Role of biochar and fungi on PAH sorption to soil rich in organic matter

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Key words: ACTIVATED BIOCHAR, AMENDMENT, HAPLIC ARENOSOL, ORGANIC MATTER, PYRENE, SORPTION

ABSTRACT

The use of biochar (BC) has been suggested for remediation of contaminated soils. This study aims to investigate the role of microorganisms in sorption of PAH to BC-amended soils. Fungi, especially the wood and litter-degrading fungi, have shown the ability for humification and to degrade recalcitrant molecules, and are thus suitable model organisms. Haplic Arenosol with high organic matter content was chosen to highlight the importance of soil organic matter (SOM) in PAH sorption, possibly to form non-extractable residue. Basidiomycetous fungi *Agrocybe praecox* and *Phanerochaete velutina* grown on pine bark were inoculated in organic matter (OM) rich Haplic Arenosol and OM poor sandy loam with either BC or chemically activated BC (ABC) and ^{14}C- labelled pyrene for 60 days. Fungi did not mineralize pyrene, but increased sorption up to 47–56% in BC-amended Haplic Arenosol in comparison with controls (13–25%) without a fungus irrespective of the presence of an adsorbent. In OM poor sandy loam only 9–12% of pyrene was sorbed to amended soil in the presence of fungus and adsorbent. Combining BC and fungi is an effective method for sorbing pyrene especially in high SOM soils.

1 Introduction

Polyaromatic hydrocarbons (PAHs) are organic contaminants widespread in the environment in many parts of the world. In Europe alone, PAHs contamination accounts for 11% of 340,000 contaminated sites (Liedekerke et al. 2014). A major source of PAHs contamination in soil is creosote, a coal-tar distillate used as a preservative for power lines and crossties (Murphy and Brown 2005). PAHs are also formed by incomplete combustion of biomass. PAHs persist in the environment due their low water solubility, complex chemical structure and reduced degradation (Winquist et al. 2014; Lamichhane et al. 2016). Nearly all PAHs are highly toxic; thus, they are of concern to all life forms in soil (Chen and Liao 2006). Bioavailability of PAHs in soil is governed by soil organic matter (SOM) content and presence of naturally occurring or added adsorbents (Macleod and Semple 2002; Cornelissen et al. 2006; Lamichhane et al. 2016). The fate of PAHs in soil depends on many physical, chemical and biological processes such as absorption, volatilization, photolysis, chemical degradation, and microbial degradation (Deng and Zeng 2017).

Biochar (BC) is produced from biomass by pyrolysis and is distinguished from other carbonaceous materials by its end use (EBC 2012). Differently from charcoal (often used for energy production), BC is used in a way that does not involve rapid mineralisation of the photosynthetically fixed carbon back to atmosphere (EBC 2012). BC has shown capacity to sorb organic contaminants (Beesley et al. 2011; Zhu et al. 2017), however, its sorption capacity is lower compared with activated carbon (AC; Hale et al. 2011). Apart from contaminant sorption, application of BC to soil offers other advantages, such as carbon sequestration (Woolf et al. 2010; Smith, 2016), soil fertility improvement (Major et al. 2010; Jones et al. 2012; Tammeorg et al. 2014a; b; Ding et al. 2016) and reduction of N_2O emissions from soil (Case et al. 2012; Angst et al. 2013; Zhu et al. 2017). Thus, BC is a relevant alternative to AC despite its moderate sorption capacity.

