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Nutrient-pollutant-interactions in cadmium-contaminated soils amended with surface-modified bone char

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WHEN YOU DO THINGS FROM YOUR SOUL,

YOU FEEL A RIVER MOVING IN YOU,

A JOY.

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List of abbreviations

AC	activated char
AC^S	sulfur-enriched activated char
ANOVA	analysis of variance
As	arsenic
BC	bone char
BC^{plus}	surface modified bone char
C	carbon
Ca	calcium
Cd	cadmium
CEC	cation exchange capacity
DAP	diammonium phosphate
E_0	reference energy
ICP-OES	inductively coupled plasma-optical emission spectroscopy
IHP	inositol hexakisphosphate
LCF	linear combination fitting
Mg	magnesium
NS	non-significant
P	phosphorus
Pb	lead
P_i	inorganic phosphorus
P_o	organic phosphorus
P_t	total phosphorus

R^2	coefficient of determination
S	sulfur
SLRI	synchrotron light research institute
SOM	soil organic matter
TSP	triple super phosphate
U	uranium
XANES	X-ray absorption near edge structure

Summary

Mineral phosphorus (P) fertilizers, produced from mined rock phosphate, a depleting non-renewable resource, fuel today's intensive agriculture, however, they contain impurities such as cadmium (Cd) and uranium (U). Consequently, their repeated application results in serious accumulation of toxic metals in soils. Sustainable agriculture, using renewable and eco-friendly fertilizers, can provide secure nutrients availability for an optimal crop production avoiding environmental hazards.

Bone char (BC), a clean, renewable and slow-release P-fertilizer, has been proved effective for promoting crop production and Cd-immobilization. One still unsolved problem is the relatively small P-solubility from the BC making precise forecasts of P-dissolution in relation to demands of growing crops difficult. This is a constraint to the general acceptance in agricultural practice. Surface modification of BC with sulfur (S) containing compounds may be an approach for improving the P-solubility. A relevant technology has been developed by scientists of the University of Rostock, and the intellectual property rights have been saved, but the agronomic impact of this technology is unknown. The most important aim of this project was to lay a scientific basis for the novel BC as an effective and safe P-source by improving the knowledge about (i) modified BC-dissolution and related Cd-immobilization mechanisms, (ii) the effect of BCs-particle size and non-equilibrium conditions on P- and Cd-release in contaminated soils and (iii) P-speciation of BCs particles and relevant treated soils by sequential fractionation and X-ray absorption near edge structure (XANES) spectroscopy. The results of individual experiments of this dissertation project are summarized as follows:

The P-dissolution and Cd-immobilizing effects of BC, surface modified BC (BC^{plus}) and diammonium phosphate (DAP) amendments in five soils, varying in pH and Cd-contamination, were evaluated by three extractants (NH_4NO_3, $NaHCO_3$ and H_2O) over 145 days of incubation. In comparison with other extraction methods, the NH_4NO_3 appeared well-suited to simultaneously assess the P- and Cd-availability. The addition of BC increased the pH in all soils whereas BC^{plus} and DAP lowered the pH in soils with pH > 5. Similar trends for NH_4NO_3-P-differences between treatments and control were observed for BC and BC^{plus} during the incubation period although BC^{plus} resulted in much larger P-concentrations. The highest Cd-immobilization was obtained in BC-treated soils. The addition of BC^{plus} and DAP decreased the Cd-concentration until 34 days of incubation in all soils and remained effective in Cd-immobilizing in soils that showed a pH raise over 145 days of incubation.

A combined incubation-leaching experiment clarified the effect of BC and BC^{plus} at different particle size fractions on P- and Cd-mobilization. Two soils each with BCs treatments were leached five times during 70 days of incubation. Over the incubation period, the P-contents in leachates were significantly increased by BC and BC^{plus} particles of 0.5-1 mm size. P-release from BCs was larger under non-equilibrium conditions in this experiment compared with a previous continuous incubation experiment. The lowest Cd-concentrations in leachate were found in soils amended with the smallest BC particles. BC^{plus} significantly increased Cd leaching, and highest amounts of Cd were leached at the smallest particle size.

Changes in chemical composition of BCs particles and treated soils of two different experiments, "incubation-leaching" and "ryegrass cultivation", were investigated by sequential P fractionation and XANES spectroscopy. The most P-increase significantly occurred in the NaOH-P and resin-P fractions in response to BC^{plus} application in both experiments. This increase of the readily available P fraction in BC^{plus}-treated soils was confirmed by linear combination fitting (LCF) analysis on P K-edge XANES spectra of BCs particles and amended soils. The proportion of Ca-hydroxyapatite decreased, whereas the CaHPO$_4$ increased in BC^{plus}-particles after amended soils had been incubated and leached or cropped by ryegrass. Based on P-XANES speciation as determined by LCF analysis, the proportion of inorganic Ca(H$_2$PO$_4$)$_2$ increased in amended soils by BC^{plus} application.

In summary, the enhanced P-dissolution achieved by surface modification of BC, reflected by higher P-concentrations in extractions, leachates and enriched soluble P-species in BC^{plus} particles and treated soils, can be explained by a combination of 1) improved intrinsic properties of BC^{plus} including lower pH and Ca-hydroxyapatite contents in favor of more soluble CaHPO$_4$, and 2) oxidation of elemental sulfur after applying BC^{plus} to the soil, which is mainly caused by microorganisms activities, resulting in the formation of protons and continuous conversion of less soluble hydroxyapatite to relatively soluble CaHPO$_4$, even after long time-periods. As the acidity of Cd-contaminated soils treated with BC^{plus} was raised, the mobilization of Cd increased. Therefore, the Cd-immobilization benefit of BC can be lost at the expense of P dissolution in BC^{plus} fertilizer, mostly depending on the pH aftereffect. Finally, the results may conclude that BC^{plus} can be offered as a clean, renewable and S-enriched P-fertilizer, which has been improved in P-dissolution compared to the original BC. The presented results may motivate further future studies exploring long-term effects of BC^{plus} on P- and Cd-speciation and response of different crops directed toward optimized fertilization efficiency and sustainable agriculture evolution.

Zusammenfassung

Mineralische Phosphor (P)-Düngemittel, die aus abgebautem Rohphosphat - einer begrenzt verfügbaren und nicht erneuerbaren Ressource - gewonnen werden, sind eine Grundlage der heutigen intensiven Landwirtschaft. Sie enthalten aber Verunreinigungen wie Cadmium (Cd) und Uran (U), die bei wiederholter Anwendung zu toxischen Schwermetallanreicherungen in Böden führen können. Eine nachhaltige Landwirtschaft, die erneuerbare und umweltfreundliche Düngemittel verwendet, muss eine sichere Nährstoffverfügbarkeit für eine optimale Pflanzenproduktion bieten und Umweltgefahren vermeiden.

Knochenkohle (KK) ist ein sauberer, erneuerbarer und langsam freisetzender P-Dünger, der sich als wirksam zur Förderung der Pflanzenproduktion und der Cd-Immobilisierung erwiesen hat. Ein noch ungelöstes Problem ist die relativ geringe P-Löslichkeit aus der KK, die exakte Vorhersagen der P-Lösung entsprechend der Anforderungen der wachsenden Pflanzenbestände schwierig macht. Dies schränkt die Akzeptanz in der Landwirtschaft ein. Eine Oberflächenmodifikation von KK mit Schwefel (S)-haltigen Verbindungen kann ein Ansatz zur Verbesserung der P-Löslichkeit sein. Eine relevante patentierte Technologie wurde von einer Gruppe von Wissenschaftlern der Universität Rostock entwickelt, aber die agronomische Wirksamkeit der mit dieser Technologie hergestellten KK ist vollständig unbekannt. Das wichtigste Ziel dieses Projektes war es, die neuartige KK als eine effektive und sichere P-Quelle zu etablieren, indem das Wissen über (i) die modifizierte KK-Auflösung und damit verbundene Cd-Immobilisierungsmechanismen erweitert wird, (ii) die Wirkung der KK-Partikelgröße und von Ungleichgewichtsbedingungen auf die P- und Cd-Freisetzung in kontaminierten Böden erforscht wird und (iii) eine P-Speziierung von KK-Partikeln und relevanten damit behandelten Böden durch sequenzielle Fraktionierung und Röntgenstrahlabsorptions-Nahkantenspektroskopie (XANES) erreicht wird. Die Ergebnisse einzelner Experimente dieses Dissertationsprojektes sind wie folgt zusammengefasst:

Die P-Auflösung und Cd-immobilisierenden Effekte von KK, oberflächenmodifizierter KK (KK^{plus}) und Diammoniumphosphat (DAP) wurden in fünf Böden, die sich in pH und Cd-Kontamination unterscheiden, durch drei Extraktionsmittel (NH$_4$NO$_3$, NaHCO$_3$ und H$_2$O) nach 145 Tagen Inkubation untersucht. Im Vergleich zu anderen Extraktionsmethoden erschien das NH$_4$NO$_3$ gut geeignet, um gleichzeitig die P- und Cd-Verfügbarkeit zu bestimmen. Die Zugabe von KK erhöhte den pH-Wert in allen Böden, während KK^{plus} und DAP den pH-Wert in Böden mit pH >5 verringerten. Ähnliche Trends für NH$_4$NO$_3$-P-

Unterschiede zwischen Behandlungen und Kontrolle wurden für KK und KK^{plus} während der Inkubationszeit beobachtet, obwohl KK^{plus} zu viel größeren P-Konzentrationen führte. Die höchste Cd-Immobilisierung wurde in KK-behandelten Böden festgestellt. Die Zugabe von KK^{plus} und DAP verringerte die Cd-Konzentration bis zu 34 Tagen Inkubation in allen Böden und blieb wirksam bei der Cd-Immobilisierung in den Böden, die über 145 Inkubationstage einen pH-Anstieg aufwiesen.

Ein kombiniertes Inkubations-Auswaschungs-Experiment untersuchte die Wirkung von KK und KK^{plus} verschiedener Partikelgrößenfraktionen auf die P- und Cd-Mobilisierung. Zwei Böden mit KK-Behandlungen wurden fünfmal während 70 Tagen Inkubation perkoliert. Während der Inkubationszeit wurden die P-Gehalte in den Sickerwässern durch Zugabe von KK und KK^{plus}-Partikeln mit einer Größe von 0,5-1 mm signifikant erhöht. Die P-Freisetzung aus KK war in diesem Experiment größer als in einem früheren kontinuierlichen Inkubationsexperiment unter Gleichgewichtsbedingungen. Die niedrigsten Cd-Konzentrationen im Sickerwasser wurden in Böden mit den kleinsten KK-Partikeln gefunden. KK^{plus} erhöhte signifikant die Cd-Auswaschung, und bei der kleinsten Partikelgröße wurden die höchsten Cd-Mengen ausgetragen.

Änderungen der chemischen Zusammensetzung von KK-Partikeln und behandelten Böden von zwei verschiedenen Experimenten, "Inkubation-Auswaschung" und "Weidelgras-gefäßversuch", wurden durch sequentielle P-Fraktionierung und XANES-Spektroskopie untersucht. Die stärksten P-Erhöhungen traten signifikant in den NaOH-P- und resin-P-Fraktionen als Reaktion auf die KK^{plus}-Anwendung in beiden Experimenten auf. Dieser Anstieg der leicht verfügbaren P-Fraktion in KK^{plus}-behandelten Böden wurde durch linear combination fitting (LCF) an P K-Kanten-XANES-Spektren von KK-Partikeln und behandelten Böden bestätigt. Der Anteil von Ca-Hydroxyapatit nahm ab, während CaHPO$_4$ in KK^{plus}-Partikeln zunahm, nachdem die behandelten Böden inkubiert und ausgewaschen oder mit Weidelgras bewachsen waren. Basierend auf P-Speziierung mit XANES und LCF-Analyse, erhöhte sich der Anteil an anorganischem Ca(H$_2$PO$_4$)$_2$ in den behandelten Böden durch KK^{plus}-Anwendung.

Zusammenfassend kann die verbesserte P-Lösung infolge der Oberflächenmodifikation von KK, die durch höhere P-Konzentrationen bei Extraktionen, Sickerwässern und angereicherten löslichen P-Spezies in KK^{plus}-Partikeln und behandelten Böden nachgewiesen wurde, durch eine Kombination von 1) verbesserten intrinsischen Eigenschaften von KK^{plus}, einschließlich

niedrigerer pH-Werte und Ca-Hydroxyapatit-Gehalte zugunsten von löslicherem $CaHPO_4$, und 2) Oxidation von elementarem Schwefel nach dem Aufbringen von KK^{plus} auf den Boden erklärt werden. Die S-Oxidation durch Mikroorganismen führt zur Bildung von Protonen und kontinuierlicher Umwandlung von weniger löslichem Hydroxylapatit zu relativ leichter löslichem $CaHPO_4$. Als die Acidität von mit KK^{plus} behandelten Cd-kontaminierten Böden erhöht wurde, nahm allerdings auch die Mobilisierung von Cd zu. Daher kann der Vorteil der Cd-Immobilisierung von KK zu Gunsten der P-Lösung in KK^{plus}-Düngern verloren gehen, was größtenteils von der pH-Wert-Nachwirkung abhängt.

Schließlich charakterisieren die hier berichteten Ergebnisse KK^{plus} als sauberen, erneuerbaren und S-angereicherten P-Dünger, der im Vergleich zur ursprünglichen KK hinsichtlich der P-Lösung verbessert wurde. Die vorliegenden Projektergebnisse können als Motivation für weitere zukünftige Studien dienen, in denen die langfristigen Auswirkungen von KK^{plus} auf die P- und Cd-Speziation und die Reaktion verschiedener Anbaukulturen für eine optimierte Düngungseffizienz und nachhaltige Landwirtschaft insgesamt untersucht werden.

1 | Introduction

1.1 Justification of the research

Increasing world population instigates raise in food demand and consequently in agricultural production that should come from more intensive and precise practices (Tilman et al., 2002). Modern farming systems have increased production mainly through greater mineral fertilizer inputs posing many risks to terrestrial ecosystems and sustainable food production (Vitousek et al., 2009). Phosphorus (P) is one of the most extensively used agricultural inputs and fundamentally required for crop growth and development due to its crucial function in plant metabolism, structure of nucleic acids and expression of information (DNA and RNA) and energy transfer pathways (Elser, 2012). Manufacturing of commercial P fertilizers is mainly based on chemical processing of mined phosphate rocks excavating from sedimentary (95%) and igneous (5%) ores estimated by U.S. Geological Survey to last for a maximum of 200 to 300 years (Pufahl and Groat, 2017). The global distribution of phosphate rock reserves is geographically restricted even more than oil in six countries (about 90%) including Morocco, China, Algeria, Syria, Jordan and South Africa (currently Morocco alone controls nearly 75% of the global reserves). The concentrated supply in few countries with unstable political situation and high demand makes importing countries vulnerable to geopolitical crises and raises concerns about availability and price of phosphate rock on the global market (Cordell and Neset, 2014). Owing to these reasons, the European Commission classified P as a critical raw material in 2014 (EU Commission, 2014). In response to growing P fertilizer demand, increasing concerns about resource depletion, mining expenses and supply risks, the global

price of rock phosphate rocketed 800% in 2008 (World Bank, 2008) causing world food crisis and many protests, disturbances and riots in developing countries (The Guardian, 2008). In addition, environmental concerns related to quality of rock phosphate and contribution of potentially hazardous trace elements such as cadmium (Cd), uranium (U), arsenic (As) and lead (Pb) are an increasingly important challenge (Mulla et al., 1980; Mortvedt, 1996; Franklin et al., 2005; Kratz and Schnug, 2006; Chen et al., 2007; Luo et al., 2009; Molina et al; 2009; Kratz et al., 2016). Consequently, repeated application of these mineral fertilizers have been reported to cause heavy metals accumulation in the soil, enhancement of potentially toxic contaminants availability to plants, transport to groundwater, and to food and ecological chains (Mendes et al., 2006). For instance, the German Federal Protection Agency estimated the addition of more than 160 t/a U to German farmlands by mineral P fertilizers (UBA, 2012). The emission of these potentially harmful substances to the soil could be reduced by setting upper thresholds for trace elements in fertilizers and also economic and policy mechanisms such as extra taxes to encourage application of eco-friendly fertilizers. Subsequently, the limited, non-replaceable and contaminated reserve of rock phosphate in the world and excessive consumption, growing demand and increasing price P has stimulated the research for recycling techniques and introducing alternative renewable sources for P-fertilizers production.

Recovering large P losses through wastewater and municipal solid waste is recently considered to be a practical option. Mihelcic et al. (2011) reported that approximately 22% of global P fertilizer demand can be achieved through P recycling from human urine and feces. Sewage sludge, a by-product of municipal, industrial and agricultural wastewater managements, is rich in N, P and organic matter (depending on its origin) which can be considered as a fertilizer if an appropriate treatment technology delivers a product of quality (Krüger et al., 2016). Over 10 million tonnes of sewage sludge are generated annually in the EU (Milieu Ltd, 2008) and Austria, Germany and Switzerland have now made a mandatory P-recovery from municipal sewage sludge (Platform E.S.P., 2017). Despite of relatively high P content (about 2 to 13% w/w depending on wastewater source and applied treatments; Günther 1997; Franz 2008; Atienza–Martínez et al., 2014; Krüger et al., 2014), sewage sludge usually contains many contaminants including heavy metals (Lundin et al., 2004; Nguyen et al., 2013), organic contaminants (Harrison et al., 2006; Loos et al., 2013) and pathogens (Sahlström et al., 2004; Cai and Zhang, 2013). Sewage sludge generally requires different recovery treatments such as composting, mesophilic and thermophilic digestion,

precipitation or crystallization as struvite, combustion and pyrolysis or combination of these procedures to maintain P in a safe and solid form that can be used as fertilizer (Dumontet et al., 1999; Pettersson et al., 2008; Guedes et al., 2014; Krüger et al., 2016; Kleemann et al., 2017). However, the agricultural use of sewage sludge is restricted by legislations due to environmental risks in several European countries such as Switzerland where the sewage sludge application in agriculture has been already banned (Franz, 2008). Thus, the exploration of new alternatives for clean and renewable P-fertilizers would be the most favorable.

In recent years remarkably interest has been focused on biochars for various environmental and agricultural advantages such as carbon sequestration (Windeatt et al., 2014), pollutants immobilization/removal (Cao et al., 2009; Beesley and Marmiroli, 2011; Cui et al., 2016) and soil fertilization (Lehmann et al., 2011). Biochar is a general term for a group of different-origin carbon-processed materials generated by thermochemical decomposition of different feedstocks biomass, e.g. agricultural lignocellulosic residues and municipal/industrial wastes, under oxygen-limited conditions. Carbon sequestration in order to improving ecological systems in agriculture and climate change mitigation has received more attention than other beneficial properties of biochars (Lehmann et al., 2008; Sohi et al., 2010). Two different approaches were followed in investigations on P-recycling performance of biochars. First, the sorption capacity of biochars for removing P from over P-loaded aqueous and terrestrial systems and second as a soil amendment to increase fertility (Streubel et al., 2012; Trazzi et al., 2016), both depending on precursors properties (Spokas et al., 2012) and pyrolysis conditions (Morales et al., 2013). Furthermore, activation processes can improve adsorptive-removal characteristic of biochars for many environmental pollutants such as sulfur dioxide (Guo and Lua, 2003), nitrate (Ota et al., 2013), chemical oxygen demand (Bansode et al., 2004) and heavy metals (Kadirvelu et al., 2001; Bohli et al., 2013; Ribeiro et al., 2015). Activation methods involve chemical impregnation agents such as $ZnCl_2$, H_3PO_4, KOH or H_2SO_4 (Caturla et al., 1991; Molina-Sabio et al., 1995; Fierro et al., 2006; Legrouri et al., 2005) and physical carbonization and gasification using oxygen, carbon dioxide and steam (Miguel et al., 2003; Ribeiro et al., 2015). Moreover, P-bearing biochar after adsorption and recovery process can be used in agricultural production as slow-release P fertilizer (Yao et al., 2013; Shepard et al., 2016). Biochars could enhance the plant availability of P in agricultural soils through promoting bioavailability of already existing P or supplying a new source of P into the soil. Blackwell et al. (2015) reported that biochars coupled with mineral

fertilizers increased mycorrhizal colonization, plant growth, and P uptake of wheat providing better P use efficiency. Similarly, biochar produced from timber residues in combination with poultry litter increased citrate-extractable P in soil which was reflected in P concentration of harvested dry beans (Gao et al., 2016). In addition, it is also expected that biochars contain different P contents depending on feedstock (e.g., poultry litter biochar = 18.2 g P kg^{-1}; wastewater sludge biochar = 20 to 40 g P kg^{-1}; Algal biochar = 3.6 g P kg^{-1}, rice husk biochar = 4.7 g P kg^{-1}, sugarcane bagasse biochar = 6.1 g P kg^{-1}, *Miscanthus x giganteus* biochar = 2.5 g P kg^{-1}; Tagoe et al., 2008; Hossain et al., 2011; Bird et al., 2011; Qian et al., 2013; Yin et al., 2013; Monterumici et al., 2015). However, the evaluation and development of biochars in order to use as P-fertilizer is not well investigated.

The highest content of P (130-200 g P kg^{-1}; Warren et al., 2009; Siebers and Leinweber, 2013; Rojas-Mayorga et al., 2015; Zwetsloot et al., 2016) among all biochars has been observed in the pyrolysis product of defatted animal bone chips, so called bone char (BC). Nutritional value of bone meal for agricultural purposes has always been recognized in last centuries (Smith, 1959). Despite of environmental and health concerns arisen with spread of disease through raw bone materials (e.g. bovine spongiform encephalopathy) and subsequent restrict EU regulations (Commission of the European Communities, 2006), it is still being used in domestic gardening and organic farms (Royal Horticultural Society, 2002; Ylivaino et al., 2008). Annual assessment of the P-budget revealed that recycling of associated P with slaughterhouse residues (meat/bone meal) and municipal sewage sludge could replace about 70% of the total P applied as mineral fertilizer in Austria (Egle et al., 2014). This illustrate that pyrolysis of animal bones can help to prevent about 339×10^6 kg of P in food sector of EU 27-countries (van Dijk et al., 2016) from being wasted, to achieve a better closed-loop P cycle and sustainably meet future P demand.

Physico-chemical properties of BC may vary widely depending on characteristics of animal bone sources and pyrolysis conditions. The light yellow colour of bone turns to brown and black during the pyrolysis at low to high temperatures (300 to 900 °C) (Reidsma et al. 2016) but Rojas-Mayorga et al. (2015) reported colour changes from black to grey and white at 800 °C and 900 °C, respectively. These colour changes originate from thermal degradation of organic matter of the bone matrix (Patel et al. 2015; Rojas-Mayorga et al. 2015). Bone chars are characterized by a mesoporous structure with a specific surface area between 42 and 114 m^2 g^{-1} (Warren et al., 2009; Siebers and Leinweber, 2013; Patel et al. 2015). The porosity and crystal structure of bone chars depend on the mineral composition (Rothwell et al., 1980),

animal species and age (Wu et al., 2003) and pyrolysis conditions (Larsen et al., 1994). The interlocked porous network remains after removal of organics from heated bones (Etok et al., 2007; Ooi et al., 2007). According to Figueiredo et al. (2010) elevated temperatures can result in higher crystallinity degrees, larger crystallite sizes and a less porous structure. Patel et al. (2015) obtained the maximum porosity at 400 °C pyrolysis temperature and with three hours residence time. Apart from common variations in basic constituents, originating from different bone sources and treatment processes, the major chemical elements of BCs are calcium (Ca: 20 to 40%), phosphorus (P: 13 to 20%) and carbon (C: 8 to 13%) (Cheung et al., 2001; Warren et al., 2009; Rojas-Mayorga et al., 2015; Zwetsloot et al., 2016). The elemental contents and ratios of BC can be influenced by the temperature and residence time of the pyrolysis. Zwetsloot et al. (2015) reported a total P enrichment with increasing pyrolysis temperature. Patel et al. (2015) obtained a mean Ca/P ration of 2.2 at pyrolysis temperatures of 400 to 600 °C and one hour residence time which increased to 2.6 at two hours residence time. Dissimilar to most biochars, BC has only about 10% w/w of C (Cheung et al., 2002; Fuller et al., 2003; Wilson et al., 2003; Medellin-Castillo et al., 2007). Based on ash-free dry matter, the C-content and the C/H and C/N ratios increased with increasing pyrolysis temperature, indicating the progressive formation of cyclic C-compounds (Rojas-Mayorga et al., 2015). Other elements such as N, Mg and S occur in low concentrations. Moreover, BC is almost free of heavy metals and contaminants (Deydier et al., 2005; Siebers and Leinweber, 2013).

