



The fate of phosphorus from bone char-based fertilizers in soil pools in a 5-year crop rotation

Jens Kruse · Kerstin Panten · Nina Siebers

Received: 18 March 2022 / Accepted: 31 August 2022 / Published online: 29 September 2022
© The Author(s) 2022

Abstract Bone char (BC) is a promising P-recycling fertilizer but with rather low P-solubility, which can be increased by modifying the BC surface with elemental sulfur (BC^{plus}), but effects on the soil P status have not been tested under field conditions yet. Hence, a long-time field experiment was started 2013 to track the fate of BC and BC^{plus} P into different soil P pools compared to a control and triple superphosphate (TSP) treatment for severely P deficient (iSPTC-A) vs. sufficiently P fertilized (iSPTC-C) soil. The fingerprint of the recent land-use history (six years grassland prior arable land) was reflected by elevated labile-P_o and NaOH-P_o concentrations at the beginning of the experiment. However, after 3 years, labile P_o concentrations converged and stabilized in

both soils at a similar level. The formation of this new equilibrium of labile P_o suggests that the rate of P_o mineralization was, to some extent, controlled by the amount of available legacy P. After the first crop rotation, the effect of P fertilization on soil-P budgets and fractions were small and mostly insignificant. Only TSP increased the available-P pools in the soil. The other pools were not affected by treatments except stable-P increased significantly after BC application in iSPTC-A. The former laboratory results of higher P solubility of BC^{plus} over BC could not be confirmed within the duration of the field trial. However, to prove that BC and BC^{plus} are capable of maintaining adequate long-term crop P supply, the continuation of this unique field trial is highly recommended.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10705-022-10228-y>.

J. Kruse · N. Siebers (✉)
Forschungszentrum Jülich GmbH, Agrosphere (IBG-3)
Institute of Bio- and Geosciences, Wilhelm Johnen Straße,
52425 Jülich, Germany
e-mail: n.siebers@fz-juelich.de

J. Kruse
Soil Science, Institute of Crop Science and Resource
Conservation (INRES), University of Bonn, Nussallee 13,
53115 Bonn, Germany

K. Panten
Julius Kühn Institute, Institute for Crop and Soil Science,
Bundesallee 69, 38116 Brunswick, Germany

Keywords Alternative fertilizers · Sequential P fractionation · Sulfur · Field trial · Phosphorus budgets

Introduction

Phosphorus (P) is essential for plant growth and cannot be replaced by any other element; thus, it is indispensable for agriculture. About 82% of the mined phosphate rock is used for the production of P fertilizers (Scholz and Wellmer, 2013); however, there are uncertainties regarding finite P rock reserves (Heckenmüller et al., 2014) and discussions on an uneven distribution of deposits. In the European Union, P and

rock phosphate is listed as one of the 30 critical raw materials and among them it is rated as one of five materials combining the highest scores for supply risk and economic importance (European Commission, 2020). Thus, efforts have increased to replace rock phosphate with secondary P resources, such as compost, manure, or animal by-products, towards an efficient and sustainable closed-loop production system.

A promising by-product from slaughterhouse industries are bone char (BC) based fertilizers (Panten and Leinweber, 2020; Siebers et al., 2014; Warren et al., 2009; Zwetsloot et al., 2016). For BC production defatted, de-gelatinized animal bone chips are pyrolyzed (600–800 °C) and produced chars contain between 130 to 150 g P kg⁻¹, mainly in the form of hydroxyapatite, as well as calcium (Ca) and magnesium (Mg), while they are free of contaminants such as cadmium and uranium (Siebers and Leinweber, 2013; Zimmer et al., 2018). The solubility of BC was found to be mainly a function of pyrolysis conditions (Biswas et al., 2021; Dela Piccola et al., 2021) and soil pH and was mostly lower than highly soluble mineral P fertilizers (Leinweber et al., 2018; Siebers et al., 2014; Warren et al., 2009). Therefore, the very porous surface of BC can be modified with up to 20% (w/w) elemental sulfur (S) (BC^{plus}; patent DE102011010525) to increase its solubility in the soil (Morshedizad et al., 2018; Zimmer et al., 2018). This is due to the so-called “*in situ digestion*”, a term introduced by Fan et al. (2003) who co-applied rock phosphates with elemental S to initiate soil acidification and foster apatite dissolution by the release of H₂SO₄ from microbial sulfoxidation. Additionally, positive effects of co-applied elemental S on P availability were also reported for a mixture of bone-wood chips biochar with sulfur-oxidizing bacteria (*Thiobacillus* Spp.) (Amin and Mihoub, 2021). The major advantage of BC^{plus} is that this *in situ* acidification occurs directly at the surface of the BC^{plus} particle. This makes it more likely that the microbially produced H₂SO₄ directly reacts with the apatitic BC^{plus} matrix leading to a more efficient and uniform release of P while reducing the risks of harmful fast pH drops and possible undesired heavy metal co-mobilization in soil, compared to, e.g., granulated S co-applied with rock phosphate (Zimmer et al., 2018). First application studies confirmed the improved P solubility of BC^{plus}, but these results were only obtained in lab incubation studies (Morshedizad et al., 2016, 2018) or one pot experiment with annual rye grass (Zimmer et al., 2019). However,

results from field application, particularly longer-term studies with annual consecutive fertilizer applications, are rare as BC^{plus} is still a relatively new fertilizer.

Recently, Panten and Leinweber (2020) evaluated data from the first BC/BC^{plus} field experiment to estimate their agronomic value for supplying P after a 5-year crop rotation. They could show that the fertilizer use efficiency was highest in the P deficient soils and plants took up higher proportions of BC^{plus} than BC. This indicated that BC^{plus} has the potential to increase available P concentrations in soils, especially with low initial P concentrations. Results from a parallel study at the same field experiment by Grafe et al. (2021), focusing on the response of the soil microbiome after application of BC and BC^{plus}, suggested that BC^{plus} influenced the bacterial P turnover by stimulating soil inherent P solubilizing bacteria, whereas BC favors P recycling from biomass and P inducible uptake systems. However, in their study, only the water-soluble P (P_{water}), calcium acetate lactate extractable P (P_{cal}), and total P (P_t) in the soil after one complete crop rotation (five years) were considered, and the influence of the repeated annual application of BC-based fertilizer on other P fractions, pools, and stocks in soil on a yearly basis was not determined. Furthermore, possible influences of the land-use change from former five years grassland to arable land at the beginning of the experiment was not examined in these studies.

In the presented study, we analyzed retained soil samples from a field experiment (Panten and Leinweber, 2020) with two BC-based fertilizers before start of the trial and annually taken during a complete crop rotation in order (i) to estimate the fate of BC-based P within various P fractions, pools, and stocks compared to highly soluble triple superphosphate (TSP) fertilizer, and (ii) to determine the short-term effects of fertilizer P on P pool dynamics, (iii) and to evaluate the possible influence and duration of this land-use change on different P pools and fractions.

Material and methods

Site description and soil sampling

Soil samples were collected from a newly established agricultural long-term experimental site (54° 3' 41.47" N; 12° 5' 5.59" E) at the Julius

Kühn Institute, Institute for Crop and Soil Science Braunschweig, Germany, which was established in 2013. The soils in the experimental field were classified as Dystric Cambisol and Haplic Luvisol (IUSS Working Group WRB, 2015) and developed from sandy loess overlying sandy fluvial sediments. The experiment was based on a former long-term P field experiment from 1985 to 2008 (for more details see Vogeler et al., 2009) following grassland from 2009 to 2012; this left randomly distributed plots with different concentrations of P_{CAL} . In 2013, all plots were plowed to a depth of 25 cm and oats were sown. After harvest, the plots were assigned to initial soil P-test classes (iSPTC) based on their topsoil P_{CAL} concentrations, i.e., iSPTC-A (severely deficient, $< 15 \text{ mg } P_{\text{CAL}} \text{ kg}^{-1}$), iSPTC-B (deficient, $15\text{--}30 \text{ mg } P_{\text{CAL}} \text{ kg}^{-1}$), and iSPTC-C (sufficient, $31\text{--}60 \text{ mg } P_{\text{CAL}} \text{ kg}^{-1}$), respectively (Wiesler et al., 2018). Afterward, the new field experiment was established in a completely randomized block design (plots $5.75 \text{ m} \times 17.5 \text{ m}$, with three field replicates) with three different iSPTC (A, B, and C), and a 5-year crop rotation of winter barley, winter oilseed rape, winter wheat, lupin, and winter rye. Three different P fertilizer treatments (45 kg ha^{-1}) were applied once a year shortly before seeding, i.e., TSP, BC, and BC^{plus} . Furthermore, a control treatment without P fertilization (control) was established for comparison. For a detailed chemical characterization of both bone char-based alternative fertilizers see Zimmer et al. (2018). Additionally, all plots were chiseled and plowed annually and received equal amounts of nitrogen (N), potassium (K), sulfur (S), and calcium (Ca) fertilizers (Table S1) to prevent deficiency in these nutrients for plant growth. For a more detailed description of the experimental design of this trial, see Panten and Leinweber (2020). Topsoil samples (0–30 cm) were taken at the start of the experiment (2013) from plots of iSPTC-A and iSPTC-C and then annually repeated in the following five years for a complete crop rotation. For this, a composite sample from eight soil cores per plot was taken after each harvest. Topsoil P stocks were calculated using a topsoil bulk density of 1.54 g cm^{-3} as estimated using the soil dry weight after drying at 105°C and the auger volume the soil samples were taken with. Soil samples were air-dried and sieved to a particle size $< 2 \text{ mm}$.