The influence of BC addition on soil microorganisms have been increasingly studied (e.g. Pietikäinen et al. 2000; Lehmann et al, 2011; Abujabhah et al. 2016; Dai et al. 2016; 2017). Pores in BC can serve as home for microorganisms (Warnock et al. 2007; Quilliam et al. 2013a) and also harbour microorganisms that are not native to soils, while surfaces can provide a platform for biofilm formation (Lehmann et al, 2011, Noyce et al. 2016). Changes in microbial composition and abundance in soil following BC addition have been reported (Pietikäinen et al. 2000; Nielsen et al. 2014; Mitchell et al. 2016; Abujabhah et al. 2016; Dai et al. 2017). O'Neill et al. (2009), Grossman et al. (2010) and Taketani et al. (2013) reported higher microbial population and an increased microbial diversity in high native black carbon Anthrosols than in adjacent soils. Increase in relative abundance of soil bacteria and a decrease in soil fungi with BC addition has been reported and the changes was due to an increase in soil carbon content as BC may have supplied labile C substrates that favored fast growing bacteria over fungi (Khadem and Raiesi 2017). BC addition stimulates biological processes such as increases in enzyme activities and respiration rates in amended soil (Al Marzooqi and Yousef 2017). Jin (2010) observed a shift in a fungal community following BC amendment. The observed changes in microbial communities following BC amendment may also be explained by increase in nutrient availability and utilization which leads to an overall increase in soil fertility (Kolton et al. 2011; Anderson et al. 2011; Nielsen et al. 2014; Pan et al. 2016). Other key factors controlling the shifts in relative abundance and diversity of microbial populations in soil following biochar addition may include; soil physicochemical properties such as organic matter content, pH and texture, type of biochar applied, incubation time and climatic conditions (Lehmann et al. 2011Farrel et al. 2013; Prayogo et al. 2013; Ogbonnaya et al. 2014; Watzinger et al. 2014; Zhu et al. 2017)

Although BC contributes greatly in sorption of PAH compounds in the BC-amended soil (Zhang et al. 2010; Chen and Yuan 2011), very little is known about the role of microorganisms especially the fungi in this sorption process (Quilliam et al. 2013b; Zhu et al. 2017). PAHs in BC-amended soil can be degraded by both bacteria and fungi in soil (Rhodes et al. 2008; Quilliam et al. 2013b) as BC can enhance action of fungi in soil (García-Delgado et al. 2015). PAHs and other aromatic compounds in the amended soil can be oxidized and incorporated into SOM as non-extractable or bound residues by enzymes produced by fungi in the soil (Dec et al. 2001; Kästner et al. 2014). The processes of enhanced sorption by BC and enhanced formation of non-extractable or bound residues from PAH compounds by fungi in soil have been adopted as effective soil remediation strategies as they reduce the bioavailability of PAH in soil (Bollag 1992; Kästner et al. 2014; Zhu et al. 2017). The possibility that both strategies, processes of enhanced sorption by BC and enhanced formation of non-extractable or bound residues from PAH compounds by fungi occurring in soil amended with BC has not been previously studied. PAH degradation in soil by fungi as well as sorption to BC-amended soil differs between soil types (Anyika et al. 2015). The fate of PAH in BC-amended soil may depend on the properties of the soil and BC as well as microbial composition of the amended soil (Anyika et al. 2015).

In this study, we investigated the role of microorganisms in sorption of PAH to BC-amended soils when combined with fungi. Pyrene, a common pollutant in PAH-contaminated soil that is not readily degraded by other microorganisms in soil was selected as a model PAH compound. The aim was to understand the role of fungi, BC and their combined effect on the environmental fate of PAH in contaminated soil using ^{14}C-pyrene-labelled compound. A particular emphasis was on the role of soil organic matter, as almost all the previous studies have been performed with OM poor soil. We hypothesized that a) the porous carbonaceous soil amendments will sorb the more PAH the higher their specific surface area and b) that PAH are more easily sorbed to biochar in low SOM soils and c) that introducing fungi to the system will further increase the sorption of PAH to high SOM soil by oxidative enzymes binding organic compounds to SOM.

