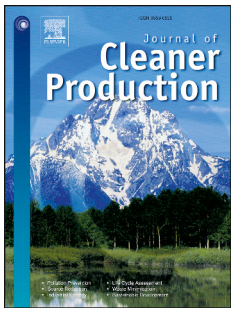
Journal Pre-proof



Hydrothermal carbonization of microalgae for phosphorus recycling from wastewater to crop-soil systems as slow-release fertilizers

Qingnan Chu, Tao Lyu, Lihong Xue, Linzhang Yang, Yanfang Feng, Zhimin Sha, Bin Yue, Robert J.G. Mortimer, Mick Cooper, Gang Pan

PII: S0959-6526(20)34671-0

DOI: https://doi.org/10.1016/j.jclepro.2020.124627

Reference: JCLP 124627

To appear in: *Journal of Cleaner Production*

Received Date: 25 May 2020

Revised Date: 13 September 2020

Accepted Date: 7 October 2020

Please cite this article as: Chu Q, Lyu T, Xue L, Yang L, Feng Y, Sha Z, Yue B, Mortimer RJG, Cooper M, Pan G, Hydrothermal carbonization of microalgae for phosphorus recycling from wastewater to crop-soil systems as slow-release fertilizers, *Journal of Cleaner Production*, https://doi.org/10.1016/j.jclepro.2020.124627.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Elsevier Ltd. All rights reserved.

Journal of Cleaner Production, Volume 283, February 2021, Article number 124627
DOI: 10.1016/j.jclepro.2020.124627

Hydrothermal carbonization of microalgae for phosphorus recycling from wastewater to crop-soil systems as slow-release fertilizers

Qingnan Chu[1,2], Tao Lyu[2,3], Lihong Xue[1,4],* Linzhang Yang,[1] Yanfang Feng[1,4], Zhimin Sha,[5] Bin Yue,[6] Robert J. G. Mortimer,[2] Mick Cooper,[2] Gang Pan[2]*

[1] *Key Laboratory of Agro-Environment in Downstream of Yangtze Plain, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China*

[2] *School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Nottinghamshire, NG25 0QF, UK*

[3] *Cranfield Water Science Institute, Cranfield University, College Road, Cranfield, Bedfordshire, MK43 0AL, UK*

[4] *School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, 212001, China*

[5] *Graduate School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai, 200240, China*

[6] *College of Geography and Environmental Engineering, Lanzhou City University, Lanzhou, Gansu 730070, China*

Corresponding authors:

*These authors contribute equally to this work

Prof. Lihong Xue (njxuelihong@gmail.com)

Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, China.

Prof. Gang Pan (gang.pan@ntu.ac.uk)

School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Nottinghamshire, NG25 0QF, UK

Journal Pre-proof

Journal Pre-proof

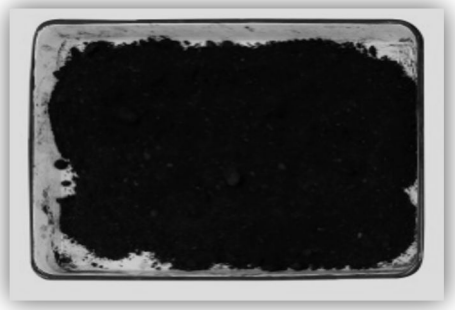
P removal by *Microcystis sp.*

(88.4% P)

Poultry Wastewater

(P 41.3ppm)

P recycle



Hydrothermal carbonization

(91.5% P recovery)

Soil P

Alkaline phosphatase

Soluble P

Exchangeable P

Wheat P

34.4% PUE

21.6% Yield

Abstract

Due to the finite stocks of phosphate rock and low phosphorus (P) use efficiency (PUE) of traditional mineral P fertilizers, more sustainable alternatives are desirable. One possibility is to culture microalgae in wastewater to recover the P and then convert the microalgae biomass into slow-release fertilizers through hydrothermal carbonization (HTC). Therefore, this study aimed to recycle P from wastewater to agricultural field using microalgae and HTC technology. *Chlorella vulgaris* (CV) and *Microcystis sp.* (MS) were cultured in poultry farm wastewater with an initial concentration of 41.3 mg P kg^{-1}. MS removed 88.4% P from the wastewater, which was superior to CV. CV- and MS-derived hydrochars were produced at 200 or 260°C, in solutions using deionized water or 1*wt%* citric acid. The MS-derived hydrochar using 1 *wt%* citric acid solution at 260 °C (MSHCA260) recovered the highest amount of P (91.5%) after HTC. The charring promoted the transformation of soluble and exchangeable P into moderately available P (Fe/Al-bound P), and using citric acid solution as feedwater increased the P recovery rate and formation of Fe/Al-bound P. With the abundant moderately available P pool, hydrochar amendment released P more slowly and enhanced the soil P availability more persistently than chemical fertilizer did, which helped to improve PUE. In a wheat-cultivation pot experiment, MSHCA260 treatment improved wheat PUE by 34.4% and yield by 21.6% more than chemical fertilizer did. These results provide a novel sustainable strategy for recycling P from wastewater to crop-soil systems, substituting the mineral P fertilizer, and improving plant PUE.

Keywords: hydrochar; microalgae technology; phosphorus fractionation; phosphorus use efficiency; sustainable development; wheat

Journal Pre-proof

Published by Elsevier. This is the Author Accepted Manuscript issued with: Creative Commons Attribution Non-Commercial No Derivatives License (CC:BY:NC:ND 4.0). The final published version (version of record) is available online at DOI:10.1016/j.jclepro.2020.124627. Please refer to any applicable publisher terms of use.

1. Introduction

Phosphorus (P) is an essential plant nutrient and makes up around 0.2% of plant dry weight (Václavková et al., 2018; Adegbeye et al., 2020). Nevertheless, soil P exists in pools of low availability and thus becomes one of the major factors limiting crop growth, affecting approximately 30% agricultural fields worldwide (Xu et al., 2019; B. Li et al., 2020). Consequently, a vast amount of P fertilizers is required for agricultural production. However, P-based synthetic fertilizers rely on P extracted from phosphate rock which is a finite non-renewable resource that might be depleted in 50-100 years (Withers et al., 2020). In addition, crops take up only 30-45% of the supplied P from synthetic P fertilizer (Shen et al., 2011; Oita et al., 2020). The P that is not incorporated into the plants is washed into waterbodies through leaching and runoff, causing environmental issues (Pan et al., 2018; Lee et al., 2020). Therefore, it is crucial to seek alternatives to chemical fertilizers and to develop methodologies that improve P use efficiency (PUE) by crops, while minimizing the negative environmental impacts.

Wastewater contains plentiful P that requires removal prior to discharge into watercourse. Microalgae have been shown to grow rapidly in such wastewater, efficiently removing P (Cabanelas et al., 2013; Subramaniyam et al., 2016; Huo et al., 2020). Microalgae are capable of absorbing inorganic P in excess through storage within their cells in the form of polyphosphate granules (Delgadillo-Mirquez et al., 2016)(Solovchenko et al., 2019). Previous studies reported that microalgae can accumulate large quantities of P (up to 2-4% of their cell dry weight), and thus have potential to be applied as fertilizer after appropriate processing (Cabanelas et al., 2013; Santos and Pires, 2018; Luo et al., 2019). Therefore, reclaiming P from wastewater streams with microalgal cultures is a sustainable and environmental-friendly solution to the shortage of phosphate rock. In the last decade, direct application of dried microalgae as an alternative to chemical P fertilizer has been evaluated (Ray et al., 2013; Mukherjee et al., 2015; Schreiber et al., 2018). A major concern is that the

Journal Pre-proof

polyphosphate-rich biomass releases the phytoavailable P too slowly into soil to satisfy the demands of growing plants. Moreover, microalgal toxins, such as microcystin and cyanotoxin, potentially threatens both soil microbial activity and plant growth if microalgae are directly applied to soil (Machado et al., 2017). These factors have driven the researchers to explore additional treatments to enhance the fertilizer values of microalgal biomass prior to its use in an agricultural context.

