The phosphorus fertilizer value of bone char for potatoes, wheat, and onions: first results

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Abstract

Bone char (BC), being rich in phosphorus (P), calcium (Ca) and magnesium (Mg), was tested for its P fertilizing potential for potato, wheat, and onion growth. The effects of different rates of crude BC and BC inoculated with *Trichoderma harzianum* to increase the P solubilization were compared to P fertilization with pure $Ca(H_2PO_4)_2$ in a pot experiment. While $Ca(H_2PO_4)_2$ increased the dry matter yield and P concentration of plants compared to the non-fertilized control, the results for the BC treatments were inconsistent. An increase in dry matter yield was only observed for potatoes after application of BC (1000 mg P kg^{-1}), whereas wheat plants showed no response to all BC application levels, and dry matter yield of onions decreased. An increase in the plant's P concentration after BC application was observed for wheat, while plant's P was unaffected in onions and decreased in potatoes. The inconsistency of the dry matter yield and P concentration of plants after BC application is most likely caused by an insufficient incubation time of the BC added to the substrate, the application of lime carbonate, and the slightly acidic pH of the substrate all leading to a reduction in P dissolution from BC. However, initial results of the P utilization for potatoes of this pot experiment demonstrate that BC might be a potential P fertilizer, but further experiments are necessary to study the effect of incubation time, type of soil, and the mode of application.

Keywords: P fertilizer, nutrient utilization, Trichoderma harzianum

Zusammenfassung

Knochenkohle als Phosphordünger für Kartoffeln, Weizen und Zwiebeln: erste Ergebnisse

In einem Gefäßversuch wurde Knochenkohle (KK), welche reich an Phosphor (P), Magnesium (Mg) und Kalzium (Ca) ist, hinsichtlich ihrer Düngeeigenschaften für Kartoffeln, Weizen und Zwiebeln getestet. Die Auswirkungen von verschiedenen Konzentrationen an KK, KK inokuliert mit *Trichoderma harzianum* zur Erhöhung der P-Löslichkeit aus KK, und $Ca(H_2PO_4)_2$ (p.a.) wurden hierzu getestet. Die Zugabe von $Ca(H_2PO_4)_2$ hatte eine Erhöhung des Trockengewichtes und der P-Konzentration in den Pflanzen zur Folge, wohingegen die Ergebnisse nach KK Zugabe uneinheitlich waren. Eine signifikante Erhöhung der Trockenmasse konnte nur für Kartoffeln nach einer Zugabe von KK (1000 mg P kg^{-1}) beobachtet werden, wohingegen für Weizenpflanzen kein Effekt und für Zwiebeln sogar eine Verringerung der Trockenmasse beobachtet werden konnte. Ähnlich verhält es sich mit der Pflanzen P-Konzentration, die in Weizen nach KK Zugabe anstieg, in Zwiebeln unverändert blieb und in Kartoffeln verringert wurde. Die uneinheitlichen Resultate bezüglich Trockenmasse und P-Konzentration der Pflanzen wurde höchstwahrscheinlich durch eine unzureichende Inkubationszeit der KK, die Zugabe von kohlensaurem Kalk und den leicht sauren pH-Wert des Substrats verursacht, was eine Verminderung der P-Lösung aus der KK zur Folge hatte. Dennoch zeigen erste Ergebnisse aus diesem Gefäßversuch die potentielle Eignung von KK für Kartoffeln, aber es sind noch weitere Versuche nötig um den Einfluss der Inkubationszeit, des Versuchsbodens, und die Form der Zugabe zu untersuchen.