Total elemental composition and P pools

Total elemental concentrations of magnesium (Mg_t) and calcium (Ca_t) were determined by digestion with aqua regia and subsequent measurements via inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Thermo Fisher iCAPTM 7600). Total nitrogen (N_t), carbon (C_t), and sulfur (S_t) were determined by dry combustion (MicroCube Elementar, Hanau, Germany). The pH values of all soil samples were measured in 0.01 M CaCl_2 (w/w 1:25). Plant available P (P_{CAL}) was extracted from the soil with calcium acetate lactate (Schüller, 1969) following ICP-OES measurements. Water-soluble P (P_{water}) was extracted in a procedure slightly modified from van der Paauw et al. (1971) (1.5 g soil in 2 mL aqua dest. for 22 h, addition of 70 mL aqua dest., overhead shaking for 60 min) before P was analyzed colorimetrically (Specord 50, Analytik Jena, Germany) using the molybdenum blue method according to Murphy & Riley (1962).

For sequential P extraction, a slightly modified Hedley et al. (1982) was used. Briefly, 0.5 g soil sample was shaken in aqua dest. for 18 h using a reciprocal shaker (20 rpm). After decantation, the next extractions were done with 0.5 M NaHCO_3 (pH 8.5), 0.1 M NaOH , and $1 \text{ M H}_2\text{SO}_4$. All extracts were centrifuged at $3500 \times g$ for 20 min and supernatants were subsequently filtered using a P-free Rotilabo Typ 601 P filter. Total P concentrations (P_t) in the obtained fractions were determined using an ICP-OES and inorganic P (P_i) using the molybdenum-blue method (Murphy and Riley, 1962) using an Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland). Organic P (P_o) was calculated as the difference between P_t and P_i . The obtained fractions can be grouped by their chemical extractability to labile P ($\text{H}_2\text{O-}P_i$ and $\text{-}P_o$ and $\text{NaHCO}_3\text{-}P_i$ and $\text{-}P_o$), moderately labile P ($\text{NaOH-}P_i$ and $\text{-}P_o$), and stable P ($\text{H}_2\text{SO}_4\text{-}P_t + \text{residual-P}$) (Negassa and Leinweber, 2009).

Mobile and potential plant-available P was also accessed via the diffusive gradients in thin films (DGT) technique. For this, DGT devices, ferrihydrite binding layers, and diffusive gel layers were purchased from DGT Research Ltd. (Lancaster, UK) and were used as described in (Davison, 2016). Briefly, all soil samples were incubated and deployed for 24 h at 22°C at 100% water holding

capacity. Following the extraction of P from the binding layer with 1 M HCl, P concentrations were analyzed with ICP-OES (iCAP 6500, Thermo Fisher, 63,303 Dreieich, Germany) and used to calculate the DGT P (P-DGT concentrations). Furthermore, to calculate P budgets and balances, we used data of P uptake from Panten and Leinweber (2020).

Statistics

All statistical analyses were performed using IBM®SPSS® Statistics Version 25. The effects of the sampling year, four fertilizer treatments, and their interaction on soil parameters were analyzed for each iSPTC separately using a mixed two-factor analysis of variance (ANOVA) with Tukey post-hoc test. One-factor ANOVA with subsequent Tukey post-hoc test was used to analyze differences between the four treatments within the sampling years. To account for plot heterogeneity in the chemical properties between treatments, all statistical analyses of time series data were performed after the normalization of the data from 2014 to 2018 with the respective values from 2013 (i.e., subtracting for each plot and year the respective value of 2013).

Results

Basic soil properties

For both iSPTCs, there was no significant treatment effect on pH and Ca_t (Table 1). However, the two lime applications (2014 and 2017) significantly increased the pH values in all treatments (pH 5 to pH 6) as well as increased Ca_t concentrations in both iSPTCs. Repeated S fertilization was also reflected in the temporal parallel course of the S concentration of all P fertilized variants. The control treatment was rather unaffected by S application and values hardly changed for both iSPTCs, which were thus significantly lower than for the other treatments. The additional input of S via BC^{plus} was not reflected in the data. Total N was also unaffected by P treatments regardless of iSPTC, but increased slightly from 2017 to 2018 (Table 1).

P budgets

All P budgets were obtained from Panten and Leinweber (2020) and relate to the first crop rotation (2014–2018). Ideally, the differences (total topsoils P stocks in 2018 minus initial P stocks 2013) equal the P budgets, assuming no additional P losses from the topsoil (0–30 cm) other than P uptake and no unidentified P inputs. However, for most treatments, calculated P stocks were higher than measured P stocks (Table 2). Mean gaps in P stocks were larger for iSPTC-C (– 12%) than for iSPTC-A (– 6%). Among all fertilized treatments, the TSP treatment showed the best agreement between calculated and measured P stocks, followed by BC, and BC^{plus} .

P fractions

The mean total P concentration in the control of the iSPTC-C soil was at the start of the field trial ~ 1.4-times higher than that in the iSPTC-A (Table 3). This was mostly due to higher concentrations of labile P_i (sum of $\text{H}_2\text{O-P}_i$, $\text{NaHCO}_3\text{-P}_i$) and moderate labile P_i (NaOH-P_o) by otherwise comparable stable P (sum of $\text{H}_2\text{SO}_4\text{-P}$, residual-P) concentrations. Concentrations of P_{cal} , P_{water} , and P_{DGT} were also about ~ 1.5–3-times higher in iSPTC-C than iSPTC-A, independent of sampling year and treatment. The general higher concentrations and proportions of total extractable P_i in iSPTC-C compared to iSPTC-A led to mostly higher P_i/P_o ratios for iSPTC-C. These P_i/P_o ratios were even widened by the decreasing trend of the initially slightly higher total P_o concentrations in all treatments of iSPTC-C at the beginning of the trial towards values being comparable to iSPTC-A. After the first year, P_{cal} decreased significantly (~ 30%) in all treatments of iSPTC-C. Then, the P_{cal} of the TSP treatment tended to increase especially in 2017. This was different for the other treatments, with decreasing P_{cal} concentrations, which increased only in 2017 followed by a decrease again in 2018. Similar but less pronounced trends were observed for P_{water} and P_{DGT} (Table 3).

Phosphorus distribution among single P fractions followed the order: $\text{P}_{\text{DGT}} = \text{P}_{\text{water}} < \text{P}_{\text{cal}}$ irrespective of iSPTCs, sampling date, and treatment. Additionally, the P distribution among obtained sequential P

Table 1 Basic chemical analyses as affected by treatments (control, TSP, BC, and BC^{plus}) and duration of the experimental time (2013 to 2018) for both initial soil P-test classes (iSPTC)-A = severely deficient, iSPTC-C = sufficient