One such potential tool for increasing PUE is the application of biochar (Anyaoha et al., 2018; Bornø et al., 2018; Fei et al., 2019; H. Li et al., 2020). Pyrolysis is the thermal treatment of biomass in absence of air at temperatures of 400-600°C, converting dry biomass into pyrochar (Foong et al., 2020). Hydrothermal carbonization (HTC) converts wet biomass to hydrochars at lower temperature (180-260 °C) (Hao et al., 2018; Cui et al., 2020). The higher hydrothermal temperature than 260°C might lead to the increased generation of noxious compounds in hydrochars, including phenols and organic acids (Hao et al., 2018). Compared with pyrolysis, HTC is generally more energy-efficient and, since it is carried out in water, wet microalgae can be directly processed without prior dehydration (Lachos-Perez et al., 2017). More importantly, the hydrolysis reaction occurring in HTC process can promote the degradation of polyphosphate into orthophosphate, with over 90% P present as orthophosphate in sewage sludge- or manure-derived hydrochars (Heilmann et al., 2014; Huang and Tang, 2016; Idowu et al., 2017). In addition, the predominant chemical P fraction in hydrochars is iron (Fe)/ aluminum (Al)-bound P (Huang and Tang, 2016; Wang et al., 2017), which is considered a moderately labile P pool for plants and acting as a buffer for available P in soil (Yao et al., 2013; Heilmann et al., 2014; Fei et al., 2019).

Biochar can also improve soil health by increasing soil electrical conductivity (EC), organic matter content, surface area, and nutrient availability (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020c). The microporous structures, surface functional groups, and intrinsic minerals of hydrochar could improve the capacity of nutrients adsorption and retention in soil (Yu et al., 2019; Chu et al., 2020a, 2020c), potentially avoiding P loss and improving plant PUE. Also, remarkable alterations of the

Journal Pre-proof

microbial community structure in biochar-amended soil have been reported (Ye et al., 2019; Lu et al., 2020), possibly by affecting phosphatase activity secreted by soil microorganisms and consequently, by P solubilization. These beneficial properties, plus the increased moderately labile P pool present within microalgae-derived hydrochars, are likely to improve the PUE, nutrients retention, and crop growth.

This study aims to achieve P recycling from wastewater to food through the recovery of P from wastewater using microalgae, converting the biomass into hydrochar by HTC, and applying the microalgae-derived hydrochars to a crop-soil system. The specific objectives of this work included 1) investigating the fate of P from wastewater to hydrochar and then to the crop-soil system; 2) screening the most suitable microalgae-derived hydrochar to improve the PUE compared to traditional synthetic P fertilizer.

2. Methods and materials

2.1. Microalgal cultivation and harvest

Chlorella vulgaris strain CCAP 211/12 and *Microcystis sp*. strain CCAP 1450/13 were used in this study and purchased from Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, Scotland. The wastewater was collected from the poultry farm at Nottingham Trent University's Brackenhurst Campus and filtered before culturing microalgae. The trials of P removal from wastewater by culturing microalgae were carried out in 3 L borosilicate bioreactors in the glasshouase of Brakenhurst campus, Nottingham Trent University, UK. The culturing conditions were: constant aeration (4 mL s^{-1}), photoperiod of 14h:10h light:dark cycles, at a controlled temperature of 25 ± 1°C under cool white fluorescent light of 10000 lux intensity. The chemical characterization of wastewater is shown in **Table S1**. The initial total P (TP) concentration in the wastewater was 41.3 mg L^{-1}. Three replicates were conducted for each microalgal strain. The dry weight (DW) of microalgae was gravimetrically assessed every two days according to standard method 2540-D (APHA, AWA, WPCF 1992) and reached the stationary phase in

Journal Pre-proof

wastewater after 14 days. Also, TP of wastewater was analyzed every two days using an auto analyzer (AQ400, SEAL Analytical GmbH, Germany) in order to monitor the P removal rate. At the end of culture total nitrogen (TN) were measured colorimetrically as nitrate after the water samples had been oxidized and total organic carbon (TOC) were measured using an organic carbon analyzed by an organic carbon analyzer (TOC-C_{CSN}, Shimadzu). Afterwards, the microalgae were collected by flocculation. The methods of flocculation were the same as detailed in our previous study (Li and Pan, 2013), and are included in the Supplementary Information. The flocculation efficiency of both CV and MS was more than 95% (**Fig. S1**).

2.2. Microalgae-derived hydrochars preparation

HTC of microalgae was conducted in a 600 mL Teflon lined stainless steel hydrothermal reactor (Parr Instruments, Moline, IL, USA), using a solid:liquid ratio of 1:9 (w/w). The wet microalgal biomass was directly mixed with the feedwater and the final solid/liquid ratio was calculated based on the moisture content. Eight types of hydrochars were produced using two different microalgae under two different feedwaters (deionized water and 1 *wt.%* citric acid solution) and two different reaction temperatures (200 and 260 °C). For each run, the reactor was heated to 200 or 260 °C at 3 °C min^{-1}, and held at the final temperature for a duration of 2 h. The pressures originating from feedwater alone at the respective reaction temperatures were not monitored. The reactor was rapidly cooled down to room temperature using a recirculating condensing engine. The solid and liquid products were initially separated by centrifugation and fully gravity-filtered through a 0.45 μm membrane. The total solid recovery rate was recorded.

2.3. Characterization of microalgae-derived hydrochars

The pH of the hydrochars was analyzed using a solid/deionized water ratio of 1:2.5 (w/v). The specific surface area (SSA) and porosity were measured using a NOVA 1200 analyzer (Anton Paar QuantaTec Inc., Graz, Austria), and were

Journal Pre-proof

calculated by the Brunauer-Emmett-Teller method (Yu et al., 2019). Total C, H, N, and S contents were determined using an Elemental Analyzer (EL III; elemental Analysensysteme GmbH, Germany). Concentrations of metallic elements, including K, Al, Ca, Fe, and Mg were determined by firstly digesting the hydrochars using HNO_3 (61%) with hydrogen peroxide and then analyzing the digests using inductively coupled plasma-optical emission spectrometry (ICP-OES), as described in a previous study (Chu et al., 2019).

The sequential extraction of the microalgae-derived hydrochars were carried out to evaluate the fractions of P present, following previous studies (Hedley et al., 1982; Bornø et al., 2018) as shown in **Fig S2**. P fractionation in chars can be separated into soluble P, exchangeable P, alkaline-dissolved and organic P, acid-dissolved and organic P, and residual P fractions. The solids were separated from the extract after each batch of extraction via centrifugation at 8000 *g* for 5 min, and the supernatant was filtered using a 0.45 μm membrane filter. The P concentrations in extracts were analyzed colorimetrically by auto-analyzer. TP concentrations of hydrochars were calculated by summation of all the P fractions. The P recovery rate was calculated according to the following formula:

$P_{recover} = (P_{total} \times \lambda / P_{feedstock}) \times 100\%$;

where P_{total} is the TP content in the hydrochar, $P_{feedstock}$ the TP content in the feedstock, and λ represents the yield of the hydrochar.