Schlüsselwörter: P-Düngung, Dünger Ausnutzungsgrad, Trichoderma harzianum

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1 Introduction

Phosphorus (P) is a major element limiting plant growth (Beileski, 1973) and up to approximately 0.2 % of a plant's dry weight is made up of P. Plants are able to take up P from soil solution only and mainly in the form of phosphates. The soil P is not available to plants to great extent and only a few soils are capable of releasing enough plant available P to support high growth rates of crops (Schachtman et al., 1998). Thus, it is necessary to add P to soils in the form of organic and mineral P fertilizer in order to increase the concentration of plant available phosphate. Today's agriculture relies on P fertilizer mostly processed from mined phosphate rock (PR), which is a depleting non-renewable resource. Several studies estimate that the world's reserves will only last for about 50 to 125 years, and many reserves are laden with impurities, e.g., toxic heavy metals such as cadmium (Cd) and uranium (U) (Gilbert, 2009). Thus, alternative renewable P sources, which are free from contaminants, are urgently needed to meet the demands for sustainable food production worldwide. Bone char (BC) is one such renewable P source, since it contains about 10 to 15 % (w/w) (Warren et al., 2009) of P and is free from Cd and U. BC is produced from animal bone chips by pyrolysis at about 600 °C. Due to its porous structure BC can be successfully inoculated with microorganisms which strengthen the resistance of plants against pests and enhance P dissolution from BC if applied to soils (Postma et al., 2010).

Generally, only 10 to 25 % of the P applied by mineral and organic fertilizers is utilized by plants in the short term (Finck, 1992). P solubilizing microorganisms naturally residing in the rhizosphere of plants can be used to increase the P availability to plants (Abd-Alla, 1994; Illmer et al., 1995; El-Komy, 2005; Krey et al., 2011). Fungi, especially *Trichoderma spp.*, have been reported to possess a great ability to solubilize P from rock phosphate by synthesizing a number of organic acids that are necessary to scavenge phosphates from soils containing bound P (Rudresh et al., 2005, Kapri and Tewari, 2010). Thus, the application of *Trichoderma spp.* for assisting in P dissolution from BC in soils is promising. In the present study, BC was inoculated with *Trichoderma harzianum* strain DAR5 in order to increase P solubility of BC. This current work was primarily planned to investigate the value of BC as a renewable and high quality P fertilizer by conducting a first pot experiment with three different crops being important for human nutrition. Additionally, the performance of *Trichoderma harzianum* assisting in P dissolution from BC was tested.

2 Experimental

2.1 Substrate and amendment characteristics

For the pot experiment a fertilized and limed bog peat based substrate was used. This was characterized by a humification degree of H3-H4 (according to Bodenkundliche Kartieranleitung (Arbeitsgruppe Boden, 2005)) pH (CaCl$_2$) of 5.90 and total P, N, and K concentrations of 230 mg P kg^{-1}, 0.86 g N kg^{-1}, and 1.17 g K kg^{-1}, respectively. When conducting the pot experiment it was not known that the bog peat was limed and hence no adjustment of the incubation time of the soil amendment and the substrate was made. In the pot experiment three different P fertilizers were tested for their effects on crop yield and P transfer characteristics: Ca(H$_2$PO$_4$)$_2$ (p.a.), BC, and BC inoculated with *Trichoderma harzianum* strain DAR5 (BC+Tri). The Ca(H$_2$PO$_4$)$_2$ is the major compound of the highly soluble triple superphosphate fertilizer and the solutions used here were grade "p.a" (= pure for laboratory analyses) to compare the BC to a P source with highest solubility and purity. The BC used has been characterized previously in more detail by Warren et al. (2009), and it contained about 150 mg P kg^{-1}, 280 mg Ca kg^{-1}, and 6.5 mg Mg kg^{-1}. The bone char was in the form of granules < 5 mm (Ø 2 mm) and mixed into the peat in the upper half of the pots having a volume of 2.5 L.

2.2 Design of pot experiment

A three-factor pot experiment was carried out to investigate the effects of: 1) type of substrate amendment (BC, BC+Tri, and Ca(H$_2$PO$_4$)$_2$); 2) different fertilization levels: (Control (C = 0 g P kg^{-1}), BC at a level of 1 g P kg^{-1} (BC 1), BC at a level of 2 g P kg^{-1} (BC 2), BC+Tri at a level of 1 g P kg^{-1} (BC+Tri 1), BC+Tri at a level of 2 g P kg^{-1} (BC+Tri 1), and Ca(H$_2$PO$_4$)$_2$ at a level of 13.2 g P kg^{-1} (P), and 3) type of crop: (potato – Molli (G2) (*Solanum tuberosum* L.), spring wheat – Kadrili (*Triticum aestivum L.*) and onion – Ch: 108 (*Allium fistulosum*). The onions were grown from seeds, with six seeds per pot planted to a depth of 2 to 3 cm. The potatoes were grown from tubers, each of five potatoes being cut into six nearly equally sized pieces so that each piece contained one eye. For each fertilizer level the six pieces from the same potato were planted in a separate pot. Twenty seeds of wheat were planted to a depth of 1 to 2 cm. After planting the pots were placed on a fleece mat in a glasshouse, arranged in a randomized complete block design, and irrigated every day. The day/night temperature was set to be 18/10 °C. Five replicates were used for the control treatment for every crop and for each potato variant and four replicates for the wheat and onion