The solubility of BC in soil can be influenced by 1) manufacturing processes which create different physicochemical properties 2) a wide range of soil characteristics leading to the chemical key reactions and 3) cropping system and field management. BC is characterized by its biological apatite-base and controlled-release properties. The lower crystalline structure and higher carbonate concentration of bone apatite directly result in greater solubility in comparison to the mineral hydroxyapatite (Wopenka and Pasteris, 2005; Pan and Darvel, 2010). The effect of pyrolysis temperature on P chemistry of BC has been evaluated by different thermal treatments. The results showed that elevated temperatures promote the decomposition of organic compounds (Ayllón et al., 2006) where the solubility decreases with increasing hydroxyapatite content (Novotny et al., 2012). The P-extractability from BC is low in water (3 to 20% of total P) but larger in formic acid (92 to 95% of total P), with a general tendency of a lower solubility in water but a larger solubility in formic acid with increasing pyrolysis temperature between 220 and 750 °C (Zwetsloot et al. 2015). The

proportions of Ca- and Mg-compounds result in alkaline pH of BC in the range of about 7 to 10 while BC dissolution is restricted at pH-values above 6. Several studies have evidenced soil pH as the most effective parameter for BC dissolution. In this regard, BCs were appeared likely insoluble in neutral and alkaline soils towards moderately soluble in acidic ranges (Warren et al., 2009; Siebers and Leinweber, 2013). Consistent with these findings, Zwetsloot et al. (2016) reported that the P availability in P-fixing soils was stimulated by root-mycorrhizae-BC interactions which may be explained by acidification in the soil rhizosphere. Warren et al. (2009) showed in an incubation experiment that the P sorption capacity of soil had an important influence on BC dissolution. This was supported by Siebers and Leinweber (2013) who revealed a positive correlation between P release from BC and the P sorption capacity of studied soils. Since BCs are essentially slow-release fertilizers highly dependent on P sorption capacity of soil, discharge of the P-saturated soil solution can lead to further P release *via* BC dissolution (Siebers and Leinweber, 2013). Moreover, different particle-sizes of fertilizer can cause various patterns of distribution and consequently P-availability in the soil (Sleight et al., 1984) and also different particle surface areas which influence fertilizer-soil reactivity (Bouldin and Sample, 1958). Thus, providing proof of optimum BC particle-size is of essential importance to insure achieving greater efficiency and higher crop yields with given fertilizers. Many studies have revealed the particle-size effects on P-release properties of different phosphate fertilizers (Sander and Eghball, 1988; Bolland and Gilkes, 1989; He et al., 2005) whereas comparatively no information exists on particle-size aspects influencing BC-dissolution.

There are few publications available about crop yield effects of BCs, and almost all are from short-term pot experiments. Siebers et al. (2012) reported yield decreases and increases for potato and wheat and yield decreases for onions compared to the controls (0 mg P kg^{-1} soil). In a forthcoming study by Siebers et al. (2014) the yield of wheat heads responded positive to BC application but lettuce and potato tubers yielded indifferent results for two soils with moderate and high P levels. Little et al. (2015) investigated the effect of BC on the growth of various weeds and arable crops in comparison to composted poultry manure in biological farming systems. They reported that BC did not adequately mimic the P supply from composted poultry manure. Zwetsloot et al. (2016) reported positive effects of BC in combination with composted poultry manure *arbuscular mycorrhizae* inoculation on maize yield in P-fixing soil. In summary, it is evident that BC sometimes but not always has a positive fertilization effect. However, reasons for inconsistent findings are not clear.

Sulfur (S) is an essential nutrient for plant metabolism and S deficiency in agricultural soils has been recognized in many European countries during the last decades (Ercoli et al., 2012). The most common form of inorganic S in soil is sulfate and can be present as SO_4^{2-} either dissolved in soil solution or adsorbed to soil particles (Barber, 1995). Leaching is potentially one of major contributors to S depletion. Thus, providing a sustainable S fertilizer is generally valuable. The oxidation of S decreases soil pH, mostly caused by soil micro-organisms activities. Many studies have reported S-oxidation by *A. ferrooxidans* and *A. thiooxidans* enhanced the solubilization of P from phosphate rock due to sulfuric acid production and in turn uptake by plants (Pathirathna et al., 1989; Besharati et al., 2007; Chi et al., 2007). In this line, Rajan (2002) found the effective dissolution of phosphate rock as a result of elemental S oxidation by *Acidithiobacillus* bacteria in alkaline soil. Bhatti and Wasim (2010) improved the P-release from phosphate rock supplemented with S-mud which was mainly attributed to the concentration of bacterially produced sulfuric acid by acidophilic iron- and sulfur-oxidizing bacteria (*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*). In order to find a way to improve P dissolution from BC, addressing the crucial role of pH, we hypothesized that blending BC with reduced S-containing compounds (approx. 30 to 50% S w/w) may promote the BC-dissolution and P-release in soils. This surface-modified bone char (BC[plus]) can be a promising approach to meet the crop P and S demands and introduce BC[plus] as an eco-friendly fertilizer. However, exactly how the surface modification may possibly influence important properties of BC has not yet been studied.

Several studies have proven that water soluble P compounds applied to the soil may redistribute mobile soil Cd into less mobile fractions and reduce the Cd uptake by plants (Naidu et al., 1994; Bolan et al., 1999; Bolan et al., 2003; Chaiyarat et al., 2011). The retention of contaminants by BC has been successfully tested for the removal of fluoride from water (Medellin-Castillo, 2007), and the adsorption increased with decreasing pH as explained by electrostatic interactions (Medellin-Castillo, 2014). Since the point of zero charge of BC is around 7.7 it can be also used for the removal of cationic contaminants from water. Experiments with Cr (III) (Flores-Cano, 2016), Cu and Zn (Wilson et al., 2003), Cd, Cu and Zn (Cheung et al. 2001, 2002), and U (Fuller et al., 2003) demonstrated the adsorption capacity of bone chars and its potential use in water purification. Thus, not surprisingly, BC has also been tested for the retention of contaminants from soil. For instance, Chen et al. (2006) showed that Pb uptake by Chinese cabbage was significantly reduced in the presence of 1.6% (w/w) of BC in soil. Siebers and Leinweber (2013) showed a reduced

Cd mobility in BC-amended soils and explained this by the increased pH. Studies on S fertilizer effects on Cd-immobilization in soil revealed no consistent results. Some researchers have reported that S application increases Cd availability in soil and Cd concentration in plants (Salardini et al., 1993; Nocito et al., 2002). Other studies, however, showed that S application could immobilize Cd in soil and decrease plant uptake (Hassan et al., 2005). It was also reported that S supply could restrain Cd uptake in rice due to the formation and precipitation of inseparable Cd-S in waterlogged paddy soils (Daskalakis and Helz, 1992; Kashem and Singh, 2001). Not surprising, the fate of Cd in following Cd-contaminated soils amended with novel BC^{plus} is completely unknown.

The critical part of soil management in modern agriculture systems is soil testing method and the ideal ones are those that narrowly reflect plant nutrient uptake, in addition, independent of soil properties. When slow release P-fertilizers are being used, extraction procedures using acidic or alkaline extractants under/overestimate the P-availability (Menon and Chien, 1995). One of the most widely used soil P tests is Olsen method (Olsen et al., 1954) that is generally accepted as an index for plant available P in both acidic and calcareous soils. Extracting solution, sodium bicarbonate ($NaHCO_3$), reacts with and dissolves P from the soil components. Some other soil P extraction methods like water and NH_4NO_3 solutions which run at approximately the original pH of the soil characteristically attain close correlations to available P values for plants when compared to other soil extraction procedures. In some European countries like Netherlands, water extraction procedure is used to approximate plant available P in the soil solution. Furthermore, the precise determination of P species is crucial for assessing the reactivity, solubility and adsorbing capability of BC and relevant treated soils. The results can improve insights into agricultural and environmental aspects of BC application. Sequential P fractionation proposed by Hedley et al. (1988) is the most commonly used procedure to characterize availability of selective organic and inorganic P pools including 1) readily available (resin-P), 2) labile ($NaHCO_3$-P), 3) fixed by Al- and Fe-oxide minerals and organic P from humic substances (NaOH-P) and 4) insoluble P bound to Ca-minerals and apatite (H_2SO_4-P). A study conducting sequential P fractionation on BC particles revealed that the majority of P was associated to the Ca and apatite fraction (72% by weight) followed by labile fraction ($NaHCO_3$-P, 1.2%), readily available fraction (resin-P, 0.3%) and adsorbed to Al- and Fe-oxide minerals (NaOH-P, 0.04%) (Zimmer, D. and Kruse, J., personal communication). In this line, Siebers et al. (2013) reported that BC application to the soil increased the H_2SO_4 extractable P fraction, mainly consisting of Ca- and Mg-

phosphates. To overcome some analytical limitations of sequential fractionations (e.g. chemically alteration of samples during successive extraction and lack of selectivity to present chemically well defined species) various novel methods were developed recently for the chemical speciation of plant nutrients and soil pollutants, among which synchrotron-based X-ray absorption near edge structure (XANES) spectroscopy has proven to be a particularly sensitive nondestructive speciation technique (Kruse et al., 2015). XANES spectroscopy enables element specific *in situ* speciation in solid and heterogeneous mediums and requires only minimal sample preparation (Beauchemin et al., 2003; Lobi and Susini, 2009). The P- and Cd-speciation in a BC-amended soil was investigated by Siebers et al. (2013) for the first time. The XANES results indicated that proportion of hydroxyapatite (main component of BC) increased in soil after BC application. Furthermore, Cd-immobilization observed in sequential extraction was confirmed as an increase in proportion of insoluble $Cd_3(PO_4)_2$ in XANES spectra. Therefore, speciation of relevant P-compounds can be well-evaluated by bringing together various analytical methods such as sequential extractions with advanced XANES technique to get new insights into the complex interactions of soil-plant system and potential use of BC^{plus} as an alternative clean P-fertilizer. To the best of our knowledge, there has been no previous study to use these multi-methodological approaches investigating P-speciation of BC and BC^{plus} particles and treated soils.

1.2   Research objectives

Since there is an urgent need to develop non-contaminated P-fertilizers from renewable resources, the present dissertation project aimed to find out the potential of newly modified BC for improving soil fertility and provide a scientific and practical reference point on BCs application as P-fertilizer and Cd-immobilizer soil amendments. More specific objectives were to:

∞ Evaluate the capability of surface modification in P-release promotion from BC-amended soils without losing the Cd-immobilization potential by three extraction methods for estimating P and Cd-availability

∞ Explore the effect of non-equilibrium condition on the P-release from BC and BC^{plus} as slow-release P fertilizers

∞ Assess the effect of particles size of BC and BC^{plus} on the P- and Cd-release in moderately Cd-contaminated soils

∞ Track the changes in P-speciation of BC and BC^{plus} particles and relevant treated soils after incubation-leaching and ryegrass cultivation practices by XANES spectroscopy and sequential fractionations

2 Effect of bone chars on phosphorus-cadmium-interactions as evaluated by three extraction procedures

_____

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2.1 Abstract

Foreseen P shortage and contamination problems have stimulated the search for renewable and contaminant-free P-fertilizers and amendments that immobilize cadmium (Cd). We investigated the P-dissolution and Cd immobilizing effect of bone char (pyrolyzed de-fatted bone chips; BC) and bone char with added reduced S compounds (BC^{plus}). Five soils varying in pH and low to high Cd-contamination were incubated with slow-release P-fertilizers (BC and BC^{plus}) and the fast P-release diammonium phosphate (DAP) and extracted with NH_4NO_3-, $NaHCO_3$-solutions and H_2O. The P-concentrations obtained by the three extractants were well correlated and NH_4NO_3 well suited to simultaneously assess the P- and Cd-solubility. The addition of BC increased pH in all soils whereas BC^{plus} and DAP lowered the pH in soils with pH > 5. Similar trends for NH_4NO_3-P differences between treatments and control were observed for BC and BC^{plus} during the incubation period although BC^{plus} resulted in much larger P-concentrations. The highest Cd-immobilization efficiency was obtained in BC treated soils. The addition of BC^{plus} and DAP decreased the Cd-concentrations until 34 days of incubation in all soils and remained effective in Cd-immobilizing in soils that showed a pH raise over 145 days of incubation. Thus, the results indicate that surface modification of BC may promote the P-dissolution along with a concomitant Cd-immobilization largely through its pH-effect but this must be confirmed in studies under non-equilibrium conditions.

Keywords: fertilizer, phosphorus release, extractant, incubation, cadmium immobilization

2.2 Introduction

Mineral P fertilizers produced from rock phosphate are limited, finite and non-renewable resources (Cordell and Neset, 2014), and they usually contain impurities such as cadmium (Cd), arsenic (As) and lead (Pb) (Franklin et al., 2005). Continuous use of these P-fertilizers may cause accumulation in soils, and enhance the contaminant availability to plants, transport to groundwater, and as a consequence to food and ecological chains (Mendes et al., 2006). Slight to moderate Cd-contamination, reflecting the legacy of intensive use phosphate fertilizers, is widespread throughout Europe (Pan et al., 2010). Since intensive crop production with high yields requires periodic P fertilization (Cordell and White, 2014), renewable but also clean alternatives to rock phosphate-based P-fertilizers are urgently needed. Thermal processing products of animal bones like bone char (BC) comprise many environmental and economic benefits to establish a sustainable agriculture (Vassilev et al., 2013). Potentially, BC is an eco-friendly alternative P-fertilizer but the P solubility is relatively small in comparison to commercial products and depends on soil pH (Warren et al., 2009). Consequently, smaller and larger effects on P uptake and crop yield were observed in different soils and in comparison to different commercial P fertilizers (Siebers et al., 2012, 2014). The P-solubility perhaps can be improved by a surface modification by adding microorganisms (Postma et al., 2010) or P-solubilizing chemicals but the fate of such surface-modified BC in Cd-contaminated soils is completely unknown.

Phosphate fertilization was found to reduce Cd-uptake by crops in heavily contaminated soils (Chaiyarat et al., 2011). In moderately contaminated soils (0.3 to 19.6 mg Cd kg^{-1} soil) the addition of BC immobilized up to 75% of mobile Cd, and this was explained by an increase in pH from the concomitant release of calcium (Ca) and magnesium (Mg) (Siebers and Leinweber, 2013). Furthermore, there is initial evidence for the precipitation of a P-Cd-phase following amendment with BC (Siebers et al., 2013) but the complex BC-P-Cd interactions in soil are far from being understood.

Soil testing methods are subject to ongoing debates and research because the ideal ones are those that narrowly reflect plant nutrient uptake and contaminant behavior. One of the most widely used soil P-tests is the sodium bicarbonate-extraction (Olsen et al., 1954) as an index for plant available P in both acidic and calcareous soils but its extraction efficiency for Cd has not yet been tested. Other extractants, e.g. H_2O or NH_4NO_3 solution, likely can be used to remove and determine plant available P along with mobile Cd. For instance, in the

Netherlands the H_2O-extraction is applied to approximate plant available P in soil (Vanderdeelen, 2002), and the NH_4NO_3-extractions is the standard method for mobile heavy metals in the German soil protection legislation (Blume et al., 2011). It is unknown, how these widely used extractants reflect the fate of P and Cd in soil after amendment with various BCs.

Therefore, the aims of present study were 1) to evaluate the three above extraction methods for estimating P- and Cd-dissolution in BC-amended soils, and 2) to find out if a surface modification of BC (BC^{plus}) enhances the P-dissolution without losing the Cd immobilization potential. Overall we want to provide a basis for developing guidelines for the application of BCs in sustainable plant production and soil amendment.

2.3 Materials and methods

2.3.1 Experimental soils

Samples from the 0 to 30 cm-layer of five soils varying in chemical and physical properties and covering the low to high range of Cd contamination were taken from grasslands (soils 1, 2 and 4) and arable sites (soils 3 and 5) in the Germany. The sources of Cd contamination (values >0.4 or >1 mg Cd kg^{-1}, depending on soil texture; Bundesministerium der Justiz, 1999), were mining activities (soils 3 and 4), industrial effluents settled in floodplains (soils 1 and 2) and a sewage farm (soil 5). The soils samples were air-dried, crushed, homogenized and sieved < 2 mm for further analyses. Fundamental physical (soil texture) and chemical properties (pH, cation exchange capacity, elemental concentrations) of soils, and the soil amendments were determined by standard methods (Blume et al., 2011) (Table 2-1).

2.3.2 Fertilizers and incubation experiment

Three fertilizers, (1) BC, produced from de-fatted and de-gelatinized animal bone chips by pyrolysis at about 800°C; (2) BC^{plus}, obtained by blending BC with reduced S-containing compounds (approx. 30 to 50% S w/w) and (3) diammonium phosphate (DAP), a commercial P fertilizer as a benchmark, were ground to pass a 90-μm mesh-sieve to minimize particle size effects. Total elemental concentrations of the fertilizers are shown in Table 2-2.

Table 2-1 Selected physical and chemical properties of the soil samples under study.

Soil	Texture			pH	CEC[a]	Organic C	Total P	Total Cd	P sorption[b]
	Sand	Silt	Clay						
	/ mass%				/ cmol kg^{-1}	/ g kg^{-1}	/ mg kg^{-1}		
1	62	14	24	6.5	30.7	56	1560	1.9	19.1
2	51	32	17	6.4	30.6	62	1202	2.6	13.2
3	50	33	17	5.9	16.6	25	1008	6.1	19.1
4	48	40	12	4.9	8.3	68	1306	15.1	27.9
5	8	89	3	4.2	6.0	32	2820	19.6	31.0

[a]Cation exchange capacity.
[b]P sorption capacity (data from Siebers and Leinweber, 2013).

Table 2-2 Chemical characteristics of bone char (BC), modified bone char (BC^{plus}), and diammonium phosphate (DAP).

Property	BC	BC^{plus}	DAP
Total P (g kg^{-1})	149.4	141.2	206.5
Total Cd (mg kg^{-1})	0.5	0.3	28
Total Ca (g kg^{-1})	184.8	219.6	8.2
Total Mg (g kg^{-1})	23.9	2.2	3.4
Total N (g kg^{-1})	7.5	8.8	185.7
Total C (g kg^{-1})	112.5	82.6	1.6
Total S (g kg^{-1})	6.3	271.4	24.8

Soils were incubated with 4 variants of P fertilization, control (0 mg P kg^{-1}), BC, BC^{plus} and DAP (P addition to reach 500 mg P kg^{-1} soil). Each soil x fertilizer variant was incubated for seven time periods (1, 3, 5, 13, 34, 70 and 145 days). For each variant (soil x fertilizer x time period, each 3 replicates) about 4 g of air-dry soil (< 2 mm) plus fertilizer were mixed and placed in 20 cm^3 polyethylene containers. The moisture content of the mixture was adjusted between 60 or 70% of field capacity. The containers were covered with a perforated lid of parafilm to prevent moisture loss while allowing aeration and were incubated in a controlled temperature room at 20ºC in the dark. The containers were watered daily by weight to recoup

evaporated moisture. The triplicate samples of each treatment were removed from the incubation after 1, 3, 5, 13, 34, 70, and 145 days, air dried, ground to pass a 2 mm-sieve, and subdivided into representative subsamples before the subsequent extractions.

2.3.3 Extraction procedures and Cd- and P-analyses

The incubated soils were extracted by (1) distilled H_2O (van der Pauw, 1971; Luscombe et al., 1979): Two g of dry soil (< 2 mm) were shaken with an end-over-end shaker with 20 ml of deionized water in a 50 mL centrifuge tube for one hour. After centrifugation for 10 minutes, the supernatant was filtered through Whatman no. 42 filter and acidified to pH 2.0 with HCl to prevent precipitation of phosphate compunds. (2) For extraction with NH_4NO_3 (He and Singh, 1993; Siebers and Leinweber, 2013) soil samples (0.5 g dry soil < 2 mm) were placed in 15-mL polymer centrifuge tubes and shaken end-over-end with 12.5 mL of 1 mol L^{-1} NH_4NO_3 solution at 20°C for 24 hours. After centrifugation at 3500×g for 10 minutes, the supernatant was carefully decanted, filtered (Whatman no. 42 filter) and stabilized with 125 μL of concentrated HNO_3. (3) For extraction with $NaHCO_3$ (Olsen et al., 1954) subsamples of 1 g of dry soil (< 2 mm) were shaken with 20 mL 0.5 M $NaHCO_3$ solution for 30 minutes. Samples were centrifuged for 10 minutes and filtered (Whatman no. 42 filter). The supernatant was diluted and acidified with HCl to destroy HCO_3^- for ICP-OES measurement.

The concentrations of Cd and P were determined with inductively coupled plasma-optical emission spectroscopy (ICP-OES, JY 238, JobinYvon, France) at wavelengths of 228.802 nm (Cd) and 214.914 nm (P).

2.3.4 Statistics

The data were analyzed by STATISTICA version 10 software. The parameters NH_4NO_3-P H_2O-P, $NaHCO_3$-P NH_4NO_3-Cd, H_2O-Cd, $NaHCO_3$-Cd and soil pH were analyzed using one-way analysis of variance (ANOVA, Tukey test) with the factors "treatment" and "days of incubation". Also a linear regression model was used to determine the significance of correlation between extraction methods.

2.4 Results and discussion

2.4.1 Chemical properties of fertilizers

The BC and BC[plus] had almost similar concentrations of total P, Cd, C and N (Table 2-2). However, BC[plus] contained much more S (27.1%) than BC and DAP (0.6 and 2.5%, respectively). DAP had much more Cd and N but less C and Ca than BC and BC[plus]. The latter were almost free of Cd (0.3-0.5 mg Cd kg^{-1}) whereas DAP contained 28.0 mg Cd kg^{-1}.

The BC used in this study agrees in composition with those characterized by Warren et al. (2009) and Siebers and Leinweber (2013), even though slight differences were observed in the chemical properties such as Ca, Mg, Cd and P contents. The BC[plus] is rich in S, an essential plant nutrient for which deficiency has been recognized in many European agricultural soils (Ercoli et al., 2012). As it is introduced here for the first time there are no data in the literature for comparison.

2.4.2 Assessment of extraction methods

The overall means of extracted P significantly varied between extractants and treatments as summarized in Fig. 2-1. The P-concentrations generally decreased in the order $NaHCO_3$-P > NH_4NO_3-P > H_2O-P. Large differences were observed for P extracted by $NaHCO_3$ as compared to H_2O-extraction in soils 1 and 2 (pH > 6). In general, the extracted P-contents in DAP largely exceeded those in BC and BC[plus] treatments, in which the differences between extractants were more pronounced as well. In the DAP treatment the $NaHCO_3$–extaction of soil 1 at day 5 of incubation yielded the maximum P content (320 mg kg^{-1}) (not shown). The H_2O-P means were roughly half those for NH_4NO_3-P and about a quarter of $NaHCO_3$-P in BC treatments. The NH_4NO_3 and $NaHCO_3$ solutions extracted rather similar P-concentrations from soils 4 and 5 which had the lowest pH (Table 2-1).