2.4. Soil incubation experiment

The soil used in the incubation experiment was collected from the top soil of Embleys farm in the UK (0-15 cm; 29% clay, 42% silt, 29% sand). The soil had the following basic properties: pH 7.7, organic matter content 2.1%, EC 0.52 mS cm^{-1}, cation exchange capacity (CEC) 2.42 cmol kg^{-1}, total N 1.2 g kg^{-1}, TP 0.63 g kg^{-1}, total K 3.2 g kg^{-1}, Olsen-P 12.1 mg kg^{-1}. Soils and hydrochars were air-dried, sieved through 2 mm mesh, and mixed to ensure a relatively homogeneous distribution. 100 g of the top soil were placed in the 200 mL transparent plastic jars for soil incubation experiments. The jars were covered with loose lids to allow air circulation but to

Journal Pre-proof

minimize water evaporation. Treatments were as follows: Untreated soil (no chemical fertilizers or hydrochars were applied), control (chemical fertilizers were applied), CV (dried powder of *Chlorella vulgaris*), CVHCA200 (CV-derived hydrochar using 1 *wt%* citrate acid solution as feedwater; 200 °C HTC), MS (dried powder of *Microcystis sp.*), MSHCA260 (MS-derived hydrochar using 1 *wt%* citric acid solution as feedwater; 260 °C HTC). CVHCA260 and MSHCA260 were selected because they had the highest P recovery rate of the *Chlorella vulgaris*- or *Microcystis sp.*-derived hydrochars. The chemical fertilization control contained 500 mg N kg soil^{-1} in the form of NH_4NO_3, 100 mg P kg soil^{-1} in the form of KH_2PO_4, and 300 mg K kg soil^{-1} in the form of K_2SO_4. In the hydrochar treatments, the application rate of hydrochars was 0.5 *wt%* of the soil and chemical fertilizers were additionally supplemented to achieve the equivalent rates with the N, P, and K rates used in the chemical fertilization control. Each treatment comprised four replicates. Incubation lasted for 120 days in an illuminated incubator at 25 °C. The soils were sampled at 0, 10, 30, 50, 80, and 120 days. During the incubation period, deionized water was added every two days to maintain a field water-holding capacity at 60% (w/w).

2.5. Wheat pot experiment

The experiments used 5L plastic pots, each of four kilograms of air-dried soil sieved to pass through a 2 mm mesh. A filter paper was placed at the bottom of the pots to prevent soil loss. Before cultivating wheat, hydrochars were mixed with soil and the pots were incubated for four weeks in a greenhouse under moderately moist conditions (60% field water-holding capacity). Wheat seeds were pre-germinated in a petri dish covered with a filter paper and kept in the dark for three days. After the preincubation period, five germinated wheat seedlings were carefully transplanted to each pot and thinned to one after one week. The design of treatments was the same as described in the soil incubation experiment (2.4). Each treatment comprised four replicates.

The wheat plants were harvested at the tillering (20 days after transplantation) and maturation stages (120 days after transplantation). In order to satisfy the

Journal Pre-proof

196 requirement of sampling at two different growth stages, two batches of experiments
197 were conducted at the same time. Rhizosphere soil samples were collected by
198 carefully cleansing the soil from the roots (Chu et al., 2017; Sha et al., 2020). The soil
199 samples were divided into two parts: one portion was freshly prepared for the
200 determination of enzyme activity and soil microbial C and P content, and another
201 portion was air-dried for analysis by sequential P fractionation. In fresh soil samples,
202 the concentration of microbial biomass C (MBC) and P (MBP) were investigated
203 using the chloroform fumigation-extraction method (Brookes et al., 1982). Acid and
204 alkaline phosphatase activities in the soil samples were determined as described in a
205 previous study (Bornø et al., 2018). In dried soil samples, the soil pH was analyzed in
206 a slurry of 1:2.5 (w/v, soil to water) using a pH-meter. Soil organic matter (SOM) was
207 measured using the potassium dichromate oxidation method. Soil total N (TN) was
208 determined by initially digesting with H_2SO_4 (98%) and then using the Kjeldahl
209 method (Chu et al., 2016a). CEC was measured using the compulsive exchange
210 method with 1.0 M ammonium acetate extraction at pH 7.0 (Brookes et al., 1982).
211 The analysis of soil P fractionation was same for the hydrochars, as described above
212 (2.3).

213 *2.6. Statistical analyses*

214 All statistical analyses were performed using SPSS version 23.0 (SPSS Inc.
215 Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate the
216 significant difference at a $P < 0.05$ probability level with Duncan's multiple range
217 test.

218 ## 3. Results

219 *3.1. Growth of microalgae and P removal in the wastewater*

220 The microbial growth curves were plotted showing the values of biomass (as
221 dry weight) in wastewater versus time (in days) (**Fig 1**). CV and MS both exhibited
222 exponential growth and reached a stationary phase after 8 days. After 14 days culture,

Journal Pre-proof

the biomass (dry matter) of MS reached the maximum value (1.14 g_{dw} L^{-1}), 8.5% higher than that of CV (**Table S2**). The average biomass productivities of CV and MS were 0.068 and 0.071 g_{dw} L^{-1} d^{-1}, respectively. The plot of P removal in the wastewater versus time (in days) is also shown in **Fig 1**. From 0 to 8 days, the P concentration in the MS culture declined from 41.3 to 4.8 mg L^{-1}, with a daily P removal rate of 2.95 mg L^{-1} day^{-1}, and in the CV culture from 41.3 to 8.8 mg L^{-1}, with a daily P removal rate of 2.32 mg L^{-1} day^{-1}(**Table S2**). After 14 days culture, the maximum P removal rate by MS was 10.7 mg L^{-1} day^{-1}, which was 23.4% higher than that of CV (**Table S2**). Overall, after 14 days culture, MS and CV removed 88.4% and 78.7% P, respectively, from an initial concentration of 41.3 mg P L^{-1}; both microalgae were demonstrated to be able to remove and enrich P from wastewater efficiently although MS was superior to CV in this respect. In addition to the P removal, after 14 days the TN concentration in CV and MS culture declined from 321.6 mg L^{-1} to 182.1 and 160.4 mg L^{-1}, TOC from 375.2 to 53.2 and 34.8 mg L^{-1}, respectively, suggesting that with the fast growth the microalgae possibly absorbed and assimilated the N and C at a high rate from the wastewater as well.

3.2. Basic physiochemical characteristics of hydrochars

The physiochemical characteristics of the microalgae and microalgae-derived hydrochars are displayed in **Table 1**. The microalgae-derived hydrochars using deionized water as feedwater all exhibited an alkaline pH after processing. Using citric acid as feedwater markedly neutralized the alkalinity of hydrochars from 7.2-8.5 to 5.7-6.6. With hydrothermal temperature decreasing from 260 °C to 200 °C, the lower pH was observed in hydrochars, irrespective of microalgae strain. Transforming the microalgae into hydrochars decreased the C, H, N, and S concentration, irrespective of microalgae strain (**Table 1**). As a consequence of the vaporization, degradation, and dissolution processes of labile fractions occurring during HTC, elements including C, H, N, and S were partially lost to feedwater, whereas conservative elements such as P and metals were retained in the hydrochars (**Table 1 and 2**). The C concentration in hydrochars using citric acid as feedwater ranged from

Journal Pre-proof

53.9-66.2%, which is 1.5-6.3% higher than that in hydrochars where deionized water was used as feedwater, while H and N concentration decreased, resulting in higher C/N and lower H/C ratio.

