Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze ambientali: tutela e gestione delle risorse naturali

Ciclo XXVII

Settore Concorsuale di afferenza: 03/A1 - CHIMICA ANALITICA

Settore Scientifico disciplinare: CHIM/01 - CHIMICA ANALITICA

TITOLO TESI

Biochar characterization for its environmental and agricultural utilization. Occurrence, distribution and fate of labile organic carbon and polycyclic aromatic hydrocarbons

Presentata da: Alessandro Girolamo Rombolà

Coordinatore Dottorato Prof. Enrico Dinelli

Relatore Prof. Daniele Fabbri

Esame finale anno 2015

1. Introduction

1.1. Biochar System

1.1.1. Biochar definition

1.1.2. Biochar regulation

1.1.3. Learning from history

1.2. Potential biomass for biochar production

1.3. Biochar production techniques

1.4. Chemical reactions behind the production of hydrochar and biochar

1.5. Biochar characteristics

1.6. Environmental impact of biochar

1.6.1 Biochar and climate change

1.6.2. Biochar and soil

1.6.3. Biochar and waste management

1.6.4. Biochar and other environmental effects

1.7. Stability of biochar

1.8. Biochar and pollutants

1.9. Biochar: some non-negligible issues

2. Aim of the thesis

3. Determination of PAHs: method development and application

3.1. Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil

3.2. Quantitative determination of PAHs in an agricultural soil treated with biochar

4. Biochar characterization for agricultural utilization

4.1. Relationships between chemical characteristics and phytotoxicity of biochar from poultry litter pyrolysis

1. Introduction

1.1. Biochar System

In the last half-century (1965-2015), the number of people on the planet has soared from 3 billion to 7 billion (United Nations Population Division), placing ever-growing pressure on the Earth and its resources.

Leading demographers, including those at the United Nations (UN) and the U.S. Census Bureau, are projecting that world population will peak at 9.5 billion to 10 billion later this century and then gradually decline as poorer countries develop. But what could happen if those projections would be too optimistic? What if population continues to soar, as it has in recent decades, and the world becomes home to 12 billion or even 16 billion people by 2100, as a high-end UN estimate has projected? Such an outcome would clearly have enormous social and environmental implications, including placing enormous stress on the world's food and water resources, spurring further loss of wild lands and biodiversity, and hastening the degradation of the natural systems that support life on Earth (Haub and Gribble, 2011). Other consequence of the rising world total population is a tremendous demand and consumption of fossil fuels for energy generation and consequently an increase in human-induced Greenhouse Gases (GHGs) emissions. The world′s total energy consumption was estimated at about 524 exajoules per year (EJ/y) and has been predicted to increase by about 27% by the year 2020 and by about 65% by 2040 (BP statistical review, 2013) and (International energy outlook, 2014). The increase in cost, depletion in availability, and deleterious environmental concerns associated with the use of fossil fuels are the main topic of debates in energy meetings.

The urgency to address these threats creates an ever increasing demand for solutions that can be implemented now or at least in the near future. These solutions need to be widely implemented both locally by individuals and through large programmes in order to produce effects on a global scale. This is a daunting and urgent task that cannot be achieved by any single technology, but requires many different and integrated approaches (Lehmann and Joseph, 2009).

Among the available options for these issues there is exploitation of chemical energy captured into biomass by thermochemical conversions into energy, fuels and bioproducts.

Biomass can be converted to biofuels and bioproducts via thermochemical processes, such as pyrolysis and gasification. The net carbon dioxide emissions from biofuel use are considered virtually zero or negative because there leased CO_2 was recycled from the

atmosphere captured during photosynthesis (Routa et al., 2012). In addition, since biomass contains a low amount of sulphur and nitrogen, combustion of biofuels leads to lower emissions of harmful gas, such as nitrous oxides (NO_x) and sulphur dioxide (SO_2), than most of fossil fuels (Vassilev et al., 2010). Such advantages of biomass make it a promising renewable energy resource.

The major products from biomass pyrolysis are a gaseous fraction (syngas), a liquid material (bio-oil) and a solid residue (biochar) with yields that depend on the process conditions. Syngas and bio-oil are considered as major intermediate products that can be used to create fuels alternative to conventional fuels. Numerous studies have been conducted involving up grading and utilization of syngas and bio-oil for various applications (Noordermeer and Petrus, 2006; Kumar et al., 2010; Mortensen et al., 2011; Swain et al., 2011).

Recently, biochar has received increasing attention for use in several applications. Biochar has unique properties that make it a valuable soil amendment to sustainably increase soil health and productivity, and also an appropriate tool for sequestering atmospheric carbon dioxide in soils for the long term in an attempt to mitigate global warming (Lehmann and Joseph, 2009). The recent broad interest in biochar has been chiefly stimulated by the discovery that biochar is the primary reason for the sustainable and highly fertile dark earths in the Amazon Basin, Terra Preta de Indio. Even though biochar has been used in many other places at other times, and has even been the subject of scientific investigation for at least a century, efforts have been isolated or regionally focused (Lehmann and Joseph, 2009).

1.1.1. Biochar definition

According to Lehmann and Joseph (2009), biochar is defined as "a carbon (C)-rich product when biomass such as wood, manure or leaves is heated in a closed container with little or unavailable air" (Lehmann and Joseph, 2009). Shackley et al. (2012) defined biochar more descriptively as "the porous carbonaceous solid produced by the thermochemical conversion of organic materials in an oxygen depleted atmosphere that has physicochemical properties suitable for safe and long-term storage of carbon in the environment". Verheijen et al. (2010) also defined biochar as "biomass that has been pyrolyzed in a zero or low oxygen environment applied to soil at a specific site that is expected to sustainably sequester C and concurrently improve soil functions under current

and future management, while avoiding short- and long-term detrimental effects to the wider environment as well as human and animal health''. The International Biochar Initiative (IBI) standardized its definition as ''a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment'' (IBI, 2012). While the European Biochar Certificate (EBC, 2014) defined biochar as "a heterogeneous substance rich in aromatic carbon and minerals. It is produced by pyrolysis of sustainably obtained biomass under controlled conditions with clean technology and is used for any purpose that does not involve its rapid mineralisation to CO_2 and may eventually become a soil amendment".

All of these definitions are directly or indirectly related to the biochar production condition and its application to soil. Lehmann and Joseph (2009) distinguished biochar operationally from charcoal. Primarily, the difference between these two terms lies in the end use. The charcoal is a source of charred organic matter for producing fuel and energy whereas the biochar can be applied for carbon sequestration and environmental management. The term hydrochar is closely related to biochar; however, it is distinguished by different condition like the hydrothermal carbonization of biomass (Libra et al., 2011). In general, biochar is produced by dry carbonization or pyrolysis and gasification of biomass, whereas hydrochar is produced as slurry in water by hydrothermal carbonization of biomass under pressure. The two chars differ widely in chemical and physical properties (Bargmann et al., 2013).

1.1.2. Biochar regulation

In 1984, Japan became the first country worldwide to approve the use of biochar as a soil conditioner. For the first time in Europe, the Swiss Federal Ministry of Agriculture officially approved the use of certified biochar in agriculture in 2013 (http://www.ithaka-journal.net/schweiz-bewilligt-pflanzenkohle-zur-bodenverbesserung?lang=en). Approval is based on strict, scientifically checked requirements with regard to the sustainability of biochar production, to biochar quality and to user protection in its application. In the EU, the use of biochar in agriculture is neither clearly regulated nor explicitly forbidden. In Germany for example, the use of biochar as animal feed is allowed. It can thus be composted with the manure and applied to fields. In addition, charcoal is allowed as an additive for fertilizers and soil conditioners. What however is missing is an exact definition

of what can be counted as biochar and which production conditions and thresholds need to be complied with. With the Swiss approval, we now have an exact definition, along with a requirement for strict quality controls. Further, the European Biochar Certificate has been developed to become the voluntary European industrial standard ensuring a sustainable biochar production and low hazard use in agronomic systems (http://www.european-biochar.org/en).

In the United States (U.S.) some biochar production systems have been recommended for generating C offsets by soil sequestration (De Gryze et al., 2010). Also, U.S. proposed federal legislation to comprehensively address energy and climate change (i.e., the American Power Act) included "projects for biochar production and use" to be considered for domestic C offset programs (Gurwick et al., 2012). Recently, IBI certified the first biochar material for effective use as a soil amendment for the development of small-scale bio-refineries for the conversion of non-food biomass into biofuels and biochar in California (http://www.biochar-international.org/certification). Biochar is commercially available in the U.S. (Biochar Supreme**,** www.biocharsupreme.com; Biochar Solutions, www.biocharsolutions.com). Further, large-scale biochar production from crop straw is now commercially available in China (Pan et al., 2011). However, the biochar price is claimed to be too much high (about 3.7 \$ kg^{-1}) and would not be balanced by the potential economic gains based on average yield improvements and current prices for CO_2 (Liu et al., 2013). Thus, biochar has not yet made a substantial entry into large-scale agricultural operations (IBI, 2014).

1.1.3. Learning from history

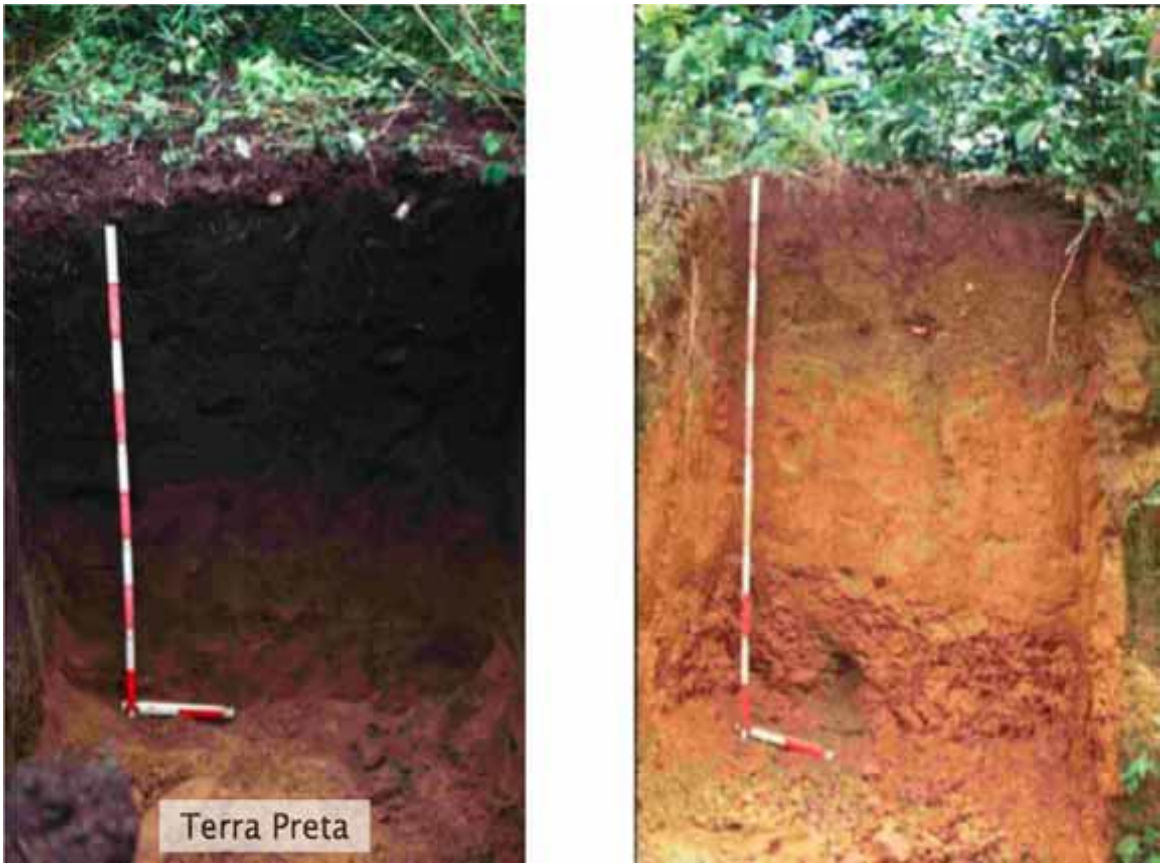
Several thousand years ago, pre-Columbian indigenous farmers used 'slash and char' to bring soils into production. 'Slash and char' sequesters approximately 50% of the carbon in the vegetation whereas 'slash and burn', still practiced by some cultures today, sequesters only about 3% of the carbon. To create 'char', vegetation cleared from new areas was smouldered at moderate temperatures in the absence of oxygen. The result was then dug into the soil. Food scraps and waste materials were also added with the result that the *terra preta* soils have not only high carbon (and are black) but also high fertility in comparison with adjacent char-free soils (Fig. 1).

The *terra preta* soils are thought to have formed over a relatively short time span – only 40-50 years. They range in depth from half a metre to two metres deep, and can

contain as much as 250 tonnes of carbon per hectare in the first 30 cm and 500 tonnes per hectare up to one meter (but different authors have different values, indicating the difficulty of assessing soil carbon, as explained in the Grassland newsletter article by Parsons and Rowarth 2009). Unimproved soils from similar parent material have approximately 60% less carbon than the 'char-enriched' soils.

Although stable, biochar is not inert – it can hold plant nutrients, including nitrogen, and often has useful supplies of potassium, sulphur and phosphate in the accompanying ash. It is probably this capacity that has resulted in reports that the addition of biochar resulted in a doubling of crop production in South America. The biochar was added with manure and food waste, as well as the ash resulting from the charring process. The ash also has a liming effect, increasing soil pH. As the ash, manure and food waste was broken down by microorganisms, the nutrients released that were not immediately immobilised (by micro-organisms) or taken up by plants, were retained by the biochar instead of being lost by leaching or, in the case of nitrogen, denitrification. Thus the biochar provided a source of nutrients that did not come from the char, but were plant available?

Figure 1. Comparison of profiles of terra preta and adjacent soils (Source: IBI website).



1.2. Potential biomass for biochar production

The biomass potentials that could be available for biochar is categorized into two types: (i) primarily produced biomass as a resource of bioenergy and biochar, and (ii) byproducts as waste biomass. However, biochar production from dedicated crops could create competition for land with any other land use option — such as food production or leaving the land in its pristine state. Therefore, biochar should be made from biomass waste materials. Appropriate biomass waste materials for biochar production include crop residues (both field residues and processing residues such as nut shells, fruit pits, bagasse, etc), as well as yard, food and forestry wastes and animal manures. Large amounts of agricultural, municipal and forestry biomass are currently burned or left to decompose and release CO_2 and methane back into the atmosphere. They also can pollute local ground and surface waters — a large issue for livestock wastes, therefore using these materials to produce biochar allows to remove them from a pollution cycle.

In theory, any C-based feedstock can be pyrolysed to produce biochar, and so biochar production has the potential to mitigate the increasing global problem of waste disposal. To date, a wide range of waste streams have been considered and tested, including biosolids (Chan and Xu, 2009), tannery wastes (Muralidhara, 1982), paper sludge (Rajkovich et al., 2011) and sewage and wastewater sludge (Bridle and Pritchard 2004; Hossain et al., 2010). The type of feedstock affects the properties of the resulting biochar (Kloss et al., 2012) in terms of crop yield effects (Jeffery et al., 2011) and recalcitrance in the soil (Zimmerman, 2010; Singh et al., 2012). Furthermore, it is likely to affect whether the resulting biochar is classified as a waste product, with implications regarding its permissibility for soil application (Sohi et al., 2010). Legislative issues surrounding biochar application to soils produced from waste products, and the classification of such biochar in terms of policy, is vital before its large-scale application can be implemented.

One readily apparent trade-off regarding choice of feedstock for biochar production is the issue of stability of the resulting biochar vs. its nutrient content. For example, evidence suggests that biochars prepared from poultry litter support greater increases in crop productivity than those obtained from wood (Jeffery et al., 2011), probably because of a higher nutrient contents in this feedstock. However, biochars from poultry litter are less stable in the soil than those prepared from wood (Singh et al., 2012).

1.3. Biochar production techniques

The conversion of biomass into biochar can be performed with the help of a variety of thermochemical processes, including pyrolysis, gasification and hydrothermal carbonization (HTC). The choice of treatment method depends on type of feedstock (wet or dry) and the desired properties of biochar for its different applications. In fact, the properties of a given biochar strongly depend on the characteristics of each process and also on the material to which the process is applied. Under all thermal treatments, biochar is generally produced by heating biomass at high temperature in the absence or limited supply of oxygen. Thermal treatments are classified based on their operating conditions such as: severity of process parameters (mainly reaction time and temperature), pre- and post-processing requirements like shaping, sizing, drying, cooling, condensation, etc. (Mosier et al., 2005; Goyal et al., 2008; Manyà et al., 2012)

Pyrolysis

The most common method to produce biochar is pyrolysis. Pyrolysis is a thermochemical decomposition process during which biomass is heated at elevated temperature (300-650 °C) in the absence of oxygen. At these temperatures, organic materials thermally decompose releasing a vapor phase and biochar. By cooling the pyrolysis vapor, polar and high-molecular-weight compounds condense out as bio-oil while low-molecular-weight volatile compounds, like CO, CO_2, CH_4 and H_2 (Brownsort, 2009; Mohan et al., 2006), remain as syngas. Biochar generally has a high carbon content, up to a half of the total carbon of the original organic matter. Bio-oil is generally a hydrophilic liquid, containing many oxygenated compounds, and can be obtained as a single aqueous phase or phase-separated (Demirbas and Arin, 2002). Syngas is generally composed by carbon dioxide, carbon monoxide, methane, hydrogen and C2 hydrocarbons in varying proportions.

Depending upon the reaction time, temperature, and heating rate the pyrolysis process is sub-divided in four categories: slow, fast, flash and intermediate pyrolysis (Bridgwater and Peacocke, 2000; Onay et al., 2003; Laird et al., 2009; Jones et al., 2009; Vamvuka, 2011).

Slow pyrolysis. Conventional or slow pyrolysis processes produces biochar by heating biomass at a low heating rate for a relatively long residence time (Table 1) and usually at lower temperature than fast pyrolysis (400°C). The target product is often the char, but this

is always accompanied by liquid and gas products although these are not always recovered. Slow pyrolysis can be divided into traditional charcoal making and more modern processes. In fact, this process has been practiced for thousands of years (Zhang et al., 2010). It continues to be widely used for production of high quality charcoal for metallurgical applications such as in the production of high grade silicon, as a leisure fuel in many developed countries, and in developing countries as an essential and storable commodity for cooking. There is widespread small scale local production in many developed countries as a cottage industry but there are limited truly commercial operations (a notable exception is charcoal production in Brazil for iron and steel production).

Several variables and factors play a critical role during the pyrolysis process, and specifically: peak temperature, pressure, vapor residence time and moisture content (Antal et al., 2003). The peak temperature is the highest temperature reached during the process. As a general rule, the charcoal yield decreases as temperature increases. However, an increase of the peak temperature results in an increase of the fixed-carbon content in biochar (Schenkel et al., 1998; Antal et al., 2000; Antal et al., 2003a). This increase is especially pronounced in the temperature range from 300 to 500 °C. In addition, the peak temperature has influence on surface area and pore size distribution (both properties generally related to specific adsorptive properties) of charcoals.

Fast pyrolysis. Fast pyrolysis produces biochar at a high heating rate (10-1000 °C s^{-1}) and short residence time (less than 10 s). The peak temperature is usually set between 500 and 550 °C in order to obtain the highest bio-oil yield (Maschio et al., 1999; Onay et al., 2001; Yanik et al., 2007; Uzun et al., 2007). In this kind of pyrolysis, biomass decomposes very quickly favouring the formation of bio-oil and inhibiting the formation of biochar (about 15% of products) (Table 1).

Intermediate pyrolysis. Intermediate pyrolysis operates between the reaction conditions of slow and fast pyrolysis, including moderate heating rates up to 200-300 °C min^{-1} and residence times for feedstock of 0.5-25 min. The product distribution generated by this process is typically 40-60% of bio-oil, 20-30% syngas and 15-25% biochar. In particular, the biochar obtained by intermediate pyrolysis is dry and has a brittle texture as it contains less tar and therefore less toxic compounds making it suitable for further applications, such as a solid fuel or as a soil amendment and/or as a fertilizer.

Flash pyrolysis. Flash pyrolysis occurs with very fast heating rates of ≥ 1000 °C s^{-1} and uses even shorter solid residence time (<0.5 s) than fast pyrolysis. The flash carbonization process has been developed by Antal and Grönli (2003) at the University of

Hawaii as an efficient way to produce biochar by the ignition of flash fire at elevated pressure in a packed bed of biomass. Air is used to pressurize a vessel to an initial pressure of 1-2 MPa, and a flash fire is ignited at the bottom of a packed bed. After a few minutes, air is delivered to the top of the packed bed and biomass is converted to charcoal. The total reaction time is less than 30 min and the temperature profile of the packed bed is conditioned by several factors: biomass feedstock, moisture content of the feedstock, heating time and the total amount of air delivered (Antal et al., 2003b). In any case, the flame front moves up the packed bed, causing the middle and top temperatures to successively increase, until reaching values near 600 °C. This procedure determines a significant improvement in yields with respect to conventional carbonization or slow pyrolysis (Antal et al., 2003b; Nunoura et al., 2006).

Gasification

Gasification is an alternative thermo-chemical conversion technology suitable for treatment of biomass or other organic matter including municipal solid wastes or hydrocarbons such as coal. Gasification primarily transforms biomass into a gaseous mixture (syngas containing CO, H_2, CO_2, CH_4, and smaller quantities of higher hydrocarbons) by supplying a controlled amount of oxidizing agent under high temperature (> than 700 °C). Although they are designed to produce gas, gasifiers under some conditions can also produce reasonable yields of char. Therefore they have been proposed as an alternative production route to pyrolysis for biochar (Brown, 2009). The typical biochar yield of gasification averages about 10wt% of biomass (Meyer et al., 2011; Qian et al., 2013). The oxidizing agent used in gasification can be oxygen, air, steam or mixtures of these gases. Air gasification produces syngas with low heating values of 4-7 MJ/Nm^3, while gasification with steam produces syngas with high heating values of 10-14 MJ/Nm^3 (Kumar et al., 2009).

Hydrothermal carbonization

Hydrothermal carbonization (HTC) of biomass takes place in water at elevated temperatures (160-800 °C). Since the water temperature is above 100 °C, the reaction pressure also must be elevated (more than 1 atm) to maintain the water in a liquid form. According to the reaction temperature, hydrothermal carbonization can be divided into high-temperature HTC (between 300 and 800 °C) and low-temperature HTC (below 300 °C) (Hu et al., 2010). Since the reaction conditions of high-temperature HTC (above 300

°C) are beyond the stability condition of most organic compounds, the dominant reaction in this case is hydrothermal gasification and the dominant products are gases, such as CH_4 and H_2 (Kruse et al., 2013). Below 300 °C, gasification is limited and carbonization of biomass to char dominates the reaction. Low-temperature HTC can mimic the natural coalification of biomass, although the reaction rate is higher and reaction time is shorter if compared to the thousand/billion years of slow natural coalification of biomass. Char yield of low-temperature biomass HTC varies from 30% to 60% depending on the feedstock properties, reaction temperature and pressure. Since HTC requires water, this may be a cost effective biochar production method for feedstock with high moisture content (Titirici et al., 2012).

Table 1. Biochar production techniques and typical yields of fraction.

	Temp & Duration	Solid (Biochar)	Liquid (Bio oil)	Gas (Syn Gas)
Slow Pyrolysis	**~ 500°C min to days**	**35%**	**30%**	**35%**
Intermediate	**450-500°C min**	**20%**	**50%**	**30%**
Fast Pyrolysis	**~ 500°C seconds**	**12%**	**75%**	**13%**
Flash Pyrolysis	**> 800°C seconds**	**10%**	**75%**	**15%**
Gasification	**> 800°C hours**	**10%**	**5%**	**85%**
HTC	**180-250°C 1-12 hours**	**70%**	**25%**	**5%**

1.4. Chemical reactions behind the production of hydrochar and biochar

During the production of biochar, biomass undergoes to a series of chemical reactions that are highly complicated and depend on both the nature of the biomass and the conditions (Glaser et al., 2001; Di Blasi, 2008; Babu, 2008; Funke and Ziegler, 2010). However, most of these chemical reactions have similar thermochemical pathways, i.e. the

degradation and depolymerization of polymeric composition of biomass take place, resulting in the formation of solid, liquid and gaseous (by-) products. The fundamental difference in various thermochemical treatments lies in the operating conditions and reaction medium that are used for the production of biochar and hydrochar. The highest reaction temperature reached during a thermochemical process is the main parameter that controls: i) the dominant reactions; ii) the reaction mechanism; iii) the physicochemical properties of char. Decarboxylation, dehydration, de-carbonylation, de-methoxylation, intermolecular rearrangement, condensation, aromatization, etc. are some of the proposed chemical reactions that can take place (Funke et al., 2010). However, in real practice, it is difficult to maintain uniform temperature profiles in pyrolysis reactors; therefore, it is most likely possible that many of the aforementioned reaction mechanisms take place simultaneously (Glaser et al., 2001).

The thermal stability of the polymeric constituent of lignocellulosic of biomass significantly depends on the reaction medium in which the process is carried out. Under standard pressure conditions (e.g. pyrolysis) the decomposition of hemicellulose takes place between 200-300 °C, followed by cellulose that decomposes at higher temperatures (300-400 °C). Lignin is the most thermo-chemically stable polymer and decomposes in a wide temperature range peaking around 600 °C (Grønliet al., 2002) In contrast, during HTC the degradation/depolymerization of biomass occurs at significantly lower temperatures than pyrolysis (Yan et al., 2009). The degradation of hemicellulose and cellulose under HTC process starts at around 160-180 °C, where most of the lignin still remains stable until near or above critical point of water (Bobleter, 1994). The polymeric degradation of biomass in the HTC process is controlled by reaction mechanisms very similar to those in the pyrolysis process. However, due to the presence of hot compressed water process the degradation of biomass during HTC is primarily initiated by hydrolysis, resulting in the cleavage of ether and ester bonds between monomeric sugars by the addition of one molecule of water (Bobleter, 1994) and thereby reducing the activation energy levels of biomass polymers (Glaser et al., 2001). During HTC, the cellulose and hemicellulose are partially or fully driven off, leaving behind a char with high lignin content.

1.5. Biochar characteristics

The composition and the chemistry of biochar can be very different according to the variety of feedstock that have been thermally degraded under a range of conditions (Antal et al. 2000; Antal and Grønli 2003; Amonette and Joseph 2009; Krull et al. 2009; Libra et al. 2011; Cantrell et al. 2012).

Kuwagaki (1990) proposed that seven properties should be measured for a quality assessment for agronomically-used biochar: pH, volatile matter, ash content, water holding capacity, bulk density, pore volume, and specific surface area. IBI and the EBC have developed a series of guidelines for biochar production and quality (IBI, 2013; EBC, 2014). For instance, IBI sets a range of 6-20 mg $kg^{-1}_{\text{dry weight (dw)}}$ as the maximum allowable threshold values (varying between different countries) for the sum of the 16 US Environmental Protection Agency's (EPA) polycyclic aromatic hydrocarbons (PAHs) in biochar (IBI, 2013). Similarly, EBC requires PAHs to be below 4 and 12 mg kg^{-1}_{dw}, in premium and basic grade biochars, respectively (EBC, 2014). Both documents also list guide values for a number of heavy metals, elemental contents (C, H, N, O) and their molar ratios, and specific surface area (SSA) (Table A1 in supplementary materials). With regard to carbon content, for instance, EBC proposed that the biochar's carbon content must be higher than $50\%_{dw}$. While IBI requires a carbon content higher than 60% and $30\%_{dw}$ for first and second class biochar, respectively.

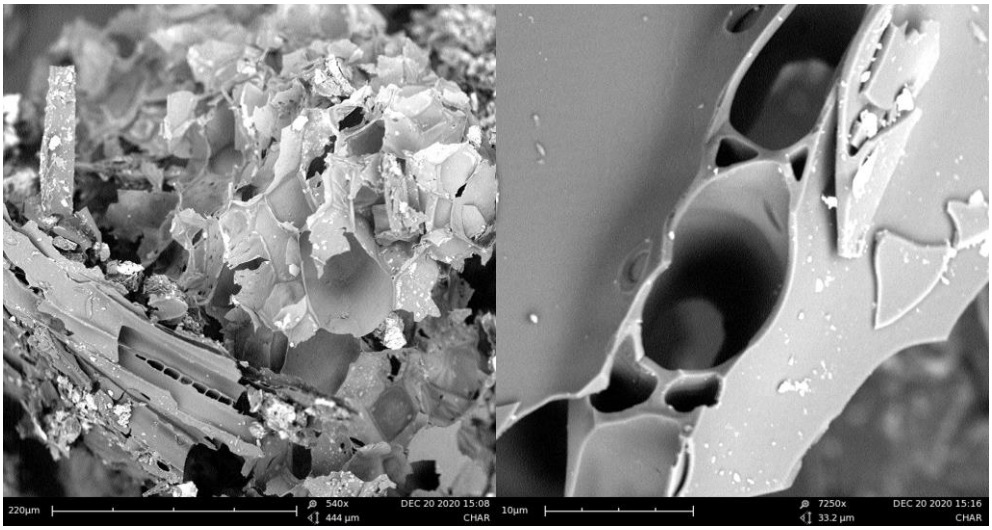
The organic carbon content of pyrolysed chars ranges between ±5% and 95% of the dry mass, dependent on the feedstock and process temperature used. Generally, biochars derived from solid biomass residues tend to have higher carbon contents (63-82%) than those derived from digestable biomass residues (35-66%) and digestates (42%) (Enders et al., 2012). For instance the carbon content of pyrolysed poultry manure is around 35% (Song et al., 2012), while that of wood is around 70-80% (Fabbri et al., 2012). When using mineral-rich feedstocks such as sewage sludge or animal manure, the pyrolysed products tend to have high ash content.

At low temperature, biochar chemical composition is closer to the original feedstock while high temperature biochar is similar to graphite (Masiello, 2004). The biochars produced at around 350 °C are mainly dominated by aromatic (aryl) carbon with small proportions of alkyl-O and alkyl-C. When the reaction temperature is further increased (>500 °C), these alkyl-O and alkyl-C were completely converted to aryl-C and these chars usually have very low H/C ratios. In general, the carbon content of biochar is inversely

related to biochar yield. Increasing pyrolysis temperature from 300 to 800°C decreases the yield of biochar from 67 to 26% and increases the carbon content from 56 to 93% (Tanaka, 1963). Beyond a certain threshold, the mass of biochar may decrease without any affect on the amount of carbon retained within it; but as mass is lost, the ash content of biochar increases (Bourke et al., 2007). Pyrolysis temperature greatly affects the surface area of pyrolysis products. In particular, the increase of pyrolysis temperature determines an increase of surface area of biochar. This effect of temperature suggest that biochar prepared at low temperature may be suitable for controlling the release of fertiliser nutrients (Day et al., 2005), whilst high temperature biochars would be more suitable as activated carbon (Ogawa et al., 2006). The surfaces of low temperature biochar are, however, hydrophobic and this may limit the capacity to store water in soil. The scanning electron microscopy images (Fig. 2) of these biochars clearing show that have a structure with voids and micropores in which water can be retained.

It is critically important to characterize biochar because its characterization will play a vital role in determining its importance and application in the industry and environment. For example, a biochar with low carbon content and high ash content is not suitable for energy product, and in the same way a biochar with low surface area and low adsorption capacity is not meant for agricultural and wastewater treatment applications.

Figure 2. Scanning electron microscopy images of orchard pruning biochar at magnification × 500 and 7000.



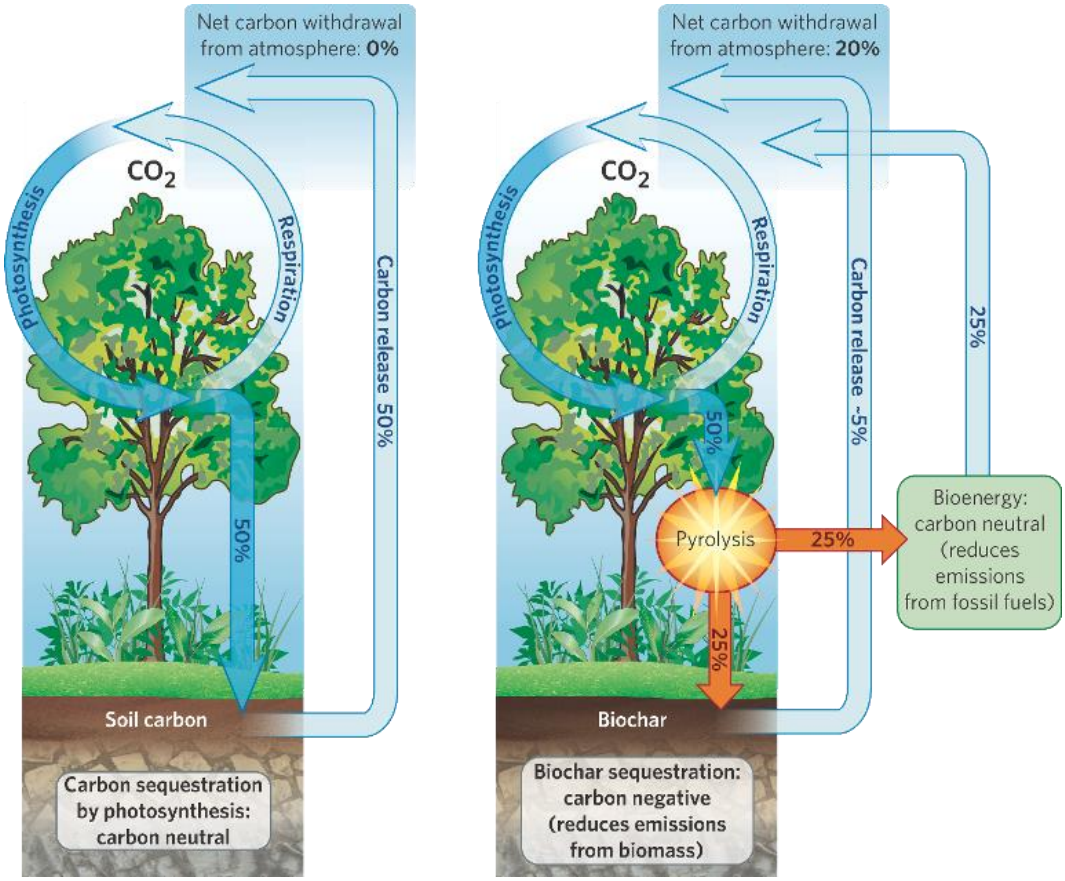
1.6. Environmental impact of biochar

1.6.1. Biochar and climate change

The greenhouse-gas (GHG) concentrations of CO_2, CH_4 and NO_x in the atmosphere have strongly risen since pre-industrial times (Ciais et al., 2013). The driver of these concentrations is an increase in human-induced GHG emissions (Ciais et al. 2013). Emissions of CO_2 are attributed to the increased use of fossil fuels, as well as to the enhanced clearing and burning of forests (Fearnside, 2000), and the expanding of agriculture. As the main cause for the rise in the global mean surface temperature (Bindoff et al. 2013), it is widely recognized that the anthropogenic GHG concentrations need to be drastically reduced to combat climate change. The world is on a trajectory that results in a level of emissions consistent with long-term average temperature increase of more than 3.5 °C (International Energy Agency, 2011). To change this trajectory, a timely and ambitious programme of mitigation measures is needed. Several studies have shown that, to stabilize global mean surface temperature, cumulative anthropogenic GHG emissions must be kept below a maximum upper limit, thus indicating that future net anthropogenic emissions must approach zero.

Basically, there are two ways to decrease atmospheric GHG concentrations – either by reducing GHG emissions into the atmosphere (climate-change mitigation) or by removing GHGs from the atmosphere (carbon-dioxide removal or carbon sequestration) (Rickels et al., 2011). Measures to reduce atmospheric GHG emissions range from energy efficiency and the replacement of fossil fuels by renewable energies to the capture of GHG emissions from industrial sites or power plants and their subsequent storage in the deep ocean or in geological formations – so-called carbon capture and storage (CCS) (Keith, 2000). In turn, potential measures to actively remove GHGs from the atmosphere include, for example, afforestation, ocean iron fertilization or enhanced weathering (Rickels et al., 2011). Likewise, the restoration of wetlands or the adoption of recommended agricultural practices, such as no-till farming, cover crops and crop rotation, are further measures to enhance carbon sequestration in natural sinks (Lal, 2008).

Figure 3. Scheme of biochar driven soil carbon sequestration. Diagram from Nature Publishing Group (Lehmann, 2007).



The production of biochar, in combination with its storage in soils, has been suggested as one possible alternative and promising strategy for CO_2 removal – a strategy that not only sequesters carbon in soils but also at the same time might improve soil quality (Lehmann et al. 2006; Sohi 2010) (Fig. 3). Biochar's climate-mitigation potential primarily stems from its highly recalcitrant nature (Cheng et al., 2008; Kuzyakov et al., 2009), which slows the rate at which photosynthetically fixed carbon (C) is returned to the atmosphere. The biochar also improves soil fertility, stimulating plant growth, which then consumes more CO_2 in a feedback effect and the energy generated as part of biochar production can displace carbon positive energy from fossil fuels.

Moreover, biochar applied to soils has been shown to reduce NO_x emissions significantly (with the added benefit of reducing nitrogen fertiliser requirements). As NO_x are approximately 320 times more effective as a GHG than CO_2, biochar could be very important in mitigating emissions. However, the mechanisms and quantities involved are

still being investigated.

Additional effects from adding biochar to soil can further reduce greenhouse gas emissions and enhance carbon storage in soil. These include: (i) Biochar reduces the need for fertilizer, resulting in reduced emissions from fertilizer production; (ii) Biochar increases soil microbial life, resulting in more carbon storage in soil; (iii) Turning agricultural waste into biochar reduces methane (another potent greenhouse gas) generated by the natural decomposition of the waste. Moreover, there may be additional benefits arising from the contribution of biochar to facilitate agricultural development and improving the socioeconomic circumstances of farmers in developing countries.

Globally, Woolf et al. (2010) estimated that the potential impact of biochar for climate-change mitigation is 12% of current anthropogenic CO_2-C equivalent (CO_2-Ce) emissions (that is, 1.8 Pg CO_2-Ce per year of the 15.4 Pg CO_2-Ce emitted annually), and that over the course of a century, the total net offset from biochar would be 130 Pg CO_2-Ce: These results are possible at current levels of feedstock availability, while preserving biodiversity, ecosystem stability and food security. They also show that conversion of all sustainably obtained biomass to maximize bioenergy, rather than biochar, production can offset a maximum of 10% of the current anthropogenic CO_2-Ce emissions. The relative climate-mitigation potentials of biochar and bioenergy depend on the fertility of the soil amended and the C intensity of the fuel being offset, as well as the type of biomass. Locations in which the soil fertility is high and coal is the fuel being offset are best suited for bioenergy production. The climate-mitigation potential of biochar (with combined energy production) is higher for all other situations.

IBI developed scenarios on carbon removal from the atmosphere by biochar. Those scenarios primarily differ in the amount of biomass that was available in a sustainable way from global Net Primary Production (NPP). The “Conservative” scenario assumes that only biomass from cropping and forestry residues that otherwise has no use (about 27% of the total residues) is available. The “Moderate” and “Optimistic” scenarios consider that 50% and 80%, respectively, of all cropping and forestry residues is available to produce biochar. For each base scenario, IBI estimated the amount of biochar produced, as well as the amounts of fossil fuel carbon emissions replaced by the energy generated during biochar production. Moreover, IBI estimated the additional amount of carbon that could be sequestered if CO_2 emissions generated during biochar production were captured and sequestered in the same way as proposed for coal combustion facilities.

The results of IBI scenarios show that the carbon sequestered in biochar can be 0.25 Gt per year by 2030 in the "Conservative" scenario, and 1 Gt annually before 2050 in the "Optimistic" scenario. An often-quoted analysis (Pacala and Socolow, 2004) shows a need to have 7 Gt of carbon per year of reduced carbon emissions by 2054 just to keep emissions at the 2004 level.

1.6.2. Biochar and soil

Biochar used as a soil amendment to improve soil fertility and plant growth has been the focus of much research in the recent past (Zhang et al., 2012; Ibrahim et al., 2013). It has shown promise as a sustainable amendment to enhance soil chemical properties (Glaser et al., 2002; Lehmann et al., 2011). Soil may become degraded due to human activities such as mining and industrial activities as well as the use of certain pesticides in agriculture.

Because of its high organic C content, biochar has the potential to serve as a soil conditioner to improve the physicochemical and biological properties of soils. Soil water retention capacity increases with increase in organic C. About 18% increase in the water holding capacity of soil containing biochar was reported (Glaser et al., 2002). Soil water holding capacity is related to hydrophobicity and surface area of biochar, and the improved soil structure following biochar application (Verheijen et al., 2010). Biochar amendments have been reported to improve soil bulk density, porosity and hydraulic conductivity (Asai et al., 2009; Jeffery et al., 2011; Abel et al., 2013). Moreover, a decrease of nutrient leaching due to biochar application has been also reported (Sohi et al., 2009; Major et al., 2010; Singh et al., 2010).

Biochar generally has a neutral to alkaline pH; however, acidic biochar has been also reported (Chan et al., 2007). The pH of biochar depends on various factors including feedstock type and the thermochemical process of production. The alkaline pH of biochar induces a liming effect on acidic soils, thereby possibly increasing plant productivity. The extent of liming effect of biochar depends on its acid neutralizing capacity that varies depending on the feedstock and pyrolysis temperature. For example, biochar derived from paper mill waste pyrolyzed at 550 °C has a liming value around 30% that one of $CaCO_3$ (Zweiten et al., 2010). Significant increases in seed germination, plant growth, and crop yields have been reported in the soils amended with biochars (Glaser et al., 2002).

The effect of biochar on microbial activity needs to be further investigated, especially when considering the possibility of large applications of biochar in agronomic systems for the purpose of increasing soil organic carbon. However, Lehmann et al. (2011) and more recently Ameloot et al. (2013) reported direct and indirect interactive effects between biochar and soil organisms. Although biochar does not provide a suitable habitat for soil microorganisms (Quilliam et al., 2013), soil microbial activity may be indirectly influenced by changes in the physicochemical properties, e.g. soil porosity, pH, cation exchange capacity (CEC) and adsorption properties. In a direct way, microorganisms can utilize a number of labile biochar constituents as an energy source (Cross and Sohi, 2011). These are presumably either relatively untransformed biomass components that have not been subjected to volatilization during pyrolysis (Ronsse et al., 2013) or volatilized compounds that have recondensed in the biochar matrix during pyrolysis (Kloss et al., 2012). However, Ameloot et al. (2014) reported that, in contrast to many short-term laboratory studies, in field experiment biochar amendment led to a lowered or equal soil microbial activity after 1-4 years incorporation in the field.

Some researches reported the potential role of biochar in reducing N losses. However, to date little is known about the effects of biochar on the soil nitrogen (N) cycle. Yanai et al. (2007) and Singh et al. (2010) have shown that biochar decreased N_2O emissions because of its ability to absorb water. In particular, Singh et al. (2010) hypothesized that reduction of N_2O emissions and ammonium leaching was determined by the increasing biochar nutrient sorption capacity due to the higher oxidative reactions on its surface over time.

Applying biochar together with organic or inorganic fertilizers can even enhance crop yields (Lehmann et al., 2002). Studies show that when biochar is applied in soil, it increases crop yield, reduces irrigation needs and enhances fertilizer efficiency (Steiner et al., 2007; Blackwell et al. 2009). However, the biochar as a soil amendment for crop production is still being investigated, and results so far are not conclusive. The application of biochar to soils can boost crop yields by up to 60% or diminish yields by up to 30%, mainly depending on the type of soil to which it is applied (Crane-Droesch et al., 2013). Spokas et al. (2012), in their biochar review article, reported that biochar application rates in research studies have range from <1 to over 100 t ha^{-1} and reported relative response to biochar compared to the treatment that receives no biochar (0 t ha^{-1}) from a reduction of 50 % to positive yield increases of ~200 %. Therefore, biochar applications affect crop yields

in highly variable ways. Such great variation likely stems from the large range of biochar application rates, crops, and soil types used. In particular, the differences in results reflect:

1. Type of feedstock for biochar and the temperature and time of pyrolysis. The application of different types of biochar can lead to very different responses (Rajkovich et al., 2012): some types of biochar can increase crop production by over 100%, and others can reduce it by a similar amount.

2. Differences in soil types. Positive effects on plant growth tend to be recorded from highly- degraded and nutrient-depleted soils (Zwieten et al., 2009). Application of biochar to fertile soils has not been shown to increase plant growth.

Therefore, the impact of biochar on the crop yield needs to be investigated further. In fact, the extent with which biochar application might increase agricultural production is an important driver in any attempt to develop systems that economically incorporate pyrolysis products within the soil. It is not the only consideration (carbon sequestration is also very important), but it requires long-term investment in agricultural experimentation.

1.6.3. Biochar and waste management

Biochar has great potential for managing the waste stream originating from animals or plants; thus, decreasing the associated pollution loading to the environment. The use of waste biomass for biochar production is not only economical but also beneficial. Making biochar from biomass waste materials should create no competition for land with any other land use option – such as food production or leaving the land in its pristine state. Therefore, the conversion of wastes into biochar through pyrolysis is potentially an effective waste management solution and economic feasibility.

Waste biomass that has been used to produce biochar includes crop residues (both field residues and processing residues such as nut shells, fruit pits, bagasse, etc), forestry waste, animal manure, food processing waste, paper mill waste, municipal solid waste and sewage sludge (Cantrell et al., 2012; Enders et al., 2012).

Large amounts of agricultural, municipal, and forestry biomass are currently burned or left to decompose and release CO_2 and methane back into the atmosphere. They also can pollute local ground and surface waters – a large issue for livestock wastes. Using these materials to make biochar not only removes them from a pollution cycle, but biochar can

be obtained as a by-product of producing energy from this biomass. Moreover, pyrolyzing the waste biomass, particularly animal manure and sewage sludge, kills any microbes present, thereby reducing the environmental health effects (Lehmann and Joseph, 2009). However, the persistence of toxic heavy metals in biochar developed from sewage sludge and municipal solid waste (Lu et al., 2012) must be carefully handled before long-term application to soils.

The annual worldwide production of wheat straw as agricultural waste was estimated to be approximately 540 million tons in 2007 (Reddy and Yang, 2007). The straw might be left on the field, burned, fed animals or used as industrial raw materials. As lignosulfonate is the main component of paper mill waste, huge amount of lignosulfonate was generated and the disposal of waste (liquid, solid and suspended matter) generated during the paper manufacturing process contributed to a very high impact on the environment, but less of them were utilized. Moreover large proportion of waste has been disposed of by burning and discharging, resulting in not only a waste of resource but also a serious environmental problem. Conversion of straw and lignosulfonate into biochar through pyrolysis has further advantages of energy and environment.

1.6.4. Biochar and other environmental effects

Biochar not only improves chemical and biological soil properties but also can help mitigate environmental issues by reducing of the mobility of heavy metals (Cu and Zn) (Hua et al., 2009) and other organic soil contaminants (i.e. insecticides, Hilber et al., 2009). The adsorption behavior of biochar for different contaminants (i.e., heavy metals, organic pollutants and other pollutants) are different and well correlated with the properties of contaminants. In addition, the adsorption mechanism may also depend on biochar's various properties including surface functional groups, specific surface area, porous structure and mineral components.