variants. The wheat seedlings were thinned to ten plants per pot after 16 days of growth. Plants were not fertilized throughout the experiment, because the fertilizers were given to the substrate before seeding or planting. Soil and plant samples of wheat and onion were taken after 42 days of growth for chemical analyses. Wheat plants were separated into ears and straw. Potato plants were harvested after haulm senescence and separated into tubers and above-ground plant parts. After washing, the fresh weight of the plants was determined for each pot. Then, plants were dried to constant weight at 70 °C, and the dry weights were recorded. For further analyses the soil samples and plant materials were finely ground.

2.3 Chemical analyses of substrate and plants

Total P concentrations of the soil samples and total P of the BC sample were determined after microwave assisted digestion with HNO_3 and HCl (CEN, 2000) (for soil and BC) or 6 mL HNO_3 and 2 mL H_2O_2 (for plants) using an inductively coupled plasma-optical emission spectroscopy (JY 238, Jobin Yvon, France). The P utilization for each crop was then calculated by the difference of the nutrient uptake of fertilized and unfertilized plants and related to the amount of nutrients in the fertilizer according to the following equation (Finck, 1992):

$$P \ utilization \ (\%) = \frac{total \ uptake - uptake \ from \ soil \ storage}{amount \ of \ nutrients \ in \ the \ fertilizer} \cdot 100$$

(1)

2.4 Statistical analyses

One-way ANOVA was used to identify significant differences in P utilization between treatments (Tuckey test *$P <$ 0.05 and **$P < 001$).

3 Results and discussion

3.1 Substrate P concentration

The application of soil amendments increased total P concentrations in the substrate (Table 2), which was determined after crop harvest.

The total P concentration in the substrate after $Ca(H_2PO_4)_2$ application was lower for the wheat and onion variants compared to the total P concentration after BC application. This result is surprising as $Ca(H_2PO_4)_2$ was applied at a level of 13.2 g P kg^{-1}, whereas BC was applied only at a level between 1 to 2 g P kg^{-1}. This is a result of the high solubility of $Ca(H_2PO_4)_2$ and thus a loss of P with the irrigation water.

Table 1:
Total P concentration of substrate (mg kg^{-1})

Variant	Potato	Wheat	Onion
		mg kg^{-1}	
C	266 ± 35	244 ± 32	182 ± 80
P	800 ± 166**	462 ± 38**	639 ± 43**
BC 1	430 ± 74**	1915 ± 705**	3702 ± 633**
BC 2	539 ± 127**	4518 ± 1182**	6302 ± 1848**
BC+Tri 1	324 ± 26**	2820 ± 231**	3048 ± 791**
BC+Tri 2	397 ± 60**	4348 ± 1301**	5564 ± 790**

Significant at *$P < 0.05$ and **$P < 0.01$ (treatment vs. control).

3.2 Dry matter yield

The effects of the different soil amendments on plant dry matter yield are presented in Table 1. Application of $Ca(H_2PO_4)_2$ increased the dry matter yield for all crops

Table 2:
Dry matter yield of plant samples (g pot^{-1})

Variant	Potato		Wheat		Onion	
	shoots	tubers	straw	ears	shoots	bulbs
				g pot^{-1}		
C	11.13 ± 0.3	19.71 ± 7.6	24.45 ± 0.3	6.77 ± 0.3	8.18 ± 0.1	6.40 ± 0.2
P	12.83 ± 0.9**	38.90 ± 14.4*	28.81 ± 0.6**	13.0 ± 0.8**	12.9 ± 1.9**	7.36 ± 0.34**
BC 1	11.75 ± 0.1**	31.58 ± 6.4*	24.76 ± 0.4	6.65 ± 0.4	8.02 ± 0.1*	6.18 ± 0.4
BC 2	11.32 ± 0.5	20.20 ± 6.3	24.76 ± 0.4	7.26 ± 0.2*	8.04 ± 0.1*	6.26 ± 0.3
BC+Tri 1	10.75 ± 0.3	15.04 ± 5.7*	24.74 ± 0.4	6.89 ± 0.5	8.32 ± 0.4	5.99 ± 0.4
BC+Tri 2	11.21 ± 0.2	19.46 ± 1.3	24.67 ± 0.4	6.62 ± 0.35	8.12 ± 0.1	6.39 ± 0.9