Moreover, the hydrochars using citric acid as feedwater showed a markedly higher concentration of metals, including Al, Ca, Fe, and Mg, irrespective of microalgal strain processed (**Table 1**). The increased abundances of these elements were possibly beneficial for P bonding in hydrochars. Additionally, as a metal with high mobility, K in hydrochars showed an opposite trend to other metals. Using citric acid as feedwater during HTC reduced K accumulation in hydrochars compared to those when using deionized water. In addition, different reaction conditions during HTC changed the adsorptive capacity of hydrochars (**Table 1**). When compared to the raw microalgae, hydrochars markedly increased the SSA and porosity. The SSA for hydrochars using citric acid as feedwater during HTC ranged from 5.8-6.7 m^2 g^{-1}, which was 16.6-18.2% higher than that in hydrochars using only deionized water as feedwater.

3.3. Recovery rate of P in hydrochars

As displayed in **Table 2**, the charring process resulted in an increased P content in the hydrochars. The P content in CV-derived hydrochars ranged from 3.4±0.2–4.3±0.4 %, which was 23.1-67.9% higher than that of raw CV, and in MS-derived hydrochars ranged from 4.2±0.3–5.8±0.4%, which was 14.7-72.2% higher than that of raw MS. Moreover, with increasing hydrothermal temperature from 200 to 260°C the TP increased from 3.2-4.1% to 3.5-4.1% in CV-derived hydrochars, and from 3.9-5.4% to 4.5-6.2% in MS-derived hydrochars. In addition, TP in MS-derived hydrochars varied from 3.9-6.2%, which was 21.9-31.9% higher than that in CV-derived hydrochars. This result might be attributed to the higher P uptake by MS in wastewater (**Fig. 1**).

With hydrothermal temperature increasing from 200 °C to 260 °C, in contrast with P recovery, the solid recovery rate of hydrochars declined from 40.7-49.8% to 37.3-45.4% in CV-derived hydrochars, and from 44.6-58.8% to 42.1-55.2% in

Journal Pre-proof

MS-derived hydrochars. This result might be ascribed to the degradation of polymeric materials (such as hemicellulose and cellulose) at higher temperatures during HTC. Moreover, using citric acid solution as feedwater during HTC markedly increased the hydrochar yield and TP, irrespective of microalgal strain. The solid recovery rate of microalgae-derived hydrochars using citric acid solution ranged from 41.1-62.0%, but that of hydrochars using deionized water ranged from 32.3-46.3%. Also, for CV, P content in CVHCA200 and CVHCA260 was 9.4-27.0% higher than that in CVHW200 and CVHW260; for MS, P concentration in MSHCA200 and MSHCA260 was 21.6-28.2% higher than that in MSHW200 and MSHW260. The highest P recovery rate for CV and MS were both attained by using citric acid as feedwater, 72.3% in CVHCA260 and 91.5% in MSHCA260.

3.4. Fractionation of P in hydrochars

The results of the sequential P fractionation of the microalgae and microalgae-derived hydrochars are presented in **Fig. 2**. The charring process significantly reduced soluble, exchangeable, and residual P fractions, but increased largely the Fe/Al-bound and Ca-bound P fractions. The soluble and exchangeable P generally correspond to the phytoavailable P. This phytoavailable P constituted 34.0% of the *Chlorella vulgaris* and 27.9% of the *Microcystis sp.*, whereas less than 20% was detected in derived hydrochars (e.g., 8.8% in MSHCA260) (**Table S5**). Additionally, with increasing hydrothermal temperature from 200 to 260 °C, the soluble P fraction decreased sharply, irrespective of microalgal strains.

However, the charring process significantly improved the Fe/Al-bound P fraction by 2.1-3.4 and 1.7-3.8 fold for CV and MS, and the Ca-bound P fraction by 2.7-4.7 and 1.4-2.5 -fold for CV and MS, respectively (**Fig. 2**). Fe/Al-bound P was the largest P fraction in microalgae-derived hydrochars, ranging from 32.8-52.7%. In addition, using citric acid solution as feedwater during HTC significantly increased the Fe/Al- and Ca-bound P fractions. The Fe/Al- and Ca-bound P in CVHCA200, CVHCA260, MSHCA200, and MSHCA260 ranged from 15.2-30.5% and 5.8-12.6%, which was 23.6-64.0% and 7.4-90.9% higher than those in CVHW200, CVHW260,

Journal Pre-proof

MSHW200, and MSHW260, respectively. Using citric acid solution as feedwater probably better facilitated the bonding between those metallic elements present and P.

Overall, HTC promoted the P transformation from readily available and recalcitrant fractions to potentially available fractions and using citric acid as feedwater sharpened such transformation. Fe/Al-bound P occupied 46.2% and 51.5% in CVHCA260 and MSHCA260, but only 19.5% and 20.1% in the raw CV and MS (**Table S5**), suggesting that P release in hydrochars will be possibly more sustainable for satisfying the long-term demand of plant growth.

3.5.P release from microalgae-derived hydrochars to soil

The raw microalgae and microalgae-derived hydrochars were incubated in the soil for 120 days, in order to investigate the release capacity of phytoavailable P, as shown in **Fig. 3**. The unfertilized soil and soil applied with chemical fertilizers were regarded as control groups. CVHCA260 and MSHCA260 were selected in this experiment because of their highest TP (**Table 2**) and highest moderately labile P pool (**Fig. 2**) among the respective microalgae-derived hydrochars. At 10 days after incubation, the available P concentration in soils treated with chemical fertilizer was remarkably and consistently higher than that in other treatments. However, after 50 days, the two hydrochar treatments, CVHCA260 and MSHCA260, significantly and persistently improved the concentration of soil available P compared to the chemical fertilizer group. At 120 days after incubation, the soil available P concentration under CVHCA260 and MSHCA260 treatment was 47.7% and 56.3% higher than that for the chemical fertilizer group, respectively. In addition, from 10 to 30 days the available P concentration in soils treated with CV and MS were consistently higher than those treated with CVHCA260 and MSHCA260, however, an opposite trend was observed from 30 to 120 days after incubation. At 120 days after incubation, soil available P concentration under CVHCA260 treatment was 10.9% higher than that under CV treatment, and under MS260 treatment was 21.0% higher than that under MS treatment. From 50 to 120 days, the highest soil available P concentration was

Journal Pre-proof

consistently detected in the MSHCA260 treatment, although a decreasing trend was detected for all the groups.

3.6. Rhizosphere soil properties

The properties of rhizosphere soils amended with microalgae or microalgae-derived hydrochars at the ripening stage of wheat are shown in **Table S6**. CVHCA260 and MSHCA260 significantly reduced soil pH by 0.4-1.6 units compared to the control. CV, MS, CVHCA260, and MSHCA260 significantly improved the SOM by 19.8%, 12.3%, 25.8%, and 26.6% respectively compared to that in control. Also, amendment by MSHCA260 significantly improved the CEC compared to the control and the two microalgae-derived hydrochars significantly improved the CEC compared to the control, and to the CV- and MS- amended soils. Moreover, with addition of CV and MS, soil total N was maintained at a similar level to that of the control, however, amendment by CVHCA260 and MSHCA260 significantly reduced soil TN by 33.3% and 42.9%, respectively, compared to the control. Because of effects on SOM and TN, CV and MS addition significantly increased soil C/N ratio compared to control, by 62.3% and 92.4% for CVHCA260 and MSHCA260 respectively.

