

# Effect of different biochars on phosphorus (P) dynamics in the rhizosphere of *Zea mays L.* (maize)

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## Abstract

**Aim** To investigate the effects of biochar on biological and chemical phosphorus (P) processes and identify potential interactive effects between P fertilizer and biochar on P bioavailability in the rhizosphere of maize. **Methods** We conducted a pot-experiment with maize in a sandy loam soil with two fertilizer levels (0 and

100 mg P kg<sup>-1</sup>) and three biochars produced from soft wood (SW), rice husk (RH) and oil seed rape (OSR). Sequential P fractionation was performed on biochar, bulk soil, and rhizosphere soil samples. Acid and alkaline phosphatase activity and root exudates of citrate, glucose, fructose, and sucrose in the rhizosphere were determined.

**Results** RH and OSR increased readily available soil P, whereas SW had no effect. However, over time available P from the biochars moved to less available P pools (Al-P and Fe-P). There were no interactive effects between P fertilizer and biochar on P bioavailability. Exudates of glucose and fructose were strongly affected by especially RH, whereas sucrose was mostly affected by P fertilizer. Alkaline phosphatase activity was positively correlated with pH, and citrate was positively correlated with readily available P.

**Conclusion** Biochar effects on biological and chemical P processes in the rhizosphere are driven by biochar properties.

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**Keywords** Biochar · Phosphorus fractionation · Root exudates · Phosphatase activity · Rhizosphere processes

## Introduction

Phosphorus (P) is an essential plant nutrient. However, today, most commercial P fertilizers originate from mined phosphate rock - a non-renewable and geographically restricted resource (Chowdhury et al. 2017). The orthophosphate ions (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>) readily

available for plant uptake are very reactive in the soil environment. Consequently, vast amounts of applied P fertilizers become unavailable for plant uptake due to strong sorption processes in the soil. Conversely, over fertilization with P can lead to runoff into surface waters resulting in eutrophication (Faucon et al. 2015). Thus, sustainable P management is of great importance to secure future crop production with a minimum negative impact on the environment.

Soil P exists in different organic and inorganic P pools with varying stability in the soil. Chemical processes such as adsorption and precipitation of phosphorus in the soil are governed by soil properties such as pH, cation exchange capacity (CEC), anion exchange capacity (AEC), organic matter content, clay content, Fe and Al (hydr)oxides, and the content of Ca, Fe, Al and Mg containing minerals (Shen et al. 2011). At low pH, P is sorbed strongly to Al and Fe (hydr)oxides, whereas P reacts mostly with Ca and Mg containing minerals when pH reaches  $\geq 6.5$  (Parfitt 1978).

The rhizosphere is a hot spot for biological and chemical processes affecting P availability in soil and plant responses to P deficiency drive many P related rhizosphere processes. Plant perception of the environment can start a cascade of signaling aimed to alter root morphology and the exudation of various compounds involved in the release of phosphate bound to soil minerals and the hydrolysis of organic phosphorus species (Raghothama 1999). Protons secreted from microbes and plant roots can acidify the soil environment releasing Ca-bound P for plant uptake. Likewise, exudates of organic anions can release P by chelating different P-binding metal species (Fe, Al and Ca) (Jones 1998). Phosphatase enzymes exuded by microbes and plant roots can hydrolyze organic P species releasing bioavailable phosphorus (Nannipieri et al. 2011). Managing soil properties can alter both biological and chemical P processes potentially affecting P bioavailability and thus enhance P-use efficiency of applied P fertilizers.

Biochar, the carbonaceous solid product from pyrolysis of organic material, has great potential as a valuable soil amendment in agriculture. Carbon (C) sequestration, the addition of mineral nutrients, improvement of soil structure and water holding capacity are some of the potential beneficial effects of biochar application to soil (Lehmann and Joseph 2015). However, biochar properties are diverse depending on production technology, production temperature, and feedstock type (Gul et al. 2015). Accordingly, different biochars can have

divergent effects on soil properties and plant growth (Manolikaki et al. 2016).

Biochar can alter important soil properties such as pH and the content of minerals (Fe, Al, Ca, and Mg) and C, potentially influencing P adsorption and precipitation processes in soil. Accordingly, biochar can either increase (Xu et al. 2014) or decrease (Jiang et al. 2015) soil P sorption affecting P bioavailability (Bornø et al. 2018). Furthermore, P originating from biochar can be taken up directly by plants depending on the availability of biochar P (Parvage et al. 2012). Roots have shown to respond to biochar amendment by changing root morphology (Abiven et al. 2015) and enhancing root biomass (Yamato et al. 2006). Yet, biochar properties (Prendergast-Miller et al. 2014) and the amount of biochar applied (Brockhoff et al. 2010) control these effects, and negative effects of biochar on root growth have also been observed (Ahmed et al. 2017). Several authors have found significant alterations of the microbial community structure in biochar-amended soil (Lehmann et al. 2011), which can affect important functional processes such as P mineralization and P solubilization (Schmalenberger and Fox 2016). Accordingly, root and microbial exudates can be affected by changes in the soil environment, and biochar has shown to increase phosphatase activity in soil incubations studies (Marzooqi and Yousef 2017) and in the field (Bera et al. 2016). However, other authors found that biochar did not affect (Zhang et al. 2017) or reduced phosphatase activity in biochar treated field plots (Foster et al. 2016), indicating that the combined effect of biochar and soil properties control changes in P processes in the rhizosphere. Therefore, increasing our knowledge of potential effects of biochars with different properties on P processes in soil can improve the efficiency of biochar application in agricultural systems.

The objective of this study was to investigate how three biochars produced from different feedstocks (soft wood pellets, rice husk, and oil seed rape) affect biological and chemical P processes in a plant-soil-biochar system with two P fertilizer regimes. We conducted a four-week maize (*Zea mays L.*) pot experiment in a soil with low P availability amended with three different biochars and two P fertilizer regimes (0 or 100 mg P kg<sup>-1</sup>) to study interaction effects between biochar and P fertilizer. Changes in inorganic and organic P pools during maize growth were studied by a modified sequential P fractionation, a method first presented by Hedley et al. (1982), where P pools with varying

availability are extracted with a sequence of acid and alkaline extractions. Biological P processes in the rhizosphere were investigated by determining acid and alkaline phosphatase activity and citrate and sugar exudates. We hypothesized that 1) biochar would affect both chemical and biological P processes in the rhizosphere of maize. However, these effects would depend on biochar properties; 2) biochar can significantly interact with P fertilizers by either increasing or decreasing P availability depending on biochar properties.

## Methods and materials

### Biochar and soil

Three standard biochars (SWP550, RH550, and OSR550) were acquired from the UK Biochar Research Centre (UKBRC), University of Edinburgh, School of GeoSciences, UK. The biochars were produced in a pilot scale rotary kiln pyrolysis unit with a nominal peak temperature of 550 °C (Mašek et al. 2018). The designated abbreviations of the biochars in this study are according to the different feedstocks: soft wood pellets (SW), rice husk (RH) and oil seed rape (OSR). The biochar materials were produced with a high level of control and extensive monitoring over the production process, which has resulted in a high degree of reproducibility. Thus, the biochars are considered as reference materials that can be applied in scientific research (UKBRC 2013).

The soil was a sandy loam originating from a nutrient depletion experiment at the University of Copenhagen's Experimental Farm in Taastrup, Denmark. The soil had been applied with 60 kg N, 60 kg K and 25 kg S ha<sup>-1</sup> y<sup>-1</sup>, and deprived of P fertilizer since 1964 resulting in a very low P availability (<10 mg P kg<sup>-1</sup>, Olsen P) (Van Der Bom et al. 2017).

