Interactive comment on "Bone char effects on soil: sequential fractionations and XANES spectroscopy" by Morshedizad et al.

We would like to thank the referees for careful review, insightful questions and very helpful comments. Overall, the reviewers have explained the importance of our research and the value of the data we present. Some critical points were about missing information, data interpretation and language. We have tried to make a point by point response, considering all suggestions and modify the manuscript accordingly. Please find our detailed responses in bold and blue color below the reviewer comments:

Referee 1: General comments

The paper investigates chemical reactions in soil after the application of bone char produced with and without treatment with reduced S compounds. This is important because bone char is a potentially very efficient soil amendment in soil with low P availability and low pH which are very common in especially tropical areas. As bones are also a widely available P source production of bone char could also be a way of improving soil fertility which is feasible. A better understanding about the chemical reaction taking place in the soil after application could help us develop bone chars specifically designed to improve specific soil types.

The paper provides this information by looking using chemical P extractions and XANES spectroscopy of both bone char samples before and after incubation in soil and on the entire soil. The changes that they observe are modest, except in a few cases, which probably reflect the fact that the changes are happening relatively slowly. In general the paper is well organized, but in some sections the language is inaccurate.

While it is usually possible to extract the meaning the reading flow becomes interrupted. Below I have indicated some places where this is the case under technical corrections, but I recommend to that the manuscript is checked by a professional copy editor.

Specific comments

Line 123: Is there a good reason for using a different soil in the plant experiment?

Response: The reason for using different soils in incubation-leaching and pot experiments was the difference in part of objectives of study. The objective of incubation-leaching study was to investigate the effect of BCs on Cd-release from moderately Cd-contaminated soil as well as P-mobilization in the soil. P mobilization was the only

research question in the pot experiment with annual ryegrass. For that reason, a soil with Cd contamination was used in incubation-leaching study, and another soil – low in P and available in large quantity – was chosen for the pot experiment. It should be noted that the same BCs particles were used in both experiments and two soils were similar in the most important factor affecting BCs solubility: acidic soil pH (4.7 and 5.2).

Line 133: Please describe how the particles were separated from the soil.

Response: As well described in sections 2.1 and 2.2, soil samples were carefully removed from columns/pots and air dried. BC 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 de-ionized water to remove adhered soil particles, allowed to dry completely at ambient conditions and finely ground for further analyses. We explained this more precisely in page 6-7, lines 145-148.

Line 175: Please indicate some more details here. How was the normalization ranges (pre and post edge) selected and what was it?

Response: We added more explanations (page 8, lines 191-194): "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 similar to those were used for the reference P *K*-edge XANES spectra (Prietzel et al. 2016) to achieve consistency in the following fitting analysis."

Line 175: How was the LCI conducted? A lot of details are needed here. What was the fitting range. What was the objective function? What was the ma number of standard allowed in the fitting, etc.

Response: We explained the LCF analysis in more details (page 9, lines 194-198): "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 (published by Prietzel et al. 2016)."

Line 207-215: Why are you describing and presenting the results from the paper by Morshedizad and Leinweber (2017) here. If they are already presented in another paper, then it should suffice to include it in the discussion to the extent that it is relevant.

Response: Since the present study focused on P speciation of treated bone char particles and amended soils which are left after different periods of incubation-leaching and pot experiments, for better understanding and interpretation of results it seems crucial and inevitable to mention that a part of P was taken up by ryegrass or leached. Also pH alteration in treated soils as most effective parameter on BCs solubility was reported here. We shifted this into the discussion part and shortened it.

Line 210: Unit appears odd for a concentration? An accumulated concentration?

Response: Yes, it is accumulated concentration. The unit implies accumulated amounts of P (mg) which were leached from treated soils (kg) during whole incubation-leaching period.

Line 238: What do you mean by insignificant? The numbers 0.01 to 0.05 does not seem to be founded on anything and the local pH changes were not measured.

Response: We rephrased (page 11, lines 256-259) to: "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)."

Line 245: It is very difficult to separate the different graph in the figures. Is there any ways that the figures with XANES spectra can be made easier to read. Especially it is difficult to distinguish the BC lines from the Ca-Phytate.

Response: We have tried different other ways to plot spectra to be more distinguishable but due to overlapping of spectra the only way to make each spectrum distinct and easier to diagnose is to plot the graph of each spectrum individually and at different adsorption intervals. However, this in turn would make it more difficult to observe changes and matching of samples and references spectra due to small differences. Besides outprints the reader especially interested in these spectra can enlarge the figure at the screen and detect differences in distinct spectra ranges.