2 Materials and methods

2.1 Chemicals

PAH mix, containing 16 United States Environmental Protection Agency (US-EPA) PAH compounds (acenaphthene, acenaphthylene, anthracene (ANT), benzo(a)anthracene, benzo(a)pyene (BaP), benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene (DBA), fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene (PYR), and carbazole at each concentration of 2 mg mL^{-1} in dichloromethane : benzene (1:1) was purchased from AccuStandard, New Haven, CT, USA. Sodium acetate (American Chemical Society certified grade), sodium chloride (analytical grade), isopropanol (analytical grade) and toluene (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1'-binaphthyl (97% purity) used as the internal standard during PAH analysis was purchased from Acros Organics, (Geel, Belgium). 4,5,9,10-^{14}C-pyrene (specific activity 2.035 GBq $mmol^{-1}$, radiochemical purity $\geq$ 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Soils and adsorbents used in the experiment

Three non-contaminated soils were used in the experiments with BC and one PAH contaminated soil was used for PAH extraction method optimization and screening of fungi. The first soil has a texture class of sandy loam and was sampled from a depth of 0–20 cm (a non-agricultural soil) from Biocenter 1 surroundings of Viikki campus of University of Helsinki, Helsinki Finland (60^{0}22′N, 25^{0}01′E). The second soil has a texture class of sand and was sampled from top 0–20 cm depth (an agricultural soil) from Viikki agricultural field, University of Helsinki, Helsinki Finland (60^{0}22′N, 25^{0}02′E). The third soil is classified as an Entic Haplocryod (Soil Survey Staff, 1998) or a Haplic Arenosol (FAO-UNESCO, 1997) and is simply referred here as Haplic Arenosol, was collected in the vicinity of Forest Field Station of the University of Helsinki in Juupajoki, (61^{0}84′ N, 24^{0}26′E) (corresponds to Hyytiälä soil in Ilvesniemi et al. 2000). Soil samples were stored at 4^{0}C before use. PAH contaminated soil which is described in detail in Winquist et al. (2014), was used to screen for fungal growth on BC-amended contaminated soil and for PAH extraction optimization.

BC was prepared from spruce wood chips as described in Tammeorg et al. (2014a). AC used was a commercial product from coconut shell charcoal (AC004; Activated Carbon Technology UK Limited, Billingham, UK). Particle size of AC was 2.36–4.75 mm. ABC was produced from BC (see the next section). Prior to the experiment with soil, BC and ABC were sieved with < 2 mm mesh. AC was used as received from the producer.

pH of adsorbents, soil and soil amended with adsorbents was measured from 1:2.5 (v v^{-1}) suspension in deionized water, while moisture contents were measured gravimetrically by drying a known mass of soil overnight at 105^0C. Soil organic matter content (SOM) was measured by placing dried soil at 550^0C for 4 hours and calculated as loss on ignition. The specific surface area of BC, ABC and AC was measured by the Brunauer, Emmett, and Teller (BET) nitrogen adsorption method measured with Micromeritics surface analyzer (Micromeritics Tristar II 3020, Norcross, GA, USA) at Tampere University of Technology, Finland. The elemental carbon, hydrogen and nitrogen content of samples was measured by using a VarioMax elemental analyzer (Elemental Analysensysteme GmbH, Hanau, Germany). Cation exchange capacity (CEC) of adsorbents and soil was analyzed by adopting a method described by Mitchell et al. (2013) with some modifications. A sample of 0.5 g of adsorbent or 1 g of soil with or without adsorbent was used. The solution was analyzed using flame photometer (Model 410, Corning, New York, USA). The contribution of native black carbon to total organic carbon content of the soil was estimated using the peroxide/weak nitric acid digestions method described by Kurth et al. (2006), except that soil was ground to 0.63 µm particle size. The total carbon remaining, estimated as oxidation-resistant elemental carbon was considered to be the soil native black carbon (Rumpel et al. 2006).