One of the characteristics of biochars is possessing large surface areas, which implies a high capacity for complexing heavy metals on their surface. Surface sorption of heavy metals on biochar has been demonstrated on multiple occasions using scanning electron microscopy (Beesley and Marmiroli, 2011; Lu et al., 2012). This sorption can be due to complexation of the heavy metals with different functional groups present in the biochar, due to the exchange of heavy metals with cations associated with biochar, such as Ca^{+2} and

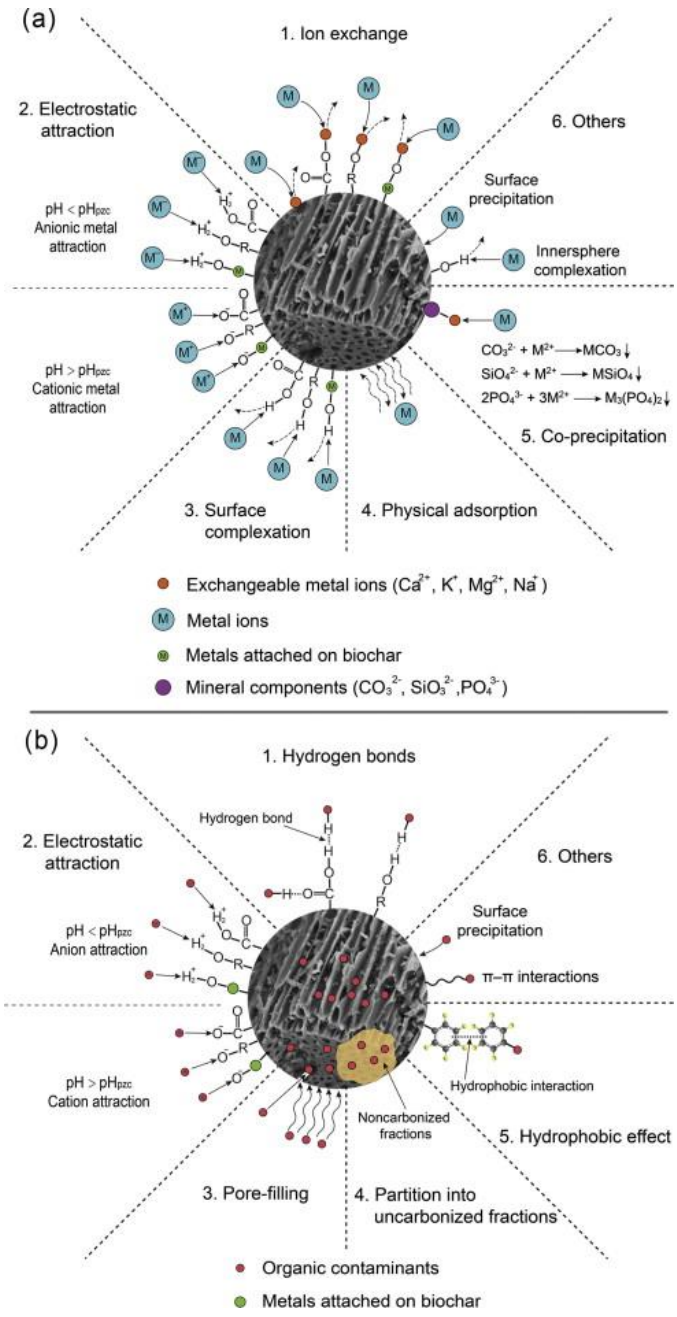
Mg^{+2} (Lu et al., 2012), K^{+}, Na^{+} and S (Uchimiya et al., 2011), or due to physical adsorption (Lu et al., 2012). Also oxygen functional groups are known to stabilise heavy metals in the biochar surface, particularly (Uchimiya et al., 2011) for softer acids like Pb^{+2} and Cu^{+2}. In addition, Méndez et al. (2009) observed that Cu^{+2} sorption was related to the elevated oxygenated surface groups, elevated superficial charge density and Ca^{+2} and Mg^{+2} exchange content of biochar. Furthermore, the surface area and porous structure of biochar can also have effects on the adsorption of heavy metals. However, as the literature data reported, the surface area and porous structure of biochar seem to have less effect on heavy metal adsorption than oxygen-containing functional groups (Ding et al., 2014). Sorption mechanisms are also highly dependent on soil type and the cations present in both biochar and soil. Some other compounds present in the ash, such as carbonates, phosphates or sulphates (Cao et al., 2009; Park et al., 2013) can also help to stabilise heavy metals by precipitation of these compounds with the pollutants. Alkalinity of biochar can also be partially responsible for the lower concentrations of available heavy metals found in biochar-amended soils. Higher pH values after biochar addition can result in heavy metal precipitation in soils. Biochar pH value increases with pyrolysis temperature (Wu et al., 2012), which has been associated with a higher proportion of ash content (Cantrell et al., 2012). Biochar can also reduce the mobility of heavy metals, altering their redox state of those (Choppala et al., 2012). As an example, biochar addition could lead to the transformation of Cr (VI) to the less mobile Cr (III) (Choppala et al., 2012). Therefore, the possible adsorption mechanisms usually involved integrative effects of several kinds of interactions including electrostatic attraction, ion exchange, physical adsorption, surface complexation and/or precipitation (Fig. 4a). However, the relative contribution of the different mechanisms to heavy metal immobilisation by different biochar remains unknown.

Fellet et al. (2011) tried to use biochar to remediate a multicontaminated mine soil. Biochar addition did not result in the decrease of the total heavy metal content of the soil; however, biochar addition reduced the bioavailability of Cd, Pb and Zn and the mobility (measured using a leaching experiment) of Cd, Cr and Pb. Uchimiya et al. (2012) analysed the effects on soil heavy meals concentrations of 10 biochars prepared from 5 feedstocks at 2 different temperatures. They observed that manures with a high or low proportion of ash or P were less effective to immobilise heavy metals. In contrast, biochars prepared at 700 °C were more effective, which could be attributed to transformations in the material, including the removal of nitrogen containing heteroaromatic and leachable aliphatic

functional groups. They found Cu and Pb relatively easy to stabilise in soil, while Cd and Ni response depended strongly on the type of biochar added to the soil.

Biochar application can also reduce the availability of organic contaminants such as phenols in the soil (Gundale and DeLuca, 2007). The sorptive capacity of biochar to organic contaminants in soil is controlled by carbonised and non-carbonised fractions and the surface and bulk properties of biochar (Obst et al., 2011). Sorptive characteristics can equally be affected by hydrophilic groups on biochar (James et al., 2005). The adsorption mechanisms by which organic contaminants bind to biochars were also combined with different kinds of interactions. In general, electrostatic interaction, hydrophobic effect, hydrogen bonds, and pore-filling may be the main mechanisms for the adsorption of organic contaminants onto biochar. The various mechanisms proposed for the interaction of biochar with organic contaminants are summarized in Fig. 4b. For instance, the adsorption of aromatic molecules such as PAHs to wood biochars is rapid and is assisted by π-π electron interactions and pore-filling mechanisms (Chen et al., 2009), multilayer adsorption, surface coverage, condensation in capillary pores, and adsorption into the polymetric matrix (Werner et al., 2005). The results of different studies collected suggests that electrostatic attraction was the dominant mechanism for adsorption of organic contaminants onto the chars, with others performed as a contributing adsorption mechanism (Inyang et al., 2014).

Figure 4. Summary of proposed mechanisms for (a) heavy metals and (b) organic contaminants adsorption on biochars (Tan et al., 2015).



Recently, the biochar has been investigated for its effectiveness in saline soil remediation. An interesting short incubation experiment (Wu et al., 2014) showed which biochar can play a more important role in saline soil remediation reducing exchangeable sodium percentage of saline soil and that biochar can improve soil fertility due to the

increment of soil organic carbon, cation exchange capacity and enhanced available phosphorus.

1.7. Stability of biochar

The stability of biochar is of fundamental importance in the context of biochar use for environmental management for two primary reasons: first, stability determines how long carbon applied to soil, as biochar, will remain in soil and contribute to the mitigation of climate change; second, stability will determine how long biochar will continue to provide benefits to soil, plant, and water quality (Lehmann et al., 2006). It is well known that a variable component of the carbon in many biochars is degradable on annual to decadal timescales and hence, only a proportion of total carbon in biochar provides long-term carbon sequestration (Bird et al., 1999; Zimmermann et al., 2012).

An increasing number of studies suggests that biochar can be degraded, by both biotic and abiotic processes (Hamer et al., 2004; Cheng et al., 2008; Guggenberger et al., 2008). However, in most of the studies the stability of biochar was assessed during laboratory incubations, with fresh biochars added to soil (Zavalloni et al., 2011; Ameloot et al., 2013). The duration of these experiments ranges from several weeks (Cross and Sohi, 2011) to several years (Kuzyakov et al., 2009; Kuzyakov et al., 2014), allowing to understand biochar stability under controlled laboratory conditions. On the contrary, there are only few studies estimating biochar degradation rates in soil (Kuzyakov et al., 2009) and the long-term stability of biochar in soils. This is because the changes of biochar content are too small for any practical experimental period. Many studies estimating the decomposition rates of biochar in soil are based on changes of CO_2 efflux after biochar application. This approach is unsuitable to estimate biochar decomposition because of the much higher contribution of soil organic matter and plant residues mineralization of the CO_2 compared to biochar.

The complexity and chemical heterogeneity of biochar has made it difficult to establish a single method suited to assessing the potential stability (Hammes et al., 2006) and hence, there is no globally established method for determination of absolute stability for biochar. However, a number of methods for comparing the relative stability of different biochar materials have emerged. These include proximate analysis using the fixed carbon as a measure of stability (ASTM Standard D3175; 2007), thermal analysis (thermogravimetry,

TG; de la Rosa et al., 2008), molecular markers by means of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS; Kaal et al., 2008, 2009, Conti et al., 2014), benzene polycarboxylic acid method (Brodowski et al., 2005), O:C or H:C molar ratios (Spokas, 2010; Enders et al., 2012; IBI Guidelines, 2012) and chemical oxidation (Cross and Sohi, 2013). Further information on the studies conducted on biochar stability can be found in the introductory section of chapters 5.1 and 5.2.

1.8. Biochar and pollutants

Biochar quality guidelines have been recently proposed such as the IBI Biochar Standard (IBI, 2013), the European Biochar Certificate (EBC, 2014) or the UK Biochar Quality Mandate (BQM, Shackley et al., 2013). In these standards, environmental risks are accounted for by the inclusion of limit values for physicochemical properties, including pollutants such as heavy metals, dioxins/furans, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). For instance, the IBI sets a range of maximum allowed threshold values (varying between different countries) for the sum of the 16 US Environmental Protection Agency's (EPA) PAHs in biochar to 6-20 mg kg^{-1} $_{\text{dry weight (dw)}}$ (IBI, 2013). Similarly, the EBC requires PAHs to be below 4 and 12 mg kg^{-1} $_{\text{dw}}$, in premium and basic grade biochars, respectively (EBC, 2014).

Research shows (Hale et al., 2012 and Oleszczuk et al., 2013) that biochar can contain dangerous inorganic contaminants (heavy metals) and organic ones (e.g. polycyclic aromatic hydrocarbons (PAH) as well as dioxins and furans (PCDD/Fs)). The presence of contaminants, therefore, poses a question mark over the common utilisation of biochars, especially for the amendment of soils used for crop plant cultures. In the case of high levels of contaminants there is the risk of their uptake by plants or migration down the soil profile to groundwaters. This may have negative effects for humans, for the environment and for living organisms. Among the threats, most frequently mentioned is the contamination of biochar with PAHs and heavy metals. While in the case of heavy metals their levels are at relatively low values (Freddo et al., 2012) and depend on the content of trace metals in the initial material, studies concerning PAHs indicate (Freddo et al., 2012; Hale et al., 2012; Hilber et al., 2012; Keiluweit et al., 2012; Oleszczuk et al., 2013; Fabbri et al., 2013) that biochars may be contaminated with those compounds to a significant degree. PAHs are formed during the pyrolysis of organic matter (including biomass) and

the content in biochars varies with relation to the feedstock, and also to the conditions of the pyrolysis process (Manyà, 2012; Wiedner et al., 2013).

The application of biochar (containing high levels of PAHs) to soils, even at small doses, may undeniably cause an increase in the soil content of those contaminants. Moreover, soils act as environmental sinks for PAHs, as they become strongly absorbed onto soil particles; degradation is mainly driven by microbial catabolism, although it is dependent on PAH bioavailability. In this respect, soil application of biochar could not only increase the concentration of soil PAH but could also affect the long-term persistence of PAHs in the environment. All these aspects need to be considered when dealing with the origin of PAHs in soil amended with biochar. Human exposure of PAHs might occur through different pathways, such as inhalation of particles generated during synthesis, handling and field applications of biochar or the ingestion of fruit/vegetables grown in biochar amended soil. Therefore, determining the content of PAHs in biochar is of utmost importance to establish risk assessment of biochar usage.

PCDD/Fs could be present in the biochars produced from feedstock that contain chlorine. Biochar feedstocks such as grasses, straws and food waste (which contains sodium chloride, i.e., salt) can be a source of chlorine. Other potential sources of chlorine in biochar feedstocks include biomass that has been exposed to salt (such as crops or trees grown near seashores), and the biomass fraction of municipal solid waste that may be contaminated with polyvinyl chloride (PVC) or other chlorine-containing plastics. Research concerning dioxin indicates that biochars may be contaminated with those pollutants (Downie et al., 2011). Moreover, in biochars from food waste have been found relatively high concentration of dioxin likely due to the high salt (sodium chloride) content in food waste. However, in the biochars studied the total dioxin concentrations (0.005-1.20 ng Kg^{-1}) (Hale et al., 2012) are lower than the guideline values for dioxin and furans in biochar of the EBC (20 ng Kg^{-1} TEQ) and IBI (17 ng Kg^{-1} TEQ). Further information on the PAH topic has been reported in the introductory part of section 3.

1.9. Biochar: some non-negligible issues

Owing to the extensive range of combinations of biochar, soils and plants, much research still needs to be undertaken to understand the large variety of resulting interactions and their effects. As research progresses, it will be possible to make

extrapolations with increasing robustness as, for example, the database upon which meta-analysis can be performed grows. Such information is vital to guide the development of certification schemes such as that proposed by the IBI, and the EBC, which is already implemented in part of Europe, as well as to guide policy.

Interaction of biochar with soil microbial communities and plants

The physical, biological and chemical processes that biochar may exert on microbial communities and their symbiotic interaction with plants, and possibly enhanced nutrient use efficiency, are not yet understood. The apparent contradiction between the high stability of biochar, soil organic matter accumulation and apparent enhancement of soil microbial activity needs to be resolved. In the future work, the effects of biochar on various soil biota groups, their diversity and functioning need to be carefully considered. Moreover, further research biochar needs to involve a careful selection of the feedstock and pyrolysis conditions to find an optimal match of biochar type to the intended ecosystem goal.

Biochar erosion, transport and fate

The loss of biochar through vertical or lateral flow is not quantified, and only recently have studies been initiated to examine movement through soil profiles and into water ways. It should be noted however that transport of biochar through the profile does not impact on its direct carbon sequestration potential.

Biochar stability

A key requirement for the use of biochar as tool for environmental management is that the carbon in the biochar is stable, meaning that a substantial fraction of the carbon sequestered is not re-mineralized on at least centennial timescales. However, a variable component of the carbon in many biochars is degradable on annual to decadal timescales and hence, only a proportion of total carbon in biochar provides long-term carbon sequestration. Although our understanding of biochar carbon stability has improved in recent years, there is limited research on process conditions to produce a biochar suitable and highly stable for the long-term carbon sequestration.

Pollutants environmental fates

The fate of contaminates in the environment is of prime importance in order to prevent severe contamination to the environment. The environmental fates of biochar-associated pollutions added to soil are still poorly understood. In particular, further research work is

required to improve understanding of the role biochar plays in sorbing PAHs and on microbial activity and how this influences the concentration of PAHs in soil and their persistence in the environment.

Synergistic effects

The interactions of biochar with soil organic matter as well as the mineral matrix need to be assessed in order to determine the nature and the environmental conditions under which synergistic effects develop.

Water holding capacity

The contribution that biochar can make to water retention, macro-aggregation and soil stability is poorly understood – yet should be of critical importance in climate change adaptation, where mitigating drought, nutrient loss and erosion are critical.

Cation exchange capacity (CEC)

While the CEC of fresh char itself is not very high biochar that has resided in soil for hundreds of years has been shown to have much higher CECs, comparable to those of zeolites. However, several studies have reported an increase in soil CEC after the application of fresh biochar. Thus, the processes that are instrumental in developing CEC over time as well as the effects that lead to an increase in CEC by addition of fresh (low CEC) biochar requires detailed understanding.

Decreased emissions of non-CO_2 greenhouse gases (e.g. N_2O and CH_4)

The currently available data on the effect of biochar additions on trace gas emission is very limited, but has a potentially great impact on the net benefit of biochar application. Therefore, further research work is required to determine the impact of biochar on the emission of N_2O and CH_4.

Soil carbon modelling

Modelling of the linked carbon and nitrogen cycles in soil with and without application of biochar is essential to understanding the fundamental mechanisms referred to above, and the impact on soil-based emissions of greenhouse gases.

Project specific Life Cycle Assessment (LCA)

The total environmental life cycle assessment has been conducted for some biochar case studies. Greenhouse balances, for example, are very project specific and hence there

is opportunity to assess the benefits over a large range of feedstock, process and biochar application scenarios.

2. Aim of the thesis

Studies on biochar are relatively recent, leaving several aspects unexplored or not fully developed. Further research on the impact of biochar in the environment in both the long and the short term is required both to avoid unforeseen consequences and to provide evidences of further potential benefits. In particular, the environmental potential and limitation of biochar in soil applications requires a full understanding of the stability and fate of carbon fractions and trace contaminants, in particular PAHs. For this reasons this thesis was focused on the assessment of biochar stability and the occurrence and fate of PAHs.

Regarding PAHs, biochar is the by-product of a thermochemical process. Therefore, the formation of PAHs from biomass pyrolysis and their occurrence in biochar is inevitable and must be considered and properly evaluated in order to avoid or limit occupational exposure, land contamination and PAHs transfer to crops. Due to the carbonaceous nature of biochar that has a great affinity for polyaromatic compounds, the analysis of PAHs is challenging, no certified reference materials are available, and standardised methods are being developed. In order to determine the level of PAHs with reliable analytical procedures and evaluate the potential negative impact, this thesis aimed at:

- developing a well characterized analytical method for the determination of PAHs in pure biochars as well as in soil-biochar matrices.
- measuring the levels of PAHs in biochars from different feedstock and process conditions, searching for causal relationships, extending the analysis to EU-PAHs (food safety) along with EPA-PAHs (environmental protection).
- assessing the long-term impacts of biochar additions, at different applications rates, on PAHs concentration in agricultural soils;
- evaluating their possible role in the phytoxicity of animal vs. plant derived biochar.

Regarding the environmental stability, this is probably the most crucial and less known among the properties of biochar of interest for assessing its benefits to soil organic carbon and CO_2 mitigation. In this topic, the thesis was focused to:

I. The determination and quantification of labile and resistant carbon fractions in biochar by hydropyrolysis (HyPy).

II. The molecular characterisation of the biochar labile fraction by HyPy combined with GC-MS.

III. The assessment of the impact of production conditions on biochar stability by pyrolysis-GC/MS and HyPy.

IV. The identification of the resistant carbon fractions and the characterization of the labile organic carbon in biochar amended soils in a four years field study.

3. Determination of PAHs: method development and application

3.1. Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil

3.1.1. Introduction

Biochar is a co-product from biomass pyrolysis that is targeted as a material with applications in environmental and agricultural management, as well as a vehicle for carbon sequestration (Sohi et al., 2010; Manyà et al., 2012). As the interest toward biochar is steeply growing, safety procedures for ensuring human health and preservation of the environment are imperative. Polycyclic aromatic hydrocarbons (PAHs) are well known carcinogenic and persistent pollutants that are ubiquitous in the environment. PAHs are formed during the pyrolysis of biomass (Fabbri et al., 2010) and their occurrence in biochar (Hale et al., 2012; Hilber et al., 2012; Schimmelpfennig and Glaser., 2012; Keiluweit et al., 2012; Freddo et al., 2012; Kloss et al., 2012) along with its possibly released into the environment need to be addressed. PAH production has also been confirmed during the production of charcoal by pyrolysis (Ré-Poppi et al., 2002; Mara Dos Santos Barbosa et al., 2006) and natural wildfires (Kim et al., 2003). Human exposure of PAHs might occur through different pathways, such as inhalation of particles generated during synthesis, handling and field applications of biochar or the ingestion of fruit/vegetables grown in biochar amended soil. Therefore, determining the content of PAHs in biochar is of utmost importance to establish risk assessment of biochar usage.

The worldwide distribution of PAHs in soils span over five orders of magnitude and is related to source (atmospheric input) and sorption ability of soil organic matter and black carbon (Nam et al., 2009). The inclusion of carbonaceous residues in soil could increase PAHs sorption on humic matter (Cornelissen et al., 2005; Oen et al., 2006; Poerschmann et al., 2007; Brandli et al., 2008) and biochar (Hale et al., 2011; Oleszczuk et al., 2012). In this respect, soil application of biochar might represent a source and/or a sink of PAHs. All these aspects need to be considered when dealing with the origin of PAHs in soil amended with biochar.

A reliable methodology of PAH analysis is a first requisite towards risk assessment. Recent studies have examined the content of PAHs in biochar (Hale et al., 2012; Hilber et al., 2012; Keiluweit et al., 2012). These results have provided a comprehensive picture on

the levels and availability of PAHs in biochar (Hale et al., 2012), the influence of pyrolysis temperature (Keiluweit et al., 2012), as well as critical aspects of validation (Hilber et al., 2012). Analytical methods described in these studies have utilized toluene as the extracting solvent. In fact, it was demonstrated that toluene is superior to other solvents for carbonaceous materials (Jonker et al., 2002). Nonetheless, extraction efficiencies are not always quantitative, especially in the case of low molecular weight (LMW) PAHs. In particular, naphthalene is problematic because of the high boiling point of toluene (111°C) which causes the loss of semi-volatile PAHs during the preconcentration step (Hilber et al., 2012; Keiluweit et al., 2012). Naphthalene is considered a possible carcinogenic to humans (IARC group 2B) and genotoxic to plants (Aina et al., 2006). Incidentally, naphthalene is often the most abundant PAH in biochar (Hale et al., 2012; Hilber et al., 2012; Schimmelpfennig and Glaser, 2012; Freddo et al., 2012; Kloss et al., 2012; Spokas et al., 2011). Naphthalene and its isotopically labelled version are often employed in studies aimed at investigating the fate of PAHs in the environment (Wild et al., 1994; Fraser et al., 1998; Kipopoulou et al., 1999; Motelay-Massei et al., 2006). In general, LMW PAHs are absorbed at higher rates than high molecular weight (HMW) PAHs (Kipopoulou et al., 1999; Motelay-Massei et al., 2006; Tao et al., 2004), and naphthalene presence could affect the growth/response of the soil microbial community (Loibner et al., 2004; Krang et al., 2007).

Although present at lower concentrations, HMW PAHs pose the highest health and environmental hazards due to the established carcinogenic potential of this class of compounds. Because of biochar's proposed use in crops and potential human exposure of biochar PAHs through bioaccumulation in agricultural products, biochar sorbed PAH concentrations could be a matter of concern (Ahn et al., 2008; Meudec et al., 2006; Rey-Salgueiro et al., 2009). On the basis of their occurrence and carcinogenicity, 15PAHs have been identified as priority hazardous substances in food by the European Union (EU) (ECR, 2006)] and 16 PAHs by US Environmental Protection Agency (USEPA) (2002), 8 of them are shared across both lists. While studies have been reported on the occurrence of USEPA PAHs in biochar due to the widespread inclusion of these compounds in worldwide environmental legislation, very limited information is available on the occurrence of EU PAHs on biochar.

In addition, recent studies were focused on the analysis of PAHs in solely biochar, but the robustness of the solvent extraction method to extract PAHs when biochar is present in the soil was not fully investigated. It is important that a method developed for the analysis

of solely biochar should be equally accurate for the biochar-soil matrix. In this context, the use of (cyclo)hexane/acetone mixtures as an extracting solvent in PAH determination in soil is rather common (e.g. Gfrerer et al., 2002; Shu et al., 2003; Beesley et al., 2010). In fact, a relatively polar solvents like acetone has been cited as beneficial for the extraction of hydrophobic PAHs from soil (Pena et al., 2007).

The present study is aimed at developing a well characterized method for the determination of PAH in biochars and soils amended with biochar by GC–MS. To this purpose, several solvent and extraction procedures were examined using the 16 EPA PAHs as targeted PAHs on a biochar utilized in agronomic field studies (Fellet et al., 2011). The method was then applied to a set of biochars investigated as soil amendments of different origin and from different process conditions (Fabbri et al., 2012). Besides the EPA PAHs, the level of EU PAHs in these biochars was investigated as well.

3.1.2. Materials and methods

3.1.2.1. Reagents and standards

Cyclohexane, acetone, acetonitrile, dichloromethane, toluene, ethyl acetate (all ultra-purity), and surrogate standard mix (for USEPA 525) containing acenaphthene-d_{10}, phenanthrene-d_{10} and chrysene-d_{12} at concentrations of 500 mg l^{-1} each in acetone were purchased from Sigma-Aldrich. PAH-Mix solution containing naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene certified at concentrations of 10 mg l^{-1} for each species in acetonitrile was purchased from Sulpeco (Belleforte, PA, USA).

PAH-Mix standards in acetonitrile (10 mg l^{-1}) of EU PAHs were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany): benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*c,d*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*c,d*]pyrene, 5-methylchrysene. Standard mix solutions containing the 15 PAHs at concentrations of 1 mg l^{-1} were prepared in acetone/cyclohexane (1:1, v/v) and stored at room temperature in the dark.

A solution of 1,3,5-tri-tert-butylbenzene (TTB, 12.7 mg l^{-1}) in acetone:cyclohexane (1:1, v/v) was prepared by weighing the pure compound purchased from Sigma-Aldrich.

3.1.2.2. Soil and biochar samples

A natural matrix soil certified reference material ERM–CC013a (manufactured by Federal Institute for Materials Research and Testing; Berlin, Germany) containing 15 PAHs with concentrations ranging from 1.14 to 12.9 mg kg^{-1} was used for the validation of the method for soil. An internal reference biochar sample (here named as reference biochar, or RB) was utilized for method optimization. This was a commercially available biochar created by the slow pyrolysis of orchard pruning, which was kindly provided by the Department of Agriculture and Environmental Sciences (DISA) University of Udine (Fellet et al., 2011). This reference biochar was homogenized and then mixed with an agricultural soil (dried and sieved 2 mm) at a 1.16% (w/w) amendment level. This concentration corresponded to an application of 36 t biochar ha^{-1} (assuming a soil with 1.2 g cm^{-3} density and 0.3 m depth) (Schimmelpfennig and Glaser., 2012; Zavalloni et al., 2011), which is within the range currently investigated for biochar use in agriculture (20–60 t biochar ha^{-1}) (Baronti et al., 2010).

Additional biochars evaluated were from an on going study on the impact of biochar additions on greenhouse gas production potentials conducted by the USDA-ARS Biochar and Pyrolysis Initiative. The full characterization of these biochars (i.e. ultimate and proximate analysis, Py-GC–MS, and microbial CO_2 production) was reported in a previous publication [40]. This group provides across-section of currently available biochars for agricultural field applications.

3.1.2.3. Sample treatment

3.1.2.3.1. Optimized sample pretreatment: soxhlet extraction and clean up

About 1 g of biochar (or 5 g soil sample) was placed into the extraction cellulose thimble, spiked with 0.1 ml of surrogate standard mix (Supelco for EPA 525 containing acenaphthene-d_{10}, phenanthrene-d_{10} and chrysene-d_{12} 5 µg ml^{-1} each in acetonitrile). The thimble was covered with cotton wool, and inserted into the Soxhlet extractor. Soxhlet extraction thimbles (and the Soxhlet apparatus) were pre-cleaned by a 4 h Soxhlet extraction with acetone/cyclohexane (1:1, v/v). Extraction was carried out with 160 ml of

extraction solvents (acetone/cyclohexane (1:1, v/v)) mixture for 36 h (4 cycles h^{-1}). The Soxhlet apparatus was covered with an aluminum foil to avoid exposure to daylight, which prevents PAH photodegradation. The extraction solvent was filtered, added with 1 ml of n-nonane, and then carefully evaporated by rotatory vacuum evaporation at 40 °C. The concentrated extract was collected and loaded onto a silica gel cartridge (6 ml, 1 g DSC-Si Supelco washed with ethyl acetate, dried and conditioned with 4 ml cyclohexane). After purification with 1 ml of cyclohexane, PAHs were eluted with 4 ml of acetone/cyclohexane (1:1, v/v). The obtained solution was then blown down to 10–50 µl under nitrogen and spiked with 10 µl of the internal standard solution (TTB at 12.7 mg l^{-1}) prior to GC–MS analysis.

3.1.2.3.2. Reflux extraction

Four different solvent systems (toluene, dichloromethane, acetone:cyclohexane 1:1 (v/v) and acetone:cyclohexane 1:5 (v/v)) were compared by means of reflux extraction. To this purpose PAHs were extracted from the biochar (2 g reference biochar added with 0.1 ml of surrogate standard mix) by refluxing for 4 h with 80 ml solvent. The extract was filtered and concentrated to ~100 µl by using rotary evaporator and then under a nitrogen stream. The obtained solution was spiked with 10 µl of internal standard (12.7 mg l^{-1} TTB) and then analyzed by GC–MS.

3.1.2.3.3. Ultrasonication extraction

Each homogenized reference biochar sample (1 g) was transferred into a Pyrex tube, and 20 ml of acetone/cyclohexane (1:1, v/v) were added. The sample was ultrasonicated for 30 min with occasional swirling. The extraction solutions were then centrifuged and the supernatant filtered into a 50 ml beaker using a 9.0 cm GF/C glass microfibre filter (Whatman International, Maidstone, UK). The obtained solutions were reduced to 2 ml using a rotary evaporator and transferred into 4 ml vials. These solutions were further reduced using nitrogen gas, spiked with 10 µl of 12.7 mg l^{-1} TTB, and analyzed by GC–MS.

3.1.2.4. GC–MS

GC–MS analyses were performed using a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a HP-5MS fused-silica capillary column (stationary phase poly[5% diphenyl/95%dimethyl]siloxane, 30 m × 0.25 mm i.d., 0.25 mm film thickness), using helium as the carrier gas. Samples (1 µl) were injected under splitless conditions (1 min, then split ratio 1:50 to the end of analysis) with an injector temperature of 280 °C. The following thermal program of the capillary column was used: 50 °C to 100 °C at 20 °C min^{-1}, then from 100 °C to 300 °C at 5 °C min^{-1}, then a hold for 2.5 min at 300 °C. The mass spectrometer operated under electron ionization (70 eV) and acquisition was performed on single ion monitoring (SIM) at the molecular ion of each PAH at the time windows corresponding to the elution region of the target PAH. Acenaphthene-d_{10} was utilized to quantify naphthalene, acenaphthylene, acenaphthene and fluorene; phenanthrene-d_{10} to quantify phenanthrene, anthracene, fluoranthene and pyrene; chrysene-d_{12} to quantify the remaining PAHs. Quantitation of EPA PAHs was based on the calibration curve (Section 3.1.2.5), while in the case of EU PAHs a single point calibration (1 mg l^{-1}, Section 3.1.2.1) was utilized.

3.1.2.5. Method validation

The figures of merit were reported for the EPA PAHs. Recovery of surrogated PAHs was determined with respect to the internal standard TTB. The procedural blank concentrations were determined as the average of five empty thimble runs. Procedural blanks were run periodically. Precision of the procedure was determined by four replicate analyses of reference biochar sample. Calibration was performed in the 0.0025–1.25 mg l^{-1} interval by serial dilutions of the 10 µg ml^{-1} EPA PAH calibration mix (Supelco). Three replicates were performed at each concentration level and the resulting instrumental response was homoscedastic for each PAH (α = 0.05, Cochran test), therefore the least-squares regression line was utilized for quantification (R^2 values were 0.993–0.999). Limit of detection (LOD) and limit of quantification (LOQ) were estimated for each analyte by using Eqs. (1) and (2)

$$LOD = 3\ s_b / a \quad (1)$$

$$LOQ = 10\ s_b / a \quad (2)$$

Table 3.1.1. Limits of detection (LOD), limits of quantification (LOD), mean concentration of EPA PAHs in reference biochar (RB) and relative standard deviations (RSD) from four replicates.

PAH	**LOD (ng g^{-1})**	**LOQ (ng g^{-1})**	**RB (µg g^{-1})**	**RSD (%)**
Naphthalene	0.08	0.2	1.75	8
Acenaphtylene	0.01	0.03	0.026	13
Acenaphthene	0.03	0.1	0.034	5
Fluorene	0.03	0.1	0.071	10
Phenanthrene	0.4	1	0.71	12
Anthracene	0.03	0.1	0.13	13
Fluoranthene	0.08	0.3	0.30	11
Pyrene	0.06	0.2	0.35	11
Chrysene	0.1	0.4	0.095	9
Benzo[*a*]anthracene	0.08	0.3	0.095	9
Benzo[*b*]fluoranthene	0.2	0.5	0.13	6
Benzo[*k*]fluoranthene	0.09	0.3	0.10	18
Benzo[*a*]pyrene	0.2	0.8	0.19	14
Indeno[1,2,3-*cd*]pyrene	0.2	0.7	0.15	16
Dibenzo[*a,h*]anthracene	0.3	0.9	0.056	15
Benzo[*ghi*]perylene	0.1	0.4	0.15	8

where s_b stands for the mean standard deviation of peak areas integrated at the retention time of the PAH from procedural blanks and *a* for the slope of the calibration curve. Results of LOD, LOQ and precision (%RSD) are listed in Table 3.1.1.

3.1.3. Results and discussion

3.1.3.1. Solvent selection

The choice of the extracting solvent is a crucial parameter in the analysis of PAHs in carbonized materials (soot, charcoal) because hydrophobic contaminants are tightly bound to the aromatic matrix (Jonker et al., 2002). In this study, the extraction ability of four different solvent systems was preliminary evaluated by means of reflux extraction under the same conditions. Toluene, solely (Hale et al., 2012; Hilber et al., 2012) or mixed with methanol (Keiluweit et al., 2012), was the solvent of choice in the determination of PAHs in biochar reported in recent literature and therefore included in this comparison. Dichloromethane is a rather common solvent in the extraction of PAHs in several matrices, including wood chars (Brown et al., 2007) and biochar (Freddo et al., 2012). Acetone/hexane mixtures were described in the analysis of PAHs in charcoal and soot samples (Jonker et al., 2002).

The recovery of surrogate PAHs for each extraction system is reported in Table 3.1.2. Toluene is the best extracting solvent in the case of spiked *d*-phenanthrene and d-chrysene. This finding is in agreement with previous studies showing the strong extraction efficiency of toluene in comparison to other solvents and solvent/mixtures (Hilber et al., 2012; Jonker et al., 2002). However, in the case of spiked *d*-acenaphthene, dichloromethane and acetone/cyclohexane 1:1 exhibited higher extraction efficiency than toluene (83 and 80% vs. 68%). The loss of LMW PAHs in the case of toluene was caused by the analytical procedure following the extraction step, as blank analysis with toluene (resulting from solvent evaporation) confirmed a recovery of 65 ± 11% of *d*-acenaphthene. A similar result was reported by Hilber et al. (2012), who suspected a cross-contamination by naphthalene possibly due to extended toluene removal. When examining the PAH concentrations as a function of solvent, the detected concentrations of the LMW PAHs were the lowest with toluene (0.84 µg g^{-1}) and highest with acetone/cyclohexane 1/1 (1.37 µg g^{-1}). Therefore, the solvent mixture of acetone/cyclohexane was selected for the method optimization, because of its superior extraction efficiency for naphthalene (the most common PAH detected on biochar; see below), its widespread use in soil analysis of PAHs, and its reduced toxicity compared to toluene and dichloromethane.

Table 3.1.2. Recovery of surrogate PAHs using different extraction procedures of reference biochar (RB).

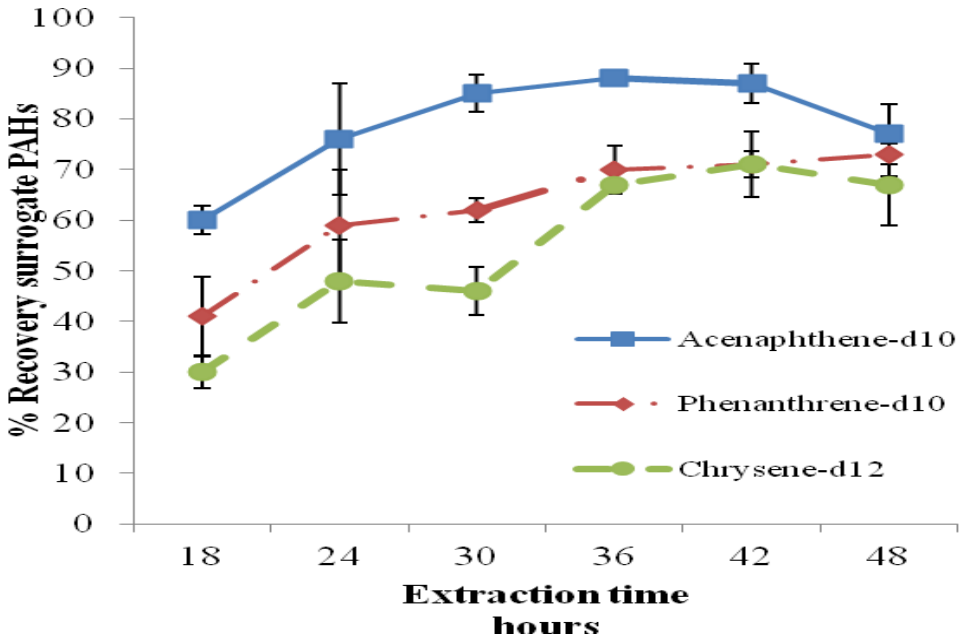
	Acenaphthene-d_{10} recovery (%)	**Phenanthrene-d_{10} recovery (%)**	**Chrysene-d_{12} recovery (%)**
Reflux extraction			
Acetone/cyclohexane 1/1	80	41	7
Acetone/cyclohexane 1/5	56	38	7
Dichloromethane	83	50	11
Toluene	68	68	58
Ultrasonication extraction			
Acetone/cyclohexane 1/1	9	4	0.4
Soxhlet extraction (18 hours)			
Acetone/cyclohexane 1/1	75	66	29
Acetone/cyclohexane 5/1	76	37	10
Acetone	84	58	29
Soxhlet extraction (36 hours)			
Acetone/cyclohexane 1/1	88	77	67

3.1.3.2. Selection of the extraction procedure

The recovery of surrogate PAHs from reflux extraction with acetone:cyclohexane 1:1 were compared with Soxhlet extraction (18 h) and ultrasonic extraction (Table 3.1.2). Ultrasonic extraction had very low recoveries (<10%) and therefore was not investigated further. As expected, the recovery of *d*-chrysene by Soxhlet extraction increased with respect to reflux conditions. Increasing (100%, v/v) or decreasing (20%, v/v) the mixing ratio of acetone with respect to the 1:1 acetone:cyclohexane mixture (i.e. 50%, v/v) did not significantly improve the recovery of the surrogate PAHs. Therefore, the acetone:cyclohexane mixture 1:1 was selected to investigate the effect of the extraction time on the recovery. The results, depicted in Fig. 3.1.1, show that the higher recoveries were achieved with longer extraction times, which is in agreement with a previous study (Hilber et al., 2012). Interestingly, the same study showed that accelerated solvent

extraction (ASE) was a less efficient than Soxhlet extraction (Hilber et al., 2012). However, prolonged extractions were problematic and did not guaranteed high recovery.

Figure 3.1.1. Recovery of deuterated PAHs vs. soxhlet extraction times with acetone:cyclohexane 1:1 v/v of reference biochar (mean values and 1 s.d. from four replicates).



We decided to focus on the behaviour of two HMW PAHs representative of five rings (benzo[*a*]pyrene) and six (indeno[1,2,3,cd]pyrene) rings as the target compounds for optimizing the extraction time. Their concentrations increased significantly when the extraction time was increased from 18 to 36 h, after which time the concentration remained almost constant. Thus, 36 h of extraction were selected for the final procedure.

3.1.3.3. Final procedure applied to reference biochar and soil

The final procedure was described in detail in Section 3.1.2.3.1. The USEPA PAH concentrations of reference biochar are reported in Table 1 along with the relative standard deviations. A typical chromatogram is presented in Fig. 3.1.2. The precision (expressed as RSD from four replicates) was good, being within the 5–18% interval. The recoveries of surrogate PAHs were satisfactory (67, 77, and 88% for *d*-acenaphthene, *d*-phenenthrene, and *d*-chrysene, respectively, Table 3.1.2). This is also considered a good result considering that PAHs are strongly associated to the aromatic carbonaceous matrix of biochar. These results are on the higher end of PAH recoveries currently reported for biochar materials. Hilber et al. (2012) reported 42–72% recovery range for several deuterated PAHs (from *d*-naphthalene to *d*12-indeno[1,2,3-cd]pyrene), and similar values (56–79%) were reported by Hale et al. (2012). The accuracy of the method developed for biochar was tested on the soil matrix by the analysis of the certified soil (ERM-CC013a). The difference between the mean measured and certified values (Table 3.1.3) were lower than the expanded uncertainty of that difference for the majority of PAHs, attesting the validity of the method for the soil matrix (Linsinger et al., 2005). Then, the ability of the method to analyze PAHs in the biochar amended soil was evaluated. The obtained concentrations of PAHs in the untreated soil and in the soil amended with biochar are presented in Table 3.1.4. The total PAH concentration in the amended soil is significantly higher than that in the untreated soil. In particular, the concentration of naphthalene is 0.0263 µg g^{-1} against 0.0098 µg g^{-1} in the untreated soil, a quite large difference due to naphthalene being the most abundant PAH in biochar at 1.75 µg g^{-1}. The excess naphthalene in the treated soil of 0.0263 – 0.0098 = 0.0165 µg g^{-1} is slightly lower than that expected from the quantity of naphthalene added with biochar corresponding to 1.75 × 1.16% = 0.0203 µg g^{-1}. Overall, the correspondence between the measured excess and expected is (0.0165–0.0203)/0.0203 = -0.19 (or -19%), which is an acceptable result and good demonstration of the accuracy of the method for LMW PAH compounds, which has been a shortcoming of some of the existing methods [i.e. 5].

Figure 3.1.2. GC-MS (SIM) chromatogram obtained from the analysis of reference biochar (RB). Peak numbers refers to PAHs listed in table 3.1.5.

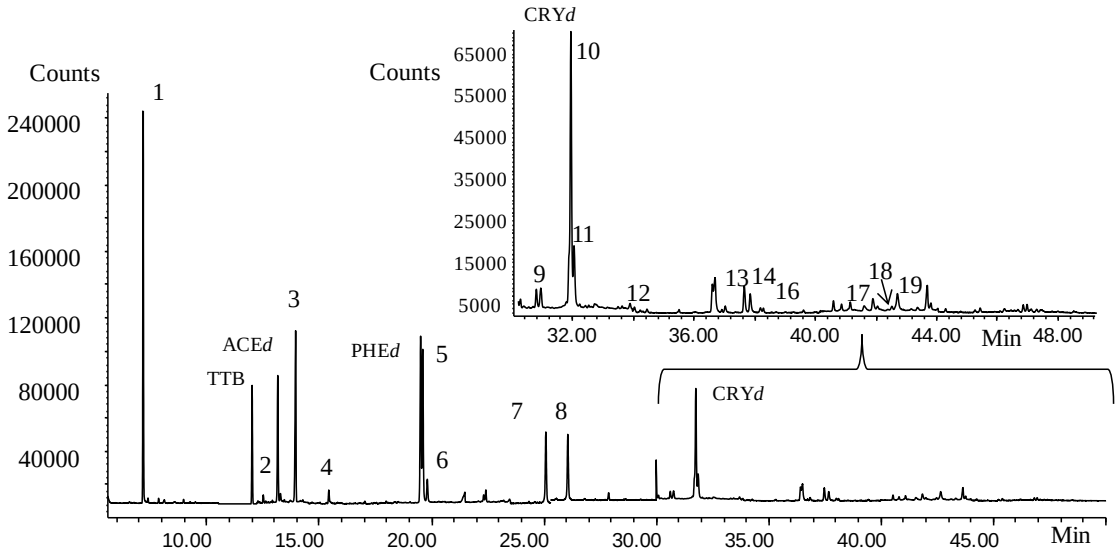


Table 3.1.3. Validation of the optimized method for the soil matrix through the analysis of the certified material ERM – CC013a.

PAH	**Measured concentration (µg g^{-1})**	**Certified value (µg g^{-1})**	**Relative error (%)**
Naphthalene	2.2 ± 0.2	2.4 ± 0.5	-9
Fluorene	1.30 ± 0.11	1.14 ± 0.11	+13
Phenanthrene	12.4 ± 0.3	12.0 ± 0.6	+2
Anthracene	1.96 ± 0.09	1.41 ± 0.22	+32
Fluoranthene	12.0 ± 0.5	12.9 ± 0.7	-9
Pyrene	8.4 ± 0.6	9.6 ± 0.3	-15
Benzo[*a*]anthracene	5.1 ± 0.3	5.6 ± 0.5	-11
Chrysene	6.3 ± 0.3	5.3 ± 0.8	+15
Benzo[*b*]fluoranthene	6.4 ± 0.4	7.1 ± 1.0	-12
Benzo[*k*]fluoranthene	4.0 ± 0.4	3.4 ± 0.4	+14
Benzo[*a*]pyrene	4.6 ± 0.4	4.9 ± 0.7	-8
Benzo[*ghi*]perylene	4.3 ± 0.7	4.6 ± 0.5	-8
Indeno[1,2,3-*cd*]pyrene	5.5 ± 0.9	5.2 ± 1.0	+3

A similar calculation was performed for the other PAHs, and the results are reported in last column of Table 3.1.4. These differences between the calculated and measured values were satisfactory for the most abundant PAHs in biochar (Table 3.1.4; at the ±20% level). These data support that the proposed method was capable to extract PAHs from a biochar amended soil, a PAH contaminated soil, and the original biochar.

Table 3.1.4. Observed concentration of PAHs in an agricultural soil and a corresponding biochar amended soil (1.16% (w/w) of reference biochar RB).

PAHs	**Soil ($\mu g\ g^{-1}$)**	**Soil + biochar ($\mu g\ g^{-1}$)**	**Difference from expected (%)**
Naphthalene	0.0098 ± 0.0002	0.0263 ± 0.0046	-19
Acenaphtylene	n.d.	n.d.	n.d.
Acenaphthene	n.d.	n.d.	n.d.
Fluorene	0.0023 ± 0.0008	0.0033 ± 0.0006	+13
Phenanthrene	0.0118 ± 0.0036	0.0212 ± 0.0063	+15
Anthracene	0.0003 ± 0.0002	0.0014 ± 0.0014	-24
Fluoranthene	0.0035 ± 0.0010	0.0075 ± 0.0030	+15
Pyrene	0.0031 ± 0.0007	0.0069 ± 0.0020	-6
Chrysene	0.0007 ± 0.0003	0.0014 ± 0.0010	-31
Benzo[*a*]anthracene	0.0039 ± 0.0007	0.0057 ± 0.0009	+60
Benzo[*b*]fluoranthene	0.0067 ± 0.0014	0.0091 ± 0.0029	+32
Benzo[*k*]fluoranthene	0.0005 ± 0.0001	0.0014 ± 0.0003	-51
Benzo[*a*]pyrene	0.0001 ± 0.0002	0.0019 ± 0.0009	-21
Indeno[1,2,3-*cd*]pyrene	0.0023 ± 0.0008	0.0040 ± 0.0022	-9
Dibenzo[*a,h*]anthracene	0.0009 ± 0.0002	0.0014 ± 0.0004	-18
Benzo[*ghi*]perylene	0.0046 ± 0.0011	0.0070 ± 0.0013	+36
Total	**0.0506 ± 0.017**	**0.0986 ± 0.019**	**-2**

Notes: Values in the tables are the mean value ±1 standard deviation from four replicates. The last column reports the relative percent difference between the measured and expected value. The expected value is the concentration calculated from the PAH concentration obtained by summing the soil and biochar contributions (Table 3.1.1). This is expressed as a relative percentage of (measured - expected)/expected × 100.

Obviously, the effect of biochar addition in soils on the level of PAHs will depend on the background level of PAHs in the soil before treatment (Nam et al., 2009; Wilcke et al., 2000), the concentration of PAHs in the original biochar, and the quantity of added biochar. Then, environmental processes (evaporation, biodegradation, or abiotic degradation) will affect the fate and levels of PAHs in amended soil. Due to the lipophilic nature of the PAHs, these compounds tend to bioaccumulate in plants (Duxbury et al., 1997; Parrish et al., 2006). Leafy vegetables typically accumulate higher levels of PAHs from the soil system than companion fruit or root crops (Lei et al., 2011). The levels of PAH observed in some of the biochars (see below) do posses levels that could be of potential health and environmental concern, depending on the application rate, original soil concentrations, and end-use for the soil.