Line 247-249: It is virtually impossible for me to see that the spectra is shifted towards HAP. To me it appears that the post-edge shoulder at 2158 eV is more pronounced in the BC samples than in any of the standards. Is that correct and if it is the case what could be the explanation?

Response: Respecting to the referee comment, if the crossed points of dashed lines with all spectra being considered, it can be clearly seen that in white line, post edge-shoulder (2158 eV) and post edge-features (2165 eV) the bone char spectra were shifted towards Ca-hydroxyapatite spectrum after incubation-leaching treatment (Fig. 1). In fact enlargement to 400% at the screen clearly shows that in the 1-2 mm fraction the BC and HAP spectra are identical the post edge-shoulder (2158 eV).

Line 256-258: In the results presented in Table 2, the same 3 standards were always used for the fitting. It that because it was always the same three standards that were selected?

Response: Yes, the LCF analysis was done to achieve the best fit using all possible combinations with at most four P standards between all references. In this case, the outputs of ATHENA were always the same three standards out of all nineteen P reference spectra.

Discussion: In general I would like you to related slightly more critically to you results of the LCF procedure. You end up with 3 standards, but how reliable is that? For example you identify Ca-phytate as a component, but in general it is difficult for distinguish different organic species P species with XANES. Does that mean that the analysis points more to organic compounds than to phytate? In general are you missing important references. In relation to this discussion, I also miss a discussion about the effect of the drying procedure on P speciation. When, for example, you dry BC particles separated from a soil, what precipitates of P are formed? When you have indications of the presence of easily soluble CaP compounds are they then formed from dissolved orthophosphate or other compounds in the process of can other compounds like HAP also be formed during the drying?

Response: We believe that the LCF results which revealed the presence of Ca-hydroxyapatite and $CaHPO_4$ as the main components of bone chars and their alterations after treatment are completely reliable and supporting for our hypothesis and objectives of study. In the case of Ca-phytate, as mentioned in discussion (page 17, lines 411-419), we agree with referee that there is uncertainty in organic P speciation by XANES analysis and also lack of reference compounds representing all P organic forms in bone char particles. It is noteworthy that all reference spectra used in this study were designed and selected for P speciation in soil by Prietzel et al. (2016). So, it could be concluded that small proportions of Ca-phytate, unspecific P references and methodic limitations may produce unreliable speciation results for the organic P compounds. Regarding the effect of drying procedure on P speciation, we agree with referee that it could have an effect on P speciation of more soluble P fractions. However, but since 1) wetting and drying cycles were a part of treating bone chars in the experimental soils and 2) all bone char particles were dried after treatments under same conditions, it was considered that drying effect did not change P speciation in bone char particles.

Line 388-390: It is difficult for me to verify from the figures that phytate appears like other CaP compounds. This reallt questions the results regarding the presence of Ca-Phytate in the samples however, which I believe could be a range of other CaP compounds.

Response: We agree with referee on Ca-phytate issue as we discussed in following sentences about uncertainty in recognition of Ca-phytate and other organic compounds in bone chars. However, it is clear that Ca-phytate has some similarities with spectra of other Ca-P compounds and bone char, especially in the white line-position. We added to the discussion that the proportion assigned to Ca-phytate also could originate from a range of other CaP compounds (page 18, lines 419-420).

Line 430: In addition to leaching of solubilized P compounds they could also have been taken up by the plant, right?

Response: In this case, the results of incubation-leaching study are discussed where no plant was grown in soil columns. We explained this in more detail (page 19, lines 454-457).

Line 456-457. I am in doubt what evidence supports this.

Response: The evidence for this statement comes from significant increases of $Ca(H_2PO_4)_2$ (LCF analysis; Tables 4 and 5), and indirect indication comes from greater amounts of resin-P (sequential P fractionation; Table 1) in soil samples treated with BC^{plus} particles.

Technical corrections

Line 60-61: This sentence is a little awkward mentioning that is it an incubation twice.

Response: Rephrased (page 3, lines 62-64) to: "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)."

Line 62-65: I would rephrase to …requirements, a considerable fraction of BC applied to the soil remains unsolluble in the short …

Response: Changed as proposed (page 3, lines 65-66).

Line 65: The last part of this (very long) sentence comes a little out of context. Please rephrase, perhaps adding another sentence spelling out the last point more precisely.