The incorporation of microalgae and microalgae-derived hydrochars to soil affected microbial activity, as shown in the results of soil phosphatase activity in **Fig. S3** and MBC and MBP content in **Fig. S4**. The introduction of C from microalgae or microalgae-derived hydrochars into soil significantly improved the soil MBC, irrespective of the growth stages, compared to the control. However, soil MBP displayed different results. At the tillering stage, no significant difference was detected for MBP, while at the ripening stage, CV and MS addition significantly reduced MBP by 26.2% and 33.9% compared to the control, and significantly lower MBP were detected in CVHCA260 and MSHCA260 treatment (reduced 71.9% and 79.5%). Different treatments affected the phosphatase activity in rhizosphere soil. No significant differences were detected for acidic phosphatase activity among treatments. However, alkaline phosphatase activity significantly improved either at tillering or

Journal Pre-proof

ripening stages for CV, MS, CVHCA260, and MSHCA260 treatments when compared to the control; alkaline phosphate activity in the rhizosphere soil under CVHCA260 and MSHCA260 treatments was 39.5-42.9% higher than that under CV and MS treatment at the tillering stage, and 51.7-56.0% higher at the ripening stage.

3.7. P fractionation in rhizosphere soil of wheat

The results of the sequential P fractionation of rhizosphere soil from a wheat pot experiment are shown in **Fig. 4**. The results of labile and stable P pools in the rhizosphere soil are shown in **Fig. S5**. Notably, the addition of CV, MS, CVHCA260, and MSHCA260 significantly reduced soluble P by 4.9-, 3.8-, 3.5-, and 3.6-fold, and exchangeable P by 47.4%, 82.6%, 80.5%, and 68.3%, respectively, at the tillering stage. However, an opposite varying trend was observed at the ripening stage; the addition of CV, MS, CVHCA260, and MSHCA260 increased soil labile P pool (sum of soluble and exchangeable P fraction) by 1.7-, 1.6-, 1.8-, and 2.1-fold compared to the control, respectively (**Fig. S5**). Compared to CV and MS, MSHCA260 treatment resulted in a higher labile P pool at the ripening stage, suggesting that addition of CVHCA260 could maintain a higher level of soil available P for a longer period.

Fe/Al-bound P is defined as the moderate P pool because the P bound to Fe and Al (hydr)oxides is not directly absorbed by plants but gradually becomes soluble. The significantly higher Fe/Al-bound P concentrations were detected at the tillering stage in the soils treated with CV, MS, CVHCA260, and MSHCA260, whereas the opposite trend was detected at the ripening stage (**Fig. 4C**). In contrast with these treatments, the Fe/Al-bound P in the control became higher as plants matured, suggesting that, at an early growth stage of wheat, the readily available P from chemical P fertilizer became gradually bound to Fe and Al (hydr)oxides. Despite the highest Fe/Al-bound P concentration (**Fig. 2**) among all hydrochars, the lowest soil Fe/Al-bound P fraction was detected in the MSHCA260 treatment at the ripening stage (**Fig. 4C**).

The sums of Ca-bound P and residual P fractions were defined as the stable P pool, because Ca-bound P usually corresponds to apatite and residual P corresponds to recalcitrant P-containing clay mineral (Hedley et al., 1982). Unlike the labile and

Journal Pre-proof

moderately available P pool, soil Ca-bound and residual P pool kept relatively stable after the addition of CV, MS, CVHCA260, or MSHCA260 (**Fig. 4D and 4E**). No significant difference was detected for soil Ca-bound P or residual P fraction among treatments except at the ripening stage, where addition of MSHCA260 significantly reduced the soil Ca-bound P fraction compared to the control.

3.8. PUE and yield of wheat

The results of PUE and yields of wheat grain are shown in **Fig. 5**. CV, CVHCA260, and MSHCA260 treatment significantly improved the plant PUE by 32.4%, 35.3%, and 34.4% compared to the control (**Fig. 5A**). Compared to the raw CV, amendment by microalgae-derived hydrochars did not significantly improved the PUE. However, among four treatments only MSHCA260 significantly improved the wheat grain yield by 21.6% (**Fig. 5B**). In addition, despite statistically insignificant difference, CVHCA260 treatment improved the grain yield by 14.5% compared to the control.

4. Discussions

4.1. Recovery of P from wastewater and conversion to microalgae-derived hydrochars

With the development of "Enhanced biological P removal", microalgae-based techniques are attracting increased attention because of the luxury uptake of P by microalgae, accumulating P up to 2-4% of their cell dry weight (Cabanelas et al., 2013; Santos and Pires, 2018; Luo et al., 2019). The cost of wastewater treatment must be counterbalanced with efficacy of P removal and production of microalgal biomass, which further produces a significant economic benefit to society (Prasad et al., 2014; Solovchenko et al., 2019). In this study, after 14 days culture in the poultry wastewater with an initial concentration of 41.3 mg P L^{-1}, MS and CV removed 88.4% and 78.7% P, respectively, showing a strong capacity to remove P from wastewater. **Table S4** compared the P removal efficiencies obtained in this study with the results in previous studies. This comparison showed that with the increased initial P

Journal Pre-proof

concentration in influent wastewater the P removal efficiency became higher. In the present study, MS and CV removed the P from wastewater (with initial P concentration of 41.3 mg L^{-1}) at 2.95 and 2.32 mg L^{-1} day^{-1}; in previous studies 8.39 mg L^{-1} day^{-1} P removal rate was observed from the wastewater at initial P concentration of 128.2 mg L^{-1} (Luo et al., 2019) and 0.55 mg L^{-1} day^{-1} P removal rate from the wastewater at initial P concentration of 8.0 mg L^{-1} (Tao et al., 2017). These results were likely attributed to the stimulated biosynthesis and storage of polyphosphate to cope with the external stress of excessive P concentration (Mujtaba et al., 2017; Shen et al., 2017; Powell et al., 2009; Solovchenko et al., 2016, 2019).

Polyphosphate is the dominant P speciation in microalgae (Powell et al., 2009; Solovchenko et al., 2019) and generally recalcitrant to degradation, making it largely unavailable for plants. HTC has been demonstrated to be able to promote degradation by polyphosphate hydrolysis in feedwater. Hence, in the present study the enriched P in microalgae was transferred to hydrochars by HTC. The P concentrations in microalgae-derived hydrochars ranged from 3.2-6.2% (**Table 2**), which were notably higher than hydrochars derived from animal manure and crop residuals (<3%) (Heilmann et al., 2014; Wang et al., 2017; Fei et al., 2019). This result demonstrated that luxury P uptake by microalgae from wastewater played an important role in producing the P-rich hydrochars. In addition, with increasing hydrothermal temperature from 200 to 260°C, the hydrochar yields were observed to decrease but the P recovery rate increased, irrespective of feedwater or microalgal strain (**Table 2**). A similar trend has been reported in the HTC treatment of swine manure (Heilmann et al., 2014), wetland plants (Cui et al., 2020), and sewage sludge (Huang and Tang, 2016). The cracking of biopolymers and P precipitation during HTC might be responsible for the higher P accumulation in higher temperature-derived hydrochars (Dai et al., 2015; Ekpo et al., 2016). The composition of feedwater was demonstrated to be an important factor for hydrochar yield and P recovery rate. Using 1% citric acid solution as feedwater significantly improved these parameters, irrespective of reaction temperature or microalgae strain. Using citric acid solution as feedwater likely promoted the bonding between metal cations and phosphate. Use of acidic feedwater

Journal Pre-proof

has previously been demonstrated to promote the release of metal cations in hydrochars (Idowu et al., 2017; Yuan et al., 2018; Cui et al., 2020) and in the transformation of organic P to inorganic P (Heilmann et al., 2014; Wang et al., 2017).

