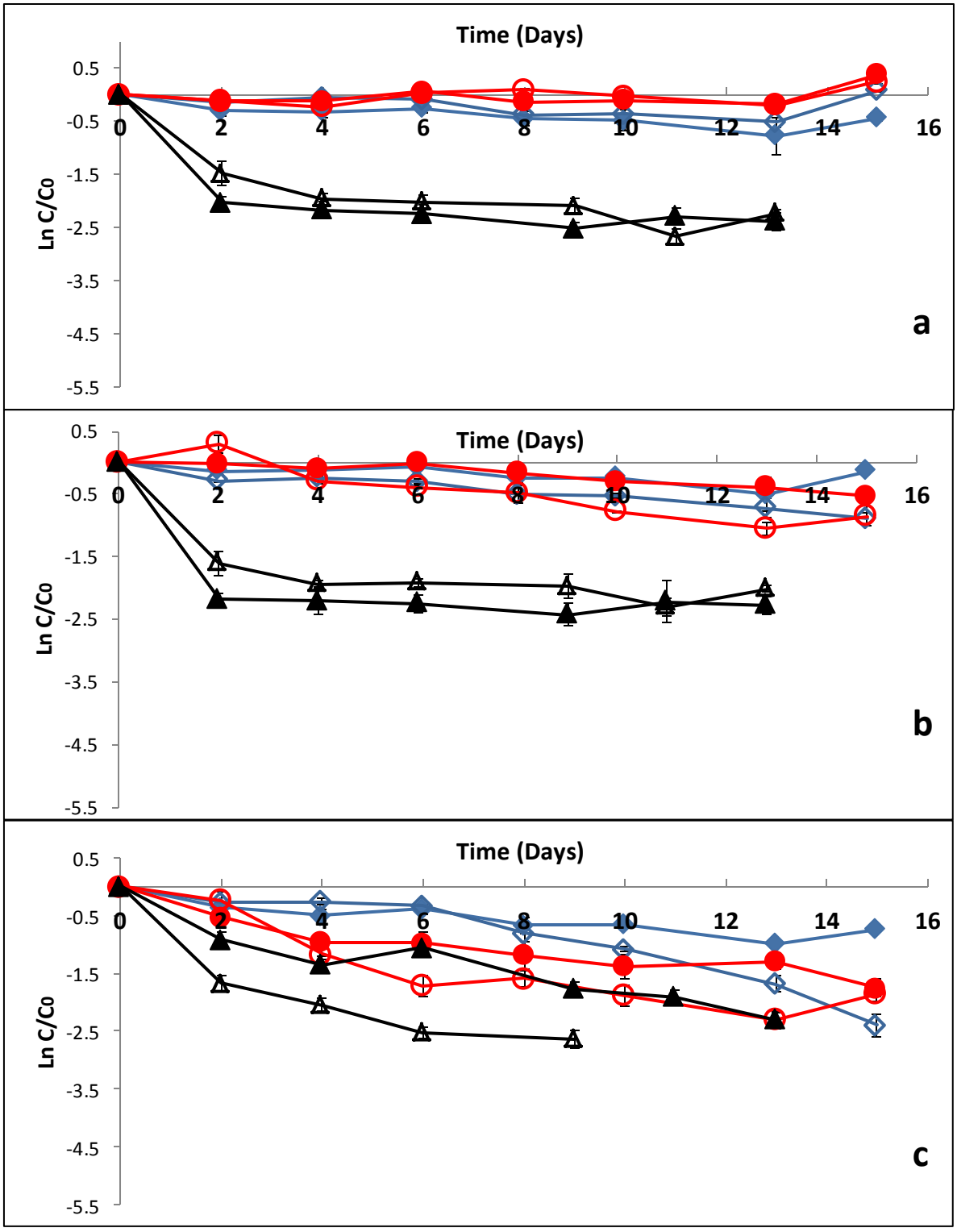

Fig. 6.1. Profile Ln C_t/C_0 in the non-nutrients amendment batches headspace for the n-hexane (a), methylcyclohexane (b) and toluene (c) in the two sets of batches, sterile (closed symbols) and live (open symbols); comparing soil (◆ ◇), soil amended with 2% biochar(● ○) and soil amended with 2% activated carbon (▲ △). Error bars: ± 1 standard deviation (SD, n=3).