In more detail, the different P-extraction methods also showed the impact of fertilization treatments and duration of incubation (Table 2-3). Significant positive differences between each treatment (BC, BC[plus] and DAP) and the corresponding controls were more often reflected by NH_4NO_3-P (n = 15) than by $NaHCO_3$-P (n = 8) and H_2O-P. However, the additional P in the BC and BC[plus] treatments always yielded larger differences in the NH_4NO_3-P than in the $NaHCO_3$-P, and this was restricted to soils with pH < 6 (soils 3, 4 and

5). Negative differences (P in control > P in treatment) were seldom significant. The H_2O extraction showed a positive effect of bone chars only five out of six cases in soils 4 and 5, and the largest difference was obtained for BC[plus] in soil 5.
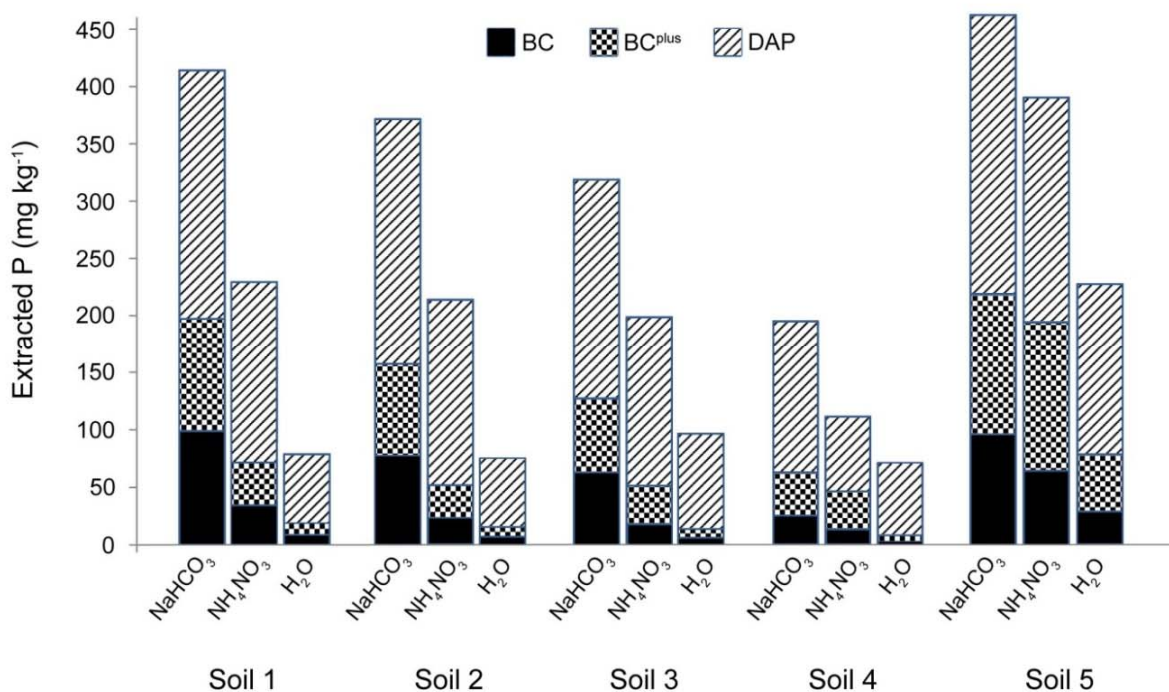
Fig. 2-1 Extracted P-concentrations as the mean value of three independent replicates during incubation period (mg kg^{-1}) using the extraction with NH_4NO_3, $NaHCO_3$ and H_2O for all soils treated with BC, BC[plus] and DAP.

Linear regression involving all data (5 soils x 4 treatments x 7 sampling dates) revealed highly significant relationships between the contents of $NaHCO_3$-P and NH_4NO_3-P ($R^2 = 0.82$, $P < 0.001$) and H_2O-P and NH_4NO_3-P ($R^2 = 0.79$, $P < 0.001$) (Fig. 2-2). The relationship between H_2O-P and $NaHCO_3$-P was significant as well at the 0.001 level ($R^2 = 0.69$) (not shown).

Table 2-3 Differences in P concentrations (mg kg^{-1}) in the fertilization treatments and the control at selected incubation days as reflected by three extraction methods. Negative values mean P concentrations smaller in treatment than in the control.[a]

Soil	Treatment	Method of extraction	Incubation period		
			Day 1	Day 34	Day 145
1	BC	NaHCO$_3$	-2.2 NS	3.9 NS	1.4 NS
		NH$_4$NO$_3$	0.3NS	3.0NS	4.9NS
		H$_2$O	-0.3 NS	0.4 NS	-0.4 NS
	BC^{plus}	NaHCO$_3$	-2.6 NS	6.6 NS	1.9 NS
		NH$_4$NO$_3$	6.9NS	4.0NS	3.5NS
		H$_2$O	0.4 NS	1.0 NS	-0.1 NS
	DAP	NaHCO$_3$	170.8**	99.4**	78.1**
		NH$_4$NO$_3$	153.3**	138.1**	87.9**
		H$_2$O	73.9**	38.8**	27.1**
2	BC	NaHCO$_3$	2.3 NS	3.1 NS	3.6 NS
		NH$_4$NO$_3$	-0.2NS	2.4NS	2.2NS
		H$_2$O	-0.1 NS	-0.1 NS	0.6 NS
	BC^{plus}	NaHCO$_3$	16.2 NS	3.5 NS	6.2 NS
		NH$_4$NO$_3$	5.8NS	7.4NS	6.9NS
		H$_2$O	0.7 NS	0.3 NS	1.5 NS
	DAP	NaHCO$_3$	164.8**	113.2**	101.4**
		NH$_4$NO$_3$	138.5**	107.8**	103.2**
		H$_2$O	65.3**	45.2**	36.6**
3	BC	NaHCO$_3$	-0.7 NS	0.6 NS	1.3 NS
		NH$_4$NO$_3$	5.5NS	10.1**	4.8NS
		H$_2$O	-0.6 NS	-0.4 NS	-0.6 NS
	BC^{plus}	NaHCO$_3$	0.8 NS	0.8 NS	2.4 NS
		NH$_4$NO$_3$	20.9**	25.9**	15.6**
		H$_2$O	0.8 NS	1.0 NS	3.4*
	DAP	NaHCO$_3$	93.9**	105.1**	82.4**
		NH$_4$NO$_3$	165.0**	121.5**	79.1**
		H$_2$O	107.2**	58.7**	43.9**
4	BC	NaHCO$_3$	1.4 NS	-1.9 NS	4.8 NS
		NH$_4$NO$_3$	4.9 NS	7.1NS	4.6NS
		H$_2$O	0.6 NS	0.7 NS	0.7 NS
	BC^{plus}	NaHCO$_3$	11.5 NS	11.8**	19.9**
		NH$_4$NO$_3$	21.5**	28.0**	20.4*
		H$_2$O	3.2**	2.9 NS	2.8*
	DAP	NaHCO$_3$	144.7**	83.3**	81.7**
		NH$_4$NO$_3$	74.3**	48.8**	36.3**
		H$_2$O	69.2**	49.1**	28.8**
5	BC	NaHCO$_3$	-10.0 NS	-22.0*	0.8 NS
		NH$_4$NO$_3$	5.0NS	18.3NS	4.9NS
		H$_2$O	-1.2 NS	-1.6 NS	-1.4 NS
	BC^{plus}	NaHCO$_3$	10.9 NS	5.6 NS	28.2*
		NH$_4$NO$_3$	84.9**	76.0**	52.1**
		H$_2$O	22.1*	17.4**	14.2**
	DAP	NaHCO$_3$	161.2**	81.1**	64.0**
		NH$_4$NO$_3$	189.4**	117.7**	88.8**
		H$_2$O	168.4**	87.4**	64.6**

[a]Tukey-Test: * Significant at $P < 0.05$; ** Significant at $P < 0.01$; NS Non-significant.
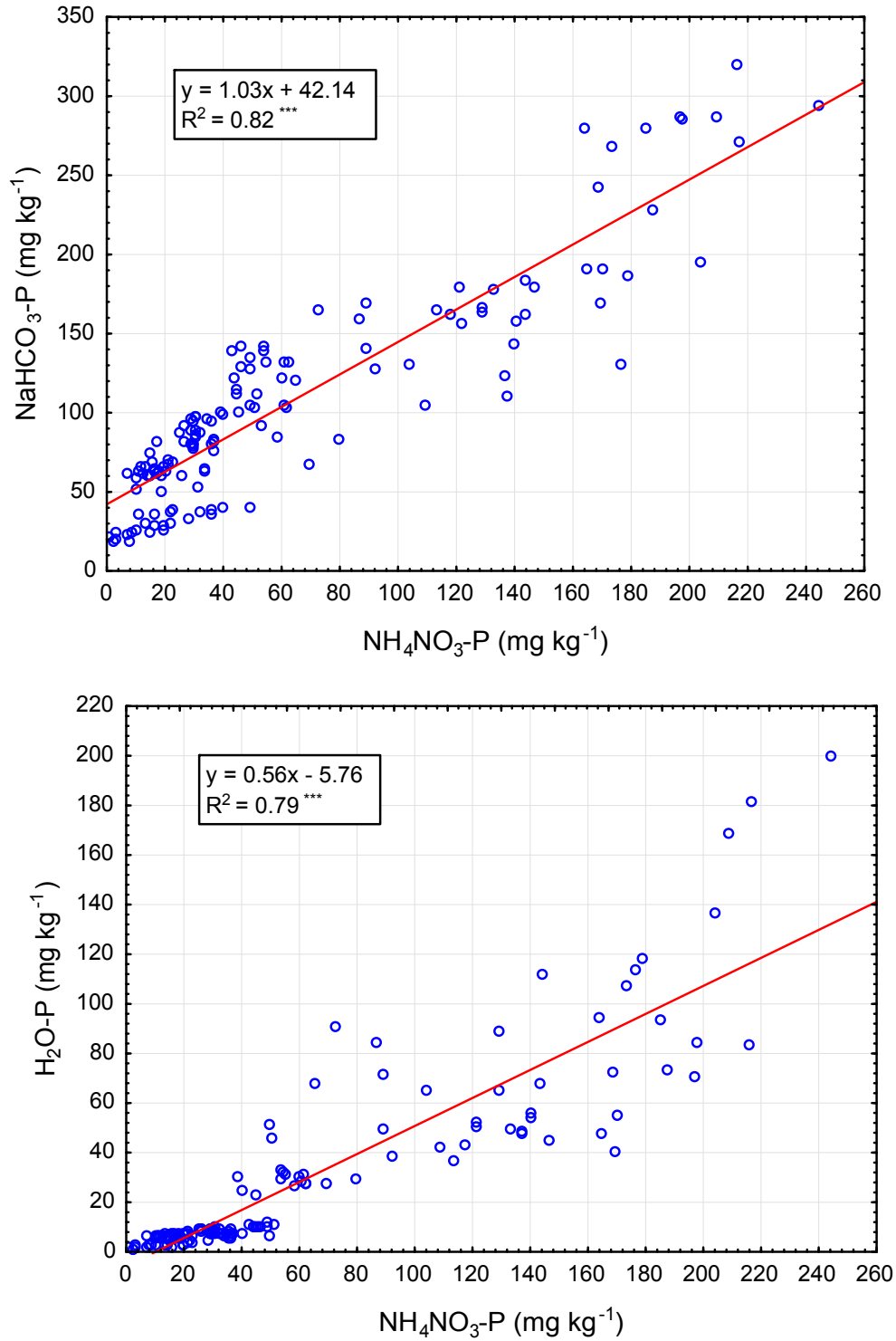
Fig. 2-2 The relationships between NH_4NO_3 extractable P (mg kg^{-1}) and NaHCO$_3$ and H$_2$O extractable P (mg kg^{-1}) for all soils and treatments. *** Significant at $P < 0.001$.

When comparing the P-extraction methods, the sequence for $NaHCO_3$-P and H_2O-P was expected and generally confirmed findings by other authors (Zhang et al., 2004; Wuenscher et al., 2015). The mean quantities of H_2O-P below those extracted by $NaHCO_3$ and NH_4NO_3 affirmed that H_2O as a weak extractant may not reveal all labile P but mainly reflects the intensity parameters, i.e. readily dissolved forms. In agreement with this, Sorn-Srivichai et al. (1988) reported that 240 mL H_2O extracted considerably less P from 2 g soil than ryegrass took up although H_2O-P was closely correlated with the plant P-uptake. The latter high correlation supports the view that represent H_2O-P is a good indicator of P readily available to the plants in a wide range of soils (Van der Pauw, 1971; Kuo, 1996). Moreover, the H_2O-P is an index for P-concentration in runoff (Pote et al., 1996) but it was not correlated with P in lysimeter leachates (Leinweber et al., 1999).

$NaHCO_3$ obviously extracts more P than H_2O (Fig. 2-1) including fractions that are not necessarily as indicators of immediate plant availability (Otabbong et al., 2004). These authors further reported that $NaHCO_3$ as a weak alkaline solution (pH 8.5) can potentially extract large amounts of organically bound P. Larger values for $NaHCO_3$-P than for the other two fractions (Figs. 2-1 and 2-2) also can be due to $NaHCO_3$ reduction effects on the Ca^{2+} and Al^{3+} activity, resulting in an elevated P-solubility in soils (Sims, 2000). In the same way, $NaHCO_3$ solution releases P bound to calcium carbonate and Fe/Al-oxide surfaces (Schoenau and O'Halloran, 2007). The $NaHCO_3$-P showed no induced P-dissolution from BC during incubation period while for DAP treatments it was and effective extractant in accordance with NH_4NO_3 (Table 2-3). Menon and Chien (1995) reported that the $NaHCO_3$ extraction is proposed as a basis for fertilizer recommendations when water soluble P-fertilizers have been used and alkaline or acidic extractants under- or overestimate the P availability. Accordingly, the P-release pattern from the two BCs during incubation was well reflected by NH_4NO_3 (Table 2-3). This extractant, used for the first time in P studies by Siebers and Leinweber (2013) revealed that the P-concentration in BC-amended soils continually increased within 34 days and subsequently decreased until 145 days of incubation.

In a study comparing 14 soil P extraction methods, Wuenscher et al. (2015) also reported that $NaHCO_3$-P was significantly correlated with H_2O-P which the correlation coefficient of the equation was 0.574 ($P < 0.001$). In agreement with this, Sorn-Srivichai et al. (1988) found strong correlations ($r = 0.90$) between $NaHCO_3$-P, H_2O-P and plant uptake of P. However, Neyroud and Lischer (2003) evaluated 16 P-extraction procedures and reported significant differences between uncorrelated P-levels extracted by $NaHCO_3$ and H_2O. In addition, the

large variability observed between results even for same methods if determined in different laboratories.

Warren et al. (2009) assessed P bioavailability of the animal bone char by extraction with the NaHCO$_3$ and reported a close correlation between NaHCO$_3$-P and dissolved-P extracted by a two-step sequential procedure using NaCl and NaOH extractants. The authors concluded that dissolved-P could be a suitable guide for available P to the plants. However, it is well known (Saggar et al., 1992; Menon and Chien, 1995; Warren et al., 2009) that NaHCO$_3$ underestimates P-availability of gradually soluble fertilizers as BCs are. For these slow-release materials (BC and BC^{plus}) NH$_4$NO$_3$ is a good alternative P-test, especially for acidic soils, and this extractant also works for fast-release P-fertilizers such as DAP (Table 2-3). However, there is only little data in the literature for direct comparisons (Siebers and Leinweber, 2013).

The most contaminated soil (soil no. 5) was selected to represent Cd-dissolution results obtained by the three extraction methods (Table 2-4). In this as well as in all other soils and treatments more Cd was extracted significantly by NH$_4$NO$_3$ than by H$_2$O and NaHCO$_3$. The widest range between extracted Cd-values with different methods was 6673 µg kg^{-1} in soil 5, where the highest measured concentration (NH$_4$NO$_3$-Cd = 7069 µg kg^{-1}) was about 18-fold greater than the lowest value (NaHCO$_3$-Cd = 396 µg kg^{-1}). The concentration of H$_2$O-Cd in most cases exceeded that of NaHCO$_3$-Cd although differences were insignificant. Comparing Cd-extractability of the three extraction methods during incubation days indicated different trend patterns for each treatment which can be attributed to chemical properties of extractant, soil and amendments interference, notably pH changes and salt concentrations.

Data in Table 2-4 show that NH$_4$NO$_3$ is an efficient extractant for soil Cd, and this can be explained by the slightly acidic effect of the NH$_4^+$ ion and the formation of soluble Cd-amine complexes (Lebourg et al., 1998; Pueyo et al., 2004). According to Pueyo et al. (2004) NH$_4$NO$_3$ extracted more Cd than CaCl$_2$ and NaNO$_3$ in 7 alkaline and neutral out of 10 soils. Correspondingly, Symeonides and McRae (1977) assessed plant-available Cd in 25 soils by various methods (e.g. NH$_4$NO$_3$, EDTA, AcOH) and found the best correlation between Cd in top grown of radish plants and soil-extracted NH$_4$NO$_3$-Cd which simulates the soil pH near the plant roots.

Table 2-4 Concentrations of Cd ($\mu g\ kg^{-1}$) in the control and different treatments during the incubation of soil 5 extracted by $NaHCO_3$, NH_4NO_3, and H_2O.[#]

Treatment	Method of extraction	Incubation days						
		1	3	5	13	34	70	145
Control	NaHCO₃	378 bA	386 bA	396 bA	414 bA	353 abA	292 aA	379 bA
	NH₄NO₃	6637 bB	6262 abB	7069 bB	6869 bB	6178 abB	6430 bB	5370 aB
	H₂O	537 aA	441 aA	532 aA	456 aA	510 aA	475 aA	447 aA
BC	NaHCO₃	358 aA	321 aA	348 aA	341 aA	307 aA	303 aA	382 aA
	NH₄NO₃	4898 aB	4992 aB	4999 aB	3785 aB	4548 aB	4015 aB	3728 aB
	H₂O	273 abA	201 aA	221 aA	286 aA	346 aA	282 abA	342 bA
BCᵖˡᵘˢ	NaHCO₃	401 bcA	459 cA	490 cA	377 abcA	290 abA	225 aA	388 abcA
	NH₄NO₃	4759 abB	5722 cB	5542 bcB	4351 aB	4319 aB	4437 aC	4764 abB
	H₂O	379 aA	388 abA	390 abA	446 abA	457 abA	465 bB	386 abA
DAP	NaHCO₃	463 bcA	511 cA	454 bcA	307 aA	263 aA	233 aA	350 abA
	NH₄NO₃	5115 bB	5239 bB	5379 bB	5151 bB	4592 abB	4425 abB	3868 aB
	H₂O	240 cdA	119 aA	135 aA	197 aA	204 bcA	216 bcA	258 dA

[#]Significant differences (α = 0.05, Tukey-Test) between rows (extraction methods) within each variant (Control., BC, BCᵖˡᵘˢ, DAP) at each day (day 1 to day 145) are marked by different upper case letters, and Significant differences (α = 0.05, Tukey-Test) between columns (day 1 to day 145) within each variant (Control., BC, BCᵖˡᵘˢ, DAP) within each extraction method ($NaHCO_3$, NH4NO3, H_2O) are marked by different lower case letters.

Menzies et al. (2007) reported that trace metal concentrations extracted by complex agents (e.g. DTPA and EDTA) or acid extractants (e.g. HCl and Mehlich-1: HCl/H_2SO_4) compared with un-buffered solutions (e.g. NH_4NO_3 and $NaNO_3$) are poorly correlated to plant phytoavailability, indicating the changes in soil-solution pH and trace metal speciation. That corresponds well with findings of many authors (Brown et al., 2005; Meers et al., 2007; Zhu et al., 2012) that NH_4NO_3-Cd correlated significantly with crop tissue Cd-accumulation. Brown et al. (2005) studied the effect of soil remediation materials including phosphorus, lime, red mud, cyclonic ashes, biosolids, and water-treatment residuals on Cd- and Zn-phytotoxicity and found the NH_4NO_3 extraction method could well predict the reduced metal phytoavailability. Thus we can summarize, that NH_4NO_3-P and NH_4NO_3-Cd are adequate predictors of available P and Cd in a wide range of soils after the slow-release P-fertilizers were applied. Nevertheless, the biouptake is the best evaluation the bioavailability estimates provided by different extractions.

2.4.3 Amendment effects on soil pH

The application of BC significantly increased the soil pH in all soils immediately after one day of incubation but during the incubation period the extent of pH-increase differed (Table 2-5). The soil pH was mostly increased within 34 days of incubation and afterwards it decreased to some insignificant differences between treatments and control until day 145. For BC^{plus}, the pH decreased continuously relative to the control in soils 1, 2 and 3 (pH > 5) throughout the incubation. In soils 4 and 5 (pH < 5), the BC^{plus} caused overall significant pH-increases but differences were insignificant for some incubation days. In the DAP treatment, the pH declined in all soils except the most acidic one (soil no. 5) where pH significantly increased. The pH increase of BC treatment for soil 5 after five days of incubation was the highest among all treatments (0.4 pH units). On the other hand, the most effective treatment for soil pH reduction was found in the BC^{plus} addition to soil 3 after 145 days of incubation (0.75 pH units).

Table 2-5 Soil pH as affected by BC, BC^{plus} and DAP fertilizers after 1 and 145 days of incubation. The pH was measured with 0.5 g soil in 1.25 ml of a 0.1 mol L^{-1} CaCl$_2$ solution using a mini-pH-meter electrode.[a]

Soil	Day 1				Day 145			
	Control	BC	BC^{plus}	DAP	Control	BC	BC^{plus}	DAP
1	6.60	6.74**	6.51**	6.49**	6.56	6.64*	6.33**	6.21**
2	6.44	6.55**	6.31**	6.39^{NS}	6.34	6.48^{NS}	6.08**	6.08**
3	5.96	6.15**	5.88**	6.00^{NS}	5.86	6.07**	5.11**	5.31**
4	4.88	5.12**	4.90^{NS}	5.07**	4.60	4.94**	4.77**	4.42**
5	4.14	4.47**	4.24**	4.44**	4.24	4.81**	4.32**	4.68**

[a]Tukey-Test: * Significant at $P < 0.05$; ** Significant at $P < 0.01$; ^{NS} Non-Significance difference.

For BC, the results in Table 2-5 confirm Siebers and Leinweber (2013) who found that the pH increase was significant in most soils after incubation for 34 days, and it was larger with higher BC application rates. These results agree closely with those reported by Ma and Matsunaka (2013) who described that soil pH increased with increasing BC application. In the BC^{plus} treated soils, the pH decrease can be explained by the microbial oxidation of the

reduced, surface-bound S-compounds to H_2SO_4, which promoted the pH decline especially in soils with pH > 5. Fuente et al. (2008) also reported a moderate reduction in soil pH (by 0.5 units) when elemental S was applied to a calcareous soil which was significantly correlated with the production of soluble sulfates and affected by the oxidation rate of S and the buffer capacity. Sulfate adsorption by soils mostly occurs at pH < 6 and the ability of S to decrease pH was influenced by the contents of clay, hydrous oxide of Fe and Al and exchangeable cations (Tabatabai, 2005).

The influence of DAP on pH alterations depended on soil pH and incubation time (Table 2-5). Similarly, McGowen et al. (2007) showed that DAP application lowered the soil pH from 7.1 to 6.5 after two months of incubation and then remained constant. Because of previous buffering of the soil with limestone, the acidification potential of DAP decreased during incubation period. Thawornchaisit and Polprasert (2009) observed a similar pH reduction in DAP-treated soils compared to the control samples. Generally, the pH-reduction by DAP in Ca^{2+}-containing soils can be explained by the below reactions (Spuller et al., 2007):

$$(NH_4)_2HPO_4 + Ca^{2+} + 2H_2O \rightarrow CaHPO_4.2H_2O + 2NH_4^+ \tag{2-1}$$

$$4CaHPO_4.2H_2O + H_2O \rightarrow Ca_4H(PO_4)_3.3H_2O + H_3PO_4 \tag{2-2}$$
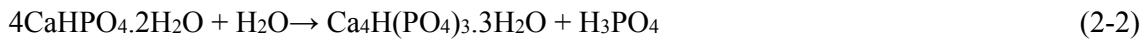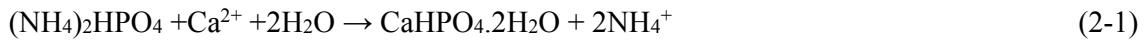
2.4.4 Release of soluble P-compounds

None of the BC treatments significantly changed the concentrations of soluble P in all soils (Table 2-3). In the majority of cases (soil x sampling date) the BC application resulted in increased NH_4NO_3-P-concentrations compared to the control. The NH_4NO_3-P concentrations increased over 34 days of incubation and decreased then to constant values until day 145. In the BC^{plus} treatments, the increase in P-concentrations was much larger but the trend in NH_4NO_3-P concentrations was similar to the BC treatments. In soils 3, 4 and 5 (pH < 6) elevated P-concentrations were obtained throughout the incubation period (Table 2-3). Including all soils and sampling dates significant negative correlations were found between the differences in NH_4NO_3-P (y = treatment minus control) and soil pH (x) (BC^{plus}: y = 158.2 − 24.3 x, R^2 = 0.65, P < 0.001; BC: y = 19.3 − 2.5 x (R^2 = 0.36, P < 0.001). All DAP treatments showed significantly increased concentrations of soluble P at day 1 of incubation but then they continuously declined during the incubation period (Table 2-3).