iSPTC-A	pH _(CaCl2)	Total C g kg ⁻¹	Total N	Total S mg kg ⁻¹	Total Ca	Total Mg	iSPTC-C	pH _(CaCl2)	Total C g kg ⁻¹	Total N	Total S mg kg ⁻¹	Total Ca	Total Mg
2013													
Control	5.1 ± 0.1	12.9 ± 0.5	0.8 ± 0	166 ± 9	1001 ± 168	863 ± 80	Control	5.2 ± 0.1	13.1 ± 0.2	0.8 ± 0	169 ± 9	1184 ± 113	839 ± 14
BC	5.1 ± 0.1	12.2 ± 0.5	0.8 ± 0	161 ± 11	886 ± 74	749 ± 136	BC	5.2 ± 0	13.2 ± 0.5	0.9 ± 0	175 ± 5	1281 ± 85	838 ± 18
BC ^{plus}	5.2 ± 0.1	12.8 ± 0.6	0.8 ± 0	171 ± 7	1072 ± 52	881 ± 56	BC ^{plus}	5.2 ± 0.1	13.2 ± 0.2	0.8 ± 0	165 ± 2	1192 ± 127	854 ± 23
TSP	5.2 ± 0.1	12.1 ± 0.4	0.8 ± 0	166 ± 12	958 ± 67	766 ± 146	TSP	5.2 ± 0	13.3 ± 0.6	0.9 ± 0	171 ± 5	1289 ± 90	853 ± 27
2014													
Control	5.4 ± 0	12.7 ± 0.2	0.8 ± 0	152 ± 10	1182 ± 114	696 ± 36	Control	5.2 ± 0.2	^a 13.6 ± 0.2	0.9 ± 0	154 ± 4	1271 ± 154	721 ± 17
BC	5.2 ± 0.1	12.4 ± 0.4	0.8 ± 0	161 ± 8	1142 ± 49	762 ± 136	BC	5.4 ± 0.2	^{ab} 12.9 ± 0.6	0.8 ± 0	163 ± 10	1286 ± 224	762 ± 180
BC ^{plus}	5.4 ± 0.2	12.7 ± 0.9	0.8 ± 0.1	168 ± 8	1194 ± 162	854 ± 63	BC ^{plus}	5.2 ± 0.1	^b 12.3 ± 0.4	0.8 ± 0	164 ± 15	1100 ± 242	726 ± 180
TSP	5.4 ± 0.2	12.1 ± 0.5	0.8 ± 0	161 ± 7	1409 ± 259	820 ± 217	TSP	5.4 ± 0.1	^b 12.8 ± 0.1	0.8 ± 0	168 ± 7	1445 ± 198	843 ± 65
2015													
Control	5.1 ± 0.3	12.5 ± 0.6	0.8 ± 0.1	160 ± 5	^a 1099 ± 216	^a 684 ± 39	Control	5.1 ± 0.1	13.2 ± 0.8	0.8 ± 0	^a 149 ± 16	1271 ± 5	718 ± 45
BC	5.1 ± 0.1	12.7 ± 0.5	0.8 ± 0	190 ± 7	^{ab} 1242 ± 103	^b 863 ± 38	BC	5.1 ± 0	13 ± 1	0.9 ± 0	^{ab} 195 ± 30	1247 ± 38	874 ± 64
BC ^{plus}	5.1 ± 0	13.2 ± 0.8	0.9 ± 0	197 ± 13	^a 1204 ± 58	^{ab} 842 ± 30	BC ^{plus}	5.1 ± 0.1	12.6 ± 1.1	0.9 ± 0	^{ab} 192 ± 10	1380 ± 152	807 ± 136
TSP	5.2 ± 0.2	12.6 ± 0.5	0.8 ± 0	190 ± 16	^b 1549 ± 381	^b 884 ± 34	TSP	5.2 ± 0.1	13.6 ± 0.9	0.9 ± 0	^b 202 ± 16	1427 ± 195	879 ± 74
2016													
Control	5.2 ± 0.3	12.6 ± 0.6	0.8 ± 0	^a 166 ± 12	1231 ± 284	777 ± 124	Control	5.2 ± 0.2	12.9 ± 1.1	0.8 ± 0.1	^a 167 ± 14	1138 ± 193	^a 690 ± 26
BC	5.3 ± 0.2	12.2 ± 0.3	0.8 ± 0	^b 201 ± 16	1412 ± 117	674 ± 41	BC	5.3 ± 0	12.3 ± 0.2	0.8 ± 0	^{ab} 202 ± 9	1775 ± 466	^b 880 ± 29
BC ^{plus}	5.3 ± 0	12.3 ± 0.9	0.8 ± 0.1	^b 203 ± 7	1319 ± 307	905 ± 42	BC ^{plus}	5.2 ± 0.2	12.1 ± 0.1	0.8 ± 0	^b 210 ± 4	1263 ± 196	^b 863 ± 50
TSP	5.4 ± 0	12.5 ± 0.6	0.8 ± 0	^b 198 ± 4	1751 ± 800	907 ± 91	TSP	5.3 ± 0.1	13.3 ± 0.3	0.8 ± 0	^b 214 ± 17	1940 ± 463	^b 943 ± 45
2017													
Control	5.9 ± 0.4	12.7 ± 0.4	0.8 ± 0	166 ± 5	1269 ± 218	^a 699 ± 15	Control	5.6 ± 0.2	13.7 ± 0.5	0.9 ± 0	172 ± 3	1436 ± 201	758 ± 60
BC	5.9 ± 0.4	13.3 ± 0.9	0.9 ± 0.1	174 ± 19	1484 ± 369	^b 949 ± 87	BC	6 ± 0.1	13.7 ± 0.5	0.9 ± 0	181 ± 24	1534 ± 187	854 ± 83
BC ^{plus}	5.9 ± 0.1	13.3 ± 1.1	0.9 ± 0.1	205 ± 29	1687 ± 457	^{ab} 1011 ± 138	BC ^{plus}	5.7 ± 0.3	13.1 ± 0.5	0.9 ± 0	181 ± 30	1566 ± 277	852 ± 26
TSP	6 ± 0.2	13.1 ± 1.2	0.9 ± 0.1	174 ± 22	1573 ± 268	^b 921 ± 13	TSP	5.8 ± 0.1	13.9 ± 0.4	0.9 ± 0	184 ± 24	1519 ± 141	875 ± 74
2018													
Control	5.9 ± 0.3	^a 13.1 ± 0.9	^a 0.8 ± 0	^a 162 ± 4	1416 ± 306	^a 744 ± 74	Control	5.9 ± 0.2	14 ± 0.3	0.9 ± 0	167 ± 4	1512 ± 82	761 ± 57
BC	6 ± 0.1	^b 14.1 ± 0.5	^b 0.9 ± 0	^b 181 ± 11	1655 ± 236	^{ab} 865 ± 56	BC	6.1 ± 0.2	14.3 ± 1.1	0.9 ± 0.1	180 ± 14	1834 ± 195	834 ± 38
BC ^{plus}	6 ± 0.3	^{ab} 13.5 ± 0.8	^c 0.9 ± 0	^b 194 ± 0	1667 ± 290	^b 885 ± 60	BC ^{plus}	5.8 ± 0.4	14 ± 0.9	0.9 ± 0.1	203 ± 32	1581 ± 173	865 ± 27
TSP	6 ± 0.3	^{bc} 13.5 ± 0.8	^{bc} 0.9 ± 0.1	^{ab} 176 ± 21	1408 ± 101	^{ab} 900 ± 22	TSP	6.1 ± 0.2	14.8 ± 1.1	0.9 ± 0	182 ± 11	1689 ± 363	961 ± 108
Year	< 0.01	0.02	< 0.01	< 0.01	0.011	0.047	Year	< 0.01	< 0.01	< 0.01	0.003	0.001	0.091
Treatment	0.94	0.23	0.063	0.035	0.11	0.021	Treatment	0.306	0.075	0.654	0.012	0.615	0.052

Table 1 (continued)

iSPTC-A	pH _(CaCl2)	Total C g kg ⁻¹	Total N	Total S mg kg ⁻¹	Total Ca	Total Mg	iSPTC-C	pH _(CaCl2)	Total C g kg ⁻¹	Total N	Total S mg kg ⁻¹	Total Ca	Total Mg
Year x treat- ment	0.96	0.42	0.06	0.512	0.421	0.126	Year x treat- ment	0.188	0.611	0.289	0.358	0.043	0.698

Values were mean ± standard deviation (n=3). Note: To account for plot heterogeneity in the chemical properties between treatments, all statistical analyses of time series data were done after normalization of the data from 2014 to 2018 with the respective values from 2013 (i.e., subtracting for each plot and year the respective value of 2013). ^{a,b,c} Significant differences between treatments ($p < 0.05$) within the same year are labeled with different lower case letters. Bold p -values highlight significant differences ($p < 0.05$) as estimated with ANOVA for the factor Year, Treatment, and Year x Treatment

fractions was also unaffected by iSPTCs, sampling date, and treatment and followed the order: $H_2O-P_o < H_2O-P_i < NaHCO_3-P_o < NaHCO_3-P_i \leq \text{Residual-P} < H_2SO_4-P < NaOH-P_o < NaOH-P_i$. Sequential P fractions were mostly unaffected by the year and/or treatment; only TSP addition increased either P concentrations or proportions or both in a few cases from which, however, no trend could be derived. During the experimental duration, concentration and proportions of $NaHCO_3-P_o$ decreased in both iSPTCs, being most pronounced in iSPTC-C and here, especially for the TSP treatment.

