Anika Zacher[1], Peter Leinweber[1], Kerstin Panten[2]

Sulfur-enriched bone char enhances P uptake by maize in a perennial pot experiment

Schwefel-angereicherte Knochenkohle erhöht P-Aufnahme von Mais in einem mehrjährigen Gefäßversuch

Affiliations
[1]University of Rostock, Faculty of Agricultue and Environmental Sciences, Rostock, Germany.
[2]Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Braunschweig, Germany.

Correspondence
Prof. Dr. agr. habil. Peter Leinweber, University of Rostock, Faculty of Agricultue and Environmental Sciences, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany, email: peter.leinweber@uni-rostock.de

Abstract

Recycling of phosphorus (P) from slaughterhouse waste, production of bone char (BC) and its use as fertilizer is a promising approach to close nutrient cycles but the fertilizer value of BC is not sufficiently clear. Therefore, two BCs (BC and sulfur-enriched BC (BC^{plus})) were tested in comparison with highly water-soluble triple superphosphate (TSP) in a perennial pot experiment with maize as test plant with high P-requirement. The fertilizers affected both the dry matter yields, and the P concentration of maize in the general order BC^{plus}, TSP > BC. The P uptake of maize in the TSP treatment accounted for 38% of the applied P in the first experimental year and decreased subsequently. By contrast, the P uptake in the BC^{plus} treatment remained quite stable over time. In conclusion, the sulfur-enriched BC^{plus} is able to maintain sufficient P availability to crops in the medium term and can be recommended as fertilizer.

Keywords

Bone char, phosphorus, phosphorus recycling, plant nutrition

Zusammenfassung

Phosphor (P)-Recycling aus Schlachtabfällen, Herstellung von Knochenkohle und deren Einsatz als Dünger ist ein vielversprechender Ansatz zum Schließen von Nährstoffkreisläufen, jedoch ist die Düngewirkung der Knochenkohle noch unklar. Deshalb wurden zwei Knochenkohlen (Knochenkohle (BC) und Knochenkohle^{plus} (BC^{plus}; schwefelangereicherte BC)) im Vergleich zu Triplesuperphosphat (TSP) in einem mehrjährigen Gefäßversuch mit Mais als stark P-abhängiger Fruchtart getestet. Die untersuchten Düngemittel beeinflussten sowohl die Trockenmasse als auch die P-Konzentration von Mais in der Reihenfolge BC^{plus}, TSP > BC. Die P-Aufnahme von Mais in der TSP-Variante erreichte im ersten Versuchsjahr 38 % des applizierten P und nahm in den Folgejahren stetig ab. Die P-Aufnahme in der BC^{plus}-Variante blieb dagegen während der Versuchsdauer relativ konstant. Daraus folgt, dass der Recycling-P-Dünger BC^{plus} eine ausreichende P-Verfügbarkeit für Nutzpflanzen langfristig aufrechterhalten kann.

Stichwörter

Knochenkohle, Phosphor, Phosphorrecycling, Pflanzenernährung

Introduction

Phosphorus (P) is essential for all living organisms. To ensure adequate P supply to arable crops, P is mainly applied as mineral fertilizer. However, geological deposits of rock phosphate for the production of mineral P-fertilizer are limited (Cordell et al., 2009; Dawson & Hilton, 2011; Heckenmüller et al., 2014) and are often contaminated with toxic heavy metals (Attallah et al., 2019). Furthermore, closing nutrient cycles is increasingly of interest from the ecological and economical perspective and has been defined as one of the elements forming the European Green Deal transforming the EU's economy for a sustainable future (Montanarella & Panagos, 2021). Therefore, alternative P fertilizers are becoming increasingly important in agricultural production (Simpson et al., 2011; Roberts & Johnston, 2015). One opportunity to conserve natural P reserves is the wider use of P-recycling products, like e.g. bone char. This is a biochar produced by technical pyrolysis of animal bones derived from slaughterhouse waste (Leinweber et al., 2019). Bone char is not only rich in minerals such as P, calcium (Ca) and magnesium (Mg) but also free of heavy metals and pharmaceuticals (Siebers et al., 2013). The solubility of P and thereby the plant availability from bone char was found to be rather low in initial studies (Siebers et al., 2012;