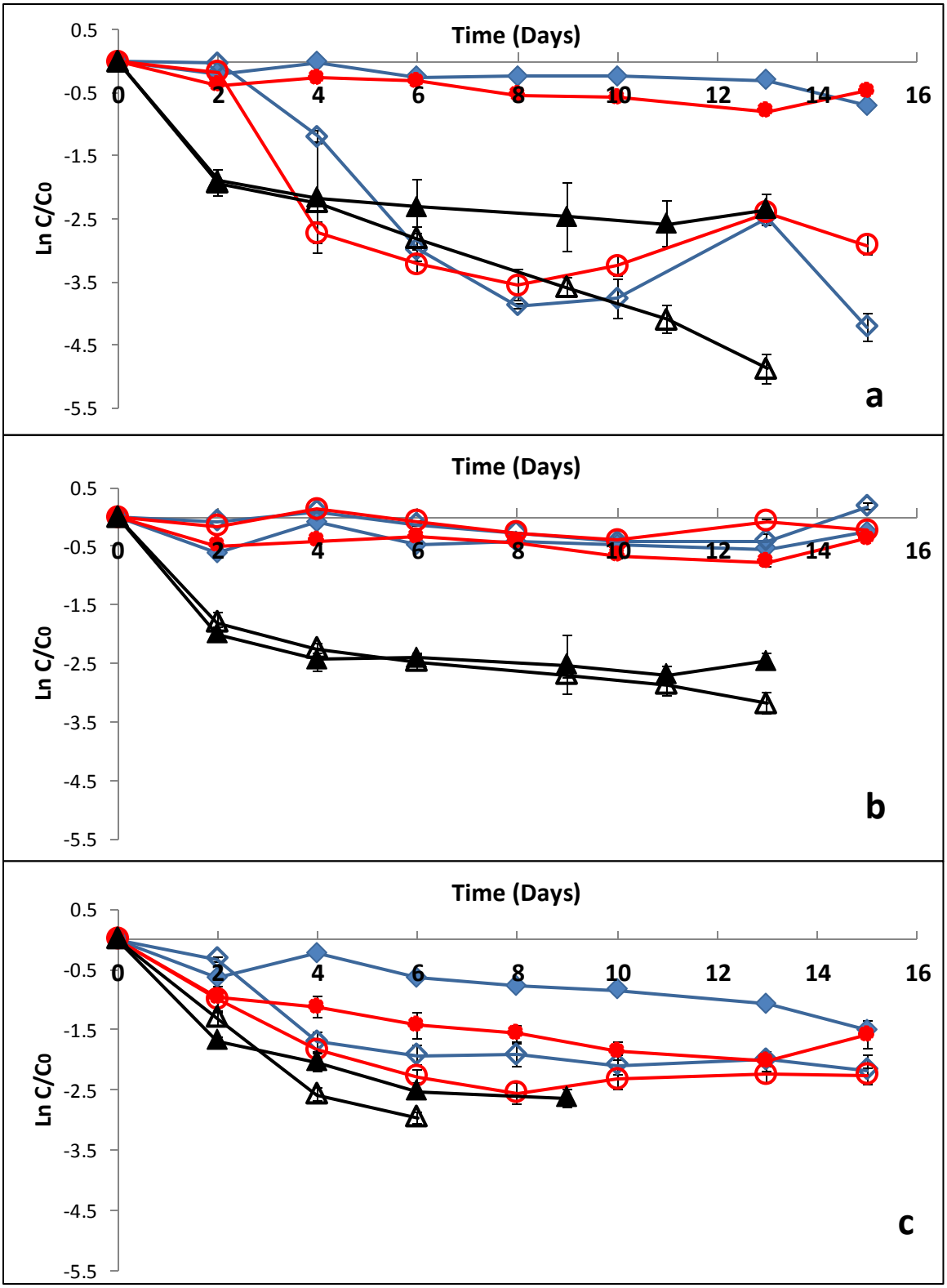

Fig. 6.2. Profile Ln C_t/C_0 in the nutrients amendment batches headspace for the n-hexane (a), methylcyclohexane (b) and toluene (c) in the two sets of batches, sterile (closed symbols) and live (open symbols); comparing soil (◆ ◇), soil amended with 2% biochar(● ○) and soil amended with 2% activated carbon (▲ △). Error bars: ± 1 standard deviation (SD, n=3).

Moreover, toluene concentration in the headspace of activated carbon amended soil batches decreased to levels below the detection limit on day 9 for live batches without nutrients and for sterilized batches with nutrients and on day 6 for live batches with nutrients, which is due to strong sorption of toluene by activated carbon and possibly also biodegradation.

The results suggested that in the batch experiments contaminated by high VPH levels (2000 ppm) using headspace concentrations of VPHs for interpretation of biodegradation processes in batches is very complicated due to equilibrium between VHPs concentration in headspace and in NAPL residuals which preserve stable concentration in the headspace by releasing VPH from the NAPL phase. Measuring CO_2 and VPH residuals may therefore give the best evaluation of the extent of VPHs biodegradation.

6.4.2 Carbon dioxide

Temporal changes of CO_2 concentrations in the two sets of batches with and without nutrients amendment are illustrated in Fig. 6.3 for soil (Fig. 6.3a), soil amended with 2% biochar (Fig. 6.3b) and soil amended with 2% activated carbon (Fig. 6.3c). Increased CO_2 levels were clearly seen in the headspace of the batches with nutrients. The CO_2 production started slowly within the first 2 days in soil with or without biochar then rose sharply to reach a peak of 1758±88 and 1850±71 µg g^{-1}, respectively. After partial replacement of CO_2 rich with fresh air on day 6 CO_2 levels fell to reach 1617±30 and 1705±16 µg g^{-1} for soil and soil with biochar respectively. This reduction in CO_2 levels could be partially attributed to the mass of CO_2 removed in the aeration process. In soil with activated carbon and nutrient batches, CO_2 production was slowly increasing over the first 9 days then increased more rapidly to reach a peak of 600±5.0 µg g^{-1} on day 15. The amount of CO_2 produced in the batches without nutrients amendments were lower in comparison with the amount of CO_2 produced in the nutrient amendment batches (see Fig. 6.3a, b and c). The CO_2 levels increased slowly in soil with or without activated carbon to reach a peak of 250±95 and 122±5.4 µg g^{-1}, respectively, during 15 days. In soil with biochar batches, these levels were increased from 6.7±0.12 µg/ g in day 0 to 715±99 µg g^{-1} in day 13. The increase in CO_2 levels of