2.3 Activated biochar production

Activated biochar (ABC) was produced from BC as described by Lalhruaitluanga et al. (2011). In brief, sieved BC was added to potassium hydroxide (KOH) solution of various concentrations (1, 10, 20, 40, 60 and 80%). The mixture was stirred for 24 h at a speed of 150 rpm. ABC was separated from KOH solution by centrifugation at 10,000 rpm for 10 min and thoroughly washed with deionized water, to remove excess KOH and other impurities that might be blocking the newly created pores on the surface of the activated biochar. The separated ABC was oven-dried at 110 °C for 24 h and then cooled at room temperature and stored in an air-tight container.

2.4 PAH sorption to soil and soil amended with adsorbents

To prepare the soil with desirable amendments, i.e. AC and ABC (1%), and BC (1 and 2%) (*w*/*w*) samples, soil was thoroughly mixed with an adsorbent. For each treatment, 10 g of soil and either AC, BC or ABC was added to a 100 mL glass bottle. Soil-adsorbent mixture in glass bottle was spiked with 125 µL of 2 g L^{-1} mixture of four PAHs, comprising ANT (3-rings), PYR (4-rings), BaP (5-rings) and DBA (5-rings) in dichloromethane benzene (1:1). Acetone (10 mL) was added to the mixture and mixed thoroughly to ensure homogeneous mixing of PAHs in soil. After mixing, acetone was allowed to evaporate in the hood for 3 days. The final concentration for each PAH in the soil was 25 mg kg^{-1} (i.e. total PAH concentration in the soil was 100 mg kg^{-1}). Soils without adsorbents were spiked with the same concentration of PAHs in order to obtain control samples. The first bottles were taken for extraction immediately after acetone had evaporated, i.e. on day 3. The remaining bottles were incubated at 21^0C in the dark for 60 days.

2.5 PAH extraction

PAH concentration of adsorbents was measured after Soxhlet extraction as described in Tammeorg et al. (2014a). Three PAH extraction methods were evaluated: ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and Soxhlet extraction (SE) for contaminated soil and details are in Fig. S1. For further analyses, ASE method was selected as it was automatic, efficient, fast, easy and environmentally safe, and PAHs were extracted as efficiently as with Soxhlet which is considered a conventional extraction method. ASE condition used are as that described by Hilber et al. (2012).

2.6 PAH analysis by gas chromatography-mass spectrometry (GC-MS)

PAHs were analysed with gas chromatography (Agilent Technologies 6890N, USA) equipped with mass spectrometry (MS Agilent 5973N) by injecting 1 µL of the extract using splitless injection mode. Separation was done on Zebron High performance ZB-5ms GC column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness) from Phenomenex, USA. A deactivated retention gap (Agilent Technologies) of 2 m length with 0.53 mm internal diameter was placed before the separation column. Helium was used as a carrier gas at constant pressure of 100 kPa. The injection temperature was set to 320^0C while the oven temperature program was as follows: 90^0C (2 min); increase of 10^0C min–1 until 320^0C; 320^0C (15 min). Detection was performed with MS in the electron impact mode with 70 eV ionization energy. The ion source temperature was 150°C and the interface between the GC and the quadruple MS was set to 320°C. Compounds were identified with the National Institute of Standard and Technology (NIST) library and quantification was done using internal standard method. Toluene mixtures containing varying amounts of analytes (concentrations = 0.1, 0.5, 1.0, 2.0, 4.0 µg mL^{-1}) and a constant amount of internal standard (1,1'-binaphthyl 5 µg $mL^{-1)}$ were used for calibration. The limits of detection for ANT, PYR, BaP and DBA were 0.02, 0.03, 0.04 and 0.06 µg mL–1, respectively.

2.7 Sorption experiment

The sorption coefficient (K_d) of ^{14}C pyrene was obtained in a batch equilibration experiment with three replicates as described by Kumari et al. (2014) with little modifications. Three millilitres from the soil solution was taken and mixed with 10 mL of scintillation cocktail (OptiPhase "HiSafe" 3® Perkin Elmer Inc.; Fisher Chemicals, Loughborough Leicestershire, England). The radioactivity was measured using a liquid scintillation counter (Wallac 1411, Scintillation Products, Wallac Oy, Turku, Finland). BC and ABC-amended soils, (sandy loam, sand and Haplic Arenosol) were used in sorption experiment with two variants; 1) non-extracted soil and 2) DOC-extracted soil. DOC was extracted from soil as described by Impellitteri et al. (2002) except that here 3 further rounds of extractions were carried out.