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Siebers et al., 2014). However, enriching bone char with reduced sulfur (S)-compounds increased the P-solubility in laboratory experiments (e.g., Morshedizad et al., 2016; Zimmer et al., 2018). Furthermore, the efficacy of bone char has been tested in field and mostly short-term pot experiments (Siebers et. al., 2012; Siebers et al., 2014; Panten & Leinweber, 2020) but the experimental basis for bone char applications as P fertilizer is rather small. Since the P release from bone char is slower than from commonly used mineral fertilizer (Warren et al., 2009; Siebers et al., 2013; Morshedizad et al., 2016) we expected rather longer-term effects on the P supply and crop yields. To test this hypothesis and generally improve the experimental background for recommending bone char, a perennial pot experiment (six growing seasons) was set up. A soilless system with gravel and sand substrate was chosen to avoid overlying effects of soil P and to allow the manual separation of bone char particles in the experimental course for further studies. Because of this substrate, it was not expected that phosphorus needs to be taken into account to differentiate between phosphorus available from the substrate and from the fertilizer. Therefore, it was decided to use as positive control the highly water-soluble triple superphosphate instead of a negative zero P control for the experiment. Maize was chosen as a very P-requiring test crop (Zicker et al., 2018; Zicker et al., 2020) and two different bone chars were tested in comparison with the highly water-soluble mineral P fertilizer triple superphosphate (TSP). In detail, we aimed at answering these questions: (1) Do the tested bone chars affect the P supply to maize? (2) How do bone chars perform in comparison with TSP? (3) How does the P supply vary over time?

Materials and methods

Experimental setup

The pot experiment was designed as a six-year experiment (2016-2021) and it was conducted in the open part of an unheated greenhouse for the first two years. Because of heavy precipitation in 2017, the pots were moved into the covered part of the unheated greenhouse, and subsequently watered with deionized water on demand. The 300 l-plastic containers (0.68 m diameter at top) were filled from the bottom to the top as follows: 18 cm stones (16-32 mm diameter) as a drainage layer, a fleece to prevent substrate material from above to shift into the drainage layer, 11 cm coarse gravel, 4 cm fine gravel and 44 cm sand. This sand had the texture 1.8% (> 2 mm), 48.0% (2 – 1 mm), 7.9% (1 – 0.63 mm), 33.9% (0.63 – 0.2 mm, and 2.4% (< 0.2 mm). To keep a closed nutrient cycle surplus rain- and irrigation water was collected through a drainage outlet at the bottom of the containers and used for subsequent irrigation. Maize (varieties DS 0331 (year 1-3), KWS Keltikus (year 4), and Limagrain LG 31.227 (year 5-6)) seeds, treated with MaximXL (Syngenta), was used as test crop. Ten seeds were sown per pot and the number of plants was reduced to seven after germination.

Treatments were replicated four times, and P fertilizers were applied as a stock fertilization for six years to an equivalent of 600 kg P ha^{-1} (21.6 g P pot^{-1}). We assumed an annual P uptake of 20 kg P ha^{-1} and an agronomic efficiency of 20%. Treatments were (1) bone char (BC) pyrolysed at about 800°C, (2) surface-modified bone char (BC^{plus}) with sulfur compounds from biogas streams (patent DE212012000046U1) adding 54.5 g S pot^{-1}, and (3) TSP. Fertilizers were used as provided with 95–100% of the particles bigger than 1 mm and mixed into the top 15 cm of the containers. Total concentration of the fertilizer elements was determined by extraction with *aqua regia* (VDLUFA, 2000). The concentrations of P (177.4 nm), Ca (318.1 nm), K (766.4 nm), Mg (279.0 nm), and Na (589.5 nm) were measured by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, icap 6000, Thermo Fisher, Cambridge, United Kingdom; wavelength given in parentheses). Trace element analyses showed that BC and BC^{plus} had much lower concentrations of As, Cd, Cr, Cu, Ni, and U than TSP fertilizer (Panten & Leinweber, 2020). Further detailed information, such as P- and S-speciation in the applied BCs, was published by Zimmer et al. (2018). All other nutrients were applied equally and annually to all pots in amounts of 21 g N pot^{-1} (divided in three doses), 6 g Ca pot^{-1} (before seeding), 10 g of K pot^{-1} (divided in two doses), 2 g S pot^{-1} (divided in two doses), 2 g Mg pot^{-1} (divided in two doses), and 8, 8, 1.6, 4, 0.8, 80 mg pot^{-1} of Zn, Mn, Cu, B, Mo and Fe, respectively (divided in two doses). The first dose of these nutrient elements was applied at seeding (N, K, S, Mg, Zn, Mn, Cu, B, Mo, and Fe), the second dose directly after the cutting of the maize plants at BBCH 32 (N, K, S, Mg, Zn, Mn, Cu, B, Mo, and Fe), and finally a third dose of N was applied during flowering.