Total content of P, K, Fe, Al, Ca, Mg, Mn, Na and S were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5100, Agilent Technologies, Manchester, UK) on ball milled and digested biochar or soil samples. Biochar samples were digested with 70% HNO<sub>3</sub>, 15% H<sub>2</sub>O<sub>2</sub> and 49% HF, and soil samples were digested with 70% HNO<sub>3</sub>, and 15% H<sub>2</sub>O<sub>2</sub>. Mineral structures of the biochars were identified with X-ray powder diffraction (XRD) using a Bruker D8 ADVANCE X-ray diffractometer (Bruker AXS GmbH, Germany), equipped with a Cu K $\alpha$

radiation source. Measurements were performed with a step length angle of 2 $\theta$ ° from 2 to 70 °C. Sequential P fractionation on biochar samples (<0.425 mm) were performed as described in the section below and Fig. S1. For additional information on remaining biochar properties see Mašek et al. (2018), UKBRC (2013). Soil pH was measured in a 1:2.5 soil/water solution using a glass electrode.

### Maize pot experiment and soil incubations

The pelletized biochars were crushed in a mortar to pass a 0.425 mm mesh screen and mixed with air-dry and sieved soil (< 2 mm) at a 2% (w/w) ratio. The biochars were crushed to a smaller particle size to ensure a relatively homogeneous distribution in the soil, and the biochar application rate corresponded to approximately 30–40 t biochar ha<sup>-1</sup>. Pots had a top diameter of 11 cm, and a volume of 0.75 L. A filter paper was placed at the bottom of the pots to prevent soil loss. A total of 450 g soil or soil/biochar mixture was applied to each pot and the pots were placed randomly in trays and watered from beneath approximately every second day. The pots were pre-incubated for eight days in the greenhouse before planting. To determine initial soil pH, CEC and sequential P fractions in bulk soil samples from all treatments (unplanted soil), 100 g soil or soil/biochar mixtures, identical to the treatments in the pots, were incubated in separate containers in replicates of six. The containers were pre-incubated together with the pots in the greenhouse for eight days.

Maize seeds were pre-germinated in a petri dish covered with a filter paper and kept in the dark for four days. After the pre-incubation period of the pots (eight days), one germinated maize seedling was carefully placed at 1 cm depth in each pot, and the topsoil layer was gently disrupted with a fork to ensure aeration of the soil. All pots and soil incubations were supplemented with nutrient solutions containing 500 mg N kg soil<sup>-1</sup> (67% as NH<sub>4</sub><sup>+</sup> and 33% as NO<sub>3</sub><sup>-</sup>), 300 mg K kg soil<sup>-1</sup>, and 200 mg S kg soil<sup>-1</sup>. Half of the replicates from each treatment (four replicates from the pot experiment and three replicates from the soil incubations) were amended with additional 100 mg P kg soil<sup>-1</sup> as dissolved KH<sub>2</sub>PO<sub>4</sub>. Nutrient solutions were applied once to the disrupted topsoil right after planting the seeds. Treatments were as follows: Control (CK-0P), control +P (CK-100P), soft wood biochar (SW-0P), soft wood biochar + P (SW-100P), rice husk biochar (RH-0P), rice

husk biochar + P (RH-100P), oil seed rape biochar (OSR-0P), and oil seed rape biochar + P (OSR-100P).

The day after planting and fertilizing the soils, soil samples from the soil incubations were collected, and pH was measured in a 1:2.5 soil/water solution using a glass electrode. Soil CEC was estimated with ammonium acetate (pH 7) followed by extraction of  $\text{NH}_4^+$  with 1 M KCl.  $\text{NH}_4$  concentration was measured colorimetrically on a flow injection analyzer (FIAstar 5000, FOSS, Sweden). Sequential P fractionation was performed on air-dry samples as described in the section below and Fig. S1. Soil samples from the soil incubations are here defined as bulk soil samples.

The maize plants were grown in the greenhouse for four weeks under ambient  $\text{CO}_2$  (400 ppm). The climatic conditions in the glasshouse cells were set to  $23/16 \pm 2$  °C day/night air temperature, 60% relative humidity, 16 h photoperiod, and  $> 500 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic active radiation (PAR) supplied by sunlight plus LED lamps.

### Harvest

Maize plants were carefully removed from the pots, and rhizosphere soil was collected on a glossy paper, by carefully cleansing the roots from the soil by hand. The rhizosphere soil was divided into two parts, one portion was air dried to be analyzed by sequential P fractionation, and a portion of fresh soil was saved for the determination of enzyme activity and pH. Citrate and sugars in the rhizosphere were extracted according to Pearse et al. (2007) and Wang et al. (2016). Briefly, the roots, still attached to the shoot, were transferred into a plastic bag containing 100 mL 0.2 mM  $\text{CaCl}_2$  solution. The plastic bag was carefully “dunked” for two minutes to mix the leftover rhizosphere soil and roots with the solution. The roots were removed from the extract and washed clean of soil particles. A subsample (~25 mL) of the  $\text{CaCl}_2$  extraction was filtered through a 0.45  $\mu\text{m}$  syringe filter, and one drop of diluted (1:1000) Micropur Forte MF 100F (Katadyn products Inc., Zürich, Switzerland) was added to the filtered sample to prevent microbial interference. The samples were stored at  $-20$  °C until analysis by ion chromatography (Metrohm AG, Herisau, Switzerland). The determination of sugars including fructose, glucose, and sucrose occurred on a Metrosep Carb 1–150 column using 100 mM sodium hydroxide as eluent; while the measurement of citric acid was done on a Carbohydrate  $\text{H}^+$

column using 0.5 mM sulfuric acid and 10% acetone as eluent. Results were calculated as  $\mu\text{mol g root}^{-1}$  dry weight. Root and shoot biomass were separated, dried at 60 °C for four days and dry weight (DW) was determined. Ball milled samples of the shoot and root material were digested with 70%  $\text{HNO}_3$ , and 15%  $\text{H}_2\text{O}_2$  and total the P content was measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5100, Agilent Technologies, Manchester, UK). Shoot N content was determined on a vario MACRO cube CNS analyzer (Elementar Analysensysteme GmbH, Germany).

### Phosphatase enzyme activity

Acid and alkaline phosphatase activity was determined according to Tabatabai (1994). Shortly, 1 g of fresh soil was added to a 50 mL Erlenmeyer flask and 4 mL modified universal buffer (MUB) adjusted to pH 6.5, or pH 11 was added to determine acid or alkaline phosphatase activity, respectively. 1 mL 0.05 M *p*-nitrophenyl phosphate (pNPP) solution was used as substrate. The flasks were carefully swirled to mix the content, covered with parafilm, and incubated in the dark at 37 °C for 1 h. After incubation 1 mL 0.5 M  $\text{CaCl}_2$  and 4 mL 0.5 M NaOH were added to each sample to stop the reaction and evoke the yellow color. The sample content was filtered through a Whatman grade 42 qualitative filter paper and the absorbance was measured on a spectrophotometer at 405 nm (Thermo Scientific™ GENESYS 10S UV-Vis, USA). Results were adjusted to a control sample that received the pNPP substrate only after the incubation period. The amount of *p*-nitrophenol (pNP) in the samples was estimated according to a standard curve containing 0, 10, 20, 30, 40 and 50  $\mu\text{g pNP}$ . Phosphatase activity was calculated as  $\mu\text{g pNP g soil}^{-1} \text{h}^{-1}$ .

### Sequential P fractionation

Sequential P fractionations were performed on biochar samples, bulk soil samples from the soil incubations and later on the rhizosphere soil collected from the maize pot experiment to detect potential changes in P pools during plant growth. The sequential P fractionation procedure was modified from Hedley et al. (1982) and Tiessen and Moir (1993). A detailed overview of the procedure is presented in Fig. S1 in the supporting information. P in extracts was measured colorimetrically on a flow

injection analyzer (FIAstar 5000, FOSS, Sweden) on neutralized samples. To determine the total P in NaHCO<sub>3</sub> and NaOH extracts, a subsample of each extract was acidified with H<sub>2</sub>SO<sub>4</sub> and digested with ammonium persulfate at 121 °C for 2 h at 1 atm in an autoclave and measured colorimetrically. Organic P (Po) in the NaHCO<sub>3</sub> and NaOH extracts were calculated by subtracting the inorganic P (Pi) from the total P in the extracts.