The P-dissolution data of the BC treatments revealed a strong dependency on the soil acidity which confirms that soil pH is a major parameter affecting P-dissolution from BCs (Warren et al., 2009; Siebers and Leinweber, 2013). However, Warren et al. (2009) found no significant differences between un-amended and treated soil with BC for higher soil pH than 6.14. The study also reported that BC dissolution could be significantly controlled by the size of P sink. In addition, the dissolution of calcium phosphate fertilizers such as BC is affected largely by P, Ca and Mg concentrations in the soil solution (Chien and Menon, 1995). It could be therefore expected that acidic soils with a high total P content and P sorption capacity strongly absorb released P from BC (high Ca and Mg content) and, consequently, mask soil pH efficiencies in P-dissolution enhancement. In this line, He et al. (2005) showed for highly acidic soils that phosphate released from P-fertilizers were sorbed by sesquioxides or precipitated as Fe/Al phosphate leaving only a portion of dissolved P available.

Modification of BC by surface-bound S compounds (BC^{plus}) promoted the P-release in soils with pH > 6 (soils 1 and 2, Table 2-3). Moreover, BC^{plus} application not only caused a pH increase in soil 5 (pH < 5) by liming effect but also improved amount and prolonged the time of P-dissolution for about 145 days compared to the control. Many studies have reported that pyrite oxidation by Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans enhanced solubilization of P from rock phosphate due to the production of H_2SO_4 and, consequently, the P uptake by plants (Pathirathna et al., 1989; Besharati et al., 2007; Chi et al., 2007). Similarly, Bhatti and Wasim (2010) improved P-release from rock phosphate by adding a S-mud and explained this by the above bacterial acid production. In addition, the C/P ratio may have an influence on the P-release. The BC^{plus} had a lower C/P ratio (0.58) than BC (0.75) (Table 2-2), and it always released more P (Table 2-3) confirming Gagnon and Simard (1999) who reported that compost with lower C/P ratio released more P during incubation of an acidic soil than compost with a higher C/P ratio. Therefore, influence of C-source for microbial populations and corresponded P-fixation by microorganism activities could result in less P-dissolution during incubation period.

2.4.5 Amendment effects on Cd concentrations

The BC amendment significantly decreased NH_4NO_3-extracted Cd in all soils after 34 days of incubation which was generally not the case in the BC^{plus} and DAP treatments (Table 2-6). For soils 3, 4 and 5, the BC application significantly lowered Cd concentration during the

whole incubation period. The addition of BC^{plus} produced an immediate significant decrease in Cd concentration after one day in soils 3, 4 and 5 and, subsequently, at day 34 in soils 1 and 2. The concentrations increased over that of control after 145 days of incubation in soils 1, 2 and 3. For the most acidic and contaminated soil 5, the pattern of Cd changes over time was similar in all treatments. The order of Cd concentrations was BC^{plus} < BC < DAP except for day 145 where the order was BC < DAP < BC^{plus} (Table 2-6).

Table 2-6 Differences in NH_4NO_3-Cd concentrations ($\mu g\ kg^{-1}$) in the fertilization treatments and the control at selected incubation days. Negative values mean P concentrations smaller in treatment than in the control.

Soil	Treatment	Incubation period		
		Day 1	Day 34	Day 145
1	BC	2.7 [NS]	-7.9[**]	-11.8 [NS]
	BC^{plus}	8.1 [NS]	-7.1[**]	10.9 [NS]
	DAP	-1.4 [NS]	-7.5[**]	-0.9 [NS]
2	BC	-6.9 [NS]	-25.0[**]	-6.9 [NS]
	BC^{plus}	11.5 [NS]	-22.9[**]	21.6[*]
	DAP	-14.5 [NS]	-24.9[**]	14.4 [NS]
3	BC	-182.7[**]	-182.4[**]	-217.9[**]
	BC^{plus}	-160.1[**]	-8.7[**]	434.2[**]
	DAP	-174.6[**]	-24.7[**]	362.9[**]
4	BC	-82.5[**]	-28.6[*]	-107.4[**]
	BC^{plus}	-65.9[**]	-11.8 [NS]	-106.5[**]
	DAP	-58.4[**]	-2.5 [NS]	-19.6 [NS]
5	BC	-1739.3[**]	-1629.6[**]	-1642.0[**]
	BC^{plus}	-1878.1[**]	-1858.6[**]	-605.4 [NS]
	DAP	-1522.4[**]	-1586.0[**]	-1501.8[**]

Tukey-Test**:** [*] Significant at $P < 0.05$; [**] Significant at $P < 0.01$; [NS] Non-Significant.

The P-dissolution (Table 2-3) coinciding with increasing pH values (Table 2-5) indicate that these two processes may contribute to the Cd immobilization in the BC treatment (Table 2-6). Siebers and Leinweber (2013) found that BC immobilized Cd but triple superphosphate did not despite of its higher P concentrations even above optimal plant growth thresholds.

Hodson et al. (2000) reported that bone meal application suitably reduced metal release from contaminated soil through the pH-increases of soil and formation of metal phosphates. The Cd-immobilization in acidic soils depends on the amount of phosphates, soluble organic substances, Fe- and Mn-oxides/hydroxides, and in alkaline soils precipitation/co-precipitation of Cd-minerals can contribute to the Cd-immobilization (Kabata, 2001). Such a newly formed Cd-P-phase ($Cd_3(PO_4)_2$) was detected by XANES spectroscopy of a strongly Cd-contaminated soil that was incubated with BC (Siebers et al., 2013).

Generally, soils can be separated into two groups according to alteration in soluble Cd after amendment. Acidic soils (4 and 5) showed the same trend of relatively constant decreases in Cd concentrations over the whole incubation period, irrespective of amendment type (Fig. 2-3, soil 5). Alkaline soils (1, 2 and 3), having generally lower Cd-concentrations (factor about 0.1) showed a similar positive amendment effect only for BC. By contrast, any Cd immobilization by BC^{plus} and DAP was only temporarily up to days 13 to 34; thereafter the Cd concentrations increased to a level above starting concentrations (Fig. 2-3, soil 3). This is explained by the pH decrease (Table 2-5) and, in case of DAP, by additional Cd inputs (28 mg Cd kg^{-1} DAP = 271 µg Cd kg^{-1} experimental soil, Table 2-2).

We tested the hypothesis that S-containing compounds in BC^{plus} may improve the BC-dissolution without losing the Cd-immobilization capacity. The results indicate that this is true for moderately acidic soils with pH ≤ 4.9 only (Fig. 2-3). The great pH-influence disagrees with Thawornchaisit and Polprasert (2009) who reported that Cd-stabilization performance of chemical P-fertilizers was correlated positively with concentration of phosphate released but not on the pH reduction in the range of pH 6 to 4. A significant pH effect was also reported by Basta and McGowen (2004) who applied limestone to prevent acidification from DAP and then found highly decreased Cd and Zn concentration in leachates from DAP treatments. Therefore, the increased Cd concentrations in the BC^{plus} and DAP treatments after 34 days (Table 2-6) may be explained by the simultaneous decrease in P-dissolution and soil pH. A Cd stabilization or precipitation by phosphate supply only may be achieved by P- applications as high as 800 mg P kg^{-1} (Hong et al., 2008) but this may produce high amounts of water soluble P and eutrophication problems.
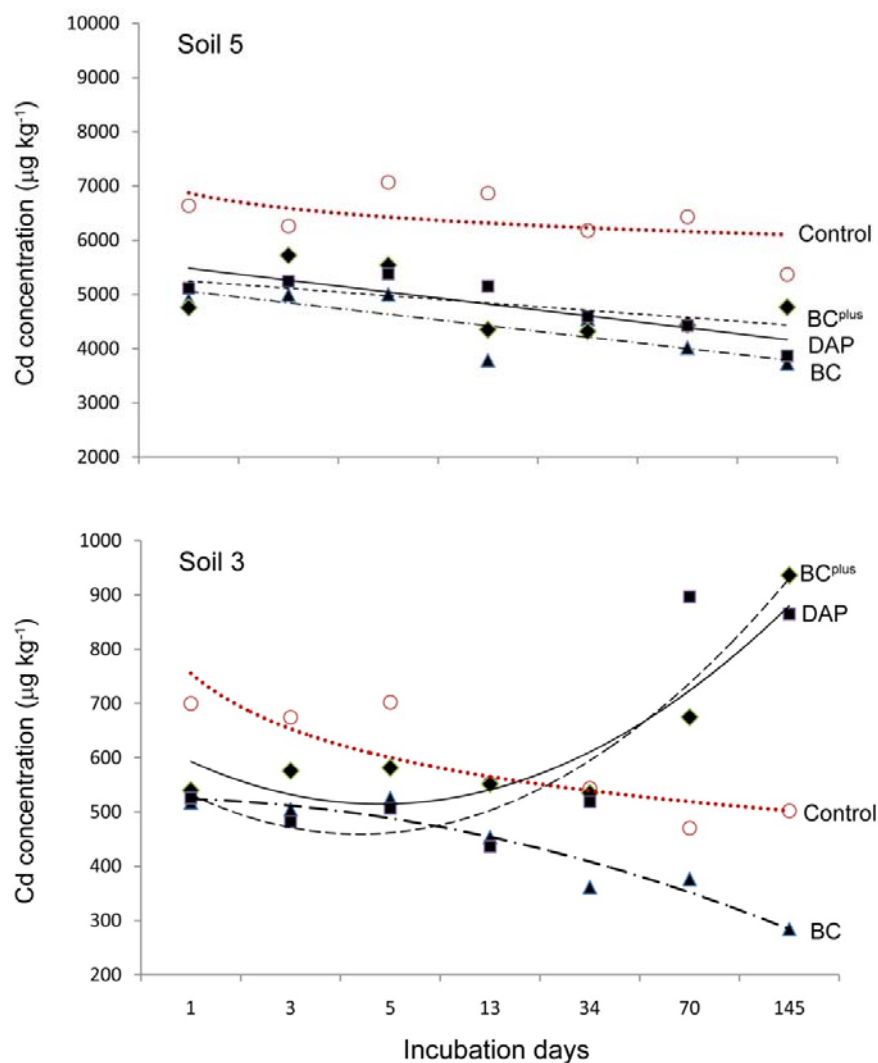
Fig. 2-3 Effect of BC, BC[plus] and DAP treatments on NH_4NO_3 extractable Cd-concentrations of soils 3 and 5 during 145 days of incubation.

2.5   Conclusions

In this study we provided evidence that NH_4NO_3 is an efficient extractant to simultaneously estimate P- and Cd-solubility in soils amended with slow- (BC and BC[plus]) and fast-release (DAP) P-fertilizers as it extracted more Cd and P than $NaHCO_3$ and H_2O. Since the P-concentrations extracted by the three methods were well correlated the novel NH_4NO_3 may be applied for different purposes such as fertilizer recommendations or soil conservation practices that traditionally rely on conventional soil P tests such as $NaHCO_3$- (Olsen) and

H_2O-extractions. However, testing the suitability of NH_4NO_3-extraction for fertilizer recommendations needs to establish how well it predicts bioavailability by correlating extraction results to plant P uptake and yield.

Furthermore, the present study introduced BC^{plus} for the first time and proved that surface modification by adding S resulted in an increased P-release from soils over a wide pH range but the effect was less pronounced in soil with pH >6. Since the Cd-immobilization by-effect was proven for moderately acidic soils (pH <6) we can recommend BC^{plus} as a slow P-release, uncontaminated and renewable fertilizer, especially for weakly Cd-contaminated soils and vulnerable crops or food products.

However, hitherto these studies were done under equilibrium conditions which incompletely represent field conditions. Therefore, the preliminary results must be confirmed in non-equilibrium laboratory studies and pot and field experiments that involve P and Cd leaching as well as plant uptake and yield determinations.

2.6 Acknowledgments

2.7 References

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3 Leaching of phosphorus and cadmium in soils amended with different bone chars

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3.1 Abstract

The benefits of bone char (BC) application may include both the mobilization of P for plant nutrition and immobilization of contaminant Cd in the soil. However, previous studies were conducted under equilibrium conditions that do not adequately reflect field conditions. Therefore, the objective was to study the effect of BC, surface-modified BC (BC^{plus}) and sulfur-enriched activated char (AC^S) application at different particle sizes on P- and Cd-mobilization under non-equilibrium conditions in a combined incubation-leaching experiment. Two soils each with the treatments (i) BC, (ii) BC^{plus}, (iii) BC + AC^S, (iv) control (0 mg P kg^{-1} soil), and (v) AC^S were leached five times during 70 days incubation. Over the complete incubation P-contents in leachates were significantly increased by BC and BC^{plus} of 0.5-1 mm size. P-release from BCs was larger under non-equilibrium condition in this experiment compared with a previous continuous incubation experiment. AC^s application reduced the P-release from BC of all particle sizes. The lowest leachate Cd-concentrations were found in soils amended with the smallest BC particles. Addition of BC^{plus} and AC^S significantly increased Cd leaching and the highest amounts of Cd were leached at the smallest particle size. In conclusion, the 0.5-1 mm size class of BCs performed best and should be introduced in practical agriculture using standard machinery.

Keywords: bone char, incubation-leaching, non-equilibrium conditions, particle size fraction, renewable fertilizer

3.2   Introduction

Modern agricultural production greatly relies on costly phosphorus (P) fertilizers from phosphate rock reserves that are being depleted (Van Kauwenbergh et al., 2013) and which may contain potentially toxic elements such as Cd, As and Pb (Jiao et al., 2012). P-recycling from human and animal waste is urgently needed to obtain contaminant-free P-fertilizers, preserve P-reserves and avoid future crises of P-fertilizer scarcity (Rhodes, 2013). Bone chars (BC) are beneficial soil amendments for safe, clean and sustainable crop production (Vassilev et al., 2013). They are biological, apatite-based P-fertilizers with low and pH-dependent solubility and slow P-release (Warren et al., 2009). Several studies, investigating the effect of BC application on labile P in soil, have provided evidence that P release differs between insoluble bone meal at soil pH >6 and moderately soluble commercial P-fertilizer at soil pH <5 (Warren et al., 2009; Siebers et al., 2012; Siebers and Leinweber, 2013; Nogalska and Zalewska, 2013). An innovative approach for promoting P-solubility from BCs in neutral to alkaline soils was a surface modification of the BC by adding sulfur (S) containing compounds (Morshedizad et al., 2016). The idea was to decrease the pH around or at the surface of BC particles by soil microbial S oxidation (Gu et al., 2011), thus promoting the particle dissolution and P release as shown for rock phosphate (Aria et al., 2010). Indeed, an incubation experiment with surface-modified BC (BC^{plus}) under equilibrium conditions revealed larger P dissolution than treatments with BC in all studied soils, even those with pH >6 (Morshedizad et al., 2016).

Particle-size can influence the P-release properties of phosphate fertilizers (Sander and Eghbal, 1988; Bolland and Gilkes, 1989; He et al., 2005). Different sizes of fertilizer particles may result in uneven distributions in soil (Sleight et al., 1984) and different particle surface areas modify reactions at fertilizer-soil-interfaces (Bouldin and Sample, 1959). Furthermore, the particle size of a fertilizer influences occupational health and safety considerations and the opportunity to use farm machinery (Hofstee and Huisman, 1990; DüMV, 2012). So far no information exists about how BC particle size affects its reactivity in soil and safety and agro-technological suitability.

Batch incubation experiments under equilibrium conditions (Warren et al., 2009; Siebers and Leinweber, 2013; Morshedizad et al., 2016) do not accurately assess the P status in fertilized soils for both agronomic (crop P fertilization) and environmental (P leaching risk) issues. This may arise because the P release and BC particle dissolution may be reduced by P

saturation and resorption (McDowell and Sharpley, 2003; Shariatmadari et al., 2006; Verloop et al., 2010). Moreover, drying and grinding of soil samples after incubation may increase the pH, resulting in precipitation and sorption of soluble and plant-available P species (Penn and Bryant, 2006). Furthermore, plant and microbial P uptake and leaching continuously maintain non-equilibrium conditions (Shariatmadari et al., 2006; Penn and Bryant, 2006; Verloop et al., 2010) that should be adapted in BC fertilization experiments.

Leaching techniques are widely used in routine soil testing such as for the determination of cation exchange capacity (CEC) (Sumner and Miller, 1996), for P-release/loss risk assessment (McDowell and Sharpley, 2001) and the determination of trace elements (Hall et al., 1998; MacDonald et al., 2004). In analogy to these methods, we developed a new laboratory-scale incubation-leaching method to test effects of different BCs on soils under non-equilibrium conditions. We hypothesize that repeated removal of P by leaching will maintain a non-equilibrium distribution between P in the solid phase and P in soil solution which, over a period of 70 days, will result in an increased P-mobilization. This can be tested comparing the results with analogous data from a previously published study (Morshedizad et al., 2016) with comparable soil samples and the same BC amendments.

Activated chars (AC) can adsorb and remove many environmental pollutants such as sulfur dioxide (Guo and Lua, 2003), nitrate (Ota et al., 2013), organics with high chemical oxygen demand (Bansode et al., 2004) and heavy metals (Kadirvelu et al., 2001; Bohli et al., 2013; Ribeiro et al., 2015). AC can be produced by treating high-carbon precursors (*e.g.* agricultural wastes) at high temperature and with chemical impregnation agents such as $ZnCl_2$, H_3PO_4, KOH or H_2SO_4 (Caturla et al., 1991; Molina-Sabio et al., 1995; Legrouri et al., 2005; Fierro et al., 2006) or by physical activation using carbonization and gasification with oxygen, carbon dioxide and steam (Miguel et al., 2003; Ribeiro et al., 2015). S-loaded AC (AC^S) is left as a waste after AC is used to remove hydrogen sulfide from biogas, and we hypothesize that adding AC^S to BC-treated soil may raise the P-release by mechanisms similar to those in BC^{plus}. Moreover, AC has a great sorption affinity for heavy metals and, thus might be more effective for Cd-immobilization in soil than BCs. However, S-addition (AC^S) could also decrease pH and reduce Cd-immobilization. These possible reactions have not yet been investigated.

The objectives of this study were to investigate the effects of (1) different particle sizes of BC and BC^{plus}, (2) non-equilibrium conditions in leached soil columns, and (3) AC^S on the P- and Cd-release from moderately Cd-contaminated soils.

3.3 Materials and methods

3.3.1 Soils

The study was performed on two top soils (A and B) of *Dystric Cambisols* (based on the FAO soil classification system) collected from an agricultural research farm of the Julius Kühn Research Institute at Braunschweig, Lower Saxony, Germany (10°27'E; 52°18'N). Soils A and B were moderately contaminated by Cd (2.0 and 2.4 mg Cd kg^{-1} soil, respectively) from previous sewage sludge application. The soils were air-dried and passed through a 2 mm sieve before physical and chemical routine analyses (Blume et al., 2011) and setup of the incubation-leaching experiments. Selected properties of the studied soils are given in Table 3-1.

Table 3-1 Selected physical and chemical properties of the soils.

Soil	Texture			Texture	pH	Organic C	Total S	Total P	Available P (NH$_4$NO$_3$)	Total Cd	Available Cd (NH$_4$NO$_3$)
	Sand	Silt	Clay								
	g/100g					----------g kg^{-1}---------		--------mg kg^{-1}----------		-----------µg kg^{-1}-----------	
A	46.2	45.3	8.5	Loam	5.4	21.2	0.6	1661	33	2002	169
B	33.0	58.5	8.5	Silt Loam	4.7	35.7	0.5	1594	14	2421	497

3.3.2 Chars used as soil amendments

Three different amendments (BC, BC^{plus} and AC^S) were used in the incubation-leaching experiment. BC, produced by pyrolyzing bovine bone chips at 800°C, was purchased from BONECHAR Carvao Ativado do Brasil, Brasil. To obtain BC^{plus} with a better P-solubility the above BC was loaded with S-containing compounds in a biogas-desulfurization process (patent application DE 212012000046U1; https://www.google.com/ patents/DE2120120000

46U1?cl=en&hl=de). The S in the BC^{plus} was composed of 60% elemental S, 30% calcium sulfate dehydrate and 10% methansulfonate; D. Zimmer et al. unpublished results of S-X-ray absorption near-edge fine structure spectroscopy. These two BC-materials were each crushed and sieved to make batches of the following particle size fractions: 0.2-0.5, 0.5-1, 1-2 and 2-4 mm. As a second approach to improve the P solubility of BC we tested a sulfur-enriched AC (AC^S). This originated from a commercial AC (Gebr. Honnens, Tarp, Germany). It had been used to remove hydrogen sulfide (H$_2$S) from a commercial biogas operation, and thus loaded with S-compounds similar to BC^{plus}. Samples of AC^S were ground to pass a 200 µm sieve to minimize the possible particle size effects. Some chemical properties of the studied chars for soil amendment are shown in Table 3-2.

Table 3-2 Chemical properties of bone char (BC), modified bone char (BC^{plus}) and sulphur-enriched activated char (AC^S).

	Property							
	Total P	Total Cd	Total Ca	Total Mg	Total C	Total S	Total N	pH
Material	--g kg^{-1}--							
BC	149.4	5×10^{-4}	184.8	23.9	112.5	6.3	7.5	7.83
BC^{plus}	122.8	3×10^{-5}	264.5	3.5	82.6	198.6	8.2	4.91
AC^S	2.2	2×10^{-5}	9.7	2.9	660.9	199.8	2.4	7.28

3.3.3 Experimental design

The amendments (BC, BC^{plus}, BC + AC^S, AC^S) were added to 30 g air-dried soil, thoroughly homogenized and placed in glass columns (2cm id × 10cm height) (Supporting Information Fig. S1). A P-free filter paper had been placed at the bottom of each column to prevent loss of solid particles. The amount of applied P (500 mg P kg^{-1} soil) was chosen as a moderate fertilization rate for slow release P fertilizers such as rock phosphate (Bolan and Hedley, 1990; He et al., 1999) and bone char (Warren et al., 2009; Siebers et al., 2012; Siebers and Leinweber, 2013) and also to achieve comparable results with our previous study (Morshedizad et al., 2016). BC and BC^{plus} were both tested in four particle size fractions (0.2-0.5, 0.5-1, 1-2, 2-4 mm). The 14 treatments were control (0 mg P kg^{-1} soil), BC (four size fraction), BC^{plus} (four size fractions), BC (four size fractions) + AC^S and AC^S alone. AC^S was always < 0.2 mm and added at 10 g kg^{-1} soil. Each treatment had five replicates.

The soil moisture content between leaching events was maintained at 60-70% field capacity by adding deionized water by weight, after permitting drainage and evaporation during the leaching process. All treated soils were incubated for 70 days. During this period, three pore volumes of deionized water were passed through each column on days 1, 5, 13, 34, and 70. A droplet irrigation system consisting of a regulated water supply and each five injection needles was used to apply water gradually to the top of the soil columns with a constant flow rate of 0.2 mL min^{-1} (Supporting Information Fig. S1). The water formed a shallow pond at the soil surface and was allowed to drain completely. The volumes of leachates were determined by weight (approximately 14-16 mL) and their P and Cd concentrations determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

3.3.4 Statistical analysis

The Shapiro-Wilk test was performed to confirm normal distribution of the data. One-way analysis of variance (ANOVA) followed by Tukey-test was used to examine significant differences between treatments. All statistics were performed using SPSS (IBM SPSS Statistics 21, 2012). Differences between means were considered to be statistically significant if $P \leq 0.05$.