3.1.3.4. Determination of EPA and EU PAHs in different biochar samples

The method developed in this study was applied to the determination of USEPA and EU PAHs in a suite of ten biochar investigated in a previous study (Fabbri et al., 2012). With the exception of biochar S-18 and S-19 (distillers grain) and S-17 (Macadamia nut shells), all the other biochars were derived from woody biomass (Table 3.1.5). Almost all 16 USEPA PAHs were detected and quantified in the biochars, as well as several EU PAHs. However, HMW EU PAHs were not detected (Table 3.1.5). The recovery of spiked deuterated PAHs ranged between 60 and 100% and for all the samples an average of 78%, 78 and 75% for *d*-acenaphthene, *d*-phenanthrene and *d*-chrysene, respectively, with ~10% RSD each. Despite the difference in feedstock and process treatment the PAH levels were quite similar (1–19 μg g^{-1}). One sample (biochar S-17) was characterized by high levels of PAHs. However, the literature reports examples of biochar with much higher concentrations, some comparable to those observed on soot (Hilber et al., 2012; Schimmelpfennig and Glaser., 2012). A large number of biochars investigated by Hale et al. (2012) exhibited total PAHs in the 0.07–3.27 μg g^{-1} interval when produced from slow pyrolysis from different biomass at temperatures between 250 and 900 °C, and higher values (45 μg g^{-1}) from gasification. These examples underline the variety of PAH levels that could find in biochars. With few exceptions (S-17), naphthalene was the most abundant PAH, in accordance to previous studies (Hale et al., 2012; Hilber et al., 2012; Schimmelpfennig and Glaser, 2012; Freddo et al., 2012; Kloss et al., 2012), followed by

phenanthrene. However, it is interesting to note that benzo[*a*]pyrene was detected in all biochars analyzed here, with concentrations ranging from 0.01 to 0.67 μg g^{-1}.

Sample S-2 was biochar obtained from the fast pyrolysis of hardwood sawdust at 500 °C, while S-3 the same biochar stored 1 year in an open drum subject to environmental conditions (Fabbri et al., 2012). Table 3.1.5 shows that the levels of LMW PAHs did not change markedly, confirming the strong sorption of PAHs to biochar. However, Hale et al. (2012) reported that artificial aging in aqueous solutions generally increased the concentration of PAHs on biochar, probably due to the leaching of hydrophilic components leaving the more hydrophobic biochar fraction.

Biochars S-18 and S-19 produced from the same feedstock (distiller grains) at similar pyrolysis temperatures (350 and 400°C, respectively) exhibited significantly different PAH concentrations (total USEPA 5.0 and 2.2 μg g^{-1}) suggesting the importance of pyrolysis conditions, as well as the role of temperature. A general trend has been observed of increasing PAH contents at shorter pyrolysis times and high pyrolysis temperatures (Hale et al., 2012). A detailed study on the presence PAHs in biochar samples produced from woody and herbaceous biomass pyrolyzed at different temperatures showed that the concentration of pyrogenic PAHs peaked at 500 °C, a common temperature in slow pyrolysis (Keiluweit et al., 2012). Chagger et al. (2000) demonstrated through modelling that PAHs are preferentially formed in a fluidized bed reactor versus a kiln style reactor, due to unstable combustion reactions present in a fluidized bed reactor. Schimmelpfennig and Glaser have underlined the importance of the particular technological process on the sorbed PAH concentrations, with wood gasifiers associated with the highest levels of PAHs on the solid residuals (Schimmelpfennig and Glaser, 2012). These authors proposed the naphthalene/phenanthrene ratio and the total PAHs concentrations as factors to differentiate pyrolysis processes between biochars. These hypotheses are also supported by our data, since biochars that are created by slow pyrolysis at longer residency times in kiln style reactors possess lower sorbed amounts of PAHs compounds.

Table 3.1.5. Concentrations of the 16 USEPA PAHs and (#) 15 EUPAHs (µg g^{-1} mean of two duplicates). (RB reference biochar; characteristics of biochars from S-2 to S-20 were published elsewhere [Fabbri et al., 2012].)

	Sample Id.	**RB**	**S-2**	**S-3**	**S-4**	**S-5**	**S-15**	**S-16**	**S-17**	**S-18**	**S-19**	**S-20**
Nr.	**PAHs**											
1	Naphthalene	1.75	1.57	1.71	2.39	0.44	0.47	0.93	2.58	0.78	0.49	3.36
2	Acenaphtylene	0.03	0.50	0.30	0.04	0.01	0.02	0.12	0.71	0.10	0.05	0.10
3	Acenaphthene	0.03	0.62	0.31	0.05	0.01	0.07	0.08	0.28	0.24	0.22	0.11
4	Fluorene	0.07	0.25	0.16	0.10	0.05	0.08	0.04	0.92	0.59	0.26	1.13
5	Phenanthrene	0.71	0.25	0.30	0.56	0.31	0.27	0.36	3.88	0.49	0.33	2.70
6	Anthracene	0.13	0.03	0.04	0.07	0.03	0.03	0.04	0.65	0.19	0.12	0.33
7	Fluoranthene	0.3	0.14	0.08	0.11	0.08	0.11	0.05	2.46	0.10	0.09	0.21
8	Pyrene	0.35	0.07	0.07	0.08	0.08	0.12	0.04	2.58	0.16	0.07	0.10
9	Cyclopenta[*c,d*]pyrene[#]	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.04	n.d.	0.03
10	Chrysene[#]	0.09	0.05	0.02	0.02	0.02	0.03	0.02	0.92	0.42	0.17	0.09
11	Benzo[*a*]anthracene[#]	0.09	0.04	0.02	0.05	0.04	0.04	0.02	0.83	0.46	0.08	0.17
12	5-methylchrysene[#]	0.01	0.11	0.04	0.02	0.02	0.02	0.09	0.27	0.21	n.d.	0.21
13	Benzo[*b*]fluoranthene[#]	0.13	0.02	0.05	0.04	0.04	0.05	0.02	0.70	0.29	0.05	0.07
14	Benzo[*k*]fluoranthene[#]	0.1	0.02	0.01	0.04	0.02	0.02	0.01	0.43	0.39	0.07	0.06
15	Benzo[*j*]fluoranthene[#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16	Benzo[*a*]pyrene[#]	0.19	0.02	0.02	0.10	0.01	0.05	0.02	0.67	0.32	0.06	0.22
17	Indeno[1,2,3-*cd*]pyrene[#]	0.15	0.02	0.01	0.13	n.d.	0.02	0.01	0.50	0.27	n.d.	0.03
18	Dibenzo[*a,h*]anthracene[#]	0.06	0.02	0.01	0.01	0.01	0.01	0.01	0.08	0.21	0.19	0.06
19	Benzo[*ghi*]perylene[#]	0.15	0.01	0.01	0.01	0.01	0.02	0.01	0.53	n.d.	n.d.	0.08
20	Dibenzo[*a,e*]pyrene[#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21	Dibenzo[*a,h*]pyrene[#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	Dibenzo[*a,i*]pyrene[#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
23	Dibenzo[*a,l*]pyrene[#]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Σ 16 EPA PAHs	**4.3**	**3.6**	**3.1**	**3.8**	**1.2**	**1.4**	**1.8**	**19**	**5.0**	**2.2**	**8.8**
	[#] Σ 15 EU PAHs	**0.97**	**0.32**	**0.2**	**0.43**	**0.18**	**0.27**	**0.22**	**5.0**	**2.6**	**0.62**	**1.0**

Given the values of total PAHs reported in Table 3.1.5, as well as those reported in the literature (Hale et al., 2012; Freddo et al., 2012) for the slow pyrolysis biochars and the level of biochar applications recommended in agriculture practices, the increased levels of elevated PAHs in biochar amended soil is not of universal concern. However, as also seen in our data and those from other studies (Keiluweit et al., 2012; Kloss et al., 2012), some biochars do have levels of sorbed PAHs that do exceed existing and proposed guidelines for the usage of specific materials (e.g. sludge, wood ash) on land (Keiluweit et al., 2012; Freddo et al., 2012; Kloss et al., 2012) including commercial biochar (Hilber et al., 2012). In addition, the bioaccumulation of PAH compounds in produce grown in biochar amended soils requires further investigation. Therefore, the development of valid analytical procedures for the determination of PAHs in biochar and biochar amended soils is critical.

3.1.3.5. Determination of EPA-PAH in EU-COST biochar samples

The method described in the previous sections was applied to three biochar samples that were part of a laboratory exercise organised within the EU COST Action TD1107 (http://cost.european-biochar.org/en). The Working Group 1.

The three biochar samples BC1, BC2 and BC3 were produced with a PYREG® 500 – III pyrolysis unit (PYREG GmbH, Dörth, Germany) from different feedstock (woodchip sievings, paper sludge - wheat husks blend, sewage sludge) at similar conditions. Information on the process is available in Bucheli et al. (2014).

The method was the same as described in the previous sections, however, two sets of analyses were run under slightly different conditions. In the first set of samples (A) about 20 g were dried at 40 °C for 48 hours and the test-sample spiked with the surrogate PAH mix (Supelco for EPA 525 containing acenaphthene-*d*10, phenanthrene-*d*10 and chrysene-*d*12; in a second set of samples (B) about 200 g were dried at 40 °C for 96 hours and spiked with 16 EPA PAHs (prepared from Dr. Ehrenstorfer PAH-Mix 9 deuterated, 10 ng μl^{-1}).

The results are reported in Tables 3.1.6, 3.1.7 and 3.1.8.

Data obtained from the few laboratories participating to the PAH determination in the ring trial are being evaluated by the WP1. Preliminary results would indicate that no major deviations in the reported data occurred for the total PAH concentrations. Naphthalene was the most dominant PAH in all the three biochars followed by phenanthrene. Naphthalene was the PAH with the largest deviations, probably because of its volatility. The longer

drying period in test B seems to have resulted in loss of naphthalene.

Table 3.1.6. Concentrations of PAHs in biochar 1 in ng g^{-1}.

	A		B	
BC1	**mean**	**sd**	**mean**	**sd**
Naphthalene	1475	56	507	84
Acenaphthylene	86	11.4	49	6
Acenaphthene	24	4.1	23	5
Fluorene	27	7.1	41	12
Phenanthrene	143	49	136	41
Anthracene	-	-	23	3
Fluoranthene	30	8	33	7
Pyrene	46	11.7	31	4
Chrysene	20	6.6	18	6
Benzo[*a*]anthracene	30	5	26	3
Benzo[*b*]fluoranthene	81	7.6	51	10
Benzo[*k*]fluoranthene	41	5.5	27	3
Benzo[*a*]pyrene	51	12.5	32	1
Indeno[1,2,3-*cd*]pyrene	n.d.	-	-	
Dibenzo[*a,h*]anthracene	n.d.	-	-	
Benzo[*ghi*]perylene	n.d.	-	-	
PAH TOTAL	2055		997	

Table 3.1.7. Concentrations of PAHs in biochar 2 in ng g^{-1}.

	A		B	
BC2	**mean**	**sd**	**mean**	**sd**
Naphthalene	1864	174	1039	90
Acenaphthylene	149	23	24	4
Acenaphthene	18	5	21	3
Fluorene	26	5.7	14	3
Phenanthrene	168	19	117	27
Anthracene	8	1.6	33	7
Fluoranthene	53	1.8	41	9
Pyrene	63	3.4	49	15
Chrysene	18	1.2	21	6
Benzo[*a*]anthracene	22	4.3	23	3
Benzo[*b*]fluoranthene	29	6.9	17	4
Benzo[*k*]fluoranthene	11	3.5	15	3
Benzo[*a*]pyrene	5.8	1.6	12	4
Indeno[1,2,3-*cd*]pyrene	6.9	0.9	11	3
Dibenzo[*a,h*]anthracene	2.3	0.8	0	
Benzo[*ghi*]perylene	8.5	2.5	12	6
PAH TOTAL	2454		1450	

Table 3.1.8. Concentrations of PAHs in biochar 3 in ng g^{-1}.

	A		B	
BC3	**mean**	**sd**	**mean**	**sd**
Naphthalene	363	83	330	33
Acenaphthylene	4.5	1.1	13	4
Acenaphthene	10.6	0.8	8	0
Fluorene	10.5	1.6	13	3
Phenanthrene	50.2	5.8	99	13
Anthracene	13.2	1.5	28	2
Fluoranthene	16.6	1.5	38	5
Pyrene	24.8	2.4	52	6
Chrysene	7.1	1.1	15	2
Benzo[*a*]anthracene	18.0	3.4	31	5
Benzo[*b*]fluoranthene	6.2	1.5	10	2
Benzo[*k*]fluoranthene	5.2	1.3	12	1
Benzo[*a*]pyrene	8.4	2.3	21	3
Indeno[1,2,3-*cd*]pyrene	5.4	1.6	17	2
Dibenzo[*a,h*]anthracene	4.2	0.8	0	
Benzo[*ghi*]perylene	9.5	3	13	1
PAH TOTAL	558		699	

3.1.4. Conclusions

A method for the determination of PAHs in biochar was developed making use of a solvent mixture (1:1 acetone:cyclohexane) in place of more toxic and/or hazardous solvents (e.g., dichloromethane, toluene) which was appropriate for the determination of LMW PAHs (including naphthalene) along with HMW PAHs. The method was validated with a certified reference soil and demonstrated its validity for the detection of PAHs deriving from biochar in a soil matrix amended with 1% biochar. Because of the strong affinity of PAHs toward biochar, solvent and duration time of the Soxhlet extraction were crucial parameters and at least 36 h was necessary to obtain a satisfactory recovery with 1:1 acetone:cyclohexane. Furthermore, this method provided satisfactory recovery when applied to a wide range of biochar samples obtained at different pyrolysis conditions from different biomass parent materials suggesting that this analytical procedure could be used successfully on different biochars. All the biochar analyzed contained the USEPA, as well

as some of the EU PAHs at detectable levels ranging from 1.2 to 19 µg g^{-1}. In particular, the presence of EU PAHs on biochar could be of concern when biochars with elevated levels of PAHs are used in human food production due to the potential of contamination. However, this aspect requires further investigations.

3.2. Quantitative determination of PAHs in an agricultural soil treated with biochar

3.2.1. Introduction

Biochar application to soils has recently emerged as a potential strategy to sequester carbon into agricultural soils, improve physical, chemical and biological properties of soil and produce renewable energy (Glaser et al., 2002; Lehmann et al., 2006; Steiner et al., 2007; Sohi et al., 2010; Uchimiya et al., 2010; Galinato et al., 2011; Vaccari et al., 2011; Ventura et al., 2013). Biochar is known to improve soil water-holding capacity (Case et al., 2012; Basso et al., 2013; Baronti at al., 2014) and plant water availability (Baronti et al., 2014), decrease nutrients leaching (Güereña et al., 2013) and bioavailability of heavy metals (Park et al., 2011), improve soil structure (Case et al., 2012) and stimulate soil microbial activity (Kolb et al., 2009; Rutigliano et al., 2014), and finally, increase the pH of soil, due to which it can be used on acidic soils with poor cultivation properties (Glaser et al., 2002; Slavich et al., 2013).

In spite of the unquestionable advantages, there are also certain threats related with the production and subsequent utilisation of biochar. Among threats, most frequently mentioned is the contamination of biochar with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. While in the case of heavy metals their levels are at relatively low values (Freddo et al., 2012) and depend on the content of trace metals in the initial material, studies concerning PAHs indicate (Freddo et al., 2012; Hale et al., 2012; Hilber et al., 2012; Keiluweit et al., 2012; Oleszczuk et al., 2013; Fabbri et al. 2013) that biochars may be contaminated with those compounds to a significant degree. PAHs are formed during the pyrolysis of organic matter (including biomass). PAHs are well known carcinogenic and persistent pollutants and 16 PAHs are classified as priority pollutants and are heavily regulated by the US EPA due to their carcinogenic, mutagenic or teratogenic properties (Keiluweit et al., 2012). Therefore, the application of biochar to agricultural soil could carry a significant risk to human health by contaminating soils. Moreover, PAHs in soil may exhibit a toxic activity towards different plants, microorganisms and invertebrates (Guo et al., 2012).

Several studies have shown that the presence of biochar in soils can influence the bioavailability and bioaccessibility of organic contaminants (Beesley et al. 2010; Gomez-Eyles et al. 2011; Zhang et al. 2013; Lattao et al. 2014). The biochar is particularly

effective at adsorbing and sequestering organic contaminants; enhanced sorption of hydrophobic organic compounds such as PAHs could actually decrease microbial mineralization by decreasing bioavailability (Rhodes et al., 2008, 2010; Xia et al., 2010), and could lead to localize PAH accumulation. On the contrary, Liang et al. (2015) reported that biochar amendment stimulates PAH-metabolizing bacterial activity by enhancing the number of gene copies related to PAH degradation and by changing the structure of soil microbial community.

Quilliam et al. (2012) reported that biochar can reduce the degradation of PAHs in two important types of agricultural soil, which in the short term could not only increase the concentration of soil PAHs but could also affect the long-term persistence of PAHs in the environment. However, the environmental fates of biochar-associated PAHs added to soil are still poorly understood. Therefore, it is necessary to improve understanding of the role biochar plays in sorbing PAHs and on microbial activity and how this influences the concentration of PAHs in soil and their persistence in the environment.

The aim of this study was quantifying the concentration of the 16 priority PAHs in soil amended with biochar at two different rates and determine the impacts of biochar additions on PAHs concentration in soils. Furthermore, the effect of biochar on PAHs levels was compared with some properties of soil, which may influence the fate of PAHs.

3.2.2. Materials and Methods

3.2.2.1. Reagents and standards

PAH-Mix solution containing each of the 16 EPA PAHs [i.e., naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene and benzo[*ghi*]perylene] certified at concentrations of 10 mg l^{-1} for each species in acetonitrile was purchased from Sulpeco (Belleforte, PA, USA).

Cyclohexane, acetone, ethyl acetate (all supra solv quality), and surrogate standard mix (for EPA 525) containing acenaphthene-d_{10}, phenanthrene-d_{10} and chrysene-d_{12} at concentrations of 500 mg l^{-1} each in acetone were purchased from Sigma-Aldrich.

A solution of 1,3,5-tri-tert-butylbenzene (TTB, 10 mg l^{-1}) in acetone:cyclohexane (1:1, v/v) was prepared by weighing the pure compound purchased from Sigma-Aldrich.

3.2.2.2. Experimental layout

The field experiment was setup in a vineyard at the "Marchesi Antinori - La Braccesca Estate" (Lat. 43° 10' 15'' N; Long. 11° 57' 43'' E; 290 m a.s.l.), located few kilometers away from Montepulciano (Tuscany, central-Italy). The vineyard has been planted in 1995 and trellis system is a single curtain with plant-row spacing of 0.8 and 2.5 m; rows orientation is East-West. The vineyard is not irrigated.

A randomized plot experiment, with three treatments and five replicates was setup in 2009. Each plot, 15 in total, had a surface area of 225 m^2 (7.5 m in width and 30 m in length) including 4 vineyard rows and 3 inter-rows. The treatments were: a single biochar application at a rate of 22 t ha^{-1} in 2009 (B); two biochar applications at a rate of 22 t ha^{-1} each, in 2009 and 2010 (BB); and a control (C). Biochar was applied with two treatments, in five replicates randomly distributed, as follows: 22 t ha^{-1} of biochar applied in 2009 (B); 22 t ha^{-1} in 2009 and further 22 t ha^{-1} in 2010 (BB) and control untreated plots (C).

Rows orientation is East-West, inter-rows are partially covered with spontaneous grass, and tilled with a chisel plow in the March–June period. The vineyard is not irrigated and it is fertilized with an inorganic fertilizer (15.0.26) twice per year (in November and April) at a rate of 120 kg ha^{-1}. Soil is acid, shallow and sandy-clay-loam textured (USDA, 2005) (Table 1) and is highly compacted below 0.4 m depth

Untreated soil (control) and soil treated by two concentrations of biochar amendment were sampled four times from 2011 to 2013 (August 2011, December 2011, May 2012 and May 2013). Sixty samples (5 replicates x 3 treatments x 4 sampling seasons) were examined, each sample was dried at 40 °C, sieved (mesh size: 2 mm) in order to obtain homogeneous samples free of stones, larger roots and other coarse fragments, and stored at - 20 °C. No losses of PAHs occur under these conditions.

3.2.2.3. Soil properties

Soils are shallow, acid, sandy-clay-loam (USDA, 2005) textured (Table 3.2.1). The total organic Carbon content (C) and Total Nitrogen content (N) were analyze by dry combustion elemental analyzer (Thermo Fisher Science) after fine grinding with a ball mill to 0.5 mm. The pH was measured potentiometrically in a 1:2.5 soil– water suspension. The CEC analysis was performed by saturation with barium–chloride at pH 8.2, displacement

of adsorbed barium by 0.05 M $MgSO_4$ and titration of the Mg remaining in solution with 0.025M EDTA (Gessa and Ciavatta, 2000).

3.2.2.4. Biochar characterization

The biochar used in the experiment is a commercial charcoal provided by "Romagna Carbone s.n.c." (Italy) obtained from orchard pruning biomass through a slow pyrolisis process at temperature of 500 °C in a transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock. The biochar at the end of the pyrolisis was crushed into particles smaller than 5 cm of diameter before the soil application. Elemental composition (C,H,N,S) was determined by combustion using a Thermo Scientific FLASH 2000 Series CHNS/O Elemental Analyzer. Ash content of the biochar was measured by heating samples in a muffle at 550 °C for 6 hours, as proposed by ANPA (2001). The oxygen content was calculated from mass balance: %O=100-% (C+H+N+ash).

As the carbonate concentration of the soils was negligible, the total measured C concentration was considered to represent total organic carbon (TOC). The TOC content was determined on 5 samples of biochar amended soil and 5 control soil according to the Ministero per le Politiche Agricole (1999), Method VII.1. Samples were pre-treated with HCl 1.5 M (40 uL in 2-3 g of sample), heated at 60 °C for 1 hour; this procedure was repeated for 4-5 times, till the samples stop reacting with HCl. Determinations were made using a Thermo Scientific FLASH 2000 Series CHNS/O Elementar Analyzer.

A mixture of biochar with deionized water at 1:10 wt/wt ratio was prepared, thoroughly mixed and pH measured at room temperature with a digital pH meter (HI 98103, Checker®, Hanna Instruments). Prior to this analyses, biochar was sieved at 2 mm and oven dried at 40 °C for 72 h.

3.2.2.5. Determination of polycyclic aromatic hydrocarbons

3.2.2.5.1. Extraction and clean up

The PAHs determined comprised of 16 compounds (US EPA List; Table 3.2.2). Analyses of PAHs were conducted as described in Fabbri et al. (2013). Briefly, about 5 g of sample was placed into the extraction cellulose thimble, spiked with 0.1 mL of surrogate PAH mix (Supelco for EPA 525 containing acenaphthene-d_{10}, phenanthrene-d_{10} and

chrysene-d_{12} 5 µg mL^{-1} each in acetonitrile). The thimble was covered with cotton wool and inserted into the Soxhlet extractor. The extraction was performed with 160 ml of extraction solvents (acetone/cyclohexane (1:1, v/v)) mixture for 36 h (4 cycles h^{-1}). The extraction solvent was filtered, added with 1 ml of *n*-nonane, and then carefully evaporated by rotatory vacuum evaporation at 40 °C.

The concentrated extract was collected and loaded onto a silica gel cartridge (6 ml, 1 g DSC-Si Supelco washed with ethyl acetate, dried and conditioned with 4 ml cyclohexane). After purification with 1 mL of cyclohexane, PAHs were eluted with 4 ml of acetone/cyclohexane (1:1, v/v). The obtained solution was then blown down to 10 – 50 µl under nitrogen, spiked with 10 µl of the internal standard solution 1,3,5-tri-tert-butylbenzene (TTB at 10 mg l^{-1}) prior to GC–MS analysis.

3.2.2.5.2. GC-MS

Samples (1 µl) were injected under a splitless condition (1 min, then split ratio 1:50 to the end of analysis) into a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30 m× 0.25 mm i.d., 0.25 mm film thickness) with the following temperature program: 50 °C to 100 °C at 20 °C min^{-1}, then from 100 °C to 300 °C at 5 °C min^{-1}, then a hold for 2.5 min at 300 °C, using helium as the carrier gas. The mass spectrometer operated under electron ionization (70 eV) and acquisition was performed on single ion monitoring (SIM) at the molecular ion of each PAH at the time windows corresponding to the elution region of the target PAH.

Acenaphthene-d_{10} was utilised to quantify naphthalene, acenaphthylene, acenaphthene and fluorene; phenanthrene-d_{10} to quantify phenanthrene, anthracene, fluoranthene and pyrene; chrysene-d_{12} to quantify the remaining PAHs. Recovery of surrogated PAHs was determined with respect to the internal standard TTB. The procedural blank was determined by going through the same extraction and cleanup procedures for each series of samples. None of the analytical blanks were found to have detectable contamination of the monitoring PAHs and thus the results were not blank corrected.

3.2.2.6. Statistical analysis

Mean and standard deviation of five replicates were used to compare results of soils and biochar amended soils. An analysis of variance (ANOVA) test was conducted with R software version 3.1.2 (2014-10-31) to evaluate significant difference between control and biochar amended soil.

3.2.3. Results and discussion

3.2.3.1. Soil and biochar characteristics

The study was carried out using an agricultural soil classified as sandy-clay-loam (USDA, 2005) textured with 70% sand, 15% silt and 15% clay. The soil characteristics were as follows: pH 5.37, total C 0.77%, total N 0.24%, total H 0.43%, and cation exchange capacity of 12.1 meq 100 g^{-1}.

Results of biochar characterizations are reported in Table 3.2.1. The biochar used for soil amendment had a total content of C, N, H, and O of 71.4%, 0.7%, 1.5%, 5.9%, respectively, an ash content of 19.9% and a pH of 9.8 (Table 3.2.1). The biochar had a molar H/C ratio of 0.26 and molar O/C ratio of 0.06, indicating a comparably high aromaticity of the biochar carbon (Zimmerman et al., 2013).

Table 3.2.1. Chemical characteristics of biochar applied in the field experiment.

	Value
C (%)	71.4
H (%)	1.54
N (%)	0.72
S (%)	0.59
O (%)	5.9
H/C (molar)	0.26
O/C (molar)	0.06
Ash (%)	19.9
pH	9.8
Charred (%)	97.6

The concentration of ∑16PAHs in the utilised biochar is 3.5 μg g^{-1} and all the US EPA PAHs were detected, with naphthalene as the most abundant species followed by phenanthrene (Table 3.2.2). With this concentration would pass current quality standards by the European Biochar Certificate (4 μg g^{-1} for premium quality and 12 μg g^{-1} for basic quality) and the International Biochar Initiative (6 μg g^{-1}). Additional details about the physicochemical properties of the biochar are presented in Baronti et al. (2014).

Table 3.2.2. BIOCHAR. Concentrations of the 16 USEPA PAHs and standard deviation (n=2) in biochar applied in the field experiment.

Sample Id.	**BIOCHAR**	
PAHs	**ng g^{-1}**	**SD**
Naphthalene	2149	658
Acenaphthylene	42	12.0
Acenaphthene	37	2.1
Fluorene	60	6.7
Phenanthrene	674	38
Anthracene	92	1.4
Fluoranthene	133	0.2
Pyrene	150	33
Chrysene	49	11
Benzo[*a*]anthracene	109	18
Benzo[*b*]fluoranthene	98	3.6
Benzo[*k*]fluoranthene	88	34
Benzo[*a*]pyrene	93	6.9
Indeno[1,2,3-*cd*]pyrene	19	5.6
Dibenzo[*a,h*]anthracene	21	2.2
Benzo[*ghi*]perylene	18	2.1
Σ 16 EPA PAHs	**3834**	**819**

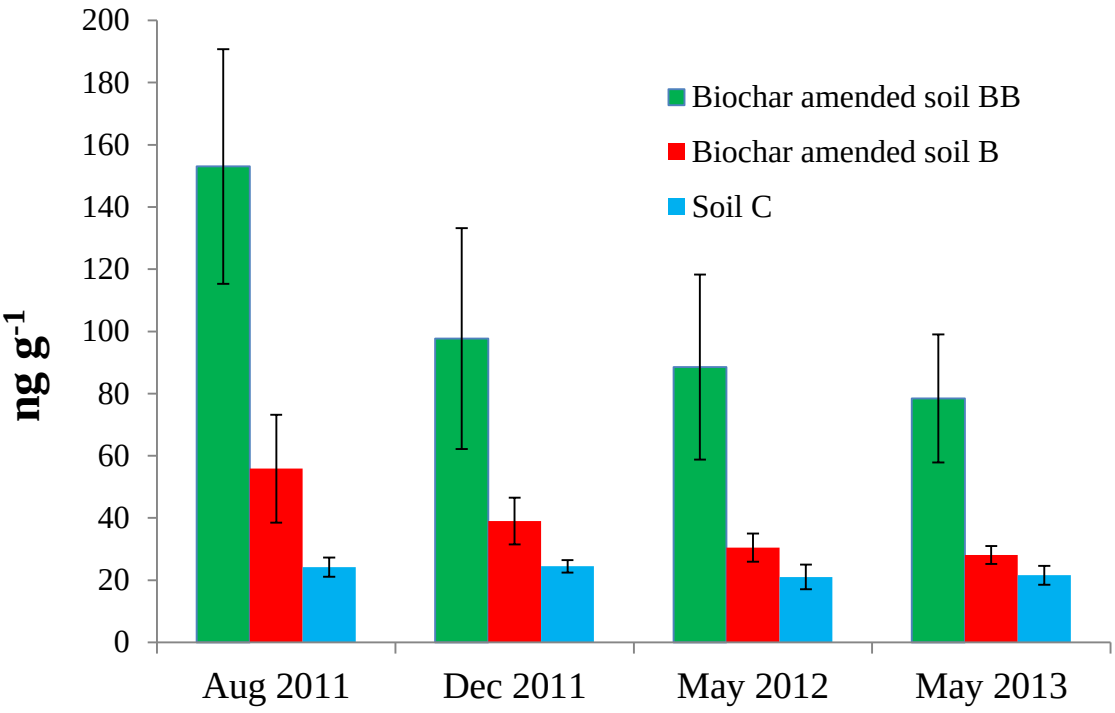
3.2.3.3. Total PAH in soil and in biochar amended soil

Almost all 16 US-EPA PAHs were detected and quantified in the amended soil samples analysed (Table 3.2.3 and Table 3.2.4). In all of the untreated soils, acenaphthylene, acenaphthene, indeno[1,2,3-cd]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene

were not detected. The recovery of spiked deuterated PAHs ranged between 60 and 100% and for all the samples an average of 78%, 78 and 75% for acenaphthene-d_{10}, phenanthrene-d_{10} and chrysene-d_{12}, respectively, with ~10% RSD each.

Total PAHs concentrations in untreated soils ranged from 18 ng^{1} to 29 ng g^{-1}, in amended soils B from 26 ng g^{-1} to 60 ng g^{-1}and in amended soils BB from 192 ng g^{-1} to 60 ng g^{-1} (Table 3.2.3 and Table 3.2.4). The total level of 16 PAHs in amended soils BB was on the same level as other data reported for agriculture soils in Europe, for example, 60-145 ng g^{-1} for arable soil in Switzerland (Bucheli et al., 2004); 187 ng g^{-1} for rural soil in United Kingdom (Wild and Jones, 1995) and 150 ng g^{-1} in Norwegian agriculture soil (Nam et al., 2008). The levels in amended soils BB, instead, were significantly lower than those in soils from non-industrial areas in China 318 ng g^{-1} and in arable lands in Poland 395 ng g^{-1} (Maliszewska-Kordybach et al., 2008).

Fig. 3.2.1. Concentrations of the 16 USEPA PAHs and standard deviation (n=5 plot) in soil, biochar amended soil BB and B at different sampling dates from the beginning of the field experiment (May 2009).



After almost one year following biochar application, the total PAHs concentrations in amended soils B resulted higher than those of the untreated soils, both in August (56 vs. 24

ng g^{-1}) and December (39 vs. 24 ng g^{-1}) (Fig. 3.2.1, Table 3.2.2 and Table 3.2.3). Moreover, the differences were statistically significant although the high dispersion of PAH values between samples withdrawn from the same parcel (n = 5) (Fig. 3.2.1). The lower concentrations observed in winter for the treated soils suggest a seasonal variability superimposed to sampling heterogeneity.

The mean concentration of total PAHs in soil amended BB, one year after the application in August 2011, was approximately 6 times higher than the control soil (153 vs. 24 ng) (Fig. 3.2.1). However, the range of total PAHs concentrations in the 5 amended soils BB sampled in August 2011 (102 ng g^{-1}-192 ng g^{-1}) remained within the range reported for Italian agriculture soils (80-304 ng g^{-1}, ARPA Piemonte - Rapporto Stato Ambiente 2009).

Four years after the addition of 44 t ha^{-1} of biochar to agricultural soils BB, the PAH concentration was significantly higher than that in unamended soil suggesting that biochar can act as a source of soil contaminants. However, the level of PAHs in the biochar amended soil soil remained within the maximum acceptable concentration for a number of European countries, 5–50 µg g^{-1} (Carlon, 2007). In addition, table 3.2.5 shows that in the amended soils BB the concentrations of the PAHs decreased significantly during the four years following biochar application.

Quilliam et al. (2013) reported that biochar can reduce the degradation of PAHs in two important types of agricultural soil, which in the short term could not only increase the concentration of soil PAHs but could also affect the long-term persistence of PAHs in the environment. On the contrary, our results suggest that the soil contamination by PAHs following biochar application is not significant at the application rates currently recommended in agriculture (20-60 t ha^{-1}) and that biochar does not decrease PAH degradation and has not long-term effects. Long-term impacts of biochar additions to soils are still not fully understood, although evidence suggests that the characteristics of soil and biochar are of central importance.

Table 3.2.3. Concentrations of the 16 USEPA PAHs and standard deviation (n=5 plot) in biochar amended soil B at different sampling dates from the beginning of the field experiment (May 2009).

Sample Id.	**Aug 2011**		**Dec 2011**		**May 2012**		**May 2013**	
PAHs	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**
Naphthalene	16.9	5.5	9.9	0.9	7.2	2.2	6.6	1.9
Acenaphthylene	2.34	1.59	0.52	0.66	n.d.	-	n.d.	-
Acenaphthene	0.32	0.48	0.73	0.54	n.d.	-	n.d.	-
Fluorene	3.5	1.72	2.8	1.78	2.8	1.26	2.6	1.13
Phenanthrene	12.1	3.6	8.4	2.0	7.5	0.7	6.5	0.8
Anthracene	2.19	0.70	1.71	0.57	1.50	0.52	1.32	0.52
Fluoranthene	5.27	2.42	4.47	1.50	3.41	0.28	2.91	0.37
Pyrene	4.74	2.06	3.66	1.29	2.76	0.30	2.64	0.20
Chrysene	1.47	0.44	1.29	0.34	0.94	0.24	0.89	0.15
Benzo[*a*]anthracene	1.72	0.68	1.32	0.29	1.17	0.11	1.20	0.20
Benzo[*b*]fluoranthene	1.69	0.51	1.53	0.42	1.25	0.25	1.48	0.22
Benzo[*k*]fluoranthene	1.36	0.94	1.33	0.94	0.99	0.72	1.00	0.61
Benzo[*a*]pyrene	0.97	0.40	0.85	0.35	0.76	0.36	0.78	0.35
Indeno[1,2,3-*cd*]pyrene	0.69	0.44	0.50	0.37	0.18	0.39	0.30	0.52
Dibenzo[*a,h*]anthracene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Benzo[*ghi*]perylene	0.51	0.51	0.28	0.28	n.d.	-	n.d.	-
Σ 16 EPA PAHs	**55.8**	**17.3**	**39.0**	**7.6**	**30.5**	**4.5**	**28.1**	**2.9**

Table 3.2.4. Concentrations of the 16 USEPA PAHs and standard deviation (n=5 plot) in biochar amended soil BB at different sampling dates from the beginning of the field experiment (May 2009).

Sample Id.	**Aug 2011**		**Dec 2011**		**May 2012**		**May 2013**	
PAHs	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**
Naphthalene	74.1	20.9	41.1	15.5	34.5	8.1	35.0	5.0
Acenaphthylene	1.7	0.3	1.1	0.4	1.0	0.4	1.0	0.5
Acenaphthene	2.0	1.0	1.4	0.5	1.5	0.4	1.1	0.2
Fluorene	4.8	1.4	2.7	0.6	2.4	0.6	2.4	0.5
Phenanthrene	29.8	11.5	17.5	5.9	18.7	5.4	14.3	5.5
Anthracene	3.5	0.6	2.2	0.9	2.2	1.0	1.5	0.4
Fluoranthene	10.3	3.3	5.9	2.3	6.8	3.5	4.9	1.7
Pyrene	10.3	3.5	5.8	2.2	6.7	3.4	4.9	1.7
Chrysene	3.0	1.0	2.0	0.6	1.4	0.8	1.2	0.3
Benzo[*a*]anthracene	2.9	0.8	2.2	0.9	2.3	0.9	1.9	0.6
Benzo[*b*]fluoranthene	3.8	1.8	3.3	1.8	2.9	1.1	2.8	1.1
Benzo[*k*]fluoranthene	2.5	1.6	1.8	0.9	1.6	0.9	1.6	0.9
Benzo[*a*]pyrene	2.3	0.6	2.0	0.4	2.2	0.6	1.8	0.3
Indeno[1,2,3-*cd*]pyrene	0.91	0.38	0.82	0.28	0.85	0.21	0.90	0.30
Dibenzo[*a,h*]anthracene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Benzo[*ghi*]perylene	1.0	0.42	0.83	0.37	0.95	0.53	0.85	0.42
Σ 16 EPA PAHs	**153.0**	**37.7**	**97.7**	**35.5**	**88.5**	**29.7**	**78.42**	**20.6**

Table 3.2.5. Concentrations of the 16 USEPA PAHs and standard deviation (n=5 plot) in soil control at different sampling dates from the beginning of the field experiment (May 2009).

Sample Id.	**Aug 2011**		**Dec 2011**		**May 2012**		**May 2013**	
PAHs	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**	**ng g^{-1}**	**SD**
Naphthalene	5.6	2.0	6.2	1.3	5.0	2.0	5.6	1.0
Acenaphthylene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Acenaphthene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Fluorene	1.55	0.3	1.61	0.42	1.74	0.14	1.52	0.27
Phenanthrene	7.8	1.4	7.7	1.2	5.66	0.7	6.7	2.0
Anthracene	0.90	0.08	0.82	0.32	0.90	0.32	0.92	0.48
Fluoranthene	2.70	1.0	2.62	0.47	2.12	0.71	1.67	0.53
Pyrene	1.83	0.89	1.81	0.20	1.82	0.57	1.37	0.31
Chrysene	0.50	0.12	0.53	0.08	0.56	0.16	0.62	0.20
Benzo[*a*]anthracene	0.98	0.25	0.84	0.16	0.80	0.13	0.73	0.16
Benzo[*b*]fluoranthene	0.89	0.14	0.82	0.28	1.24	0.46	1.05	0.40
Benzo[*k*]fluoranthene	0.74	0.16	0.79	0.14	0.59	0.10	0.55	0.10
Benzo[*a*]pyrene	0.75	0.03	0.79	0.16	0.63	0.16	0.80	0.18
Indeno[1,2,3-*cd*]pyrene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Dibenzo[*a,h*]anthracene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Benzo[*ghi*]perylene	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Σ 16 EPA PAHs	**24.2**	**3.08**	**24.5**	**2.01**	**21.0**	**3.97**	**21.6**	**3.01**

3.2.3.4. Individual PAH concentration and degradation

The individual concentrations of 16 US EPA PAHs in control soils and in soils amended by two concentrations of biochar are presented in Tables 3.2.3, 3.2.4 and 3.2.5. The PAHs with 2–3 rings composed the majority of PAHs in control soil and in amended soil samples while PAHs with 4–6 rings only accounted for 24-40% of ∑PAHs on average.

A detailed analysis of the contribution of the individual PAHs in amended soils indicated the dominance of naphthalene (40 ± 3.5% BB and 26 ± 3.2% B of the total PAHs) and phenanthrene (20 ± 1.3% BB and 25 ± 1.5% B of the total PAHs) in all the samples studied.

In the control soils, phenanthrene, naphthalene and fluoranthene dominated the PAH profiles, supplying 30 ± 2.4%, 25 ± 1.3%, and 10 ± 1.5% of the total PAH concentrations, respectively. In this control soil, the observed fingerprints of PAHs are in agreement with data in the literature, where the same compounds were reported to be the dominating ones in soil samples (Bucheli et al., 2004; Zhang et al., 2006; Kwon et al., 2014). Table 3.2.6 and 3.2.7 show that in the amended soils B and BB the concentrations of the PAHs decreased significantly during the four years following biochar application. The total PAH decreases by about 50% compared to that found the first year. However, the degradation was generally higher for low molecular weight PAHs. Up to 61 and 56% in soils B and BB, respectively, whereas for PAHs with five and six rings the corresponding figures varied between 1 and 37%. In particular, naphthalene reduces its first year value to 61% in B and 53 in BB. Acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and chrysene were all reduced to approximately 40-50% of their initial values (concentration found the first year). The PAHs with higher molecular weights, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-c,d]pyrene and benzo[*ghi*]perylene showed a decrease between 1 and 37%.

In a few cases, an increase in concentration of PAHs is observed, which is likely caused by the heterogeneity of the soil. High molecular weight PAHs are well known for their recalcitrance to biodegradation because of their low bioavailability. PAH molecule stability and hydrophobicity are two primary factors which contribute to the persistence of HMW PAHs in the environment. This could due to the higher biodegradation rate of soil bacteria in utilizing lower than higher ring PAHs as energy (Olson et al., 2008). However, it is interesting to note that benzo[*a*]pyrene, considered to be representative for the group of cancerogenic PAHs, was detected in all amended soil analyzed here, with concentrations from 0.8 to 2.3 ng g^{-1}. This levels of benzo[*a*]pyrene are lower than 3-13 ng g^{-1} reported for agriculture soils in Norway, Poland, Czech Republic and China (Nam et al., 2008; Gusev et al., 2008; Maliszewska-Kordybach et al., 2009; Cao et al., 2013). Moreover, the level of benzo[*a*]pyrene varied in untreated soils from 3 to 3.7%, in amended soils B and BB from 1.5 to 2.8%. The presented results for control soil are in agreement with other data reported in the literature (3-5%) concerning soils from non-industrial areas (Desaules et al., 2008; Maliszewska-Kordybach et al., 2009; Cao et al., 2013).

Table 3.2.6. Reduction PAH concentration (%) in amended soils B compared to concentration found the first year (August 2011) at different sampling dates from the beginning of the biochar amendment (April 2009).

Sample Id.		**Dec 2011**	**May 2012**	**May 2013**
PAHs	**Time after amendment**	**27 moths**	**37 moths**	**49 moths**
	Reduction (%)			
Naphthalene		41	57	61
Acenaphthylene		78	-	-
Acenaphthene		-128	-	-
Fluorene		21	21	26
Phenanthrene		31	38	46
Anthracene		22	31	40
Fluoranthene		15	35	45
Pyrene		23	42	44
Chrysene		12	36	40
Benzo[*a*]anthracene		23	32	30
Benzo[*b*]fluoranthene		10	26	13
Benzo[*k*]fluoranthene		2	27	26
Benzo[*a*]pyrene		12	22	19
Indeno[1,2,3-*cd*]pyrene		28	74	57
Dibenzo[*a,h*]anthracene		-	-	-
Benzo[*ghi*]perylene		45	-	-
Total PAHs		**30**	**45**	**50**

Table 3.2.7. Reduction PAH concentration (%) in amended soils BB compared to concentration found the first year (August 2011) at different sampling dates from the beginning of the biochar amendment (April 2009).

Sample Id.		**Dec 2011**	**May 2012**	**May 2013**
PAHs	**Time after amendment**	**27 moths**	**37 moths**	**49 moths**
	Reduction (%)			
Naphthalene		45	53	53
Acenaphthylene		33	40	41
Acenaphthene		32	26	43
Fluorene		44	51	51
Phenanthrene		41	37	52
Anthracene		38	38	56
Fluoranthene		42	34	52
Pyrene		44	35	52
Chrysene		33	52	61
Benzo[*a*]anthracene		26	21	37
Benzo[*b*]fluoranthene		14	25	28
Benzo[*k*]fluoranthene		28	35	37
Benzo[*a*]pyrene		11	5	19
Indeno[1,2,3-*cd*]pyrene		10	6	1
Dibenzo[*a,h*]anthracene		-	-	-
Benzo[*ghi*]perylene		-	-	-
Total PAHs		**36**	**42**	**49**

Table 3.2.8. Reduction PAH concentration (%) in soils C compared to concentration found the first year (August 2011) at different sampling dates from the beginning of the biochar amendment (April 2009).

Sample Id.		**Dec 2011**	**May 2012**	**May 2013**
PAHs	**Time after amendment**	**27 moths**	**37 moths**	**49 moths**
	Reduction (%)			
Naphthalene		-1	11	0
Acenaphthylene		-	-	-
Acenaphthene		-	-	-
Fluorene		-3	-12	2
Phenanthrene		2	27	13
Anthracene		9	-1	-3
Fluoranthene		3	22	38
Pyrene		1	0	25
Chrysene		-7	-12	-25
Benzo[*a*]anthracene		14	18	25
Benzo[*b*]fluoranthene		8	-39	-18
Benzo[*k*]fluoranthene		-7	20	25
Benzo[*a*]pyrene		-5	17	-7
Indeno[1,2,3-*cd*]pyrene		-	-	-
Dibenzo[*a,h*]anthracene		-	-	-
Benzo[*ghi*]perylene		-	-	-
Total PAHs		**-1**	**13**	**11**

3.2.2.5. Molecular diagnostic ratios

PAH diagnostic ratios have been used to determine the source of PAH and the relative importance of combustion and petroleum derived PAH in sediments and in soils (Yunker et al., 2002; Tobiszewski and Namieśnik, 2012; Vane et al., 2013). PAH diagnostic ratios may be an efficient supporting tool in studying the fate of PAH in the soil and assessing the influence of the biochar on the PAH degradation/leaching. Mutsazawa et al. (2001) investigated the photodegradation of PAHs emitted with diesel particles deposited on soils and found that fluoranthene and pyrene were rather stable, but that pyrene degraded faster

on most of the model soils. Under natural conditions, the photodegradation of PAHs bound to diesel particles deposited on soils is expected to be very slow.

The literature provides descriptions of more than ten different molecular diagnostic ratios (Katsoyiannis et al. 2011; Tobiszewski and Namieśnik 2012). In this study, three molecular ratios were used: anthracene/(phenanthrene+anthracene), fluoranthene/(fluoranthene+pyrene), naphthalene/(naphthalene+phenanthrene). Anthracene/(phenanthrene+anthracene) and fluoranthene/(fluoranthene+pyrene) diagnostic ratios are frequently applied to soil samples; on the contrary naphthalene/(naphthalene+phenanthrene) is not used in literature. Schimmelpfennig and Glaser (2012) have found that naphthalene and phenanthrene can be specific of the source of different biochars.

The results of calculations of molecular diagnostic ratios for the soil, biochar amended soil and biochar samples are presented in Table 3.2.9 and in Fig. 3.2.2 in the form of the so-called cross plots. The values of anthracene/(phenanthrene+anthracene) and fluoranthene/(fluoranthene+pyrene) in the biochar amended soil B and BB were of 0.10-0.17 and 0.50-0.55, respectively, characteristic for contaminants of pyrogenic origin, and thus biochar. Moreover, those results are in agreement with the results of Kuśmierz et al. (2014) for soils situated in the vicinity of biochar production sites (Table 3.2.9). In addition, are similarly with molecular ratios calculated for various biochars on the basis of literature data (Table 3.2.9). PAH cross plots for the ratios of fluoranthene/(fluoranthene+pyrene) vs. naphthalene/(naphthalene+phenanthrene) shows that biochar addition to the soil induced a decrease of these molecular diagnostic ratios (Fig. 3.2.2). In particular, the biochar amendment at 44 t ha^{-1} caused a considerable similarity of these diagnostic ratios to those of the used biochar.