2.8 Fungal strains, inoculation and screening

Fungal strains used in this experiment were obtained from the Fungal Biotechnology Culture Collection (FBCC) of the Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland, and were grown on 2% malt extract agar plates and incubated at 25 °C before the use in experiments. Basidiomycetous fungi were chosen as model organisms due to their unique ability to decontaminate PAH contaminated soil by mineralization and humification of PAH compounds. The fungal species with FBCC number (isolation number is given in parenthesis) were: *Agrocybe dura* 478 (Mn71-2), *Agrocybe praecox* 476 (Tm70.84), *Phanerochaete velutina* 941 (T244i), *Obba rivulosa* 939 (T241i) (formerly known as *Physisporinus rivulosus*), *Rhodocollybia butyracea* 626 (K209) and *Stropharia coronilla* 480 (stock gram B). Fungal liquid cultures were prepared according to Anasonye et al. (2015). Fungi were incubated on autoclaved Scots pine (*Pinus sylvestris*) bark, until the surface of the bark was fully covered with fungal mycelium. The screening of fungal strains was performed in PAH contaminated soil amended with either 1% or 2% (w/w) BC. Petri-dishes of 9 cm in diameter were filled with 50 g of soil and 2.5 g pine bark with fungal mycelium was placed on top of the soil. Fungal growth was examined visually once a week during 90 days of incubation at room temperature in the dark. Moisture content of the soil was kept constant by adding water if needed.

2.9 Sorption of ^{14}C-pyrene

Two fungi were selected for further experiments, namely *A. praecox* and *P. velutina*. Non-sterile sandy loam or Haplic Arenosol (10 g) was placed in a 100 mL glass bottle (Schott Duran laboratory glassware, Mainz, Germany) and ^{14}C-labelled pyrene was added uniformly to soil (radioactivity 2,100 Bq per bottle) and thoroughly mixed to ensure homogeneous result. Sand was not used in this experiment as two soil types with marked difference in SOM was preferred. The soil was amended with either BC or ABC, 1% for sandy loam and 2% for Haplic Arenosol (w/w). Soil moisture content was adjusted to 40% of maximum water holding capacity using deionized water and 5 g of fungal inoculum growing on bark was added on top of the soil. For control bottles, uninoculated bark was added on top of spiked soil.

Abiotic control bottles were included to account for the impact of indigenous microorganisms on pyrene sorption and degradation. For abiotic control, soil was autoclaved before spiking with ^{14}C-pyrene and adding adsorbent. All treatments with three replicates were incubated at 21^0C in the dark for 60 days. The bottles were flushed with moist air for 15 minutes once a week. Mineralized fraction, i.e. evolved $^{14}CO_2$ was trapped during the aeration period into 10 mL of 1 M NaOH. Radioactivity was measured from 1 mL of trapping solution mixed with 10 mL of scintillation cocktail (OptiPhase "HiSafe"). After incubation, bark with or without fungus was separated from soil by sieving. Pyrene was extracted from the soil with ASE as described earlier. The radioactivity was measured with liquid scintillation counter from 1 mL of toluene extract mixed with 10 mL of the scintillation cocktail. After toluene extraction, soil and sieved bark were combusted separately with Junitek Oxidizer (Junitek Oy, Turku, Finland). During combustion, all the carbon in the sample forms CO_2, which is trapped with a mixture of 16 mL Lumasorb2 II and Carboluma2 scintillation liquids (1:1 v/v; Lumac LSC, Belgium) and radioactivity is measured with liquid scintillation counter. The mass balance of ^{14}C-pyrene was determined as in Valentin et al. (2013) with modifications: (1) toluene extraction (available fraction), (2) fraction bound to soil (unavailable fraction), and (3) fraction bound to bark.