3.4 Results and discussion

3.4.1 Effect of BC particle size on P- and Cd-leaching

Compared to the control, all BC particle size fractions in both soils significantly increased the cumulatively leached P-contents over the incubation period of 70 days (Fig. 3-1). After 70 days of incubation, BC treatments of the 0.5-1 mm size fraction released the largest P-amounts only in soil B while the BC^{plus} treatment released significantly larger P-amounts in both soils. If the particle size was larger than 1 mm, the P-release decreased in BC and BC^{plus} treatments (Fig. 3-1). The enhancing effect of the 0.5-1 mm fraction on P-release from BC^{plus} was highest after 13 days of incubation compared with the other incubation periods. The BC^{plus} treatment showed a higher dependency of P-release on particle size and incubation period than BC. In 0.5-1 mm size fractions, the BC amendment increased the P-concentration in the leachate more than BC^{plus} after 1 day in soil A and after 13 days in soil B. In other cases, especially after 70 days of incubation, the P-release into the leachate was much higher

in the BC[plus]-amended soils. However, although the BC[plus] caused a pH decrease in all treatments, the P-release exceeded that of BC only for two finer size fractions in soil A and the 0.5-1 mm-fraction in soil B.

Fig. 3-1 The cumulative leached P-concentrations as the mean value of five independent replicates (mg P kg^{-1} soil) after 1, 5, 13, and 70 days of incubation for soils A and B treated with different size fractions of BC and BC[plus]. Significant differences ($\alpha = 0.05$, Tukey-test) between particle size fractions within BC or BC[plus] amendments are marked by different letters. Asterisks indicate differences of each treatment compared with the control. [*] Significant at $P < 0.05$ level; [**] Significant at $P < 0.01$ level; [***] Significant at $P < 0.001$ level.

Although there were moderate differences in chemical properties of BC and BC[plus] (Table 3-2) all treatments jointly showed similar trends on P-release in response to particle size. In the case of slow/controlled release fertilizers such as BCs, it was expected that a smaller particle size would increase fertilizer dissolution. Trenkel (1997) reported that nitrogen release rate from urea-crotonaldehyde (slow release N-fertilizer) was the larger the finer the particles were. The effect of particle sizes on the fate of P-fertilizers in the soil is perhaps more complicated because many factors influence the fate of applied P in soils, *e.g.* contents of calcium carbonate and Fe-/Al-oxides, pH, redox potential, organic matter which all influence the sorption, desorption and precipitation mechanisms. Sander and Eghball (1988) investigated the effect of different ammonium polyphosphate particle sizes (particle weights of 0.00019, 0.0009, 0.025, 0.93, and 22 mg) on winter wheat yield. The maximum fertilizer efficiency was obtained at intermediate particle size (0.025 mg) which is comparable with our results. This might be related to the optimum conditions for continuous P-release during growth season with minimal P-fixation in the soil (adsorption and/or precipitation reactions). However, nano- or micro-sized P-fertilizers provide large specific surface areas that promote their possible attachment to solid soil surfaces (He et al., 2013). The results of the present incubation-leaching study are also consistent with those from Ma and Matsunaka (2013) who examined three size fractions (<1 mm, 1-2, and 2-4 mm) of biochar (pyrolyzed meat and bone meal from dairy cattle) in a pot experiment. The <1 mm size-fraction of biochars was most effective to increase P-dissolution and subsequently promote plant growth. Our findings provide more detailed information on the <1 mm size fraction since we investigated two sub-classes of particle sizes. Results showed larger P-release from 0.5-1 mm BC-particles than from the 0.2-0.5 mm fraction.

The total leachable Cd contents were affected by particle size in BC and BC[plus] treatments (Fig. 3-2). Compared to the control, all BC amendments in soil A and the 0.2-0.5 and 0.5-1 mm size fractions in soil B significantly decreased Cd-release into the leachates. The addition of BC[plus] with the two finer particle size fractions (0.2-0.5 and 0.5-1 mm) increased the Cd-release but the coarse size fractions had no influence (1-2 and 2-4 mm). For BC and BC[plus] treatments, leached Cd contents and particle size fractions followed different trends: no size effect (soil A) or increased Cd-release with increasing particle size (soil B) in the BC treatment vs. reduction in Cd-release with increasing particle size in the BC[plus]-treatment. The BC treatments with 0.2-0.5 mm particle size gave the lowest Cd release. In the BC treatment, the leached Cd was reduced by the factor ~1.3 compared to the control, meaning that this

amount of Cd was immobilized. Conversely, the highest Cd-release was obtained at 0.2-0.5 mm sized BC^{plus} after 70 days of incubation. In this treatment the mobilized Cd amount is reflected by the Cd-leaching larger by factor 1.7 (soil A) and factor 1.4 (soil B) compared to the controls.
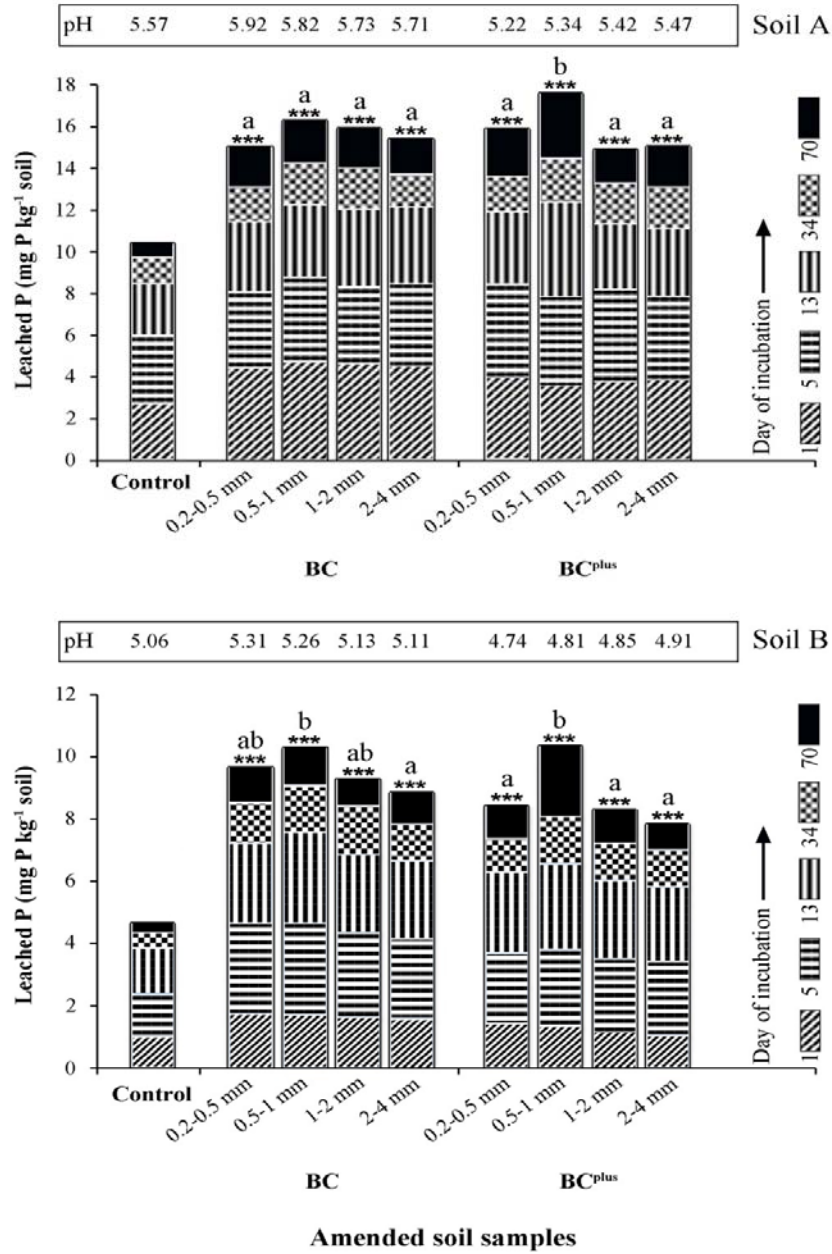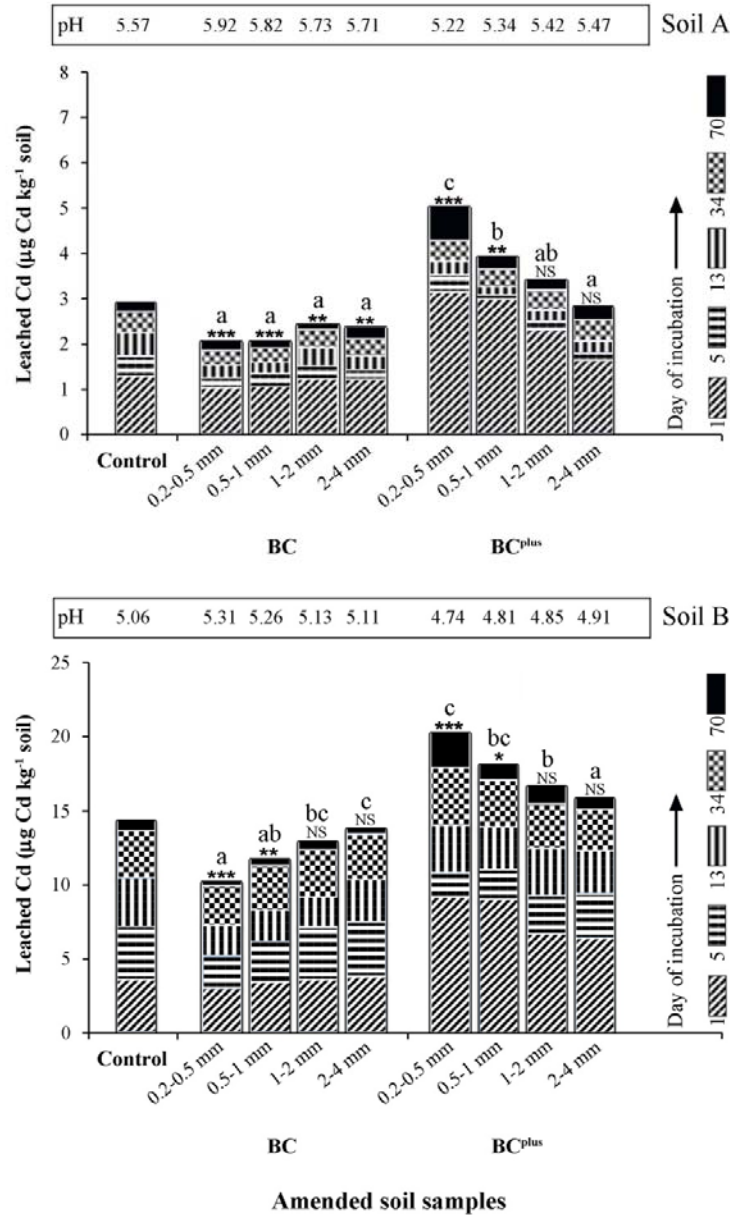

Fig. 3-2 The cumulative leached Cd-concentrations as the mean value of five independent replicates (μg Cd kg^{-1} soil) after 1, 5, 13, and 70 days of incubation for soils A and B treated with different size fractions of BC and BC^{plus}. Significant differences ($\alpha = 0.05$, Tukey-test) between particle size fractions within BC or BC^{plus} amendments are marked by different letters. Asterisks indicate differences of each treatment compared with the control. * Significant at $P < 0.05$ level; ** Significant at $P < 0.01$ level; *** Significant at $P < 0.001$ level.

The contrasting effects of BC and BC[plus] on Cd-release were expected. Many studies reported the pH as a main factor controlling the Cd-availability in soil (Seaman et al., 2001; Basta et al., 2005). The liming effect of BC, measured as pH increase (Fig. 3-2), probably activated the Cd-adsorptive capacity of soil surfaces (Siebers and Leinweber, 2013; Siebers et al., 2013). These results are in agreement with a laboratory leaching experiment with contaminated soils from mine tailings, in which Sneddon et al. (2006) observed Cd immobilization and significantly reduced Cd-concentrations in leachates following bone meal application. Morshedizad et al. (2016) reported that surface modification of BC by reduced S-compounds weakened the immobilizing effect of BC or even mobilized Cd due to a slight acidification of treated soils. In agreement with that previous study, assessment of leachates and amended soils after incubation with BC and BC[plus] revealed the same pattern of pH alterations. Importantly, as the particle size of BC and BC[plus] was reduced, the corresponding expected effects on soil pH were intensified (Figs. 3-1 and 3-2). This means that the highest pH increase was associated with the smallest BC particle size fraction and the highest pH decrease was found in soils with the smallest BC[plus] particle size fraction (Fig. 3-2).

3.4.2 Evaluation of the incubation-leaching method

To evaluate the incubation-leaching method used in the present study, proportions of released P determined by the new method described here were compared with results of the previous incubation-extraction method described in Morshedizad et al. (2016). In that study, two soils were investigated which had similar physico-chemical properties (pH, total P and texture) and the same BC treatments under same incubation conditions (between leaching events) as described in the present study. Comparison of the P-proportions released by the two different methods revealed the effects of the method (equilibrium vs. non-equilibrium), incubation period and soil amendments (Fig. 3-3). On the first day of incubation, the P-proportion extracted by H_2O from soil 4 (1.2 and 0.61% of total P (P_t) for amendment with BC and BC[plus], respectively (Morshedizad et al., 2016)) exceeded the P-proportion that was leached from the corresponding soil B (0.34 and 0.2% of P_t for amendment with BC and BC[plus], respectively). After 13 days of incubation, a higher proportion of P was released by incubation-leaching compared with incubation-extraction. On day 70 of the incubation-leaching experiment, BC and BC[plus] released approximately twice as much P as in the previous incubation-extraction experiment. These differences occurred in both comparable

soil pairs (soils 3 and A, soils 4 and B) and seemed to be more pronounced in soils 3 and A with larger pH values. Irrespective of incubation time, the incubation-extraction method resulted in larger P-proportions in the BC^{plus} treatment compared with the BC treatment.
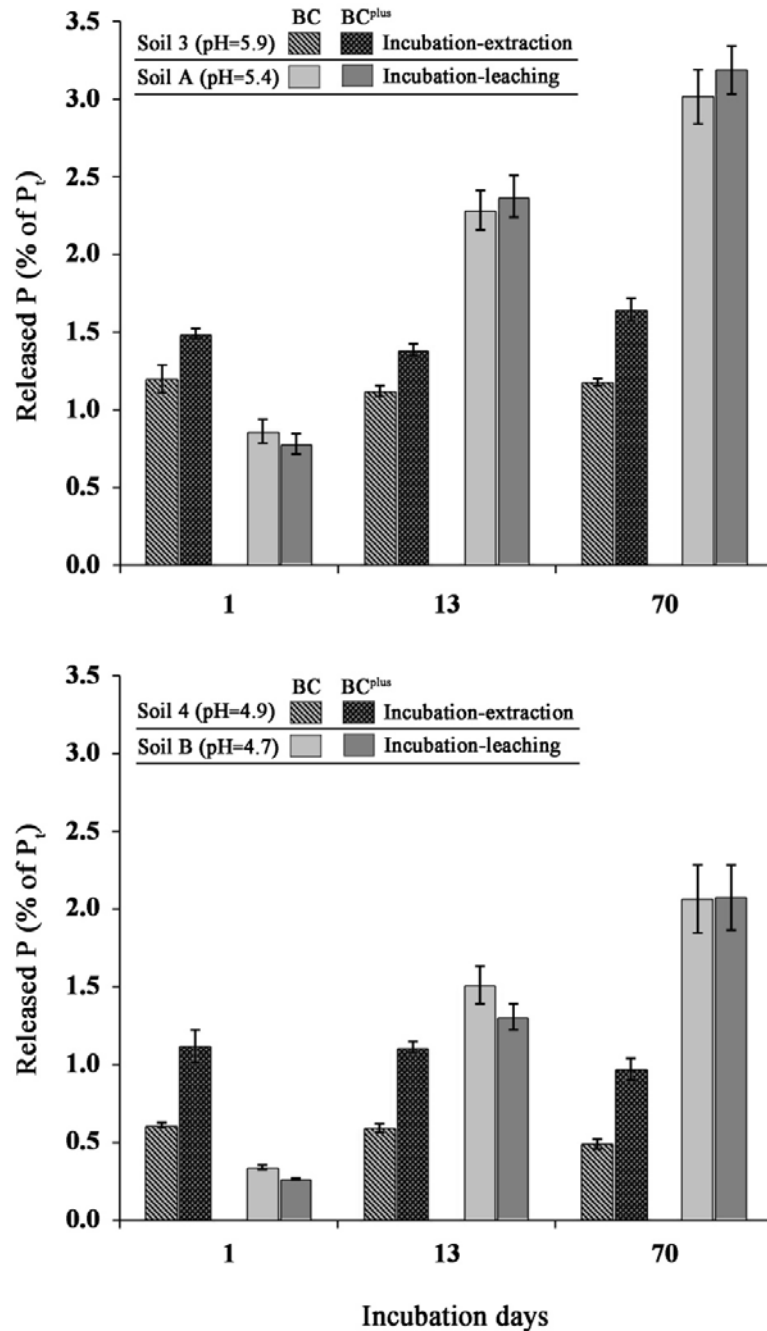
Fig. 3-3 Proportions of released P (as percentage of total P added) from BC-amended soils obtained by incubation-extraction in a previous study (soils 3 and 4, Morshedizad et al., 2016) and by incubation-leaching in the present study (soils A and B).

The differences found at the start of the experiment (day 1: extraction > leaching) can be explained by the physically more vigorous extraction by shaking (Matschonat and Vogt, 1996; Muukkonen et al., 2009). After a longer period of time, the P-proportions released with the extraction method remained relatively constant because of increased ionic strength of the solution under equilibrium conditions. Furthermore, drying and disaggregation by grinding at the end of incubation prior to extraction may have resulted in a reduced H_2O-extractability of P that may have been mobilized before (Olsen and Court, 1982; Horn and Taubner, 1989; Arai and Sparks, 2007). In contrast, larger P-proportions obtained by the leaching method in the longer term (days 13 and 70) indicate that keeping the soil wet and its aggregate structure undisturbed most likely prevents re-sorption or precipitation of released P. Furthermore, complete renewal of the soil solution by the five leaching events during 70 days created temporary non-equilibrium conditions which promote the P-release from BCs. These findings are in agreement with McDowell and Sharpley (2003) who reported two steps of P-release, a first rapid and short step with greater release rate followed by a second slower and longer P-release, which is controlled by diffusion kinetics and resorption processes. The P-release results of the present study (Fig. 3-3) are in agreement with Hantschel et al. (1988) who reported that the solution obtained by percolating water through undisturbed soil cores was more realistic for evaluation of chemical soil-solution composition than saturation extracts. Although the soil columns for incubation-leaching were disturbed and re-packed, and not intact like those of Hantschel et al. (1988), it is considered that the chemical composition of water leached through incubated soil aggregates in the present study better represents field conditions than H_2O-extracts from dried, considerably altered and dispersed soils after the incubation-extraction procedure of the previous experiment (Morshedizad et al., 2016). Furthermore, the incubation-leaching method would be better suitable to include field conditions such as preferential placement of fertilizer particles in large pores and inter-aggregate spaces as well as growth of plant roots in voids/aggregates-interfaces with weak strength (Dexter, 1978).

3.4.3 Amendment with Sulfur-loaded activated char (AC^S)

The addition of AC^S to BC amended soils generally decreased the P-release, whereas it increased the Cd-removal (Table 3-3). The P-decrease in relation to the treatments without AC^S ranged from 0.66 mg kg^{-1} soil (control) to 4.46 mg kg^{-1} soil (BC size fraction of 0.5-1 mm) in soil A. In soil B, there was a small P-increase in the control (0.56 mg kg^{-1} soil) but

not in BC treatments where the reduction of P-release peaked at the 0.5-1 mm of BC size fraction (4.20 mg kg^{-1} soil). AC^S increased the cumulative contents of leached Cd in all treatments, ranging from 1.87 to 4.68 µg kg^{-1} soil (soil A) and 3.18–7.50 µg kg^{-1} soil (soil B). Unexpectedly, the H$^+$ concentrations decreased in all treatments, meaning that the pH values were larger at the end of the incubation period.

Table 3-3 Differences in the cummulative leached P (mg kg^{-1} soil) and Cd (µg kg^{-1} soil) and [H$^+$] concentrations (mol L^{-1}) from treatments with and without AC^S application after 70 days of incubation.

Soil	Parameter	Treatment				
			BC particle size fraction			
		Control	0.2-0.5 mm	0.5-1 mm	1-2 mm	2-4 mm
A	ΔP	-0.66^{NS}	-3.92***	-4.46***	-4.15***	-2.91***
	ΔCd	2.91***	1.87***	2.58***	2.42***	4.68***
	Δ[H$^+$]	-1.8×10^{-7}	-3.5×10^{-7}	-3.7×10^{-7}	-8.2×10^{-7}	-5.7×10^{-7}
B	ΔP	0.56*	-2.47**	-4.20***	-3.15***	-2.67***
	ΔCd	5.84***	6.71**	7.50**	3.18*	6.04**
	Δ[H$^+$]	-11.2×10^{-7}	-7.3×10^{-7}	-9.3×10^{-7}	-11.0×10^{-7}	-16.1×10^{-7}

Negative quantites mean the values were smaller in AC^S treatments than in those without AC^S.

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

NS, non-significant.

The lower values of leached P caused by AC^S application can be explained by the increase in pH values. However, this pH effect shown by negative differences in [H$^+$] (Table 3-3) was unexpected considering the large S-concentration of AC^S (Table 3-2). One reason for [H$^+$] reduction may be the leaching of soluble acidic S-compounds followed by the well-known liming effect of biochars (Hartley et al., 2006; Houben et al., 2013). Another unexpected result was the elevated amounts of Cd-release despite the pH increases. The reason for this discrepancy is not known, but it could be attributed to the facilitated transport of Cd bound to AC^S-colloids, which may pass the filter at the bottom of the column. This suggestion arises from observation of a high sorption affinity of AC for Cd (Kadirvelu et al., 2001) and obvious S-concentrations observed in the ICP-OES spectra of AC^S-leachates (data not shown).

3.5   Conclusions

Since in the medium and long term more P was mobilized under non-equilibrium than under equilibrium conditions of previous studies, the new incubation-leaching approach better reflected the fate of fertilizer P in aggregated soils and plant P requirements during the growing season. This research showed that the optimum particle size of BCs to release a maximum of P along with relatively small Cd concentrations into soil solution was not necessarily the smallest size fraction, but the 0.5-1 mm particle size, for both BC and BC^{plus}. Particles of this size are acceptable for use in commercial fertilizer machinery. A smaller particle size would require pelleting according to German occupational health and safety regulations (DüMV, 2012). Therefore, along with ongoing pot experiments to simulate plant P uptake and leaching, the experimental basis is established for testing BCs as clean, renewable P-fertilizer under field conditions. S-loaded AC (AC^S) so far cannot be recommended for amendment of Cd-contaminated soils because it mobilizes Cd and may increase the risk of Cd uptake by agricultural crops and Cd transfer into the food chain or Cd leaching to groundwater.

3.6   Acknowledgements

3.7   References

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4

Bone char effects on soil: sequential fractionations and XANES spectroscopy
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4.1 Abstract

Acceptability of novel bone char fertilizers depends on their P release but reactions at bone char surfaces and impacts to soil P speciation are insufficiently known. By sequential fractionation and synchrotron-based X-ray absorption near edge structure (XANES) spectroscopy we investigated whether and how the chemical composition of bone char particles has been altered in soil and, consequently, has affected the P speciation of amended soils. Therefore, two different kinds of bone char particles (BC, produced by pyrolysis of degreased animal bone chips at 800 ºC and BC^{plus}, a BC enriched with reduced sulfur compounds) were manually separated from the soil at the end of two different experiments: "incubation-leaching" and "ryegrass cultivation". Sequential P-fractionation of amended soils showed P-enrichment in all fractions as compared to the control. The most P increase between all treatments significantly occurred in the NaOH-P and resin-P fractions in response to BC^{plus} application in both, incubation-leaching and ryegrass cultivation experiments. This increase of the readily available P fraction in BC^{plus} treated soils was confirmed by linear combination fitting (LCF) analysis on P K-edge XANES spectra of BCs particles and amended soils. The proportion of Ca-hydroxyapatite decreased, whereas the proportion of CaHPO$_4$ increased in BC^{plus} particles after amended soils had been incubated and leached and cropped by ryegrass. Based on P XANES speciation as determined by LCF analysis, the proportion of inorganic Ca(H$_2$PO$_4$)$_2$ increased in amended soils after BC^{plus} application. These results indicated that soil amendment with BC^{plus} particles leads to elevated P concentration and maintains more soluble P species than BC particles even after 230-days of ryegrass cultivation.