Differing reaction temperatures and feedwater composition in the HTC process affected the P fractionation in the resulting hydrochars (**Fig. 2**). The charring of microalgae significantly reduced the soluble, exchangeable, and residual P fractions, but increased the Fe/Al-bound and Ca-bound P fractions. Similar results have been reported where 44.3% readily available P was detected in the raw sewage sludge but only 7.5% was detected in the derived hydrochars (Fei et al., 2019). During HTC some orthophosphate dissolved into the feedwater and was lost, whereas most other P species bonded and adsorbed with various metals to increase retention on the hydrochars (Zhang et al., 2016). A larger proportion of microalgal P was chemisorbed by Fe/Al (hydr)oxides compared to that by Ca-containing compounds, which is comparable with hydrochars derived from sewage sludge (Huang and Tang, 2016; Fei et al., 2019), but extremely different from animal manure and other plant biomass where Ca-bound P is dominant (Heilmann et al., 2014; Dai et al., 2015; Bornø et al., 2018; Cui et al., 2020). Because PAC was used as a reagent to flocculate and collect the microalgae after culturing in wastewater (**Fig S1**), a larger amount of $AlCl_3$ was possibly remained in the microalgae and promoted the formation of the Al-bound P fractions, as reflected in the Al concentration of hydrochars (0.9-2.8%). Notably, using 1% citric acid solution as feedwater significantly increased the Fe/Al-bound P fraction in these hydrochars, irrespective of microalgal strains or reaction temperature, achieving 46.2% and 51.5% in CVHCA260 and MSHCA260. Importantly, these Fe/Al-bound P fractions can be desorbed in soil and slowly released as phytoavailable species (Yao et al., 2013; Heilmann et al., 2014; Fei et al., 2019), avoiding the P leaching or runoff due to overly fast dissolution, which occurs with chemical P fertilizer (Koppelaar and Weikard, 2013; Sha et al., 2018; Liu et al., 2020). The Fe/Al-bound P fractions are considered as moderately available and act as a buffer for available P in soils (Wang et al., 2014; Zhang et al., 2016; Cui et al., 2020). The loss of a readily-available P pool during the HTC was more than made up for by a much

Journal Pre-proof

larger increase in Fe/Al-bound P that eventually can be slowly released into the soil to support plant growth.

4.2. Application of microalgae-derived hydrochars to a crop-soil system as slow-release P fertilizer

Soil incubation and wheat pot experiments both revealed that the microalgae-derived hydrochars supplied the soil with a pool of slowly-releasable P, and consequently, the plants with more sustainable P nutrition than did traditional chemical P fertilizer. In the soil incubation experiment, amendment with CVHCA260 and MSHCA260 persistently improved the soil available P from 50 to 120 days (**Fig 3**). In the wheat pot experiment, CVHCA260 and MSHCA260 amendment significantly improved the soil soluble and exchangeable P fractions (**Fig. 4A and 4B**). The abundant Fe/Al-bound P pool in hydrochars could be an important reason for the observed slow-release of P. Sewage sludge-derived hydrochars have been demonstrated to transform the available P fraction from raw sludge to an Fe/Al-bound P fraction after HTC, possessing a strong capacity to release P in electrolyte solution (Huang and Tang, 2016; Fei et al., 2019). Similar results were also reported in the persistent increase in the soil labile P pool, following amendment with crop residue-derived biochars (Xu et al., 2016; Bornø et al., 2018). These results suggest that chemical P fertilizer was beneficial for increasing soil P availability at an early stage of plant growth whereas microalgae and microalgae-derived hydrochars were able to supply P to plants over a longer term. However, prior to utilization by plants the initial pulse of fast-release P from a chemical P fertilizer could possibly be lost by leaching, runoff, and assimilation by soil organisms, as reflected by the significantly higher soil MBP in the control (**Fig. S4**).

As the wheat grew, it is possible that the pool of moderately available P treated with microalgae-derived hydrochars gradually transformed to the labile P pool. This effect is similar with the application of organic and slow-release fertilizer (Chu et al., 2016b; Václavková et al., 2018). Root activity, leading to the exuding of organic acids into soil, and phosphatase excreted by soil microorganisms might have driven such

Journal Pre-proof

transformation (Shen et al., 2018). In the present study, the amendment of CVHCA260 and MSHCA260 greatly improved the alkaline phosphatase activity in the rhizosphere both at tillering and ripening stages (**Fig. S3**). HTC promoted the hydrolysis of macromolecules from microalgae cells, such as polyphosphate and proteins, to produce a large amount of low weight molecules in the resulting hydrochars (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020b). It is speculated that these low weight molecules were readily assimilated by soil microorganisms and thus increased microbial activity or, possibly caused a shift in the composition of the microbial community, resulting in increased levels of alkaline phosphatase, concomitantly increasing soil available P. Moreover, the charring process significantly improved the SSA and porosity (**Table 1**). Higher SSA and increased porous volume levels are extremely important for improving nutrient retention in soil because they can facilitate higher mass transfer fluxes and adsorption loading of soil nutrients (Bornø et al., 2018; Chu et al., 2020a; Lu et al., 2020), which might be another reason for the maintaining higher soil available P over a long term.

With the persistently improved soil available P pool in the rhizosphere, microalgae-derived hydrochar treatments significantly improved plant PUE (**Fig. 5A**). Notably, however, compared to CV, CVHCA260 and MSHCA260 were observed not to significantly improve plant PUE. A possible reason might be that improved P availability due to hydrochar addition exceeded the demand of plant growth. In the soil incubation experiment, the labile P pool of soils treated with hydrochars were still far higher than those of most arable soils (< 20 mg kg^{-1}) and even in excess of the recommended P application rate (40-50 mg kg^{-1}) in agricultural fields (Sha et al., 2018; Václavková et al., 2018; H. Li et al., 2020). Therefore, in future studies, a reduced application rate of hydrochars will be attempted. In addition, despite all four treatments increasing PUE, only MSHCA260 significantly increased the yield of wheat grain (**Fig. 5B**), perhaps because as well as improved soil P availability, increases soil C/N, SOM, CEC could also be contributing (**Table S6**). These factors are beneficial for nutrient mineralization and retention in soil and root morphology (Shen et al., 2011; Xu et al., 2014; Lu et al., 2020), which might also help promote

Journal Pre-proof

wheat yield production. In addition, a slightly alkaline soil (pH 7.7) was used in the present study and the CHVCA260 and MSHCA260 addition lowered the soil pH to 6.4 and 6.6, respectively (**Table S6**). The lowering soil pH was beneficial for dissolving Ca-bound P and facilitating hydrochars to provide more adsorption sites between Fe/Al and P compounds. In the present study only deionized water and 1% citric acid were used as feedwater. In case the feedwater with higher pH is attempted to produce hydrochars, e.g., 2-5% citric acids, would be acidic and thus possibly aggravate the soil acidification. Although lowering soil pH might be helpful for the increase of Fe/Al-bound P pool, the soil acidification is also harmful for root respiration and growth, as shown in the previous study that poplar sawdust-hydrochar with pH of 3.7 inhibited rice growth and yield greatly (Yu et al., 2019). Further studies should carry on to investigate the effects of different feedwater pH on P recovery in hydrochars.