### Statistical analysis

The effect of biochar treatment (CK, SW, RH, and OSR), P fertilizer (0-P or 100-P) and the interaction between the two factors were estimated with two-way ANOVA on all the measured data, the results are presented in Table S1 in the supporting information. In addition, one-way ANOVA and Tukey test were performed individually on the data for each P fertilizer level (0-P and 100-P). Levene's test was used to test for homogeneity of variance before any ANOVA was performed. If the data did not fulfill the requirement for homogeneity of variance, the data was log transformed and tested again. The significance of correlations was tested using Pearsons Product-Moment correlation. All statistical analyses were run in R studio version 3.3.2.

## Results

### Biochar characteristics

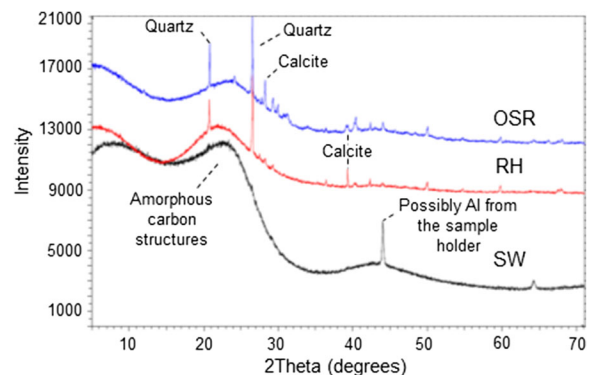
The biochars differed in chemical and mineralogical properties. SW had a lower pH and ash content, and a higher C content compared to RH and OSR. RH had the highest ash content and lowest C content, whereas OSR had intermediate contents regarding these variables (Table 1). Higher ash contents in RH and OSR compared to SW were further reflected in the X-ray diffraction patterns of the biochars (Fig. 1), where RH and OSR exhibited more distinct peaks compared to SW, indicating the presence of crystalline minerals. OSR and RH spectra had distinct peaks identified as quartz and calcite; whereas the most distinct peak in the SW spectra was assigned to Al from the sample holder (Singh and Raven 2017). All three biochars exhibited amorphous C structures, yet these structures were most apparent in the SW spectra and less pronounced in the OSR spectra.

**Table 1** Soil and biochar properties

	Soil	SW	RH	OSR
Nominal Peak Temperature (°C)	–	550	550	550
Surface area (m <sup>2</sup> g <sup>-1</sup> )	–	26.4	20.1	7.3
Total ash (%)	–	1.25	47.93	19.50
EC (dS m <sup>-1</sup> )	–	0.09	0.48	2.27
pH	5.2	7.91	9.71	9.78
CEC (cmol + kg <sup>-1</sup> )	10.2	–	–	–
Biochar C stability (%) <sup>a</sup>	–	69.62	95.28	97.31
C (%)	0.94	85.52	48.69	68.85
H (%)	–	2.77	1.24	1.82
O (%)	–	10.36	2.47	8.91
H:C	–	0.39	0.28	0.32
O:C	–	0.09	0.04	0.1
N (%)	0.14	< 0.1	1.04	1.59
Mineral N (mg kg <sup>-1</sup> )	–	< 3	< 3	> 3
P (g kg <sup>-1</sup> )	0.37	0.28	2.18	2.57
K (g kg <sup>-1</sup> )	19.20	2.58	11.68	37.10
Fe (g kg <sup>-1</sup> )	13.43	0.34	1.66	0.78
Al (g kg <sup>-1</sup> )	14.55	0.14	1.61	1.22
Ca (g kg <sup>-1</sup> )	2.37	3.07	2.89	9.98
Mg (g kg <sup>-1</sup> )	2.34	0.51	1.52	3.36
Mn (g kg <sup>-1</sup> )	0.33	0.33	0.63	0.03
Na (g kg <sup>-1</sup> )	4.07	0.11	0.33	0.19
S (g kg <sup>-1</sup> )	0.23	0.20	0.41	0.94

Biochar abbreviations are SW = Soft wood biochar, RH = Rice husk biochar, OSR = Oil seed rape biochar. P, K, Fe, Al, Ca, Mg, Mn, Na and S ( $n=2$ ), soil pH and CEC ( $n=3$ ). Remaining properties see UKBRC (2013)

<sup>a</sup>Biochar C stability % refers to the long-term stability 100–1000 years (Cross and Sohi et al. 2013)



**Fig. 1** XRD pattern of SW, RH and OSR biochars. Biochar abbreviations see Table 1

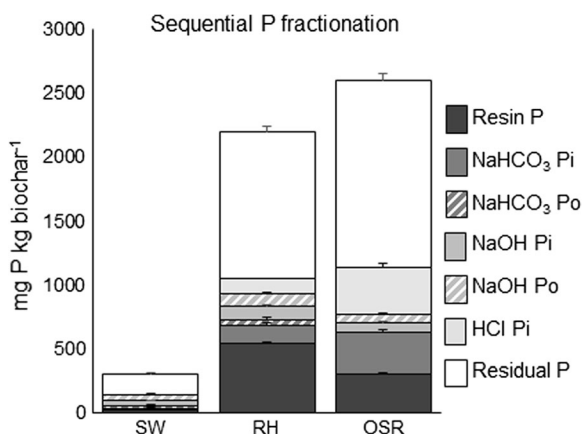


### Biochar P fractionation

The total phosphorus content was 7–9 times lower in SW compared to RH and OSR (Table 1). The results from the sequential P fractionation of the biochars are presented in Fig. 2. Approximately half of the total P in the biochars could be extracted with the different extracts. The readily available Pi pool (Resin P + NaHCO<sub>3</sub> Pi) was 37 mg P kg<sup>-1</sup>, 686 mg P kg<sup>-1</sup>, and 629 mg P kg<sup>-1</sup> in SW, RH, and OSR, respectively. The extractable organic P fractions (NaHCO<sub>3</sub> Po + NaOH Po) were highest in SW where 20% of the total P was assigned as extractable organic P, whereas only 2 and 6% of total P were assigned as extractable organic P in OSR and RH, respectively. The NaOH Pi fraction representing Fe and Al phosphates was 14%, 5 and 3% in SW, RH, and OSR, respectively. Finally, the HCl Pi fraction representing Ca phosphate was highest in OSR where 14% of total P could be extracted with 1 M HCl while only 5 and 2% of total P were recovered in RH and SW, respectively.

### Biochar effects on soil properties

Initially, all biochars significantly increased soil pH (Table 2), however, after plant growth the pH was only significantly higher in OSR-0P and OSR-100P treatments compared to all other treatments (Table S1 and Table 2). The two-way ANOVA revealed a marginal effect of biochar on soil CEC (Table S1). However, this effect was not evident from the one-way ANOVA performed on 0-P and 100-P treatments separately (Table 2).



**Fig. 2** Sequential P fractionation of SW, RH, and OSR. Error bars indicate S.E. ( $n = 3$ ). Biochar abbreviations see Table 1

### Maize growth and P uptake

Maize shoot and root biomass increased in RH-0P and OSR-0P, yet not in SW-0P compared to CK-0P (Fig. 3). However, only RH-100P resulted in significantly higher root biomass compared to CK-100P (Fig. 3). The root to shoot ratio was lower in 100-P treatments compared to 0-P treatments (Fig. 3), and the two-way ANOVA revealed that P fertilizer had a stronger effect on the root to shoot ratio compared to biochar (Table S1).