2.10 Statistical analyses

The effects of experimental treatment combinations on the percentage of PAH sorbed were tested with a two-way analysis of variance (ANOVA) with soil type, treatment, and their interactions as fixed effects for both incubation times (3 and 60 days). The effects of experimental treatment combinations on the K_d and mass balance were tested with a two and three-way analysis of variance (ANOVA), respectively with soil type, treatment, and their interactions as fixed. Means were compared using the Tukey HSD multiple pair-wise comparison test at $p < 0.05$. The normal distribution of the residuals from the models was tested with Shapiro-Wilk test and the homogeneity of variances was tested with Levene's test. If the data failed to meet the assumptions for parametric statistics, Box-Cox transformation was used (Box and Cox, 1964). Statistical analyses were carried out with the software package PASW v 20.0 (SPSS Corp., Chicago, USA).

3 Results

3.1 Physicochemical properties of soils and adsorbents

Haplic Arenosol had lower pH and significantly higher DOC and SOM contents compared with sandy loam and sand (Table 1). The highest content of native black carbon was recorded in Haplic Arenosol (2% of soil total mass) while the lowest was in sandy loam soil. Elemental compositions of BC, ABC and AC were similar, but specific surface area of

AC was considerably higher than that of ABC and BC (Table 2). Total PAH (US-EPA 16 PAH compound) concentration of adsorbents were below or within the recommended maximum limit set by international BC initiative (6—30 µg g^{-1}; IBI 2015) and below the limit by European Union (12 µg g^{-1}; EBC 2012) for use as a soil amendment in agriculture.

3.2 Sorption capacity of BC

Soil types, treatments and interactions affected the percentage of PAH sorbed for all four PAHs used in this experiment (Table 3). ABC-amended sandy loam sorbed (95-100%) PAHs in 3 days (Table S1). However, the effect seemed to be only short-lived as the sorption efficiency of ABC had decreased notably after 60 days of incubation. In sandy loam significantly, more pyrene was sorbed than in other soil types. AC addition to sandy loam and sand significantly enhanced pyrene sorption ($p < 0.001$, Table 3, Table S1) compared to BC and ABC during a 60-day incubation period.

3.3 Role of dissolved organic carbon (DOC)

BC and ABC addition significantly (Figure 1, $p < 0.001$, Table S2) increased ^{14}C-pyrene sorption to both sandy loam and sandy soils and the increase with ABC in both soils were larger compared with BC. In Haplic Arenosol, BC and ABC addition had little or no effect on pyrene sorption. When DOC was removed from the soil, pyrene sorption to control sandy loam and sand without amendments significantly (Fig 1, $p < 0.001$) decreased although it significantly improved in Haplic Arenosol. BC and ABC additions to DOC-extracted soil significantly (Fig 1, $p < 0.001$, Table S2) increased pyrene sorption to sandy loam soil.

3.4 Fungal treatment

All the screened fungi grew to the soil amended with BC except *Obba rivulosa* that grew only on bark used as a carrier material in inoculation (data not shown). Regardless of whether BC was added at 1 or 2 %, (*w*/*w*) there was no difference in growth of fungi. However, two fungi, *A. praecox* and *P. velutina* were chosen for further experiment, as they have shown in previous studies to have the capability to colonize the soil, compete favorably with indigenous microorganisms and to degrade PAH in soil (Steffen et al. 2002; Winquist et al. 2014).