Sampling and analyses

Each year two samplings were performed in six vegetation periods (2016 – 2021): at BBCH stage 32, two plants were taken for intermediate harvest and the remaining five plants were taken for harvest at BBCH 85. To determine plant dry matter yield the samples were dried at 60°C until constant weight in a ventilated oven. Dried samples were ground ≤ 0.5 mm with an ultracentrifugal mill (Retsch ZM 100 or 200, 42781 Haan, Germany). About 0.5 g of plant material was digested with 6 ml nitric acid and 1.5 ml hydrogen peroxide in a microwave (CEM MARS, Metthews, USA). Total element concentrations were determined by ICP-OES (icap 6000, Thermo Fisher, Cambridge, United Kingdom): P (177.4 nm), K (766.4) nm, Ca (317.9 nm), Mg (280.2 nm), Mn (257.6 nm) and Zn (218.3 nm). Contents of total carbon, nitrogen and sulfur (C, N, S) were determined in a 10 mg subsample using the CNS-analyser (Vario EL cube, Elementar Analysensysteme GmbH, 63505 Langenselbold, Germany).

Data and statistical evaluation

Data were tested for normal distribution using the Shapiro-Wilk test. In case of normal distribution, significance of P fertilizers was tested using one-way analysis of variance (ANOVA, Tukey test). Non-normal distributed data were analysed with the Kruskal-Wallis-test. Differences were considered significant at $p \leq 0.05$. Statistical analyses were performed with R version 3.6.1 (R Core Team, 2019) and R package agricolae version 1.3-1 (De Mendiburu, 2016).

Results

There were significant differences in dry matter of maize, both in whole plants at BBCH 32 and in straw and grain yields at BBCH 85 in six experimental years (2016 – 2021) (Tables 1 and 2). The fertilizer type affected the dry matter yields and often followed the order BC^{plus}, TSP > BC. The yield component “thousand grain weight” of maize at BBCH 85 was lowest in the BC-treatments, while weights were similar in the BC^{plus} and TSP treatments (Tables 1 and 2).

The P concentrations showed the order BC^{plus}, TSP > BC at most of the sampling dates (Tables 1 and 2). The concentrations of other nutrient elements remained stable during the duration of the experiment (Table S1).

The total P uptake of maize, cumulated for each experimental year, ranged from 0.05 g per pot (2021, BC) to 8.67 g per pot (2016, TSP) (Fig. 1). The BC treatments had lowest P uptake in each experimental year. The TSP and the BC^{plus} treatments showed opposite developments for the P uptake along the experimental years. While TSP application caused a higher P uptake than BC^{plus} in 2016 it was the other way around since 2019. The values were similar for both treatments in 2017 and 2018.

Discussion

The highest yields in the first experimental year 2016, following the order TSP > BC^{plus} > BC (Table 1), indicate that P supply was a yield-limiting factor, which is not surprising for the P-poor substrate. The general order, BC^{plus}, TSP > BC in the following samplings (Tables 1 and 2) confirms findings in a pot experiment conducted by Zimmer et al. (2019), in which TSP and BC^{plus} caused insignificantly higher ryegrass yields compared to BC or zero P. Different from this, Panten & Leinweber (2020) did not report P fertilizer effects on grain yield in a five-year field experiment. In that study, BC and BC^{plus} reached 97

Table 1. Mean yield components (dry matter (g) and thousand grain weight (g)) and phosphorus concentration of maize in a pot experiment at BBCH 32 and 85 in 2016 – 2018 as affected by type of P-fertilizer (TSP (triple superphosphate), BC (bone char), BC^{plus} (sulfur enriched bone char). Low letters indicate significant differences (standard deviation in brackets, $p < 0.05$, Kruskal-Wallis test or Tukey test in dependence of normal distribution of data).