The P concentrations can be further differentiated into three P pools of different availability, i.e., labile P (sum of H_2O-P_{i+o} and $NaHCO_3-P_{i+o}$), moderately labile P ($NaOH-P_{i+o}$), and stable P (H_2SO_4-P and residual-P) (Fig. 1). Total P concentration in the pools generally followed the order of labile P < stable P < moderately labile P for all sampling times and for both iSPTCs. With increasing experimental duration, there was a trend of decreasing labile P concentrations visible for the control, BC, and BC^{plus} treatments, being more pronounced in the iSPTC-C soil (Fig. 1). Generally, the treatment effects were small; only TSP significantly increased labile P concentration compared with BC (2016), BC, and BC^{plus} (2017), or control and BC (2018) for iSPTC-A. The other pools were not affected by treatments except for a significant increase in stable P in 2018 after BC application compared to the control for iSPTC-A (Fig. 1).

Discussion

BC and BC^{plus} application had no effect on pH and main nutrient elements

The observed changes in basic soil properties over the experimental duration were independent from fertilizer treatments and iSPTCs, and thus did not reflect fertilizer effects but rather changes in overall field management. For instance, the observed increase in N_i in all treatments in 2018, despite omitted N fertilization, is explained by the cultivation of *Lupinus angustifolius* L., which is known to fix atmospheric N_2 (e.g., Pueyo et al., 2021). Also liming resulted in a significant increase in pH and also Ca_i concentrations in all treatments, which masked a possible

Table 2 P budgets and calculated difference of total P stocks in the topsoil before (2013) and after the one crop rotation cycle (2018)

	iSPTC-A			iSPTC-C		
	P budget ¹	² Difference P stocks 2018–2013 (kg ha ⁻¹)	³ Recovery calculated stock (%)	P budget	² Difference P stocks 2018–2013 (kg ha ⁻¹)	³ Recovery calculated stock (%)
Control	– 68	– 175 ± 54	93 ± 4	– 85	– 225 ± 191	94 ± 8
BC	151	57 ± 68	94 ± 4	138	– 236 ± 168	85 ± 6
BC ^{plus}	150	– 46 ± 176	89 ± 9	140	– 318 ± 251	82 ± 10
TSP	143	151 ± 137	101 ± 8	137	– 80 ± 134	91 ± 5

¹All P budgets were obtained from (Panten and Leinweber, 2020)

²Differences of total P stocks in the topsoil (30 cm) between the sampling year 2013 and 2018

³Proportion (%) of measured total P stocks in 2018 in relation to calculated total P stocks in 2018 (P stocks 2013 + P budget)

pH increase due to BC or BC^{plus} addition as often described in the literature (Morshedizad et al., 2016; Siebers and Leinweber, 2013). Furthermore, despite the high S_t contents of BC^{plus}, 270 g kg⁻¹ (Zimmer et al., 2018), led to the highest proportional increase in soil S_t among all treatments, an even more pronounced effect of BC^{plus} on soil S_t concentrations and S fertilization of plants was masked by the S fertilization all treatments annually received. However, although this led to an underestimation of the positive effects and co-benefits of BC and especially BC^{plus} on soil and yields, both liming and additional S fertilization were necessary as pH and S_t were below recommendation. Without this, the effects of P fertilization were not differentiable from pH and S effects.

Treatment induced changes in P_t stocks were masked in the standard deviations of P_t stock

As indicated by the positive P budget of all fertilized treatments, P fertilization in both iSPTCs was above the plant requirements, which however was not reflected in the corresponding increase in the soil's P_t stocks. Such underestimation of topsoils P stocks — when considering the individual P budgets (Table 2) — were also reported in other studies based on long-term P fertilization trials (Siebers et al., 2021; Zimmer et al., 2018). For example, Siebers et al. (2021) reported that, despite positive P budgets, most of the P treatments of four different long-term P fertilization trials showed negative P balances for the topsoil (30 cm) in the range between – 17 to – 46%. Siebers et al. interpreted the fact that for some sites and treatments, the gradual inclusion of even deeper soil

layers in the calculation improved the P balance as an indication of the site- and soil-specific leaching of excess fertilizer P from the topsoil to the subsoil. Due to the higher P_t concentration in iSPTC-C, it is likely, that the P sorption capacity of the topsoil in iSPTC-C is more exhausted than in iSPTC-A and thus a higher risk of leaching exists. However, the present soil texture (36% sand, 57% silt, 7% clay; (Panten and Leinweber, 2020)) and the fact that the treatments with the highly soluble TSP even showed the lowest gap in the balance suggest that P transport via leaching plays only a minor role. Translocation of fertilizer P by plant roots or root material below 30 cm depth is also a known factor responsible for gaps in P budgets (e.g., Bauke et al., 2017). It is reasonable that such mechanisms at least partly explain the results from the recent study as crops grew within the mean rooting depth of > 30 cm. However, when taking the relatively large standard deviations of the P_t stocks in 2013 and 2018 (~8% iSPTC-A; 5%; iSPTC-C) into account, likely, the proportion of the calculated accumulated P budgets on the overall P_t stocks (iSPTC-A: 9%, iSPTC-C: 6%) was still too small. Hence, at this stage of the field experiment, treatment-induced changes in the P_t stocks are probably still masked in the standard deviations of P_t stock.

Land-use change and available legacy P control amount and mineralization of P_o

It is known that various fractions of soil P respond more and faster to contrasting field management and P fertilization treatments than P_t concentrations or P_t stocks in the soil. For instance, short-term effects

Table 3 Total phosphorus (P_t), plant available calcium acetate extractable-P (P_{CAL}), water soluble P (P_{water}), and sequentially extracted Hedley-P fractions as affected by treatments (Control, TSP, BC, and BC^{plus}) and time (2013 to 2018) for initial soil P-test class A (ISPTC-A) and C (ISPTC-C)

ISPTC-A	P _t	P _{cal}	P _{water}	P _{DGT}	Total extractable		H ₂ O-P		NaHCO ₃ -P		NaOH-P		H ₂ SO ₄ -P		Residual-P	
					P _i	P _o	P _i /P _o	P _i	P _o	P _i	P _o	P _i	P _o			
														mg kg ⁻¹ (%)		
2013																
Control	374±36	19±4 (5)	3±1 (1)	3±1 (1)	191±31 (51)	146±4 (39)	1.3±0.2	5±1 (1)	2±0 (0)	29±5 (8)	26±3 (7)	87±15 (23)	119±4 (32)	70±10 (19)	37±1 (10)	
BC	331±9	13±1 (4)	2±0 (1)	4±2 (1)	157±4 (47)	142±1 (43)	1.1±0	4±1 (0)	2±0 (1)	22±2 (7)	24±1 (7)	70±5 (21)	116±1 (35)	62±4 (19)	32±6 (10)	
BC ^{plus}	368±46	18±5 (5)	3±1 (1)	5±1 (1)	194±26 (53)	141±12 (38)	1.4±0.1	5±1 (1)	3±1 (1)	27±8 (7)	25±4 (7)	88±13 (24)	114±11 (31)	74±3 (20)	34±7 (9)	
TSP	326±12	12±1 (4)	2±0 (1)	5±2 (1)	160±5 (49)	137±9 (42)	1.2±0.1	4±1 (0)	3±0 (1)	20±2 (6)	23±1 (7)	70±5 (22)	111±9 (34)	66±5 (20)	28±6 (9)	
2014																
Control	367±60	15±5 (4)	2±1 (1)	3±2 (1)	189±38 (51)	145±18 (40)	1.3±0.1	4±1 (2)	2±0 (1)	31±10 (8)	21±4 (6)	85±19 (23)	122±16 (33)	69±11 (19)	33±6 (9)	
BC	351±5	12±1 (4)	2±0 (1)	1±0 (0)	178±2 (51)	141±5 (40)	1.3±0.1	3±1 (1)	2±0 (1)	24±1 (7)	22±1 (6)	77±3 (22)	117±6 (33)	74±0 (21)	31±3 (9)	
BC ^{plus}	412±29	18±7 (4)	3±1 (1)	2±1 (1)	206±59 (50)	175±42 (43)	1.3±0.6	4±1 (2)	3±1 (1)	36±16 (9)	18±2 (4)	95±21 (23)	154±41 (38)	71±21 (17)	32±2 (8)	
TSP	350±19	13±1 (4)	2±0 (1)	2±0 (1)	179±15 (51)	140±5 (40)	1.3±0.1	5±1 (0)	1±0 (0)	26±7 (8)	19±3 (5)	81±5 (23)	120±6 (34)	66±9 (19)	32±2 (9)	
2015																
Control	365±62	16±5 (4)	^a 2±0 (1)	2±0 (1)	179±42 (49)	149±24 (41)	1.2±0.3	3±1 (1)	3±0 (1)	30±9 (8)	^a 22±1 (6)	86±17 (24)	124±23 (34)	59±19 (16)	37±5 (10)	
BC	373±71	13±0 (4)	^{ab} 2±0 (1)	4±2 (1)	170±16 (46)	176±51 (47)	1±0.2	1±0 (0)	5±1 (1)	25±3 (7)	^{ab} 21±2 (6)	81±8 (22)	151±53 (39)	63±8 (17)	26±5 (7)	
BC ^{plus}	426±39	18±7 (4)	^a 2±1 (1)	2±1 (1)	210±44 (49)	187±48 (44)	1.2±0.4	2±0 (1)	4±0 (1)	34±11 (8)	^{ab} 24±5 (6)	100±26 (23)	158±49 (37)	74±10 (17)	30±5 (7)	
TSP	377±52	15±3 (4)	^b 2±0 (1)	3±2 (1)	191±31 (51)	158±32 (42)	1.2±0.3	2±0 (1)	3±0 (1)	27±8 (7)	^b 23±1 (6)	89±14 (24)	132±33 (35)	71±12 (19)	29±4 (8)	
2016																
Control	375±28	17±6 (4)	3±1 (1)	3±2 (1)	200±42 (53)	134±25 (36)	1.6±0.6	4±1 (2)	2±0 (1)	28±16 (7)	21±3 (6)	107±11 (29)	111±26 (30)	61±18 (16)	41±9 (11)	
BC	325±12	13±1 (4)	2±0 (1)	2±0 (1)	167±8 (51)	131±4 (40)	1.3±0.1	2±1 (0)	1±0 (0)	17±3 (5)	18±1 (6)	80±3 (25)	111±3 (34)	67±13 (21)	28±6 (9)	