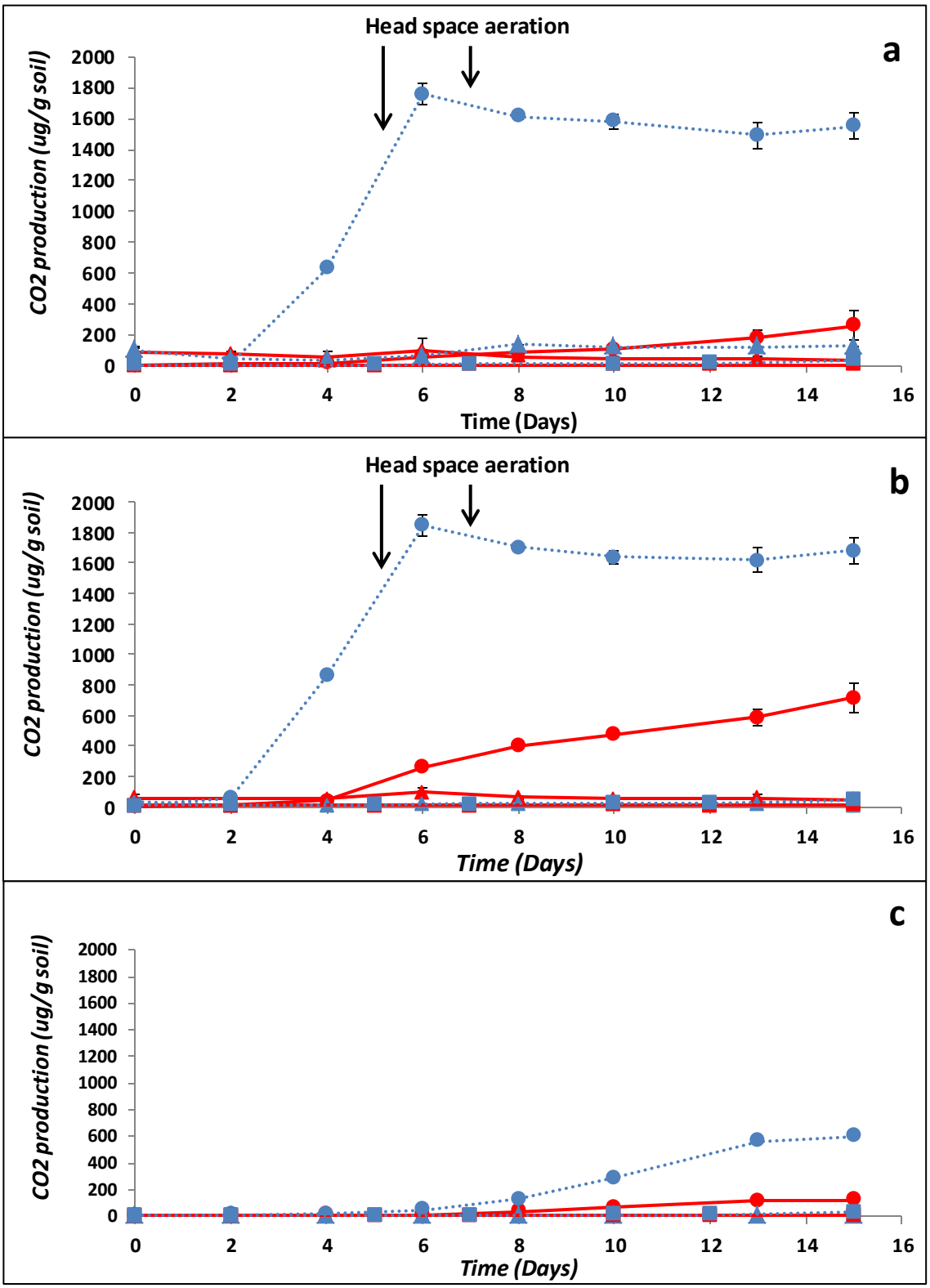

Fig. 6.3. CO_2 production for the soil (a), soil amended with 2% biochar (b) and soil amended with 2% activated carbon (c) in the two sets of batches, non-nutrients amendment (lines) and nutrients amendment (broken lines) of live with VPHs (● ●), live without VPHs (■ ■) and abiotic with VPH (▲ ▲) batches. Error bars: ± 1 standard deviation (SD, n=3).

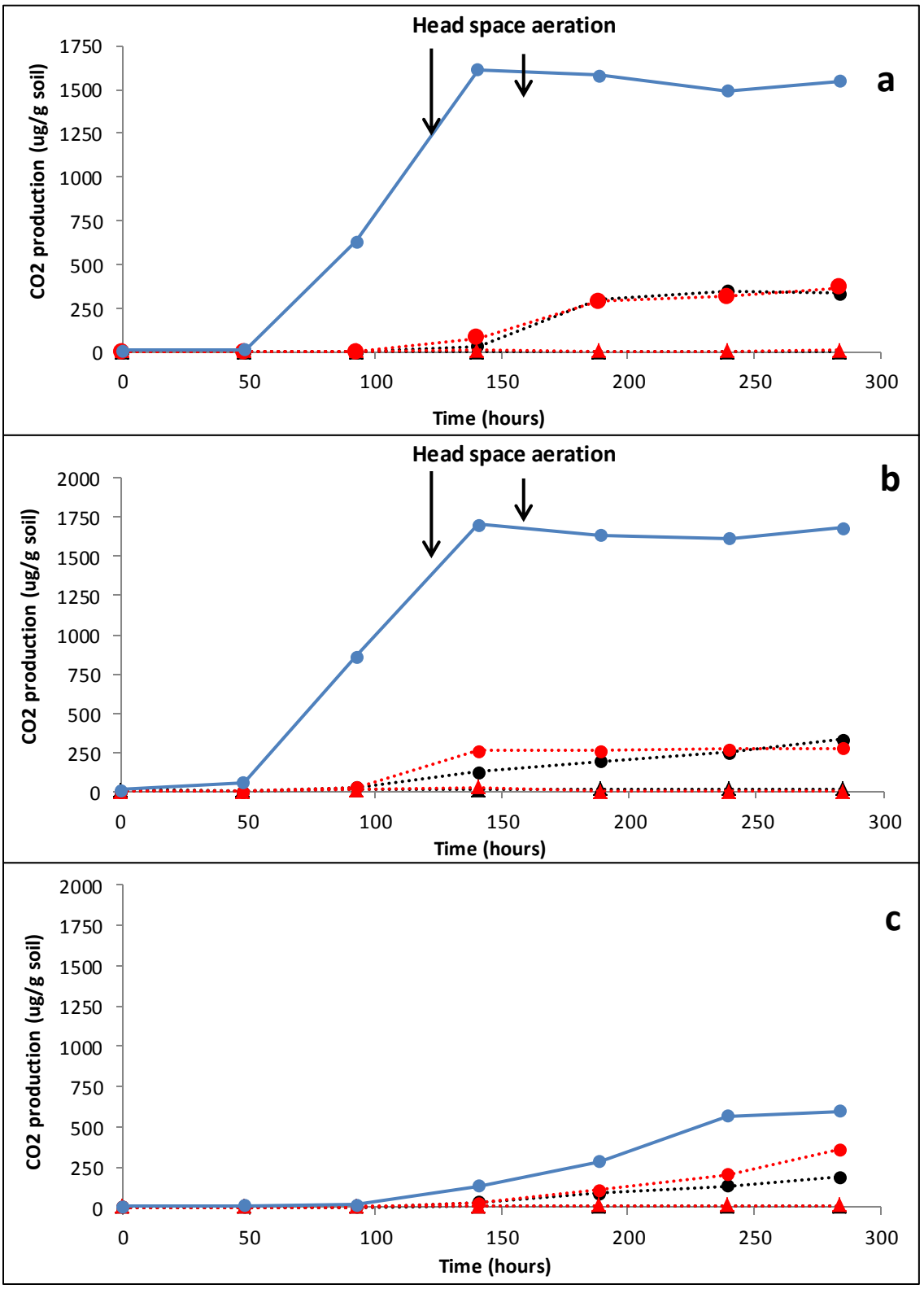

Fig. 6.4. CO_2 production for the soil (a), soil amended with 2% biochar (b) and soil amended with 2% activated carbon (c) in the three sets of batches, nutrients amendment (——), nitrogen amendment (·····), and phosphate amendment (—·—·—) of live (● ● ●) and abiotic (▲ ▲ ▲) batches. Error bars: ± 1 standard deviation (SD, n=3).