Keywords: bone char, P speciation, sequential fractionation, X-ray absorption near edge structure spectroscopy

4.2   Introduction

At the global scale readily-available sources of phosphorus (P), a crucial macro-nutrient element for agricultural production, are being faced with scarcity and overpricing (Scholz et al., 2013; Van Vuuren et al., 2010). Environmental pollutions frequently arise from their impurities (Cd, U) (Hartley et al., 2013; Kratz et al., 2016), and from over-fertilization (Rubaek et al., 2013). Further problems are the quick formation of stable and inaccessible compounds that limit plant P uptake (Shen et al., 2011) and the low agronomic efficiency of not more than 15% of fertilizer P in the first year of application (Schnug et al., 2003). Many recent studies have targeted sustainable agriculture through improving P-availability from applied fertilizers (Delgado et al., 2002; Schröder et al., 2011), increasing P-uptake efficiency from organic and inorganic P-pools in the soil (Kaur and Reddy, 2014) and developing new technologies for P-recycling from human and animal waste (Siebers and Leinweber, 2013; Herzel et al., 2016). Particular attention has been paid to the oxidation process, *e.g.* by thiobacilli of elemental sulfur to sulfuric acid, in order to enhance the solubility of non water-soluble P from rock phosphates (Powers, 1923; Lee et al., 1987; Fan et al., 2002) or meat and bone ashes (Schnug et al., 2003).

As an economically and environmentally attractive example, pyrolyzed animal bone chips branded as "bone char" (BC), a slow-release apatite-based P-fertilizer, have been surface modified by sulfur (S) compounds to enhance its solubility in neutral to alkaline soils. Incubation-leaching and pot experiments confirmed that surface-modification was an effective approach in P-release promotion from BC fertilizer (Morshedizad et al., 2016; Zimmer, D. and Panten, K., personal communication). Such an "in situ" digestion of an apatitic phosphate with elemental S was first described by Fan et al. (2002; 2012).

Despite these attempts to raise dissolution and use-efficiency of BC in supplying P for crop requirements, a considerable fraction of applied BC-P to the soil remains insoluble in the short term and is not taken up by plants over the entire cropping period. A detailed P speciation can clarify the fate of insoluble P from BC which has not been done before.

Chemical speciation is described as analytical identification of chemical species of defined elements and measuring their quantities in the system (Templeton et al., 2000). The precise characterization of various P species in the soil as a dynamic response to non-equilibrium conditions imposed by human activities such as fertilization can support a better understanding of reactivity, stability and particularly plant-accessibility of different P-forms

and provide a basis for best management practices. Several techniques such as sequential fractionation (Dieter et al., 2010; Condron and Newman, 2011), nuclear magnetic resonance (NMR) spectroscopy (Liu et al., 2009; Vestergren et al., 2012; Ahlgren et al., 2013), Raman spectroscopy (Lanfranco, 2003; Vogel et al., 2013) and chromatography coupled to mass spectroscopy (De Brabandere et al., 2008; Paraskova et al., 2015) have been developed for P speciation analysis in soil and sediments. Each one of these techniques can offer specific advantages and disadvantages depending on phase and complexity of sample matrixes (Kruse et al., 2015). Complementarily, X-ray absorption near edge structure (XANES) spectroscopy is well-suited for identification of various P species through fingerprinting of molecular structures in solid and heterogeneous mediums based on fine feature and position of absorbing edges (Kelly et al., 2008; Kizewski et al., 2011). The advantages of XANES spectroscopy for soil samples make it a promising technique for direct and *in situ* P-speciation with no pretreatment and minimum sample manipulation (Toor et al., 2006; Kelly et al., 2008).

According to the best of our knowledge, no studies have characterized P-speciation changes in BC particles over non-equilibrium conditions in the soil system, and only few investigations have been reported on the P-release from BC and alteration in P-species of the soil. Siebers et al. (2013) investigated *K*-edge XANES spectroscopy on BC-incubated soil samples and provided evidence that the increase in extractable Ca- and Mg-phosphate fractions were related to the contribution of hydroxyapatite (HAP) increase after BC application. Accordingly, the objective of this study was to provide practical information on the fate and alteration of P-species in BC and novel surface modified BC (BC^{plus}) particles and their treated soils under incubation-leaching and ryegrass cultivation practices, using sequential P-fractionation and P-XANES spectroscopy.

4.3 Materials and methods

4.3.1 Incubation-leaching experiment

Two particle size fractions(1-2 and 2-4 mm) of bone chars (BC, produced by pyrolysis of degreased animal bone chips at 800ºC and BC^{plus} as a surface modified BC obtained by blending with reduced S-containing compounds composed of 60% elemental S, 30% calcium sulfate dehydrate and 10% methanesulfonate (Zimmer et al. unpublished results of S X-ray absorption near-edge fine structure spectroscopy) in a commercial biogas desulfurization

process; patent application DE 212012000046U1;www.google.com/patents /DE212012000046U1?cl=en&hl=de)were incubated with a silt loam soil. The soil was classified as *Dystric Cambisol* (FAO) with pH of 4.7 (measured in 0.01 mol L^{-1} $CaCl_2$ solution) and total (digestion with HNO_3 and analyzed using ICP-OES; USEPA, 1997) and available (extracted by 1 mol L^{-1} NH_4NO_3 and analyzed using ICP-OES; He and Singh, 1993) P-contents of 1.6 g P kg^{-1} and 14 mg P kg^{-1}, respectively. The BC and BC^{plus} contained total P of 149 and 123 g kg^{-1}, total calcium (Ca) 185 and 265 g kg^{-1}, total S 6 and 199 g kg^{-1} and had average of pH_{CaCl2} values 7.8 and 4.9, respectively.

The BCs were added to 30 g of air dry soil (<2mm) at the levels of 0 mg P kg^{-1} soil (control) and 500 mg P kg^{-1} soil in five replicates. The soil and BCs mixture was homogenized and packed into glass columns with 10 cm length and inner diameter of 2 cm. A P-free filter (MN 616 G; Macherey-Nagel GmbH & Co., KG Düren, Germany) was placed at the bottom of each column to avoid any particle losses. The amended soils were incubated for 70 days at 20ºC in the dark and constant soil moisture between 60-70% of soil water holding capacity. During the incubation period, the soil columns were leached with three pore volumes of deionized water added by a droplet irrigation simulator system. The leaching process was repeated in five steps, each one after 1, 5, 13, 34 and 70 days. The P-concentrations in collected leachates were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES). Outcomes of the leaching experiment were described in Morshedizad and Leinweber (2017). After the incubation-leaching experiment, the treated soil samples were carefully removed from the glass columns, air dried and BCs particles were manually separated from the soils very gently. The BCs particles were delicately washed with deionized water to remove adhered soil particles, allowed to dry completely at ambient conditions and finely ground for further analyses.

4.3.2 Pot experiment with annual ryegrass

The same BC and BC^{plus} as described for the incubation-leaching experiment were used in original sizes (mostly between 1 to 5 mm) for P fertilization of annual ryegrass in a pot experiment. The experiment was set-up using an acidic sandy silt soil with available P-content of 24.2 mg P kg^{-1} and pH of 5.2. The pot experiment was set up by adding BC and BC^{plus} at the levels of 0 mg P kg^{-1} (control) and 280 mg P kg^{-1} into the 6 kg of the soil dry matter in each pot and in four replicates arranged in a complete randomized block. After four

weeks of incubation at field capacity water content and ambient temperature conditions, 30 seeds of annual ryegrass per each pot were sown on 13th of May 2016. The experiment was conducted in a glasshouse under ambient air and temperature conditions and the soil moisture was maintained at field capacity during the whole experiment. All other essential nutrients were sequentially added at sufficient levels before seeding and after each six cuts of ryegrass between 23rd of June and 3rd of November 2016. Finally, after the last harvest (7th), plant parts (shoots and roots) were dried at 60 °C and BCs particles were manually separated from the soils as they could be detected visually by their size and dark color very gently, using tweezers. Then these particles were washed delicately with deionized water to remove attached soil particles, allowed to dry completely at ambient conditions and finely ground to fine powders for further analyses.

4.3.3 Sequential phosphorus fractionation

Soil samples were sequentially extracted based on chemical solubility in order according to a modified Hedley et al. (1982) procedure. After BC particles detachment, duplicate 0.5 g fine-ground and air-dried soil samples were weighed into 50-mL centrifuge tubes. In summary, chemical P fractionation includes the following steps:

1) The mobile and readily available P fraction was extracted with resin strips (saturated in 0.5 M NaHCO$_3$) after 18 hours end-over-end shaking in 30 mL deionized water. The resin strips were separated from solids/solution and washed using 50 mL of 1 M HCl to remove absorbed P. The soil suspension was centrifuged at 2500 × g for 20 minutes and the supernatant was decanted.

2) Next, the labile inorganic and organic fractions weakly absorbed to mineral surfaces and some microbial P were extracted by 30 mL of 0.5 M NaHCO$_3$, 18 hours end-over-end shaking and centrifugation at 2500 × g for 20 minutes. The supernatant was filtered (Whatman no. 42 filter) and collected for measurements.

3) The inorganic P adsorbed and bound to Al- and Fe-oxide minerals and organic P from humic substances were extracted using 30 mL of 0.1 M NaOH solution and repeating the second step procedure as described above.

4) The relatively insoluble fraction of P bound to Ca and Mg minerals and apatite was extracted by 30 mL of 1 M HCl in the same way as for the previous steps.

Total P concentrations (P_t) and inorganic P (P_i) in all extracts were measured by ICP-OES and colorimetrically (molybdenum blue method; Murphy and Riley, 1962), respectively. The organic P (P_o) concentrations were calculated by $P_t - P_i$.

4.3.4 Phosphorus K-edge XANES analysis

The XANES data collection for characterizing P-species in all soil samples and BCs particles was acquired at the Synchrotron Light Research Institute (SLRI) in Nakhon Ratchasima, Thailand, on the beamline 8 (BL8) of the electron storage ring with a covering photon energy from 1.25 to 10 KeV, electron energy operated at 1.2 GeV and beam current of 80-150 mA (Klysubun et al., 2012). The P K-edge XANES spectra were collected from dried and very finely ground treated soils and particulate BCs samples which had been diluted to P concentrations < 10 mg P kg^{-1} with SiO_2 powder (to eliminate self absorption effects; Prietzel et al., 2013), again ground in agate stone mini-mortar and spread uniformly as a thin layer on P-free kapton tape (Lanmar Inc., Northbrook, IL, USA). Data collection was operated in standard conditions comprising the energy calibration by standard pure elemental P and allocating the reference energy (E_0) at 2145.5 eV using the maximum peak of spectrum. All spectra were recorded at photon energies between 2045.5 and 2495.5 eV in step sizes of 5 eV (2045.5 to 2105.5 eV and 2245.5 to 2495.5 eV), 1 eV (2105.5 to 2135.5 eV and 2195.5 to 2245.5 eV) and 0.25 eV (2135.5 to 2195.5 eV) with a 13-channel germanium detectorin fluorescence mode. At least three scans were collected and averaged for each sample.

The P-XANES spectra were normalized and after merging replicates, a linear combination fitting (LCF) was performed using the ATHENA software package (Ravel and Newville, 2005). All XANES spectral data were baseline corrected in the pre-edge region between 2115–2145 eV and normalized in the post-edge region of 2190–2215 eV. The same ranges were used for the reference P K-edge XANES spectra to achieve consistency in the following fitting analysis (Prietzel et al. 2016). To achieve the best compatible set of references with each specified sample spectrum, LCF analysis was performed in the energy range between - 20 eV and +30 eV relative to the E_0 using the combinatorics function of ATHENA software to attain all possible binary, ternary and at most quaternary combinations between all

nineteen P reference spectra. The following set of reference P K-edge XANES spectra, all recorded in SLRI under the same adjustments by Werner and Prietzel (2015) and Prietzel et al. (2016), were used for fitting and calculations; Ca-, Al- and Fe-phytate, non-crystalline and crystalline $AlPO_4$, non-crystalline and crystalline $FePO_4.2H_2O$, Ca-hydroxyapatite ($Ca_5(OH)(PO_4)_3$), inositol hexakisphosphate (IHP), ferrihydrite-IHP, montmorillonite-Al-IHP, soil organic matter (SOM)-Al-IHP, ferrihydrite-orthophosphate, montmorillonite-Al-orthophosphate, SOM-Al-orthophosphate, bohemite-IHP, bohemite-orthophosphate, $CaHPO_4$, $Ca(H_2PO_4)_2$ and $MgHPO_4$. To select the best possible combination fit between the sample spectrum and the P-reference spectra, the lowest reduced chi value (χ^2) and R-factor were chosen.

4.4 Results

4.4.1 Effect of BCs on sequentially extracted P after incubation-leaching

After 70 days of incubation-leaching, the sequential P-fractionation of amended soils showed variations in the amount and distribution of various P-fractions between different treatments (Table 4-1). For all treatments, NaOH extracted the majority of fractionated P (62.4 to 66.5% of total fractionated P), followed by the labile P fraction ($NaHCO_3$, 19.2 to 20.0%), HCl-P (9.5 to 13.9%) and the readily available P (resin strips, 4.1 to 4.8%). The BCs addition increased the total soil P pools although the difference was significant only for the $BC^{plus}_{1-2 mm}$ and BC_{1-2mm} treatments. The largest increase in total fractionated P_t (resin-P_t + $NaHCO_3$-P_t + NaOH-P_t + HCl-P_t) occurred in $BC^{plus}_{1-2 mm}$ (133.8 mg P kg^{-1} soil) followed by $BC_{1-2 mm}$ (118.6 mg P kg^{-1} soil), $BC_{2-4 mm}$ (67.1 mg P kg^{-1} soil) and $BC^{plus}_{2-4 mm}$ (35.7 mg P kg^{-1} soil), compared to the control soil.

The proportion of P-enrichment in each fraction varied between different treatments in the order NaOH-P > HCl-P > $NaHCO_3$-P > resin-P for $BC^{plus}_{1-2 mm}$ and $BC^{plus}_{2-4 mm}$. For the $BC_{1-2 mm}$ and $BC_{2-4 mm}$ treatment the order was HCl-P > NaOH-P > $NaHCO_3$-P > resin-P. In all treatments, the P_i proportions in each of the P fractions were greater than the P_o proportions. Compared to the control soil, the most P_i increase was observed in NaOH-P_i and resin-P_i in response to BC^{plus}_{1-2mm} application (Table 4-1). Moreover, after 70 days of incubation-leaching, soil pH increased in BC treatments whereas BC^{plus} amendments had an acidifying effect. Soil pH levels of BC_{1-2mm} and $BC_{2-4 mm}$ increased by 0.07 and 0.05 units and

decreased for $BC^{plus}_{1-2\ mm}$ and $BC^{plus}_{2-4\ mm}$ treatments by 0.21 and 0.15 units compared to unamended control soil (pH = 5.06).

Table 4-1 Distribution of inorganic P (P_i), organic P (P_o) and total P (P_t) concentrations (mg P kg^{-1} soil) of sequentially extracted P-fractions in the soils as affected by different treatments (treated with two particle size fractions (1-2 and 2-4 mm) and original sizes of BC and BC^{plus} or unfertilized soils (control) after incubation-leaching and ryegrass cultivation experiments.

Treatment	Resin-P			NaHCO$_3$-P			NaOH-P			HCl-P		
	P_i	P_o	P_t	P_i	P_o	P_t	P_i	P_o	P_t	P_i	P_o	P_t
Incubation-leaching												
Control	47	5	52	160	99	259	565	294	859	113	10	123
BC$_{1-2}$	56^{NS}	7^{NS}	63^{NS}	163^{NS}	108^{NS}	271^{NS}	578^{NS}	303^{NS}	881^{NS}	140^{NS}	56^{NS}	196^{NS}
BC$_{2-4}$	50^{NS}	7^{NS}	57^{NS}	161^{NS}	105^{NS}	266^{NS}	574^{NS}	301^{NS}	875^{NS}	121^{NS}	40^{NS}	161^{NS}
BC$^{plus}_{1-2}$	61*	7^{NS}	68^{NS}	172^{NS}	111^{NS}	283^{NS}	593*	313^{NS}	906*	131^{NS}	37^{NS}	170^{NS}
BC$^{plus}_{2-4}$	50^{NS}	7^{NS}	57^{NS}	160^{NS}	104^{NS}	264^{NS}	574^{NS}	298^{NS}	872^{NS}	115^{NS}	21^{NS}	135^{NS}
Ryegrass cropping experiment												
Control	4	4	8	25	27	52	75	121	196	28	5	33
BC	2^{NS}	5^{NS}	7^{NS}	24^{NS}	32^{NS}	56^{NS}	79^{NS}	125^{NS}	204^{NS}	30^{NS}	6^{NS}	36^{NS}
BC^{plus}	6*	5^{NS}	11*	35^{NS}	27^{NS}	62^{NS}	85**	128^{NS}	213^{NS}	34^{NS}	7^{NS}	41^{NS}

* Significant at $P<0.05$; ** Significant at $P<0.01$.
^{NS} Non-significant difference (treatment vs. control; Tukey-Test).

4.4.2 Effect of BCs on sequentially extracted P after ryegrass cropping

Sequentially extracted P fractions in soil varied between different treatments after 230 days of ryegrass cropping (Table 4-1). In all treatments (control, BC and BC^{plus}), NaOH-P was the largest P pool mainly associated with Al- and Fe-oxide minerals and humic substances (65.0 to 67.5% of total fractionated P) followed by the NaHCO$_3$-P (18.2 to 19.0%), HCl-P (11.5 to 12.6%) and resin-P (2.2 to 3.4%) fractions. Enrichments of P fractions in BC^{plus} treatments were more pronounced than in treated soils with BC particles. In this treatment the concentrations of readily available and labile inorganic P fractions were insignificantly smaller than in the control. Additionally, a significant increase in P concentration was obtained only in resin-P_i and NaOH-P_i fractions of the BC^{plus} treated soil (Table 1). The maximum increase of total fractionated P was obtained in BC^{plus} treatment (37.6 mg P kg^{-1} soil). In comparison to incubation-leaching results, a similar sequence was observed for the

order of increasing magnitude of P fractions in response to BC and BC^{plus} amendments (NaOH-P > NaHCO$_3$-P > HCl-P > resin-P). However, for BC treatment, the total P extracted by resin strips was lowered in comparison with the control. In the control and BC treatments, P$_o$ was the predominant form in NaOH-P and NaHCO$_3$-P fractions while for BC^{plus} it was only in the NaOH-P fraction. Each P fraction was highest under BC^{plus} application, except for NaHCO$_3$-P$_o$ of BC treatment.

Separately, the effect of BC and BC^{plus} application on ryegrass yield parameters was examined in the 230-days pot experiment. The results indicated that P uptake, ryegrass yield and apparent nutrient recovery efficiency (ANR) of BC^{plus} treatments exceeded that of BC and control treatments and increased to values comparable with triple super phosphate (TSP) fertilizer (Zimmer, D. and Panten, K., personal communication). Addition of BC and BC^{plus} did not significantly change the bulk soil pH, although local acidification around BC^{plus} particles (pH 4.9; Morshedizad and Leinweber, 2017) probably can lower soil pH in small scale areas compared to BC treatments (pH about 8).

4.4.3 XANES analysis of BCs particles

All spectra from BCs were characterized by an intense white-line peak, post-edge position and without a distinct pre-edge which corresponded to calcium phosphate compounds including Ca-hydroxyapatite, dicalcium phosphate (CaHPO$_4$) and Ca-phytate (Fig. 4-1). The P K-edge XANES results indicated no obvious alterations in spectral features of BCs particles after the incubation-leaching experiment. After 70-days of incubation-leaching, the BC spectra were shifted towards Ca-hydroxyapatite, and this was more pronounced for the 2-4 mm than for the 1-2 mm BC-particles. The opposite trend was the case for BC^{plus} particles where the white-line signal intensity decreased after incubation-leaching period and the post-edge of spectra tended more to dicalcium phosphate. This effect was stronger for BC^{plus}-particle size reduction from 2-4 mm to 1-2 mm.
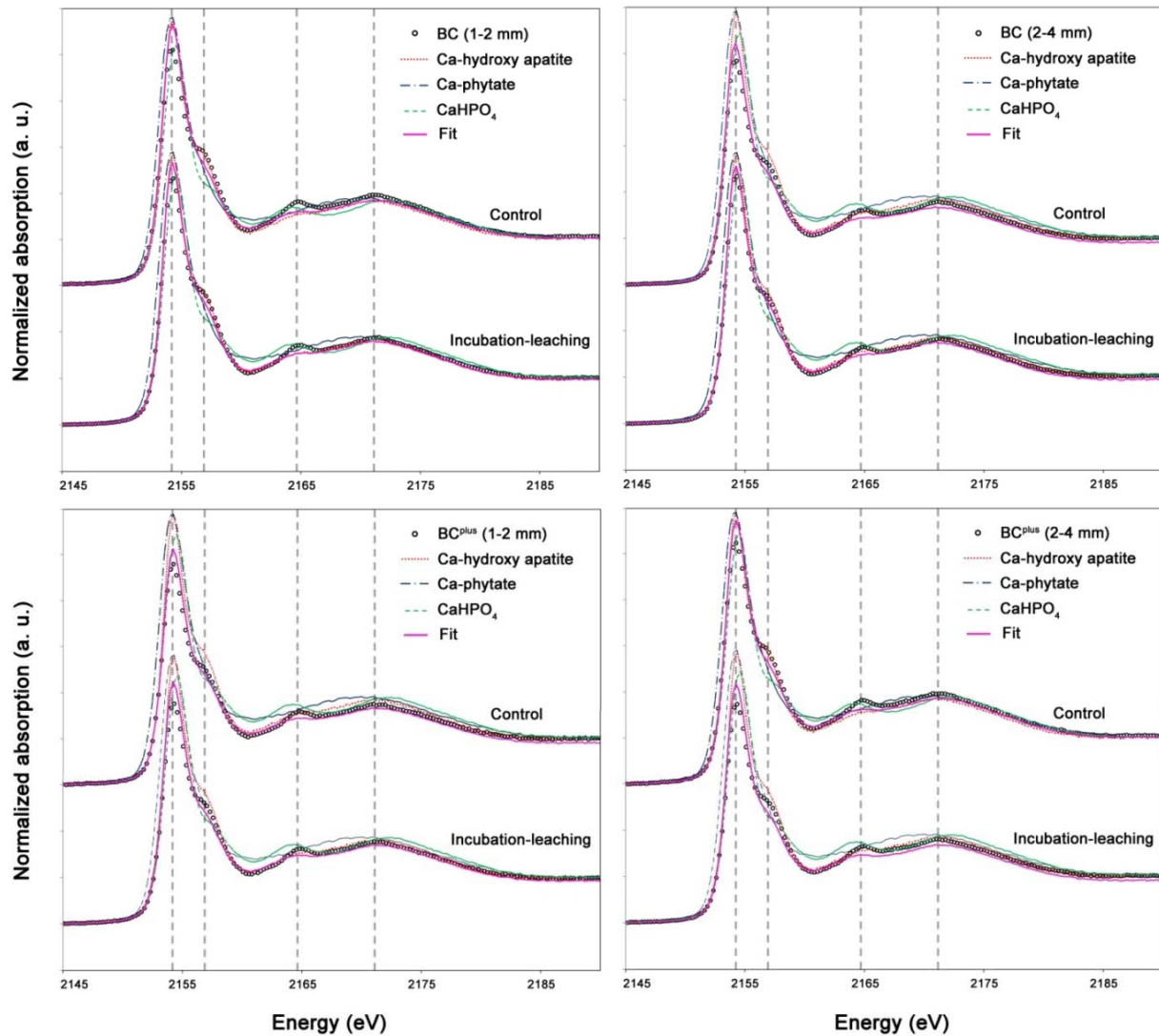
Fig. 4-1 Normalized P K-edge XANES spectra of different BC and BC^{plus} particle sizes (1-2 and 2-4 mm) before (control) and after 70-days incubation-leaching experiment compared to the reference compounds selected using LCF method.