There was significant difference in the levels of pyrene that was sorbed to the soils used in the study ($p < 0.001$, Table 4). The role of fungal action to pyrene sorption in BC or ABC-amended soils was evaluated in two soil types. Higher levels of ^{14}C-pyrene was sorbed to Haplic Arenosol incubated with fungus (47–56%) compared with sandy loam (up to 19%). The lowest levels of pyrene sorption in the soil were observed in autoclaved soils with BC or ABC (9–15%), highlighting the role of indigenous microorganisms in PAH sorption to soil. Three-way analysis of variance (ANOVA) also showed that soil type significantly influenced pyrene sorption to the bark used in the experiment ($p < 0.001$, Table 4). Significantly higher levels of pyrene sorbed to sandy loam (27–36%) than to Haplic Arenosol (2–7%). There was no significant difference between the two fungal strains used in the way they caused change in sorption of pyrene to BC-amended soil. Both fungi significantly increased the sorption of pyrene to Haplic Arenosol-BC complex (Table 4). Similarly, the two adsorbents used had no significant difference in the way they cause changes in sorption of pyrene to soil incubated with fungi. Haplic Arenosol amended with either of the adsorbents showed increased capacity to sorb pyrene compared to OM poor soil.. HaplicThere was no evolution of $^{14}CO_2$ in either control or treated soil during the entire incubation period.

4 Discussion

PAH sorption was maximum mostly with ABC in the beginning of incubation regardless of soil type. This is particularly interesting considering that AC had almost double BET surface area compared with ABC or BC, thus our first hypothesis was only partially supported by the evidence. The phenomenon could be explained by the formation of alkaline surface sites on BC during activation with potassium hydroxide or wider pore size of ABC compared to AC and BC (de Andrés et al. 2013). Chemically activated char is known to form surface sites leading to enhanced sorption capacity (de Andrés et al. 2013; Lamichhane et al. 2016) and less time is needed for PAH to access sorption sites in ABC than AC or BC, if pore size in ABC is wider. Also, alkali modified BC enhances BC sorption capacity through the formation of π-π electron donor acceptor interaction between modified BC and aromatic rings of PAH (Liu et al. 2012; Zhu et al. 2017) Hence, BET surface area is not the only factor controlling PAH sorption, but other chemical and physical properties as well as the aliphatic regions of adsorbents play a role in contaminantssorption (Park et al. 2013). However, when ABC was aged for 60 days in soil, it was unable to retain most of the sorbed PAH. During a prolonged incubation in soil, the surface chemistry and sorption characteristics of ABC may have been altered (Cheng and Lehmann 2009; Gibson et al. 2016), resulting in the release of already sorbed PAH. This phenomenon is typical to char produced by chemical activation with potassium hydroxide and such effect was not detected with AC and BC. In a field scale experiment, Martin et al. (2012) observed release of already sorbed diuron and atrazine from BC in soil during aging, but when BC dose was increased, sorption remained stable. Thus, the ability of an adsorbent to retain the contaminant over time in soil may depend on adsorbent dose used. In our study, two doses were used, but very little or no difference was observed between doses. Further research will be required to determine whether PAH-ABC-soil complex is more stable with increased ABC dose and aging.

We investigated the role of DOC in PAH sorption to BC or ABC-amended soil. As hypothesized, higher levels of sorption were observed with sandy loam and sand, which had lower DOC concentrations in comparison with Haplic Arenosol. The result is in line with previous findings (Zhang et al. 2010; Kumari et al. 2014) where enhancement of PAH sorption in soil with BC was clearly observed in soils with low DOC and organic carbon contents. DOC associates strongly with BC surfaces in soil (Hale et al. 2011; Zhang et al. 2010), and SOM may block the pores of BC and reduce sorption of organic contaminants (Pignatello et al. 2006; Lian et al. 2015). After DOC was removed from soil there was enhanced PAH sorption to the Haplic Arenosol, not only to soil amended with BC, but also to control soil. This phenomenon in Haplic Arenosol could be explained by high native black carbon content compared with sandy loam and sand.