Only P fertilizer had a significant effect on shoot and root P concentration, yet there was a significant interaction between P fertilizer and biochar on root P concentration (Table S1). One-way ANOVA and Tukey test revealed that RH-0P and OSR-0P slightly, yet significantly, increased the P concentration in the roots compared to CK-0P (Fig. 3). Total maize P uptake significantly increased in RH-0P and OSR-0P treatments compared to CK-0P. However, this effect was absent in the 100-P treatments (Fig. 3).

### Sequential P fractionation in bulk soil and rhizosphere soil samples

The results of the sequential P fractionation of bulk soil incubations and rhizosphere soil are presented in Fig. 4. Initially in the bulk soil, resin P increased in RH-0P and OSR-0P compared to CK-0P. However, RH and OSR did not increase resin P in the 100-P treatments. No significant differences between any of the biochar treatments and CK were observed in NaHCO<sub>3</sub> Pi, NaOH Pi or NaOH Po fractions in bulk 0-P or 100-P treatments. However, NaHCO<sub>3</sub> Po (0-P level) and HCl Pi (100-P level) were slightly increased in the biochar treatments compared to the controls.

After plant growth, 43 mg P kg soil<sup>-1</sup> on average was lost from the Resin P pool in the 100-P treatments, while there were no changes in the resin P pools in the 0-P treatments. In the NaHCO<sub>3</sub> Pi fractions approximately 7 mg P kg<sup>-1</sup> and 14 mg P kg<sup>-1</sup> were lost in the 0-P and 100-P treatments, respectively. Furthermore, the NaHCO<sub>3</sub> Pi fraction was significantly higher in RH-0P and OSR-0P treatments compared to CK-0P after plant growth. In contrast to the resin P and NaHCO<sub>3</sub> Pi fractions, a significant increase in the NaOH Pi fraction of 15 mg P kg soil<sup>-1</sup> on average was observed in SW-0P, RH-0P, and OSR-0P and 26 mg P kg soil<sup>-1</sup> on average in CK-100P and RH-100P after plant growth. No significant P loss was observed from the NaHCO<sub>3</sub> Po or

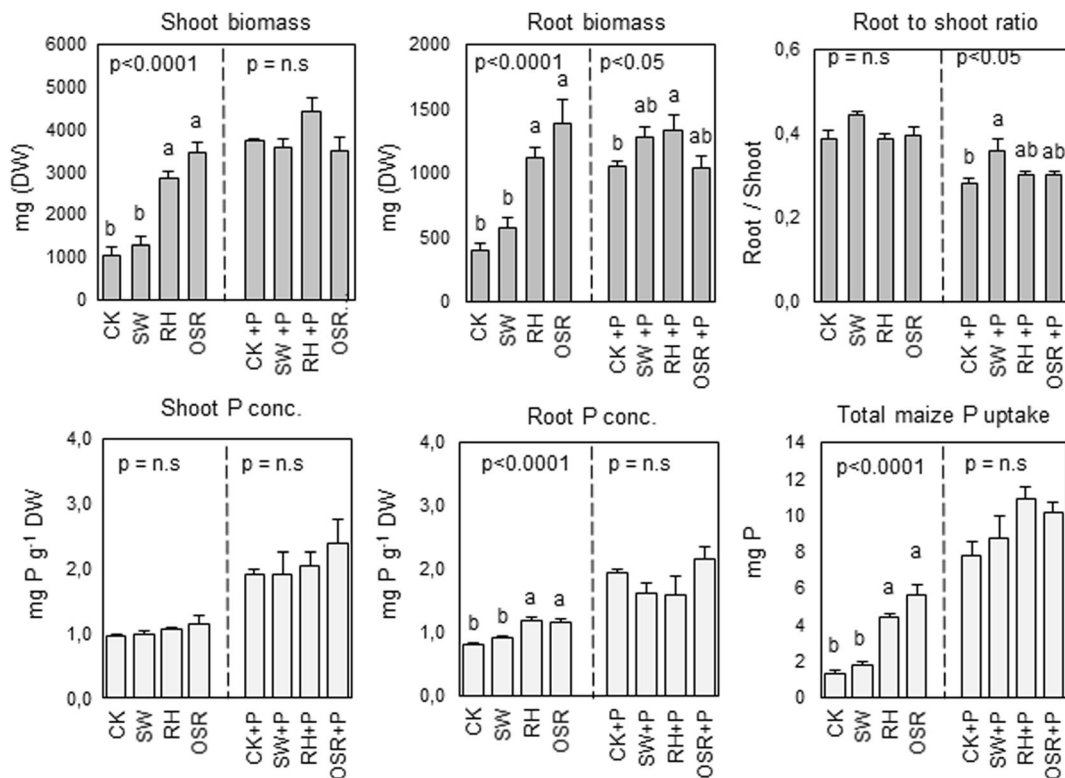
**Table 2** Bulk soil CEC and pH, and rhizosphere soil pH ( $\pm$ S.D), CEC ( $n = 3$ ), bulk soil pH ( $n = 3$ ), Rhizosphere soil pH ( $n = 4$ )

Treatments	Bulk soil CEC ( $cmol^+ kg^{-1}$ )	Bulk soil pH	Rhizosphere soil pH
CK	10.2 ( $\pm$ 0.7)	5.2 ( $\pm$ 0.08) c	4.9 ( $\pm$ 0.04) c
SW	9.9 ( $\pm$ 0.2)	5.5 ( $\pm$ 0.01) b	5.0 ( $\pm$ 0.02) b
RH	12.5 ( $\pm$ 1.0)	5.7 ( $\pm$ 0.02) b	5.0 ( $\pm$ 0.02) bc
OSR	10.5 ( $\pm$ 0.2)	6.6 ( $\pm$ 0.06) a	5.5 ( $\pm$ 0.04) a
ANOVA	$p = n.s$	$p < 0.0001$	$p < 0.0001$
CK + P	11.5 ( $\pm$ 0.5)	5.3 ( $\pm$ 0.04) c	4.9 ( $\pm$ 0.03) b
SW + P	9.9 ( $\pm$ 0.1)	5.6 ( $\pm$ 0.02) b	5.1 ( $\pm$ 0.03) b
RH + P	11.2 ( $\pm$ 0.4)	5.7 ( $\pm$ 0.03) b	5.0 ( $\pm$ 0.02) b
OSR + P	12.4 ( $\pm$ 1.0)	6.6 ( $\pm$ 0.03) a	5.8 ( $\pm$ 0.03) a
ANOVA	$p = n.s$	$p < 0.0001$	$p < 0.0001$

One-way ANOVA and Tukey tests were performed on soil parameters for 0-P and 100-P treatments separately. Significant differences between treatments are indicated with different letters. Biochar abbreviations see Table 1