In recent years the synergistic effects of hydrochar application together with chemical fertilizer on crop yield have been widely reported (Bornø et al., 2018; Yu et al., 2019; Chu et al., 2020a, 2020c). However, importantly, this study for the first time demonstrated a substitutive role of hydrochars over chemical P fertilizer to improve crop production.

5. Conclusions

The microalgae, CV and MS, both showed a strong ability for the removal of P from P-rich wastewater, and MS was superior to CV in this respect. After 14 days culture in wastewater, MS removed 88.4% P from wastewater at 2.65 mg L^{-1} day^{-1}. Then 91.5% P were recovered from the raw MS to the hydrochar MSHCA260. The P-enriched microalgae-derived hydrochars behaved as a slow-release P fertilizer to satisfy the long-term demand of growing wheat. MSHCA260 amendment improved the plant PUE by 34.4% and yield production by 21.6%. The findings from this study can be used to develop sustainable and eco-friendly strategies to recycle P from wastewater to agricultural fields for food production, which has the positive dual

Journal Pre-proof

effects of saving the cost of wastewater treatment and the production of a valuable slow-release P fertilizer to alleviate the possible future shortage of phosphate rock. A limitation of the present study is that the microalgal culture was conducted using a batch experiment under a fixed initial P concentration. The large-scale outdoor experiments where the influent flow of wastewater keeps feeding are worthy of carrying out to simulate the industrial wastewater process and quantify the P removal efficiency by microalgae. Also, the microalgae-derived hydrochars did not improve significantly the PUE compared to the raw microalgae. Thus, follow-up works are required to carry out before the large-scale field trials. Further modifications such as loading metal cations to ameliorate the P chemisorption can be attempted in addition to using citric acid solution as HTC feedwater.

Conflicts of interests

The authors declare no competing financial interest

Journal Pre-proof

Acknowledgement

We appreciate the funding by National Natural Science Foundation of China (41807099, 41877090) and Jiangsu Agricultural Science and Technology Innovation Fund (CX(19)1007). We also acknowledge the financial support of Hunan Zhongke Water Environmental Management Co., Ltd. And Yantai HABs Control and Ecological Restoration Technology Co., Ltd through their cooperation projects with NTU.

Author contributions

QC designed the experiments; GP and LX acquired the funding and supervised the research; QC, TL, BY, and MC performed the experiments; QC analyzed the data; YF and LY visualized the work; QC wrote the manuscript; TL, RM, LY, and MC reviewed and edited the manuscript; QC, GP, and LX finalized the manuscript.

595 **Supplementary Information**

596 Detailed information about the materials and methods for microalgae flocculation,
597 chemical properties of wastewater, the maximum biomass concentration, maximum
598 biomass productivity, average biomass productivity, maximum P removal rate, and
599 average P removal rate determined for CV and MS, the concentration of TN, TP, and
600 TOC in the effluent of wastewater after culturing CV and MS, comparisons between P
601 removal efficiencies and average P removal rated obtained in this study and previous
602 studies reporting microalgal in different wastewater effluents, mass content and
603 relative abundance of different P fractions in raw microalgae and microalgae-derived
604 hydrochars, the properties of rhizosphere soils amended with microalgae or
605 microalgae-derived hydrochars at ripening stage of wheat, flocculation efficiency and
606 zeta potential for flocculating microalgae, overview of the sequential P fractionation
607 procedure performed on hydrochar and soil samples, acid and alkaline phosphatase
608 activity in the rhizosphere soil, microbial biomass C and P in the rhizosphere soil,
609 labile and stable P pool in the rhizosphere soil, are presented in the **Supplementary**
610 **Information**.

Journal Pre-proof

References

Adegbeye, M.J., Ravi Kanth Reddy, P., Obaisi, A.I., Elghandour, M.M.M.Y., Oyebamiji, K.J., Salem, A.Z.M., Morakinyo-Fasipe, O.T., Cipriano-Salazar, M., Camacho-Díaz, L.M., 2020. Sustainable agriculture options for production, greenhouse gasses and pollution alleviation, and nutrient recycling in emerging and transitional nations - An overview. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.118319

Anyaoha, K.E., Sakrabani, R., Patchigolla, K., Mouazen, A.M., 2018. Critical evaluation of oil palm fresh fruit bunch solid wastes as soil amendments: Prospects and challenges. Resour. Conserv. Recycl. https://doi.org/10.1016/j.resconrec.2018.04.022

Bornø, M.L., Eduah, J.O., Müller-Stöver, D.S., Liu, F., 2018. Effect of different biochars on phosphorus (P) dynamics in the rhizosphere of Zea mays L. (maize). Plant Soil 431, 257–272. https://doi.org/10.1007/s11104-018-3762-y

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. https://doi.org/10.1016/0038-0717(82)90001-3

Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour. Technol. 131, 429–436. https://doi.org/10.1016/j.biortech.2012.12.152

Chu, Q., Sha, Z., Maruyama, H., Yang, L., Pan, G., Xue, L., Watanabe, T., 2019.

Journal Pre-proof

Metabolic reprogramming in nodules, roots, and leaves of symbiotic soybean in response to iron deficiency. Plant. Cell Environ. https://doi.org/10.1111/pce.13608

Chu, Q., Sha, Z., Nakamura, T., Oka, N., Osaki, M., Watanabe, T., 2016a. Differential Responses of Soybean and Sorghum Growth, Nitrogen Uptake, and Microbial Metabolism in the Rhizosphere to Cattle Manure Application: A Rhizobox Study. J. Agric. Food Chem. 64, 8084–8094. https://doi.org/10.1021/acs.jafc.6b03046

Chu, Q., Sha, Z., Osaki, M., Watanabe, T., 2017. Contrasting Effects of Cattle Manure Applications and Root-Induced Changes on Heavy Metal Dynamics in the Rhizosphere of Soybean in an Acidic Haplic Fluvisol: A Chronological Pot Experiment. J. Agric. Food Chem. 65, 3085–3095. https://doi.org/10.1021/acs.jafc.6b05813

Chu, Q., Watanabe, T., Shinano, T., Nakamura, T., Oka, N., Osaki, M., Sha, Z., 2016b. The dynamic state of the ionome in roots, nodules, and shoots of soybean under different nitrogen status and at different growth stages. J. Plant Nutr. Soil Sci. 179, 488–498. https://doi.org/10.1002/jpln.201600059

Chu, Q., Xu, S., Xue, L., Liu, Y., Feng, Y., Yu, S., Yang, L., Xing, B., 2020a. Bentonite hydrochar composites mitigate ammonia volatilization from paddy soil and improve nitrogen use efficiency. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137301

Chu, Q., Xue, L., Cheng, Y., Liu, Y., Feng, Y., Yu, S., Meng, L., Pan, G., Hou, P.,

Journal Pre-proof

Duan, J., Yang, L., 2020b. Microalgae-derived hydrochar application on rice paddy soil: Higher rice yield but increased gaseous nitrogen loss. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137127

Chu, Q., Xue, L., Singh, B.P., Yu, S., Müller, K., Wang, H., Feng, Y., Pan, G., Zheng, X., Yang, L., 2020c. Sewage sludge-derived hydrochar that inhibits ammonia volatilization, improves soil nitrogen retention and rice nitrogen utilization. Chemosphere 245, 125558. https://doi.org/10.1016/j.chemosphere.2019.125558

Cui, X., Lu, M., Khan, M.B., Lai, C., Yang, X., He, Z., Chen, G., Yan, B., 2020. Hydrothermal carbonization of different wetland biomass wastes: Phosphorus reclamation and hydrochar production. Waste Manag. https://doi.org/10.1016/j.wasman.2019.10.034

Dai, L., Tan, F., Wu, B., He, M., Wang, W., Tang, X., Hu, Q., Zhang, M., 2015. Immobilization of phosphorus in cow manure during hydrothermal carbonization. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2015.04.009

Delgadillo-Mirquez, L., Lopes, F., Taidi, B., Pareau, D., 2016. Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. Biotechnol. Reports. https://doi.org/10.1016/j.btre.2016.04.003

Ekpo, U., Ross, A.B., Camargo-Valero, M.A., Fletcher, L.A., 2016. Influence of pH on hydrothermal treatment of swine manure: Impact on extraction of nitrogen and phosphorus in process water. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2016.05.012

Fei, Y. heng, Zhao, D., Cao, Y., Huot, H., Tang, Y. tao, Zhang, H., Xiao, T., 2019.