Table 3 (continued)

iSPTC-A	P _t	P _{cal}	P _{water}	P _{DGT}	Total extractable			H ₂ O-P		NaHCO ₃ -P		NaOH-P		H ₂ SO ₄ -P		Residual-P
					P _i	P _o	P _i /P _o	P _i	P _o	P _i	P _o	P _i	P _o	P _i	P _o	
mg kg ⁻¹ (%)																
BC ^{plus}	361±61	18±8 (5)	3±1 (1)	4±2 (1)	198±56 (54)	132±6 (37)	1.5±0.4	3±1 (3)	2±0 (1)	25±12 (7)	19±2 (5)	104±27 (29)	111±7 (31)	65±13 (18)	31±3 (9)	
	TSP	359±21 (5)	3±0 (1)	4±2 (1)	192±15 (53)	135±6 (38)	1.4±0.1	4±1 (1)	4±1 (1)	26±3 (7)	19±1 (5)	96±3 (27)	113±7 (32)	68±14 (19)	31±3 (9)	
2017																
Control	361±40	^a 19±8 (5)	3±2 (1)	3±0 (1)	188±32 (52)	134±8 (38)	1.4±0.2	4±1 (2)	2±0 (1)	31±11 (9)	19±2 (5)	94±20 (26)	114±8 (32)	60±1 (17)	38±7 (10)	
BC	334±8	^{ab} 14±1 (4)	2±0 (1)	2±0 (1)	168±9 (50)	133±6 (40)	1.3±0.1	2±1 (0)	4±0 (1)	17±2 (5)	19±3 (6)	81±2 (24)	110±4 (33)	67±6 (20)	32±3 (10)	
BC ^{plus}	364±64	^{ab} 23±9 (6)	3±1 (1)	6±3 (1)	200±21 (55)	132±41 (36)	1.6±0.4	4±1 (2)	2±0 (1)	28±11 (7)	18±5 (5)	90±16 (25)	112±37 (30)	78±6 (22)	32±3 (9)	
TSP	444±81	^b 26±4 (6)	4±0 (1)	3±0 (1)	248±52 (56)	162±30 (37)	1.5±0.2	5±1 (0)	2±0 (1)	35±6 (8)	19±3 (4)	116±20 (26)	141±29 (32)	92±29 (20)	34±6 (8)	
2018																
Control	^a 336±44	^a 15±5 (4)	^a 2±1 (1)	2±1 (1)	174±24 (52)	130±18 (39)	1.3±0.1	^a 3±1 (1)	4±0 (1)	^a 24±5 (7)	20±2 (6)	86±10 (26)	106±15 (32)	61±10 (18)	32±5 (9)	
BC	^{ab} 344±7	^b 13±1 (4)	^{ab} 2±1 (1)	2±1 (1)	173±3 (51)	133±8 (39)	1.3±0.1	^a 2±1 (0)	3±1 (1)	^a 20±3 (6)	20±2 (6)	77±11 (23)	110±4 (32)	74±7 (22)	37±2 (11)	
BC ^{plus}	^{ab} 358±50	^{abc} 20±8 (5)	^{ab} 3±2 (1)	3±1 (1)	193±48 (53)	132±2 (37)	1.5±0.4	^a 4±1 (2)	2±0 (0)	^{ab} 30±15 (8)	18±2 (5)	91±28 (25)	112±1 (32)	69±4 (19)	34±2 (10)	
TSP	^b 359±25	^c 23±2 (6)	^b 4±1 (1)	3±1 (1)	201±12 (56)	126±14 (35)	1.6±0.1	^b 6±2 (0)	2±0 (0)	^b 33±6 (9)	17±2 (5)	96±5 (27)	107±13 (30)	66±16 (18)	32±2 (9)	
Year	0.05	<0.001	0.007	0.175	0.226	0.011	0.239	< 0.001	0.1	0.036	0.02	0.041	0.027	0.196	0.36	
Treat- ment	0.115	0.028	0.015	0.883	0.105	0.35	0.331	0.267	0.38	0.156	0.348	0.137	0.357	0.119	0.762	
Year x treatment	0.039	< 0.001	0.012	0.081	0.026	0.756	0.087	0.193	0.338	0.062	0.612	0.09	0.646	0.131	0.247	

Table 3 (continued)

iSPTC-C	P _t	P _{cal}	P _{water}	P _{DGT}	Total Hedley extractable			H ₂ O-P		NaHCO ₃ -P		NaOH-P		H ₂ SO ₄ -P		Residual-P
					P _i	P _o	P _i /P _o	P _i	P _o	P _i	P _o	P _i	P _o	P _i	P _o	
mg kg ⁻¹ (%)																
2013																
Control	519±18	51±4 (10)	10±1 (2)	11±3 (2)	318±8 (61)	165±8 (32)	1.9±0	16±3 (2)	2±0 (0)	71±3 (14)	32±3 (6)	153±4 (30)	131±7 (25)	77±10 (15)	36±2 (7)	
BC	514±17	51±4 (10)	10±1 (2)	8±1 (2)	308±14 (60)	171±11 (33)	1.8±0.2	16±3 (0)	4±1 (1)	62±6 (12)	38±8 (7)	155±5 (30)	129±6 (25)	76±14 (15)	36±2 (7)	
BC ^{plus}	525±9	48±3 (9)	9±1 (2)	11±3 (2)	310±23 (59)	177±15 (34)	1.8±0.3	16±3 (2)	2±0 (0)	57±27 (11)	43±19 (8)	149±4 (28)	133±4 (25)	87±8 (17)	37±1 (7)	
TSP	520±12	48±4 (9)	9±1 (2)	8±1 (2)	299±20 (58)	183±10 (35)	1.6±0.2	15±3 (0)	3±1 (1)	48±18 (9)	49±15 (9)	150±6 (29)	130±4 (25)	86±14 (17)	37±1 (7)	
2014																
Control	511±21	38±5 (^{ab} 7)	7±0 (1)	8±2 (2)	309±20 (60)	164±5 (32)	1.9±0.1	11±2 (1)	5±0 (1)	62±7 (12)	30±3 (6)	153±13 (30)	129±4 (25)	83±6 (16)	^a 38±2 (7)	
BC	473±51	35±3 (^a 8)	7±1 (1)	6±1 (1)	314±6 (67)	126±51 (26)	2.9±1.6	14±3 (2)	2±0 (0)	64±5 (14)	25±4 (5)	145±5 (31)	98±55 (20)	92±4 (19)	^{ab} 34±3 (7)	
BC ^{plus}	445±26	33±5 (^{ab} 8)	6±1 (1)	5±2 (1)	280±25 (63)	136±6 (31)	2.1±0.2	9±2 (3)	1±1 (0)	56±10 (13)	27±1 (6)	133±8 (30)	108±7 (24)	82±16 (18)	^b 29±3 (7)	
TSP	485±29	36±3 (^b 7)	7±1 (1)	6±1 (1)	295±24 (61)	158±8 (33)	1.9±0.1	12±2 (4)	3±0 (1)	55±13 (11)	35±7 (7)	141±9 (29)	120±10 (25)	86±7 (18)	^{ab} 32±2 (7)	
2015																
Control	498±16	^a 36±4 (^{ab} 7)	5±0 (1)	4±0 (1)	278±25 (^a 56)	^a 182±18 (^a 37)	^a 1.5±0.3	9±2 (0)	4±0 (1)	61±6 (12)	25±1 (5)	137±11 (^a 28)	153±20 (31)	71±8 (14)	37±1 (7)	
BC	453±36	^{ab} 34±4 (^a 8)	5±1 (1)	3±0 (1)	297±7 (^{ab} 66)	^{ab} 123±41 (^{ab} 27)	^{ab} 2.6±0.8	7±2 (1)	4±1 (1)	59±6 (13)	21±4 (5)	143±11 (^{ab} 32)	98±44 (21)	88±5 (20)	33±3 (7)	
BC ^{plus}	463±47	^{ab} 33±3 (^{ab} 7)	4±0 (1)	4±2 (1)	269±13 (^a 58)	^a 162±37 (^a 35)	^a 1.7±0.3	7±1 (0)	4±0 (1)	57±5 (12)	25±4 (5)	131±5 (^{ab} 28)	132±36 (28)	75±11 (16)	33±4 (7)	
TSP	430±24	^b 39±3 (^b 9)	7±0 (2)	4±2 (1)	306±19 (^b 71)	^b 92±24 (^b 21)	^b 3.5±0.9	10±2 (0)	2±0 (0)	60±11 (14)	25±3 (6)	154±9 (^b 36)	65±21 (15)	83±2 (19)	32±3 (7)	
2016																
Control	^a 462±11	^a 33±5 (7)	5±1 (1)	5±1 (1)	267±27 (58)	161±34 (35)	1.7±0.6	^{ab} 8±2 (^{ab} 2)	3±0 (1)	57±5 (12)	24±2 (5)	134±21 (29)	134±37 (29)	67±1 (15)	35±5 (8)	
BC	^{ab} 428±26	^a 32±4 (7)	5±1 (1)	6±1 (1)	266±31 (62)	133±8 (31)	2±0.3	^a 7±2 (^a 1)	5±0 (1)	45±7 (10)	18±3 (4)	138±19 (32)	110±4 (26)	77±6 (18)	29±3 (7)	