year	BBCH maize	harvest	fertilizer type	dry matter (g)		thousand grain weight (g)		P (g kg^{-1})	
2016	32	whole plant	TSP	11.68 (1.23)	a	-	-	11.14 (0.71)	a
			BC	1.17 (0.61)	c	-	-	1.32 (0.29)	c
			BC^{plus}	4.57 (1.52)	b	-	-	4.26 (1.44)	b
	85	straw	TSP	933.36 (175.57)	a	-	-	3.76 (0.30)	a
			BC	258.20 (153.45)	b	-	-	1.33 (0.32)	c
			BC^{plus}	885.18 (42.40)	a	-	-	0.79 (0.22)	b
		grain	TSP	1090.29 (510.09)	a	221.87 (27.61)	a	3.09 (0.07)	a
			BC	227.79 (240.90)	b	162.04 (18.18)	b	2.96 (0.55)	a
			BC^{plus}	999.85 (327.53)	a	233.91 (10.20)	a	2.81 (0.24)	a
2017	32	whole plant	TSP	18.76 (4.61)	a	-	-	4.59 (1.34)	a
			BC	6.73 (2.50)	b	-	-	1.80 (0.08)	b
			BC^{plus}	14.09 (5.93)	ab	-	-	4.08 (0.31)	a
	85	straw	TSP	636.65 (52.57)	a	-	-	1.64 (0.88)	a
			BC	461.57 (39.19)	b	-	-	0.48 (0.07)	b
			BC^{plus}	679.77 (35.18)	a	-	-	1.45 (0.23)	ab
		grain	TSP	673.04 (33.03)	a	233.68 (17.22)	a	2.75 (0.22)	a
			BC	276.27 (56.04)	b	186.53 (16.53)	b	1.88 (0.53)	a
			BC^{plus}	679.77 (35.18)	a	222.96 (7.48)	a	2.57 (0.18)	a
2018	32	whole plant	TSP	21.10 (4.73)	ab	-	-	2.62 (0.21)	a
			BC	11.44 (5.37)	b	-	-	2.09 (0.38)	b
			BC^{plus}	23.22 (6.79)	a	-	-	2.88 (0.05)	a
	85	straw	TSP	584.88 (59.62)	a	-	-	0.89 (0.13)	ab
			BC	370.43 (160.95)	a	-	-	0.65 (0.16)	b
			BC^{plus}	623.47 (57.53)	a	-	-	1.03 (0.11)	a
		grain	TSP	804.93 (81.72)	a	320.47 (18.34)	a	2.45 (0.23)	a
			BC	468.25 (295.61)	a	277.44 (32.95)	a	1.89 (0.13)	b
			BC^{plus}	884.39 (51.44	a	315.33 (15.38)	a	2.81 (0.18)	a

Table 2. Mean yield components (dry matter (g) and thousand grain weight (g)) and phosphorus concentration of maize in a pot experiment at BBCH 32 and 85 in 2019 – 2021 as affected by type of P-fertilizer (TSP (triple superphosphate), BC (bone char), BC^{plus} (sulfur enriched bone char). Low letters indicate significant differences (standard deviation in brackets, $p < 0.05$, Kruskal-Wallis test or Tukey test in dependence of normal distribution of data).

year	BBCH maize	harvest	fertilizer type	dry matter (g)		thousand grain weight (g)		P (g kg^{-1})	
2019	32	whole plant	TSP	35.40 (9.30)	a	-	-	2.06 (0.25)	a
			BC	9.60 (9.61)	b	-	-	1.52 (0.16)	b
			BC^{plus}	33.37 (5.25)	a	-	-	2.25 (0.15)	a
	85	straw	TSP	698.18 (84.78)	a	-	-	0.84 (0.25)	ab
			BC	257.97 (225.55)	b	-	-	0.56 (0.08)	b
			BC^{plus}	782.35 (62.72)	a	-	-	1.15 (0.12)	a
		grain	TSP	366.63 (44.65)	a	285.59 (9.16)	a	2.30 (0.11)	b
			BC	131.15 (105.51)	b	164.43 (85.89)	b	2.21 (0.47)	b
			BC^{plus}	278.98 (65.06)	ab	298.82 (54.20)	a	3.01 (0.24)	a
2020	32	whole plant	TSP	19.10 (5.55)	a	-	-	2.31 (0.13)	b
			BC	3.29 (0.86)	b	-	-	1.35 (0.40)	c
			BC^{plus}	24.23 (7.63)	a	-	-	2.58 (0.07)	a
	85	straw	TSP	506.20 (47.72)	a	-	-	0.46 (0.13)	a
			BC	158.40 (102.70)	b	-	-	0.74 (0.21)	a
			BC^{plus}	617.39 (46.98)	a	-	-	0.51 (0.17)	a
		grain	TSP	641.69 (35.60)	a	264.40 (10.55)	b	1.67 (0.24)	a
			BC	155.86 (139.64)	b	141.13 (64.58)	c	2.29 (0.78)	a
			BC^{plus}	617.39 (46.98)	a	284.93 (8.04)	a	2.49 (0.28)	a
2021	32	whole plant	TSP	37.55 (5.33)	a	-	-	2.04 (0.13)	b
			BC	3.20 (0.89)	b	-	-	1.67 (0.18)	b
			BC^{plus}	34.21 (9.16)	a	-	-	2.49 (0.26)	a
	85	straw	TSP	406.96 (65.27)	b	-	-	0.81 (0.07)	b
			BC	113.24 (143.59)	c	-	-	1.00 (0.05)	a
			BC^{plus}	504.45 (36.33)	a	-	-	0.88 (0.04)	b
		grain	TSP	537.84 (48.77)	a	257.19 (18.40)	a	1.54 (0.10)	a
			BC	47.13 (76.51)	b	108.60 (44.88)	b	1.85 (0.24)	a
			BC^{plus}	556.39 (115.96)	a	245.94 (31.97)	a	1.70 (0.21)	a