Response: We rephrased the last part of the sentence and added a separate sentence (page 3, lines 66-68): "A detailed P speciation can clarify the fate of insoluble P from BC which has not been done before."

Line 66: "has often been described in terms of". Do you mean "Is defined as" or"consists of" or "is"

Response: Changed (page 3, line 69) to: "Chemical speciation is described as analytical identification…"

Line 68: "Variable" is not a good word. Maybe you mean "various".

Response: Changed as proposed (page 4, line 71).

Line 69: Fertilization is not a non-equilibrium condition as you claim in this sentence.
Please rephrase.

Response: Rephrased (page 4, lines 71-72) to: "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 …"

Line 78: …is well suited for identification of …

Response: Changed as proposed (page 4, line 82).

Line 81: …soil samples makes it a promising …

Response: Changed (page 4, line 85) to: "soil samples make it a promising…

Line 88: …soil samples and provided evidence that the increase …

Response: Changed as proposed (page 4, line 92).

Line 90: …not clear what you mean by "applicable information"

Response: Changed (page 4, line 94) to: "practical information"

Line 97: Poorly constructed sentence. You probably mean "Two particle fractions (1-2 mm abd 2-4 mm) of two bone char"

Response: We rephrased the sentence (page 5, line 101): "Two particle size fractions (1-2 and 2-4 mm) of bone chars (BC, produced by pyrolysis of degreased animal bone chips at 800°C) and …"

Line 98: Please give some more information on the S treatment. What reduced compounds was used? How was I blended and under what conditions?

Response: We rewrote the sentence and explained that in more details (page 5, lines 102-107): "… 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 % methansulfonate; Zimmer et al. unpublished results of S X-ray absorption near-edge fine structure spectroscopy) in a commercial biogas purification process (patent application DE 212012000046U1;www.google.com/patents/DE212012000046U1?cl=en&hl=de) were incubated with a silt loam soil."

Line 98: Poorly constructed sentence. You seem to claim that the S compounds are characterized in detail in the former paper, but I don't believe that is the case.

Response: We rewrote the sentence and explained in more details (see above).

Line 101-102: It that the extractants in parenthesis. Please give more details and a reference.

Response: We explained in more detail and added the references (page 5, lines 107-111): "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."

Line 103 and in the following …total P of 149

Response: Corrected accordingly (page 5, line 111).

Line 106-108: Poorly constructed sentence.

Response: The long sentence was divided into three sentences and simplified (page 5, lines 114-118): "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 filter (MN 616 G; Macherey-Nagel GmbH & Co., KG Düren, Germany) was placed at the bottom of each column to avoid any particle losses."

Line 110: 3 pore volumes were added or leached through?

Response: Three pore volumes were added (page 5, line 120): "During the incubation period, the soil columns were leached with three pore volumes of deionized water added using a droplet irrigation simulator system…"

Line 111: Not clear what a leaching step is.

Response: We clarified that in a separate sentence (page 6, lines 121-122): "The leaching process was repeated in five steps, each one after 1, 5, 13, 34 and 70 days of incubation.

Line 113-114: Not clear what the outcome in this paper is and how it is different from the outcome described in this paper. Please be more specific.

Response: To avoid bringing results in "Materials and methods" section, we explained some specific and relevant outcomes in the "Discussion" section.

Line 127: Not clear what natural temperature conditions are.

Response: Changed to "ambient" (page 6, line 138).

Line 129: Again, what do you mean by natural air conditions?

Response: Changed to "ambient" (page 6, line 140).

Line 164: Please indicate around here what samples you analyzed with XANES.

Response: We explained that as proposed (page 8, lines 178-179): "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 lower P concentrations…"

Line 174: I suppose the spectra were recorded in fluorescence yield model, but please indicated this.

Response: The referee is right; we added this information (page 8, 187).

Line 220: Sudden change in writing style. Before you called it NaOH-P.

Response: NaOH-P was introduced as inorganic P adsorbed and bound to Al- and Fe-oxide minerals previously in lines 164-166 but we rephrased this by starting with NaOH-P as largest pool and added explanation afterwards (page 10, 236-238).

Line 281: …period in the ryegrass pot …

Response: Corrected as proposed (page 13, line 300).

Line 321: …was identified by LCD analysis as the dominant…

Response: Changed as proposed (LCF) (page 14, line 341).

Line 341: What is positive loadings?