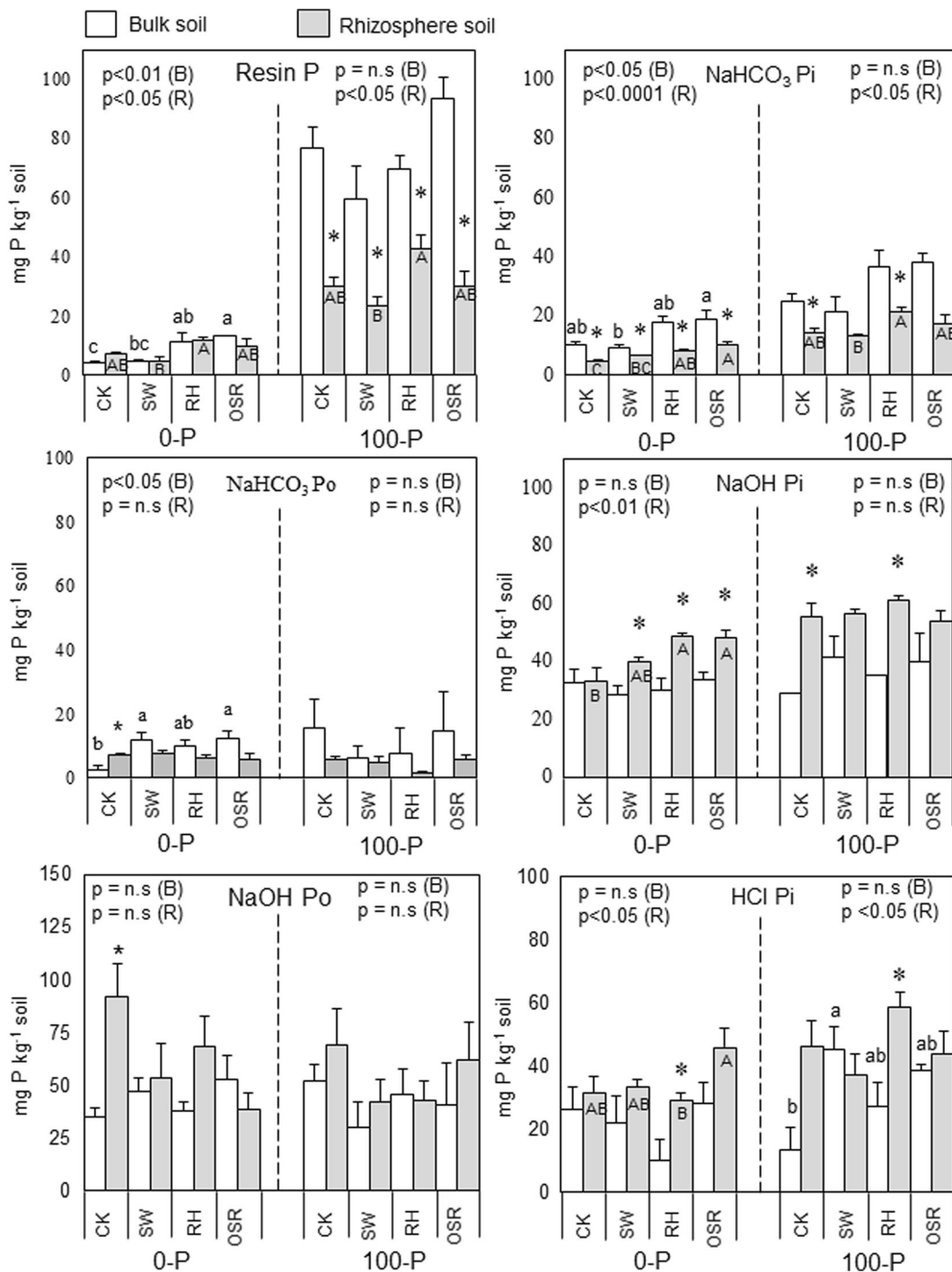
NaOH Po fractions after plant growth, though, significant increases in the  $NaHCO_3$  Po and NaOH Po fractions was observed in the CK-0P treatment after plant

growth. However, the estimated NaOH Po concentrations had a large variation, and no clear effect of biochar or P fertilizer could be deduced (Fig. 4 and Table S1).



**Fig. 3** Maize root and shoot biomasses and P concentrations, total P uptake and root to shoot ratios in all treatments. Error bars indicate S.E. ( $n = 4$ ). One-way ANOVA and Tukey’s test were performed for each P fertilizer regime (0-P or 100-P) to test for

significant differences between treatments. Significant differences between treatments are indicated with different letters. Treatment abbreviations see Table 1 (+P = 100 mg P  $kg\ soil^{-1}$ )



**Fig. 4** Sequential P fractionation of bulk soil ( $n=3$ ) and rhizosphere soil ( $n=4$ ). Error bars indicate S.E. One-way ANOVA and Tukey test were performed for each P fraction in the 0-P and 100-P treatments separately for bulk and rhizosphere soil samples. ANOVA  $p$  values for bulk (B) and rhizosphere (R) samples are indicated for each fraction at each fertilizer level. Small letters

indicate significant differences between bulk soil samples and capital letters indicate significant differences between rhizosphere soil samples. One-way ANOVA significance ( $p < 0.05$ ) to test for significant differences between bulk and rhizosphere soil samples for each treatment is indicated by \*. Biochar abbreviations see Table 1

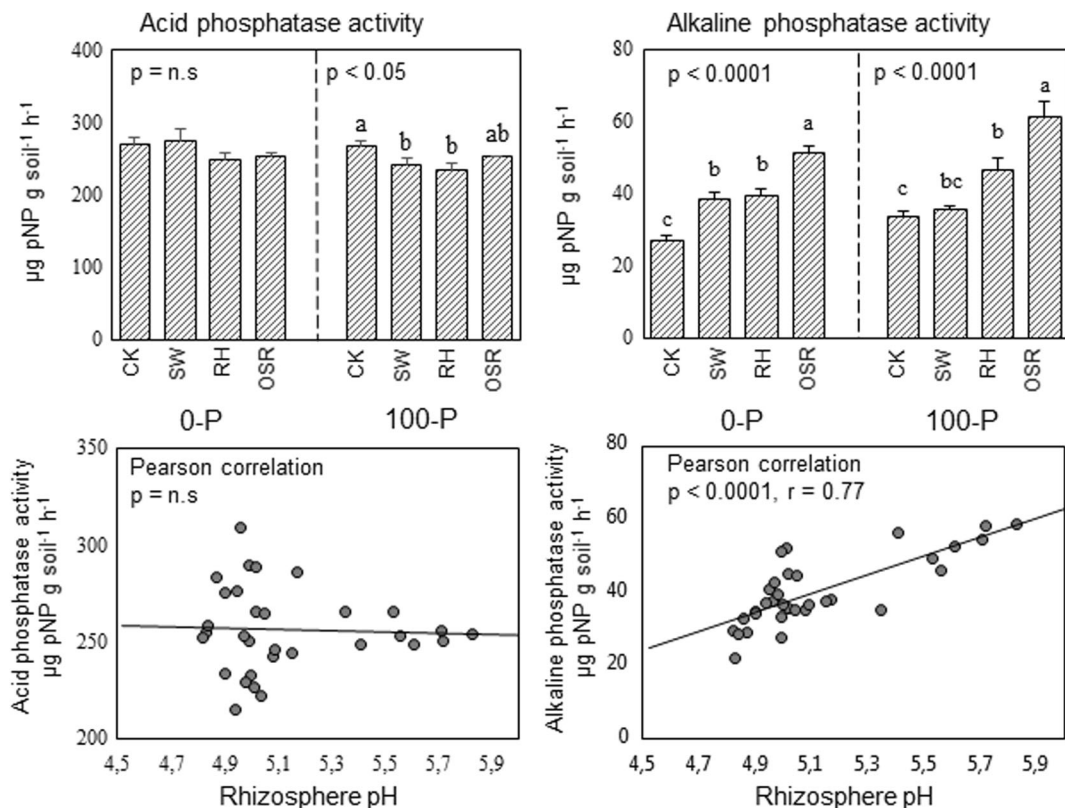


Likewise, there was a pronounced variability in the estimated HCl Pi fractions. However, a significant increase in HCl Pi was observed in the RH-0P and RH-100P treatments after plant growth.

Overall, both biochar and P fertilizer affected the readily available P fractions (Resin P + NaHCO<sub>3</sub> Pi), yet, P fertilizer had a stronger effect than biochar application according to the two-way ANOVA for both bulk soil samples and rhizosphere samples (Table S1). There were no interactions between biochar and P fertilizer on any P fractions in bulk soil samples, whereas a significant interaction was found only in the HCl Pi fraction in the rhizosphere samples (Table S1). RH and OSR increased readily available P, whereas SW had no marked effect. After plant growth, P was lost from the readily available P fractions, and P in the less available P pools (NaOH Pi) increased in treatments where biochar and/or P fertilizer was applied.