and 95% of the TSP induced yield, respectively. The similarity in yield responses in pot experiments (Table 1 and Zimmer et al. (2019)) but difference to the outcome of a field experiment (Panten & Leinweber, 2020) are explained by the different conditions, especially rooting zone, exposure to weather conditions and the crops grown.

The concentrations of major nutrient elements in maize (Tables 1 and 2, Supplemental Table 1) were in agreement with results by e.g. Ferreira et al. (2014) and Özpinar & Özpinar (2009). The P concentrations, generally similar in the BC^{plus} and the TSP but above the BC treatments (Tables 1 and 2), are explained by the interplay between P-solubility, P uptake and biomass formation. Similarly, the concentrations of other major nutrient elements reflect the intended non-shortage achieved by the annual fertilizer application. The general low P-solubility of BC as reported by, e.g., Warren et al. (2009), Robinson et al. (2018) and Zimmer et al. (2018) is the best explanation for the low P concentration (Tables 1 and 2), P uptake (Fig. 1) and yield of maize (Tables 1 and 2) in the BC treatments of this experiment.

The temporal decrease in yearly P uptake in the TSP treatments and relative constancy in the BC^{plus} treatments (Fig. 1) agrees with Zimmer et al. (2019) who also recorded increased P uptake in the BC^{plus} and TSP treatments in comparison to BC. The proportions of total applied P, taken up by plants over the whole experimental duration, increased in the order BC (17.8%) < BC^{plus} (66.2%) < TSP (82.3%). This large exploitation of added P, especially in the TSP treatment, is explained by the fact that the substrate provided no P at all. In analogy as discussed above for the yields, the field experiments by Panten & Leinweber (2020) did not reveal BC^{plus} induced effects on P uptake in course of the crop rotation. This disagreement between pot and field experiment results can be explained by the different crops grown that have species-specific P mobilization strategies (e.g. Palomo et al., 2006; Mat Hassan et al., 2012; Maltais-Landry et al., 2014), nutrient uptake and