Also the third hypothesis of ours was supported by the evidence as both *A. praecox* and *P. velutina* increased sorption of pyrene to the Haplic Arenosol so that the bound fraction was approximately half of total pyrene. There seems to be a synergistic effect between BC and fungi in decreasing bioavailability and sorption of PAH, which was earlier confirmed by García-Delgado et al. (2015) with low SOM soil, and our results further emphasize the role of SOM in the process. White-rot and litter-decomposing fungi are known to produce oxidative enzymes, such as laccase and manganese peroxidase, which are known to bind organic compounds to SOM through oxidative coupling (Berry and Boyd, 1984; Bollag 1992; Held et al. 1997) as well as mineralize them (Steffen et al. 2002, Tuomela et al. 1999). However, we measured no evolution of $^{14}CO_2$ during the entire incubation period. SOM influence has been observed to reduce mineralization of pentachlorophenol (Tuomela et al. 1999) and the increase of aromatic rings and addition of BC probably enhances the phenomenon as has been proved with ^{14}C-labelled synthetic lignin and phenanthrene (Tuomela et al. 2002, Rhodes et al. 2008). If a contaminant is bound irreversibly to soil or BC, this can be considered as relevant remediation action (Bollag 1992). In our earlier studies (Anasonye et al. 2014 and 2015) of soil system where a white-rot fungus was grown (including *P. velutina*), manganese peroxidase was the main oxidative enzyme found with little or no laccase activity, but García-Delgado et al. (2015) detected both laccase and manganese peroxidase from PAH contaminated soil with *Pleurotus ostreatus*. BC addition did not affect the enzyme production of the fungus. In addition to BC and SOM, bark acted as an adsorbent especially when organic matter content of soil was low, also previously reported (Olivella et al. 2013, Winquist et al. 2014,). In addition to binding sites of the bark, bark extractives are capable of sorbing PAH (Olivella et al. 2013).

The results obtained from sterile soil with BC or ABC were compared with those obtained from non-sterile soil with similar amendments in order to show the difference in roles of biotic versus abiotic processes in enhancement of pyrene sorption in amended soil. The presence of native microbes in soil had higher impact on pyrene sorption to the amended-Haplic Arenosol compared with the amended-sandy loam soil. Soil with high organic carbon tends to favor fungal colonization over bacteria (Zhang et al. 2015), thus Haplic Arenosol, which is rich in lignocellulosic materials, was probably more colonized with fungi capable of enhancing pyrene sorption as detected with *A. praecox* and *P. velutina*.

Stability of bound compound is very important as slow and continuous leaching to groundwater will pose a constant risk to the environment (Bollag 1992). In our system, the soil was subjected to strong extraction procedure with organic solvent to obtain the freely available fraction. The ^{14}C-pyrene fraction remaining in soil after strong chemical treatment is most probably bound to adsorbents-SOM complex through covalent bonds, which are the most resistant to extraction and degradation (Khan et al. 1978), and should remain in soil for a very long time.

Conclusions

We found that PAH can effectively be sorbed to carbonaceous soil amendments and thus be a promising tool for remediating polluted sites. The effectiveness of biochar to sorb PAH can further be increased when activating the biochar thus improving not only its BET surface area, but also its pore morphology and surface chemistry. The method was shown to be even further enhanced if biochar addition is combined with treatment with basidiomycetous fungi, especially in high SOM soils. Sorption of pyrene to BC-SOM complex enhanced by fungi could help reduce the leaching of pyrene from soil to groundwater, but the results are yet to be validified under field conditions

Acknowledgements

The authors thank Jussi Heinonsalo, Kati Hakala and Kari Steffen for providing the experimental soils, Kaj-Roger Hurme for providing the guidance in working with labeled compounds. This research was funded by Maj and Tor Nessling Foundation.

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Figure caption

Figure 1 Sorption parameter (K_d) (L kg^{-1}) of ^{14}C-pyrene in soil with (on left) and without DOC (on right) with or without 1% (w/w) BC or ABC. Dissolved organic matter (DOC) was extracted from the soil with deionized water three times