### Acid and alkaline phosphatase activity

Acid and alkaline phosphatase activity in the rhizosphere soil is presented in Fig. 5. Acid phosphatase activity was unaffected by biochar application without P fertilizer. However, in the 100-P treatments, the acid phosphatase activity was slightly higher in the CK-100P compared to the biochar treatments (Fig. 5 and Table S1). In contrast, the alkaline phosphatase activity increased significantly in the biochar treatments. In both 0-P and 100-P treatments OSR nearly doubled the alkaline phosphatase activity, whereas RH increased the alkaline phosphatase activity by one third. However, SW only increased the alkaline phosphatase activity compared to CK in the 0-P treatments and not in the 100-P treatments. Accordingly, biochar had a stronger effect on alkaline phosphatase activity compared to P fertilizer according to the two-way ANOVA (Table S1).



**Fig. 5** Acid and alkaline phosphatase activity in fresh rhizosphere soil samples (top), error bars indicate S.E (n=4). Relationship between acid and alkaline phosphatase activity and rhizosphere pH (bottom). One-way ANOVA and Tukey test were performed for the acid and alkaline phosphatase activity in 0-P and 100-P treatments separately. ANOVA p values are indicated on the

graphs. Significant differences between treatments are indicated with different letters. Correlation significance between rhizosphere pH and acid and alkaline phosphatase activity were tested with Pearsons Product-Moment correlation. Biochar abbreviations see Table 1

## Citrate, glucose, fructose, and sucrose in the rhizosphere

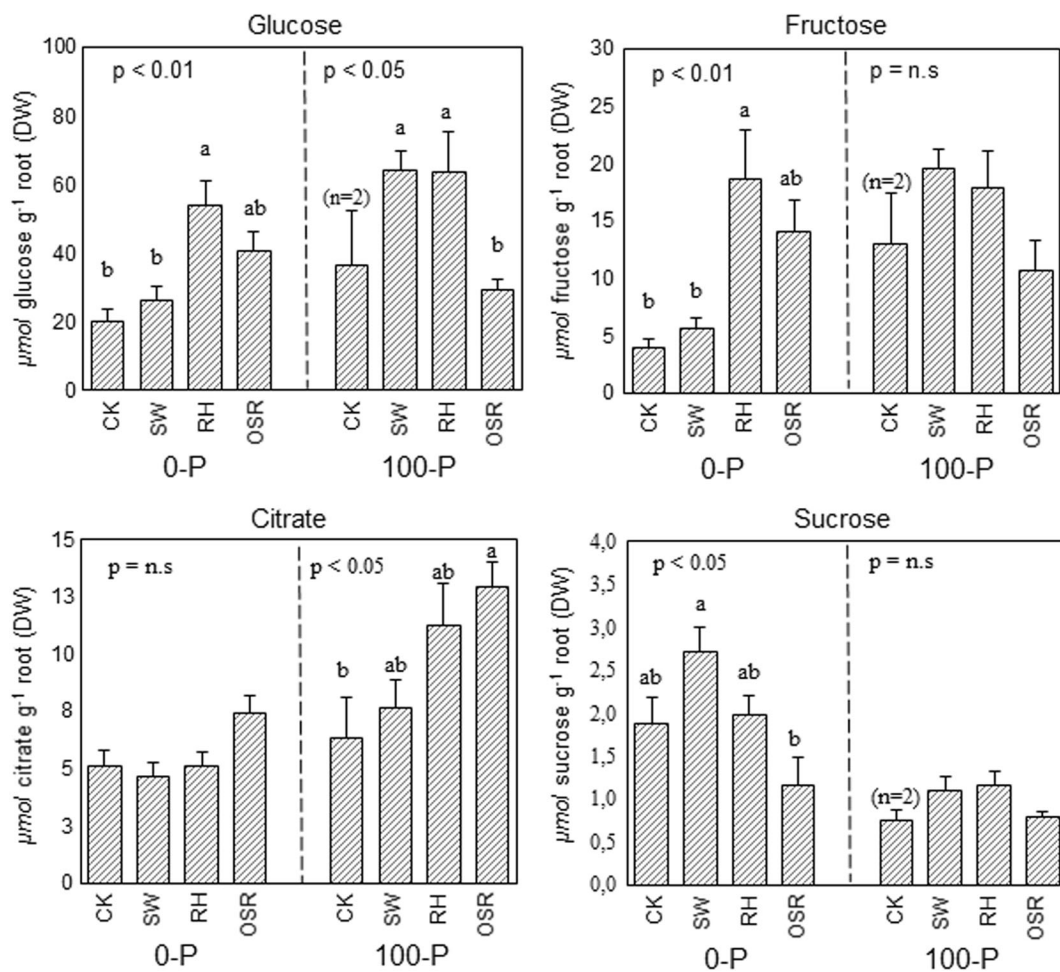
The citrate content in the rhizosphere was unaffected by biochar amendment in the 0-P treatments. However, the citrate content was significantly higher in OSR-100P ( $13 \pm 2.2 \mu\text{mol citrate g}^{-1} \text{ root (DW)}$ ) compared to CK-100P ( $6 \pm 3.8 \mu\text{mol citrate g}^{-1} \text{ root (DW)}$ ) (Fig. 6). RH-0P significantly increased the content of glucose and fructose relative to CK-0P, whereas SW-0P and OSR-0P did not. RH-100P and SW-100P had a significantly higher glucose content than OSR-100P (Fig. 6). According to the two-way ANOVA (Table S1) biochar application, P fertilizer, and the interaction between the two all had significant effects on the glucose and fructose content in the rhizosphere, where biochar had a stronger effect than P fertilizer. In

contrast, P fertilizer had a stronger effect than biochar on the rhizosphere sucrose content (Table S1). Accordingly, the sucrose content was generally lower in the 100-P treatments compared to the 0-P treatments. Also, biochar affected the sucrose content, and SW-0P treatments had a significantly higher rhizosphere sucrose content compared to OSR-100P.

## Discussion

## Biochar characteristics and effects on soil properties

Gul et al. (2015) reviewed a range of biochar properties and found that biochars produced from crop residues had a higher nutrient content, pH, and less stable C



**Fig. 6** Rhizosphere exudates of sugars and citrate. Error bars indicate S.E. (n=4). One-way ANOVA and Tukey test were performed for glucose, fructose, sucrose, and citrate in 0-P and

100-P treatments separately. ANOVA p values are indicated on the graphs. Significant differences between treatments are indicated with different letters. Biochar abbreviations see Table 1

compared to biochars produced from woody feedstock with more lignocelluloses. This complies well with a higher P content and pH in RH and OSR compared to SW (Table 1). However, the content of stable C was highest in OSR (67%) compared to SW (60%) and RH (46%) (Table 1). High ash content is often associated with a higher content of crystalline minerals (Wu et al. 2016). Correspondingly, RH and OSR had distinct XRD patterns and high elemental contents (P, K, Fe, Al, Ca, Mg, Mn, Na, and S) compared to SW (Fig. 1 and Table 1). A higher content of alkaline metals ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^{+}$ ) possibly in the form of oxides and carbonates explain the high pH of RH and OSR compared to SW (Table 1). However, despite the high ash content and pH of RH, bulk soil pH only increased by 0.5 pH units, and no pH effect could be detected after plant growth (Table 2). In contrast, OSR increased soil pH by 1.4 pH units, and soil pH was still 0.9–0.6 pH units higher than CK treatments after plant growth, indicating a higher pH buffer capacity after OSR amendment compared to RH or SW. The biochars had no clear effect on soil CEC (Table 2), which might be due to the fact that biochars produced at higher temperatures generally have a lower CEC due to the loss of volatile organic matter (VM) (Mukherjee et al. 2011). Thus, the higher pH buffer capacity in OSR amended soils could be a result of slow mineral dissolution and precipitation processes.