Table 3 (continued)

iSPTC-C	P _t	P _{cal}	P _{water}	P _{DGT}	Total Hedley extractable			H ₂ O-P		NaHCO ₃ -P		NaOH-P		H ₂ SO ₄ -P		Residual-P
					P _i	P _o	P _i /P _o	P _i	P _o	P _i	P _o	P _i	P _o	P _i	P _o	
BC ^{plus}	^b 429±2	^{ab} 32±3 (7)	5±1 (1)	5±1 (1)	258±15 (60)	143±14 (33)	1.8±0.3	^a 8±2 (^{ab} 1)	3±0 (1)	45±4 (10)	21±4 (5)	128±16 (30)	120±15 (28)	76±7 (18)	28±4 (6)	
TSP	^{ab} 477±31	^b 41±6 (8)	7±1 (1)	7±2 (1)	308±22 (64)	138±7 (29)	2.2±0.1	^b 11±2 (^b 1)	2±0 (0)	56±4 (11)	19±3 (4)	157±16 (33)	117±3 (25)	85±4 (18)	32±4 (7)	
2017																
Control	448±47	41±8 (9)	^a 6±2 (1)	6±1 (1)	269±40 (60)	141±7 (32)	1.9±0.2	^a 6±1 (^a 2)	1±0 (0)	57±12 (13)	21±1 (5)	143±22 (32)	119±8 (^a 27)	63±9 (14)	37±2 (8)	
BC	473±20	44±6 (9)	^{ab} 8±1 (2)	6±0 (1)	298±14 (63)	144±20 (30)	2.1±0.3	^{ab} 11±2 (^{ab} 0)	4±0 (1)	53±4 (11)	17±1 (4)	149±14 (32)	123±19 (^{ab} 26)	85±5 (18)	32±6 (7)	
BC ^{plus}	441±35	43±15 (10)	^{ab} 7±1 (1)	6±1 (1)	268±40 (61)	140±9 (32)	1.9±0.4	^a 8±2 (^{ab} 2)	3±1 (1)	45±16 (10)	21±7 (5)	141±22 (32)	117±11 (^{ab} 27)	73±9 (17)	32±3 (7)	
TSP	502±10	53±2 (11)	^b 10±1 (2)	8±0 (2)	328±6 (65)	140±3 (28)	2.3±0.1	^b 14±3 (^b 1)	5±0 (1)	61±2 (12)	18±1 (4)	167±3 (33)	117±5 (^b 23)	86±1 (17)	34±6 (7)	
2018																
Control	470±24	^a 38±4 (^a 8)	6±1 (1)	5±0 (1)	287±32 (61)	147±14 (31)	2±0.4	11±2 (1)	4±0 (1)	51±10 (11)	27±10 (6)	144±12 (31)	116±4 (25)	81±13 (17)	36±4 (8)	
BC	463±30	^a 39±5 (^{ab} 8)	7±1 (1)	6±1 (1)	290±29 (63)	136±4 (30)	2.1±0.2	9±2 (3)	2±0 (0)	54±7 (12)	19±6 (4)	131±8 (28)	116±2 (25)	96±18 (21)	37±6 (8)	
BC ^{plus}	456±61	^{ab} 40±6 (^{ab} 9)	6±1 (1)	6±1 (1)	285±63 (62)	138±3 (31)	2.1±0.5	10±2 (2)	3±1 (1)	55±11 (12)	19±5 (4)	133±23 (29)	115±5 (26)	87±32 (19)	33±2 (7)	
TSP	503±30	^b 55±9 (^b 11)	10±3 (2)	7±0 (1)	327±15 (65)	138±30 (27)	2.5±0.6	14±3 (2)	5±0 (1)	64±7 (13)	17±3 (3)	158±15 (32)	116±25 (23)	91±0 (18)	37±7 (7)	
Year	0.129	<0.01	<0.01	0.003	0.072	0.902	0.481	0.002	0.792	0.029	<0.01	0.106	0.824	0.017	0.074	
Treat- ment	0.309	0.71	0.078	0.28	0.238	0.004	0.025	0.145	0.842	0.348	0.409	0.183	0.149	0.324	0.073	
Year x treatment	0.071	0.216	0.190	0.155	0.284	0.081	0.08	0.028	0.112	0.228	0.489	0.182	0.11	0.929	0.646	

Values were mean ± standard deviation (n=3). Values in brackets were proportions of each P fraction to total P. Note: To account for plot heterogeneity in the chemical properties between treatments, all statistical analyses of time series data were done after normalization of the data from 2014 to 2018 with the respective values from 2013 (i.e., subtracting for each plot and year the respective value of 2013). ^{a,b,c} Significant differences between treatments ($p < 0.05$) within the same year are labeled with different lower case letters. Bold p -values highlight significant differences ($p < 0.05$) as estimated with ANOVA for the factor Year, Treatment and Year x Treatment

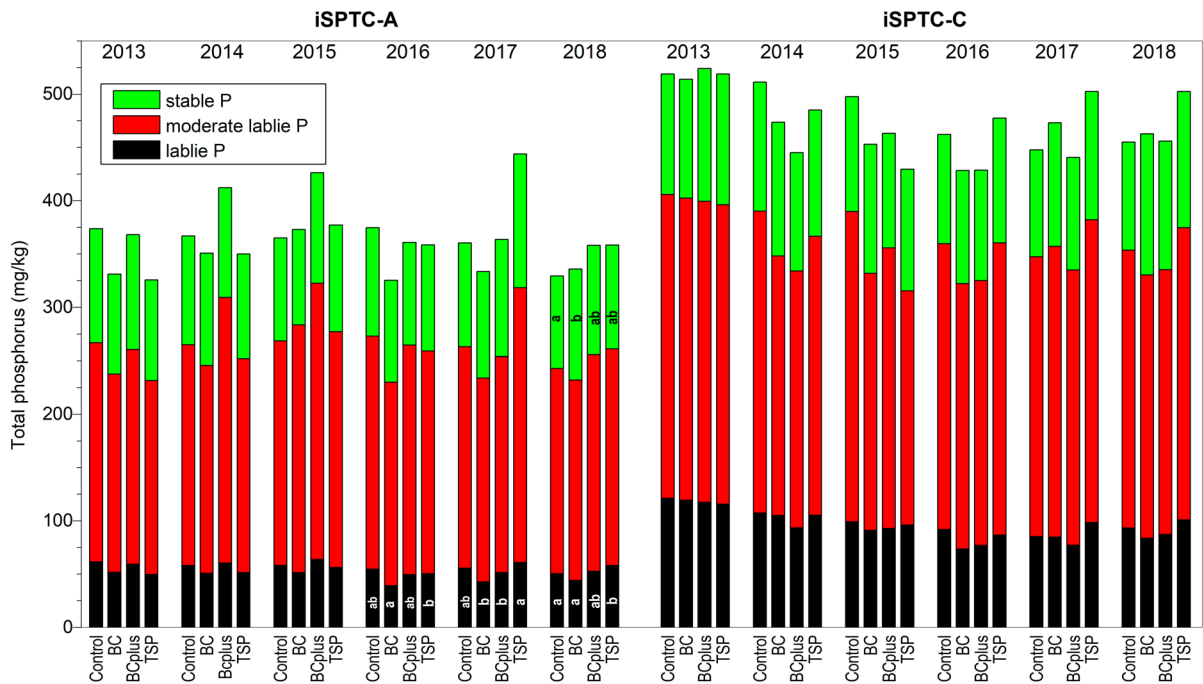


Fig. 1 Development of the mean ($n=3$) concentrations of labile phosphorus (P) (resin $P_{i+o} + NaHCO_3-P_{i,o}$), moderate labile P ($NaOH-P_{i,o}$), and stable P ($H_2SO_4-P + \text{residual P}$) after annual (2013 to 2018) P fertilization (control = NoP, BC = bone char, and BC^{plus} = sulfur modified bone char) in two soils