Significant at *$P < 0.05$ and **$P < 0.01$ (treatment vs. control).

compared to the unfertilized controls. The results for the various BC applications were inconsistent. For a few treatments the dry matter yield increased after addition of BC, whereas dry matter yield of onion shoots decreased. The addition of BC+Tri had no effect on plant biomass compared to the control.

The P in $Ca(H_2PO_4)_2$ is highly water soluble and thus to a great extent available for plants, which explains the increase in dry matter yield after $Ca(H_2PO_4)_2$ application. In most of the samples the plant biomass was not altered after BC application, being a result of several factors. Firstly, the incubation time of the BC with the substrate was inadequate for significant P dissolution from BC into the substrate. Warren et al. (2009) studied P dissolution from BC and TSP and found that immediately after application a limited amount of plant available P was released, whereas the highly soluble triple superphosphate fertilizer increased the plant available P fraction to a much higher extent immediately after application. Thus, in this study the incubation time of BC seemed to not be adequate to release sufficient P available to plants. Secondly, the slightly acidic pH of the substrate may have caused a reduced P dissolution from BC. This can be concluded from Warren et al. (2009) who found that P was not released at a soil pH (water) above 6.14. In the present study the substrate had an initial pH ($CaCl_2$) of 5.90. Since the pH determined with the $CaCl_2$ method is generally lower compared to the water method (Minasny et al., 2011) the pH of the substrate used in present study might have exceeded the cut-off pH found by Warren et al. (2009) for a significant P dissolution. Thirdly, the form of lime that was applied to the substrate may also have inhibited the P dissolution from BC, as was shown by Rex and Kühn (2008). In their study, converter lime and lime carbonate were applied to BC amended soil but a fertilizing effect was observed for converter lime only. In that study lime carbonate applied to the soil might have reduced the P dissolution from BC. Thus, in order to definitely prove the plant growth enhancing effect of BC a new pot experiment was set-up to further study the factors of incubation time, soil pH, and application of other amendments.

3.3 Plant P concentration

The addition of $Ca(H_2PO_4)_2$ increased plant P concentration for all crops, whereas the BC 2 and BC+Tri 2 applications led to an increase in the P concentration of wheat (Figure 1). Furthermore, BC addition did not affect the P concentration in onions and reduced potato plant P for the BC 2, BC+Tri 1, and BC+Tri 2 treatments.
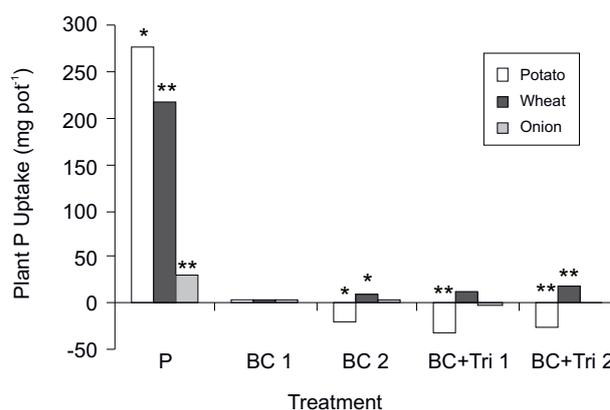

Figure 1:
Plant P uptake response to substrate amendment. Significant at $*P < 0.05$ and $**P < 0.01$ (treatment vs. control).

All crops with increased dry matter yield due to $Ca(H_2PO_4)_2$ application were also responsive to plant P concentration as a result of the high solubility and thus good P availability. Onion P concentration in the BC 1 and BC 2 treatments showed no treatment response even though the dry matter yield was reduced. Higher concentration of plant available P after biomass ash supply were also found in a study of Schiemenz and Eichler-Löbermann (2010). However, this increase did not always result in higher plant P uptake.