To quantify the P speciation of BC and BC^{plus} particles, LCF analyses using all possible combinations were performed on all P K-edge XANES spectra (Table 4-2). The fitting results indicated that untreated BC and BC^{plus} particles before the experiment contained on average 61 and 60% Ca-hydroxyapatite, 22 and 30% CaHPO$_4$ and 18 and 10% Ca-phytate. After 70-days of incubation-leaching, the proportion of Ca-hydroxyapatite increased to the average of 80% in BC while it remained unchanged in BC^{plus} particles. The CaHPO$_4$ proportion increased in BC^{plus} particles to the average of 34% whereas the lower content was assigned in

the spectra of BC particles accounting for 10% of total P species. Moreover, the Ca-phytate proportion decreased slightly in BC and BC^{plus} particles from about 18 and 10% to averages of 11 and 7%, respectively.

Table 4-2 Results of linear combination fitting (LCF) conducted on P K-edge XANES spectra of bone char (BC) and surface modified bone char (BC^{plus}) particles before and after a 70-days incubation-leaching period. These best fits were achieved using all possible combinations with nineteen spectra of P reference compounds.

Reference compound	Before experiment				After 70 days incubation-leaching			
	BC		BC^{plus}		BC		BC^{plus}	
	1-2 mm	2-4 mm	1-2 mm	2-4 mm	1-2 mm	2-4 mm	1-2 mm	2-4 mm
Ca-hydroxyapatite (%)	58± 6	64±5	62± 5	58± 5	75± 4	85± 3	59± 5	60± 6
CaHPO$_4$ (%)	24± 5	19± 4	28± 4	32± 6	14± 3	5± 2	33± 4	35± 5
Ca-phytate (%)	18± 4	17± 4	10± 3	10± 3	11± 3	10± 2	8± 4	5± 4
R-factor	0.012	0.008	0.007	0.009	0.005	0.002	0.009	0.010

Spectra of BC and BC^{plus} particles, before and after 230-days of ryegrass cultivation, were characterized by a sharp white-line followed by a shoulder and then a post-edge feature between 2160 and 2175 eV which was divided into two peaks (Fig. 4-2). These features were most similar to P K-edge XANES spectra of Ca-hydroxyapatite, CaHPO$_4$ and Ca-phytate standard compounds. Treated BC particles had a white-line with higher intensity which appeared more similar to the Ca-hydroxyapatite spectrum. In contrast, BC^{plus} particles under ryegrass cultivation showed a weaker white-line exhibiting the shoulder and post-edge feature more comparable to the CaHPO$_4$ spectrum.

Some differences in proportions of P species observed between BCs particles before and after the cropping period in the ryegrass pot experiment are presented in Table 4-3. The LCF results revealed overall contributions of 63 and 70% Ca-hydroxyapatite, 29 and 29% CaHPO$_4$ and 8 and 1% Ca-phytate in the original BC and BC^{plus}, respectively. After the cropping period, the percentage of Ca-hydroxyapatite was increased in BC particles. In the BC^{plus} treatment, the percentage of CaHPO$_4$ increased from 29 to 43, while the percentage of Ca-hydroxyapatite was reduced from 70 to 49%. The Ca-phytate proportion remained unchanged in BC particles while that of BC^{plus} increased from 1 to 8% after the ryegrass cultivation period.
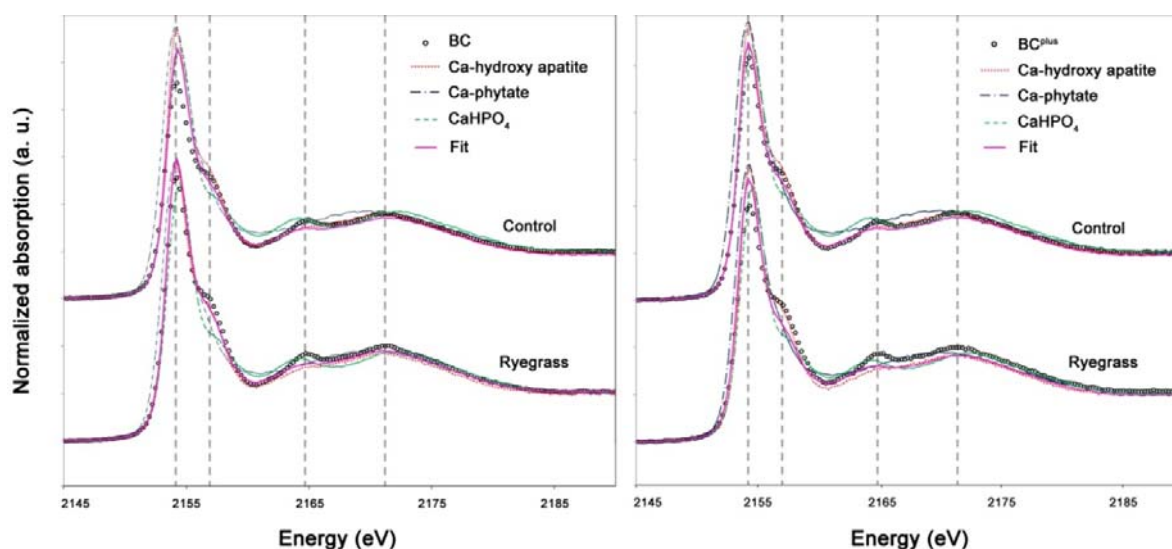
Fig. 4-2 P *K*-edge XANES spectra of BC and BC[plus] particles before (control) and after 230-days of ryegrass cultivation compared to the reference compounds selected by LCF method.

Table 4-3 Results of linear combination fitting (LCF) conducted on P *K*-edge XANES spectra of bone char (BC) and surface modified bone char (BC[plus]) particles before and after 230-days of ryegrass cultivation in a pot experiment. These best fits were achieved using all possible combinations with nineteen spectra of P reference compounds.

Reference compound	Before experiment		After 230 days ryegrass cultivation	
	BC	BC[plus]	BC	BC[plus]
Ca-hydroxyapatite (%)	63± 6	70± 4	75± 4	49± 8
CaHPO$_4$ (%)	29± 5	29± 3	17± 4	43± 6
Ca-phytate (%)	8± 4	1± 3	8± 3	8± 5
R-factor	0.012	0.005	0.006	0.018

4.4.4 XANES analysis of soil samples

The P *K*-edge XANES spectra of soil samples from the incubation-leaching experiment showed two dominant features including 1) a strong white-line lacking pre-edge and shoulder and 2) a tailed post-edge feature (Fig. 4-3). The most similarity to these features was seen in XANES spectra of amorphous AlPO$_4$, FePO$_4$ and SOM-Al-IHP compounds. Distinct differences appeared between the control and treated soil with BC[plus], not with BC treatments. This was reflected by slightly lower intensities of both white-line and post-edge features.

Fig. 4-3 P *K*-edge XANES spectra of unfertilized (control) and fertilized soils with BC and BC^plus particles under 70-days incubation-leaching experiment compared to the reference compounds selected by LCF method.

Table 4-4 Results of linear combination fitting (LCF) conducted on P *K*-edge XANES spectra of unfertilized (control) and fertilized soils with bone char (BC) and surface modified bone char (BC^plus) particles in the 70-days incubation-leaching experiment. These best fits were achieved using all possible combinations with nineteen spectra of P reference compounds.

Reference compound	Control	BC treatment		BC^plus treatment	
		1-2 mm	2-4 mm	1-2 mm	2-4 mm
Ca-hydroxyapatite (%)	8± 1	4± 1	8± 1	0	0
AlPO$_4$ amorphous (%)	42± 1	42± 2	40± 1	27± 1	24± 1
FePO$_4$ (%)	29± 1	31± 2	31± 1	27± 1	26± 1
SOM-Al-IHP (%)	21± 2	23± 4	21± 3	26±2	21± 1
Ca(H$_2$PO$_4$)$_2$ (%)	0	0	0	20± 1	29± 1
R-factor	0.0003	0.0007	0.0003	0.0005	0.0004

The P species of treated soils in the incubation-leaching experiment were determined by LCF analysis to select at most four reference compounds in combinatorics of all possible fitting combinations (Table 4-4). The fitting results indicated that P in the control soil and BC treatments occurred dominantly as AlPO$_4$ amorphous ($\approx$ 40%), FePO$_4$ ($\approx$ 30%) and SOM-Al-IHP ($\approx$ 20%) compounds. In BC^plus treated soils, the average proportion of amorphous AlPO$_4$

decreased to 26% and instead, $Ca(H_2PO_4)_2$ was identified with an average of 25% which did not appear in the control and BC treatments. The LCF results showed that the soil treated with BC[plus] had no detectable Ca-hydroxyapatite which was found in the control and BC treatments.

The XANES spectra recorded from treated soil samples in the ryegrass pot experiment showed the presence of an intense white-line in the energy range of 2152 to 2158 eV and a stretched post-edge feature approximately from 2165 to 2178 eV (Fig. 4-4). Decreases in white-line and post-edge intensities of the soil samples appeared as an effect of BC[plus] application. Visual inspection of P *K*-edge spectra revealed no indication of specific alteration in spectral features in response to the BC treatment.
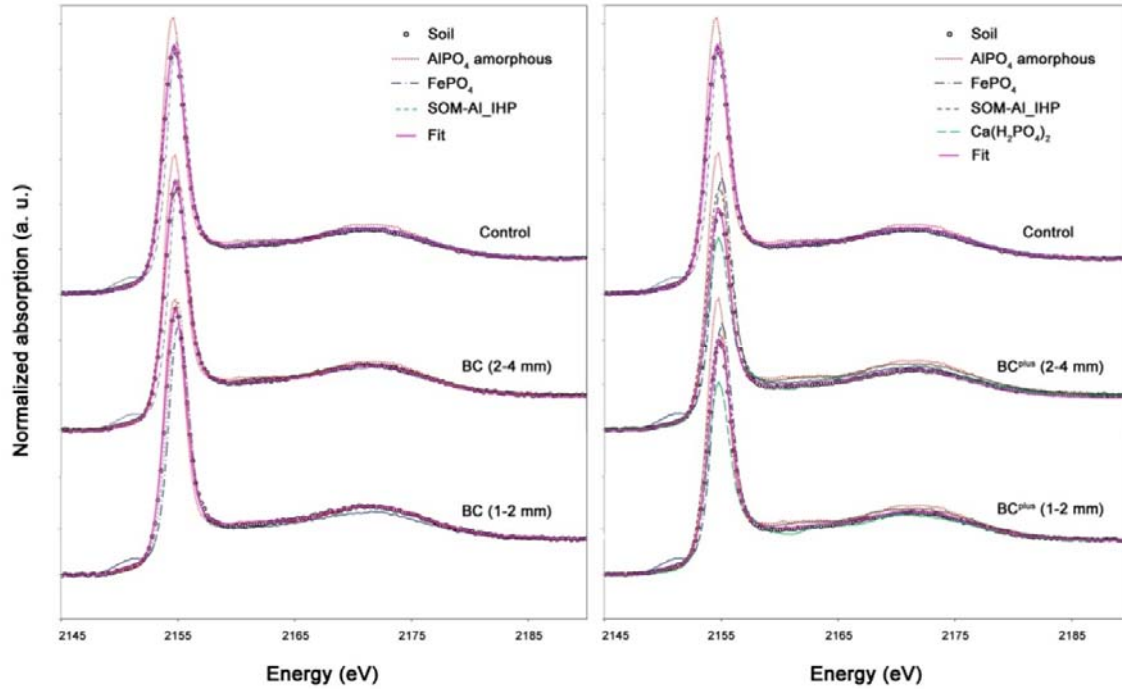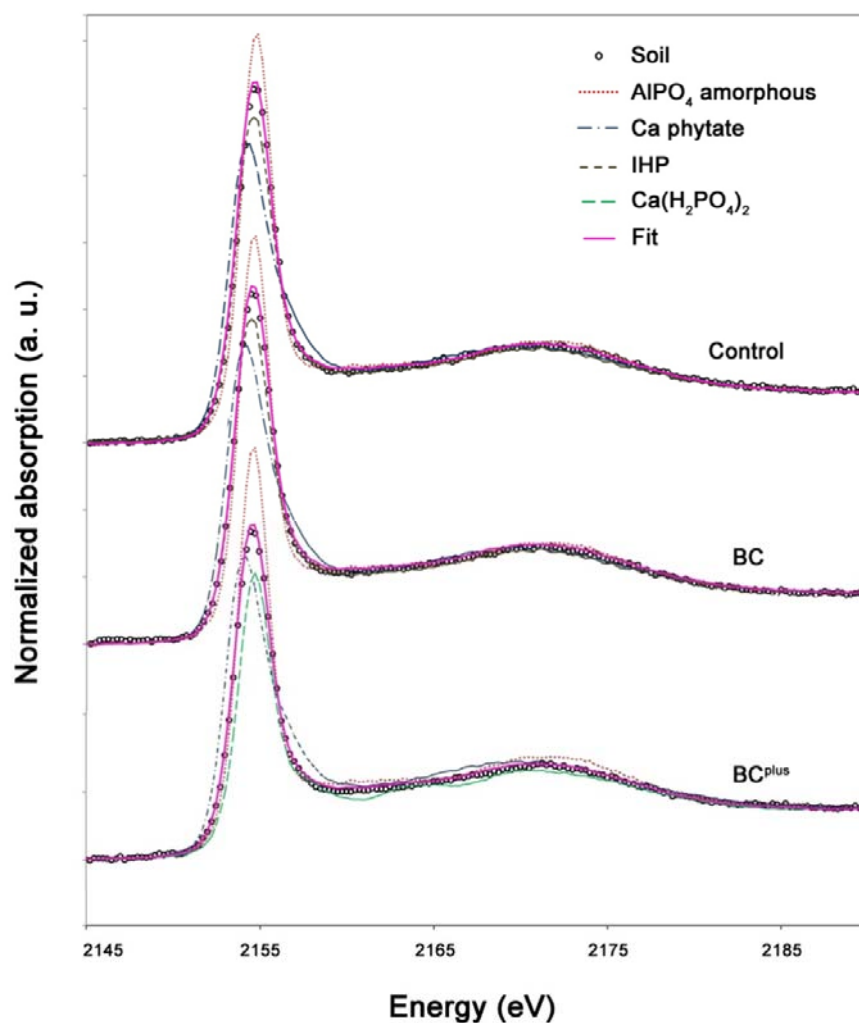

Fig. 4-4 P *K*-edge XANES spectra of unfertilized (control) and fertilized soils with BC and BC[plus] particles under 230-days of ryegrass cultivation compared to the reference compounds selected by LCF method.

Amorphous $AlPO_4$ was identified by LCF analysis as dominant component ($\approx$ 35%) in all treated soil samples from the ryegrass pot experiment (Table 4-5). The second major P form in the control soil was IHP (29%) followed by Ca-phytate (27%), with the latter also as pronounced as that observed for BC and BC[plus] treatments. All treated soils varied in proportions of free or bound IHP forms. The Mont-Al-PO_4 and $Ca(H_2PO_4)_2$ compounds were only assigned in the control and BC[plus] treatments, respectively.

Table 4-5 Results of linear combination fitting (LCF) conducted on P *K*-edge XANES spectra of unfertilized (control) and fertilized soils with bone char (BC) and surface modified bone char (BC[plus]) particles under 230-days of ryegrass cultivation in a pot experiment. These best fits were achieved using all possible combinations with nineteen spectra of P reference compounds.

Reference compound	Control	BC treatment	BC[plus] treatment
$AlPO_4$ amorphous (%)	35± 3	35± 3	34± 1
Ca-phytate (%)	27± 3	28± 3	27± 1
IHP (%)	29± 5	21± 7	0
Mont-Al-IHP (%)	0	16± 1	0
SOM-Al-IHP (%)	0	0	25± 2
Mont-Al-PO_4 (%)	9± 1	0	0
$Ca(H_2PO_4)_2$ (%)	0	0	14± 2
R-factor	0.0006	0.0008	0.0006

4.5 Discussion

4.5.1 P availability as revealed by sequential fractionation

The sequence of P distribution between sequentially extracted P-fractions was in accordance with findings by many studies (Cross and Schlesinger, 1995; McDowell and Stewart, 2006; Hashimoto and Watanabe, 2014), reflecting the general status of different P-pools in acidic soils. The results indicated that the largest P proportion was found in the NaOH fraction reflecting P fixed to Fe- and Al-oxides as followed by the $NaHCO_3$-P fraction assigned to weakly P absorbed on crystalline Fe- and Al-oxides or surface of minerals. Guo et al. (2000) reported that the NaOH-P fraction may support the labile $NaHCO_3$-P fraction as a buffering P-pool in highly weathered and acidic soils. According to soil pH values (4.7 and 5.2), the

larger proportions of $NaHCO_3$-P even than HCl-P can be explained by the abundance and surface loadings of Fe- and Al-oxides that support the electrostatic binding of phosphate ions and a scarcity of Ca and Mg minerals or soluble ions. As expected, the lowest P proportions were found in the mobile and readily available P fraction extracted by resin strips in agreement to many comparable studies (Cross and Schlesinger, 1995; Bauchemin et al., 2003; Sharpley et al., 2004; Siebers et al., 2013). Among the two soils which were used in the two different experiments, the largest proportions of inorganic P were achieved in the soil after incubation-leaching experiment, while the organic P forms were considerably more abundant in the soil samples after ryegrass cultivation (Table 4-1). These differences may be due to the microbial activities in the rhizosphere of grasses and transformation of P_i to more stable P_o fractions during longer plant cultivation period (230 days) than in the non-cropped incubation-leaching experiment (70 days).

In general, all P fraction concentrations were elevated by adding BC and BC[plus] particles which appeared to follow the same pattern in both soils under two different experimental conditions. However, significant differences were found only between the control and BC[plus] treated soils (1-2 mm in the incubation-leaching experiment) for the resin-P and NaOH-P fractions. Since the BCs particles were separated from the soils before chemical analysis, it was expected that partly dissolved BCs would have a limited impact on different P-fractions rather than totally ground and mixed BCs. This is consistent with the study of Siebers et al. (2013) according to which the BC application (<90 µm BC thoroughly mixed to soil) significantly increased the insoluble P proportion (H_2SO_4-P). Additionally, our study confirmed previous findings concerning the effect of particle sizes on the P release from BCs (Morshedizad and Leinweber, 2017) and consequently the P status of treated soils (Ma and Matsunaka, 2013). Sequentially extracted P contents increased with decreasing size of BC particles whereby BC[plus] treatments appeared more dependent on particle size than BC treatments. The results of sequential P fractionation of BC[plus] treatments in the incubation-leaching experiment indicated that the P increase was more pronounced for P fixed to Al- and Fe-oxides (NaOH-P) than other fractions, whereas for BC treatments the largest increase occurred in P bound to Ca and Mg minerals (HCl-P). It seems that local pH changes in soil associated with BC and BC[plus] amendments could eventually lead to a different distribution of released P into differently soluble or insoluble P-pools which are generally controlled by pH (Arai and Sparks, 2007). However, due to lower fertilization level and longer period of experiment in ryegrass cultivation compared to incubation-leaching, it appears that the

chemical equilibrium has been established in the soil (no significant change in bulk soil pH) and, accordingly, the soil P fractions were altered minimal.

4.5.2 P speciation of BCs particles by XANES

The predominance of Ca-hydroxyapatite in BCs as evidenced by P K-edge XANES analysis is consistent with findings reported by previous studies (Warren et al., 2009; Siebers et al., 2013). The mineral phase of bone consists mainly of hydroxyapatite, and its contribution to bone and bone char compositions depends on species and the age of animals (Wu et al., 2003) and carbonization temperature and residence time (Novotny et al., 2012). Bone crystallinity might be improved through structural modifications on poorly crystalline fresh bone samples (such as mineral maturity over periods of time or intensive carbonization) which can also result in increased proportions of hydroxyapatite and accordingly a decrease in P solubility (Novotny et al., 2012). Based on LC fittings, the second major component of BCs particles was $CaHPO_4$, in good agreement with the results of Rajendran et al. (2013) who indicated the heated bones at 400 ºC contained some more soluble phosphates such as $CaHPO_4$ and CaH_2PO_4 in addition to the hydroxyapatite fraction. The authors reported that spectra of calcined bone samples at 700 ºC had white-line at 2154 eV and two post-edge peaks at 2162 eV and 2169 eV with no pre-edge peaks and appeared similar to $CaHPO_4$ and CaH_2PO_4 spectra. Our LCF also assigned Ca-phytate in BCs samples which seems to be controversial as a component of animal bone materials. The P K-edge spectrum of Ca-phytate is very similar to other Ca-bound P compounds with a distinct white-line and lack of a pre-edge feature, although it is likely distinguishable due to specific shape of white-line tailing and absence of post-edge signal at 2164 eV (Prietzel et al., 2016). Moreover, some inaccuracies in LCF estimations have to be considered because of 1) uncertainty in speciation of organic P forms by K-edge XANES, 2) lack of reference compounds representing all P forms in BCs and 3) smaller Ca-phytate proportions than proposed 10 to 15% of P_t as detection limit for reliable XANES fittings (Beauchemin et al., 2003). Therefore, the P proportions assigned to Ca-phytate also could originate from a range of other CaP compounds.

In both experiments, incubation-leaching and ryegrass cropping, changes in proportions of Ca-hydroxyapatite and $CaHPO_4$ in BC particles followed an opposite trend than in BC[plus] particles. After placement of BC particles in the soil, Ca-phosphate seemed to be released gradually over time which provides a locally lime-saturated condition. Due to elevated pH

surrounding the BC particles, dissolved P can be resorbed to maintain solubility and the Ca-P equilibrium constant which likely resulted in a decreased proportion of soluble $CaHPO_4$ and possibly the transformation into the relatively insoluble Ca-hydroxyapatite fraction. In contrast, if BC[plus] particles were applied to soils, larger proportions of $CaHPO_4$ at the expense of Ca-hydroxyapatite could be explained by soil acidification through the microbial oxidation of released S (Lee et al., 1987; Fan et al., 2002). This effect was more pronounced over the longer time period in the ryegrass cropping pot experiment, favoring a greater $CaHPO_4$ than Ca-hydroxyapatite fraction. This implies that BC[plus] can actively supply P with predominance of soluble over insoluble P forms in the long-term and, thus, meet crop requirements.