only differing in their initial soil P-test class (iSPTC). iSPTC-A = severely deficient, $< 15 \text{ mg calcium acetate lactate extractable P (P}_{\text{CAL}} \text{ kg}^{-1})$, iSPTC-C (sufficient, $31\text{--}60 \text{ mg P}_{\text{CAL}} \text{ kg}^{-1}$). Significant differences between treatments within the same year ($p < 0.05$) are labeled with different lower case letters

of the conversion from grassland to arable are usually reflected in a temporary boost in the activity or amount of microbial biomass due to increased soil aeration, the incorporation of fresh organic material into the soil, and break-down of soil aggregates and the associated the release of occluded C and also other nutrients (Kalhor et al., 2017; Siebers and Kruse, 2019). This may lead to priming effects with increased soil organic matter mineralization (Kuzakov et al., 2000), which most likely explains the temporally increased contents of labile and moderately labile P_i and P_o at the beginning of the 2013 experiment.

The observed lower $NaOH-P_o$ and $NaHCO_3-P_o$ concentrations for iSPTC-A in 2013 (Table 3) indicate that these effects can be expected to be less pronounced for iSPTC-A than for iSPTC-C because of the smaller amount of accumulated incorporated organic matter – as indicated by the significantly lower C_t concentration – and a general lower microbial activity/biomass in this P deficit soil. The

successive mineralization of these P_o compounds with increasing experimental duration lead to the observed subsequent trend of decreasing of $NaOH-P_o$ and especially $NaHCO_3-P_o$ in both soils, which is less stable and therefore more easily mineralized than $NaOH-P_o$ (Cross and Schlesinger, 1995; Turner et al., 2005). The fact that from 2016 the concentrations of $NaHCO_3-P_o$ (to some extent also $NaOH-P_o$) of both iSPTCs converged and stabilized in the following years at a similar level suggests that a new equilibrium for P_o has been established in the top-soil that is independent of the iSPTCs. This indicates that the effect of land-use change from grassland to arable on P_o concentrations mainly diminished after around three years. However, $NaHCO_3-P_o$ concentrations of the two iSPTCs leveled off at the same time despite $\sim 70\%$ higher mean concentrations of $NaHCO_3-P_o$ for iSPTC-C compared to iSPTC-A at the start of the experiment. This suggests that microbial activity and thereby the rate of P_o mineralization was to some extent controlled by the amount of

available P, e.g., P_{cal} , which was around twofold higher for iSPTC-C compared to iSPTC-A (Table 3). However, data indicate that P treatment had no further effect on P_0 mineralization as treatment-induced changes in available P were still too small within the experimental duration. The observed slight trends of decreasing plant-available P, i.e., P_{cal} , labile P ($\text{H}_2\text{O-P} + \text{NaHCO}_3\text{-P}$), P_{water} , and P_{DGT} in iSPTC-C followed in varying degree the above-mentioned trend of labile P_0 in the first two to three years of the field experiment as this fraction of P_0 is partly included in the applied plant-available P tests. Therefore, the larger loss in $\text{NaHCO}_3\text{-P}_0$ concentration in iSPTC-C explains the observed higher losses in P_{cal} , labile P, P_{water} , and P_{DGT} in the iSPTC-C compared to A.

BC and BC^{plus} could not compensate for the demand of the plants for labile P

A closer look at the composition of labile P indicates stagnating or a tendency towards decreasing concentrations of $\text{NaHCO}_3\text{-P}_i$ in the control and both BC treatments in the first three years of the field experiment. This suggests that the triple application of both BC fertilizers did not affect plant-available P pools in the soil of both iSPTCs. Thus, the higher demand for labile P of crops compared to grassland was mostly compensated by soil legacy P. In contrast, the observed slight increasing trend in the TSP treatments indicated a starting accumulation of excess fertilizer P_i in that fraction, which were also reflected in the P_{cal} concentrations. This agrees with the higher bio-availability of TSP compared to BC-based fertilizers, as already described in the literature (e.g., Leinweber et al., 2018; Siebers et al., 2014; Zimmer et al., 2019). However, in 2017 these trends were interrupted briefly after the cultivation of lupine, which raised the available P concentrations (e.g., P_{cal} , P_{water} , and $\text{NaHCO}_3\text{-P}_i$) in almost all treatments due to its known ability to increase the availability of soil P (Alamgir et al., 2012). The again decreased concentrations of available P in the control and the BC-based treatments in 2018 indicated that this pool of mobilized P was almost depleted in the subsequent year.

After the first crop rotation with five annual P applications, the effects on soil P fractions were still small and only the TSP treatments showed significantly higher available P concentrations compared to the unfertilized control. However, only for iSPTC-A,

this led to higher cumulative yields (control 90%, BC 94%; BC^{plus} 95%; TSP 100%) whereas if initially sufficient available soil P was available (iSPTC-C), no yield effects were observed (Panten and Leinweber, 2020). The approximately 10% reduced relative cumulative yields in the control treatment of iSPTC-A implies that already after five years of cultivation, the demand of plant-available P in the test plots cannot be provided by soil legacy P. For iSPTC-C the pool was still sufficient to maintain even similar yields as those obtained for the TSP treatment. This suggests a general lower contribution of fertilizer P on plant nutrition for iSPTC-C than iSPTC-A as reflected in the higher apparent nutrient recovery in grains of all iSPTC-A treatments (Panten and Leinweber, 2020). This higher P use efficiency in iSPTC-A also reflects, to some extent, the higher P solubilization potential of the microbial community in iSPTC-A soils, which is better adapted to low levels of available P compared to iSPTC-C.

The field trial did not confirm the former laboratory results on the benefits of in situ digestion of BC^{plus}

The fact that despite P application both BC-based treatments showed a similar decreasing trend in available soil P suggests that most of the available fertilizer P was directly taken up by the plants and did not accumulate in the soil. Since BC and BC^{plus} mainly consist of insoluble apatite (Dela Piccola et al., 2021; Zimmer et al., 2018), solubilization of P was expected to be slow. However, the expected corresponding accumulation of excessive BC or BC^{plus} in the stable P pool ($\text{H}_2\text{SO}_4\text{-P} + \text{residual P}$) was not reflected in the data, most likely due to the still small proportional change.

It was surprising that no difference in P availability between BC and BC^{plus} treatments was observed, since the previous batch and pot experiments proved higher solubility of BC^{plus} due to the modification of its surface with elemental S (Morshedizad et al., 2018, 2016; Zimmer et al., 2019). However, our field results fit to results of Panten and Leinweber (2020) reporting no difference in relative cumulative yields for iSPTC-C and only marginal higher yields for the BC^{plus} (95%) compared to the BC treatment in iSPTC-A (BC 94%). This lack of benefit from sulfur-modified BC^{plus} can be explained by the short duration of the experiment and thus by small accumulated

changes in soil available P concentrations, as well as by the masking and attenuation of the P mobilizing effect of S-induced in situ digestion of BC^{plus} particles by the additional S fertilizer applied. Furthermore, the necessary repeatedly liming continuously increased pH toward the target value of ~pH 6.5 and therewith to less favorable values for the dissolution of BC-based fertilizers. Therefore, it is reasonable to assume that the potential solubility of BC will continue to decrease, but the benefit of local H₂SO₄ formation and pH reduction around the BC^{plus} particles will be more significant in subsequent experimental years.