PAHs present in soil can be degraded by native bacteria and fungi (Zhang et al., 2006), resulting in a (possibly selective) decrease of concentration over time, with rates depending on soil type, organic carbon and nutrient content, humidity and aeration (Sabaté et al., 2006; Zhang et al., 2006). The results of microbial PAH degradation studies indicate that phenanthrene may be degraded faster than anthracene, and fluoranthene faster than pyrene (Sabaté et al., 2006). Moreover, PAHs may undergo desorption: fluoranthene and pyrene are desorbed at similar rates, but phenanthrene is desorbed faster than anthracene (Enell et al., 2005). The change of molecular ratios as a function of time for the biochar amended soils B and BB are presented in Table 3.2.10. It is interesting to note that PAHs molecular ratios have remained largely unchanged during the four years following biochar

application. Therefore, these resultants may indicate that PAH degradation in biochar amended soil is influenced by biochar.

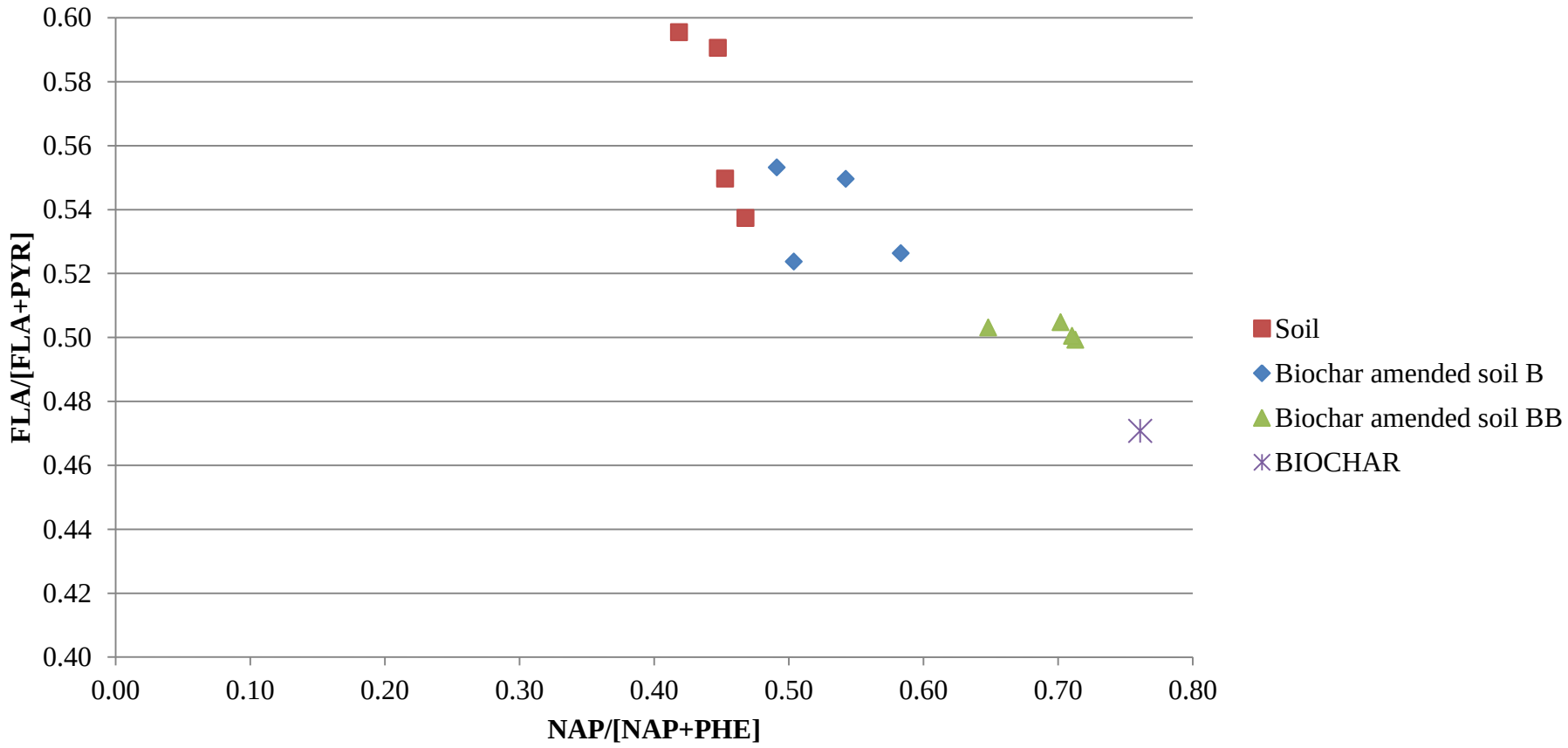
Table 3.2.9. Calculated anthracene/[phenanthrene+anthracene] (ANT/[ANT+PHE]), fluoranthene/[fluoranthene+pyrene] (FLA/[FLA+PYR]), naphthalene/[naphthalene+phenanthrene] (NAP/[NAP+PHE]) ratios in biochar amended soil B and BB, soil C, biochar sample and literature data.

Sample name	ANT/[ANT+PHE]	FLA/[FLA+PYR]	NAP/[NAP+PHE]	
				Present work
Soil C	0.1143	0.5683	0.4467	
Soil B	0.1646	0.5382	0.5302	
Soil BB	0.1038	0.5019	0.6934	
Biochar	0.1201	0.4707	0.7611	
				Literature
Wood 400°C	0.1006	0.3715	-	Keiluweit et al. (2012)
Wood 500 °C	0.1238	0.3302	-	
Wood 600 °C	0.0683	0.3667	-	
Biochar 1	0.1923	0.4627	0.7714	Hilber et al. (2012)
Biochar 2	0.1571	0.5304	0.7330	
Biochar 3	0.1668	0.5840	0.7876	
Biochar 4	0.1705	0.5495	0.7621	
Hardwood 500 °C	0.1071	0.6666	0.8626	Fabbri et al. (2013)
Wood waste 550 °C	0.0882	0.5000	0.5866	
Wood waste 470 °C	0.0879	0.5789	0.8101	
Soil W1a	0.2396	0.4951	-	Kuśmierz et al. (2014)
Soil W1b	0.1969	0.5303	-	
Soil W1c	0.2139	0.4916	-	

Table 3.2.10. Calculated anthracene/[phenanthrene+anthracene] (ANT/[ANT+PHE]), fluoranthene/[fluoranthene+pyrene] (FLA/[FLA+PYR]), naphthalene/[naphthalene+phenanthrene] (NAP/[NAP+PHE]) ratios in biochar amended soil B and BB and soil C sampled four times from 2011 to 2013 (August 2011, December 2011, May 2012 and May 2013).

Sample name	ANT/[ANT+PHE]	FLA/[FLA+PYR]	NAP/[NAP+PHE]
Soil C			
Aug 2011	0.1032	0.5955	0.4186
Dec 2011	0.0964	0.5907	0.4475
May 2012	0.1378	0.5373	0.4679
May 2013	0.1199	0.5497	0.4528
Biochar amended soil B			
Aug 2011	0.1532	0.5264	0.5833
Dec 2011	0.1696	0.5496	0.5424
May 2012	0.1668	0.5532	0.4912
May 2013	0.1688	0.5237	0.5038
Biochar amended soil BB			
Aug 2011	0.1044	0.4993	0.7130
Dec 2011	0.1099	0.5048	0.7019
May 2012	0.1041	0.5031	0.6482
May 2013	0.0969	0.5005	0.7104

Fig. 3.2.3. PAH cross plots for the ratios of fluoranthene/[fluoranthene+pyrene] (FLA/[FLA+PYR]) vs. naphthalene/[naphthalene+phenanthrene] (NAP/[NAP+PHE]).



3.2.3.6. Effects of biochar on soil properties and relationship with PAHs

The chemical and physical characteristics of the soil and biochar amended soil B and BB during the three years of studies are reported in Tables 3.2.11, 3.2.12 and 3.2.13. Biochar amended soil pH, C content and CEC were increased and soil bulk density decreased under biochar amendments in both treatments, being more or less in proportional to the amendment rates for pH and CEC. On the contrary, the changes in C content and soil bulk density in all years were not corresponding with the biochar amendment rates. In particular, biochar amendment at 44 t ha^{-1} caused a consistent decrease in soil bulk density by 0.15 mg m^{-3} in 2010 year and by 0.06 g cm^{-3} in 2011 and 2012 years as compared to the corresponding control, respectively. Moreover, the biochar amendment at 44 t ha^{-1} caused a consistent increase in organic C by 4.7% in 2010 year, by 3.9% in 2011 year and by 3.4% in 2012 year as compared to the corresponding control, respectively. This effect, however, also if less consistent is present in the biochar amended soil B. In these soils the biochar amendment at 22 t ha^{-1} caused an increase in organic C by 0.68% in 2010 year, by 0.49% in 2011 year and by 0.37% in 2012 year as compared to the corresponding control, respectively.

Biochar addition to the soil induced a significant increase of soil CEC. This increase of CEC in the biochar amended soils B and BB compared to control C (Table 3.2.11, 3.2.12 and 3.2.13) is proportional to the amendment rates. The increase of CEC in soils amended can be attributed to the presence of retained oxygen content in biochar used in this experiment, as previously published (Lee et al., 2010). The retained oxygen content presents itself on the biochar as primarily carbonyl, carboxyl, and phenolic groups, all of which in part facilitate CEC through electrostatic interactions. Furthermore, this data shows that the biochar sample has the capability of not only serving as a long-term carbon sequestration agent, but also has the potential to increase soil CEC.

The soil pH was clearly modified by the amendments in both treatments. In particular, the pH of the soil the first year after application significantly increased with the concentration of biochar amendment, starting from 5.50 in the control to 6.47 in the biochar amended soil B and 7.18 in the BB (Table 3.2.11, 3.2.12 and 3.2.13). A similar trend was observed by Fellet et al. (2011) using the same concentrations of biochar application. Compared to the first year of biochar amendment, biochar amended soil pH almost unchanged in the 2011 and 2012 in both treatments. The increase in soil pH after the application of biochar may be attributed to the alkaline nature of biochar.

PAH degradation in the soil is slow; however, PAHs may be degraded through properly stimulated soil microorganisms by mineralization, co-metabolic degradation and non-specific radical oxidation (Wetzel et al., 1997). Soils inherently contain complex autochthonous microbial communities, which have PAH degrading abilities (Ding et al., 2010). The acidity of soils can control conditions for microbial degradation and regulate the sorptive capacity of organic matter (e.g. by protonation of acidic functional organic matter groups), thus it may contribute in different ways to soil matrix effects on PAH degradation (Maliszewska-Kordybach, 1999; Bucheli et al., 2004). Therefore, in biochar amended soil BB the higher pH may have been important for influencing the fate of PAHs. Moreover, the biochar has been shown to increase microbial activity in soil (Steinbeiss et al., 2009) which can stimulate PAH degradation. Our results showed that total PAH in BB soil decreases by about 50% in two years, and therefore the addition of biochar could have increased the degradation of PAHs.

Table 3.2.11. Chemical characteristics of soil C in the field experiment.

	Soil			
	Jun 10	**Feb 11**	**Jun 11**	**Jun 12**
C (%)	0.67±0.02	0.73±0.08	0.82±0.07	0.93±0.04
pH	5.50±0.22	5.18±0.30	5.25±0.15	5.39±0.26
EC (meq 100 g^{-1})	12.6±0.4	11.8±0.9	11.5±1.5	11.9±1.9
Bulk density (mg m^{-3})	1.44±0.05	1.44±0.10	1.45±0.06	1.44±0.03

Notes: Values in the tables are the mean value ± standard deviation from five replicates.

Table 3.2.12. Chemical characteristics of biochar amended soil B in the field experiment.

	Biochar amended soil			
	Jun 10	**Feb 11**	**Jun 11**	**Jun 12**
C (%)	1.35±0.25	1.33±0.30	1.31±0.29	1.30±0.29
pH	6.47±0.24	6.54±0.25	6.32±0.14	6.34±0.24
EC (meq 100 g^{-1})	18.16±0.96	18.32±1.05	18.14±0.83	18.22±0.78
Bulk density (mg m^{-3})	1.42±0.03	1.42±0.07	1.40±0.03	1.40±0.06

Notes: Values in the tables are the mean value ± standard deviation from five replicates.

Table 3.2.13. Chemical characteristics of biochar amended soil BB in the field experiment.

	Biochar amended soil			
	Jun 10	**Feb 11**	**Jun 11**	**Jun 12**
C (%)	5.4±1.2	5.11±0.96	4.76±0.53	4.3±1.4
pH	7.18±0.11	6.76±0.18	6.59±0.20	6.61±0.30
EC (meq 100 g^{-1})	26.0±3.7	24.3±1.8	24.1±1.8	22.9±1.8
Bulk density (mg m^{-3})	1.29±0.18	1.38±0.06	1.38±0.25	1.38±0.09

Notes: Values in the tables are the mean value ± standard deviation from five replicates.

3.2.4. Conclusions

The fate of PAHs in biochar amended soil is relevant in order to prevent severe contamination to the environment. The results presented in this study show that the biochar addition determines an increase of the amounts of PAHs. However, the results corresponding to the amendment of 22 and 44 t ha^{-1} suggest that the soil contamination by PAHs following biochar application is not significant at the application rates currently recommended in agriculture (20-60 t ha^{-1}). In fact, the levels of PAHs in the soil remained within the maximum acceptable concentration (5–50 μg g^{-1}) for a number of European

countries. Moreover, biochar amendment in four years does not increase the concentration of soil PAH. Therefore, the biochar does not reduce the degradation of PAHs in agricultural soil and does not affect the persistence of PAHs in the environment. However, the impact of biochar on the fate of PAHs needs to be investigated further for different soils, biochars, over longer periods, and also under different field conditions.

4. Biochar characterization for agricultural utilization

4.1. Relationships between chemical characteristics and phytotoxicity of biochar from poultry litter pyrolysis

4.1.1. Introduction

Biochar is the carbonaceous product of biomass pyrolysis which can be used as soil additive capable to mitigate a variety of agro-environmental stresses through the permanent storage of biomass carbon, pH correction, reduced synthetic fertilizer use, decreased runoff of fertilizers and agrochemicals (Glaser et al., 2002; Lehmann et al., 2006; Steiner et al., 2007; Sohi et al., 2010; Uchimiya et al., 2010; Galinato et al., 2011; Vaccari et al., 2011; Ventura et al., 2013).

The effect of adding biochar to soils may result in increased plant growth, productivity and yield (Graber et al., 2010, Vaccari et al., 2011, Joseph et al., 2013) attributed to the improvement of soil water-holding capacity (Case et al., 2012; Basso et al., 2013; Baronti at al., 2014), lower disease incidence in crops (Matsubara et al., 2002; Elad et al., 2010; Elmer and Pignatello, 2011), reduced bioavailability of heavy metals (Park et al., 2011), increased nitrogen and carbon bioavailability (Scharenbroch et al., 2013). Preventing loss of nutrient leaching may reduce the needs of fertilizer use (Liang et al., 2006; Laird et al., 2010). Because of its basicity, biochar can be used in acidic soils with poor cultivation properties (Glaser et al., 2002; Slavich et al., 2013). However, the effects of biochar are highly variable depending on the feedstock, thermochemical process conditions, application rate, soil characteristics, environmental conditions, and plant species (Chan and Xu, 2009; Jeffery et al., 2011, Schulz and Glaser, 2012), explaining the variety of outcomes reported in literature that range from a boost in plant productivity to evident phytotoxicity (Jeffery et al., 2011).

Prompted by the urgency to find applications alternative to its disposal and management, poultry litter has been investigated as a substrate in the preparation of biochar (Chan et al., 2008; Van Zwieten et al., 2013; Novak et al., 2014). Possible benefits of amending soils with poultry litter biochar have been reported and attributed to an improved nitrogen availability (Chan 2008; Van Zwieten et al., 2013). Lower N_2O emissions with respect to the raw poultry litter and the elimination of potential pathogens has advocated its pyrolytic conversion (Van Zwieten et al., 2013). However, the use of

biochar especially from animal origin has raised concerns related to its possible toxicity and studies have been recently conducted to explore physiological effects on biota (Bastos et al., 2014; Smith et al., 2013; Oleszczuk et al., 2012).

Bioassays based on seed germination and early stage seedling growth is a simple and commonly used ecotoxicological test for evaluating the impact of biochar amendment on crop growth (Solaiman et al., 2012). The test of phytotoxicity of the biochars was made in the absence of soil due to the large soil–char interactions observed in some studies (Zimmerman et al., 2011) and because Solaiman et al. (2012) demonstrated that growing seedlings in pure biochar materials is a valid tool in assessing the effect of biochar application rate on germination.

Seed germination, one of the most important phases in the life cycle of a plant, is highly responsive to existing environment (Kuriakose et al., 2008). Factors such as heavy metals (Wollan et al., 1978), PAHs (Rogovska et al., 2012), ammonia (Wong et al., 1983), salts (Adriano et al., 1973) and low molecular weight fatty acids (Zucconi et al., 1985) have been shown to be responsible for inhibitory effects.

Some studies have examined the effect of biochar on seed germination (Free et al., 2010; Solaiman et al., 2012; Busch et al., 2013). Rogovska et al. (2012) reported that biochars contain phytotoxic compounds that inhibit germination of maize. In contrast, Free et al. (2010) reported that maize seed germination was not significantly affected by biochars made from a range of organic sources. Solaiman et al. (2012) showed that biochars generally increased germination at low application rates (10-50 t ha^{-1}), whereas higher application rates of 100 t ha^{-1} had no effect or decreased germination rate. Alburquerque et al. (2014) observed that different biochars exerted a positive effect on seed germination also to high application rates instead.

Recent studies have also suggested different methods for reducing the toxicity of biochar (Bargmann et al., 2013; Buss and Masek, 2014). Washing biochar with water or an organic solvent has been successfully tested to reduce phytotoxicity of biochar (Bargmann et al., 2013; Bernardo et al., 2010; Rogovska et al. 2012). Meanwhile, Kołtowski et al. (2015) demonstrated significant reduction of biochars toxicity by drying them at various temperatures (100–300 °C) for 24 h.

While the published and ongoing investigations are providing increasing data helpful to understand the relationships between biochar characteristics and seed germination (Bargmann et al., 2013; Kołtowski et al., 2015), further studies are needed to better clarify the role played by the chemical properties in determining the plant toxicity in order to

forecast strategies in biochar synthesis or post-treatments. Biochars from different feedstock and process conditions may exhibit a wide range of plant response, from growth inhibition to stimulation. The relatively simple seed germination test is a valid and fast tool to compare several biochars obtained from different starting materials and under different pyrolysis conditions. Since the test is performed in short time and without the buffering effect of soil, it could be considered a kind of precautionary procedure that highlights intrinsic phytotoxicity of the tested materials.

The aim of this study was to evaluate phytotoxicity of biochar from poultry litter by means of standard germination tests and to identify possible relationships with its chemical characteristics. To this purpose, germination tests with cress (*Lepidium sativum* L.) were conducted to poultry litter biochars synthesised at different pyrolysis conditions. Besides manure, poultry litter typically contains bedding materials made up of lignocellulosic residues. Therefore, a comparison was made with biochars from a representative herbaceous residue, corn stalk, prepared under the same conditions. The effect of solvent extraction and biological conditioning on seed germination was tested on a selected poultry litter biochar prepared upon pyrolysis at 400 °C (PL400). The chemical composition of mobile constituents in this sample capable to be potentially released in the water and air compartments was investigated by solvent extraction and solid-phase microextraction (SPME).

4.1.2. Materials and Methods

4.1.2.1. Biochar synthesis

Cornstalk was described in a previous study (Cordella et al., 2012). Granular poultry litter was a marketed organic fertilizer obtained after processing raw poultry litter collected from local broiler farms by pasteurizing at 80–110°C, milling, and pelletizing. Biomass was air dried at 60°C, milled and sieved at 2 mm before pyrolysis.

Batch pyrolysis experiments were conducted under nitrogen with a fixed bed tubular quartz reactor placed into a refractory furnace (see Conti et al., 2014 for details) with about 20 g cornstalk or 35 g poultry litter exactly weighed and uniformly placed onto a sliding quartz boat; nitrogen flow was set at 1500 cm^3 min^{-1} and when the temperature inside the reactor, measured with a thermocouple, reached the selected value, the boat was pushed into the oven and left for a given residence time before pulling it back into the unheated

part of the reactor. Pyrolysis were performed under three different conditions based on a previous study (Conti et al. 2014) of temperature/residence time: 400°C/20 min, 500°C/10 min and 600°C/5 min. In accordance to the original biomass (cornstalk, CS; poultry litter, PL) and pyrolysis temperature, the obtained biochar samples were named CS400, CS500, CS600, PL400, PL500 and PL600. Chemicals were purchased by Sigma Aldrich. SPME Carboxen-PDMS fibers and the fiber holder were purchased by Supelco.

4.1.2.2. Biochar Characterization

Elemental composition (HCNS) was determined by combustion using a Thermo Scientific Flash 2000 series analyzer. Ash was determined as the residual mass left after exposure at 600 °C for 5 hours. The oxygen content was calculated from the mass balance: O%=100-(C+H+N+ash)%.

Analytical pyrolysis (Py-GC-MS) were conducted at 900 °C for 100 seconds with a CDS 5250 pyroprobe interfaced to a Varian 3400 GC-Saturn 2000 MS. GC-MS conditions and the determination of indicators of carbonisation % charred and toluene/naphthalene ratio were described in Conti et al. (2014).

The content of the 16 EPA priority PAHs was measured in triplicate as described in Fabbri et al., (2013). Briefly, about 0.5 g of biochar were spiked with 0.1 mL of surrogate PAH mix (Supelco for EPA 525 containing acenaphthene-*d*10, phenanthrene-*d*10 and chrysene-*d*12 5 µg mL^{-1} each in acetonitrile) and soxhlet extracted with acetone/cyclohexane (1:1, v/v) for 36 hours. The solution was filtered, added with 1 ml of *n*-nonane (keeper), carefully evaporated by rotatory vacuum evaporation at 40 °C and cleaned up by solid phase extraction onto a silica gel cartridge before analysis with a Agilent HP 6850 GC coupled to a Agilent HP 5975 quadrupole mass spectrometer; GC-MS conditions were those detailed in Fabbri et al., (2013). Recovery of surrogate PAHs was determined with respect to the internal standard tri-*tert*-butylbenzene added prior to GC-MS analysis.

Volatile fatty acids (VFAs) were determined by the single drop extraction procedure as described in Torri et al., (2014). About 200 mg of biochar exactly weighed was added with 0.1 ml of internal standard solution (1.0 g l^{-1} 2-ethylbutyrate in deionised water) and thoroughly mixed with 0.2 ml of saturated aqueous $KHSO_4$. After centrifugation, a drop of dimethyl carbonate (1.2 µl) from a 10 µl chromatography microsyringe was exposed into the supernatant aqueous solution. After 20 min exposure the drop was retracted and

injected into a GC-FID (injection temperature 250°C) equipped with polar GC column (Agilent Q7221J&W nitroterephthalic-acid-modified polyethylene glycol DB-FFAP 222 30 m, 0.25 mm, 0.2 µm) with the following thermal program: 80°C for 5 min, then 10°C/min to 250°C. Calibration was performed by applying the same procedure to standard solutions containing known concentration of each VFA (namely: acetic, propionic, isobutyric, butyric, isovaleric and valeric acid).

For the determination of N-$NH4^{+}$, about 10 g of biochar were placed in an end-to-end shaker for 2 h with 1 N KCl (1:10 dw:v) followed by centrifuging at 4500 x g for 20 m and passing through a 0.45 µm paper filter.

4.1.2.3. SPME of mobile compounds

The SPME was applied to aqueous extracts following the procedure under development by Ghidotti et al. 2014 using a Carboxen-PDMS fiber direclty immersed (DI-SPME) into the test solution added with KH_2PO_4/Na_2HPO_4 phosphate buffer 2M at pH 5.3 and internal standard (*o*-eugenol and 2-ethyl butyric) under magnetic stirring for 30 minutes followed by thermal desoprtion at 250 °C and GC-MS analysis.

Head space (HS) analysis was performed following the procedure described by Spokas et al., 2011 modified for the SPME sampling with Carboxen-PDMS fiberv (HS-SPME) utilising *o*-eugenol as internal standard (Ghidotti et al. 2015).

Separation of thermally desorbed compounds was conducted with a DB-FFAP polar column (30m lenght, 0.25mm i.d, 0.25µm film thickness).

4.1.2.4. Biochar post-treatments. Aqueous extraction

About 2 g of PL400 was extracted with 50 mL of deionised water in a 100 mL flask at room temperature for 12 hours with mechanical shaking. The aqueous phase was separated by filtration through a 0.22 mm paper filter and used as such for the germination test, while an aliquot was kept at –20 °C for SPME-GC-MS analysis (see above). The solid biochar residue left after water extraction was further extracted with 50 mL of methanol under reflux for 12 hours. The methanol was separated by filtration and an aliquot corresponding to the 40g/L suspension of biochar was poured into petri dishes and dried overnight at 70

°C under vacuum to remove all the methanol. Thereafter deionised water was added to perform germination tests. The final solid biochar residue left after water and methanol extraction was dried overnight at 100°C under vacuum and utilized for germination tests.

4.1.2.5. Biochar post-treatments. Biological treatment

Microbial treatment of PL400 was conducted for 14 days with an activated sludge. The sludge was obtained from a municipal wastewater treatment plant located in Ravenna after centrifugation at 6000 rpm (20% w/w volatile suspended solids). A suspension containing 0.5 g of the concentrated sludge and 250 ml of 40 g l^{-1} PL400 in deionised water was thoroughly mixed under laminar shake at 120 rmp overnight. An aliquot of 10 ml of this suspension was added in petri dishes and stored at 14 days at 25°C before performing germination test as shown above.

4.1.2.6. Germination tests

The germination tests were conducted in four replicates by incubating 50 seeds of cress (*Lepidium sativum* L.) with 5 g of a mixture containing biochar and deionized water onto sterilized cellulose filter paper (Whatman No. 1) placed in a petri dish sealed with paraffin film. Three levels of biochar concentration were tested 2, 5 and 40 g L^{-1}. These rates were equivalent to 2, 5 and 40 t ha^{-1} on an area basis of 10 cm soil depth and a dry bulk density of 1.5 kg m^{-3}. Germination tests were also performed on the fractions obtained from the chemical and biological post-treatments of PL400 described above. The quantities of these fractions were adjusted to correspond to the concentration level of 40 g l^{-1} of the original biochar. Before incubation, the samples were shaken at 150 r.p.m. on a platform shaker at room temperature for 24 h. pH and electrical conductivity (EC) were determined. The pH was directly measured placing the glass-electrode into the suspension with a pH-meter Mettler Toledo SG 2-ELK. The electrical conductivity (EC) was measured with a Delta OHM HD 8706 conductimeter in the supernatant obtained by centrifuging the suspension and filtered at 0.45 micron.

Phytotoxicity tests were performed on biochar:deionized water mixtures (wetted biochar) according to the procedure described in UNI 11357:2010. The experiments were conducted with 50 seeds of cress which were incubated with 10 g of biochar saturated with deionized water according to value of the water holding capacity (table 4.1.1) on sterilized

cellulose filter paper placed in a petri dish. All Petri dishes were covered and incubated in room thermostat at 25 ± 2° C for 72 ± 0.5 hours in the dark. Similarly, a control was prepared with deionised water.

After 72 h of exposure, a visible root development was used as the operational definition of seed germination. Data were reported as percentage relative seed germination (RSG) with respect to the control (deionised water):

RSG = (number of seeds germinated in the sample/ number of seeds germinated in control) * 100

4.1.2.7. Statistical analysis

All the experiments were conducted at least in duplicate. Results of germination tests were evaluated statistically using Analysis of Variance (ANOVA) performed with STATISTICA (StatSoft Italia, 2011) and GMAV (Underwood and Chapman, 1997) followed by Student-Newman-Keuls post hoc tests.

4.1.3. Results and Discussion

4.1.3.1. Characterization of biochar

Bulk analysis

The yields and characteristics of biochars obtained from the pyrolysis of poultry litter (PL400, PL500, PL600) under three different pyrolysis conditions are reported in Table 4.1.1 and compared with those of biochar from corn stalk (CS400, CS500, CS600). As expected, the chemical characteristics of the biochars were dependent on the original biomass and the pyrolysis conditions. In particular, the H/C and O/C ratios decreased with increasing pyrolysis temperature, while the content of ash increased as observed with the same pyrolysis unit under the same conditions (Conti et al. 2014). Biochar from poultry litter contained higher levels of nitrogen, sulphur and ash, derived from the manure fraction, as demonstrated by comparative studies on manure and lignocellulosic biochars (Novak et al., 2014). In general, the elemental composition, ash content and the trends with pyrolysis conditions of the poultry litter chars here investigated were comparable to those reported in the literature (Chan et al. 2008, Cimò et al., 2014, Song et al., 2012, Van Zwieten et al., 2013).

Extractable compounds

The concentrations of specific potentially toxic extractable compounds, namely PAHs, VFAs and ammonium are reported in Table 4.1.2. Solvent extractable PAHs occurred within the range of 0.7 - 1.7 mg kg $^{-1}$, values that were typical of biochars from different origins (Hale et al., 2012; Fabbri et al., 2013; Hiber et al., 2012). PAHs concentration can be considered negligible for acute effect lower than typical values in soils, Bucheli et al., 2004. Generally, naphthalene was the most abundant PAHs followed by phenanthrene and fluorene, the level of benzo[*a*]pyrene was in the 5-65 ng g^{-1} range.

The concentration of VFAs was significant higher in poultry litter biochars (4-9 mg kg^{-1}) in comparison to those from cornstalk. (2-4 mg kg^{-1}). Acetic acid was always the most abundant VFA. VFAs derived from feedstock fermentation during silage in poultry litter and the new formation of fatty acids from the thermal degradation of lipids.

Ammonium was not detected in cornstalk biohar, whereas it was abundant in biochars from poultry litter with higher concentrations in the less carbonised biochars.

Table 4.1.1. Yields, water-holding capacity, elemental analysis and ash (% wt dry weight mean values ± s.d. n=4) and elemental molar ratios of biochars from the pyrolysis of corn stalk (CS) and poultry litter (PL) at different conditions (400 °C/20 min, 500 °C/10 min, 600 °C/5 min).

Biochar	Yield (% wt)	water-holding capacity (%)	Elemental content (%)					Ash (%)	Molar ratios
			C	H	N	O	S		H/C
CS400	38.3±0.9	69.5	50±2.1	3.3±0.1	0.96±0.03	15±1.9	0.07±0.01	28.95±0.01	0.79
CS500	33±1.6	81.1	51±1.6	2.7±0.1	0.91±0.04	14.9±0.1	0.03±0.04	30.14±0.02	0.63
CS600	31.4±0.5	73.7	50.7±0.3	2.4±0.1	0.81±0.03	13±1.6	-	32.30±0.02	0.57
PL400	49±3.4	88.6	33±4.7	2.7±0.5	3.6±0.8	11±2.1	1.7±0.5	46.64±0.01	0.98
PL500	41.4±0.9	94.1	33±1.0	2.1±0.1	3.4±0.1	6.5±0.9	2.2±0.1	52.29±0.04	0.76
PL600	39.5±0.5	92.3	31.4±0.5	1.7±0.1	3.2±0.5	4.6±0.3	2.3±0.1	56.82±0.01	0.65

Table 4.1.2. Molecular analysis of extractable compounds and volatile matter by Py-GC-MS of biochar from corn stalk (CS) and poultry litter (PS) (mean values and s.d. from two replicates, T/N toluene/naphthalene ratio).

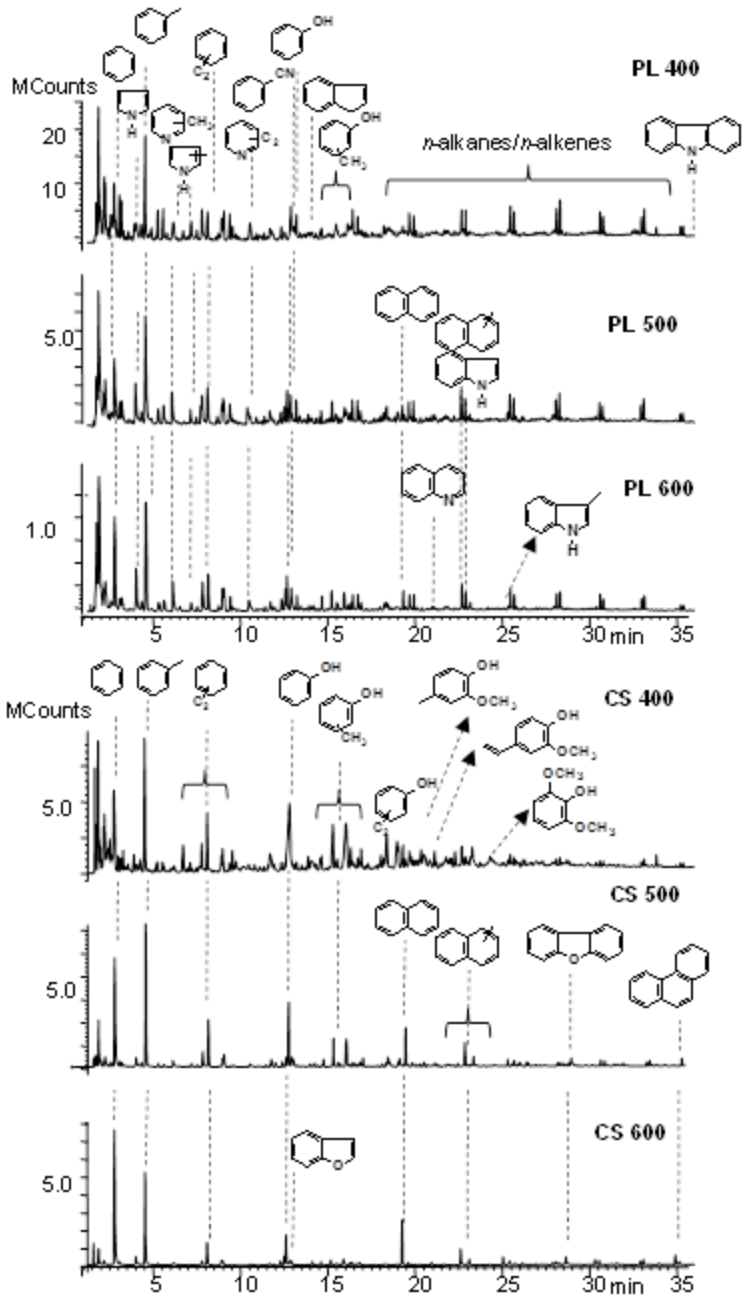
	Extractable			Py-GC-MS	
Biochar	**PAHs**	**VFAs**	**NH_4^+**		
				%	**T/N**
	mg kg^{-1}	**mg g^{-1}**	**mg kg^{-1}**	**charred**	
CS400	0.72±0.06	3.8±1.2	-	53±3	8.5±0.7
CS500	1.09±0.05	0.9±0.3	-	80±3	5.4±1.5
CS600	0.84±0.01	2.6±0.1	-	92±3	3.0±1.7
PL400	1.7±0.2	9.3±0.3	45	88±9	13±10
PL500	0.88±0.05	4.3±2.0	25	90±3	12±6
PL600	0.79±0.01	6.8±1.8	14	88±3	11±2

Py-GC-MS. Thermolabile fraction

The molecular composition of the thermally labile fraction could be inferred from the structural identification of the compounds identified in the pyrolysates (Conti et al., 2014, Fabbri et al., 2013, Kaal and Rumpel, 2009). The pyrolysate of CS400 was characterised by a complex pattern of compounds dominated by phenols and methoxyphenols associated to the presence of partially charred lignin, while the pyrolysate of CS600 contained few peaks due to the hydrocarbons associated to more heavily charred fraction (Fig. 4.1.1). Proxies of the degree of carbonisation established in previous studies (Conti et al., 2014) for lignocellulosic biomass were confirmed in this study for corn stalk biochar: the toluene/naphthalene ratio and the relative abundance of compounds representative of the charred fraction (% charred) exhibited a clear trend with H/C ratios (Table 4.1.2). However, biochar samples from poultry litter did not exhibit significant changes with the H/C ratios and the variability was higher. This finding would suggest that the pyrolysis proxies developed for lignocellulosic biochar could not be valid for biochar containing charred proteins and lipids.

The occurrence of partially charred components from proteins and lipids were clearly evidenced in the pyrolysates of biochar. Phenols and methoxyphenols were detected in PL400 as well as in CS400 in accordance to the fact that the original substrates contained a

Figure 4.1.1. Total ion chromatograms from Py-GC-MS of poultry litter (PL) and cornstalk (CS) biochars.



lignocellulosic component. The distinctive signature of PL pyrolysate was the occurrence of nitrogen-containing compounds (NCCs) from proteins and a pattern of *n*-alkanes/*n*-alk-1-enes assigned to the thermal cracking of bound or free fatty acids. The occurrence of saturated alkyl domains was confirmed by ^{13}C-NMR studies on poultry manure biochars that disappear after carbonisation and may play a role in the availability of sorbed compounds (Cimò et al., 2014). Among the tentatively identified NCCs, pyrrole, pyridine, (iso)quinoline and carbazole along with their alkyl derivatives are indicative of partially charred proteinaceous matter. It worthwhile to note that NCCs were also identified in the volatile fraction by SPME as described in the next section.

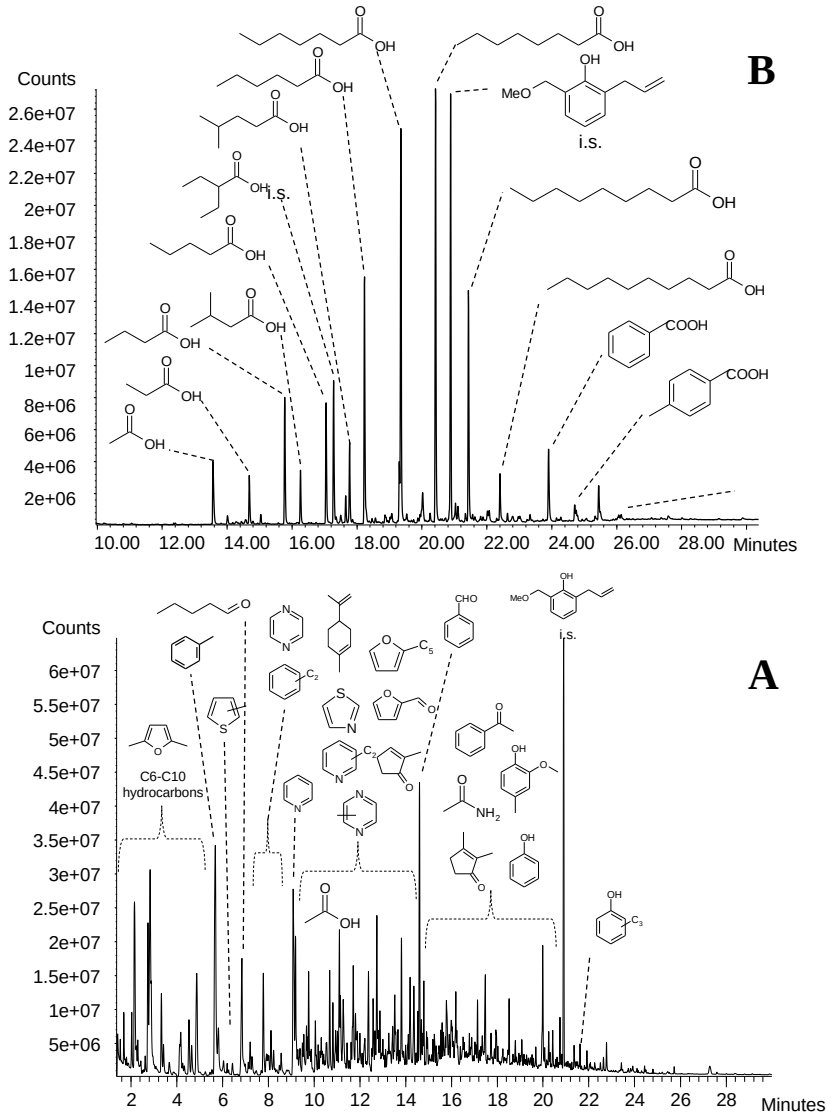
SPME-GC-MS. Volatile and water soluble compounds

Information on the molecular characteristics of the mobile fraction was gathered by HS-SPME (volatile) and DI-SPME (water soluble). The attention was specifically focused to organic compounds, being reported that heavy metals are generally present below the limits causing adverse effects and loosely bioavailable (Cely et al., 2015). Moreover, Bastos et al. 2014 argued that in aqueous extracts PAHs and metals might occur at concentrations below the level to pose detrimental effects, at least for woody biochar up to 80 t ha^{-1}; however, it is to be remarked that the biological response depended on the organisms selected in the bioassay (Bastos et al., 2014).

SPME was applied to sample PL400 that was utilised in post-treatment studies. The results are shown in figure 2 for the analysis of volatile organic compounds (VOCs) by HS-SPME (fig. 4.1.2A) and DI-SPME of aqueous extracts (fig. 4.1.2B), respectively. The VOCs were characterised by the presence of a wide array of compounds deriving from the thermal degradation of polysaccharides (e.g. cyclopentenones, furans), lignin (e.g. 4-vinylphenol, guaiacol), NCCs (e.g. pyrroles, pyridines, indole), lipids (e.g. VFAs, acetic acid is also derived from hemicellulose). Alkylated pyrazines and acetamide were probably derived from Maillard reactions between carbohydrates and proteins.

Notably, a suite of short chain *n*-alkanes/alkenes was identified supporting Py-GC-MS results and literature data about the occurrence of aliphatic components in poultry litter biochar (Cimò et al., 2014). It is expected that the polar fraction of VOCs will be preferentially distributed into the aqueous phase in comparison to non-polar constituents. In fact, the SPME-GC-MS analysis of the PL400 water extract showed a predominance of organic acids, including C_2-C_{10} aliphatic and C_7-C_9 aromatic acids (Figure 4.1.2B).

Figure 4.1.2. Total ion chromatograms obtained after (A) HS-SPME of volatile organic compounds and (B) DI-SPME of water extract of poultry litter biochar (PL400).



4.1.3.2. Effect of biochar on seed germination

The pH and EC of the biochar/water suspensions utilized in germination tests are reported in Table 4.1.3. The pH values were higher for the suspensions with the more carbonized biochars from the same feedstock and increased for each biochar type with increasing concentration. Under the same conditions, the pH was higher in the suspensions with poultry litter biochar, in accordance to previous studies (Novak et al., 2014).

The EC values of the suspensions increased with increasing biochar concentration and, at the same concentrations, the biochar from poultry litter had much higher (from 4 to 40

times for the same condition) EC values than the CS suspensions. Salinity can have a detrimental effect on seed germination and plant growth, especially in the seedling stage, though the response of various plant species to salinity differs considerably (Mengel and Kirkby, 1987). In general, salinity effects are mostly negligible in extracts with EC readings of 2000 μS cm^{-1} or less (Hoekstra et al. 2002). This critical level was exceeded in poultry litter biochar at 40 g L^{-1}. The toxicity of inorganic nitrogen results mainly from ammonia (NH_3) which affects plant growth and metabolism at low concentration levels at which NH_4^+ is not harmful (Mengel and Kirkby, 1987). At concentrations of 0.15-0.20 mM, which are comparable to those calculated in the 40 g l^{-1} biochar/water mixtures, NH_3 could be toxic (Bennett and Adams, 1970).

The effect of the biochar suspensions on seed germination of cress (*Lepidium sativum* L.) is presented in Table 4.1.4 in terms of percent seed germination with respect to control (deionised water only).

The assay results in this work suggested that all the cornstalk biochar suspensions had little impact on seed germination as one-way ANOVA analysis showed no significant difference between control group and test groups ($p > 0.05$). Noticeably, CS400 was almost non-toxic to germination even if used as the growth substrate (UNI test, table 4.1.3).

On the contrary, all the biochar samples from poultry litter inhibited significantly the seed germination at the highest level of 40 g L^{-1} in water suspensions. At the harsh conditions of the UNI test the germination was totally suppressed.

The comparison with cornstalk suggested that the toxicity of biochar from poultry litter could be explained by some distinctive chemical components originated from this feedstock. Compounds derived from lignin and cellulose/hemicellulose could be excluded on the ground that biochar samples from corn stalk did not suppress seed germination in water suspensions. The suspensions of biochars from both substrates presented similar pH values, thus this parameter is not involved in toxicity. This in accordance to the findings by Gell et al. 2011 who did not evidenced clear trends of pH and short term phytotoxicity in biochars of different origins, at least under neutral/basic conditions. Similarly, the concentration of solvent extractable PAHs was similar in PL and CS biochars, thus PAHs cannot be responsible of the observed toxicity. Acetic acid was present in all the biochars and at similar levels, partly due to the decomposition of cellulose/hemicellulose. The suite of alkanes/alkenes characterising the Py-GC-MS pyrolysates of poultry litter biochar

samples would suggest the presence of a lipid fraction producing shorter chain fatty acids by thermal degradation as confirmed by SPME-GC-MS on PL400.

The main differences between the CS and PL biochars were the higher content of elemental nitrogen (table 4.1.1) and ammonium (table 4.1.2), and the presence of a thermally labile fraction derived from proteins and lipids (Py-GC-MS data).

Table 4.1.3. Results from chemical analysis of biochars and relative water suspensions.

Biochar	pH			Electrical conductivity (mS cm^{-1})		
	2 g l^{-1}	5 g l^{-1}	40 g l^{-1}	2 g l^{-1}	5 g l^{-1}	40 g l^{-1}
CS400	7.6	8.0	8.5	16	75	$1.8\ 10^3$
CS500	8.2	8.5	9.3	72	76	$1.9\ 10^3$
CS600	8.4	8.9	10.1	$1.9\ 10^2$	$3.5\ 10^2$	$1.9\ 10^3$
PL400	8.0	9.0	9.7	$7.1\ 10^2$	$1.3\ 10^3$	$7.3\ 10^3$
PL500	8.4	9.3	10.2	$8.7\ 10^2$	$1.5\ 10^3$	$7.7\ 10^3$
PL600	9.4	9.8	10.3	$9.3\ 10^2$	$2.0\ 10^3$	$8.1\ 10^3$

4.1.3.3. Germination tests after biochar post-treatment

A selected sample of poultry litter biochar (PL400) was extracted with water followed by methanol extraction, the extracts and the residue were utilized in germination tests. Germination tests were also performed to PL400 after treatment with sewage sludge to assess the effect of biodegradation. The quantity of extracts and the residues corresponded to the biochar loading level of 40 g l^{-1}. The results are presented in figure 4.1.3. The cress germination rate in the water extracts was similar to that of the original biochar suspensions indicating inhibition due to some components in the water extracts. The germination rates increased significantly to values similar to the control when the suspension was made with the biochar left after solvent extraction. These observations are supported by the results of Rogovska et al. (2012), who showed that growth inhibition no longer occurred when biochars were washed prior to germination.

Biochar suspensions treated with an active sludge for almost two weeks displayed a germination rate similar to the extracted biochar (figure 4.1.3). The reduced toxicity could be ascribed to microbial degradation of some noxious components as suggested by Bargmann et al. (2013).

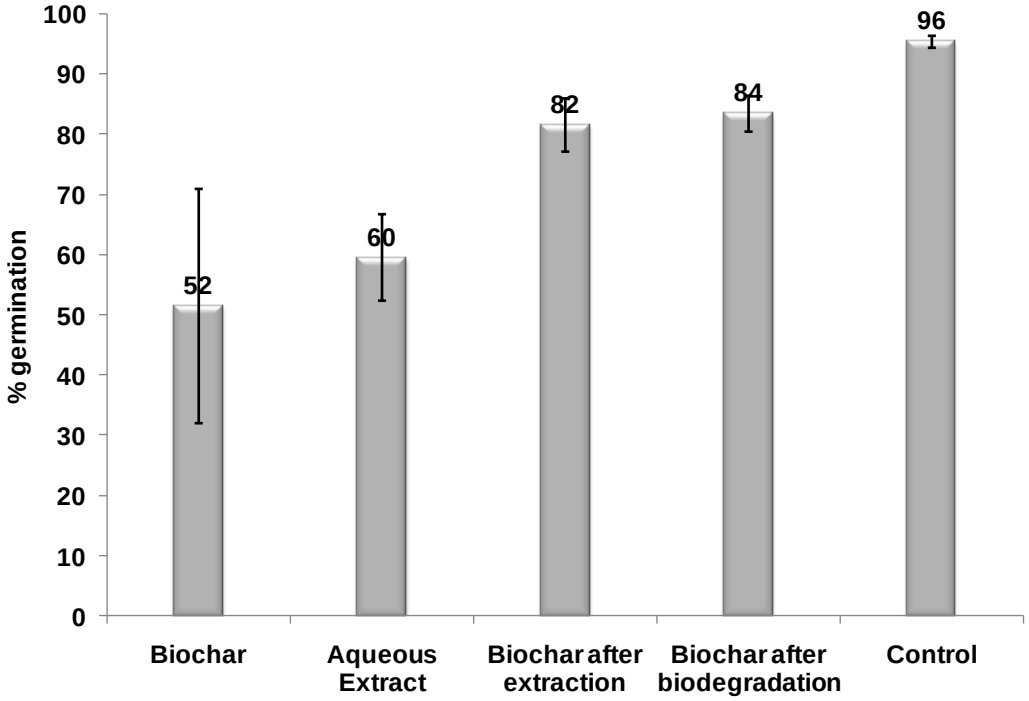
Table 4.1.4. Relative seed germination (% of control) of cress (*Lepidium sativum* L.) in biochar:deionized water suspensions (2, 5, 40 g l^{-1}) and phytoxicity tests (% seed germination with respect to control) according to UNI 11357:2010 (mean values and s.d. from four replicates). The percent seed germination in pure deionised water (control) is also reported.