Response: "… surface loadings that support the electrostatic binding of phosphate ions." We rephrased this and explained in more detail (page 15, lines 359-362).

Line 346: What do you mean most inorganic? Either it is organic or inorganic. Please rephrase.

Response: Rephrased (page 15, line 366) to: "the largest proportion of inorganic P"

Line 376: Wu et al 2003 is missing in the reference list.

Response: We added it to reference list (page 28, lines 671-674): "Wu, Y., Ackerman, J. L., Strawich, E. S, Rey, C., Kim, H-M., and Glimcher, M. J.: Phosphate ions in bone: identification of a calcium–organic phosphate complex by ^{31}P solid-state NMR spectroscopy at early stages of mineralization, Calcif. Tissue Int., 72, 610–26, doi:10.1007/s00223-002-1068-8, 2003."

Line 377-380: You seem to argue that if you increase crystallinity of the sample the amount of HAP will increase. But you can have both amorphous and crystalline HAP so how can you my make that conclusion?

Response: As we mentioned in these lines, both crystallinity and abundance of hydroxyapatite depend on bone properties and pyrolysis conditions. However, we argue that increase in crystallinity and amount of hydroxyapatite (relative to other amorphous Ca-phosphates in bone and bone char) under any circumstances can decline bone char solubility. We rephrased the text accordingly (page 17, lines 401-405).

Line 434: Regarding the XANES results of the ryegrass cultivation…

Response: Changed as proposed (page 19, line 460).

Line 435: In the control soil…

Response: Corrected as proposed (page 19, line 461).

Line 445: What do you mean "practices" is "treatments" a better word?

Response: "treatments in the two different experiments" refers to leaching-incubation experiment and ryegrass cultivation. We changed this accordingly (page 20, line 471).

Line 452: …through addition of S compounds proved more…

Response: Changed as proposed (page 20, line 479).

Referee 2: General comments

The paper is concerned with changes in phosphorus (P) chemistry following application of bone char to soil as an alternative to conventional P fertiliser. The research addresses the important need to develop strategies for increasing the sustainability of P management in agriculture, coupled with increasing demands to recycle P from renewable materials previously regarded as wastes. An important feature of the paper is the analysis of P both in the bone char particles and soil, pre- and post-amendment period. Also, the paper compares two types of bone char; one having received a novel treatment with reduced S compounds. Further pertinent factors under consideration are bone char particle size and scale of experimental approach (incubation/leaching vs plant bio-assay). Conventional chemical extraction techniques and XANES spectroscopy areused to study the soil P chemical changes, whilst XANES is also used to investigate the corresponding changes to Pchemistry in the bone chars.

Specific comments

Lines 101, 124. Can the authors explain why different soils wereused for the two experiments? Ideally, the same soil should have been used for both approaches.

Response: The reason for using different soils in incubation-leaching and pot experiments was the difference in part of objectives of study. The objective of incubation-leaching study was to investigate the effect of BCs on Cd-release from moderately Cd-contaminated soil as well as P-mobilization in the soil. P mobilization was the only research question in the pot experiment with annual ryegrass. For that reason, the soil with Cd contamination was used in incubation-leaching study, and another soil – low in P and available in large quantity – was chosen for the pot experiment. It should be noted that the same BCs particles were used in both experiments and two soils were similar in the most important factor affecting BCs solubility: acidic soil pH (4.7 and 5.2).

Line 110. What is the rationale for leaching with three pore volumes? Is this number based on conventional methodology, and/or represent typical drainage discharge?

Response: In routine leaching experiments, depending on soil texture, approximate three pore volumes (loam texture) water have been used to completely displace the soil pore water.

Line 133. I think more detail is needed on how the particles were 'manually' separated from the soil.

Response: As well described in sections 2.1 and 2.2, soil samples were carefully removed from columns/pots and air dried. BC 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 adhered soil

particles, allowed to dry completely at ambient conditions and finely ground for further analyses. We explained this more precisely in page 6-7, lines 145-148.

Line 148. Filtered through what pore size, or paper type?

Response: We added more information about filter type: in page 5, lines 116-117: "MN 616 G P-free filter (Macherey-Nagel GmbH & Co., KG Düren, Germany)" and in page 7, line 163: "Whatman no. 42 filter"

Line 193-194. In Table 1, I don't see any significant increases in soil P pools following the BC1-2mm additions.