soil with biochar batches was higher in comparison with soil with or without activated carbon which may be attributed to biochar contents of nitrogen and phosphorus stimulating microbial activity or lower biomass yield coefficients of microorganisms thriving in biochar amended soil. The CO_2 concentration in sterile batches and control live batches with and without biochar or AC amendment, but without VPH addition remained low and fairly constant. Comparison between types of nutrients is shown in Fig. 6.4 for soil (Fig. 6.4a), soil amended with 2% biochar (Fig. 6.4b) and soil amended with 2% activated carbon (Fig. 6.4c). From this figure, it can see that CO_2 production started slowly within the first 6 days then rose to reach a peak of 370±59 and 350±41 μg g^{-1} soil with only nitrogen or with only phosphate respectively and to reach a peak of 362±79 and 191±12 μg g^{-1} soil with activated carbon and only nitrogen or with activated carbon and only phosphate, respectively. Although these increases in CO_2 production were higher in comparison with CO_2 production in soil with or without activated carbon treatment, these increases in CO_2 production were lower than CO_2 production in same soil with both nitrogen and phosphate supplements. CO_2 production in soil with and without biochar amended with only nitrogen or phosphate were lower in comparison with in same treatment with both nitrogen and phosphate. The increase in CO_2 production and reduction in VPHs residual in soil with or without biochar indicated that biodegradation took place in the both sets of batches. However, nutrients content controlled the degree of biodegradation and thus CO_2 production and VPHs residual percentages. Moreover, although sorption of VPH on biochar could reduce availability of both nutrients and VPHs, there were enough available nutrients and VPHs for some microbial activity. In soil with activated carbon, very strong sorption on activated carbon particles reduced availability both of nutrients and VPHs. As a result of that the CO_2 production was lower and percentage residual of most VPHs was higher. The results suggested sorption was main factor controlling the biodegradation rate of total VPHs in soil with activated carbon. However, in soil with or without biochar, the nutrients availability was the main factor controlling biodegradation rate of VPHs, whereas compound-specific sorption and preferential biodegradation by microorganisms using different VPH compounds as carbon source were secondary factors affecting of biodegradation rate of VPHs. Moreover, the results indicated that microorganisms need two nutrients (nitrogen and phosphate) for

degrading VPHs, and providing one of them it is not enough to reach optimal biodegradation rates.

6.4.3 VPHs residual in soil

The initial total volatile petroleum hydrocarbon content of any experiment batch was 0.6 mg C/g soil before incubation. Total VPHs residuals in sterile batches relative to total VPHs added, when extracted after 15 days, ranged from 68 % to 102% and the average was 84.0±14.0%. Total VPH residuals in non-nutrient amendment batches were significantly higher in comparison with those in nutrient amendment batches (ANOVA-Fisher's test, $P<0.002$). Total mass of VPH residuals in batches without nutrient amendment after 15 days was 0.39±0.09 mg C g^{-1} soil, 0.31±0.04 mg C g^{-1} soil and 0.48±0.08 mg C g^{-1} soil for soil without and with biochar and with activated carbon respectively, while in nutrients batches, the total mass of VPH residuals was 0.13±0.04 mg C g^{-1} soil, 0.11±0.006 mg C g^{-1} soil and 0.44±0.03 mg C g^{-1} soil for soil without and with biochar and with activated carbon respectively. Without nutrient addition, the total mass of n-octane, *m*-xylene, and 1,2,4-trimethylbenzene in soil with biochar is lower in comparison with soil by 45.9%, 50.1% and 58.6%, respectively; whereas the total residual mass of toluene was lowest at 16.4 % in soil and higher in soil with biochar at 41.8%. To compensate for the mass of VPH lost by incomplete extraction, sample clean-up and sorption to glass and lid, the mass of each compound of VPH was divided by the respective mass recovered from sterile batches and was called VPHs residual relative to sterile batches, which is illustrated in Fig. 6.5, without (Fig. 6.5a) and with nutrients amendment (Fig. 6.5b) for soil with or without biochar or activated carbon. Fig. 6.5 shows that in soil with or without biochar, the total VPHs residual in nutrients amendment batches were lower in comparison with those of without nutrients batches, especially for the group of monoaromatics and the group of straight-chain alkanes. In soil with activated carbon, total residuals of hexane and toluene are statistically significant lower in nutrient amended batches (ANOVA-Fisher's test, $P<0.01$), while for the other compounds the difference between batches with or without nutrients amendment are not statistically significant. The results suggest that VPH biodegradation in the investigated soils was limited by inorganic nutrient availability, but for some compounds VPH biodegradation in activated carbon