4.5.3 P speciation of treated soils by XANES

Differences between characteristics of two soils, dissimilar mechanisms of incubation-leaching and plant uptake besides different experiment time durations complicate the joint interpretation of the P-XANES data. In unfertilized soil of the incubation-leaching experiment, the proportions of P species followed the order $AlPO_4$ > $FePO_4$ > SOM-Al-IHP > Ca-hydroxyapatite which did not vary despite partial changes in some proportions after application of both size fractions of BC particles. In general, these results concur with the findings by Siebers et al. (2013) that Ca-hydroxyapatite proportion was slightly increased by BC application. This could be attributed to irreversibly mixing finely-ground BC to the soil samples whereas in the present experiments the BC particles were separated from the soils before P speciation. Furthermore, these XANES data (Table 4-4 and Table 4-5) are in agreement with sequential P fractionation results (Table 4-1) which indicated the dominance of inorganic over organic P forms and showed the P fractions almost unchanged after BC application. Implications of low solubility of BC particles observed in this work are consistent with previous studies showing a slow release P from BCs (Warren et al., 2009; Siebers et al., 2013; Morshedizad et al., 2016). Besides reducing the $AlPO_4$ and Ca-hydroxyapatite proportions, BC[plus] particles introduced highly soluble $Ca(H_2PO_4)_2$ to soils in the incubation-leaching experiment. These results imply that considerable changes in P speciation were more attributed to pH reductions and, accordingly, leaching out solubilized P-forms compared with P enrichment by BC[plus] dissolution. This is supported by results from a previous publication in which two particle sizes of BC[plus] gave a significant rise in the leached P-concentration after 1, 5, 13, 34 and 70 days of incubation along with reductions in

soil pH (Morshedizad and Leinweber, 2017). This is in line with Sato et al. (2005) who found that increasing soil pH in a naturally acidic soil (pH = 4.32) was an effective approach to minimize P leaching, while pH decrease resulted in transformation of stable to soluble and more leachable P species. Regarding the XANES results of the ryegrass cultivation experiment (Table 4-5), the effect of BC^{plus} treatment can be explained better. In the control soil, the presence of AlPO$_4$ and increasing abundance of organic P-forms (Ca-phytate and IHP compounds) (Table 4-5) were consistent with the appearance of NaOH-P and HCl-P fractions by sequential extraction (Table 4-1). In the BC treatment the proportions of AlPO$_4$ and Ca-phytate did not change compared to the control but the contribution of organic P increased by Mont-Al-IHP formation. The stability of different P fractions can be favored by the pH effect (Gustafsson et al., 2012) likewise the dependence of BC particles solubility on the soil pH (Siebers et al., 2013). In agreement with incubation-leaching results (Table 4-4), Ca(H$_2$PO$_4$)$_2$ was detected as a result of BC^{plus} amendment even though similar proportions of AlPO$_4$ and Ca-phytate were observed between the control and BC^{plus} treatment. However, the date in Table 4-5 on the presence/absence of Ca(H$_2$PO$_4$)$_2$ in soils of ryegrass experiment may have been influenced by small proportions (<10-15%; reliable detection limit by XANES, Beauchemin et al., 2003) of other simple calcium phosphates that have a spectrum similar to the one of Ca(H$_2$PO$_4$)$_2$ in LCF analysis. The results of sequential P fractionation and XANES analyses on treatments in the two different experiments presented here demonstrated that surface modification of BC particles effectively improved soluble P fractions in BC^{plus} particles and, consequently, in amended soils.

4.6   Conclusions

In the present study, the P speciation by a sequential P fractionation and P K-edge XANES spectroscopy revealed the noticeable alteration in the P-pools of treated soil samples. Results of incubation-leaching and ryegrass cultivation experiments indicated that BC^{plus} produced by surface-modification of BC through addition of S compounds provided more soluble and plant-available P than non-modified BC during the growth season. The S oxidation and thereby the soil pH decrease seems to stimulate the P release from BC^{plus} particles. The P K-edge XANES analyses of BC^{plus} particles revealed more soluble CaHPO$_4$ than in BC particles at the expense of Ca-hydroxyapatite. This was associated with the addition Ca(H$_2$PO$_4$)$_2$ or similar simple Ca-P-compounds to amended soils, as indicated by sequential P fractionation

and XANES analyses. Future studies will be directed to validate the beneficial effects of BC[plus] at the field scale with different soils and to optimize the surface modification of BCs.

4.7 Data availability

All compiled data of this study are published in figures and tables. Detailed primary data including the incubation-leaching and ryegrass cultivation results and the acquired XANES spectra will be saved and published in the BonaRes Data Center (https://www.bonares.de/research-data) and get the BonaRes DOI prefix (10.20387).

4.8 Competing interests

The authors declare that they have no conflict of interest.

4.9 Acknowledgments

4.10 References

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5 Summarizing discussion, conclusions, and outlook

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5.1 Introduction

This dissertation investigated and outlined several objectives to evaluate a novel BC fertilizer which aimed to address potentials of new recycling sources and help moving toward a closed-loop P supply. In this chapter the obove mentioned objectives and hypothesis are followed by relevant results and combined with discussions on how this project has answered the research questions along with particular emphasis on rational and consistent connection between the chapters. The overall conclusion also highlights where forthcoming studies would improve knowledge and eliminate lack of certainty in areas that need more accurate analysis or even were beyond the scope of the present project.

5.2 Surface-modified bone char as a new alternative P-fertilizer

The increasing concerns about upcoming scarcity and high toxic-element concentrations of rock phosphate, the main source for the manufacturing of P-fertilizers, invigorate research efforts to discover clean and renewable P sources and develop innovative technologies for their introduction into agricultural practice. Consequently, P management policies have been focused more prominently on P-recovery and -recycling strategies. The application of bone char (BC; rich in P and almost free of contaminants) in soil for *in-situ* Cd-remediation and also in order to increase P fertility has been proved by recent studies (Warren et al., 2009; Siebers and Leinweber, 2013; Siebers et al., 2014). Despite these promising results, it remained a controversial issue to determine the ability and effectiveness of BC to release and supply crop-available P demand in a reliable way. Regarding the fact that P-release from BC (apatite-base material) is controlled by the ability of soil-biota to provide protons, a [technical] surface modification of BC with the aid of sulfur containing compounds and following oxidation by *thiobacilli* to sulfuric acid in soil can be a promising approach for improving the P-solubility in BCs. As the first and fundamental step for the assessment of surface-modification performance, the P-solubilizing and Cd-immobilizing abilities of a novel BC[plus] [provided to the doctoral candidate by the tutor] and the primary BC were compared in different soils that cover a wide range of pH-values and Cd-contamination. Moreover, the soil testing area, as a basis for appropriate fertilization/remediation practices, where information is lacking specially on application of BC fertilizers, was highlighted and characterized to some extent by evaluation of three routine extractants (**Chapter 2**). Results revealed that surface modification of BC lowered the pH in soils with pH> 5 during

incubation period and resulted in an excess of P-dissolution. Although the BC^{plus} increased the pH of the two most acidic soils (pH values of 4.2 and 4.9), the most significant increase in P-concentrations also occurred in these soils compared to BC and control treatments. This was a result of BC^{plus} improvement in both intrinsic properties and secondary aspects (after application) which would undoubtly determine its final soil amendment potential. While the pH effect has been demonstrated as the most important parameter regarding P-release from BCs, some discrepancies make the exact estimation of available P based on soil pH values difficult. Results of available P produced by BCs amendments in three comparable incubation experiments and over two different pH-ranges are shown in Table 5-1. It can be observed that the change trends of P-dissolution are not always consistent with soil pH alterations. This implies that the variability in released P from BCs in almost similar pH values can be related to other unknown mechanisms. However, the promising results obtained in **Chapter 2** indicate that surface modification promotes P-release in both pH ranges.

Table 5-1 Change in available P in BCs-treated soils as a percentage of applied P (500 mg P kg^{-1} soil) compared to the control (0 mg P kg^{-1} soil) after 145 days of incubation.

Fertilizer	Soil pH	ΔP [% of added P]	Reference
BC	6.8	+ 0.4	Warren et al., 2009
BC	6.4	+ 1.3	Siebers and Leinweber, 2013
BC	6.4	+ 0.4	Chapter 2; Morshedizad et al., 2016
BC^{plus}	6.4	+ 1.4	Chapter 2; Morshedizad et al., 2016
BC	3.3	+ 27.1	Warren et al., 2009
BC	3.4	+ 0.9	Siebers and Leinweber, 2013
BC	4.2	+ 3.7	Chapter 2; Morshedizad et al., 2016
BC^{plus}	4.2	+ 15.2	Chapter 2; Morshedizad et al., 2016

In **Chapter 2**, the highest Cd-immobilization efficiency was obtained in BC treated soils, and surface modification decreased the beneficial Cd-immobilizing effect largely through a pH-decrease. The positive effect of soluble P-compounds on Cd-immobilization appeared in BC^{plus} and DAP treatments in a similar pattern over 34 days of incubation but did not last until 145 days of incubation, except for most acidic soils as a consequence of raise in pH values (Table 2-6 and Fig. 2-3; **Chapter 2**). Nevertheless, in contrast with mineral fertilizers such as DAP, the BC^{plus} can increase soil fertility and quality along with a concomitant Cd-immobilization without any integration and input of contaminants. Regarding to well-correlated P-concentrations achieved by NH_4NO_3 with results of routine P-extractants and

also high efficiency in Cd extraction, the application of NH_4NO_3 for simultaneous estimation of P- and Cd-availability in soil can help to decrease costs and save the time.

According to these results, two further objectives were required to get a better impression of overall surface modification performance. First of all, since previous studies on BCs were performed under equilibrium conditions, which are simple to set up but eventually do not adequately reflect field conditions, the influence of non-equilibrium conditions on P- and Cd-release from incubated BCs treatments were investigated by addition of leaching steps to remove dissolved products from equilibrated soil-solution phase and stimulate more BCs-dissolution. Secondly, the effect of particle size, one of the most important physical properties which can influence surface area, reactivity, machinery application and distribution of BCs in soil, was evaluated to find an optimum size-fraction depending on P-dissolution and Cd-immobilization rates. The results demonstrated that non-equilibrium conditions mobilized more P from bone chars (essentially as slow-release fertilizers and highly dependent on P sorption capacity of soil) through repeated leaching steps and discharge of the P-saturated soil solution. Furthermore, this research also evidenced that the optimum size of both BC and BC^{plus} particles, corresponding to most P-release into the leachates, was not necessarily the finest (0.2-0.5 mm) but the 0.5-1 mm size fraction. The effect of particle size on pH revealed that the most changes were associated with the smallest BC and BC^{plus} size fractions. Accordingly, as a function of pH and in agreement with the previous study (**Chapter 2**), Cd-immobilization occurred in BC treatments and was intensified as particle sizes became smaller whereas BC^{plus} increased the Cd-release from treated soils with two finer particle size fractions (0.2-0.5 and 0.5-1 mm) or remained unchanged in particle sizes >1 mm. The results indicated that particle size was more effective on P-release from BC^{plus} than BC treatments. The results of **Chapter 3** provided important information to make comparisons between laboratory and field conditions to some extent and achieve reliable estimation of BCs potential for P-fertilization and Cd-immobilization of soil.

As the next and final step, assessing alteration in solubility and fractionation of P-species in BC, BC^{plus} and relevant treated soils after an experimental incubation-leaching period and a long-term ryegrass cultivation was crucial to offer insights into how surface modification can improve BC-dissolution process. Therefore, in **Chapter 4**, sequential P fractionation and X-ray absorption near edge structure (XANES) spectroscopy were applied and this approach successfully clarified P-speciation of BCs and revealed how treated soils were affected consequently. Sequential fractionation revealed P-enrichments in the studied fractions of BCs

treated soils. However, significant increases were observed in inorganic P bound to Al- and Fe-oxide (NaOH-P) and readily available P (resin-P) fractions in response to BC^{plus} application. These results support the promoted P-dissolution of BC^{plus} treatments as reported in **Chapter 2** and provide evidence for enhanced P-uptake, ryegrass yield and apparent nutrient recovery efficiency (ANR) of BC^{plus} treatments compared to BC and control treatments (Zimmer and Panten, unpublished). More pronounced P fixed to Al- and Fe-oxides (NaOH-P) and bound to Ca and Mg minerals (HCl-P) in BC^{plus} and BC treatments, respectively, were associated with different pH changes by BCs (Table 2-5 and Fig. 3-1; **Chapters 2** and **3**). These changes in P-speciation were associated to the subsequent distribution of released P into different P-pools, which are generally controlled by pH values, as well. Consistent with previous findings and research hypotheses, the P K-edge XANES analysis evidenced that Ca-hydroxyapatite was the predominant mineral constituent of BC and BC^{plus} particles. An improved quality of BC^{plus} through surface modification was concluded from the enlarged proportion of soluble P-species ($CaHPO_4$) at the expense of low-/in-soluble Ca-hydroxyapatite. Besides that, tracking the P-speciation changes of BCs particles, treated in incubation-leaching and ryegrass cropping experiments, revealed the continuous increase in $CaHPO_4$-proportions along with reductions of Ca-hydroxyapatite proportions in BC^{plus} particles while the opposite trend was found for BC. This improvement in P-solubility was reflected by $Ca(H_2PO_4)_2$ and resin-P increases in BC^{plus}-amended soils as identified by XANES and sequential fractionation analyses. The results demonstrate that BC^{plus} can actively supply P with a predominance of soluble over insoluble P-species, even after long-term cultivation and meet crop requirements. In this line, the relative agronomic efficiency (RAE, yield of intended fertilizer compared to a standard fertilizer) was calculated from few available studies reporting BC effect on crop yields and very recent long-term pot experiment conducted on BC and BC^{plus} fertilizers (Table 5-2). The results reveal that the addition of BC sometimes lead to an increased crop yield. The fertilization effect of BC seems to be more prominent for grasses and grain crops with relatively long vegetation period and intensive root system enabling the exploitation of slowly soluble P-sources more than vegetables and potato which stronger depend on fast P-supply from easily soluble sources such as TSP. Thus, an additional consideration for BCs application is the effect of cropping system on P efficiency. More importantly, the ryegrass cultivation results provide consistent evidence for improved solubility and plant availability of P after surface modification of BC.

Table 5-2 Effect of BCs fertilizers on relative agronomic efficiency (RAE) of different crops. RAE = (difference between yield of BCs-treated soil and non-fertilized soil / difference between yield of control fertilizer (TSP) treated soil and non-fertilized soil) × 100.

Fertilizer	Crop	RAE [%]	Reference
BC	Onion	-22.9 – -3.4	Siebers et al., 2012
BC	Potato	2.6 – 61.9	Siebers et al., 2012
BC	Wheat	-1.93 – 7.11	Siebers et al., 2012
BC	Lettuce	-91.7 – 9.3	Siebers et al., 2014
BC	Potato	-248.9 – 36.5	Siebers et al., 2014
BC	Wheat	11.3 – 173.0	Siebers et al., 2014
BC	Maize	60 – 65	Zwetsloot et al., 2016
BC	Ryegrass	-44.4 – 45.5	Zimmer and Panten, unpublished
BCplus	Ryegrass	1.6 – 177.3	Zimmer and Panten, unpublished

5.3 Implications for future research

The present dissertation project considered a range of experimental works and analyses to assess the performance of surface modification on fertilization properties of bone char. However, those works were constrained by financial support and time period of study for a PhD thesis. In future, other important factors which can affect BCs in soil-plant system should be further explored in forthcoming studies in order to indentify how additional and advanced techniques can help to maintain, justify and improve the value of BC as a clean and renewable P-fertilizer.

As we proved in **Chapter 3** that results obtained by routine incubation-extraction experiments (equilibrium condition; **Chapter 2**) are imperfect to simulate field conditions, the innovative incubation-leaching method should be also improved by considering more parameters such as unsaturated water flow, exuded enzymes and microbial activities in the vicinity of the roots, temperature changes and mechanical forces in the soils. This can help to identify mechanisms of P- and Cd-adsorption/desorption under controlled laboratory conditions and achieve better simulation on fate of BCs in soil. Moreover, regarding the performance of NH_4NO_3 extractant in estimation of P- and Cd-availability in soil (**Chapter 2**), it is advisable to perform pot/field experiments to verify the comparability of extraction results with crop uptake. Since different particle sizes of BCs influenced soil pH, P- and Cd-dissolution differently, it would be of great interest to use different size fractions of BCs for a range of soil science approaches such as soil fertilization, conservation and contaminants remediation. More importantly, the 0.5-1 mm size class of BCs (performed best in P-release; **Chapter 3**) should be tested in field experiments using standard farm machinery to introduce into practical agriculture. Except of soil pH and P sorption capacity, the other soil parameters

responsible for the P- and Cd-release from BCs-treated soils require further investigations if advanced understanding of BCs effects in soil are required as well. Abundant amounts of activated chars loaded with P, S and other nutrient elements after different purification and filtration processes are attractive options that should be well characterized in order to improve soil fertility, physical quality, remediation and carbon sequestration strategies.

The P-speciation results presented here evidenced some capacity of XANES analysis and sequential fractionation procedures to illustrate alteration of different P forms in BCs particles and relevant treated soils. There are potentials to improve XANES analysis through extension of standard set of spectra (additional P-references as predictor compounds) to represent major P species in BCs and treated soils. P-speciation approaches must also be improved by applying complementary NMR-investigations (^{31}P NMR; a key analytical technique to identify P-compounds in complex environmental samples). The general knowledge about surface modified BC should be improved by XANES speciation of related P- and S-compounds which as a prerequisite it is necessary to build up and/or complete spectra libraries of S K-/L-edge spectra. The methodological study of detailed speciation of P, S and Cd in BCs-treated soils will eventually lead to a better understanding how these chemical parameters reflect the BCs properties and consequently to modified process for producing solubility-comparable BCs with commercial fast-release P-fertilizers.

The proved potential of BC^{plus} to release more P (immobilize less Cd) than BC should be explored further in a wide range of soil properties and long-term field experiments for various common agricultural crops with different root system and P-demand during growth stages in order to identify how long the effect of BC and BC^{plus} amendments can persist. Also effects of BCs on soil quality, especially physical properties (aggregation and increase water holding capacity regarding to carbon sequestration), should be examined only over long time intervals. In addition, use of drainage lysimeters and concurrent evaluation of P-, S- and Cd-leachability from these field experiments will be of high interest for contaminant management and sustainable agriculture practices.

In summary, the present dissertation project has systematically clarified some important aspects of BC-surface modification, for the first time, and demonstrated that BC^{plus} is a promising renewable P-fertilizer which due to improved P-solubility efficiency, economical advantages and being free of contaminants can be an outstanding alternative for mineral P fertilizers.

5.4 References of Chapters 1 and 5

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7 Proof of individual contribution

Manuscript included in the thesis	Author (M. Morshedizad)	Co-authors
Morshedizad, M.; Zimmer, D.; Leinweber, P: *Effect of bone chars on phosphorus-cadmium-interactions as evaluated by three extraction procedures.* ***Journal of Plant Nutrition and Soil Science.*** (2016). 179. pp. 388-398 **Chapter 2**	Generating research idea, generating and evaluating data, writing the manuscript.	**D. Zimmer** (assistance during data evaluation, correcting the manuscript) **P. Leinweber** (generating research idea, correcting the manuscript)
Morshedizad, M.; Leinweber, P: *Leaching of phosphorus and cadmium in soils amended with different bone chars.* ***Journal of CLEAN – Soil, Air, Water.*** (2017). 45. pp. 1–7 **Chapter 3**	Generating research idea, generating and evaluating data, writing the manuscript.	**P. Leinweber** (generating research idea, correcting the manuscript)
Morshedizad, M.; Panten, K.; Klysubun, W.; Leinweber, P: *Bone char effects on soil: sequential fractionations and XANES spectroscopy.* ***Journal of SOIL.*** (2018). 4. pp. 23–35. **Chapter 4**	Generating research idea, generating and evaluating data, writing the manuscript.	**K. Panten** (assistance during ryegrass pot experiment) **W. Klysubun** (assistance during XANES measurements, correcting the manuscript) **P. Leinweber** (generating research idea, correcting the manuscript)

8 Theses

Nutrient-pollutant-interactions in cadmium-contaminated soils amended with surface-modified bone char

Presented by Mohsen Morshedizad

I Rationale and objectives of the research

Phosphorus (P) is an indispensable vital element for living organisms, plant growth and food productions. The P-fertilizers are mostly derived from phosphate rock, a potentially critical non-renewable resource connected with major problems *e.g.* supply risks, price fluctuation, quantitative and qualitative depletion of resources, and concomitant toxic impurities such as cadmium and uranium. Recent concerns over future P scarcity and food security have sparked interests in P-recovery and -recycling strategies.

Cadmium (Cd) is non-essential and highly toxic trace element to plants, animals, humans, and even micro-organisms (causing hazards *e.g.* disturbing enzyme activities, reduction in chlorophyll content, itai-itai disease and damaging human vital organs). Enrichment of Cd in agricultural soils mainly occurs from application of contaminated P-fertilizers posing high risk of Cd-uptake by plants and animals. Among several restoration and remediation strategies which have been proved to secure food safety and human health, *in-situ* Cd-stabilization by clean and organic amendments has shown great potential and cost-effective performance.

Bone char (BC), a P-rich organic material, is emerging as a clean, renewable and economical substitute for rock phosphate origin P-fertilizers to improve soil fertility and immobilize Cd availability. However, appearing slow-release and highly pH-dependent, may raise reasonable doubt about its sufficient capability to meet crop demand. Therefore, a surface modification approach with the aid of sulfur (S) containing compounds for promoting BC dissolution was developed to address this important issue.

In order to establish the novel BC as non-contaminated P source, the surface modification effects on P-fertilizing and Cd-immobilizing characteristics of BC must be understood in detail, and this can be achieved by 1) evaluating the P- and Cd-dissolution in Cd-contaminated soils incubated with BC, surface modified BC (BC^{plus}), and diammonium phosphate (DAP) by means of NH_4NO_3, $NaHCO_3$ and H_2O extractants, 2) simulation of field non-equilibrium conditions using a new-designed incubation-leaching approach toward a better estimation of P- and Cd-release from soils treated with different particle sizes of BC and BC^{plus}, and 3) employment of P-speciation methods to explore chemical alterations of BCs particles and relevant treated soils after incubation-leaching and ryegrass cultivation experiments.

II Main research results

The incubation experiment conducted on five soils with low to moderate Cd-contamination revealed that surface modification of BC was effective to increase P-dissolution over a wide range of soil pH. Besides significant correlation between P-contents extracted by different procedures, the NH_4NO_3 was well-suited to simultaneously assess the P- and Cd-availability. The highest Cd-immobilization efficiency as well as most pH increase occurred in BC treatments. The addition of BC^{plus} and DAP reduced Cd-concentration until 34 days of incubation in all soils and remained effective in Cd-immobilizing in the soils with pH raise over incubation period. The results indicate that availability of both P and Cd in BC and BC^{plus} treatments is strongly dependent on soil pH values.

The novel combined incubation-leaching experiment clarified the effects of non-equilibrium condition and particle size on BCs efficiency. The cumulative P-release from BC and

BC^{plus} after 70 days of incubation-leaching (non-equilibrium condition) was larger by factor 2 than in the previous incubation-extraction experiment. The treatment with 0.5-1 mm BC- and BC^{plus}-particles released the largest amounts of P into the leachates. Smallest BC-particles immobilized most Cd in soil resulting in minimum Cd-concentration in the leachates. The addition of BC^{plus} significantly increased the Cd-concentration in the leachates so that the more Cd was leached as particle-size became smaller. Thus, the 0.5-1 mm size class of BCs must be prescribed for large-scale field experiments using standard farm machinery to introduce this clean, renewable P fertilizer into practical agriculture.

Synchrotron-based X-ray absorption near edge structure (XANES) spectroscopy and sequential P fractionation methods revealed reliable evidence on P-species of BCs particles and relevant treated soils before and after incubation-leaching and ryegrass cultivation experiments. Application of BC and BC^{plus} enriched all sequentially extracted P-fractionations compared to the control. A significant increase occurred only in resin-P and NaOH-P fractions of the BC^{plus}-treated soil. Linear combination fitting (LCF) analysis on P K-edge XANES spectra proved increase of the readily available P fraction by detecting elevated proportions of $CaHPO_4$ in BC^{plus} particles as well as increased inorganic $Ca(H_2PO_4)_2$ in amended soils. Overall, implications of P-speciation results demonstrated that surface modification of BC effectively improved soluble P fractions in BC^{plus} particles and, consequently, in treated soils over incubation-leaching and ryegrass cultivation experiments.

III Conclusions and outlook

The first ever surface modification of BC, relying on promoting P-release through S-oxidation and thereby soil pH decrease, was introduced and successfully characterized over different experiments. The results of present dissertation project lead to the wider approval of "bone chars" as clean, renewable, cost-beneficial, fertility enhancer and Cd-immobilizer soil amendments. Depending on which advantage of BCs has been determined by priority and soil properties affecting BCs efficiency (*i.e.* pH, P sorption capacity, Cd-contamination level), an appropriate particle size of BC or BC^{plus} would be wisely selected for optimizing P-fertilization and Cd-immobilization practices.

The future studies should consider three fundamental and highly interlinked research areas to evaluate, improve and establish BCs application. First, the long-term field experiments with taking into account different soil and crop properties should be conducted to assess soil-BC-plant interactions. The second line of forthcoming investigations must be improvement of methodological approaches for P, S and Cd-speciation in BCs-treated soils *e.g.* extending standard set of XANES spectra and applying complementary techniques (*e.g.* ^{31}P NMR) for identification of the complex mixture of P-compounds. Finally, the BC-modification approaches have to be optimized in order to enhance BC^{plus}-efficiency as a clean alternative P-fertilizer in promoting crop yield, improving chemical and physical soil properties and balance agricultural and environmental functions.