Biochar Id.	**Germination (%)**			
	2 g l^{-1}	**5 g l^{-1}**	**40 g l^{-1}**	**UNI**
Control	96±2	97±2	98±2	94±2
CS400	98±2	93±1	96±4	81±3
CS500	97±5	98±1	96±2	41±3
CS600	95±4	95±2	96±1	14±4
Control	92±2	92±2	92±2	94±1
PL400	83±4	74±3	53±19	no germination
PL500	77±2	73±2	47±10	no germination
PL600	77±3	74±3	53±7	no germination

4.1.4. Discussion

The results showed in Figure 4.1.3 indicated that the relative seed germination of water extracts is low and comparable to that of the original biochar strongly supporting the hypothesis that the polar/ionic constituents ending up in water are responsible to the observed biochar toxicity. Similarly, Bargmann et al., 2013 applying germination tests to hydrochars from various origins demonstrated that the inhibiting effects were caused by some water soluble substances. These authors hypothesized that organic acids could be possibly responsible of the toxicity of the water extractable fractions. The potential of microbial detoxification was evidenced by Busch et al. (2013) who observed that the genotoxicity of hydrochar mixed with compost became lower than that of pure hydrochar.

Figure 4.1.3. Seed germination rates relative to control of original poultry litter biochar (PL400), water extracts and PL400 after post-treatments (solvent extraction and treatment with active sludge). Gerimantion tests referred to a biochar load of 40 g l^{-1}. The germination rate of control (water only) is reported for comparison.



Gell et al., 2011 showed that the short term phytotoxicity of biochar is dependent on the feedostock and is probably associated to ionic water soluble constituents rather than the less polar organic compounds composing tars. In accordance, the methanolic extract of biochar after water extraction exhibited a seed germination rate of 93% (not reported in figure 4.1.3) higher than that measured in the water extracts. Interestingly, among the various biochars investigated by Gell et al. (2011) those obtained from poultry biochar exhibited positive effects (radish roosh elongation) and acted in decreasing phytotoxicity of digestates. The calculated concentrations of VFAs in biochar suspensions (from data of table 4.1.2) were higher than those that may cause detrimental effects, for instance calculated PL400 VFAs at 40 g l^{-1} (374 µg g^{-1}) was higher than 252 µg g^{-1} EC50 values for plant growth (Himanen et al., 2012). Probably because different factors are governing the physiological response of VFA including pH and bio-availability (Paavola and Rintala, 2008; Himanen et al., 2012). In addition, the occurrence of aromatic acids was identified by DI-SPME along with VFAs.

These results of this study suggested that toxic compounds responsible for the toxicity of PL400 were water extractable and biodegradable. The SPME analysis of the water extracts (figure 4.1.3) evidenced that aliphatic and aromatic carboxylic acids were the dominant compounds. Py-GC-MS and HS-SPME analyses evidenced the presence of NNCs that seemingly were not partitioned into the water phase. The role of PAHs can be excluded as they are not water soluble and occurred at low, and comparable to cornstalk, levels in PL400.

Biochar has a potential as a soil amendment for improving soil quality, decrease fertilizers losses and store carbon into the soil. Nevertheless, as soil additive, the absence of phytotoxicity is the minimal requirement. Biochar from poultry litter may exert negative effect at least at the relatively high level of soil amendment (40 t ha^{-1}) due to the presence of water soluble and biodegradable components, probably derived from the thermal decomposition of proteins and lipids. However, the toxicity can drastically be reduced by means of washing with water or mixing with biologically active material. Whereas leaching (accompanied by wastewater generation) would be not an applicable option, biological treatment (e.g. composting or mixing with activated sludge) of phytotoxic biochars could be a simple and economic solution for increase the agronomic performance of biochar characterized by toxicity issues. Results obtained shows that biochar are not an "intrinsically safe" material, and every biochar (from different process and/or feedstock) has to be evaluated, checked and eventually treated before the agronomic application.

5. Application of analytical pyrolysis methods to the characterization of organic carbon in biochar

5.1. Characterisation of soil and biochar amended soil by hydropyrolysis

5.1.1. Introduction

Strategies to improve soil quality and increase the soil organic carbon (SOC) in agricultural soils receive a lot of attention. SOC is an important soil constituent influencing soil and water quality, farming practices and ultimately food production (Bruce et al., 1998). Besides its significance to soil quality and food production, soil carbon pool plays an important role in the overall global carbon budget.

A possible way to increase SOC content is to add biochar to soil (Lehmann et al., 2006; Lehmann, 2007). Biochar is carbonaceous product of biomass pyrolysis which attracts research interest due to its potential value for long-term carbon sequestration with additional agronomic benefits. The application of biochar to soil has been proposed for increasing the SOC and restraining the growth of atmospheric CO_2 concentration (Lehmann, 2007). Although our understanding of biochar stability has improved in recent years (Ameloot et al., 2013), there is limited research on the effects of biochar on native SOC and biochar carbon stability in soils in environmental conditions over a longer time-scale. It is well known that a variable component of biochar is labile (degradable on annual/decadal timescales) and hence, only a proportion of total carbon in biochar provides long-term carbon sequestration.

Actually, an increasing number of observations suggests that biochar can be degraded, by both biotic and abiotic processes (Hamer et al., 2004; Cheng et al., 2008; Guggenberger et al., 2008). However, in most of the studies the stability of biochar was assessed during laboratory incubations, with fresh biochars added to soil (Zavalloni et al., 2011; Ameloot et al. 2013). The duration of these experiments ranges from several weeks (Cross and Sohi, 2011) to several years (Kuzyakov et al., 2009 and Kuzyakov et al., 2014), allowing to understand biochar stability under controlled laboratory conditions. Moreover, in recent studies various analytical techniques have been applied to investigate stability of biochar (De la Rosa et al., 2008; Kaal et al., 2008, 2009; McBeath and Smernik, 2009; Michel et al., 2009; Conti et al., 2014).

However, there are only few studies estimating biochar degradation rates in soil (Kuzyakov et al., 2009; Hilscher and Knicker, 2011) and the long-term stability of biochar

in soils. This is because the changes of biochar content are too small for any practical experimental period. Many studies estimating the decomposition rates of biochar in soil are based on changes of CO_2 efflux after biochar application. This approach is unsuitable to estimate biochar decomposition because of the much higher contribution of soil organic matter and plant residues mineralization of the CO_2 compared to biochar.

Therefore, our study addresses the separation and determination of labile and resistant carbon fractions in soils and biochar amended soils for the quantification of stabile fraction (black carbon). An emerging pyrolytic approach isolating and quantifying BC in soils and chars is hydropyrolysis combined with GC-MS. This analytical method has not yet been applied to biochar amended soils in long term studies.

HyPy is pyrolysis assisted by high hydrogen pressures (150 bar) in presence of a dispersed sulphided molybdenum catalyst. Application of HyPy to sediments, soils or organic matter results in the reductive removal of all labile organic matter (defined as non-BC_{HyPy}) (Wuster et al., 2012), so isolating a highly stable portion of the BC (BC_{HyPy}) that is predominantly composed of >7 ring aromatic domains (Meredith et al., 2012). The high hydrogen pressure and slow heating rate employed, together with the presence of a sulphided molybdenum catalyst, prevent the generation of secondary char (Love et al., 1995) that is encountered with other chemical or thermal oxidative methods. In general, HyPy offers a potential mean to discriminate between bound and adsorbed organic species. As a result, the technique has been used to remove adsorbed products, facilitating analysis of organic carbon in samples (Brocks et al., 2003).

It was also observed that HyPy appeared able to discriminate between relatively labile biochars reporting low BC_{HyPy} values and more refractory, high-BC_{HyPy} soot in pure samples, and between environmental samples from industrial sites with BC predominantly derived from combustion of fossil fuels and agricultural sites dominated by the burning of vegetation (Meredith et al., 2012).

The aim of the study consists of the identification of the BC and characterization of the labile organic carbon in biochar amended soils in a four years field study. Here, for the first time, we present the molecular composition of labile fraction of soil with biochar by HyPy.

5.1.2. Materials and methods

5.1.2.1. Soils collection and incubation experiment

The field experiment was setup in a vineyard at the "Marchesi Antinori - La Braccesca Estate", Montepulciano, Tuscany, Italy (43°10′15″ N, 11°57′43″ E). A randomized plot experiment, with three treatments and five replicates, was setup in 2009. Each plot, 15 in total, had a surface area of 225 m^2 (7.5 m in width and 30 m in length) including 4 vineyard rows and 3 inter-rows. The treatments were: two biochar applications at a rate of 22 t ha^{-1} each, in 2009 and 2010 (BB); and a control (C). Biochar was applied in the inter-row space of the vineyard using a spreader and it was incorporated into the soil using a chisel plow tiller at 0.15 m depth.

Untreated soil (control) and soil treated by biochar amendment were sampled four times from 2011 to 2013 (August 2011, December 2011, May 2012 and May 2013). Forty samples (5 replicates x 2 treatments x 4 sampling seasons) were examined, each sample was dried at 40 °C, sieved (mesh size: 2 mm) in order to obtain homogeneous samples free of stones, larger roots and other coarse fragments, and stored at - 20 °C.

5.1.2.2. Soils, biochar amended soils and biochar characterization

The soil is a sandy-clay-loam (USDA, 2005) from the 0−30 cm horizon of the vineyard. It was air-dried (72 h) and then sieved (2 mm). The contents of carbon, nitrogen, hydrogen and sulfur of soil and biochar amended soil were determined by an elemental analyzer (Thermo Scientific, FLASH 2000 Series). As the carbonate concentration of the soils was negligible, the total measured C concentration was considered to represent TOC. The pH, was measured potentiometrically in a 1:2.5 soil–water suspension. The CEC analysis was performed by saturation with barium–chloride at pH 8.2, displacement of adsorbed barium by 0.05 M $MgSO_4$ and titration of the Mg remaining in solution with 0.025M EDTA (Gessa and Ciavatta, 2000). The texture of the vineyard soil was composed of 15% clay, 15% silt, and 70% sand.

The biochar used in the experiment is a commercial charcoal provided by "Romagna Carbone s.n.c." (Italy) obtained from orchard pruning biomass through a slow pyrolisis process at temperature of 500 °C in a transportable ring kiln of 2.2 m in diameter and

holding around 2 t of feedstock. The biochar at the end of the pyrolisis was crushed into particles smaller than 5 cm of diameter before the soil application.

The contents of carbon, nitrogen, hydrogen and sulfur of biochar were determined by combustion using a Thermo Scientific FLASH 2000 Series CHNS/O Elemental Analyzer. Ash content of the biochar was measured by heating samples in a muffle at 550 °C for 6 hours, as proposed by ANPA (2001). The oxygen content was calculated from mass balance: %O=100-% (C+H+N+ash). The pH of biochar was measured (1:10 wt/wt ratio of biochar with deionized water) by a digital pH meter (HI 98103, Checker®, Hanna Instruments) at room temperature. Prior to this analyses, biochar was sieved at 2 mm and oven dried at 40 °C for 72 h.

5.1.2.3. Hydropyrolysis

Hydropyrolysis (HyPy) tests were performed using the procedure described in detail in a number of publications (e.g. by Ascough et al., 2009; Meredith et al., 2012). Briefly, 50-100 mg of biochar sample and 3-4 g of biochar amended soil were loaded with a Mo catalyst using an aqueous/methanol 0.2 M solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$]. Catalyst weight was ~ 5% of the sample weight for soil and biochar amended soil, ~ 10% for biochar. The catalyst loaded biochar samples were placed within shortened borosilicate pipette ends (20 mm long), plugged at each end with pre-cleaned quartz wool and then placed in the HyPy reactor. The catalyst loaded soil and biochar amended soil samples instead were placed directly in the reactor with steel wool on the bottom. We used the recommended temperature program previously optimized for pyrogenic carbon quantification where the samples are heated at rate of 300°C min^{-1} from 50 to 250°C, then heated at 8 °C min^{-1} from 250°C until the final temperature of 550°C for 2 min (Ascough et al., 2009; Meredith et al., 2012), all under a hydrogen pressure of 15 MPa. A hydrogen sweep gas flow of 5 L min^{-1}, measured at ambient temperature and pressure, ensured that the products were quickly removed from the reactor vessel, and subsequently trapped in a silica gel-filled trap cooled by dry ice.

5.1.2.4 Black carbon quantification

The BC (reported as BC_{HyPy}) content of each sample was derived by comparing the organic carbon (OC) content of the catalyst loaded samples prior to HyPy with those of

their HyPy residues (Eq. (1)). Elemental composition (HCNS) was determined by combustion using a Thermo Scientific Flash 2000 series analyzer.

$$BC_{HyPy}\ (BC{=}OC\%) = \frac{\text{Residual OC (mg C in HyPy residue including spent catalyst)}}{\text{Initial OC (mg C in sample including catalyst)}} \text{ x } 100$$

As the carbonate concentration of the soils was negligible, the total measured C concentration was considered to represent total organic carbon (TOC). The TOC content was determined on 5 samples of biochar amended soil and 5 control soil according to the Ministero per le Politiche Agricole (1999), Method VII.1. Samples were pre-treated with HCl 1.5 M (40 uL in 2-3 g of sample), heated at 60 °C for 1 hour; this procedure was repeated for 4-5 times, till the samples stop reacting with HCl. Determinations were made using a Thermo Scientific FLASH 2000 Series CHNS/O Elementar Analyzer.

5.1.2.5. Non-BC_{HyPy} fraction characterisation

The non-BC_{HyPy} fraction (hydropyrolysate) from the soil, biochar amended soil and biochar samples were desorbed from the silica recovered from the trap with 10 ml aliquots of n-hexane and dichloromethane (DCM). The eluents were evaporated to 1 ml at room temperature for 12 h prior to analysis. GC–MS analyses in full scan mode (m/z 35–650) were performed on 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer (EI mode, 70 eV), equipped with an autosampler and a split/splitless injector. Analytes were separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95%dimethyl]siloxane, 30 m × 0.25 mm i.d., 0.25 mm film thickness), using helium as the carrier gas, and an oven programme of 50°C (hold for 2 min) to 300°C (hold for 33 min) at 5°C min^{-1}. Samples (1 µl) were injected under splitless conditions (1 min, then split ratio 1:50 to the end of analysis) with an injector temperature of 280°C. The abundance of the individual *n*-alkanes were quantified from the m/z 57 mass chromatograms, and for the PAHs the mass chromatograms of the molecular ion of each compound was used, following the addition of 100 µl of hexatriacontane (100 mg l^{-1}, Sigma-Aldrich) and 100 µl of 1,3,5-tri-tert-butylbenzene (TTB, 100 mg l^{-1} Sigma-Aldrich) respectively as internal standards, assuming a response factor for each compound of 1.

The PAHs were identified by matching the retention times of each peak in the sample chromatogram with those of a standard solution. Interfering coelution problems were evaluated in the samples by comparing mass spectra of the samples with those of the standards as well as with those from the NIST mass spectra library (NIST MS Search r. 2.0).

5.1.2.6. Statistical analysis

Quantitative data are presented as mean values ± standard deviation (n = 5). An analysis of variance (ANOVA) was undertaken to determine significant difference between control and soil with biochar. A significant difference was statistically considered at level of $p < 0.05$.

5.1.3. Results and discussions

5.1.3.1. Properties of the soil and biochar

The study was carried out using an agricultural soil classified as sandy-clay-loam (USDA, 2005) textured with 70% sand, 15% silt and 15% clay. The soil characteristics were as follows: pH 5.37, total C 0.77%, total N 0.24%, total H 0.43%, and cation exchange capacity of 12.1 meq 100 g^{-1}

Results of biochar characterizations are reported in Table 5.1.1. The biochar used for soil amendment had a total content of C, N, H, and O of 71.4%, 0.7%, 1.5%, 5.9%, respectively, an ash content of 19.9% and a pH of 9.8 (Table 5.1.1). The biochar had a molar H/C ratio of 0.26 and molar O/C ratio of 0.06, indicating a comparably high aromaticity of the biochar carbon (Zimmerman et al., 2013). All the US EPA PAHs were detected in the utilised biochar and summed up to 3.5 μg g^{-1}, with naphthalene as the most abundant species followed by phenanthrene. With this concentration would pass current quality standards by the European Biochar Certificate (2013, 12 μg g^{-1}) and the International Biochar Initiative (2012, 20 μg g^{-1}). Additional details about the physicochemical properties of the biochar are presented in Baronti et al. (2014).

Table 5.1.1. Chemical characteristics of biochar applied in the field experiment.

	Value
C (%)	71.4
H (%)	1.54
N (%)	0.72
S (%)	0.59
O (%)	5.9
H/C (molar)	0.26
O/C (molar)	0.06
Ash	19.9
pH	9.8
Charred (%)	97.6

5.1.3.2 Stable carbon fraction

It is important to determine how much of the carbon contained in biochar is potentially stable over long periods of time as there are likely to be various fractions, differing in their stability, ranging from very unstable (labile) fractions to very recalcitrant (stable) fractions. The HyPy method has been demonstrated to remove almost all labile organic carbon, leaving a residue of highly stable with the average ring structures greater than 7 fused rings (Meredith et al., 2012; Wuster et al., 2012). The low molecular weight non-BC_{HyPy} that are removed by HyPy along with any other residual labile organic compounds are unlikely to be stable on centennial timescales due to their susceptibility to biological and chemical oxidation (Ascough et al., 2008).

We used the HyPy to quantify the effect of biochar addiction in soils on the level of BC. Obviously, the effect of biochar addition in soils on the level of BC will depend on the background level of BC in the soil before treatment, the BC in the original biochar, and the quantity of added biochar. Then, environmental processes (evaporation, biodegradation, or abiotic degradation) will affect the fate and levels of BC in amended soil.

The high BC_{HyPy} value found for biochar sample (83±3.3%) and the very high stability of this fraction under HyPy conditions (17±1.2%) seen in this study suggest that it is

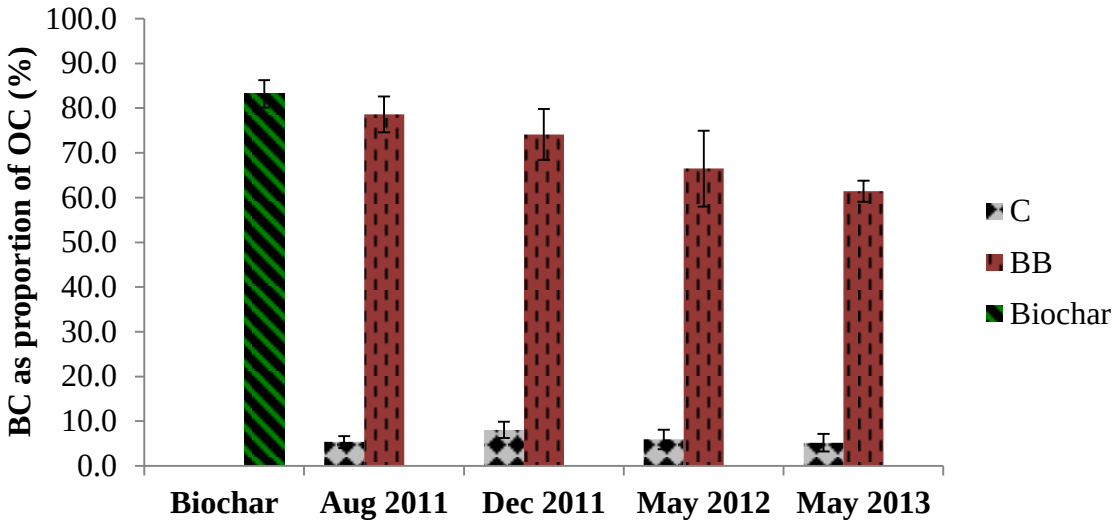
composed predominantly of soot-derived BC and that carbon added with biochar should be quite recalcitrant to degradation.

Table 5.1.2. Weight loss (%) of the soil and biochar amended soil during HyPy treatment.

Sample	Weight loss (%)			
	Aug 2011	**Dec 2011**	**May 2012**	**May 2013**
Biochar amended soil	6.2±0.7	6.9±0.4	5.8±0.8	7.7±1.2
Soil	7.0±0.8	7.1±0.5	7.4±0.3	6.6±0.4

Mass losses during HyPy of the samples were c. 7.7–5.8% w/w and no difference was found observed between biochar amended soil and soil without biochar (Table 5.1.2). On the contrary, the results show a significant difference in BC_{HyPy} concentration during four years of biochar experiment (Fig. 5.1.1). The BC_{HyPy} concentration in amended soils, one year after the application in August 2011, was approximately 20 times higher than the control soil (79% ± 4.0% vs. 5.4% ± 1.3%). For the soil samples the BC_{HyPy} content is 6.1% (±2.1%), which is comparable with range of BC contents reported by Hammes et al. (2007) for a sand-rich soil (Chernozem) and a clay-rich soil (Vertisol). The carbon present in control without biochar was very labile during HyPy, with complete conversion apparent at 550°C, and therefore a BC_{HyPy} content very low (BC/OC = 5.2-8.0%). The low BC_{HyPy} reported for soil samples suggests that is composed predominantly of lignocellulosic material and humic acids.

Figure 5.1.1. Black carbon (BC) as proportion of organic carbon (OC) as measured by HyPy of the soil (C), biochar amended soil (BB) and biochar.



The BC_{HyPy} concentration in biochar amended soils in the 4-year study ranged between 79% ± 4.0% and 61% ± 2.4% (Fig. 5.1.1) The change BC_{HyPy} as a function of time for the biochar amended soils and reference soils without biochar are presented in Fig. 5.1.1. It is interesting to note that the labile carbon during the first year was 21% and that really 21% and 24% of total organic carbon has been lost after 2 e 3 years, respectively (Fig. 5.1.1). However, table 5.1.2 shows that in biochar amended soil the BC_{HyPy} decreased during the three years following biochar application. The BC_{HyPy} loss during the experiment was 13 ± 8.5% after 2 years and 18 ± 2.4% after 3 years comparing with the first year value. Therefore, one possible explanation for the organic carbon decrease is the organic degradation combined with the leaching of a small part of the BC_{HyPy} fractions in the biochar amended soil after four years of weathering.

5.1.3.3. Labile fraction

The non-stable fraction is also very important and, in addition to the quantification of the BC_{HyPy} fraction, HyPy also allows the molecular characterisation of the labile fraction defined as non-BC_{HyPy} fraction. The labile fraction is the part of the biochar that during its storage in soil is released by predominantly microbial activity within the first few weeks or months after the application of biochar (Masek et al., 2013). Therefore, the labile fraction that evolves from biochar during its storage in soil is highly likely to impact on microbial

activity, and therefore affects the functioning of the soil as a whole. Some studies have examined the non-BC_{HyPy} fraction from soils (Meredith et al., 2013). These fractions are likely to be dominated by products of decomposition of labile organic matter such as lignocellulosic material and humic acids. However, there are not researches that identifie labile organic compounds in biochar amended soils. Moreover, the HyPy allows to characterize these materials with better preservation than typically encountered with more traditional pyrolytic methods.

5.1.3.4 Aromatic hydrocarbon non-BC_{HyPy} fraction

As well as isolating the BC_{HyPy} fraction, HyPy also allows the characterisation of the non-BC_{HyPy} material at a molecular level by GC-MS analysis. The remaining material that was labile under HyPy conditions, as so is defined as non-BC_{HyPy} can be recovered and characterised. The mass chromatograms of the non-BC_{HyPy} fraction (Fig. 5.1.2) derived from soil and biochar amended soil contain a high abundance of PAHs, predominantly fluoranthene and pyrene. The PAHs released and trapped following HyPy treatment can be considered as part of the BC continuum. Their presence in the non-BC_{HyPy} fraction will be due to their greater volatility relative to the larger more condensed and refractory aromatic domains which form the BC_{HyPy}.

Fig. 5.1.2. Total ion chromatograms from HyPy of biochar amended soil and soil (non-BC_{HyPy} fraction).

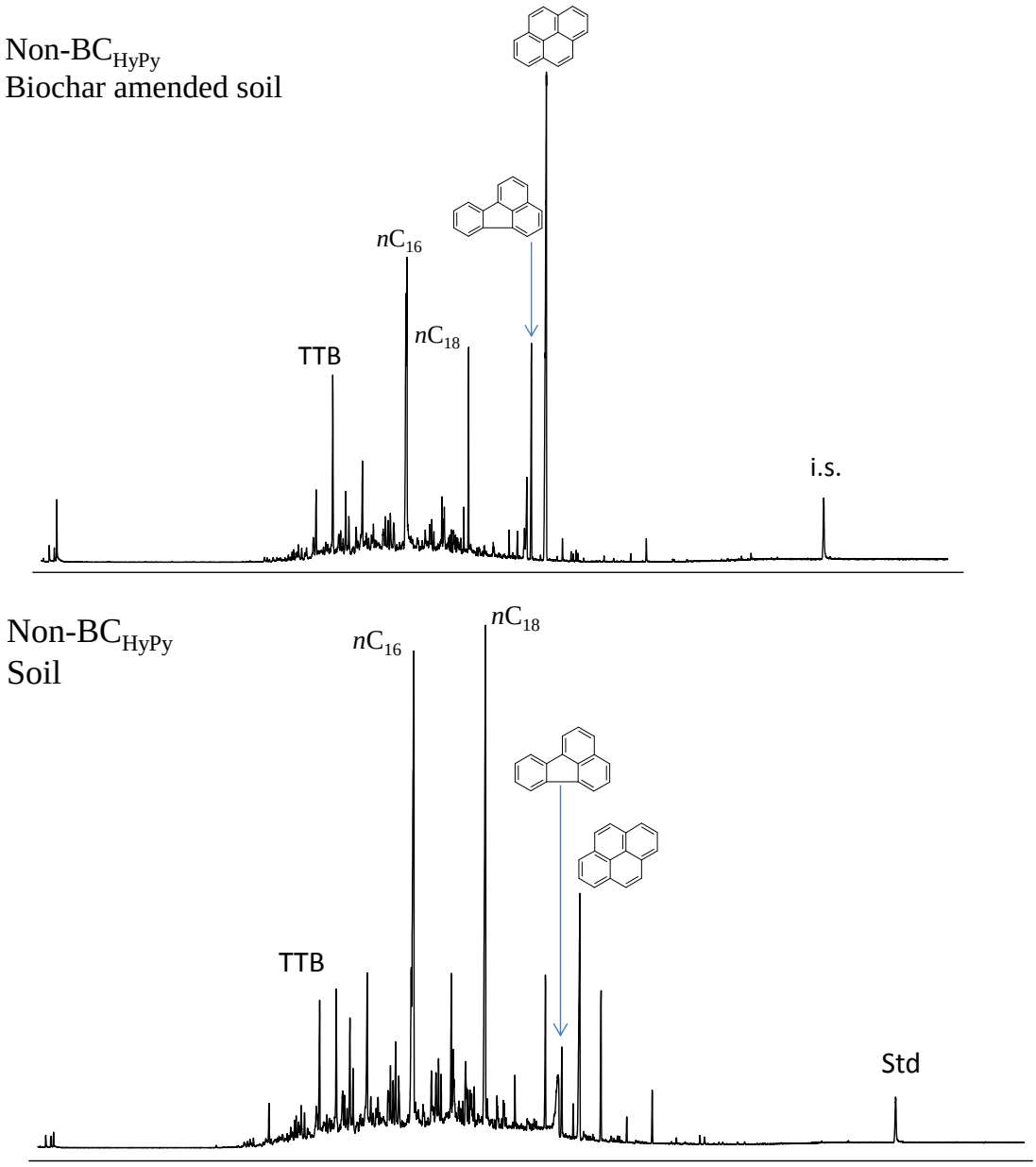


Table 5.1.3. Concentration of PAHs (μg g^{-1}) released by the HyPy of biochar amended soil.

	Biochar amended soil			
PAHs	**Aug 2011**	**Dec 2011**	**May 2012**	**May 2013**
Naphthalene	0.15±0.32	n.d.	n.d.	n.d.
Acenaphthene	0.33±0.32	n.d.	n.d.	n.d.
Fluorene	0.13±0.06	0.10±0.13	0.09±0.02	0.08±0.12
Phenanthrene	1.4±1.1	1.7±1.7	0.89±0.68	0.91±0.42
Anthracene	0.05±0.03	0.05±0.02	0.08±0.03	0.14±0.07
Fluoranthene	3.0±1.5	3.3±1.4	2.9±0.9	2.4±1.1
Pyrene	33.2±17.6	36.3±15.5	38.0±14.3	30.3±12.8
Chrysene	n.d.	n.d.	n.d.	n.d.
Benzo[*a*]anthracene	n.d.	n.d.	n.d.	n.d.
Methylchrysene	0.43±0.12	0.56±0.34	0.55±0.39	0.43±0.06
Benzo[*b*]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*k]*fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*a*]pyrene	n.d.	n.d.	0.02±0.04	n.d.
Indeno[1,2,3-cd]pyrene	n.d.	n.d.	0.15±0.33	n.d.
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	n.d.
Benzo[ghi]perylene	0.29±0.25	0.40±0.37	0.30±0.21	0.18±0.23
Total PAHs	38.9±20.5	42.4±19.3	42.9±16.4	34.5±14.3

Notes: Values in the tables are the mean value ± standard deviation from five replicates.

Table 5.1.4. Concentration of PAHs (μg g^{-1}) released by the HyPy of soil.

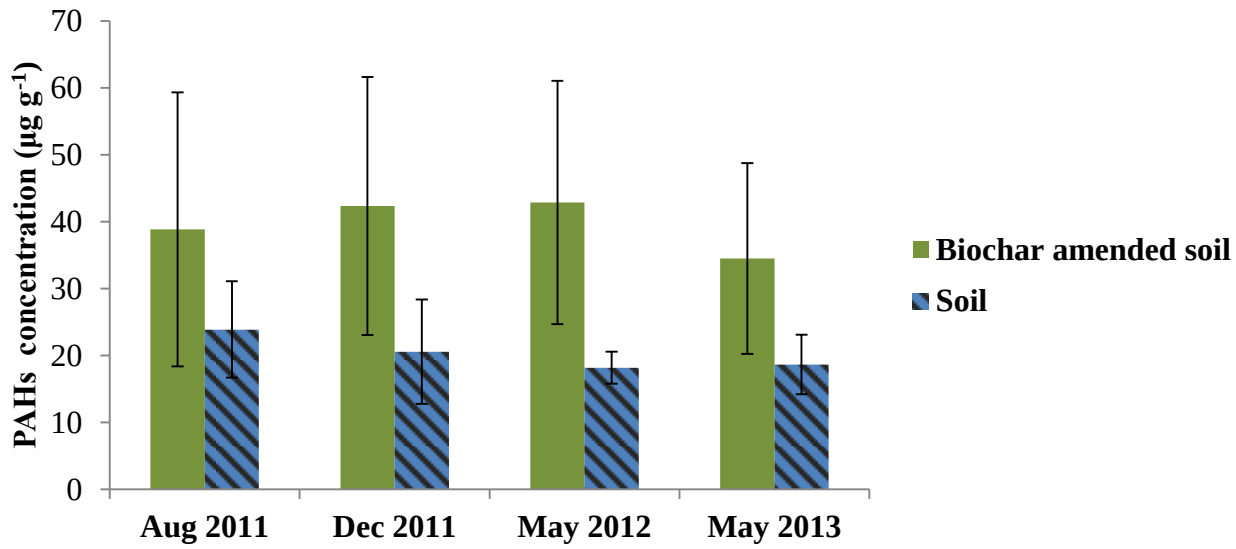
PAHs	Soil			
	Aug 2011	**Dec 2011**	**May 2012**	**May 2013**
Naphthalene	n.d.	n.d.	n.d.	n.d.
Acenaphthene	n.d.	n.d.	n.d.	n.d.
Fluorene	0.13±0.02	0.13±0.05	n.d.	n.d.
Phenanthrene	0.75±0.17	0.44±0.13	0.27±0.07	0.22±0.10
Anthracene	0.09±0.05	0.09±0.08	0.05±0.01	0.06±0.02
Fluoranthene	1.84±0.53	1.57±0.62	1.55±0.18	1.51±0.32
Pyrene	20.5±6.3	17.5±6.6	15.9±2.2	16.7±4.2
Chrysene	n.d.	n.d.	n.d.	n.d.
Benzo[a]anthracene	n.d.	n.d.	n.d.	n.d.
Methylchrysene	0.27±0.11	0.39±0.21	0.30±0.04	0.13±0.08
Benzo[*b*]fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*k]*fluoranthene	n.d.	n.d.	n.d.	n.d.
Benzo[*a*]pyrene	n.d.	n.d.	n.d.	n.d.
Indeno[1,2,3-cd]pyrene	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	n.d.
Benzo[ghi]perylene	0.17±0.15	0.18±0.08	0.11±0.09	n.d.
Total PAHs	23.9±7.2	20.6±7.8	18.2±2.4	18.7±4.4

Notes: Values in the tables are the mean value ± standard deviation from five replicates.

The PAHs detected and quantified in amended and untreated soils ranged from 2-ring compounds (naphthalene) to 6-ring compounds (benzo[*ghi*]perylene), with the 4-ring compound pyrene being the most abundant (Table 5.1.3 and Table 5.1.4). In almost all of the soils, fluorene, phenanthrene, anthracene, fluoranthene and pyrene were detected. This range of ring size is consistent with the PAHs distribution found in the non-BC_{HyPy} fraction generated by the HyPy of soil samples (Meredith et al., 2013), and the definition of BC_{HyPy} as being composed of PAHs with >7 rings proposed by Meredith et al. (2013). However, in Meredith et al. (2013) has been reported a bigger average ring distribution.

We found that biochar amendment influence soil PAHs concentration during the incubation period (Fig. 5.1.3). The total mean concentration value of PAHs in the amended soils resulted higher than those of untreated soils during the 4 years of experiment (Fig. 5.1.3). The total PAHs concentration in untreated soils ranged between 10.4 and 31.9 µg g^{-1} and in biochar amended soils between 16.1 and 75.2 µg g^{-1} (Table 5.1.3 and Table 5.1.4). In addition, in the amended soils the concentrations of the PAHs did not decrease during the four years following biochar application (Fig. 5.1.3). However, the differences were not statistically significant due to the high dispersion of PAH values between samples withdrawn from the same parcel (n = 5) (Table 5.1.3).

Figure 5.1.3. Concentration of PAHs (µg g^{-1}) released by the HyPy of soil and biochar amended soil



A detailed analysis of the contribution of the individual PAHs in biochar not subject to environmental degradation and biochar amended soil indicated a similar distribution profile in the non-BC_{HyPy} fraction, with dominance of fluoranthene and pyrene in all the samples studied (Table 5.1.3 and Table 5.1.5). However, also in the control soils, fluoranthene and pyrene dominated the PAH profiles, supplying 7.6 ± 2% and 85 ± 8% of the total PAH concentrations, respectively. Therefore, the distribution profiles of PAHs, which comprise approximately 5-10% by weight of the non-BC_{HyPy}, do not reflect biochar modification.

Moreover, these long term field trials did not allowed to observe systematic changes in the PAH distribution of the non-BC_{HyPy} fractions. Four years after the addition of biochar

to agricultural soils, the PAHs not were degraded and the distribution profile is the same during the 4 years of experiment.

Table 5.1.5. Observed concentration of PAHs released by the HyPy of biochar and a corresponding biochar amended soil (2.22% (w/w) of reference biochar).

PAHs	**Biochar ($\mu g\ g^{-1}$)**	**[a]Soil + biochar ($\mu g\ g^{-1}$)**
Naphthalene	n.d.	n.d.
Acenaphthene	4.7±0.6	0.24
Fluorene	17±3.5	0.30
Phenanthrene	419±79.0	5.45
Anthracene	10±1.7	0.19
Fluoranthene	149±23.3	3.41
Pyrene	468±110	23.28
Chrysene	n.d.	n.d.
Benzo[a]anthracene	n.d.	n.d.
Methylchrysene	7.0±1.6	0.35
Benzo[*b*]fluoranthene	n.d.	n.d.
Benzo[*k]*fluoranthene	n.d.	n.d.
Benzo[*a*]pyrene	n.d.	n.d.
Indeno[1,2,3-cd]pyrene	n.d.	n.d.
Dibenzo[a,h]anthracene	n.d.	n.d.
Benzo[ghi]perylene	n.d.	0.13
Total PAHs	1075±217	33.2

Notes: Values in the tables are the mean value ±1 standard deviation from five replicates. The last column reports the relative percent difference between the measured and expected value. [a]The expected value is the concentration calculated from the PAH concentration obtained by summing the soil and biochar contributions.

5.1.3.Aliphatic hydrocarbons non-BC_{HyPy} fraction

Fig. 5.1.2. shows mass chromatograms of the non-BC_{HyPy} fraction derived from soil and biochar amended soil, which was found to contain, in addition to PAHs, a high abundance of *n*-alkanes, predominantly even numbered homologs. The *n*-alkanes comprise approximately 10-20% by weight of the non-BC_{HyPy} fraction.

The concentrations and diagnostic indices of *n*-alkanes in non-BC_{HyPy} fraction of the soil and biochar amended soil are presented in Table 5.1.6. and Table 5.1.7. The labile fraction generated by the HyPy of soil (Fig. 5.1.4.) and biochar amended soil (Fig. 5.1.5) is dominated by *n*-alkanes in the range nC_{13} to nC_{27} (the low carbon number compounds having been lost to evaporation), with a distribution having maxima at nC_{16} to nC_{18}, and a even/odd predominance (C_{14} to C_{26} homologues CPI = 0.21 soil, CPI = 0.23 amended soil). The total concentrations of *n*-alkanes in soil were higher than ones in the biochar amended soil during the 4 years of experiment (Fig. 5.1.6). In untreated soils concentrations of *n*-alkanes (nC_{13} to nC_{27}) ranged from 64.2 µg g^{-1} to 130.7 µg g^{-1} and in amended soils from 18.2 µg g^{-1} to 72.9 µg g^{-1} (Table 5.1.6. and Table 5.1.7). After almost one year following biochar application, the total mean concentration values of *n*-alkanes in biochar amended soils was approximately 3 times higher than the control soil, both in August (37.2 vs. 106.7 µg g^{-1}) and December (44.4 vs. 102.6 µg g^{-1}) (Fig. 5.1.6).

Figure 5.1.4. Soil GC-MS mass chromatograms. Reconstructed ion chromatogram m/z 57 showing the n-alkane distribution present in the non-BC_{HyPy} fraction.

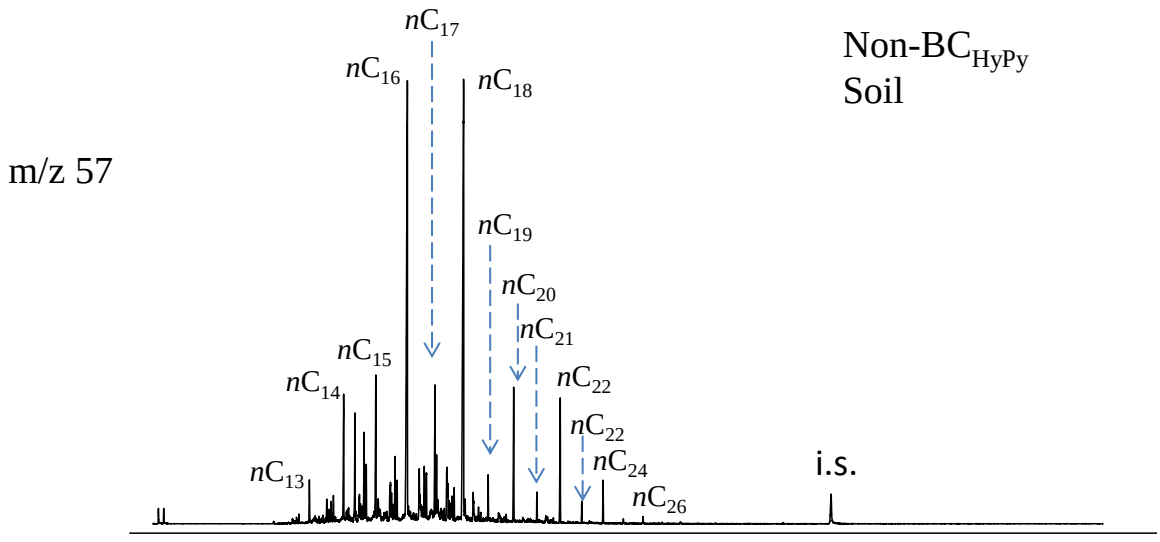


Fig. 5.1.5. Biochar amended soil GC-MS mass chromatograms Reconstructed ion chromatogram m/z 128 + 154 + 166 + 178 + 202 + 231 + 242 + 276 showing the major PAHs present in thenon-BC_{HyPy} fraction.

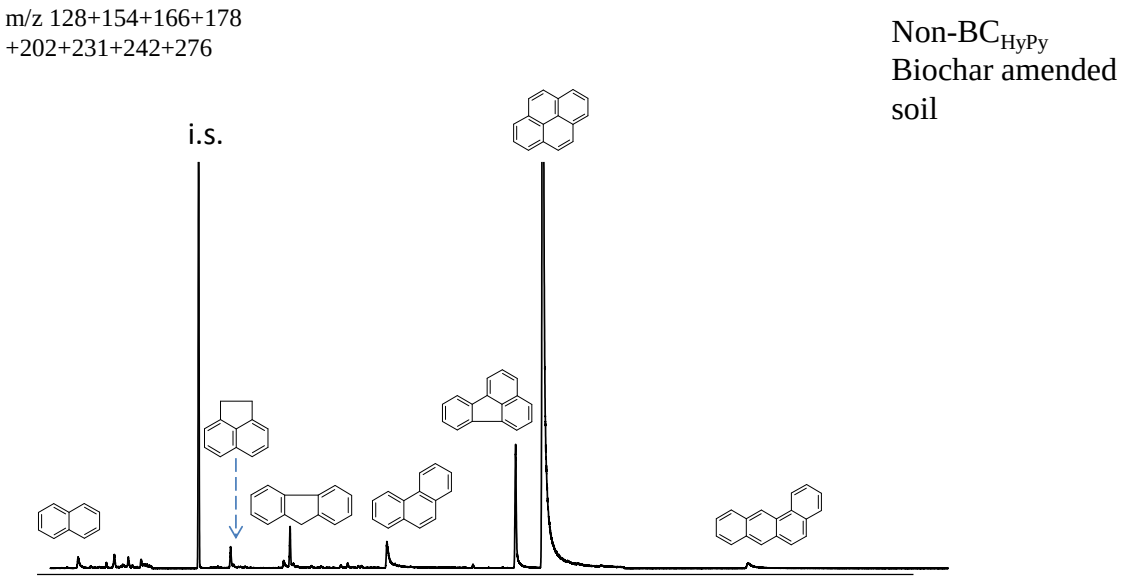
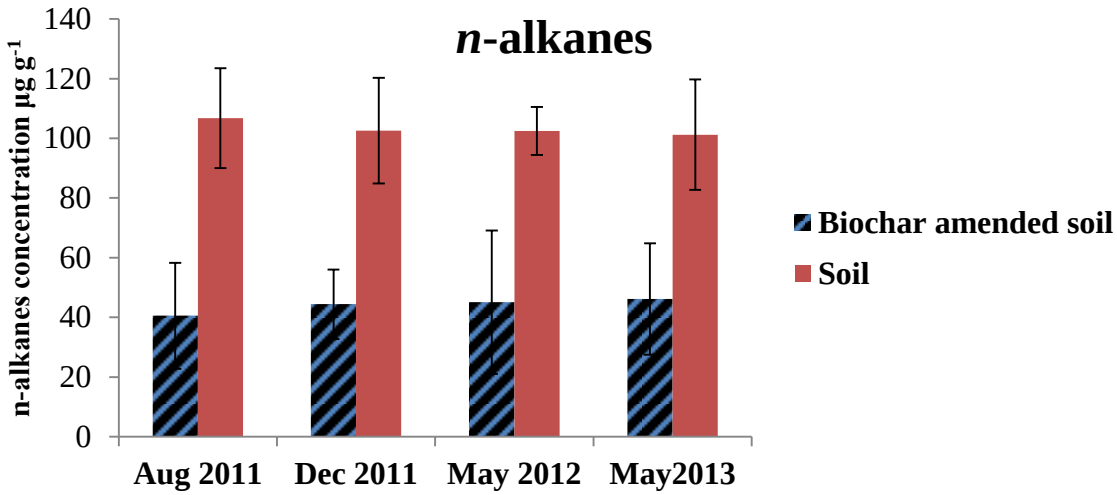


Figure 5.1.6. Concentration of *n*-alkanes (µg g^{-1}) released by the HyPy of soil and biochar amended soil.



The characteristic relative distribution of *n*-alkanes in soil (Fig. 5.1.4) is not affected by biochar modification. The effect of biochar on soil reduces the *n*-alkanes concentrations, but both biochar amended soils and untreated soils are dominated by the same aliphatic compounds. In particular, *n*-alkanes are characterized by a higher abundance of medium chain homologues (nC_{16}, nC_{18}, nC_{20}, nC_{22} and nC_{24}) and are dominated by nC_{16} and nC_{18}.

The Carbon Preference Indices (CPI) which expresses the ratio of odd-carbon numbered to even-carbon-numbered n-alkanes is useful to determine the degree of biogenic versus petrogenic input (Simoneit and Mazurek, 1982; Simoneit, 1989; Zheng et al., 2000; Young, 2002). (Mazurek and Simoneit, 1984). *n*-alkanes originate from epicuticular waxes of terrestrial plants and exhibit high values of CPI (CPI >1), whereas CPI values for vehicular emissions and other anthropogenic activities are close to unit (CPI ~1).

The calculated CPI values for soil (0.21) and for biochar amended soil (0.23) are similar (Table 5.1.8) and comparable to those observed for soil dichromate residue in Meredith et al. (2013). This even numbered distribution, with a CPI of the C_{14} to C_{24} homologues of 0.21 and 0.23 indicates the importance of anthropogenic activities. These *n*-alkanes were not predominantly original constituents of the soil organic matter. The *n*-alkanes found in terrestrial soils are commonly dominated by odd-carbon-numbered *n*-alkanes in the nC_{23}–nC_{33} range derived from the epicuticular waxes of higher plants (Zelles et al., 1999). However, a more probable source is from biolipids which exhibit an even/odd preference, and typically include a high abundance of the C_{16} homologue, hexadecanoic (palmitic) acid, in the short-chained (C_{10} to C_{20}) fraction which is predominantly derived from microbial biomass. The acidic species are known to be hydrogenated under HyPy conditions to form the corresponding even-numbered *n*-alkanes (Meredith et al., 2006).

Table 5.1.6. Concentration of *n*-alkanes (µg g^{-1}) released by the HyPy of biochar amended soil.