The results of the sequential P fractionation of the biochars were comparable to those of Xu et al. (2016a) for crop residue biochars produced at similar temperatures. However, Xu et al. (2016a) found that the HCl-extractable Ca-P fraction ranged between 13 and 25% and Schneider and Haderlein (2016) found that the Ca-P fraction ranged from 43 to 86% of total P in wood chip, grass and pruning residue biochars pyrolyzed at 700 °C. These values are considerably higher than what was found in this study (Fig. 2). A high Ca-P may relate to a generally higher Ca content, higher temperature during pyrolysis (Schneider and Haderlein 2016), or differences in the feedstock (Xu et al. 2016a). Accordingly, OSR had the highest HCl-Pi content, consistent with a higher content of elemental Ca compared to RH and SW (Table 1 and Fig. 2). The readily available inorganic P contents (Resin-P +  $\text{NaHCO}_3$ -Pi) were 17–18 times higher in RH and OSR compared to SW (Fig. 2). Thus, the P fertilizer value is considerably higher in these crop residue biochars. The organic P fractions ( $\text{NaHCO}_3$ -Po +

$\text{NaOH}$ -Po) were marginal in all three biochars. Correspondingly, Xu et al. (2016a) showed that organic P decomposes and more stable inorganic P species are formed during pyrolysis of crop residues.

#### Biochar and P fertilizer effects on maize growth and P uptake

Maize plants were able to utilize P originating from RH and OSR biochars in the 0-P treatments, which was reflected by the shoot and root biomass and total maize P uptake (Fig. 3). Similarly, Xu et al. (2016b) found that wheat straw biochar enhanced plant biomass to the same level as P fertilizer. Nevertheless, except for the root biomass (Fig. 3), the effect of biochar was generally masked by co-application of P fertilizer, and no additive effects on maize growth or P uptake were observed in the P-amended treatments (Table S1 and Fig. 3). This may relate to the low amount of readily available P (resin P and  $\text{NaHCO}_3$  Pi) added with the biochars, which only amounted to 1, 13 and 14 mg P kg soil<sup>-1</sup> for SW, RH, and OSR respectively, compared to the high amount of P added in the P fertilized treatments (100 mg P kg<sup>-1</sup>). On the other hand, we found no evidence for any negative interactions between P fertilizer and biochar application on P uptake due to P sorption by biochar particles (Fig. 4), as was previously found (Bornø et al. 2018; Xu et al. 2016b). Negative interactions between P fertilizer and biochar have previously been assigned to P sorption processes controlled by a high pH (Xu et al. 2016b) and calcite content of biochar (Bornø et al. 2018), while in this study, the biochars had only limited effect on soil pH and relatively low calcite content (Fig. 1 and Table 2).

Considering the total amount of P added with the biochars compared to the P fertilizer, a larger fraction of the total P applied was taken up by the plants in the biochar treatments. Thus, the P use efficiency of the biochar P was relatively high in treatments without additional P fertilizer. However, this should be considered in regard to the use of low P soil and it is possible that the biochar P was more effectively used by the plant in the P depleted soil. Thus, as biochar would mostly be co-applied with fertilizers in the field, the use efficiency of biochar may not be as high as found here.

## Biochar effects on soil P pools and the interaction with P fertilizer

Only few studies have investigated dynamic changes of P pools in biochar-amended soils using sequential P fractionation. Similar to our results for RH and OSR application, Xu et al. (2016b) found an increase in readily available P pools ( $\text{H}_2\text{O Pi}$  and  $\text{NaHCO}_3\text{Pi}$ ) following amendment with crop residue biochars in a saline-sodic soil, yet, they found no effect on  $\text{NaOH Pi}$  even after 90 days of incubation. An increase in  $\text{NaOH Pi}$  in biochar-amended soil after plant growth, as was found in the present study, is likely related to soil type, as a greater proportion of P dissolved from the biochar will bind to Fe and Al (hydr)oxides in acid soil compared to alkaline soil (Parfitt 1978). Alternatively, Wang et al. (2014) assigned the increase in  $\text{NaOH Pi}$  in biochar amended, sandy, slightly acidic soil to intrinsic biochar P belonging to the  $\text{NaOH Pi}$  fraction. However, the  $\text{NaOH Pi}$  content of the biochars in our study was relatively low (Fig. 2), and increases in  $\text{NaOH Pi}$  was only observed after plant growth, implying the interaction between several processes in the soil. Possibly, acidification of the soil during plant growth leading to dissolution or desorption of biochar P could result in re-adsorption of P to soil or biochar Fe and Al containing particles, consequently increasing the  $\text{NaOH Pi}$  pools.

Coinciding with the findings of other studies, P fertilizer was the main driver for observed changes in P pools in biochar-amended soils fertilized with P (Farrell et al. 2014; Xu et al. 2016b). Thus, as discussed above, P fertilizer seemed to initially mask most biochar effects in the bulk soil, and only after plant growth, the effect of biochar became more visible in the P fertilized treatments, where RH had significantly higher contents of readily available P compared to SW treatments, as was also observed in the 0-P treatments (Fig. 4). Higher readily available P in RH treatments may be related to a greater resin P content of RH biochar compared to the other biochars. Possibly, the readily available P from RH could only be detected after the plant had taken up P from the P fertilizer and additional P was adsorbed by soil particles. In contrast to this study,  $\text{NaOH Po}$  was previously found to increase after P fertilization (Richards et al. 1995; Vu et al. 2009). Thus, it is possible that the increase in  $\text{NaOH Po}$  in CK-0P after plant growth (Fig. 4) was caused by the unintended presence of root fragments in the soil samples releasing organic P.

Altogether, biochar affected the dynamic P pools in the soil, and these effects were dependent on biochar feedstock. Readily available P from the biochar affected soil P pools (Fig. 4), and even though P fertilizer initially masked the effects of biochar on readily available P in the bulk soil, biochar effects were visible after plant growth. However, it appears that especially soil texture and pH combined with biochar feedstock and production temperature will affect the dynamic changes in soil P pools (Farrell et al. 2015; Wang et al. 2014; Xu et al. 2016b). Therefore, different combinations of biochars and soil types need to be further investigated for the optimal use of biochar in agricultural systems.

## Rhizosphere sugars and organic anions – Response to biochar and P fertilizer

Sugars and organic anions are non-stable compounds in the rhizosphere, and microbial degradation, sorption processes, and reuptake by roots affect the estimation of rhizosphere exudates (Oburger et al. 2013). Therefore, estimates of rhizosphere sugar and citrate content related to the root biomasses are not absolute values and should be seen as a snapshot of a dynamic system.

Sugars have been estimated to constitute 72–73% of root exudates secreted by soil grown maize (Azaizeh et al. 1995). The efflux of sugars into the rhizosphere is assumed to be controlled by passive diffusion from the roots to the soil solution (Jones et al. 2004). Translocation of sucrose from shoots to roots is believed to function as a signaling mechanism affecting root morphogenesis in P deficient plants by increasing the root to shoot ratio (Hammond and White 2008). Accordingly, a positive correlation between sucrose content in the rhizosphere and root to shoot ratio was observed in the present study (Pearson corr.  $p < 0.0001$ ,  $r = 0.61$ ). Both biochar and P fertilizer had a significant effect on sucrose content in the rhizosphere (Table S1 and Fig. 6) and OSR resulted in a significantly lower sucrose content compared to SW in 0-P treatments possibly due to the increased P availability in OSR-0P treatments (Fig. 3 and Table S1).