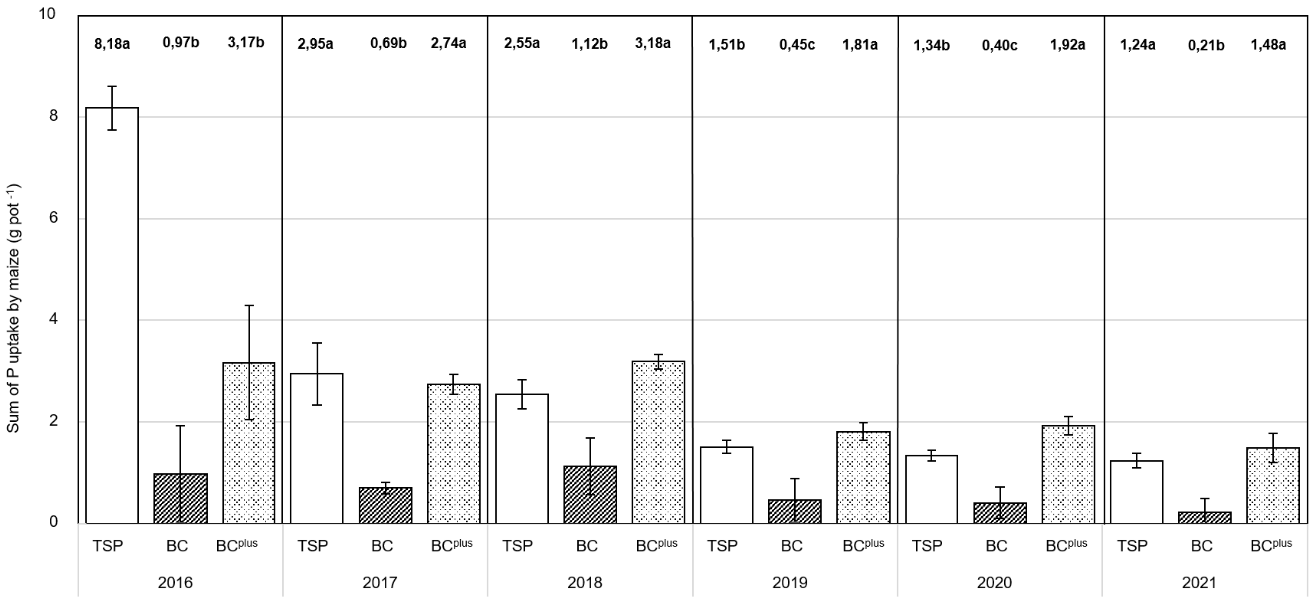

Figure 1. Mean cumulated P uptake (sum of harvests at BBCH 32 [whole plant] and 85 [straw + grain]) and standard deviations (g per pot) of maize in a pot experiment in 2016 – 2021 as affected by type of P-fertilizer (TSP: triple superphosphate), BC: bone char), BC^{plus}: sulfur enriched bone char). Low letters indicate significant differences ($p < 0.05$, Kruskal-Wallis test or Tukey test in dependence of normal distribution of data).

biomass formation in addition to the above discussed effects of growth conditions.

The higher P concentrations and P uptake in the TSP and BC^{plus} treatments compared to BC (Fig. 1), that agreed with higher yields (Tables 1 and 2), are first of all proving the P demand of the maize crops that was not met by the experimental substrate. The high P uptake in the TSP treatment plausibly is caused by the P solubility in substrate, as previously explained by Zimmer et al. (2019). The decreased P uptake in the TSP treatment from year to year probably originates from the very high P uptake of 38% of the applied P in the first experimental year that likely resulted in a comparably low level of internal P cycling. Because of the soilless substrate, no P except the applied P at the beginning of the experiment became plant available. Additionally, P was applied intentionally only at the beginning of the experiment, even though it is well known that in soils with a good P status yield gains also can be achieved by freshly applied P (Buczko et al., 2018). The increased P uptake in BC^{plus} with duration of the experiment probably is due to the so-called "*in situ* digestion effect" (Fan et al., 2012) from the oxidizing S-compounds of BC^{plus}. Biological oxidation of S even in the non-sterile gravel substrate by active S-oxidizing bacteria (Chaudhary et al., 2019), formation of H_2SO_4 and the resulting pH decrease in soil may have stimulated the destruction of bioapatite and release of P from the BC^{plus} particles. This effect became more important with longer duration of a P experiment in the laboratory (Morshedizad et al., 2016).

Conclusions

The perennial pot experiment conducted in the present study was useful to assess the effects of different P-fertilizers (triple superphosphate (TSP), bone char (BC) and sulfur-enriched bone char (BC^{plus})) on yield parameters, P concentration and P uptake of maize.

(1) Since sulfur-enriched BC^{plus} increased yield and P supply to maize in comparison to BC the "*in situ* digestion" by added S is necessary to make bone char a potential P sources in a closed loop cycling economy.
(2) From the two BCs under study the BC^{plus} showed similar P supply to maize as TSP, except in the first experimental year. On the contrary, untreated BC supplied minor and fluctuating amounts of P to maize. Therefore, this material cannot be recommended as fertilizer to P demanding crops.
(3) The decreased P-supply to maize in the TSP treatment and the constant P-supply in the BC^{plus} treatment conclusively demonstrates that BC^{plus} can be considered as a moderately slow-release P fertilizer. Therefore, a P recycling economy essentially must involve a low-cost, dissolution supporting S-enrichment to BCs to produce a long-term P fertilizer.
(4) Forthcoming studies are directed to better understand the dissolution kinetics and microbial processes at the individual fertilizer particle scale, and the possible duration of stock fertilization for different crop rotations.

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Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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