	Biochar amended soil			
***n*-alkanes**	**Aug 2011**	**Dec 2011**	**May 2012**	**May 2013**
nC_{13}	0.52±0.26	0.003±0.002	0.003±0.001	0.024±0.003
nC_{14}	2.8±1.0	0.037±0.017	0.002±0.001	1.04±10.02
nC_{15}	3.5±0.9	1.62±0.9	0.19±0.11	2.8±1.7
nC_{16}	14±5.5	11.3±6.9	7.6±5.3	16±6.7
nC_{17}	2.8±1.2	2.9±0.9	3.4±1.6	3.2±1.1
nC_{18}	12±6.9	18±6.9	20±10	14±5.8
nC_{19}	0.78±0.38	1.4±0.5	1.9±0.9	1.1±0.4
nC_{20}	1.73±1.18	3.2±1.3	4.1±2.6	2.5±1.1
nC_{21}	0.46±0.29	0.81±0.30	1.08±0.7	0.68±0.31
nC_{22}	1.0±1.0	2.9±1.3	3.98±1.9	2.46±1.24
nC_{23}	0.28±0.21	0.60±0.37	0.71±0.55	0.51±0.28
nC_{24}	0.61±0.50	1.1±0.8	1.34±1.42	1.02±0.48
nC_{25}	0.11±0.07	0.14±0.06	0.24±0.20	0.21±0.05
nC_{26}	0.13±0.10	0.16±0.09	0.30±0.26	0.27±0.10
nC_{27}	0.05±0.03	0.17±0.07	0.16±0.15	0.17±0.13
Total nC_{13}-nC_{27}	40.5±17.8	44.4±11.6	45.1±24.0	46.1±18.6
CPI nC_{12}-nC_{24} [a]	0.26	0.20	0.23	0.20

[a] Carbon preference index (CPI) formula of Bray and Evans (1961).

Table 5.1.7. Concentration of *n*-alkanes (μg g^{-1}) released by the HyPy of soil.

	Soil			
***n*-alkanes**	**Aug 2011**	**Dec 2011**	**May 2012**	**May 2013**
nC_{13}	0.59±0.07	0.91±0.58	0.004±0.003	0.064±0.06
nC_{14}	2.6±0.95	3.1±2.1	0.025±0.014	1.1±0.7
nC_{15}	5.3±1.3	5.7±1.2	1.1±0.3	3.0±0.9
nC_{16}	30±5.6	26±2.0	18.8±0.4	25±5.1
nC_{17}	5.3±3.0	5.6±0.8	6.06±0.5	6.1±1.5
nC_{18}	34±8.9	29±3.6	36±2.4	35±11.4
nC_{19}	2.5±0.4	2.1±0.7	2.99±0.3	2.42±1.1
nC_{20}	4.2±3.6	8.0±1.4	9.78±0.8	7.2±3.3
nC_{21}	2.38±0.6	2.4±0.5	3.04±0.4	1.7±0.9
nC_{22}	10±4.2	11±3.1	14±2.3	7.6±4.4
nC_{23}	2.2±1.0	2.1±0.8	2.2±1.3	1.4±0.9
nC_{24}	4.8±2.7	4.9±2.5	6.3±1.1	2.8±1.8
nC_{25}	0.73±0.41	0.66±0.42	0.92±0.19	0.41±0.29
nC_{26}	1.11±0.72	1.01±0.65	1.27±0.3	0.56±0.38
nC_{27}	0.37±0.23	0.32±0.20	0.47±0.33	0.17±0.10
Total nC_{13}-nC_{27}	107±16.7	103±17.7	102±8.0	93.8±18.6
CPI nC_{12}-nC_{24} [a]	0.22	0.24	0.19	0.19

[a] Carbon preference index (CPI) formula of Bray and Evans (1961).

5.1.4. Conclusions

A short-term incubation study was carried out to investigate the effect of biochar addition to soil on the level of organic carbon and to study the persistence and resistance of biochar in the environment. Although the carbon added with biochar should be quite recalcitrant to degradation, it is well known that a variable component of biochar is labile (degradable on annual/decadal timescales) and hence, only a proportion of total carbon in biochar provides long-term carbon sequestration.

Previous studies reported that HyPy is potentially a precise method for BC measurements in soil, lignocellulosic material, coals, and petroleum source rocks. The results presented in this study suggest that HyPy provides a rapid and convenient technique for the quantification of BC and determination of the stable carbon in soil treated with biochar. The ability of the method to determine BC in the biochar amended soil was evaluated. The findings of this study showed that biochar amendment significantly influence soil BC concentration during the incubation period. In particular, the obtained concentrations of BC in the amended soil are significantly higher than that in the untreated soil. Obviously, the effect of biochar addition in soils on the level of BC will depend on the BC in the original biochar. The high BC_{HyPy} value found for biochar (83±3.3%) used in this study suggests that it should be quite recalcitrant to degradation.

Moreover, the HyPy allowed the characterisation on a molecular level of labile fraction defined as non-BC_{HyPy} fraction. In addition to a number of PAHs, the non-BC_{HyPy} fraction was also found to contain a significant abundance of *n*-alkanes, with a marked predominance of even-numbered homologues. These compounds are probably derived from lipids, hydrogenated during HyPy. However, further researches are need to characterise the BC_{HyPy} residues and more fully confirm that they are entirely free of non-BC material.

5.2. Characterization of biochar stability by hydropyrolysis and pyrolysis-GC/MS

5.2. Biochar stability characterization by hydropyrolysis and pyrolysis-GC/MS

5.2.1. Introduction

Biochar is carbonaceous solid formed by the pyrolysis of biomass which attracts research interest due to its potential value for long-term carbon sequestration. The addition of biochar to soil has been proposed as strategy that not only sequesters carbon in soils but also at the same time mitigates different environmental issues. Research has demonstrated that biochar has considerable potential as a sustainable tool for carbon sequestration, soil amelioration, greenhouse gas emissions reduction and fertilizer runoff reduction, as well as waste management (Glaser et al., 2002; Lehmann et al., 2009; Sohi et al., 2010; Woolf et al., 2010; Zavalloni et al., 2011; Galinato et al., 2011; Kookana et al 2011; Yao et al., 2012).

A key requirement for the use of biochar as tool for environmental management is that the carbon in the biochar is stable, meaning that a substantial fraction of the carbon sequestered is not re-mineralized on at least centennial timescales (Gurwick et al., 2013). However, a variable component of the carbon in many biochars is degradable on annual to decadal timescales and hence, only a proportion of total carbon in biochar provides long-term carbon sequestration (Bird et al., 1999; Zimmermann et al., 2012). Although our understanding of biochar carbon stability has improved in recent years (Ameloot et al., 2013), there is limited research on process conditions to produce a biochar suitable and highly stable for the long-term carbon sequestration (Conti et al., 2014; McBeath et al., 2015). The properties of biochar, including stability, depend on the type of feedstock, pyrolysis temperature and pyrolysis method (Labbe et al., 2006; Nguyen and Lehmann, 2009; Singh et al., 2012). However, biochar stability depends also on the environmental factors (temperature, rainfall, soil type) of the site where the biochar is incorporated into the soil (Czimczik and Masiello, 2007).

A number of approaches have been proposed to assess biochar stability. However, there is no agreed methodology for determining the long-term stability of biochar yet. The methods which have been proposed to assess biochar stability include solid state nuclear

magnetic resonance spectroscopy (solid state ^{13}C NMR) (McBeath and Smernik, 2009), Fourier transform infrared spectroscopy (FTIR) (Michel et al., 2009), proximate analysis using the fixed carbon as a measure of stability (ASTM Standard D3175; 2007), thermal analysis (thermogravimetry, TG; de la Rosa et al., 2008), molecular markers by means of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS; Kaal et al., 2008, 2009, Conti et al., 2014), benzene polycarboxylic acid method (Brodowski et al., 2005), O:C or H:C molar ratios (Spokas, 2010; Enders et al., 2012; IBI Guidelines, 2012) and chemical oxidation (Cross and Sohi, 2013). Many studies reported that chemical oxidants, such as $K_2Cr_2O_7$, $KMnO_4$, HNO_3, and H_2O_2, could be used to evaluate the oxidative nature of biochar and reflect the long-term stability of biochar (Trompowsky et al., 2005; Knicker et al., 2007; Calvelo Pereira et al., 2011; Li et al., 2014). In Masek et al. (2013) stable carbon in biochar was determined using an accelerated ageing assay. This assay involved the thermal and chemical oxidation of milled biochar samples. Samples were placed in 5% hydrogen peroxide and heated to 80 °C, and carbon stability then was calculated gravimetrically using the %C data of samples before and after oxidation. This approach is considered to be more representative of the degradation processes to which biochar would be subjected in the environment. The results of the accelerated ageing experiments have demonstrated for a range of biochars chemical behaviours consistent with the hypothesis that biochars produced at higher temperatures exhibit more resistance to oxidative degradation carbon (black carbon) fractions in biochar. Black carbon (BC) is an important component of organic carbon (OC), and it is defined as the carbon-rich (>60%) product of the incomplete combustion of fossil fuels and biomass (Goldberg, 1985), that includes a range of products such as char, charcoal, ash, and soot (Preston and Schmidt, 2006).

An emerging pyrolytic approach isolating and quantifying BC in a range of environmental matrices is hydropyrolysis (HyPy) combined with GC-MS (Ascough et al., 2009; Meredith et al., 2012; Wurster et al., 2013). These studies have shown that the HyPy method removes all labile organic matter (defined as non-BC_{HyPy}) (Wuster et al., 2012), so isolating a highly stable portion of the BC (BC_{HyPy}) predominantly composed of >7 ring aromatic domains (Meredith et al., 2012). The high hydrogen pressure and slow heating rate employed, together with the presence of a sulphided molybdenum catalyst, prevent the generation of secondary char (Love et al., 1995) encountered with other chemical or thermal oxidative methods. In addition to the quantification of the BC_{HyPy} fraction, HyPy

also allows the molecular characterisation of the biochar labile fraction defined as non-BC_{HyPy} fraction.

The molecular signature of the thermally labile fraction of biochar was also examined by pyrolysis-GC/MS and its association to the stability of the carbon in biochar investigated (Pereira et al., 2011; Conti et al., 2014). In particular, the GC-MS traces (pyrograms) of biochar were featured by peaks associated with benzene, toluene, naphthalene, biphenyl, dibenzofuran and benzonitrile (Kaal et al., 2009). These pyrolysis products were assumed to represent the charred fraction abundant of aromatic structures that occur in a thermally labile form in the carbonaceous matrix. In addition, pyrolysis product ratios representing the relative abundance of alkylated and parent compounds (e.g., benzene/toluene peak area ratio) were proposed as indicators for the presence of saturated alkyl bridges between polyaromatic structures and hence a measure of the charring intensity (Kaal et al., 2012). Therefore, Py-GC-MS is able to provide molecular indices of biochar stability. However, there are no studies aimed at comparing these indices to those arising from HyPy.

The aim of the present study is to assess the impact of production conditions on biochar stability, providing moreover a comparison between molecular analysis by Py-GC-MS and HyPy on biochar samples produced from three feedstock and the same pyrolysis unit. Different process conditions, charring temperature and residence time, were utilised to obtain biochars with different degrees of charring.

5.2.2. Experimental section

5.2.2.1. Samples

Three different types of biomass were used as feedstock materials: pine wood chips – with an average size of 3 cm × 2 cm × 0.5 cm – from Robeta Holz OHG, Milmersdorf, Germany; beech wood spheres – with a diameter of 25 mm –, provided by Meyer and Weigand Gmbh, Nordlingen, Germany; corn digestate derived from maize silage.

Biochar samples were produced by pyrolysis of sample using a stainless steel fixed-bed reactor of 102.5 cm height and 22 cm of internal diameter. The inert atmosphere is provided by a N_2 flow entering the reactor (20 L min^{-1} and 50 L min^{-1}) from the bottom through a stainless steel grate to get an uniformly distributed flow. The samples (in the range of kilograms) were uniformly placed inside the reactor in a stainless steel container

of 21 cm of diameter and 56 cm height which is placed directly on the previously mentioned grate. The reactor is externally heated with a wire heater with a maximum power of 3000 W placed on the external reactor wall. Both flanges in the reactor are also heated and insulated to reduce heat losses. The N_2 flow is preheated before entering the reactor as well. The temperature operation of this preheater is 600 °C.

Pyrolyses were performed at three different temperatures, 340 °C, 400 °C and 600 °C. The biochar samples obtained were labeled as PW ID 1, PW ID 2, BW ID 1, BW ID 2, CD EU 1 and CD EU 2 (where PW, BW, CD stand for pine wood, beech wood and corn digestate, respectively; 1 and 2 indicated the highest and lowest pyrolysis temperatures, respectively; ID and EU, see Table 5.2.1).

Table 5.2.1. Biomass feedstock and pyrolysis conditions of biochar samples. (# : Sample identifiers).

#	**Raw material**	**Volatiles %**	**Max T °C**	**N_2 flow L/min**
PW ID 1	Pine wood	10.30	600	20
PW ID 2	Pine wood	33.70	400	20
CD EU 1	Corn digestate	12.66	600	20
CD EU 2	Corn digestate	15.93	400	20
BW ID 1	Beech wood	9.93	600	20
BW ID 2	Beech wood	-	340	50

5.2.2.2. Biochar bulk characterization

The pH of the biochar samples was determined by adding biochar to deionized water at 1:10 wt/wt mass ratio and pH measured at room temperature with a digital pH meter (HI 98103, Checker®, Hanna Instruments). Elemental composition (HCNS) was determined by combustion using a Thermo Scientific Flash 2000 series analyzer. Ash was determined as the residual mass left after exposure at 600 °C for 5 hours. The oxygen content was calculated from the mass balance: Oxygen (%) = 100 - Ash content (%) - C (%) – H (%) - N (%). Moisture contents were determined (ASTM D-3173) at 105 °C.

5.2.2.3. Polycyclic aromatic hydrocarbons (PAHs)

The content of PAHs in biochars was measured in triplicate as described in Fabbri et al., (2013), but using 16 PAHs surrogate of each of the 16 US EPA PAHs instead of 3 PAHs surrogate. The measured PAHs included naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*ah*)anthracene, benzo(*ghi*)perylene, and indeno(1,2,3-*c,d*)pyrene.

Briefly, about 0.5 g of biochar was spiked with 0.1 mL of a 5 mg l^{-1} solution of surrogate 16 EPA PAHs (prepared from Dr. Ehrenstorfer PAH-Mix 9 deuterated, 10 ng μl^{-1}) and soxhlet extracted with acetone/cyclohexane (1:1, v/v) for 36 hours. The solution was filtered, added with 1 ml of *n*-nonane (keeper), carefully evaporated by rotatory vacuum evaporation at 40 °C and cleaned up by solid phase extraction onto a silica gel cartridge before analysis with a Agilent HP 6850 GC coupled to a Agilent HP 5975 quadrupole mass spectrometer; GC-MS conditions were those detailed in Fabbri et al., (2013). Recovery of surrogate PAHs was determined with respect to the internal standard tri-*tert*-butylbenzene. Results are reported as averages of three replicates analyses.

5.2.2.4. Pyrolysis-GC/MS

Py-GC-MS analyses were performed using an electrically heated platinum filament CDS 1000 pyroprobe valved interfaced to a Varian 3400 GC equipped with a GC column (HP-5-MS; Agilent Technologies 30 m × 0.25 mm, 0.25 μm) and a mass spectrometer (Saturn 2000 ion trap, Varian Instruments) set at an electron ionization at 70 eV in full scan acquisition (10–450 *m/z*). A quartz sample tube containing of weighed biochar sample (5-10 mg) added with 1 μL of internal standard solution (*o*-isoeugenol at 1000 mg L^{-1} in methanol) was inserted into the Py-GC interface (300 °C) and then pyrolysed at 900 °C (set temperature) for 100 s with helium as carrier gas (100 ml min^{-1}). The following thermal program was used: 35 °C to 310 °C at 5°C min^{-1}.

Yields were estimated from the ratio of the peak area integrated in the mass chromatogram of a characteristic ion of the selected pyrolysis product and the peak area of the internal standard, the quantity of added internal standard and the amount of sample pyrolysed (Torri et al., 2010). An unitary relative response factor was assumed for all the quantified compounds on the basis that our objective was the comparison between samples

on a quantitative base rather than the knowledge of the absolute yield of each pyrolysis product. Total yields were the summed yields of all the selected pyrolysis products.

A set of 38 pyrolysis products among the most abundant and representative of biological precursors was selected on the basis of a previous work (Fabbri et al., 2012) (Table 5.2.2). On each biochar sample was carried out a single analysis and the pyrolysis products were quantified both in terms of yields (μg g^{-1}) and relative abundance considering a relative standard deviation (RSD) between 8-36 % that is typical of Py-GC-MS analysis.

Table 2. Pyrolysis products of biochar, the mass to charge ratio (m/z) of the quantitation ion and their predominant origin: C, charred biomass; H holocellulose (sugars); L, lignin; P, proteins (nitrogen-containing compounds).

#	Pyrolysis product	m/z	origin	#	Pyrolysis product	m/z	origin
1	Benzene	78	C	20	2-ethylphenol	122	L
2	Hydroxyacetone	75	H	21	2,5-dimethylphenol	122	L
3	Dimethylfuran	96	H	22	2,3-dimethylphenol	122	L
4	Pyrrole	67	P	23	3-ethylphenol	122	L
5	Toluene	91	C	24	Naphthalene	128	C
6	2-Methyltiophene	98	P	25	Catechol	110	L
7	o-Xylene	91	C	26	2-methylnaphthalene	142	C
8	Fufurilic alcool	98	H	27	1-methylnaphthalene	142	C
9	m/p-Xylene	91	C	28	4-methylguaiacol	138	L
10	Styrene	104	C	29	4-vinylguaiacol	150	L
11	Ethylbenzene	91	C	30	Syringol	154	L
12	Phenol	94	L	31	Biphenyl	154	C
13	Benzofuran	118	C	32	4-ethylguaiacol	137	L
14	Benzonitrile	103	C	33	4-methylsyringol	168	L
15	Indole	117	P	34	Fluorene	164	C
16	3-methylphenol	108	L	35	Phenanthrene	178	C
17	4-methylphenol	107	L	36	Anthracene	178	C
18	Guaiacol	109	L	37	Fluoranthene	202	C
19	Methyl-benzofurans (3 isomers)	132	C	38	Pyrene	202	C

5.2.2.5. Hydropyrolisis

Hydropyrolysis (HyPy) tests were performed using the procedure described in detail in a number of publications (e.g. by Ascough et al., 2009; Meredith et al., 2012). Briefly, 50-100 mg of biochar sample were loaded with a Mo catalyst using an aqueous/methanol 0.2

M solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$]. Catalyst weight was ~ 10% of the sample weight. The catalyst loaded biochar samples were placed within shortened borosilicate pipette ends (20 mm long), plugged at each end with pre-cleaned quartz wool and then placed in the HyPy reactor. We used the recommended temperature program previously optimized for pyrogenic carbon quantification where the samples are heated at rate of 300°C min^{-1} from 50 to 250°C, then heated at 8 °C min^{-1} from 250°C until the final temperature of 550°C for 2 min (Ascough et al., 2009; Meredith et al., 2012), all under a hydrogen pressure of 15 MPa. A hydrogen sweep gas flow of 5 L min^{-1}, measured at ambient temperature and pressure, ensured that the products were quickly removed from the reactor vessel, and subsequently trapped in a silica gel-filled trap cooled by dry ice.

5.2.2.6. Black carbon quantification

The BC (reported as BC_{HyPy}) content of each sample was derived by comparing the organic carbon (OC) content of the catalyst loaded samples prior to HyPy with those of their HyPy residues (Eq. (1)). Elemental composition (HCNS) was determined by combustion using a Thermo Scientific Flash 2000 series analyzer.

$$BC_{HyPy}\ (BC{=}OC\%) = \frac{\text{Residual OC (mg C in HyPy residue including spent catalyst)}}{\text{Initial OC (mg C in sample including catalyst)}} \times 100 \qquad (1)$$

5.2.2.7. Non-BC_{HyPy} fraction characterisation

The non-BC_{HyPy} fraction (hydropyrolysate) from the soil, biochar amended soil and biochar samples were desorbed from the silica recovered from the trap with 10 ml aliquots of n-hexane and dichloromethane (DCM). The eluents were evaporated to 1 ml at room temperature for 12 h prior to analysis. GC–MS analyses in full scan mode (m/z 35-650) were performed on 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer (EI mode, 70 eV), equipped with an autosampler and a split/splitless injector. Analytes were separated by a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95%dimethyl]siloxane, 30 m × 0.25 mm i.d., 0.25 mm film thickness), using helium as the carrier gas, and an oven programme of 50°C (hold for 2 min) to 300°C (hold for 33 min) at 5°C min^{-1}. Samples (1 µl) were injected under splitless conditions (1 min, then split ratio 1:50 to the end of analysis) with an injector

temperature of 280°C. For the quantification of PAHs the mass chromatograms of the molecular ion of each compound was used, following the addition of 100 µl of 1,3,5-tri-tert-butylbenzene (TTB, 100 mg l^{-1} Sigma-Aldrich) as internal standards, assuming a response factor for each compound of 1.

The PAHs were identified by matching the retention times of each peak in the sample chromatogram with those of a standard solution. Interfering coelution problems were evaluated in the samples by comparing mass spectra of the samples with those of the standards as well as with those from the NIST mass spectra library (NIST MS Search r. 2.0).

5.2.2.8. Statistical Analysis

Quantitative data are presented as mean values ± standard deviation (n = 2). Recovery of surrogate PAHs was (mean ± %RSD for all the data set): 80% ± 6% naphthalene-d_8, 69% ± 27% acenaphthylene-d_8, 91% ± 4% acenaphthene-d_{10}, 88% ± 21% fluorene-d_{10}, 90% ± 17% phenanthrene-d_{10}, 70% ± 23% anthracene-d_{10}, 88% ± 10% fluoranthene-d_{10}, 87% ± 9% pyrene-d_{10}, 83% ± 18% chrysene-d_{12}, 84% ± 10% benzo(*a*)anthracene-d_{12}, 87% ± 11% benzo(*b*)fluoranthene-d_{12}, 79% ± 15% benzo(*k*)fluoranthene-d_{12}, 79% ± 23% benzo(*a*)pyrene-d_{12}, 75% ± 22% indeno(1,2,3-*c*,*d*)pyrene-d_{12}, 82% ± 12% dibenz(*ah*)anthracene-d_{14} and 77% ± 19% benzo(*ghi*)perylene-d_{12}. Student t tests were conducted with Excel (2011) to evaluate significant difference between two parameters of biochar. Linear (Pearson) correlation coefficient between two variables r(df), where df stands for degrees of freedom, was determined for all the investigated parameters. Two set of data were assumed to be correlated when the absolute value of r was larger than the critical value at the level of significance p = 0.01 for two-tailed test.

5.2.3. Results and discussion

5.2.3.1 Biochar characteristic

Results of biochar characterizations are reported in Tables 5.2.3 and 5.2.4. The biochars had a range of 46.4–91.6% carbon; 1.1–47.3% ash; 0.2–1.9% nitrogen; 3.8–22.9% oxygen. The biochars showed profound differences in properties, depending on feedstock and pyrolysis temperature (Table 5.2.3). Pyrolysis temperature showed significant effect on

elemental compositions of wood biochars and to a lesser extent on that of corn digestate biochars. In particular, the data showed that carbon content of wood biochar increased with temperature, while the oxygen and hydrogen contents decreased. This resulted in lower H/C and O/C atomic ratio values at increasing final temperature (Table 5.2.3).

The degree of carbonisation of chars is generally expressed by molar H/C (Calvelo Pereira et al., 2011; Enders et al., 2012) or O/C ratios (Spokas et al., 2010; Brodowski et al., 2005). The O/C ratios here investigated chars ranged from 0.04 (PW ID 1) to 0.24 (PW ID 2) in accordance to the loss of oxygenated functionalities with increasing carbonisation (Krull et al., 2009), and were strongly correlated with molar H/C ratios (R = +0.96, Table 5.2.3).

However the carbon and oxygen contents of beech and pine wood biochar was higher compared to that of corn digestate biochar. On the contrary, the corn digestate biochar showed higher ash and nitrogen contents. The differences in ash and carbon contents can be linked to the chemical composition differences between wood and corn digestate. Wood contains more cellulose and hemicelluloses and during high temperature pyrolysis (> 500 °C), the components are reduced to carbon thus the higher carbon content in wood biochar (Ahamedna et al., 2000, Keiluweit et al., 2010, Al-Wabel et al., 2013).

The ash content of biochar samples was influenced mainly by feedstock and to a lesser extent by pyrolysis temperature with ash content increasing with pyrolysis temperature. The increase in ash content should result from progressive concentration of minerals and destructive volatilization of lignocelluloses matters as temperature increased (Tsaia et al., 2012). However, the ash content of corn digestate biochar was much higher (up to 47.27%) than that in beech and pine wood (up to 1.51%).

Table 5.2.3. Elemental composition of biochar samples (# : Sample identifiers). Mean values and % relative standard deviation (rsd) from two replicates.

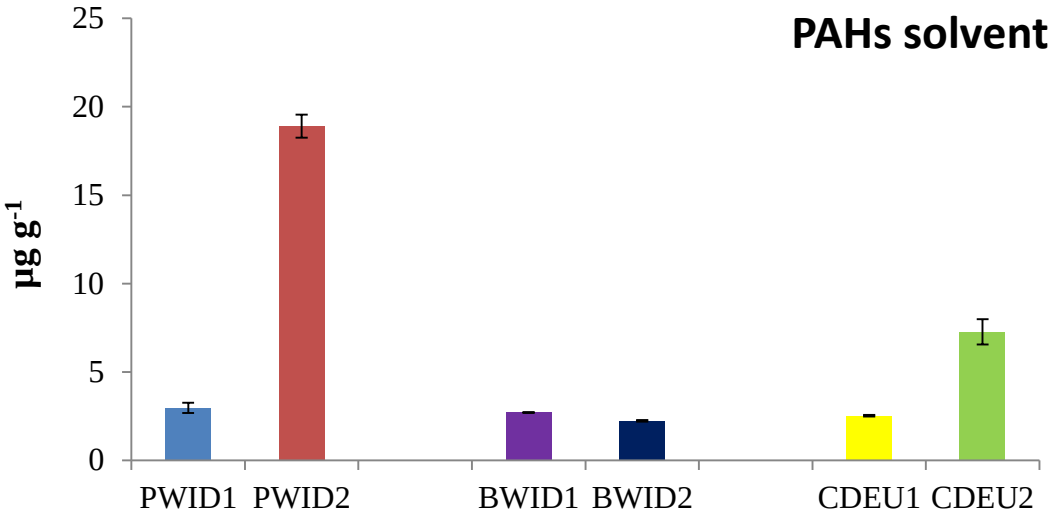
#	H/C (molar)	O/C (molar)	C (%)	H (%)	N (%)	O (%)	Ash (%)	Moisture (%)
PW ID 1	0.33	0.04	91.6 ± 2.3	2.50 ± 0.069	0.21 ± 0.017	4.4 ± 2.4	1.32 ± 0.04	0.10 ± 0.001
PW ID 2	0.70	0.24	71.7 ± 0.81	4.20 ± 0.064	0.18 ± 0.011	22.9 ± 0.89	1.06 ± 0.02	0.19 ± 0.026
CD EU 1	0.22	0.06	46.4 ± 0.37	0.86 ± 0.019	1.58 ± 0.004	3.87 ± 0.43	47.27 ± 0.04	0.13 ± 0.014
CD EU 2	0.30	0.06	48.0 ± 0.82	1.21 ± 0.074	1.91 ± 0.048	3.80 ± 1.0	45.07 ± 0.05	0.08 ± 0.004
BW ID 1	0.29	0.08	87.6 ± 2.8	2.14 ± 0.18	0.19 ± 0.047	9.3 ± 2.0	1.51 ± 0.04	0.13 ± 0.006
BW ID 2	0.72	0.23	71.9 ± 2.4	4.33 ± 0.092	0.18 ± 0.005	22.4 ± 2.4	1.14 ± 0.08	0.18 ± 0.014

The concentrations of PAHs ranged between 2.2 (BW ID 2) and 18.9 μg g^{-1} (PW ID 2) (Table 5.2.4. and Figure 5.2.1.), thus not all the biochars were below the levels recommended by IBI or EBC (4-12 μg g^{-1}). However, it is interesting to note that despite the difference in feedstock at 600 °C the PAH levels were quite similar (2.2-2.9 μg g^{-1}), while were significantly different at low temperature (340-400°C). Therefore, the influence of feedstock type on PAHs concentration is evidenced by results obtained from biochar produced at low temperature. PAH levels tended to increase with decreasing H/C ratios, indicating that a multitude of factors could influence the occurrence of PAHs in biochar (Schimmelpfennig and Glaser, 2012). In fact, different trends were reported in the literature (Hale et al., 2012; Keiluweit et al., 2012; Schimmelpfennig and Glaser, 2012), such as decreasing or increasing PAH concentrations with increasing pyrolysis time/temperature for slow and fast pyrolysis, respectively (Hale et al., 2012), or PAH concentrations peaking at 500 °C in grass biochars produced in the 100-700 °C pyrolysis interval (Keiluweit et al., 2012). For beech wood and corn digestate biochar, naphthalene was the most abundant PAH, in accordance to previous studies (Hale et al., 2012; Hilber et al., 2012; Schimmelpfennig and Glaser, 2012; Freddo, et al., 2012; Fabbri et al., 2013), followed by phenanthrene. While for the pine wood biochar the most abundant PAH was phenanthrene, followed by naphthalene in BW ID 1 and by fluoranthene and pyrene in BC ID 2.

Table 5.2.4. Concentrations of the 16 US-EPA PAHs in biochar. Mean values in ng g^{-1} d.w. and standard deviation from two replicates.

Sample Id.	**PW ID 1**	**PW ID 2**	**CD EU 1**	**CD EU 2**	**BW ID 1**	**BW ID 2**
PAHs	***ng g^{-1}***		***ng g^{-1}***		***ng g^{-1}***	
Naphthalene	617 ± 8.1	423 ± 64	1512 ± 15	6039 ± 635	1132 ± 12	1204 ± 3.2
Acenaphthylene	49 ± 11	145 ± 29	243 ± 4.5	613 ± 52	326 ± 41	173 ± 31
Acenaphthene	119 ± 16	546 ± 24	144 ± 7.2	113 ± 16	176 ± 18	273 ± 57
Fluorene	273 ± 4.9	745 ± 2.4	124 ± 0.6	148 ± 6.5	292 ± 19	147 ± 22
Phenanthrene	778 ± 188	10244 ± 233	135 ± 19	130 ± 0.6	274 ± 37	183 ± 34
Anthracene	81 ± 7.1	824 ± 77	23 ± 23	22 ± 0.1	20 ± 2.3	23 ± 0.8
Fluoranthene	207 ± 46	3355 ± 135	61 ± 13	41 ± 0.3	74 ± 0.8	41 ± 4.2
Pyrene	45 ± 9.1	1732 ± 209	48 ± 9.3	44 ± 12	81 ± 4.3	48 ± 9.3
Chrysene	229 ± 12	181 ± 18	41 ± 6.6	12 ± 3.3	94 ± 7.8	6.4 ± 0.7
Benzo[*a*]anthracene	183 ± 24	341 ± 62	27 ± 5.9	23 ± 1.6	8.0 ± 0.5	20 ± 0.7
Benzo[*b*]fluoranthene	288 ± 30	197 ± 4.0	89 ± 18	33 ± 2.7	79 ± 15	32 ± 8.0
Benzo[*k*]fluoranthene	76 ± 15	37 ± 2.4	34 ± 3.0	35 ± 3.0	40 ± 6.8	12 ± 2.1
Benzo[*a*]pyrene	19 ± 2.6	25 ± 5.0	44 ± 7.7	20 ± 1.7	117 ± 11	79 ± 15
Indeno[1,2,3-*cd*]pyrene	n.d.	38 ± 6.0	n.d.	n.d.	n.d.	n.d.
Dibenzo[*a,h*]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo[*ghi*]perylene	n.d.	70 ± 5.2	n.d.	n.d.	n.d.	n.d.
Σ 16 EPA PAHs	**2964 ± 290**	**18905 ± 290**	**2524 ± 41**	**7274 ± 710**	**2730 ± 14**	**2240 ± 45**

Figure 5.2.1. Concentrations of the 16 US-EPA PAHs in biochar. Mean values in µg g^{-1} d.w. and standard deviation from two replicates.



5.2.3.2. Biochar carbon thermal stability stability determination via HyPy

It is important to determine how much of the carbon contained in biochar is potentially stable over long periods of time as there are likely to be various fractions, differing in their stability, ranging from very unstable (labile) fractions to very recalcitrant (stable) fractions. The HyPy method has been demonstrated to remove almost all labile organic carbon, leaving a highly stable residue with average polycondensed structures greater than 7 fused rings (Meredith et al., 2012; Wuster et al., 2012). The low molecular weight non-BC_{HyPy} that are removed by HyPy along with any other residual labile organic compounds are unlikely to be stable on centennial timescales due to their susceptibility to biological and chemical oxidation (Knicker et al., 2008; Ascough et al., 2008).

Table 5.2.5 and Figure 5.2.2 show the results obtained from analysis of the biochar samples using HyPy. It can be seen a significant difference in BC_{HyPy} concentration and mass losses during HyPy of the biochar samples. The feedstock source and pyrolysis temperature clearly influenced the proportion of BC and the degree of condensation of aromatic C (Table 5.2.5 and Figure 5.2.2). The wood biochars produced at 340-400 °C contained the lowest proportions of BC_{HyPy} fraction (15.7 – 20.5%), with the pine wood biochar having lower proportions (15.7 ± 0.54%) than the biochar produced from beech

wood (20.5 ± 5.0%) (Table 5.2.5). The BC_{HyPy} fraction of the 600 °C wood biochars (Figure 5.2.2) contained the highest proportions of BC_{HyPy} fraction (89.7-95.9%), with the pine wood biochar again having higher proportions (95.9 ± 0.69%) than the biochar produced from beech wood (89.7 ± 1.7%) (Table 5.2.5). Therefore, the fraction of BC as proportion of total organic carbon, as determined by the HyPy method, increases from 15.7% at 400 °C to 95.9% at 600 °C for wood biochars, demonstrating that pyrolysis temperature exerts a strong control on the formation of BC (Figure 5.2.2 and Table 5.2.5).

Table 5.2.5. Weight loss (%) during HyPy treatment and black carbon (BC) from HyPy of the biochars. Mean values and standard deviation from two replicates.

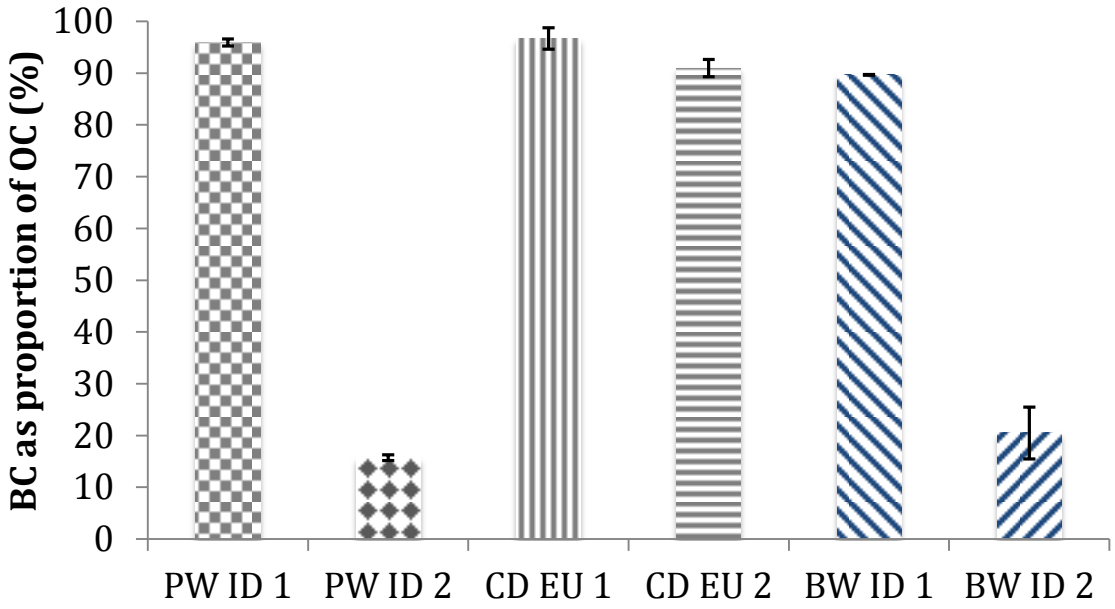
Sample	**BC_{HyPy} (BC/OC)**		**Weight loss**	
	%	**sd**	**%**	**sd**
PWID1	95.9	0.69	20.0	2.2
PWID2	15.7	0.54	82.9	0.11
BWID1	89.7	1.7	16.1	3.2
BWID2	20.5	5.0	76.4	3.1
CDEU1	96.7	2.1	12.7	3.2
CDEU2	91.0	1.7	16.1	4.0

The relatively low BC_{HyPy} contents of the BW ID 2 and PW ID 2 biochars are to be expected given that the temperature of formation for these samples was only 340 and 400 °C, respectively, which may have allowed for products of the incomplete thermal degradation of cellulose and lignin to be preserved in biochar (Hammes et al., 2007). These findings are consistent with those of some researches (McBeath and Smernik, 2009; Nguyen et al., 2010; Masek et al., 2013) who also observed an increase in aromaticity and aromatic condensation of biochars with increasing pyrolysis temperature. However, PW ID 2 and BW ID 2 biochars do not meet the quality criteria of the European Biochar Certificate, which sets a range of minimum allowed threshold values for the black carbon content in the biochars (10 - 40% of overall carbon).

The influence of feedstock type on the degree of carbonisation is evidenced by results HyPy obtained from biochar of corn digestate. This feedstock at 400 °C produced a thoroughly carbonised biochar, compared with BW ID 2 and PW ID 2. This was reflected in (i) a low mass losses during HyPy; (ii) a high value of black carbon by HyPy; (iii) a low value of non-BC_{HyPy} fraction produced (Figure 5.2.2).

The BC_{HyPy} content was inversely correlated with H/C and O/C ratios (R = -0.99 in both cases, Table 5.2.3) confirming as biochars with low H/C and O/C values are graphite-like materials (i.e. soot, black carbon, activated carbon), they are expected to be more stable and less prone to degradation (Masiello, 2004).

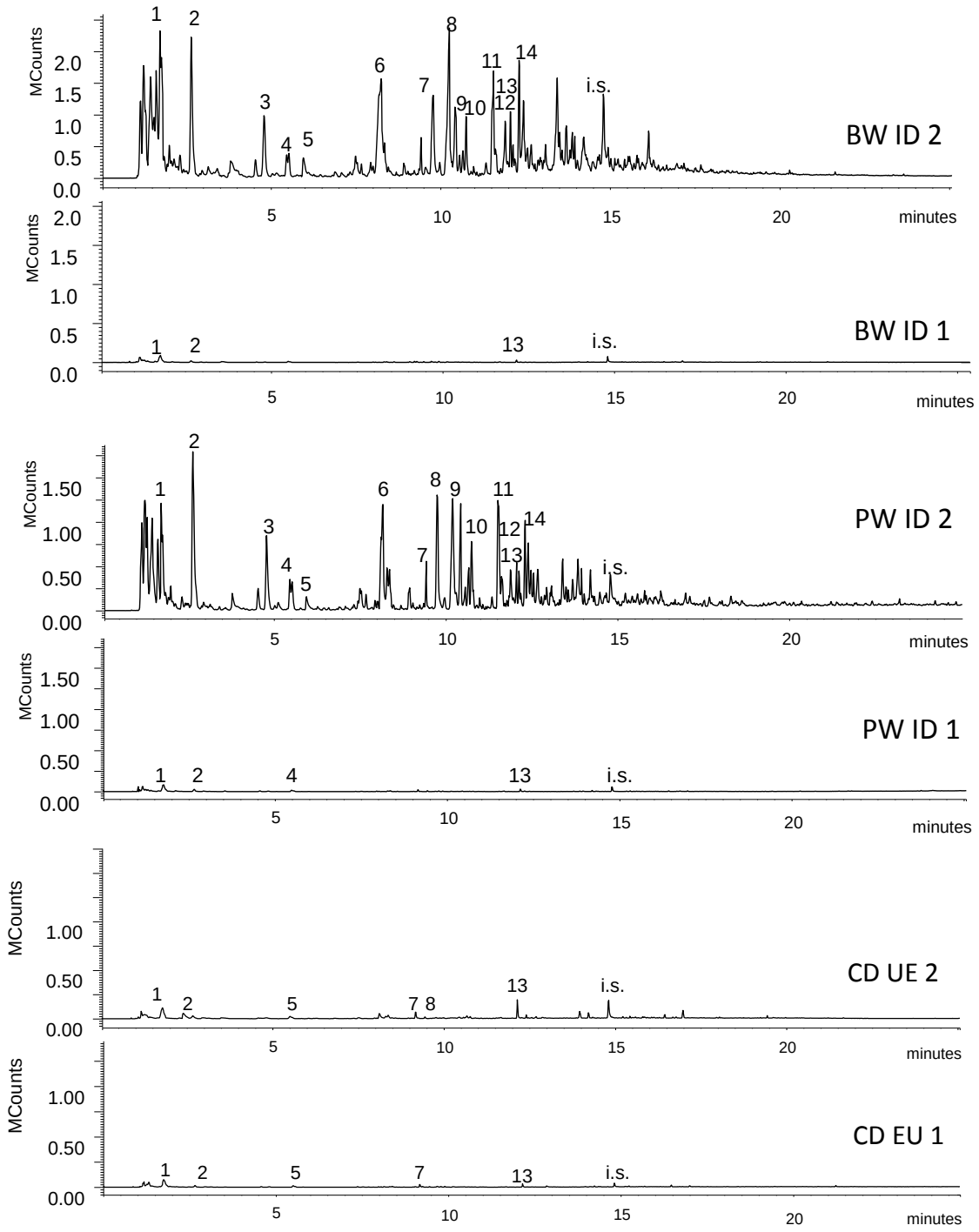
Figure 5.2.2. Black carbon (BC) as proportion of organic carbon (OC) as measured by HyPy of the biochar.



5.2.3.3. Biochar carbon thermal stability stability determination Py-GC-MS

The analysed biochar samples produced different pyrolysate patterns when subjected to Py-GC-MS. Some typical pyrograms resulting from Py-GC-MS of biochar samples are depicted in Fig. 5.2.3, the numbers in which refer to the pyrolysis product list (Table 5.2.2). Samples with low BC_{HyPy} values produced complex pyrolysates with intense peaks assignable to the pyrolysis products of hemicelluose, cellulose or lignin, on the contrary samples with high BC_{HyPy} ratios produced simple pyrograms with weak peaks of aromatic hydrocarbons.

Figure 5.2.3. Total ion chromatograms from Py–GC–MS of biochar BW ID 2, BW ID 1, PW ID 1, PW ID 2, CD EU 2 and CD EU 1. Peak attribution: (1), benzene; (2), toluene; (3), m,p-xylene; (4), styrene; (5) ethylbenzene; (6), phenol; (7), indole; (8), 3-methylphenol; (9). 4-methylphenol (10), guaiacol; (11), 2,5-dimethylphenol; (12), 2,3-dimethylphenol; (13), naphthalene; (14), catechol. Internal standard: (i.s.) o-isoeugenol.



The list of 38 pyrolysis products with corresponding retention times, *m*/*z* fragments used for quantification and the total yields are provided in Table 5.2.2. These pyrolysis products, as described in Conti et al. (2014), were grouped into three thermolabile class fractions: highly carbonised (charred), weakly carbonised hemi/cellulose and weakly carbonised lignin (see experimental part). The grouping of pyrolysis products into the fraction was specified in Section 5.2.2.4.

The quantity of evolved pyrolysis products was expressed in terms of "yield" to give a rough estimate of the mass fraction that was analysed by GC–MS. Table 5.2.6 shows that the summed yields varied over three orders of magnitude, spanning from 2,6 10^6 µg g^{-1} (PW ID 2, pyrolysis of pine wood at 400 °C) down to 192 µg g^{-1} for the biochar characterized by the highest temperature production and the highest content of ash (corn digestate biochar). Not surprisingly, the highest yields were obtained with low temperatures (340 and 400 °C). The biochar synthesized at 600°C and at high ash contents (corn digestate) gave very weak signals (Figure 5.2.3) because of high thermal stability and therefore limited the ‘‘pyrolysability’’ of large polyaromatic clusters. Fig. 5.2.3 shows more details on the yield of biochar as a function of temperature, as well as the charred content of biochar produced under the different conditions. It can be seen that the yield of biochar decreases while the BC increases with the pyrolysis temperature in the studied range.

The influence of feedstock type on the degree of carbonisation is evidenced by results Py-GC-MS obtained from biochar of corn digestate. This feedstock at 400 °C produced a thoroughly carbonised biochar with the high charred percentage and the almost complete disappearance of phenol and methoxyphenols from the pyrolysates (Fig. 5.2.3). This result could be explained also by the fact that the corn digestate biochar is characterized by the relatively high content of ash. Moreover, it is interesting to note that biochars more instable (PW ID 2 and BW ID 2) are those with the higher level of PAHs (PW ID 2) and lower level (BW ID 2).

In summary, these two techniques provided detailed and consistent information concerning the chemical characterisation and the stability of biochar samples. For pyrolysis-GC/MS, the sum of the charred products is a representative parameter of the relative proportion of BC in biochar. While for HyPy, the stable fraction of biochar can be defined as the portion of the biochar stable under HyPy conditions (BC_{HyPy}). The degree of carbonisation, based mainly on the characterisation of the samples using Py-GC-MS and

HyPy, increased in the order PWID2 ~ BWID2 < BWID1 ~ CDEU2 < PWID1 ~ CDEU2; his was corroborated by the elemental analysis. It can also be inferred from the results that, for a more complete carbonisation of the biochar, it is important the feedstock type and the final pyrolysis temperature, but also that at high temperature the chemical characteristics of biochar are influence to a lesser extent by feedstock. In particular, the concentrations of PAHs ranged between 2.2 (BW ID 2) and 2.9 µg g^{-1} (PW ID 1), thus always below the levels recommended by International Biochar Initiative or European Biochar Certificate (4-12 µg g^{-1}). Therefore, pyrolysis processes will need to be set up to maximise the overall benefit, not only the yield of stable biochar, and will therefore be very case specific.

Table 5.2.6. Yields in µg g^{-1} and benzene/toluene ratio (B/T) from Py–GC–MS of biochars.

Sample	Temp. °C	Py-GC-MS yields (µg g^{-1})	Charred %	Lignin %	Proteins %	Holocellulose %	B/T
PW ID 1	600	192	99.5	-	0.5	-	3.2
PW ID 2	400	2654434	36.6	61.9	0.2	1.4	0.49
CD EU 1	600	572	86.6	13.4	-	-	2.6
CD EU 2	400	283	96.1	3.6	0.3	-	6.0
BW ID 1	600	502	99.8	-	0.2	-	8.9
BW ID 2	340	66152	37.4	62.5	0.001	0.001	0.49

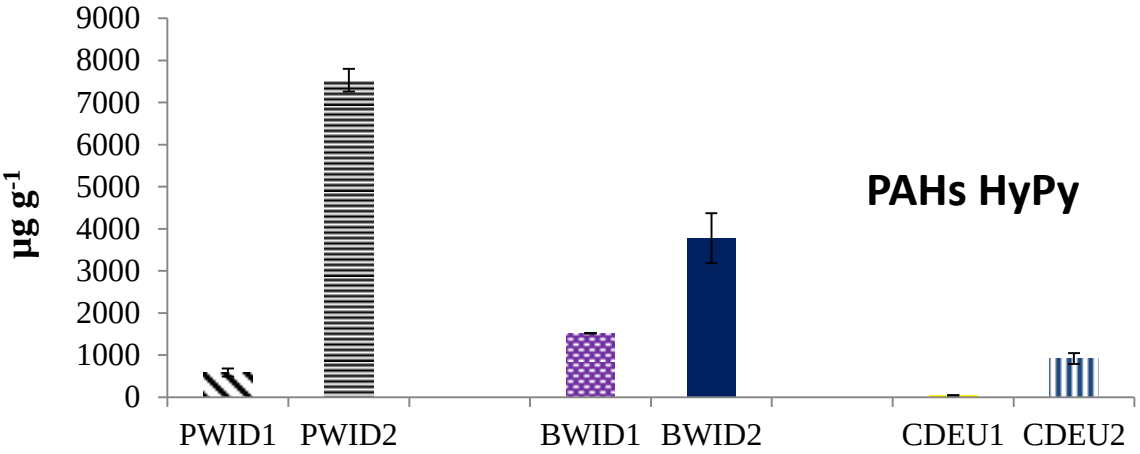
5.2.3.4. HyPy and Py-GC-MS: molecular characterization of labile fraction of biochar

As highlighted in the previous section, the stable fraction of biochar is one of the key parameters to be considered in defining biochar production conditions. However, the labile fraction, which evolves during its storage in soil, is also very important. This fraction is highly likely to impact on microbial activity (Lehmann et al., 2011, Ameloot et al., 2013), and therefore affects the functioning of the soil as a whole, including the balance of indigenous labile pools (de Graaff et al., 2010, Ameloot et al., 2014). In fact, the microorganisms can utilize a number of labile biochar constituents as an energy source (Cross and Sohi, 2011). These are presumably either relatively untransformed biomass components that have not been subjected to volatilization during pyrolysis (Ronsse et al.,

2013) or volatilized compounds that have recondensed in the biochar matrix during pyrolysis (Imam and Capareda, 2012; Kloss et al., 2012). Moreover, many biochar associated labile components have biocidal activity (Graber et al., 2010), which may increase its stability against biotic decomposition.