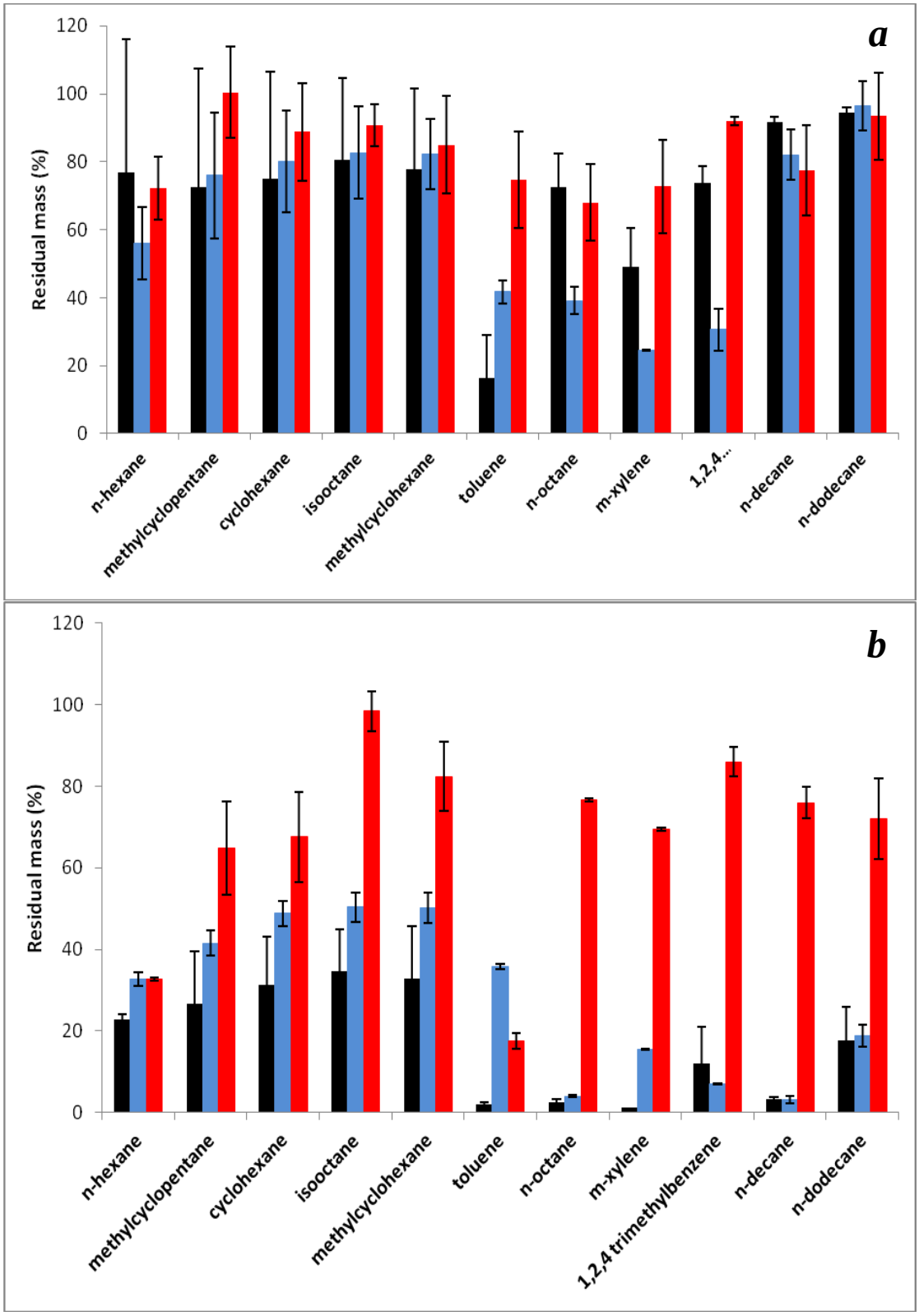

Fig. 6.5. VPHs residual relative to sterile batches for the batches without (a) and with nutrients amendment (b); comparing soil (■), soil amended with 2% biochar (■) and soil amended with 2% activated carbon (■) Error bars: ± 1 standard deviation (SD, n=3).