Before plants can utilize sucrose as a source of carbon and energy in the roots sucrose needs to be cleaved into hexoses (glucose and fructose) a process catalyzed by invertases or sucrose synthase enzymes (Sturm 1999). Thus, increased hexoses in the roots have been shown to correlate to root elongation rate, and high concentrations of hexoses have been found in fast-



growing roots (Freixes et al. 2002; Muller et al. 1998). Furthermore, studies found that glucose and fructose increased in root exudates of maize under P deficient conditions in a hydroponic study (Carvalhais et al. 2011), and in the roots of P deficient white lupine (Müller et al. 2015) and barley (Sicher 2005). However, in this study, we found no correlation between any indicators of P deficiency (P conc. in shoot or root or root to shoot ratio) and rhizosphere content of glucose or fructose ( $p > 0.05$ ). Accordingly, biochar had a stronger effect than P fertilizer on the glucose and fructose contents (Table S1), and especially RH seemed to increase glucose and fructose in the rhizosphere independently of P fertilizer (Table S1 and Fig. 6). Thus, a higher content of glucose and fructose in the rhizosphere could relate to root growth rate at the time of sampling, which is also supported by a larger root biomass in these treatments (Fig. 3). However, more detailed studies need to be conducted to confirm this hypothesis. Furthermore, plant exudates secreted into the rhizosphere can significantly affect the microbial community structure and activity affecting important processes in the soil such as nutrient cycling (Dessaux et al. 2016). Therefore, the effects of biochar on plant internal and external exudates of sugars need more attention.

Increased root exudation of citrate under low P levels have been reported for hydroponic studies of maize (Jones and Darrah 1995; Gaume et al. 2001). However, under soil grown conditions the relationship between citrate and P nutrition seems more devious, and soil properties seem to have a substantial influence on the citrate concentration in the rhizosphere (Gerke 2015). In this study, rhizosphere citrate concentration was strongly affected by P fertilizer (Table S1), yet P addition did not decrease the rhizosphere citrate concentration as would be expected from hydroponic studies with maize (Gaume et al. 2001) (Fig. 6). Wouterlood et al. (2006) found that the citrate content in the rhizosphere of two soil grown cultivars of chickpea increased with increasing P fertilization. In accordance, we found that citrate concentration was positively correlated with resin P (Pearson corr.  $p = 0.0027$ ,  $r = 0.51$ ) and  $\text{NaHCO}_3$  Pi (Pearson corr.  $p = 0.0003$ ,  $r = 0.61$ ). Gerke (2015) reviewed that soil sorption capacity and sorption mechanism may control the extent of water extractable citrate in the rhizosphere, where the presence of Fe and Al (hydr)oxides results in lower extractable citrate compared to soils where Ca-citrate precipitates dominate the sorption mechanism. Furthermore, citrate sorption

to goethite was found to decrease in the presence of phosphate and with increasing pH (Geelhoed et al. 1998). Accordingly, Wouterlood et al. (2006) reported that the highest rhizosphere concentration of carboxylates was found in soil with high total-P, high Colwell-P, low phosphorus retention index (PRI), and low reactive Fe concentration. Thus, a tendency towards a higher rhizosphere citrate concentration in P treatments might be related to the competition between phosphate and citrate for sorption sites. Accordingly, extractable  $\text{NaHCO}_3$ -Pi and  $\text{NaOH}$ -Pi in the rhizosphere increased with increasing P addition, indicating a stronger occupation of P at these sorption sites with (Fig. 4). Furthermore, the highest rhizosphere citrate concentration was found in RH-100P and OSR-100P treatments, thus, an increase in pH by application with especially OSR might have further decreased the citrate sorption. Lastly, an increase in microbial activity and changes in the microbial community structure with increasing nutrient availability and pH could have affected the citrate secretion by microorganisms; however, no such data was determined in this study.

#### Biochar and P fertilizer effects on phosphatase activity in the rhizosphere

In a review by Margalef et al. (2017) the authors found that acid phosphatase activity was positively correlated with available organic P, whereas alkaline phosphatase activity was positively correlated with Olsen-P (plant available inorganic P) in natural soils. Accordingly, we found a significant relationship between acid phosphatase activity and  $\text{NaHCO}_3$  Po (Pearson corr.  $p = 0.04$ ,  $r = 0.32$ ) (Fig. 4), whereas alkaline phosphatase activity correlated positively with resin P (Pearson corr.  $p = 0.04$ ,  $r = 0.31$ ) and  $\text{NaHCO}_3$  Pi (Pearson corr.  $p = 0.002$ ,  $r = 0.52$ ). However, studies have found that application of inorganic P can suppress phosphatase activity due to the inhibition of PHO genes (Nannipieri et al. 2011 and references therein). Yet, we found no indications for an inhibitory effect of inorganic P on acid or alkaline phosphatase activity in this study (Fig. 5). On the contrary, in compliance with our results increased alkaline phosphatase activity in biochar amended soils relate to an increase in soil pH (Bera et al. 2016; Marzooqi and Yousef 2017). Consequently, a significant positive correlation between alkaline phosphatase activity and rhizosphere pH was observed, while acid phosphatase did not correlate with pH (Fig. 5). Furthermore,



it appears, that in studies where biochar application decreased or had no effect on alkaline phosphatase activity, the bulk soil pH was relatively high (>8) (Foster et al. 2016; Zhang et al. 2017; Liu et al. 2017). An initial high soil pH may reduce the effect of biochar application or biochar may increase soil pH far above the optimum for alkaline phosphatase activity. This indicates that the positive effects of biochar on alkaline phosphatase activity are more likely to occur in acid to neutral soils when pH increases. However, even though OSR had the strongest effect on alkaline phosphatase activity (50% increase) and resulted in the greatest initial increase in pH (1.4 units), the alkaline phosphatase activity also increased by approximately 30% in the SW-0P compared to the CK-0P even though the initial increase in pH was only 0.3 pH units (Table 2 and Fig. 5). Correspondingly, factors other than pH such as increased microbial biomass (Li et al. 2017) or a potential shift in the microbial community structure (Ventura et al. 2014) may be responsible for the increased alkaline phosphatase activity in the presence of biochar.

## Conclusion

In compliance with our first hypothesis, biochar affected both biological and chemical P processes in a plant-soil-biochar system, and these effects were driven by biochar properties. RH and OSR increased the readily available P pools (Resin P and  $\text{NaHCO}_3$  Pi), whereas SW did not have any pronounced effects on any P pools. The increase in readily available P was reflected in maize biomass and P uptake, suggesting that crop residue biochars have a higher P fertilizing effect compared to SW biochar. P fractionation results after plant growth indicated that some of the dissolved P from the biochars moved to less available P pools over time. In P-fertilized treatments, small differences between the biochar treatments with regard to readily available P pools could only be observed after plant growth. Thus, contradictory to our second hypothesis, a significant interaction between P fertilizer and biochar potentially increasing or decreasing P bioavailability was not observed in this study.

Increased alkaline phosphatase activity in biochar treatments was positively related to soil pH. However, even a small change in pH induced by SW resulted in a significant increase in alkaline phosphatase activity, suggesting that a combination of biochar properties and not only the pH effect might be responsible for this positive response

to biochar. Root exudates of especially glucose and fructose in the rhizosphere were strongly affected by biochar application, and different biochars had contrasting effects on the content of these sugars, indicating that the plant response to biochar application can alter the composition of root exudates secreted into the rhizosphere. However, more studies are needed to understand these mechanisms fully. Rhizosphere citrate concentrations were highest in OSR and RH treatments when the biochars were co-applied with P fertilizer. Thus, we suggest that both the soluble P content and soil pH affected the citrate content in the rhizosphere, due to competition between citrate and P for sorption sites.

In conclusion, an increase in readily available P is not the only beneficial effect of biochar application when considering P dynamics in a soil-plant-biochar system, and also increased phosphatase activity and rhizosphere citrate concentration was observed. However, the interaction between biochar properties, soil type, and P fertilizers needs to be further investigated before we can draw more general conclusions about these effects.

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