Response: Corrected (page 9, line 215) as "total soil P pools" and explained more in following sentences (summed P_t values and statistical significances are not shown in table).

Line 207-215. These detailed leachate data from the authors' related work should not be presented here. Instead, the relevant information should be integrated within the Discussion. However, it would be useful in the current paper to see the final soil pH data, as these are directly relevant to the soil P chemistry. These additional data would require a minor amendment to the Materials and Methods section.

Response: Since the present study focused on P speciation of treated bone char particles and amended soils which are left after different periods of incubation-leaching and pot experiments, for better understanding and interpretation of results it seems crucial and inevitable to mention that a part of P was taken up by ryegrass or leached. Also pH alteration in treated soils as most effective parameter on BCs solubility was reported here. We shifted this into the discussion part and shortened it.

Line 222-224. I think it is misleading to state 'Both BC and BC^{plus} amendments led to enrichments of P fractions, except for the readily available and labile inorganic P concentrations in the BC treatments.' It appears that the BC treatments fail to change any of the P fractions with any statistical significance. Similar to the comment on Lines 193-194, above, I think the authors should check through the Results section that all statements concerning treatment effects are accurately qualified in terms of their statistical significance.

Response: We agree, and we rephrased (page 10, lines 239-242) to: "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."

Line 231-232. This reads like an afterthought. Does the statistical significance of this increase warrant an earlier mention in the section?

Response: We moved the sentence to line 242 following the rephrased sentence (see above).

Line 237-240. As commented previously, I think the soil pH data are too important to be omitted from the paper.

Response: The effects of BC treatments on soil pH are reported for incubation-leaching experiment in lines 228-232 and for ryegrass pot experiment in lines 256-259. Also we rephrased the sentence (page 11, lines 256-259) to: "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)."

Line 362-364. Given the lack of statistical significance, I think this sentence misrepresents the data. Maybe rephrase along the lines of the data suggesting that there is more evidence for a positive effect of particle size on P fractions in the BCplus treatments…?

Response: The statement on particle size effect was proven in lines 216-219: "The largest increase in total fractionated P_t (resin-P + $NaHCO_3$-P + NaOH-P + HCl-P) occurred in $BC^{plus}_{1\text{-}2\ mm}$ (133.8 mg P kg^{-1} soil) followed by $BC_{1\text{-}2\ mm}$ (118.6 mg P kg^{-1} soil), $BC_{2\text{-}4\ mm}$ (67.1 mg P kg^{-1} soil) and $BC^{plus}_{2\text{-}4\ mm}$ (35.7 mg P kg^{-1} soil), compared to the control soil."

Line 366-377. I am not sure the data adequately support this ('largest increase...in …HCl-P') in the ryegrass experiment.

Response: The referee is right. We rephrased the sentence to support leaching-incubation results (page 16, lines 385-386): "The results of sequential P fractionation of BC^{plus} treatments in the incubation-leaching experiment indicated that…"

Line 367-370. The pH data must be presented in support of this discussion point. Line 399-402. As above.

Response: We added a paragraph to explain the effect of pH changes on soil P fractions in ryegrass experiment (pages 16-17, lines 391-394): "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".

Line 403-406, and further into the Discussion. The authors discuss possible acidifying effects on the P speciation. In order to develop the discussion a little more broadly, it might be useful to explore any evidence for acid producing reactions (in terms of pH, and / or P fractions and species) in the rhizosphere, per se; i.e. by comparing the control treatments between the incubation and ryegrass experiments.

Response: We added the pH values of BC (7.8) and BC^{plus} (4.9) amendments and comparison between control treatments of incubation and ryegrass experiments into the discussion. Moreover, chemical reactions around BC particles and in rhizosphere causing

possible pH alteration have not been measured and only can be stated as speculation or suggestion for forthcoming studies.

Line 408-410. This sentence is confusing. I recommend removing it.

Response: Removed as proposed.

Lines 444-446. Here or earlier in the Discussion. Given the underlying challenge of enabling bone products to dissolve in neutral to alkaline soils (Lines 57-59), I think the Discussion needs to try and link briefly the current observations, obtained using acid soils, to higher soil pH scenarios.

Response: We explained more about pH effects in discussions.

Although the paper is well constructed, with an appropriate balance among its various components, there are several sentences where the scientific English could be improved. I would be happy to help with suggestions for these improvements using Track Changes, if a Word version of the paper was made available.

Response: We greatly appreciate this generous offer of the reviewer.