Conclusions

This study is the first to compare the short-term effects of P fertilization with TSP, BC, and BC^{plus} and their effects on soil P stocks and P fractions at the field scale, and thus represents the next necessary step following previous laboratory and pot experiments. Data from this study and previous studies suggest that differences in the available legacy P, i.e., iSPTC, and the associated difference in the P solubilization potential of adapted microorganism communities controlled the P uptake from both BC-based fertilizers and thus its fate into the soil. While this suggests that BC-based fertilizers are a sustainable P resource in the future, especially for P-deficient soils, further long-term evaluations are needed to prove if BC and especially BC^{plus} can maintain adequate crop P supply over the long-term, also in non-acidic soils. Since in subsequent cropping cycles the masking effects of grassland conversion will be less relevant, the continuation of this unique field trial is highly recommended. However, subsequent evaluations of this field trial should consider the observed effects of land-use change on P fractions during the first three years of the trial. Future research should also focus on the chemical and physical persistence of BC particles in soils and their fate in different soil size fractions. Tracking the changes in physical and chemical properties of individual BC and especially BC^{plus} particles in the field over time will also help to better understand the processes of S-induced in situ digestion.

Acknowledgements The authors gratefully acknowledge the German Federal Ministry of Education and Research (BMBF)

for funding the BonaRes project InnoSoilPhos [031B0509D, 031A558G, 031B0509E] and S. Hohenberger (University of Bonn) for extraordinary help in the lab and P. Narf for technical support. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding Open Access funding enabled and organized by Projekt DEAL. The research leading to these results received funding for the BonaRes project InnoSoilPhos from the German Federal Ministry of Education and Research (BMBF) under Grant Agreement No 031B0509D, 031A558G, 031B0509E.

Declarations

Conflict of interests The authors have no competing interests to declare that are relevant to the content of this article.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alamgir M, McNeill A, Tang C, Marschner P (2012) Changes in soil P pools during legume residue decomposition. *Soil Biol Biochem.* <https://doi.org/10.1016/j.soilbio.2012.01.031>
- Amin AEEAZ, Mihoub A (2021) Effect of sulfur-enriched biochar in combination with sulfur-oxidizing bacterium (*thiobacillus* spp.) on release and distribution of phosphorus in high calcareous P-fixing soils. *J Soil Sci Plant Nutr.* <https://doi.org/10.1007/s42729-021-00500-5>
- Biswas PP, Liang B, Turner-Walker G, Rathod J, Lee YC, Wang CC, Chang CK (2021) Systematic changes of bone hydroxyapatite along a charring temperature gradient: an integrative study with dissolution behavior. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2020.142601>
- Commission E (2020) Study on the Eu's list of critical raw materials - critical raw materials factsheets. *Crit Raw Mater Factsheets.* <https://doi.org/10.2873/11619>
- Cross AF, Schlesinger WH (1995) A literature review and evaluation of the. Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197–214. [https://doi.org/10.1016/0016-7061\(94\)00023-4](https://doi.org/10.1016/0016-7061(94)00023-4)

- Dela Piccolla C, Hesterberg D, Muraoka T, Novotny EH (2021) Optimizing pyrolysis conditions for recycling pig bones into phosphate fertilizer. *Waste Manag.* <https://doi.org/10.1016/j.wasman.2021.06.012>
- Fan X, Habib L, Fleckenstein J, Haneklaus S, Schnug E (2003) In situ digestion: a concept to manage soil phosphate in organic farming. pp. 219–228
- Grafe M, Kurth JK, Panten K, Raj AD, Baum C, Zimmer D, Leinweber P, Schlöter M, Schulz S (2021) Effects of different innovative bone char based P fertilizers on bacteria catalyzing P turnover in agricultural soils. *Agric Ecosyst Environ.* <https://doi.org/10.1016/j.agee.2021.107419>
- Heckenmüller M, Narita D, Klepper G (2014) Global Availability of Phosphorus and Its Implications for Global Food Supply: an economic Overview. Kiel Work Pap
- Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations I. *Soil Sci Soc Am J* 46:970. <https://doi.org/10.2136/sssaj1982.03615995004600050017x>
- Kalhor SA, Xu X, Chen W, Hua R, Raza S, Ding K (2017) Effects of different land-use systems on soil aggregates: a case study of the Loess Plateau (Northern China). *Sustain.* <https://doi.org/10.3390/su9081349>
- Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biol Biochem.* [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)
- Leinweber P, Hagemann P, Kebelmann L, Kebelmann K, Morshedizad M (2018) Bone char as a novel phosphorus fertilizer. *Phosphorus Recovery and Recycl.* https://doi.org/10.1007/978-981-10-8031-9_29
- Morshedizad M, Zimmer D, Leinweber P (2016) Effect of bone chars on phosphorus-cadmium-interactions as evaluated by three extraction procedures. *J Plant Nutr Soil Sci.* <https://doi.org/10.1002/jpln.201500604>
- Morshedizad M, Panten K, Klysubun W, Leinweber P (2018) Bone char effects on soil: sequential fractionations and XANES spectroscopy. *SOIL.* <https://doi.org/10.5194/soil-4-23-2018>
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Negassa W, Leinweber P (2009) How does the hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: a review. *J Plant Nutr Soil Sci.* <https://doi.org/10.1002/jpln.200800223>
- Panten K, Leinweber P (2020) Agronomic evaluation of bone char as phosphorus fertiliser after five years of consecutive application. *J Cultiv Plants* 72:561–576. <https://doi.org/10.5073/JfK.2020.12.02>
- Pueyo JJ, Quiñones MA, Coba de la Peña T, Fedorova EE, Lucas MM (2021) Nitrogen and phosphorus interplay in lupin root nodules and cluster roots. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2021.644218>
- Scholz RW, Wellmer FW (2013) Approaching a dynamic view on the availability of mineral resources: what we may learn from the case of phosphorus? *Glob Environ Chang.* <https://doi.org/10.1016/j.gloenvcha.2012.10.013>
- Schüller H (1969) Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphates in Böden. *Zeitschrift Für Pflanzenernährung Und Bodenkd* 123:48–63
- Siebers N, Kruse J (2019) Short-term impacts of forest clear-cut on soil structure and consequences for organic matter composition and nutrient speciation: a case study. *PLoS ONE* 14:e0220476. <https://doi.org/10.1371/journal.pone.0220476>
- Siebers N, Leinweber P (2013) Bone char: a clean and renewable phosphorus fertilizer with cadmium immobilization capability. *J Environ Qual.* <https://doi.org/10.2134/jeq2012.0363>
- Siebers N, Godlinski F, Leinweber P (2014) Bone char as phosphorus fertilizer involved in cadmium immobilization in lettuce, wheat, and potato cropping. *J Plant Nutr Soil Sci.* <https://doi.org/10.1002/jpln.201300113>
- Siebers N, Wang L, Funk T, von Tucher S, Merbach I, Schweitzer K, Kruse J (2021) Subsoils—a sink for excess fertilizer P but a minor contribution to P plant nutrition: evidence from long-term fertilization trials. *Environ Sci Eur.* <https://doi.org/10.1186/s12302-021-00496-w>
- Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. *Talanta.* <https://doi.org/10.1016/j.talanta.2004.11.012>
- Vogeler I, Rogasik J, Funder U, Panten K, Schnug E (2009) Effect of tillage systems and P-fertilization on soil physical and chemical properties, crop yield and nutrient uptake. *Soil Tillage Res.* <https://doi.org/10.1016/j.still.2008.10.004>
- Warren GP, Robinson JS, Someus E (2009) Dissolution of phosphorus from animal bone char in 12 soils. *Nutr Cycl Agroecosystems.* <https://doi.org/10.1007/s10705-008-9235-6>
- Wiesler F, Appel T, Dittert K, Eertseder T, Müller T, Nätscher L, Olf, H-W, Rex M, Schweitzer KDS, Taube F, Zorn W (2018) Phosphordüngung nach Bodenuntersuchung und Pflanzenbedarf: VDLUFA-Standpunkt [WWW Document]. URL https://www.vdlufa.de/Dokumente/Veroeffentlichungen/Standpunkte/2018_Standpunkt_P-Duengung.pdf Access: 10.02.2022
- Zimmer D, Kruse J, Siebers N, Panten K, Oelschläger C, Warentin M, Hu Y, Zuin L, Leinweber P (2018) Bone char vs. S-enriched bone char: multi-method characterization of bone chars and their transformation in soil. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2018.06.076>
- Zimmer D, Panten K, Frank M, Springer A, Leinweber P (2019) Sulfur-enriched bone char as alternative P fertilizer: spectroscopic, wet chemical, and yield response evaluation. *Agric.* <https://doi.org/10.3390/agriculture9010021>
- Zwetsloot MJ, Lehmann J, Bauerle T, Vanek S, Hestrin R, Nigussie A (2016) Phosphorus availability from bone char in a P-fixing soil influenced by root-mycorrhizae-biochar interactions. *Plant Soil.* <https://doi.org/10.1007/s11104-016-2905-2>