3.3 P utilization

The data for P utilization from $Ca(H_2PO_4)_2$ and BC for different fertilizer treatments and crops are presented in Table 3. In the present study, only the $Ca(H_2PO_4)_2$- and BC 1 treatments to potatoes reached a degree of P utilization of 9.42 and 15.96 %, respectively. For the other treatments the degree of P utilization was only in the range between -0.34 to 5.86 % and therefore lower than the general short term P utilization of mineral and organic P fertilizer (Finck, 1992). The low degree of P utilization for the highly soluble $Ca(H_2PO_4)_2$ can be explained by P leaching with the irrigation water, as equation (1) considers the amount of nutrients applied with the fertilizer but does not take leaching into account. Therefore, the P utilization from $Ca(H_2PO_4)_2$ might be underestimated. The application of $Ca(H_2PO_4)_2$ was equivalent to a highly soluble mineral P fertilizer and was used as control treatment to evaluate the P utilization from BC. The P utilization for potatoes and the BC 1 treatment was increased by 169.4 % relative to the P utilization from $Ca(H_2PO_4)_2$ serving as control treatment. This outstanding performance was not observed for the other treatments with a P utilization in the range of -3.6 to 26.8 % compared to the $Ca(H_2PO_4)_2$ treatment. However, more consistent results were obtained for wheat, with a

P utilization in the range between 15.2 to 42.7 % of the Ca(H$_2$PO$_4$)$_2$ treatment. BC inoculated with *Trichoderma harzianum* showed a slightly higher P utilization performance with 42.7 % (BC+Tri 1) and 40.4 % (BC+Tri 2) compared to the BC 1 and BC 2 treatments. This was different for the onion variants, for which P utilizations rates of 86.3 and 53.9 % for the treatments BC 1 and BC 2 were achieved, respectively, whereas the performances of BC+Tri 1 and BC+Tri 2 were -10.8 and 2.9 %, respectively.

Table 3:

P utilization (%) for different variants and crops

	P utilization (%)		
Variant	Potato	Wheat	Onion
P	9.42	5.86	1.02
BC 1	15.96**	0.91**	0.88**
BC 2	2.52**	1.53**	0.55**
BC+Tri 1	-0.34**	2.50**	-0.11**
BC+Tri 2	0.91**	2.37**	0.03**

Significant at *$P < 0.05$ and **$P < 0.01$ (BC amendments vs. P treatment).

The choice of the strain of *Trichoderma sp.* is of crucial importance for the P solubilizing performance. In a study conducted by Kapri and Tewari (2010), various *Trichoderma sp.* cultures were tested for their P solubilizing potential from tricalcium phosphate. After an incubation of 96 h the highest P solubilization was obtained for the isolate of *Trichoderma sp.* DRT-1 (352.8 µg P mL^{-1}), whereas the isolate ORT-4 solubilized only 251.9 µg P mL^{-1} above the baseline level of 51.3 µg P mL^{-1} for the control treatment. To the best of our knowledge, the strain used in this study has not been evaluated for its P solubilization capacity. In a previous study, no difference in acid phosphatase activity in soil was found between BC and BC inoculated with *Trichoderma harzianum* strain DAR5 (Baum et al., 2008). Thus, usage of BC inoculated with *Trichoderma harzianum* strain DAR5 combined with an insufficient incubation time might explain the inconsistent P utilization data for different crops.

4 Conclusions

The results of this study suggest that the P utilization from BC can be comparable to a highly soluble P source under some circumstances. Therefore, BC is potentially suitable as Cd- and U-free "ecological" P fertilizer. The attempt to enhance the P solubility by incorporating BC inoculated with *Trichoderma harzianum* into the substrate used for pot experiments was ineffective or counterproductive to increase the plant's P concentration and dry matter yield. In terms of environmental risks, especially water contamination, the application of BC compared to a highly soluble P compound is advantageous as P release is not that rapid and, thus, less prone to leaching. Another pot experiment was conducted to determine if the inconsistent performance of BC as P fertilizer for different crops was due to the experimental set-up of this pot experiment. Initial results suggest that changes in the experimental set-up with respect to lime addition to the substrate and mode of application influence the P fertilizing effect of BC.

Acknowledgements

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