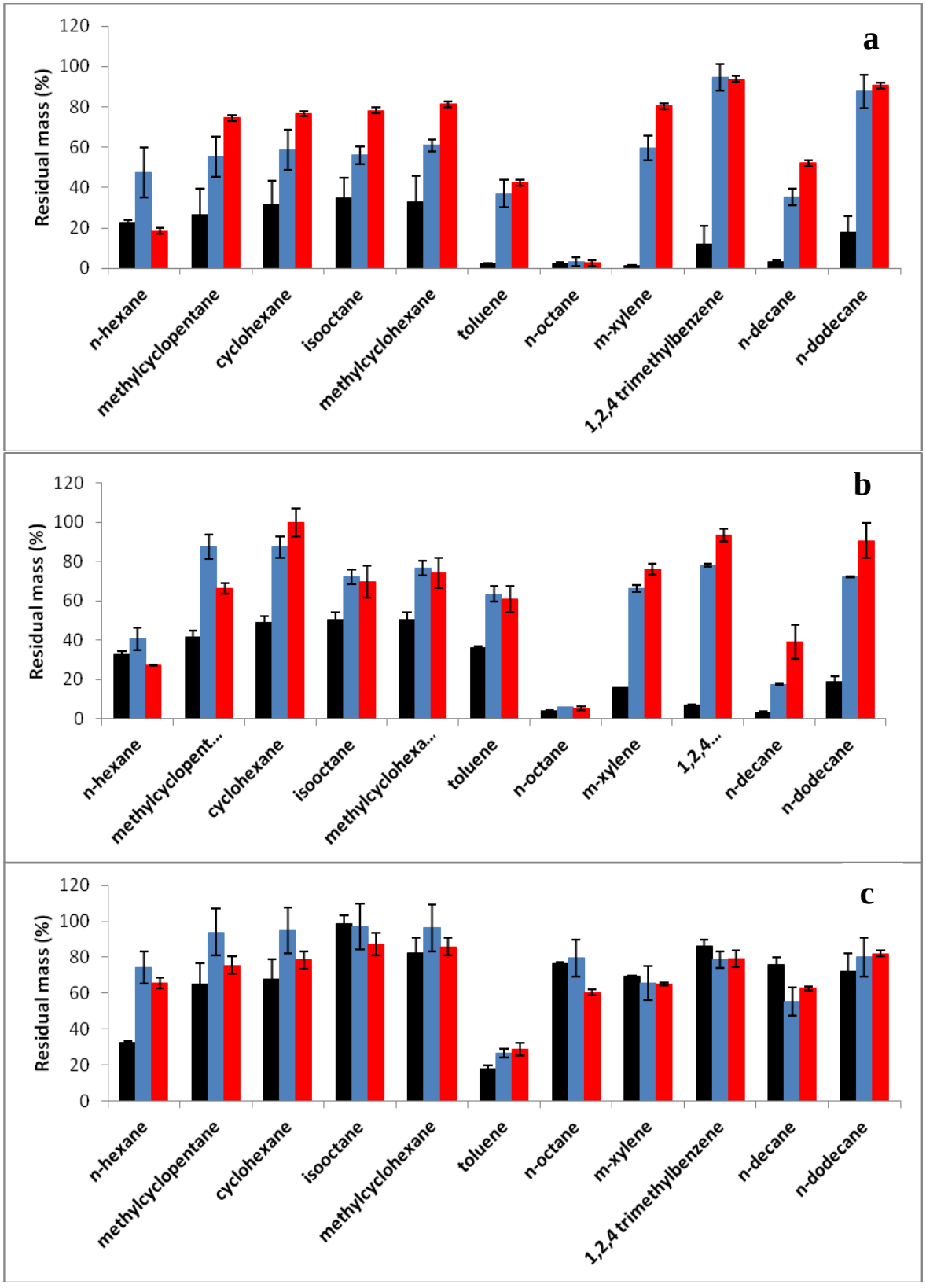

Fig. 6.6. VPHs residual relative to sterile batches for soil (a), soil amended with 2% biochar (b) and soil amended with 2% activated carbon(c); comparing nutrients amendment (■), nitrogen amendment (■) and phosphate amendments (■). Error bars: ± 1 standard deviation (SD, n=3).

amended soil appears to be limited by VPH bioavailability. Fig. 6.5a shows the effects of sorbent material types on total VPHs residual in batches without nutrient addition. The difference in total residual of hexane, methylcyclopentane, methylcyclohexane, cyclohexane, isooctane, n-octane, n-decane and n-dodecane are not a statistically significant in the batches containing soil with or without biochar or activated carbon. However, the differences are a statistically significant for the monoaromatic compounds toluene, *m*-xylene and 1,2,4-trimethylbenzene (ANOVA-Fisher's test, $P<0.01$). Toluene residuals were higher in soil with biochar due to particularity strong sorption of toluene by the surface of the biochar thus reducing the availability of toluene for biodegradation. For *m*-xylene and 1,2,4-trimethylbenzene additional methyl-groups on the aromatic ring appear to interfere with these interatomic interactions (McBeath and Smernik, 2009) and therefore the biodegradation of these compounds was less affected by biochar amendment. In the soil with activated carbon batches, the total residual of methylcyclopentane, toluene, *m*-xylene and 1,2,4-trimethylbenzene were higher in comparison with soil which can be attributed to strong sorption of these compound to the surface area of activated carbon. The effects of inorganic nutrients types on total VPHs residual in batches was illustrated in Fig. 6.6, for soil (Fig. 6.6 a), soil with biochar (Fig. 6.6 b) and soil with activated carbon (Fig. 6.6 c). From this graph it can be seen that total VPHs residuals in soil with or without biochar amended with only nitrogen or only phosphate were higher in comparison with the same soils amended with both nitrogen and phosphate (Fig. 6.6 a,b) while the differences in total VPHs residual between only nitrogen or phosphate amended batches and both nutrients amended batches in soil with activated carbon were not statistically significant (ANOVA-Fisher's test, $P<0.354$). The results support the well-known phenomenon that availability of inorganic nutrients controls the biodegradation of carbon rich and nutrient poor substrates such as VPHs and degrading microorganisms need both nitrogen and phosphate to get optimal biodegradation rates.

The increases in the CO_2 production and the reductions in VPHs residual in soil with and without biochar amended with nitrogen and phosphate are support the earlier studies (Shabir *et al.*, 2008; Singh *et al.*, 2009; Styriakova *et al.*, 2009; Jin *et a*l., 2010;

Tiehm *et a*l., 2010), which found that the biodegradation of petroleum hydrocarbons enhanced by providing nutrients. Moreover, These results are also consistent with Swindool *et al.*(1998) and Baraddock *et al.*(1997) findings who concluded that the concept of a single limiting nutrient may not applicable to degrading microorganisms and they need two nutrients (nitrogen and phosphate) for degrading VPHs, and providing one of them it is not enough to reach optimal biodegradation rates. The current work show low CO_2 production and high VPHs in soil with activated carbon. This may be attributed to very strong sorption on activated carbon particles which reduced availability both of nutrients and VPHs. This result are consistent with those of Carmichael *et al.* (1997), Jensen *et al. (*2004), Rhodes *et al. (*2008), Werner *et al. (*2010) and Jones *et al.*(2011) who concluded that there is a significant inverse relationship between sorption strength of petroleum hydrocarbons and their biodegradation. Because of aromatic nature of biochar surface area, a grater toluene residual and low toluene biodegradation was observed in the biochar amended soil with or without nutrients compared to *m*-xylene and 1,2,4-trimethylbenzene. This result supports McBeath and Smernik (2009) and Kelly *et al.* (1996) findings who pointed out that the biodegradation of xylene was more complete and faster than toluene due to interfere between methyl-groups on *m*-xylene and interatomic surface of biochar.

6.5 Conclusions:

This chapter investigated the impact of biochar or activated carbon amendment (2% on dry weight basis) on the biodegradation of a mixture of 12 volatile petroleum hydrocarbons (VPHs) in an aerobic sandy soil. Biochar amendment resulted in decreased toluene degradation, but enhanced degradation of the other petroleum compounds. CO_2 production in biochar amended soil was roughly three times higher than in un-amended soil. Nutrient addition increased biodegradation of all VPHs, and the total VPHs biodegradation rate was significantly less in nutrient and biochar amended soil as compared to nutrient amended soil without biochar, while CO_2 production was comparable in nutrient amended soil with and without biochar amendment. Activated carbon amended soil had the lowest CO_2 production and VPHs biodegradation with and without nutrient amendment. We conclude that nutrient

availability was the main factor controlling biodegradation rates of total petroleum hydrocarbons in the soil investigated, whereas sorption to biochar was a secondary factor influencing the biodegradation of monoaromatic compounds, in particular toluene. Reduced biomass yields in biochar amended soils could explain the observation of greater CO_2 production per mass of VHPs degraded. Activated carbon amendment slowed VPHs biodegradation because of the exceptionally strong sorption of VPHs to the activated carbon.