As well as isolating the BC_{HyPy} fraction, HyPy also allows the characterisation of the non-BC_{HyPy} material at a molecular level by GC-MS analysis. Fig. 5.2.5 shows mass chromatograms of the non-BC_{HyPy} fraction derived from biochars, which was found to contain a high abundance of PAHs. The PAHs detected and quantified (Table 5.2.7) in the biochars ranged from 2-ring compounds (naphthalene) to 7-ring compounds (coronene), with the 4-ring compound pyrene being the most abundant. This range of ring size is consistent with the PAHs distribution found in the non-BC_{HyPy} fraction generated by the HyPy of 5 archaeological charcoals (Ascough et al., 2010), and the definition of BC_{HyPy} as being composed of PAHs with >7 rings proposed by Meredith et al. (2012). Their presence in the non-BC_{HyPy} fraction will be due to their greater volatility relative to the larger more condensed and refractory aromatic domains which form the BC_{HyPy}.

Figure 5.2.5. Concentrations of PAHs released by the HyPy of biochar. Mean values in µg g^{-1} d.w. and standard deviation from two replicates.



The total mean concentration value of PAHs in biochars (HyPy determination) ranged between 7531 (PW ID 2) and 43 µg g^{-1} (CD EU 1) (Table 5.2.7 and Fig. 5.2.5). However, it is interesting to note that at high temperature also the concentrations of PAHs in labile

fraction of biochar determinate by HyPy are influence to a lesser extent by feedstock. Moreover, the biochars produced at 600 °C, as for the PAHs concentration determined by soxhlet extraction, have the PAH levels lower (43-1520 µg g^{-1}) than that at low temperature (920-7531 µg g^{-1}).

Almost all PAHs were detected and quantified in the biochars at lower temperature (Table 5.2.2), while in all of the biochars at 600 °C, naphthalene, acenaphthylene, acenaphthene, methylchrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*a,h*)anthracene, benzo(*ghi*)perylene, indeno(1,2,3-*c,d*)pyrene and coronene were not detected. The individual concentrations of the PAHs in biochars are presented in Table 5.2.7 and typical distribution profiles are shown in Fig. 5.2.6. A detailed analysis of the contribution of the individual PAHs in biochars produced at 600 °C indicated the dominance of phenanthrene (8-32% of the total PAHs), fluoranthene (15-28% of the total PAHs) and pyrene (36-61% of the total PAHs) in all the samples studied. While in the wood biochars at lower temperature the PAHs with 5–7 rings composed almost the majority of PAHs (PW ID 2 = 48%, BW ID 2 = 55%). Therefore, it can be assumed that biochar generated at a temperature of 340-400 °C will have an aromatic structure that is not sufficiently condensed to be entirely captured in the analytical window of HyPy. However, also in the biochar CD EU 2 (400 °C), phenanthrene, fluoranthene and pyrene dominated the PAH profiles, supplying 28 %, 14 % and 40% of the total PAH concentrations, respectively. This result could be explained also by the fact that the corn digestate biochar is characterized by the relatively high content of ash.

The distribution of pyrolysis products is sensitive to the feedstock, as well as production conditions (Figure 5.2.4 and Table 5.2.8). The pyrolysates of all biochar samples were featured by the presence of aromatic hydrocarbons including benzene, benzene derivatives, and polycyclic aromatic hydrocarbons (PAHs; e.g., naphthalene, phenanthrene). Aromatic hydrocarbons (e.g., benzene, toluene, C2-benzenes, naphthalene, phenanthrene, diphenyl) along with benzofurans were grouped into a single family of compounds representing the charred fraction of biochar (C in Table 5.2.5). The high proportion of these products in the pyrolysates (% charred) which ranged from 36.6% (PW ID 2) to >99% (PW ID 1 – BW ID 1), indicative of charred biomass. This is in accordance to Kaal et al. (2009) who proposed that benzene, toluene, naphthalene, diphenyl and benzofuran could be associated specifically to the charred fraction of BC.

In addition, the degree of de-alkylation might be a proxy of thermal alteration (Kaal et al., 2012). The de-alkylation degree can be estimated in Py–GC–MS from the ratio of parent/alkylated compound, such as benzene/toluene (B/T), ratios. B/T ratios for the biochar pyrolysates ranged between 0.49 and 8.9 (Table 5.2.5) and tended to increase with decreasing overall yields and with increasing the relative abundance of pyrolysis products indicative of charring (% charred).

The pyrolysis products lignin markers, represented by 2-methoxyphenols (guaiacols), 4-vinylguaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-methylsyringol and 2,5-dimethoxyphenols (syringols), were abundant in the pyrolysate of PW ID 2 and BW ID 2 biochar, while these lignin markers (Ralph and Hatfield, 1991) were not detected in PW ID 1, BW ID 1, CD EU 1 and CD EU 2. The phenols and methylphenols, which are less specific lignin markers, were abundant in PW ID 2 and BW ID 2, but were detected also in corn digestate biochars. In this case, the phenols and methylphenols are therefore of little diagnostic value with respect to highly or weakly pyrolysed lignin.

Figure 5.2.6. Total ion chromatograms from HyPy of biochars PW ID 2 and BW ID 2 showing the PAHs present in the non-BC_{HyPy} fraction.

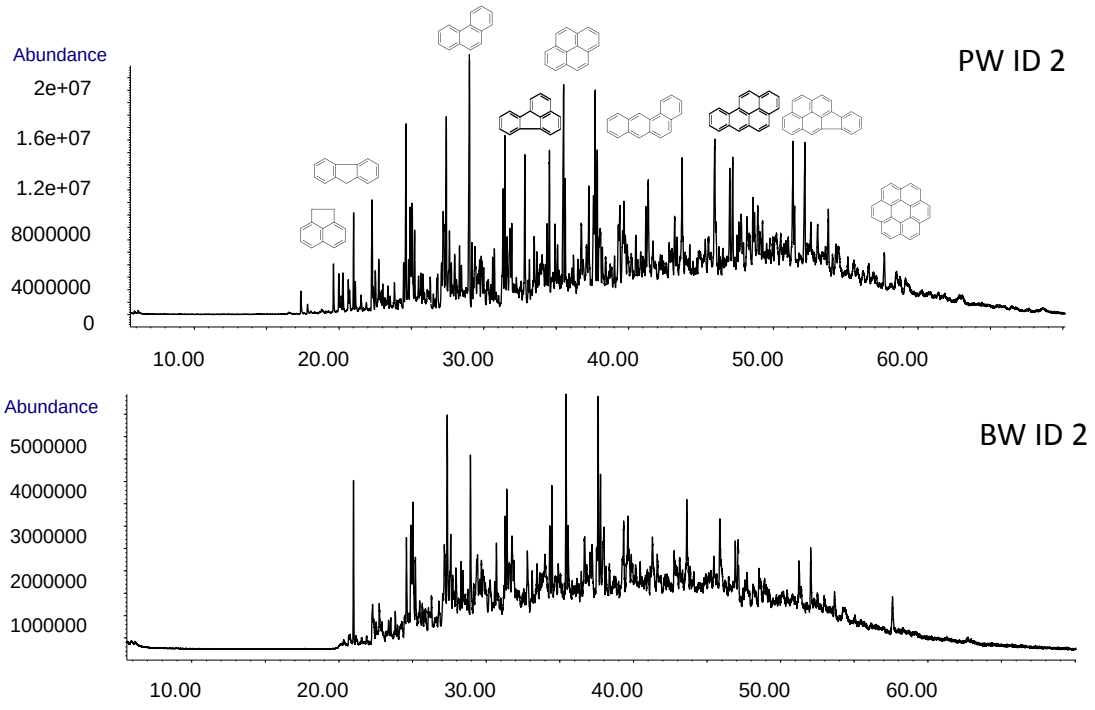


Table 5.2.7. Observed concentration of PAHs released by the HyPy of biochar samples.

PAHs	Mol. Wt.	Rings	PW ID 1 (µg g^{-1})	PW ID 2 (µg g^{-1})	CD EU 1 (µg g^{-1})	CD EU 2 (µg g^{-1})	BW ID 1 (µg g^{-1})	BW ID 2 (µg g^{-1})
Naphthalene	128	2	n.d.	57 ± 30	n.d.	n.d.	n.d.	8.0 ± 3.8
Biphenyl	154	2	n.d.	96 ± 24	n.d.	n.d.	n.d.	28.0 ± 0.5
Acenaphthene	154	3	n.d.	77 ± 11	n.d.	n.d.	n.d.	47.5 ± 2.2
Fluorene	166	3	3.70 ± 0.7	416 ± 45	n.d.	14.2 ± 0.9	14.4 ± 0.16	161 ± 33.9
Phenanthrene	178	3	186 ± 41.4	1019 ± 32	3.4 ± 0.07	257 ± 46.1	579 ± 9.2	335 ± 80.4
Anthracene	178	3	20.5 ± 0.7	166 ± 9	1.3 ± 0.01	13.4 ± 2.1	18.4 ± 0.3	74 ± 14.7
Fluoranthene	202	4	111 ± 7.3	548 ± 27	11.9 ± 2.8	126 ± 5.2	225 ± 7.1	289 ± 61.1
Pyrene	202	4	239 ± 49.2	938 ± 14	26.0 ± 6.3	406 ± 71.6	540 ± 14.5	432 ± 102
Chrysene	228	4	19.8 ± 1.4	265 ± 24	n.d.	5.87 ± 0.7	53.2 ± 3.5	186 ± 45
Benzo[a]anthracene	228	4	10.7 ± 1.3	346 ± 13	n.d.	18.3 ± 3.3	19.0 ± 0.4	150 ± 26.0
Methylchrysene	242	4	n.d.	311 ± 6	n.d.	10.0 ± 1.5	14.0 ± 0.2	234 ± 22.7
Benzo[*b*]fluoranthene	252	5	n.d.	734 ± 30	n.d.	21.5 ± 1.0	32.9 ± 0.9	384 ± 59.9
Benzo[*k]*fluoranthene	252	5	n.d.	470 ± 18	n.d.	17.4 ± 1.8	24.4 ± 1.7	266 ± 58.6
Benzo[*a*]pyrene	252	5	n.d.	463 ± 26	n.d.	8.1 ± 1.2	n.d.	243 ± 45.0
Indeno[1,2,3-cd]pyrene	276	6	n.d.	601 ± 56	n.d.	4.76 ± 0.7	n.d.	346 ± 28.8
Dibenzo[a,h]anthracene	278	6	n.d.	159 ± 16	n.d.	2.65 ± 0.04	n.d.	102 ± 7.8
Benzo[ghi]perylene	276	6	n.d.	682 ± 86	n.d.	15.8 ± 1.2	n.d.	378 ± 51.8
Coronene	300	7	n.d.	186 ± 16	n.d.	n.d.	n.d.	115 ± 2.1
Total PAHs			**591 ± 93.7**	**7531 ± 270**	**43 ± 9.1**	**920 ± 131**	**1520 ± 10.8**	**3778 ± 593**

Table 5.2.8. Concentrations of the pyrolysis products of biochar. Mean values (µg g^{-1}) and standard deviation from two replicates.

Sample Id.	**BW ID 1**	**BW ID 2**	**PW ID 1**	**PW ID 2**	**CD EU 1**	**CD EU 2**
Compound name	***µg g^{-1} pyrolysed***		***µg g^{-1} pyrolysed***		***µg g^{-1} pyrolysed***	
Benzene	341 ± 83	4592 ± 771	109 ± 6.8	265527 ± 39092	237 ± 10	91 ± 16
Hydroxyacetone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dimethylfuran	n.d.	773 ± 23	n.d.	17.2 ± 1.5	n.d.	n.d.
Pyrrole	n.d.	n.d.	n.d.	30 ± 18	n.d.	n.d.
Toluene	38 ± 9.5	9452 ± 1106	34 ± 5.6	544318 ± 61563	91 ± 42	15.3 ± 1.3
2-Methyltiophene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furaldehyde	n.d.	136 ± 34	n.d.	n.d.	n.d.	n.d.
o-Xylene	3.9 ± 1.1	909 ± 40	6.3 ± 1.4	51.4 ± 4.2	14.4 ± 1.0	1.18 ± 0.01
m-p-Xylene	3.7 ± 0.2	3274 ± 385	4.8 ± 1.2	183801 ± 16905	15.2 ± 0.3	1.55 ± 0.05
Styrene	22.8 ± 1.1	830 ± 34	7.9 ± 0.7	51.4 ± 1.9	23 ± 5.4	10.7 ± 2.3
Ethyl-benzene	n.d.	79 ± 5.1	n.d.	n.d.	n.d.	n.d.
Phenol	n.d.	15620 ± 2701	n.d.	662373 ± 470703	n.d.	n.d.
Benzofuran	n.d.	1345 ± 137	5.5 ± 0.6	79.1 ± 2.5	17 ± 3.0	n.d.
Benzonitrile	n.d.	n.d.	0.90 ± 0.14	n.d.	17.6 ± 0.9	35 ± 8.0
Indole	2.4 ± 0.3	99.0 ± 0.7	0.39 ± 0.02	4.8 ± 0.2	n.d.	0.94 ± 0.36
3-Methylphenol	n.d.	4340 ± 249	n.d.	279125 ± 86793	17 ± 13	2.84 ± 0.08
4-Methylphenol	n.d.	7307 ± 92	n.d.	497598 ± 265920	27 ± 26	4.24 ± 0.18
Guaiacol	n.d.	1563 ± 41	n.d.	81.3 ± 1.2	n.d.	n.d.
Methyl-benzofuran(1)	n.d.	319 ± 12	0.34 ± 0.08	19.3 ± 1.7	n.d.	n.d.
Methyl-benzofuran(2)	n.d.	660 ± 1	1.1 ± 0.22	18.5 ± 1.6	n.d.	5.3 ± 1.3
Methyl-benzofuran(3)	n.d.	911 ± 43	1.1 ± 0.22	51.7 ± 3.8	n.d.	6.7 ± 1.8
2-Ethylphenol	n.d.	312 ± 11	n.d.	18.6 ± 7.5	n.d.	n.d.
2,5-Dimethylphenol	n.d.	2910 ± 18	n.d.	220813 ± 88639	11 ± 8.3	0.92 ± 0.11
2,3-Dimethylphenol	n.d.	2082 ± 13	n.d.	25.6 ± 10.1	10 ± 7.5	1.16 ± 0.15
3-Ethylphenol	n.d.	620 ± 22	n.d.	27.4 ± 6.3	11 ± 7.6	0.93 ± 0.09
Naphthalene	63 ± 8.7	863 ± 74	15.5 ± 0.05	50.4 ± 9.3	50 ± 4.9	76 ± 20
Catechol	n.d.	4149 ± 1047	n.d.	129 ± 13.1	n.d.	n.d.
2-Methylnaphthalene	5.2 ± 0.8	526 ± 53	1.60 ± 0.03	31.9 ± 8.5	6.4 ± 2.1	14.1 ± 3.3
1-Methylnaphthalene	5.6 ± 0.4	264 ± 29	1.92 ± 0.07	17.0 ± 5.6	6.4 ± 1.7	9.4 ± 1.9
4-Vinylguaiacol	n.d.	46.1 ± 0.1	n.d.	2.3 ± 0.2	n.d.	n.d.
4-Methylguaiacol	n.d.	1001 ± 192	n.d.	125 ± 31.6	n.d.	n.d.
Syringol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Biphenyl	8.7 ± 1.1	44.5 ± 1.5	1.53 ± 0.09	4.1 ± 1.6	9.4 ± 0.8	4.38 ± 0.99
4-Ethylguaiacol	n.d.	995 ± 111	n.d.	31.9 ± 11.7	n.d.	n.d.
4-Methylsyringol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
o-Isoeugenol (i.s.)	71 ± 1.1	60 ± 3.1	22 ± 2.3	15.7 ± 4.8	61 ± 9.3	74 ± 18
Fluorene	2.4 ± 0.12	94 ± 7.1	n.d.	3.1 ± 0.5	3.9 ± 0.1	0.93 ± 0.11
Phenanthrene	2.5 ± 0.05	16.8 ± 0.6	n.d.	6.2 ± 0.8	3.9 ± 1.9	0.25 ± 0.02
Anthracene	2.4 ± 0.20	12.4 ± 2.0	n.d.	1.3 ± 0.2	n.d.	n.d.
Fluoranthene	n.d.	3.8 ± 1.1	n.d.	0.9 ± 0.1	n.d.	n.d.
Pyrene	n.d.	3.4 ± 1.0	n.d.	0.7 ± 0.1	n.d.	n.d.

5.2.4. Conclusions

The aim of this study was to examine molecular proxies of the thermal stability of the carbon in biochar produced from different feedstocks and under different production process and in the context of its carbon sequestration potential. HyPy was applied to isolate the carbon component most likely stable in the environment on centennial timescales and the Py-GC-MS providing qualitative and quantitative information on stability of biochar.

The results presented in this study demonstrated that HyPy is a rapid and convenient technique for the quantification of BC and determination of the stable carbon in biochar. In support of HyPy method, a strong relationship was found between the H/C and O/C values of biochar samples and the BC_{HyPy} values found. Moreover, the ability of the HyPy method to determine BC in the biochar was evaluated comparing the results with Py-GC-MS. The results showed that these two techniques provided detailed and consistent information concerning the chemical characterisation and the stability of biochar samples. As well as isolating the BC_{HyPy} fraction, HyPy also allows the characterisation of the non-BC_{HyPy} material at a molecular level by GC-MS analysis.

The influence of feedstock type and pyrolysis conditions on the degree of carbonisation and other biochar properties was evidenced by HyPy and Py-GC-MS. In particular, the fraction of BC in wood biochar increases with increasing pyrolysis temperature levelling the biochar characteristics from the feedstock. The levels of solvent extractable PAHs and HyPy evolved PAHs were higher in biochars produced at the lower temperatures. This means that biochar exposed to higher pyrolysis temperatures contains a higher proportion of the stable fraction than biochar produced at low temperatures suggesting that, from a carbon sequestration point of view, high temperature pyrolysis biochar is preferable. However, studies indicate that from the point of view of sequestering maximum amount of carbon per unit of feedstock, low-temperature conversion processes might perform as effectively as higher temperature pyrolysis processes. Therefore, this aspect requires further investigations.

6. Conclusions

The study presented in this thesis was targeted to expand our understanding on the application of biochar in the environment. In particular, focusing on the potential risks associated to the application of biochar in soil biochar due to the presence of harmful substances as well on the stability of biochar.

To the purpose of evaluating the potential risks arising from the occurrence of polycyclic aromatic hydrocarbons (PAHs) sorbed onto biochar, an analytical method was developed for the determination of the 16 USEPA-PAHs in the original biochar and soil containing biochar. The concentration of these PAHs along with the 15 EU-PAHs, priority hazardous substances in food, was determined in a suite of currently available biochars for agricultural field applications, which were derived from a variety of parent materials and pyrolysis conditions. The method consisted in surrogate PAH spiking, prolonged soxhlet extraction with acetone-cyclohexane, SPE clean-up and GC-MS analysis. The method was successfully validated with a certified reference material for the soil matrix. In the absence of commercially available reference materials for charcoal the method could not be fully validated for biochar. However, the recoveries of surrogate (perdeuterated) PAHs were satisfactory for almost all of the many investigated biochars from different substrates and synthesis conditions. The participation to a laboratory exercise within the EU-COST TD1107 enabled a comparison of the method with methods in use in other laboratories.

All the biochars analyzed in this thesis contained the USEPA, as well as some of the EU-PAHs at detectable levels ranging from 1.2 to 19 μg g^{-1}. Results have indicated that, considering an application of 20-60 t biochar ha^{-1}, the degree of PAH contamination will be dependent on both the presence of background PAHs in soil and the concentrations of sorbed PAHs on the biochar. Moreover, along with PAH levels determined in other studies, our data suggested that biochars produced by slow pyrolysis from woody biomass possessed the lowest level of sorbed PAHs (< 10 μg g^{-1}).

Once spread in the soil, biochar could be a source or a sink of PAHs. The environmental fate of biochar-associated PAHs is still poorly understood due to the paucity of long-term in-field studies on this topic. Therefore, it is necessary to improve knowledge of the role biochar plays in sorbing PAHs and on microbial activity and how this influences the concentration of PAHs in soil and their persistence in the environment.

The changes in PAH content and distribution was examined in a four year study following biochar addition in soils in a vineyard (CNR IBIMET). The obtained results

showed that the biochar addition determined an increase of the amount of PAHs. However, the levels of PAHs in the soil remained within the maximum acceptable concentration for a number of European countries. Moreover, the biochar did not reduce the degradation of PAHs in the investigated agricultural soil, a conspicuous fraction of PAHs was degraded bringing the PAH levels close to those of the untretaed soil. The absence of an increasing concentration trend with time indicated that biochar did not act as a sink of environmental (e.g. atmospheric) PAHs. Therefore, the impact attributable to PAHs following biochar application to soil can be minimal.

The four years sampling of vineyard soil performed by CNR-IBIMET was exploited to study the environmental stability of biochar and its impact on soil organic carbon. In the literature, several approaches have been proposed to assess biochar stability. Yet, there is no agreed methodology for determining the long-term stability of biochar. In this research, the stability of biochar produced from different feedstock and under different production processes was investigated by analytical pyrolysis (Py-GC-MS) and pyrolysis in the presence of hydrogen (HyPy). In particular, HyPy was applied to isolate the carbon component most likely to be stable in the environment on a centennial timescales The findings of this study showed that biochar amendment significantly influence soil stable carbon fraction concentration during the incubation period. In particular, the obtained concentrations of stable carbon fraction in the amended soil are significantly higher than those in the untreated soil. Obviously, the effect of biochar addition in soils on the level of stable carbon will depend on the BC in the original biochar. The high stable carbon value found for biochar (83±3.3%) used in this study suggests that it should be quite recalcitrant to degradation.

Moreover, the HyPy allowed the characterisation on a molecular level of labile carbon fraction defined as non-BC_{HyPy} fraction. In addition to a number of PAHs, the non-BC_{HyPy} fraction was also found to contain a significant abundance of *n*-alkanes, with a marked predominance of even-numbered homologues. These compounds are probably derived from lipids, hydrogenated during HyPy.

The results presented in this study demonstrated that HyPy is a valid technique for isolating and quantifying stable carbon in soil matrices treated with biochar. Moreover, the ability of the HyPy method to determine stabile carbon fraction in the biochar was evaluated comparing the results with flash analytical pyrolysis (Py-GC-MS) on a variety of biochars. In fact, Py-GC-MS can provide information on the thermal labile fraction of

biochar at a molecular level. HyPy and Py-GC-MS were applied to biochars deriving from three different feedstock (woody, herbaceouse and digestate biomass) at two different pyrolysis temperatures. HyPy and Py-GC-MS evidenced the influence of feedstock type and pyrolysis conditions on the degree of carbonisation and other biochar properties. In particular, the stable fraction in wood biochar increases with increasing pyrolysis temperature levelling the biochar characteristics from the feedstock. This means that, from a carbon sequestration point of view, a high temperature pyrolysis biochar is preferable.

In general, the obtained results showed that these two techniques provided detailed and consistent information concerning the chemical characterisation and the stability of biochar samples. By isolating the stable fraction, HyPy also allowed the characterisation of the labile carbon fraction at a molecular level by GC-MS analysis.

Biochar has potential as soil amendment for improving soil quality, decreasing fertilizers losses and store carbon into the soil. Nevertheless, as soil additive, the absence of phytotoxicity is the minimal requirement. We have showed above that the concentration of PAHs in biochars from slow pyrolysis of lignocellulosic feedstock is generally sufficiently low to keep the degree of contamination in soils at safe levels. However, biochars from sources other than woody or herbaceous biomass could exhibit detrimental effects on plants. In this context, biochar from poultry litter was investigated in this thesis. Biochars were prepared by intermediate pyrolysis at different temperatures and compared with biochars from corn stalk prepared under the same pyrolysis conditions. The phytotoxicity of these biochars was estimated by means of seed germination tests on cress (*Lepidium sativum* L.).

Results obtained show that biochar from poultry litter may exert negative effect at least at the relatively high level of soil amendment (40 t ha^{-1}). The role of PAHs in the inhibition of seed germination was excluded. Instead, potential candidates of toxicity were identified in water soluble and biodegradable components, probably derived from the thermal decomposition of proteins and lipids. In supporting this hypothesis, the toxicity was drastically reduced by water extraction or mixing with biologically active materials, while the water extracts inhibited the germination. Therefore, biochar is not an "intrinsically safe" material, and every biochar from different processes and/or feedstock has to be evaluated, checked and possibly treated before the agronomic application.

References

Abel, S.; Peters, A.; Trinks, S.; Schonsky, H.; Facklam, M.; Wessolek, G. Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. Geoderma 2013, 202-203, 183–191.

Adriano, D.C.; Chang, A.C.; Pratt, P.F.; Sharpless, R. Effect of soil application of dairy manure on germination and emergence of some selected crops. J. Environ. Quality 1973, 2, 396–399.

Ahamedna, M.; Marshall, W.M.; Rao, R.M. Production of granular activated carbons from selected agricultural by-products and evaluation of their physical, chemical and adsorptive properties. Bioresour Technol 2000, 71 113-123.

Ahn, S.; Werner, D.; Luthy, R.G. Modeling PAH mass transfer in a slurry of contaminated soil or sediment amended with organic sorbents. Water Res. 2008, 42, 2931–2942.

Aina, R.; Palin, L.; Citterio, S. Molecular evidence for benzo[a]pyrene and naphthalene genotoxicity in Trifolium repens L. Chemosphere 2006, 65, 666–673.

Alburquerque, J.A.; Calero, J.M.; Barrón, V.; Torrent, J.; del Campillo, M.C.; Gallardo, A.; Villar, R. Effects of biochars produced from different feedstocks on soil properties and sunflower growth. J. Plant Nutr. Soil Sci. 2014, 177, 16–25.

Al-Wabel, M.I., Al-Omran, A.; El-Naggar, A.H.; Nadeem, M.; Usman, A.R. Pyrolysis temperature induced changes in characteristics and chemical composition of biochar produced from conocarpus wastes, Bioresour. Technol. 2013, 131, 374-379.

Ameloot, N.; Graber, E.R.; Verheijen, F.G.A.; De Neve S. Interactions between biochar stability and soil organisms: review and research needs. European Journal of Soil Science 2013, 64, 379-390.

Ameloot, N.; Sleutel, S.; Case, S.D.C. Alberti, G.; McNamara N.P.; Zavalloni, C.; Vervisch, B.; delle Vedove, G.; De Nevea, S. C mineralization and microbial activity in four biochar field experiments several years after incorporation. Soil Biology & Biochemistry 2014, 78, 195-203.

Amonette, J. E. and Joseph, S. Characteristics of biochar: Microchemical properties. In: Biochar for environmental management: Science and technology. Lehmann, J. and S. Joseph (eds). Earthscan, London, UK and Sterling, 2009, 33-52.

Antal, M. J.; Allen, S. G.; Dai, X.; Shimizu, B.; Tam, M. S.; Gronli, M. Attainment of the theoretical yield of carbon from biomass. Ind. Eng. Chem. Res. 2000, 39, 4024 – 4031.

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

Antal, M. J.; Mochidzuki, K.; Paredes, L. S. Flash carbonization of biomass. Ind. Eng. Chem. Res. 2003b, 42, 3690−3699.

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

Ascough P.; Bird M. I.; Wormald P.; Snape C. E.; Apperley D. Influence of pyrolysis variables and starting material on charcoal stable isotopic and molecular characteristics. Geochim. Cosmochim. Acta 72 2008, 6090–6102.

Ascough, P.L.; Bird, M.I., Brock, F.; Higham, T.F.G.; Meredith, W.; Snape, C.E.; Vane C.H. Hydropyrolysis as a new tool for radiocarbon pre-treatment and the quantification of black carbon. Quaternary Geochronology 2009, 4, 140-147.

ASTM Standard D3175, Standard test method for volatile matter in the analysis sample of coal and coke, West Conshohocken, 2007.

ARPA Piemonte, Rapporto sullo Stato dell'Ambiente in Piemonte, 2009.

Bobleter, O. Hydrothermal degradation of polymers derived from plants. Prog Polym Sci 1999, 19, 797–841.

Babu, B.V. Biomass pyrolysis: a state-of-the-art review. Biofuels, Bioprod Bioref 2008, 2, 393–414.

Bargmann, I.; Rilling, M.C.; Buss, W.; Kruse, A.; Kuecke M. Hydrochar and biochar effects on germination of spring barley. J. Agro. Crop Sci., 2013, 199, 360–373.

Baronti, S.; Vaccaria, F.P.; Miglietta, F.; Calzolari, C.; Lugato, E.; Orlandini, S.; Pini, R.; Zulian, C.; Genesio, L. Impact of biochar application on plant water relations in Vitis vinifera (L.). Europ. J. Agronomy 2014, 53, 38-44.

Baronti, S.; Alberti, G.; Delle Vedove, G.; Di Gennaro, F.; Fellet, G.; Genesio, L.; Miglietta, F.; Peressotti, A.; Vaccari, F.P. The biochar option to improve plant yields: first results from some field and pot experiments in Italy. Italian J. Agronomy 2010, 5, 3–11.

Basso, A.S.; Miguez, F.E.; Laird, D.A.; Horton, R.; Westgate, M. Assessing potential of biochar for increasing water-holding capacity of sandy soil. Global Change Biol. Bioenergy 2013, 5, 132-143.

Bastos, C.; Keizer, J.J., Prodana, M.; Abrantes, N.; Soares, M.V.M.; Loureiro S. Potential risk of biochar-amended soil to aquatic systems: an evaluation based on aquatic bioassays. Ecotoxicology 2014, 23, 1784–1793.

Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L. Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 2010, 158, 2282–2287.

Beesley, L. and Marmiroli, M. The immobilization and retention of soluble arsenic, cadmium and zinc by biochar. Environmental Pollution 2011, 159, 474–480.

Bernardo, M.; Lapa, N.; Gonçalves, M.; Barbosa, R.; Mendes, B.; Pinto, F.; Gulyurtlu, I. Toxicity of char residues produced in the co-pyrolysis of different wastes. Waste Manage 2010, 30, 628–635.

Bennet, A.C.; Adams, F. Concentration of NH_3(aq) required for incipient NH_3 toxicity to seedlings. Soil Sci. Soc. Am. Proc. 1970, 34, 259–263.

Bindoff, N.L., Stott, P.A.; AchutaRao, K.M.; Allen, M., Gillett, N.; Gutzler, D.; Hansingo, K.; Hegerl, G.; Hu, Y.; Jain S.; Mokhov, I.; Overland, J.; Perlwitz, J.; Sebbari, R.; Zhang, X. Detection and attribution of climate change: from global to regional. In: Climate change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, 2013.

Bird, M.I.; Moyo, C.; Veenendaal, E.M.; Lloyd, J.; Frost, P. Stability of elemental carbon in a savanna soil. Glob Biogeochem Cycles 1999, 13, 923-932.

Blackwell, P.; Riethmuller, G.; Collins M. Biochar Application to Soil (Chapter 12), in: J. Lehmann and S. Joseph (Eds.), Biochar for Environmental Management: Science and Technology, Earthscan, London, UK. 2009, 207.

Bourke, J.; Manley-Harris, M.; Fushimi, C.; Dowaki, K.; Nonoura, T.; Antal, M.J. Do all carbonized charcoals have the same chemical structure? 2. A model of the chemical structure of carbonized charcoal. Industrial and Engineering Chemistry Research 2007, 46, 5954-5967

BP Statistical Review of World Energy, 2013. British Petroleum 2013, 1–48 (bp.com/statisticalreview).

Brandli, R.C.; Hartnik, T.; Henriksen, T.; Cornelissen, G. Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil. Chemosphere 2008, 73, 1805–1810.

Bray, E.E.; Evans, E.D. Distribution of n-paraffins as a clue to recognition of source beds. Geochimica et Cosmochimica Acta 1961, 22, 2–15.

Bridgwater, A. and Peacocke G. Fast pyrolysis processes for biomass. Renew Sustain Energy Rev, 2000, 4, 1–73

Bridle, T.R. and Pritchard, D. Energy and nutrient recovery from sewage sludge via pyrolysis. Water Science Technology 2004, 50, 169–175.

Brocks, J. J.; Love, G.D.; Snape, C.E.; Logan, G.A.; Summos, R.E.; Buick, R. Release of bound aromatic hydrocarbons from late Archean and Mesoproterozoic kerogens via hydropyrolysis. Geochimica et Cosmochimica Acta 2003, 67, 1521-1530.

Brodowski, S.; Rodionov, A.; Haumaier, L.; Glaser, B.; Amelung, W. Revised black carbon assessment using benzene polycarboxylic acids, Organic Geochemistry 2005, 36 1299-1310.

Brown, R.A.; Kercher, A.K.; Nguyen, T.H.; Nagle, D.C.; Ball, W.P. Production and characterization of synthetic wood chars for use as surrogates for natural sorbents. Org. Geochem. 2007, 37, 321–333.

Brown, R. Biochar production technology, in Biochar for Environmental Management, ed by Lehmann J and Joseph S. Earthscan, London 2009, 127–146.

Brownsort, P.A. Biomass pyrolysis processes: performance parameters and their influence on biochar system benefits (MASc thesis), The University of Edinburg, 2009.

Bruce, J.P.; Frome, M.; Haites, E.; Janzen, H.; Lal, R.; Paustian. K. Carbon sequestration in soils. J. Soil Water Conservation 1998, 54, 382-389.

Bucheli, T.D.; Blum, F.; Desaules, A.; Gustafsson, Ö. Polycyclic aromatic hydrocarbons, black carbon, and molecular markers in soils of Switzerland. Chemosphere 2004, 56, 1061-1076.

Bucheli, T.D.; Bachmann, H.J.; Blum, F.; Bürge, D.; Giger, R.; Hilber, I.; Keita, J.; Leifeld, J.; Schmidt H.-P. On the heterogeneity of biochar and consequences for its representative sampling. Journal of Analytical and Applied Pyrolysis 2014, 107, 25–30.

Busch, D.; Stark, A.; Kammann, C.I.; Glaser, B. Genotoxic and phytotoxic risk assessment of fresh and treated hydrochar from hydrothermal carbonization compared to biochar from pyrolysis. Ecotoxicol. Environ. Saf. 2013, 97, 59–66.

Buss, W. and Masek, O. Mobile organic compounds in biochar – A potential source of contamination – Phytotoxic effects on cress seed (Lepidium sativum) germination. Journal of Environmental Management 2014, 137, 111–119.

Calvelo Pereira, R.; Kaal, J.; Camps Arbestain, M.; Pardo Lorenzo, R.; Aitkenhead, W.; Hedley, M.; Macías, F.; Hindmarsh, J.; Maciá-Agulló, J.A. Contribution to characterisation of biochar to estimate the labile fraction of carbon. Org. Geochem. 2011, 42, 1331-1342.

Cantrell, K. B.; Hunt, P.G.; Uchimiya, M.; Novak, J.M.; K. S. Ro., K.S. Impact of pyrolysis temperature and manure source on physicochemical characteristics of biochar. Bioresource Technology 2012, 107, 419- 428

Cao, X.; Ma, L.; Gao, B.; Harris, W. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ. Sci. Technol 2009. 43, 3285–3291.

Cao, X.F.; Liu, M.; Song, Y.F.; Ackland, M.L. Composition, sources, and potential toxicology of polycyclic aromatic hydrocarbons (PAHs) in agricultural soils in Liaoning, People's Republic of China. Environ Monit Assess 2013, 185, 2231-2241.

Carlon, C. Derivation Methods of Soil Screening Values in Europe. A Review and Evaluation of National Procedures Towards Harmonization. European Commission, Joint Research Centre, EUR 22805-EN. 2007, 306.

Case, S.D.; Whitaker, J.; McNamara, N.P.; Reay, D.S. The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil. The role of soil aeration. Soil Biol. Biochem. 2012, 51, 125-134.

Cely, P.; Gascó, G.; Paz-Ferreiro, J.; Méndez, A. Agronomic properties of biochars from different manure wastes. Journal of Analytical and Applied Pyrolysis 2015, 111, 173–182.

Chagger, H.K.; Jones, J.M.; Pourkashanian, M.; Williams, A. The formation of VOC, PAH and dioxins during incineration.Trans I.Chem.E. 2000, 78, 53-59.

Chan, K.Y.; Van Zwieten, L.; Meszaros, I.; Downie A.; Joseph, S. Agronomic values of greenwaste biochar as a soil amendment. Soil Research 2007, 45, 629–634.

Chan, K.Y.; Van Zwieten, L.; Meszaros, I.; Downie, A.; Joseph, S. Using poultry litter biochars as soil amendments. Australian Journal of Soil Research 2008, 46, 437–444

Chan, K.Y. and Xu, Z. Biochar: nutrient properties and their enhancement. In Biochar for Environmental Management: Science and Technology; Lehmann, J., Joseph, S., Eds.; Publisher: Earthscan, London, 2009.

Chen, B. and Chen, Z. Sorption of naphthalene and 1-naphthol by biochars of orange peels with different pyrolytic temperatures. Chemosphere 2009, 76, 127–133.

Cheng, C-H.; Lehmann, J.; Engelhard, M.Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. Geochimica et Cosmochimica Acta 2008, 72, 1598–1610.

Choppala, G. K.; Bolan, N. S.; Megharaj, M.; Chen, Z.; Naidu, R. The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. J. Environ. Qual. 2012, 41, 1175–1184.

Ciais, P.; Sabine, C.; Bala, G.; Bopp, L.; Brovkin, V.; Canadell, J.; Chhabra, A.; DeFries, R.; Galloway, J.; Heimann, M.; Jones, C.; Le Quéré, C.; Myneni, R. B.; Piao, S.; Thornton, P. Carbon and Other Biogeochem. Cy. in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Cimo', G.; Kucerik, J.; Berns, A.E.; Schaumann, G.E.; Alonzo, G.; Conte, P. Effect of Heating Time and Temperature on the Chemical Characteristics of Biochar from Poultry Manure. J. Agric. Food Chem. 2014, 62, 1912-1918.

Conti, R.; Rombolà, A.G.; Modelli, A.; Torri, C.; Fabbri. D. Evaluation of the thermal and environmental stability of switchgrass biochars by Py-GC-MS. Journal of Analytical and Applied Pyrolysis 2014, 110, 239–247.

Cordella, M.; Torri, C.; Adamiano, A.; Fabbri, D.; Barontini, F.; Cozzani, V. Bio-oils from biomass slow pyrolysis: a chemical and toxicological screening. J. Hazard. Mater. 2012, 231–232, 26–35.

Cornelissen, G. and Gustafsson, O. Importance of unburned coal carbon, black carbon, and amorphous organic carbon to phenanthrene sorption in sediments. Environ. Sci. Technol. 2005, 39, 764–769.

Crane-Droesch, A.; Abiven, S.; Jeffery, S.; Torn, M.S. Heterogeneous global crop yield response to biochar: a meta-regression analysis. Environmental Research Letters 2013, 8, 044049.

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

Cross, A. and Sohi, S.P. A method for screening the relative long-term stability of biochar. GCB Bioenergy 2013, 5, 215-220.

Czimczik, C.I. and Masiello, C.A. Controls on black carbon storage in soils, Glob. Biogeochem. Cycles 2007, 21, GB3005.

Day, D.; Evans, R.J.; Lee, J.W.; Reicosky, D. 'Economical CO_2, SO_x and NO_x capture from fossil-fuel utilization with combined renewable hydrogen production and large-scale carbon sequestration'. Energy 2005, 30, 2558–2579.

de Graaff, M.-A.; Classen, A.T.; Castro, H.F.; Schadt, C.W. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. The New phytol 2010, 188, 1055-1064.

De Gryze, S.; Cullen, M.; Durschinger, L.; Lehmann, J.; Bluhm, D.; Six, J. Evaluation of opportunities for generating carbon offsets from soil sequestration of biochar. An issues paper commissioned by the Climate Action Reserve, final version, (2010) http://www.terraglobalcapital.com/press/Soil_Sequestration_Biochar_Issue_Paper1.pdf

de la Rosa, J.M.; Knicker, H.; López-Capel, E.; Manning, D.A.C.; González-Perez, J.A.; González-Vila, F.J. Direct detection of black carbon in soils by Py–GC/MS, ^{13}C NMR spectroscopy and thermogravimetric techniques. Soil Science Society of America Journal 2008, 72, 258-267.

Demirbas, A. and Arin, G. An Overview of Biomass Pyrolysis. Energy Sources 2002, 5, 471-482.

Desaules, A.; Ammann, S; Blum, F.; Brändli, R.C.; Bucheli, T.D.; Keller, A. PAH and PCB in soils of Switzerland–status and critical review. J Environ Monit 2008, 10, 1265-1277.

Di Blasi, C. Modelling chemical and physical processes of wood and biomass pyrolysis. Prog Energ Combust Sci 2008, 34, 47–90.

Ding, W.; Dong, X.; Ime, I.M.; Gao, B.; Ma, L.Q. 2014. Pyrolytic temperatures impact lead sorption mechanisms by bagasse biochars. Chemosphere 2014, 105.

Ding, G.C.; Heuer, H.; Zühlke, S.; Spiteller, M.; Pronk, G.J.; Heister, K.; Kögel-Knabner, I.; Smalla, K. Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes by using a novel PCR detection system. Appl. Environ. Microbiol. 2010, 76, 4765-4771.

Downie, A. E.; L. Van Zwieten, Smernik, R.J.; Morris, S.; Munroe P.R. "Terra Preta Australis: Reassessing the carbon storage capacity of temperate soils." Agriculture, Ecosystems & Environment 2011, 140, 137-147.

Duxbury, C.; Dixon, D.G.; Greenberg, B.M. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemna gibba. Environ. Toxicol.Chem. 1997, 16, 1739–1748.

ECR, European Commission Regulation No 1881/2006 of 19th December (2006). Setting maximum levels for certain contaminants in foodstuffs. Available at: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF. Accessed 12 March 2015.

Elad, Y.; David, D.R.; Harel, Y.M.; Borenshtein, M.; Kalifa, H.B.; Silber, A.; Graber, E.R. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Disease Control and Pest Management 2010, 100, 913–921.

Elmer, W.H. and Pignatello, J. Effect of biochar amendments on mycorrhizal associations and Fusarium crown and root rot of Asparagus in replant soils. Plant Disease 2011, 95, 960–966.

Enell, A.; Reichenberg, F.; Ewald, G.; Warfvinge, P. Desorption kinetics studies on PAH-contaminated soil under varying temperatures. Chemosphere 2005, 61, 1529-1538.

Enders, A.; Hanley, K.; Whitman, T.; Joseph, S.; Lehmann, J. Characterization of biochars to evaluate recalcitrance and agronomic performance. Bioresource Technology 2012, 114, 644-653.

European Biochar Certificate Guidelines for a Sustainable Production of Biochar. Version 5 of 1st December 2014 (2014). http://www.european-biochar.org/biochar/media/doc/ebc-guidelines.pdf Accessed 5 March 2015.

Fabbri, D.; Rombolà, A.G.; Torri, C.; Spokas, K.A. Determination of polycyclic aromatic hydrocarbons in biochar and biochar amended soil. J. Anal. Appl. Pyrol. 2013, 103, 60-67.

Fabbri, D.; Torri, C.; Spokas, K.S. Analytical pyrolysis of synthetic chars derived from biomass with potential agronomic application (biochar). Relationships with impacts on microbial carbon dioxide production. J. Anal. Appl. Pyrol. 2012, 93, 77-84.

Fabbri, D.; Adamiano, A.; Torri, C. GC-MS determination of polycyclic aromatic hydrocarbons evolved from pyrolysis of biomass, Anal. Bioanal. Chem. 2010, 397, 309–317.

Fearnside, P. M. Global warming and tropical land-use change: greenhouse gas emissions from biomass burn-ing, decomposition and soils in forest conversion, shifting cultivation and secondary vegetation. Climatic Change 2000, 46, 115–158.

Fellet, G.; Marchiol, L.; Delle Vedove, G.; Peressotti, A. Application of biochar on mine tailings: effects and perspectives for land reclamation. Chemosphere 2011, 83, 1262–1297.

Freddo, A.; Cai, C.; Reid B.J. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environ. Pollut. 2012, 171, 18-24.

Flores-Cervantes, D.X.; Largeau, C.; Rouzaud, J.-N.; Rumpel, C.; Guggenberger, G.; Kaiser, K.; Rodionov, A.; Gonzalez-Vila, F.J.; Gonzalez-Perez, J.A.; de la Rosa, J.M.; Manning, D.A.C.; López-Capél, E.; Ding, L. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. Global Biogeochemistry Cycles 2007, 21, GB3016.

Fraser, M.P., Cass, G.R.; Simoneit, B.R.T.; Rasmussen, R.A. Air quality model evaluation data for organics. 5. C6 – C22 nonpolar and semipolar aromatic compounds. Environ. Sci. Technol. 1998, 32, 1760–1770.

Free, H.F.; McGill C.R.; Rowarth J.S.; Hedley M.J. The effect of biochars on maize (Zea mays) germination. New Zeal. J. Agr. Res. 2010, 53, 1–4.

Freddo, A.; Cai, C.; Reid, B.J. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environ. Pollut. 2012, 171, 18-24.

Funke, A. and Ziegler, F. Hydrothermal carbonization of biomass: a summary and discussion of chemical mechanisms for process engineering. Biofuels Bioprod Biorefining 2010, 4, 160–177.

Galinato, S.P.; Yoder, J.K.; Granatstein, D. The economic value of biochar in crop production and carbon sequestration. Energy Policy 2011, 39, 6344-6350.

Gessa, C. and Ciavatta, C. Complesso di scambio. In: Metodi di analisi chimica del suolo, Ministero delle Politiche Agricole, Alimentari e Forestali Coord. P. Violante. Ed. F. Angeli 2000, 13, 1-31.

Gfrerer, M.; Serschen, M.; Lankmayr, E. Optimized extraction of polycyclic aromatic hydrocarbons from contaminated soil samples. J. Biochem. Biophys. Method. 2002, 53, 203–216.

Ghidotti, M.; Conti, R.; Fabbri, D.; Hornung A. Molecular analysis of extractable fraction of biochar. Ecomondo, a mediterranean platform for the sustainable growth. Fabio Fava Ed. Rimini (I) November 5-8, Maggioli, 2014, 49-54.

Ghidotti, M.; Fabbri, D.; Hornung A."SPME-GC-MS determination of mobile compounds in biochar" submitted to the Biorefinery I: Chemicals and Materials From Thermo-Chemical Biomass Conversion and Related Processes. A New ECI Conference Series September 27-October 2, 2015. Chania (Crete), Greece

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

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

Goldberg, E.D. Black Carbon in the Environment. Properties and Distribution. Wiley-Interscience, 1985, New York.

Gomez-Eyles, J. L.; Sizmur, T.; Collins, C. D.; Hodson, M. E. Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environ. Pollut. 2011, 158, 278-284.

Goyal, H.B.; Seal, D.; Saxena, R.C. Bio-fuels from thermochemical conversion of renewable resources: a review Renew Sustain Energy Rev 2008, 12, 504–517.

Graber, E.R.; Harel, Y.M.; Kolton, M., Cytryn, E.; Silber, A.; David, D.R.; Tsechansky, L.; Borenshtein, M.; Elad, Y. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant and Soil 2010, 337, 481-496.

Grønli, M.G.; Várhegyi, G.; Di Blasi, C. Thermogravimetric analysis and devolatilization kinetics of wood. Ind Eng Chem Res 2002, 41, 4201–4208.