Chapter 7: Conclusion and future work

7.1 Effects of biochar on soil chemical properties

Biochar is not only carbon rich, but also has a high total nitrogen and phosphorus content, and affects soil electrical conductivity and pH. Therefore, biochar amendments may alter the chemical properties of amended soils. The increases in total nitrogen and total phosphorus depend on the biochar application rate and soil properties. Readily available nitrogen and phosphate are much lower in comparison with total nitrogen and phosphate. Biochar amended soil actually decreased readily available nitrogen concomitant with an increasing biochar application rate, because the sorption of ammonium and nitrate by biochar was found to be very high. Readily available phosphate was also decreased. This reduction is not only attributed to the high maximum phosphate amount adsorbed (q_{max}) by biochar, but it is also speculated to be due to the accelerated precipitation of phosphate as calcium phosphate due to increases in the soil pH in biochar amended soil. The availability of nutrients in soil with or without biochar (2% and 10%) was decreased with increasing prior contact period between soil and biochar from one day to 30 days. These reductions were variably attributed to the immobilization of available nitrogen and phosphate because of its high C/N ratio in soil amended with biochar, and/or to increased nitrification rates, and/or to increased precipitation of phosphate as calcium phosphates with increasing contact period. It may also have been due to the sorption of more of these nutrients to the biochar surface area with increasing contact period. The effect of contact period was higher in sandy soil due to its pH being higher, which can accelerate the precipitation of phosphate; the reduction in readily available phosphate was lower in clayey loam soil due to its lower pH of 5.7-6.0. Moreover, readily available nitrogen was shifted from nitrate to ammonium in biochar amended soil with an increasing contact period, which is attributed to the sorption capacity of ammonium being higher compared to nitrate. Therefore, nitrate was both more available and consumed by microbes, possibly denitrifying bacteria, over the contact period. An important conclusion from this work is that biochar amendment will not always enhance inorganic nutrient availability in soil, especially in closed systems such as the laboratory batches and columns investigated in this study. In the field, however, enhanced nutrient sorption will enhance nutrient retention in soil by reducing nutrient

leaching with infiltrating water to groundwater, and the effect of biochar on nutrient availability in soil may insofar be more beneficial under field conditions.

7.2 Effects of biochar on biogenic gases

In order to better understand the secondary environmental impacts of biochar-based remediation efforts, two batch experiments were conducted to examine the impact of biochar on denitrification activity and methane oxidation. The first was related to the effects of biochar amendment on denitrification activity, compared to two sets of batches, with or without the substrate supplements. Sandy soil, clayey loam soil, sandy loam soil and loamy soil with two application rates of biochar (2% and 10%) were used to investigate the biochar impacts. The N_2O productions in biochar amended soils varied and were dependent on the soil properties and provision of the substrate supplements. Without provision of the substrate supplements, the N_2O production in the first incubation period was higher compared to the second incubation treatment. This higher N_2O production is due to higher nitrogen availability in the first incubation period before the apparent depletion of the available nitrogen forms over the 30 day contact period.

Provision of substrate supplements accelerates the N_2O production in soils supplied with nitrate, glucose and glutamic acid, and this production was ten times higher in comparison with the same soil without supplements. There were significant differences in denitrification activity between biochar application rates, soils and contact periods, but no consistent biochar effects. The different responses to biochar may be attributed to different soil properties, such as the availability of nitrate and soil pH. For example, the effects of the two contact periods on the N_2O production in loamy soil were not significant, which may be attributed to the difference in the availability of nitrate between two incubation periods not being significant in this soil. Moreover, while biochar improved the soil pH of clayey loam soil, the soil pH became even more alkaline in sandy soil with biochar amendment, which may negatively affect denitrifying microorganisms. Biochar does not seem to have a consistent impact on denitrification.

The second set of batch experiment was related to the effects of biochar amendment on methane oxidation. Sandy soil, clayey loam soil, sandy loam soil and loamy soil with biochar (2% and 10%) were used to investigate the biochar impacts. The methane oxidation in biochar amended soils varied and was again dependent on soil properties; these have the most significant influence on methane oxidation and this could explain the different soil responses to biochar. Except for clayey loam soil amended with biochar (10%), there was no methane oxidation in soils amended with the highest dose of biochar (10%), indicating an inhibition. An opposite effect in clayey loam soil could be attributed to enhanced pore size distribution and the improved soil aeration following biochar amendment. Furthermore, since there were no differences between the two soil-biochar contact periods, the results show that this variable did not influence methane oxidation rates, despite of its effects on available nutrients.

7.3 Effects of biochar or activated carbon on VPH sorption and implications for the biodegradation rates of VPHs in soil porewater (batch experiment).

The impact of biochar or activated carbon on the soil solids-water distribution coefficient (K_d) and the first-order biodegradation rate in porewater (k_w) was investigated by conducting batch experiments. Sorbent amendments greatly increased the values of K_d and these increases in activated carbon amended soil were greater compared to biochar amended soil, because the activated carbon had both a higher surface area and volume of micropores. The aromatic compounds have the greatest enhancement in K_d value in biochar amended soil compared to straight-chain, cyclic and branched alkanes, due to their ability to interact via $\pi-\pi$ electron forces with the aromatic surface of the biochar. The estimated biodegradation rates in soil pore water of most VPHs were faster in the soil amended with activated carbon compared to the soil with or without biochar. However, the biodegradation of n-octane in activated carbon amended soil was not detectable, due to the very strong sorption inhibiting the dissolution of this compound in soil pore water.

7.4 Effects of biochar or activated carbon on the attenuation of volatile petroleum hydrocarbons in soil column studies

In order to determine the effects of biochar and activated carbon amendments on the fate, vapour migration and volatilization of VPHs in sorbent amended soil without nutrient supplements, three different column experiments were conducted under aerobic conditions. The effect of activated carbon amendments on the gas phase concentration of VPHs was clearly greater compared to biochar amendments. Biochar and activated carbon amendments reduced the early contaminant break-through in the lag phase. These amendments slowed the vapour migration and thereby early volatilization of VPHs in the first few days of the column experiments, especially in the activated carbon column. The prediction of the sorbent amendment impacts on the biodegradation was challenging, but some effects were obvious. The activated carbon amendment slowed the availability of both VPHs and nutrients in the growth phase period, when VPH degrading bacteria presumably increased in abundance, resulting in a smaller amount of degraded VPH and CO_2 produced in this particular period. In a subsequent stationary phase, however, nutrient recycling likely limited the biodegradation of petroleum hydrocarbons and a limited amount of petroleum hydrocarbons could be degraded per volume of soil and time. Activated carbon induced reductions in the availability of contaminants and their slower vapour migration and better chromatographic separation reduced an apparent overloading of the soils VPH biodegradation capacity in the later part of the experiment and thereby facilitate the biodegradation of less preferred carbon substrates, such as the cyclic and branched alkanes. Reduced VPH bioavailability in activated carbon amended soil decreased the impacts of substrate-substrate inhibition by more readily degradable substrates, such as linear alkanes and monoaromatic hydrocarbons, which was evident in soil without sorbent amendment. The activated carbon amendment greatly improved the ratio of VPH mass degraded relative to VPH mass emanating from soil, if one considers the pollution attenuation over the entire length of the soil column. Slower VPH migration in activated carbon amended soil means more time is available to degrade the pollutants, and more time means more biodegradation, if VPH biodegradation relies on the recycling of a limited amount of inorganic nutrients between decaying and newly formed microbial biomass. The biodegradation of VPHs in the columns does not correspond to the first-order kinetics model prediction, due to

a complex dependence of the biodegradation on many factors which are not easily represented in numerical models. Consequently, it is difficult to simulate the biodegradation kinetics.

Overall, to our knowledge, this work is the first study to investigate effects of biochar or activated carbon amendments on the attenuation of VPHs in soil. Biochar and activated carbon amendments are potentially a sustainable remediation strategy for dealing with volatile petroleum hydrocarbons pollution. These sorbents are able to reduce the risk of VPHs to biota and the also surrounding environments without using large scale, energy intensive and treatment processes.

7.5 Effects of biochar or activated carbon and nutrients on the biodegradation of volatile petroleum hydrocarbon (batch experiment)

In order to compare the effect of biochar and activated carbon with and without nutrient addition on the fate of volatile petroleum hydrocarbons in soil where NAPL is present, batch experiments were conducted to examine the impact of nutrients and biochar, or activated carbon, on the biodegradation and residual of volatile petroleum hydrocarbons, compared to two sets of batches, with or without nutrients. An aerobic sandy soil with and without biochar or activated carbon (2%) was used. The impact of the interaction between nutrients and carbonaceous sorbents on the biodegradation of VPHs was dependent on the type of sorbent. The nutrient availability was the main factor controlling total petroleum hydrocarbons biodegradation and their residual in soil with or without biochar, whereas sorption to biochar was a secondary factor influencing the biodegradation of monoaromatic compounds, in particular toluene, in the biochar amended soil. The strong sorption of VPHs to the activated carbon was the main factor controlling total VPH biodegradation and the residual of VPHs in activated carbon amended soil. From these findings, it would appear the nutrient amendment is a more promising strategy than sorbent amendment for the remediation of VPH polluted soil. However, nutrient amendment has its own disadvantages such as the risk of nutrient leaching into groundwater and the need for repeated applications of

nutrients if water passes through soil. Indeed, sorbents such as biochar or activated carbon could help with nutrient retention in soil, given the nutrient sorption capacity of biochar and activated carbon demonstrated in this study, and combined nutrient and sorbent amendment may therefore provide mutual benefits under field conditions which were not seen in the batch study. While nutrient supplements can optimize the conditions for microbial growth, sorbent amendments may help retain pollutants and nutrients within bioactive zones, i.e. before they escape to the atmosphere and/or leach into groundwater. The choice of the best remediation strategy will be site-specific and for instance depend on the remediation goals. If the main goal is a rapid reduction in the total pollution levels in soil, sorbent amendment would not be recommended, but if the remediation focus is instead on minimizing risks of spreading pollution beyond site boundaries and from soil into other environmental compartments such as groundwater and the atmosphere, then sorbent amendment may well be beneficial.

7.6 Future work

This work has provided some useful results but also highlights some issues where no definite conclusions could be drawn from the present findings. It is therefore recommended that research should be continued into the impacts of carbonaceous sorbents on the availability, degradation and transport of VPHs and also on inorganic nutrient retention and cycling. Specific examples of areas where further work is needed are discussed next.

An expansion of the column experiments should look at the impact of carbonaceous sorbents on the availability, degradation and mobility of VPH in treated soil under different aeration conditions, and different nutrient regimes. Soil samples should be taken from the column during different times of the experiment, to investigate changes in nutrient availability and the abundance and diversity of the bacteria population during the experimental period. Future experiments should encompass a variable amount of biochar and activated carbon variables (application rate and particle size). For more accurate prediction of VPH transport and biodegradation in biochar or

activated carbon amended soil, further modelling efforts should be carried out focussing on an assessment of the impact substrate-substrate inhibition on biodegradation and nutrient recycling between decaying biomass and growing biomass. The information obtained in this work will be the starting point for the design of better models which would also use data obtained with quantitative molecular tools to analyse further the abundance and dynamic of the VPH-degrading bacteria population in biochar or activated carbon amended soils, and which will also focus on microbial diversity under different conditions.

To understand completely the impacts of carbonaceous sorbents on the nutrient availability in amended soils, different forms of phosphate and nitrogen in amended soil should be analysed by sequential extraction, and the effects of different contact periods between carbonaceous sorbents and soil on the availability of nutrients should be investigated. Future work may concentrate on the competition between different nutrients and ions on the sorption areas of sorbents, and the effects of the former on the availability of these nutrients should be investigated by carrying out competitive sorption isotherms experiments.

To evaluate more completely the impact of biochar on biogenic gases production and oxidation in biochar amended soils, batch experiments should be conducted for longer contact periods and should also encompass a wider range of soils with varying physicochemical properties (especially the soil pH, soil salinity and clay content). However, it appears to be clear from the current study that responses will be soil type specific, and one should therefore attempt to more reliably link soil properties with biochar effects on biogenic gases. Better insights into biochar effects on biogenic gases will also necessitate analysis of microbial population compositions and dynamics.

Field experiments should be carried out to examine the efficacy of the proposed techniques under field conditions. It would be particularly important to gain a better understanding of how biochar and activated carbon may assist with nutrient retention in soil.

Further research is also needed to investigate the potential for using biochar as a tool of carbon capture and storage as carbonate in biochar amended soil, since there are some indications that biochar may facilitate carbonate precipitation.

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