Güereña, D.; Lehmann, J.; Hanley, K.; Enders, A.; Charles Hyland, C.; Riha, S. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. Plant Soil 2014, 365 (1–2), 239-254.

Guggenberger, G.; Rodionov, A.; Shibistova, O.; Grabe, M.; Kasansky, O.A.; Fuchs, H.; Mikheyeva, N.; Zhazhevskaya, G.; Flessa, H. Storage and mobility of black carbon in permafrost soils in the forest tundra ecotone in northern Siberia, Global Change Biology 2008, 1, 1367–1381.

Gundale, M. J. and T. H. DeLuca, T.H. Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/douglas fir ecosystem. Biol Fertil Soils 2007, 43, 303-311.

Guo, J.S.; Ntakirutimana, T.; Gao, X.; Gong, D.C. Sources, toxicity, potential cancer risk assessment and analytical methods for monitoring of polycyclic aromatic hydrocarbons. Adv. Mater. Res. 2012, 524-527, 1739-1750.

Gurwick, N. P.; Kelly, C.; Elias, P The Scientific Basis for Biochar as a Climate Change Mitigation Strategy: Does it Measure Up? Union of Concerned Scientists 2012. Available

at: http://www.ucsusa.org/global_warming/solutions/forest_solutions/biochar-climate-change-mitigation-strategy.html.

Gurwick, N.P.; Moore, L.A.; Kelly, C.; Elias, P. A systematic review of biochar research, with a focus on its stability in situ and its promise as a climate mitigation strategy. PLoS One 2013, 8. Doi:10. 1371/journal.pone.0075932.

Gusev, A.; Rozovskaya, O.; Shatalov, V.; Sokovyh, V.; Aas, W.; Breivik, K.; Halse, A.K. Persistent Organic Pollutants in the Environment. EMEP Status Report 2008, 3.

Hale, S.E.; Hanley, K.; Lehmann, J.; Zimmerman, A.; Cornelissen, G. Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar. Environ. Sci. Technol. 2011, 45, 10445–10453.

Hale, S.E.; Lehmann, J.; Rutherford, D.; Zimmerman, A.R.; Bachmann, R.T.; Shitumbanuma, V.; O'Toole, A.; Sundqvist, K.L.; Arp, H.P.H.; Cornelissen, G. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. Environ. Sci. Technol. 2012, 46, 2830-2838.

Hamer, U.; Marschner, B.; Brodowski, S.; Amelung, W. Interactive priming of black carbon and glucose mineralization. Organic Geochemistry 2004, 35, 823-830.

Hammes, K.; Smernik, R.J.; Skjemstad, J.O.; Herzog, A.; Vogt, U.F.; Scminidt, M.W.I. Synthesis and characterization of laboratory-charred grass straw (Oryza sativa) and chestnut wood (Castenea sativa) as reference materials for black carbon quantification. Org. Chem. 2006, 37, 1629-1633.

Hammes, K.; Schmidt, M.W.I.; Smernik, R.J.; Currie, L.A.; Ball, W.P.; Nguyen, T.H; Louchouarn, P.; Houel, S.; Gustafsson, Ö.; Elmquist, M.; Cornelissen, G.; Skjemstad, J.O.; et al. Temperature sensitivity of black carbon decomposition and oxidation. Environmental Science and Technology 2010, 44, 3324-3331.

Hardy Schulz, H.; Glaser, B. Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. J. Plant Nutr. Soil Sci. 2012, 175, 410–422

Haub, C. and Gribble J. Population Bulletin. Population Reference Bureau 2011, 60 (2).

Hilber, I.; Wyss, G.S.; Mäder, P.; Bucheli, T.D.; Meier, I.; Vogt, L.; Schulin. R. Influence of activated charcoal amendment to contaminated soil on dieldrin and nutrient uptake by cucumbers. Environ. Pollut. 2009, 157, 2224–2230.

Hilber, I.; Blum, F.; Leifeld, J.; Schmidt, H.-P.; Bucheli, T.D. Quantitative determination of PAHs in biochar: a prerequisite to ensure its quality and safe application. Journal of Agricultural and Food Chemistry 2012, 60, 3042–3050.

Hilscher, A. and Knicker, H. Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology & Biochemistry 2011, 43 (2), 261-270.

Himanen, M.; Prochazka, P.; Hänninen, K.; Oikari, A. Phytotoxicity of low-weight carboxylic acids. Chemosphere 2012, 88, 426–431.

Hoekstra, N.J.; Bosker, T.; Lantinga, E.A. Effects of cattle dung from farms with different feeding strategies on germination and initial root growth of cress (Lepidium sativum L.) Agriculture. Ecosystems and Environment 2002, 93, 189–196.

Hossain, M.K.*;* Strezov, V.*;* Yin Chan, K.*;* Nelson, P.F. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (Lycopersicon esculentum). Chemosphere *2010,* 78, 1167–1171.

Hu, B.; Wang, K.; Wu, L.; YuS-H.; Antonietti, M.; Titirici, M-M. Engineering carbon materials from the hydrothermal carbonization process of biomass. Adv Mater 2010, 22, 813–828.

Hua, L.; Wu, W.X.; Liu, Y.X.; McBride, M.; Chen, Y.X. Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. Environ. Sci. Pollut. Res. 2009, 16, 1-.9

Ibrahim, H. M.; Al-Wabel, M. I.; Usman, A. R. A.; Al-Omran, A. Effect of Conocarpus biochar application on the hydraulic properties of a sandy loam soil. Soil Sci. 2013, 178, 165–173.

International Biochar Initiative (IBI), Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used In Soil, 2012.

International Energy Agency, 2011. World Energy Outlook 2011.

International energy outlook, 2014. U.S. Energy Information Administration 2014.

Inyang, M.; Gao, B.; Zimmerman, A.; Zhang, M.; Chen, H. Synthesis, characterization, and dye sorption ability of carbon nanotube–biochar nanocomposites. Chem. Eng. J. 2004, 236, 39–46.

Imam, T.; Capareda, S. Characterization of bio-oil, syn-gas and bio-char from switchgrass pyrolysis at various temperatures. Journal of Analytical and Applied Pyrolysis 2012, 93 170-177.

James, G.; Sabatini, D.A.; Chiou, C.T.; Rutherford, D.; Scott, A.C. Karapanagioti, H.K. Evaluating phenanthrene sorption on various wood chars. Water Res. 2005, 39, 549–558.

Jeffery, S.; Verheijen, F.G.A.; Van der Velde, M., Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems and Environment 2011, 144, 175–187.

Jeffrey M. Novak, Keri B. Cantrell, Donald W. Watts, Warren J. Busscher, Mark G. Johnson. Designing relevant biochars as soil amendments using lignocellulosic-based and manure-based feedstocks. J Soils Sediments, 2014, 14, 330–343.

Jonker, M.T.O. and Koelmans, A.A. Extraction of polycyclic aromatic hydrocarbons from soot and sediment: solvent evaluation and implications for sorption. Environ. Sci. Technol. 2002, 36, 4107–4113.

Jones, S.B; Valkenburg, C; Walton, C.W.; Elliott, D.C.; Holladay, J.E.; Stevens, D.J; Kinchin, C; Czernik, S. Production of gasoline and diesel from biomass via fast pyrolysis, hydrotreating and hydrocracking: a design case. Pacific Northwest National Laboratory, Richland, WA (2009).

Joseph, S.; Graber, E.R.; Chia, C.; Munroe, P.; Donne, S.; Thomas, T.; Nielsen, S.; Marjo, C.; Rutlidge, H.; Pan, G.X.; Li, L.; Taylor, P.; Rawal, A.; Hook, J. Shifting paradigms: development of high-efficiency biochar fertilizers based on nano-structures and soluble components. Carbon Manag. 2013, 4, 323–343.

Kaal, J.; Brodowski, S.; Baldock, J.A.; Nierop, K.G.J.; Martínez Cortizas, A. Characterisation of aged black carbon using pyrolysis–GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarisation ^{13}C nuclear magnetic resonance (DP/CP NMR) and the benzene polycarboxylic acid (BPCA) method. Organic Geochemistry 2008, 39, 1415-1426.

Kaal J., Rumpel C. Can pyrolysis-GC/MS be used to estimate the degree of thermal alteration of black carbon? Organic Geochemistry 2009, 40, 1179–1187.

Kaal, J.; Schneider, M.P.W.; Schmidt, M.W.I. Rapid molecular screening of black carbon (biochar) thermosequences obtained from chestnut wood and rice straw: a pyrolysis–GC/MS study. Biomass Bioenergy 2012, 45, 115-129.

Katsoyiannis, A.; Sweetman, A.J.; Jones, K.C. PAH molecular diagnostic ratios applied to atmospheric sources: a critical evaluation using two decades of source inventory and air concentration data from the UK. Environ Sci Technol 2011, 45, 8897-8906.

Kealan, G.; van Groenigen, J.W.; Cayuela, M.L. Residues of bioenergy production chains as soil amendments: Immediate and temporal phytotoxicity. Journal of Hazardous Materials 2011, 186, 2017–2025.

Keiluweit, M.; Kleber, M; Sparrow, M.A.; Simoneit, B.R.T.; Prahl, F.G. Solvent-Extractable Polycyclic Aromatic Hydrocarbons in Biochar: Influence of Pyrolysis Temperature and Feedstock. Environ. Sci. Technol. 2012, 46, 9333-9341.

Keiluweit, M.; Nico Pete, S.; Johnson Mark, G.; Kleber, M. Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environ Sci Technol 2010, 44, 1247-1253.

Kim, E.-J.; Oh, J.-E; Chang Y.-S. Effects of forest fire on the level and distribution of PCDD/Fs and PAHs in soil. Sci. Total Environ. 2013, 311, 177–189.

Kipopoulou, A.M.; Manoli, E.; Samara, C. Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environ. Pollut. 1999, 106, 369–380.

Kloss, S.; Zehetner, F.; Dellantonio, A.; Hamid, R.; Ottner, F.; Liedtke, V.; Schwanninger, M; Gerzabek, M.H.; Soja, G.; Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. Journal of Environmental Quality 2012, 41, 990–1000.

Kolb, S.E.; Fermanich, K.J.; Dornbush, M.E. Effect of charcoal quantity on microbial biomass and activity in temperate soils. Soil Sci. Soc. Am. J. 2009, 73 (4), 1173-1181.

Kloss, S.; Zehetner, F.; Dellantonio, A.; Hamid, R.; Ottner, F.; Liedtke, V.; Schwanninger, M.; Gerzabek, M.H.; Soja, G. Characterization of Slow Pyrolysis Biochars: Effects of Feedstocks and Pyrolysis Temperature on Biochar Properties. J. Environ. Qual. 2012, 41, 990-1000.

Knicker, H.; Müller, P.; Hilscher, A. How useful is chemical oxidation with dichromate for the determination of ‘‘black carbon’’ in fire-affected soils? Geoderma 2007, 142, 178-196.

Kołtowski, M.; Oleszczuk, P. Toxicity of biochars after polycyclic aromatic hydrocarbons removal by thermal treatment. Ecological Engineering 2015, 75, 79–85.

Kookana, R.S.; Sarmah, A.K.; Van Zwieten, L.; Krull, E.; Singh, B. Chapter three - biochar application to soil: agronomic and environmental benefits and unintended consequences, In: Donald LS, editor. Advances in agronomy. San Diego: Academic Press, 2011, 103-143.

Krang, A.S. Naphthalene disrupts pheromone induced mate search in the amphipod Corophium volutator (Pallas). Aquat. Toxicol. 2007, 85, 9–18.

Krull, E.S.; Baldock, J.A.; Skjemstad, J.O.; Smernik, R.J. Characteristics of biochar: Organo-chemical properties. In: Biochar for environmental management: Science and technology. Lehmann, J. and S. Joseph (eds). Earthscan, London, UK and Sterling, 2009, 53-65.

Kumar, A.; Jones, D.; Hanna, M. Thermochemical biomass gasification: a review of the current status of the technology. Energies 2009, 2, 556–581.

Kuriakose, S.V.; Prasad, M.N.V. Cadmium stress affects seed germination and seedling growth in Sorghum bicolor L. Moench by changing the activities of hydrolyzing enzymes. Plant Growth Regul. 2008, 54, 143–156.

Kruse, A.; Funke, A.; Titirici, MM. Hydrothermal conversion of biomass to fuels and energetic materials. Curr Opin Chem Biol 2013, 17, 515–521.

Kusmierz, M. and Oleszczuk, P. Biochar production increases the polycyclic aromatic hydrocarbon content in surrounding soils and potential cancer risk. Sci. Pollut. Res Int. 2014, 21, 3646-3652.

Kuzyakov, Y.; Subbotina, I.; Chen, H.Q.; Bogomolova, I.; Xu, X.L. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 2009, 41, 210-219.

Kuzyakov, Y.; Bogomolova, I.; Glaser B. Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. Soil Biol. Biochem. 2014, 70, 229–236.

Kwon, H.O.; Choi, S.D. Polycyclic aromatic hydrocarbons (PAHs) in soils from a multi-industrial city, South Korea. Sci. Total Environ. 2014, 470–471, 1494-1501.

Labbe, N.; Harper, D.; Rials, T.; Elder, T. Chemical structure of wood charcoal by infrared spectroscopy and multivariate analysis. Journal of Agricultural and Food Chemistry 2006, 54, 3492-3497.

Laird, D.A.; Brown, R.C.; Amonette, J.E.; Lehmann, J. Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels Bioprod Biorefining 2009, 3, 547–562.

Laird, D.; Fleming, P.; Wang, B; Horton, R.; Karlen, D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 2010, 158, 436–442.

Lal, R. Sequestration of atmospheric CO_2 in global carbon pools. Energy & Env. Sci. 2008, 1, 86-100.

Lattao, C.; Cao, X.; Mao, J.; Schmidt-Rohr, K.; Pignatello, J. J. Influence of molecular structure and adsorbent properties on sorption of organic compounds to a temperature series of wood chars. Environ. Sci. Technol. 2014, 48 (9), 4790-4798.

Lee, J.W.; Kidder, M.; Evans, B.R.; Paik, S.; Buchanan III, A.C.; Garten, C.T.; Brown, R.C. Characterization of Biochars Produced from Cornstovers for Soil Amendment. Environ. Sci. Technol. 2010 , 44, 7970-7974.

Lehmann, J.; Gaunt, J.; Rondon, M. Bio-char sequestration in terrestrial ecosystems - a review. Mitig. Adapt. Strategy Glob, Change 2006, 11, 403-427.

Lehmann, J. A Handful of carbon. Nature 2007, 447, 143–144.

Lehmann, J. and Joseph, S. Biochar for environmental management: An introduction. In: J. Lehmann and S. Joseph, editors, Biochar for environmental management: Science and technology. Earthscan, London. 2009, 1-12.

Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota – A review. Soil Biology & Biochemistry 2011, 43, 1812–1836

Lei, F.-F.; Huang, J.-Y.; Zhang, X.-N.; Liu, X.-J.; Li, X.-J. Determination of polycyclic aromatic hydrocarbons in vegetables by headspace SPME-GC. Chromatographia 2011, 74, 99–107.

Li, F.; Cao, X.; Zhao, L.; Wang, J.; Ding, Z. Effects of Mineral Additives on Biochar Formation: Carbon Retention, Stability, and Properties. Environ. Sci. Technol. 2014, 48 (19), 11211-11217.

Liang, B.; Lehmann, J.; Solomon, D.; Kinyangi, J.; Grossman, J.; O'Neill, B.; Skjemstad, B.J.O.; Thies, J.; Luizão, F.J.; Petersen, J.; Neves E.G. Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 2006, 70, 1719–1730.

Libra, J.A.; Ro, K.S.; Kammann, C.; Funke, A.; Berge, N.D.; Neubauer, Y.; Titirici, M.M.; Fühner, C.; Bens, O.; Kern, J.; Emmerich, K.H. Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. Biofuels, 2011, 2, 89–124.

Linsinger, T. Comparison of a measurement result with the certified value, Application Note 1 EC-JRS IRMM (European Commission-Joint Research Center Institute for Reference Materials and Measurements), 2005 revision Jan 2010, Geel Belgium, 2 pp (available at: http://irmm.jrc.ec.europa.eu/).

Liu, P.; Liu, W.-J.; Jiang, H.; Chen, J.-J.; Li, W.-W.; Yu, H.-Q. Modification of biochar derived from fast pyrolysis of biomass and its application in removal of tetracycline from aqueous solution. Bioresour. Technol 2012, 121, 235–240.

Liu, L.; Chen, P.; Sun, M.; Shen, G.; Shang, G. Effect of biochar amendment on PAH dissipation and indigenous degradation bacteria in contaminated soil. Journal of Soils and Sediments 2015, 15, 313-322.

Liu, X.; Zhang, A., Ji; C.; Joseph, S.; Bian, R.; Li, L.; Pan, G.; Paz-Ferreiro, J. Biochar's effect on crop productivity and the dependence on experimental conditions—a meta-analysis of literature data. Plant Soil 2013, 373, 583–594.

Loibner, A.P.; Szolar, O.H.J.; Braun, R.; Hirmann, D. Toxicity testing of 16 priority polycyclic aromatic hydrocarbons using Lumistox. Environ. Toxicol. Chem. 2004, 23, 557–564.

Love, G.D.; Snape, C.D.; Carr, A.D.; Houghton, R.C. Release of covalently-bound alkane biomarkers in high yields from kerogen via catalytic hydropyrolysis. Organic Geochemistry 1995, 23, 981-986.

Lu, H; Zhang, Y.Y.; Huang, X.; Wang, S; Qiu, R. Relative distribution of Pb^{2+} sorption mechanisms by sludge-derived biochar. Wat Res 2012, 46, 854–862.

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

Maliszewska-Kordybach, B.; Smreczak, B.; Klimkowicz-Pawlas, A.; Terelak, H. Monitoring of the total content of polycyclic aromatic hydrocarbons (PAHs) in arable soils in Poland. Chemosphere 2008, 73, 1284-1291.

Maliszewska-Kordybach, B.; Smreczak, B.; Klimkowicz-Pawlas, A. Concentrations, sources, and spatial distribution of individual polycyclic aromatic hydrocarbons (PAHs) in agricultural soils in the Eastern part of the EU: Poland as a case study. Science of the Total Environment 2009, 407, 3746-3753.

Manyà J.J. Pyrolysis for biochar purposes: a review to establish current knowledge gaps and research needs. Environ Sci Technol 2012, 46, 7939–7954.

Mara Dos Santos Barbosa, J.; Ré-Poppi, N.; Santiago-Silva, M. Polycyclic aromatic hydrocarbons from wood pyrolyis in charcoal production furnaces. Environ. Res. 2006, 101, 304–311.

Maschio, G.; Koufopanos, C.; Lucchesi, A. Pyrolysis, a promising route for biomass utilization. Bioresour. Technol. 1992, 42, 219 − 231.

Mašek, O.; Brownsort, P.; Cross, A.; Sohi, S. Influence of production conditions on the yield and environmental stability of biochar. Fuel 2013, 103, 151–155.

Masiello, C.A New directions in black carbon organic geochemistry. Mar. Chem. 2004, 92, 201–213.

Matsubara, Y.; Hasegawa, N.; Fukui H. Incidence of fusarium root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. Journal of Japanese Society of Horticultural Science 2002, 71, 370–374.

Mazurek, M.A. and Simoneit, B.R.T. Characterization of biogenic and petroleum derived organic matter in aerosols over remote, rural and urban areas. In: Identification and Analysis of Organic Pollutants in Air, ACS Symp., L. H. Keith (ed.) Ann Arbor Science/Butterworth Publishers, Woburn, MA., 1984, 353-370.

McBeath, A.V.; Smernik, R.J. Variation in the degree of aromatic condensation of chars. Organic Geochemistry 2009, 40, 1161-1168.

McBeath, A.V.; Wurster, C.M.; Bird, M.I. Influence of feedstock properties and pyrolysis conditions on biochar carbon stability as determined by hydrogen pyrolysis. Biomass and Bioenergy 2015, 73, 155-173.

Méndez, A.; Barriga, S.; Fidalgo, J. M.; Gascó, G. Adsorbent materials from paper industry waste materials and their use in Cu(II) removal from water. J. Hazard. Mater. 2009, 165, 736–743.

Mengel, K.; Kirkby, E.A. Principles of Plant Nutrition, 4th Edition. International Potash Institute, Bern, 1987, 745.

Meudec, A.; Dussauze, J.; Deslandes, E.; Poupart, N. Evidence for bioaccumulation of PAHs within internal shoot tissues by a halophytic plant artificially exposed to petroleum-polluted sediments. Chemosphere 2006, 65, 474–481.

Meyer, S.; Glaser, B.; Quicker, P. Technical, economical, and climate-related aspects of biochar production technologies: a literature review. Environ Sci Technol 2011, 45, 9473–9483.

Meredith, W.; Ascough, P.L.; Bird, M.I.; Large, D.J.; Snape, C.E.; Sun, Y.; Tilston, E.L. Assessment of hydropyrolysis as a method for the quantification of black car-bon using standard reference materials. Geochimica et Cosmochimica Acta 2012, 97, 131-147.

Meredith, W.; Ascough, P.L.; Bird, M.I.;. Large, D.J.; Snape, C.E.; Song, J.; Sun, Y.; Tilston, E.L. Direct evidence from hydropyrolysis for the retention of long alkyl moieties in black carbon fractions isolated by acidified dichromate oxidation. J. Anal. Appl. Pyrolysis 2013, 103, 232–239.

Michel, K.; Terhoeven-Urselmans, T.; Nitschke, R.; Steffan, P.; Ludwig, B. Use of near- and mid-infrared spectroscopy to distinguish carbon and nitrogen originating from char and forest-floor material in soils. Journal of Plant Nutrition and Soil Science 2009, 172, 63-70.

Mohan, D.; Pittman, C.U.; Steele, P.H. Pyrolysis of wood/biomass for bio-oil: a critical review. Energy Fuels, 2006, 20, 848–889.

Mortensen, P. M.; Grunwaldt, J. D.; Jensen, P.A.; Knudsen, K.G.; Jensen A.D. A review of catalytic upgrading of bio-oil to engine fuels. Appl Catal A 2011, 407, 1–19.

Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 2005, 96, 673–686

Motelay-Massei, A.; Garban, B.; Tiphagne-larcher, B.; Chevreuil, M. Ollivon, D. Mass balance for polycyclic aromatic hydrocarbons in the urban watershed of Le Havre (France): Transport and fate of PAHs from the atmosphere to the outlet. Water Res. 2006, 40, 1995–2006.

Muralidhara, H.S. Conversion of tannery waste to useful products. Resources and Conservation *1982,* 8, 43–59.

Mutsazawa, S.; Nasser-Ali, L.; Garrigues, P. Photolytic Behavior of polycyclic aromatic hydrocarbons in diesel particulate matter deposited on the Ground. Environmental Science and Technology 2001, 35, 3139-3143.

Nam, J.J.; Thomas, G.O.; Jaward, F.M.; Steinnes, E.; Gustafsson, O.; Jones, K.C. PAHs in background soils from Western Europe: influence of atmospheric deposition and soil organic matter. Chemosphere 2008, 70, 1596-1602.

Nam, J.J.; Sweetman, A.J.; Jones, K.C. Polynuclear aromatic hydrocarbons (PAHs) in global background soils. J. Environ. Monit. 2009, 11, 45–48.
Nguyen, B.T.; Lehmann, J. Black carbon decomposition under varying water regimes. Org Geochem 2009, 40, 846-853.

Noordermeer, M.A. and Petrus, L. Biomass to biofuels, a chemical perspective. Green Chem 2006, 8, 861–867.

Nunoura, T.; Wade, S. R.; Bourke, J.; Antal, M. J. Studies of the flash carbonization process. 1. Propagation of the flaming pyrolysis reaction and performance of a catalytic afterburner. Ind. Eng. Chem. Res. 2006, 45, 585−599.

Obst, M.; Grathwohl, P.; Kappler, A.; Eibl, O.; Peranio, N; Gocht T. Quantitative high-resolution mapping of phenanthrene sorption to black carbon particles. Environ. Sci. Technol. 2011, 45, 7314–7322.

Oen, A.M.P.; Cornelissen, G.; Breedveld, G.D. Relation between PAH and blackcarbon contents in size fractions of Norwegian harbor sediments. Environ. Pollut. 2006, 141, 370–380.

Ogawa, M.; Okimori, Y.; Takahashi, F. Carbon sequestration by carbonization of biomass and forestation: Three case studies. Mitigat. Adaptat. Strateg. Global Change 2006, 11, 429–444.

Oleszczuk, P.; Hale, S.E. Lehmann, J.; Cornelissen, G. Activated carbon and biochar amendments decrease pore-water concentrations of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge. Biores. Technol. 2012, 111, 84–91.

Oleszczuk, P.; Josko, I.; Kusmierz, M. 2013. Biochar properties regarding to contaminants content and ecotoxicological assessment. J. Hazard. Mater. 2013, 260, 375-382.

Olson, P.E.; Castro, A.; Joern, M.; Du Teau, N.M.; Pilon-Smits, E.; Reardon, K.F. Effects of agronomic practices on phytoremediation of an aged PAH-contaminated soil. Journal of Environmental Quality 2008, 37, 1439-1446.

Onay, Ö.; Beis, S. H.; Koçkar, Ö. M. Fast pyrolysis of rape seed in a well-swept fixed bed reactor. J. Anal. Appl. Pyrolysis 2001, 58 −59, 995 − 1007.

Onay, O.; Kockar, O.M. Slow, fast and flash pyrolysis of rapeseed Renew Energy 2003, 28, 2417–2433

Paavola, T.; Rintala, J. Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour. Technol 2008, 99, 7041–7051.

Pacala, S. and R. Socolow, R. Stabilization wedges: Solving the climate problem for the next 50 years with current technologies. Science 2004, 305, 968-972.

Pan, G. X.; Lin, Z. H.; Zhang, A. F.; Zheng, J. W.; Zhang, X. H. Perspective on biomass carbon industrialization of organic waste from agriculture and rural areas in China. J. Agric. Sci. Tech. 2011, 13, 75–82.

Park, J. H.; Choppala, G. H.; Lee, S. J.; Bolan, N.; Chung, J. W.; Edraki, M. Comparative sorption of Pb and Cd by biochars and its implication for metal immobilization in soil. Water Air Soil Poll. 2013, 224, 1711.

Park, J.H.; Choppala, G.K.; Bolan, N.S.; Chung, J.W.; Chuasavathi, T. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant and Soil 2011, 348, 439–451.

Parrish, Z.D.; White, J.C.; Isleyen, M.;Gent, M.P.N.; Iannucci-Berger, W. ; Eitzer, B.D.; Kelsey, J.W.; Mattina, M.I. Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species. Chemosphere 2006, 64, 609–618.

Parsons, T. and Rowarth, J. Grassland newsletter 2009.

Pena, M.T.; Casais, M.C.; Mejuto, M.C.; Cela, R. Optimization of the matrix solid-phase dispersion sample preparation procedure for analysis of polycyclic aromatic hydrocarbons in soils: Comparison with microwave-assisted extraction. J. Chromatogr. A 2007, 1165, 32–38.

Poerschmann, J.; Fabbri, D.; Górecki, T. Investigation of the solvent extracts of humic organic matter (HOM) isolated from the Ravenna Lagoon to study environmental pollution and microbial communities. Chemosphere 2007, 70, 206–214.

Preston, C.M. and Schmidt, M.W.I. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeoscience 2006, 3, 397–420.

Qian, K; Kumar, A; Patil, K.; Bellmer, D; Wang, D.; Yuan, W.; Huhnke, R.L. Effects of biomass feedstocks and gasification conditions on the physiochemical properties of char. Energies 2013, 6, 3972–3986.

Quilliam, R.S.; Rangecroft, S.; Emmett, B.A. Is biochar a source or sink for polycyclic aromatic hydrocarbon (PAH) compounds in agricultural soils? GCB Bioenergy 2013, 5, 96-103.

Ralph, J. and Hatfield, R.D. Pyrolysis-GC-MS characterization of forage materials. J. Agric. Food Chem. 1991, 39, 1426-1437.

Rajkovich, S.; Enders, A.; Hanley, K.; Hyland, C.; Zimmerman, A.R.; Lehmann, J. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biology and Fertility of Soils 2011, 48, 271-284.

Reddy, N.; Yang, Y. Preparation and characterization of long natural cellulose fibers from wheat straw. Journal Agricultural & Food Chemistry 2007, 55(21), 8570-8575.

Ré-Poppi, N.; Santiago-Silva, M.; Identification of polycyclic aromatic hydrocarbons and methoxylated phenols in wood smoke emitted during production of charcoal. Chromatographia 2002, 55, 475–481.

Rey-Salgueiro, L.; Martínez-Carballo, E.; García-Falcón, M.S.; González-Barreiro, C.; Simal-Gándara, J. Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. Food Chemistry 2009, 115, 814–819.

Rhodes, A.H.; Carlin, A.; Semple, K.T. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science & Technology 2008, 42, 740-745.

Rhodes, A.H.; McAllister, L.E.; Chen, R.; Semple, K.T. Impact of activated charcoal on the mineralisation of 14C-phenanthrene in soils. Chemosphere 2010, 79, 463-469.

Rickels, W.; Klepper, G.; Dovern, J.; Betz, G.; Brachatzek, N.; Cacean, S.; Gussow, K.; Heintzenberg, J.; Hiller, S.; Hoose, C.; Leisner, T.; Oschlies, A.; Platt, U.; Proel□, A.; Renn, O.; Schafer, S.; Zurn, M. Large-Scale Intentional Interventions into the Climate System? Assessing the Climate Engineering Debate. Scoping report conducted on behalf of the German Federal Ministry of Education and Research (BMBF). Kiel Earth Institute, Kiel, 2011.

Rogovska, N.; Laird, D.; Cruse, R.M.; Trabue, S.; Heaton, E. Germination tests for assessing biochar quality. J. Environ. Qual. 2012, 41, 1014-1022.

Ronsse, F.; van Hecke, S.; Dickinson, D; Prins, W. Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions. Global Change Biology and Bioenergy 2013, 5, 104-115.

Routa, J.; Kellomaki, S.; Strandman, H. Effects of forest management on total biomass production and CO_2 emissions from use of energy biomass of norway spruce and scots pine. Bioenerg Res 2012, 5, 733–747.

Rutigliano, F.A.; Romano, M.; Marzaioli, R.; Baglivo, I.; Baronti, S.; Miglietta, F.; Castaldi, S. Effect of biochar addition on soil microbial community in a wheat crop. Eur. J. Soil Biol. 2014, 60, 9-15.

Sabaté, J.; Viñas, M.; Solanas, A.M. Bioavailability assessment and environmental fate of polycyclic aromatic hydrocarbons in biostimulated creosote contaminated soil. Chemosphere 2006, 63, 1648-1659.

Scharenbroch, B.C.; Meza, E.N.; Catania, M.; Fite, K. Biochar and biosolids increase tree growth and improve soil quality for urban landscapes. J. Environ. Qual. 2013, 42, 1372.

Schenkel, Y.; Bertaux, P.; Vanwijnbserghe, S.; Carre, J. An evaluation of the mound kiln carbonization technique. Biomass Bioenergy 1998, 14, 505−516.

Schimmelpfennig, S.; Glaser, B. One step forward toward characterization: some important material properties to distinguish biochars, J. Environ. Qual. 2012, 41, 1001-1013.

Shackley, S.; Carter, S.; Knowles, T.; Middelink, E.; Haefele, S.; Sohi, S.; Cross, A.; Haszeldine S. Sustainable gasification-biochar systems? A case-study of rice-husk gasification in Cambodia, Part 1: Context, chemical properties, environmental and health and safety issues. Energy Policy 2012, 42, 49–58.

Shackley, S.; Ibarrola, R.; Hammond, J.; Hopkins D. Biochar Quality Mandate. British Biochar Foundation (BRAF), UK (2013).

Shu, Y.Y.; Lai, T.L.; Lin, H.; Yang, T.C.; Chang, C. Study of factors affecting on the extraction efficiency of polycyclic aromatic hydrocarbons from soils using open-vessel focused microwave-assisted extraction. Chemosphere 2003, 52, 1667–1676.

Simoneit, B.R.T. and Mazurek, M. Organic matter of the troposphere-II. Natural background of biogenic lipid matter in aerosols over the rural Western United States. Atmospheric Environment 1982, 16, 2139–2159.

Simoneit, B. R.T.; Organic matter of the troposphere-V. Application of molecular marker analysis to biogenicemissions into the troposphere for source reconciliations. Journal of Atmospheric Chemistry 1986, 8, 251–275

Singh, B.P.; Hatton, B.J.; Balwant, S.; Cowie, A.L.; Kathuria, A. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. Journal of Environmental Quality 2010, 39, 1224–1235.

Singh, B.P.; Cowie, A.L.; Smernik, R.J. Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. Environ Sci Technol 2012, 46, 11770-11778.

Slavich, P.G.; Sinclair, K.; Morris, S.G.; Kimber, S.W.L.; Downie, A.; Zwieten, L.V. Contrasting effects of manure and green waste biochars on the properties of an acidic ferralsol and productivity of a subtropical pasture. Plant Soil 2013, 366, 213-227.

Smith Cameron R., Rachel L. Sleighter, Patrick G. Hatcher, and James W. Lee. Molecular Characterization of Inhibiting Biochar Water Extractable Substances Using Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Environ. Sci. Technol., 2013, 47, 13294-13302.

Solaiman, Z.M.; Murphy, D.V.; Abbott, L.K. Biochars influence seed germination and early growth of seedlings. Plant Soil 2012, 353, 273–287.

Sohi, S.; Lopez-Capel E.; Krull, E.; Bol, R. Biochar, climate change and soil: A review to guide future research. CSIRO Land and Water Science Report 05/09. Highett: CSIRO, 2009.

Sohi, S.P.; Krull, E.; Lopez-Capel, E.; Bol, R. A review of biochar and its use and function in soil. Advances in Agronomy 2010, 105, 47–82.

Song, W.; Guo, M. Quality variations of poultry litter biochar generated at different pyrolysis temperatures. Journal of Analytical and Applied Pyrolysis 2012, 94, 138–145.

Spokas, K.A. Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Management 2010, 289-303.

Spokas, K.A., Novak, J., Stewart, C., Cantrell., K., Uchimiya, M., DuSaire, M., Ro, K., Qualitative analysis of volatile organic compounds on biochar. Chemosphere 2011, 85, 869–882.

Spokas, K.A., Cantrell, KB.; Novak, J.M; Archer, D.A.; Ippolito, J.A.; Collins, H.P.; Boateng, A.A.; Lima, I.M.; Lamb, M.C.; McAloon, A.J.; Lentz, R.D.; Nichols. K.A.; Biochar: A synthesis of its agronomic impact beyond carbon sequestration. J. Environ. Qual. 2012, 41, 973–989.

Steinbeiss, S.; Gleixner, G.; Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biology & Biochemistry 2009, 41, 1301-1310.

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

Swain, P.K.; Das, L.M.; Naik, S.N. Biomass to liquid: a prospective challenge to research and development in 21st century. Renew Sustain Energy Rev 2011, 15, 4917–4933.

Tan, X.; Liu, Y.; Zeng, G.; Wang, X.; Hu, X; Gu, Y; Yang, Z. Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere 2015, 125, 70–85.

Tanaka, S. Fundamental study on wood carbonization. In: Bulletin of Experimental Forest of Hokkaido University. 1963.

Tao, S.; Cui, Y.H.; Xu, F.L.; Li, B.G.; Cao, J.; Liu, W.X.; Schmitt, G.; Wang, X.J.; Shen, W.R.; Qing, B.P.; Sun, R. Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin. Sci. Total Environ. 2004, 320, 11–24.

Titirici, M-M; White, R.J.; Falco, C.; Sevilla, M. Black perspectives for a green future: hydrothermal carbons for environment protection and energy storage. Energy Environ Sci 2012, 5, 6796–6822.

Tobiszewski, M.; Namieśnik, J. PAH diagnostic ratios for the identification of pollution emission sources. Environ Pollut 2012, 162, 110-119.

Torri, C.; Fabbri, D. Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. Bioresource Technology 2014, 172, 335–341

Trompowsky, P.M.; Benites, V.; Madari, B.E. de M; Pimenta, A.S.; Hockaday, W.C.; Hatcher, P.G. Characterization of humic like substances obtained by chemical oxidation of eucalyptus charcoal. Organic Geochemistry 2005, 36, 1480-1489.

Tsai, W.T.; Liu, S.C.; Chen, H.R.; Chang, Y.M.; Tsai, Y.L. Textural and chemical properties of swine-manure-derived biochar pertinent to its potential use as a soil amendment, Chemosphere 2012, 89, 198-203.

Uchimiya, M.; Lima, I.M.; Klasson, K.T.; Wartelle, L.H. Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere 2010, 80, 935-940.

Uchimiya, M.; Chang, S. C.; Klasson, K. T. Screening biochars for heavy metal retention in soil: Role of oxygen functional groups. J. Hazard. Mater. 2011, 190, 432–444.

Uchimiya, M.; Cantrell, K. B.; Hunt, P. G.; Novak, J. M.; Chang, S. C. Retention of heavy metals in a Typic Kandiudult amended with different manure-based biochars. J. Environ. Qual. 2012, 41, 1138–1149.

Underwood A.J. and Chapman M.G. GMAV 5 for Windows, 1997. University of Sydney, Sydney

USDA, 2005. U.S.D.A. (United States Department of Agriculture). Natural Resources Conservation Service. National Soil Survey Handbook 2005.

US EPA. Polycyclic Organic Matter. Environmental Protection Agency, Washington, DC (2002). Available at: http://www.epa.gov/ttn/atw/hlthef/polycycl.html.

Uzun, B. B.; Pütün, A. E.; Pütün, E. Composition of products obtained via fast pyrolysis of olive-oil residue: effect of pyrolysis temperature. J. Anal. Appl. Pyrolysis 2007, 79, 147 – 153.

Vaccari, F.P.; Baronti, S.; Lugato, E.; Genesi, L.; Castaldi, S.; Fornasier, F.; Miglietta, F. Biochar as a strategy to sequester carbon and increase yield in durum wheat. Eur. J. Agron. 2011, 34, 231-238.

Vamvuka, D. Bio-oil, solid and gaseous biofuels from biomass pyrolysis processes—an overview. J Energy Res 2011, 35, 835–862.

Vane, C.H.; Rawlins, B.G.; Kim, A.W.; Moss-Hayes, V.; Kendrick, C.P.; Leng, M.J. Sedimentary transport and fate of polycyclic aromatic hydrocarbons (PAH) from managed burning of moorland vegetation on a blanket peat, South Yorkshire, UK. Science of the Total Environment 2013, 449, 81-94.

Van Zwieten, L; Kimber, S.; Morris, S; Chan, K.Y.; Downie, A; Rust, J; Joseph, S.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility Plant and Soil 2010, 327, 235–246.

Van Zwieten, L; Kimber, S.W.L.; Morris, S.G.; Singh, B.P.; Grace, P.R.; Scheer, C.; Downie, A.E.; Cowie, A.L.; Rust, J. Pyrolysing poultry litter reduces N_2O and CO_2 fluxes. Science of the Total Environment 2013, 465 279–287.

Vassilev, S.V.; Baxter, D.; Andersen L.K.; Vassileva, C.G. An overview of the chemical composition of biomass. Fuel 2010, 89, 913–933.

Ventura, M.; Sorrenti, G.; Panzacchi, P.; George, E.; Tonon, G. Biochar Reduces Short-Term Nitrate Leaching from A Horizon in an Apple Orchard. J. Environ. Qual. 2013, 42, 76-82.

Verheijen, F.; Jeffery, S.; Bastos, A.C.; van der Velde, M.; Diafas, I. Biochar application to soils. A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. European Commission, Italy. 2010.

Werner, D.; Karapanagioti, H.K. Comment on "modeling maximum adsorption capacities of soot and soot-like materials for PAHs and PCBs". Environ. Sci. Technol. 2005, 39, 381–382.

Wetzel, C.; Banks, M.K.; Schwab, A.P. Rhizosphere effects on the degradation of pyrene and anthracene in soil E.L. Kruger, T.A. Anderson, J.R. Coats (Eds.), Phytoremediation of

Soil and Water Contaminants. ACS Symposium 664, American Chemical Society, Washington, DC 1997, 254-262.

Wiedner, K.; Naisse, C.; Rumpel, C.; Pozzi, A.; Wieczorek, P.; Glaser, B. Chemical modification of biomass residues during hydrothermal carbonization – What makes the difference, temperature or feedstock? Organic Geochemistry 2013, 54, 91–100.

Wilcke, W. SYNOPSIS Polycyclic Aromatic Hydrocarbons (PAHs) in Soil – a Review. J. Plant Nutrition Soil Sci. 2000, 163, 229–248.

Wild, S.R.; Jones, K.C. The significance of polynuclear aromatic hydrocarbons applied to agricultural soils in sewage sludges in the UK. Waste Manag. Res. 1994, 2, 49–59.

Wild, S.R.; Jones, K.C. Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source inventory and budget. Environ. Pollut. 1995, 88, 91-108.

Wollan, E.; Davis, R.D.; Jenner, S. Effects of sewage sludge on seed germination. Environ. Pollut. 1978, 17, 195–205.

Wong, M.H.; Cheung, Y.H.; Cheung, C.L. The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of Brassica parachinensis. Environ. Pollut. Ser. 1983, 30, 109–123.

Woolf, D.; Amonette, J.E.; Street-Perrott, F.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. Nature Communications 2010, 56.

Wu, W.,;Yang, M.; Feng, Q.; McGrouther, K.; Wang, H.; Lu, H.; Chen, Y. Chemical characterization of rice straw-derived biochar for soil amendment. Biomass Bioenerg. 2012, 47, 268–276.

Wu, Y.; Xu, G., Shao, H. B. Furfural and its biochar improve the general properties of a saline soil. Solid Earth 2014, 5, 665–671,

Wurster, C.M.; Lloyd, J.; Goodrick, I.; Saiz, G.; Bird, M.I. Quantifying the abundance and stable isotope composition of pyrogenic carbon using hydrogen pyrolysis. Rapid Commun Mass Spectrom 2012, 26, 2690-2696.

Wurster, C.M.; Saiz, G.; Schneider, M.P.W.; Schmidt, M.W.I.; Bird, M.I. Quantifying pyrogenic carbon from thermosequences of wood and grass using hydrogen pyrolysis. Org Geochem 2013, 62, 28-32.

Xia, X.; Li, Y.; Zhou, Z.; Feng, C. Bioavailability of adsorbed phenanthrene by black carbon and multi-walled carbon nanotubes to Agrobacterium. Chemosphere 2010, 70, 1329-1336.

Yan, W.; Acharjee, T.C.; CCoronella, C.J.; Vásquez, V.R. Thermal pretreatment of lignocellulosic biomass. Environ Prog Sustain Energy 2009, 28, 435–440.

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

Yanik, J.; Kornmayer, C.; Saglam, M.; Yuksel, M. Fast pyrolysis of agricultural wastes: characterization of pyrolysis products. Fuel Process. Technol. 2007, 88, 942−947.

Yao, Y.; Gao, B.; Zhang, M.; Inyang, M.; Zimmerman, A. Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. Chemosphere 2012, 89, 1467-1471.

Yin Chan, K.; Xu, Z. Biochar: nutrient properties and their enhancement. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management. Earth-scan, U.S.A. 2009.

Young, L. and Wang, C. Characterization of n-alkanes in $PM_{2.5}$ of the Taipei aerosol. Atmospheric Environment 2002, 36, 477–482.

Yunker, M.B.; Macdonald, R.W.; Vingarzan, R.; Mitchell, R.H.; Goyette, D.; Sylvestre, S. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Organic Geochemistry 2002, 33, 489-515.

Zavalloni, C.; Alberti, G.; Biasiol, S.; Delle Vedove, G.; Fornasier, F.; Liu, J.; Peressotti, A. Microbial mineralization of bio-char and wheat straw mixture in soil: a short-term study. Appl. Soil Ecol. 2011, 50, 45-51.

Zelles, L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 1999, 29, 111–129

Zhang, A.F.; Liu, Y.M.; Pan G.X.; Hussain, Q.; Li, L.Q.; Zheng, J.W., Zhang, X.H. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from central China Plain. Plant Soil 2012, 351, 263–275.

Zhang, L.; Chunbao, X.; Champagne, P. Overview of recent advances in thermo-chemical conversion of biomass. Energy Convers. Manage 2010, 51, 969−982.

Zhang, X.K.; Wang, H.L.; He, L.Z.; Lu, K.P.; Sarmah, A.; Li, J.W.; Bolan, N.S.; Pei, J.C.; Huang H.G. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci Pollut Res 2013, 20, 8472-8483.

Zhang, X.-X.; Cheng, S.-P.; Zhu, C.-J.; Sun, S.-L. Microbial PAH-Degradation in soil: degradation pathways and contributing factors. Pedosphere 2006, 16, 555-565.

Zheng, M.; Fang, M.; Wang, F.; To, K.L. Characterization of the solvent extractable organic compounds in $PM_{2.5}$ aerosols in Hong Kong. Atmospheric Environment 2000, 34, 2691–2702.

Zimmerman, A.R. Abiotic and microbial oxidation of laboratory-produced black carbon. Environmental Science & Technology 2010, 44, 1295–1301.

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

Zimmerman, A.R. and Gao, B. The Stability of Biochar in the Environment. In: Biochar and Soil Biota, (N. Ladygina and F. Rineau, eds.), CRC Press, Boca Raton, FL 2013.

Zimmermann, M.; Bird, M.I.; Wurster, C.; Saiz, G.; Goodrick, I.; Barta, J.; Capek, P.; Santruckova, H.; Smernik, R. Rapid degradation of pyrogenic carbon. Global Change Biology 2012, 18, 3306-3316.

Zucconi, F.; Monaco, A.; Forte, M.; De Bertoldi, M. Phytotoxins during the stabilization of organic matter. In Composting of Agricultural and Other Wastes; Gasser, J.K.R., Ed.; Publisher: Elsevier, London, UK, 1985, 73–86.

Acknowledgments

First of all, I would like to express my sincere gratitude to my supervisor, Prof. Daniele Fabbri. His invaluable support in my research and constant encouragement during all my PhD have been essential for the completion of this thesis.

Secondly, my acknowledgements to Prof. Colin E. Snape (University of Nottingham) and to Dr. Will Meredith (University of Nottingham) for hosting me and supervising my work, organizing the hydropyrolysis experiments of biochar and soil.

Surely, I could not forget Dr. Silvia Baronti (Istituto di Biometeorologia – CNR, Firenze) for soil and biochar amended soil used for analysis described in Chapters 3.2 and 5.1 and Dr. Alba Dieguez-Alonso (Technische Universität Berlin) for biochars used for analysis described in Chapter 5.2. I am grateful to Dr. H.-P. Schmidt who coordinated the WP1 of the EU-COST TD1107 for the participation to the ring trial. Part of the activity was conducted in collaboration with the Unità Operativa Biomasse within the framework of the APQ Ricerca Intervento a "Sostegno dello sviluppo dei Laboratoridi ricerca nei campi della nautica e dell'energia per il Tecnopolo di Ravenna" "Energia, parte Biomasse" between Università di Bologna and Regione Emilia Romagna (Italy). The biochars used in the characterizations reported in Chapter 3.1 are part of the USDA-ARS Biochar and Pyrolysis Initiative and USDA-ARS GRACENet (Greenhouse Gas Reduction through Agricultural Carbon Enhancement Network) Programs.

I would still like to acknowledge all professors and colleagues which contributed to the work presented in this thesis: Prof. Kurt Spokas (USDA) for biochars collection and characterization, Dr. Cristian Torri (University of Bologna, Department of Chemistry G. Ciamician) for his work in biochar characterization and germination test, Dr. Roberto Conti (University of Bologna, Fraunhofer UMSICHT) for synthesis and characterization of biochar, Dr. Michele Ghidotti (University of Bologna, CIRI EA) Dr. for SPME analysis, Dr. Dennis Zannoni for ammonium analysis (University of Bologna, CIRSA) and Dr. Giovanni Marisi for some of the experiments of section 4.

During my PhD, I had the privilege of meeting and collaborating with many precious people, who helped and supported me in different ways. No words to truly express my gratitude to Dr. Chiara Samorì (University of Bologna, CIRSA), Dr. Danilo Malferrari (University of Bologna, CIRSA) and Dr. Chiara Lorenzetti (University of Bologna, CIRSA).

Finally, I would like to deeply thank my wife Mariarita, whose unconditional and endless love has always been a propellant energy for all my achievements.