Journal Pre-proof

Phosphorous retention and release by sludge-derived hydrochar for potential use as a soil amendment. J. Environ. Qual. 48, 502–509. https://doi.org/10.2134/jeq2018.09.0328

Foong, S.Y., Liew, R.K., Yang, Y., Cheng, Y.W., Yek, P.N.Y., Wan Mahari, W.A., Lee, X.Y., Han, C.S., Vo, D.V.N., Van Le, Q., Aghbashlo, M., Tabatabaei, M., Sonne, C., Peng, W., Lam, S.S., 2020. Valorization of biomass waste to engineered activated biochar by microwave pyrolysis: Progress, challenges, and future directions. Chem. Eng. J. https://doi.org/10.1016/j.cej.2020.124401

Hao, S., Zhu, X., Liu, Y., Qian, F., Fang, Z., Shi, Q., Zhang, S., Chen, J., Ren, Z.J., 2018. Production Temperature Effects on the Structure of Hydrochar-Derived Dissolved Organic Matter and Associated Toxicity. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.7b04983

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Sci. Soc. Am. J. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Heilmann, S.M., Molde, J.S., Timler, J.G., Wood, B.M., Mikula, A.L., Vozhdayev, G. V., Colosky, E.C., Spokas, K.A., Valentas, K.J., 2014. Phosphorus reclamation through hydrothermal carbonization of animal manures. Environ. Sci. Technol. https://doi.org/10.1021/es501872k

Huang, R., Tang, Y., 2016. Evolution of phosphorus complexation and mineralogy during (hydro)thermal treatments of activated and anaerobically digested sludge:

Journal Pre-proof

Insights from sequential extraction and P K-edge XANES. Water Res. 100, 439–447. https://doi.org/10.1016/j.watres.2016.05.029

Huo, S., Liu, J., Addy, M., Chen, P., Necas, D., Cheng, P., Li, K., Chai, H., Liu, Y., Ruan, R., 2020. The influence of microalgae on vegetable production and nutrient removal in greenhouse hydroponics. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.118563

Idowu, I., Li, L., Flora, J.R.V., Pellechia, P.J., Darko, S.A., Ro, K.S., Berge, N.D., 2017. Hydrothermal carbonization of food waste for nutrient recovery and reuse. Waste Manag. 69, 480–491. https://doi.org/10.1016/j.wasman.2017.08.051

Koppelaar, R.H.E.M., Weikard, H.P., 2013. Assessing phosphate rock depletion and phosphorus recycling options. Glob. Environ. Chang. https://doi.org/10.1016/j.gloenvcha.2013.09.002

Lachos-Perez, D., Brown, A.B., Mudhoo, A., Martinez, J., Timko, M.T., Rostagno, M.A., Forster-Carneiro, T., 2017. Applications of subcritical and supercritical water conditions for extraction, hydrolysis, gasification, and carbonization of biomass: A critical review. Biofuel Res. J. https://doi.org/10.18331/BRJ2017.4.2.6

Lee, E.K., Zhang, X., Adler, P.R., Kleppel, G.S., Romeiko, X.X., 2020. Spatially and temporally explicit life cycle global warming, eutrophication, and acidification impacts from corn production in the U.S. Midwest. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.118465

Li, B., Yin, T., Udugama, I.A., Dong, S.L., Yu, W., Huang, Y.F., Young, B., 2020.

Journal Pre-proof

Food waste and the embedded phosphorus footprint in China. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.119909

Li, H., Li, Y., Xu, Y., Lu, X., 2020. Biochar phosphorus fertilizer effects on soil phosphorus availability. Chemosphere. https://doi.org/10.1016/j.chemosphere.2019.125471

Li, L., Pan, G., 2013. A universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand. Environ. Sci. Technol. https://doi.org/10.1021/es305234d

Liu, Xuewei, Yuan, Z., Liu, Xin, Zhang, Y., Hua, H., Jiang, S., 2020. Historic Trends and Future Prospects of Waste Generation and Recycling in China's Phosphorus Cycle. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.9b05120

Lu, H., Yan, M., Wong, M.H., Mo, W.Y., Wang, Y., Chen, X.W., Wang, J.J., 2020. Effects of biochar on soil microbial community and functional genes of a landfill cover three years after ecological restoration. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137133

Luo, L. zao, Shao, Y., Luo, S., Zeng, F. jian, Tian, G. ming, 2019. Nutrient removal from piggery wastewater by Desmodesmus sp.CHX1 and its cultivation conditions optimization. Environ. Technol. (United Kingdom). https://doi.org/10.1080/09593330.2018.1449903

Machado, J., Campos, A., Vasconcelos, V., Freitas, M., 2017. Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: A review of their relevance for agricultural plant quality and public health. Environ. Res.

Journal Pre-proof

https://doi.org/10.1016/j.envres.2016.09.015

Mujtaba, G., Rizwan, M., Lee, K., 2017. Removal of nutrients and COD from wastewater using symbiotic co-culture of bacterium Pseudomonas putida and immobilized microalga Chlorella vulgaris. J. Ind. Eng. Chem. https://doi.org/10.1016/j.jiec.2017.01.021

Mukherjee, C., Chowdhury, R., Ray, K., 2015. Phosphorus recycling from an unexplored source by polyphosphate accumulating microalgae and cyanobacteria-a step to phosphorus security in agriculture. Front. Microbiol. 6, 1–7. https://doi.org/10.3389/fmicb.2015.01421

Oita, A., Wirasenjaya, F., Liu, J., Webeck, E., Matsubae, K., 2020. Trends in the food nitrogen and phosphorus footprints for Asia's giants: China, India, and Japan. Resour. Conserv. Recycl. 157, 104752. https://doi.org/10.1016/j.resconrec.2020.104752

Pan, G., Lyu, T., Mortimer, R., 2018. Comment: Closing phosphorus cycle from natural waters: re-capturing phosphorus through an integrated water-energy-food strategy. J. Environ. Sci. (China). https://doi.org/10.1016/j.jes.2018.02.018

Powell, N., Shilton, A., Chisti, Y., Pratt, S., 2009. Towards a luxury uptake process via microalgae - Defining the polyphosphate dynamics. Water Res. https://doi.org/10.1016/j.watres.2009.06.011

Prasad, P., Pullar, D., Pratt, S., 2014. Facilitating access to the algal economy: Mapping waste resources to identify suitable locations for algal farms in Queensland. Resour. Conserv. Recycl.

Journal Pre-proof

https://doi.org/10.1016/j.resconrec.2014.01.008

Ray, K., Mukherjee, C., Ghosh, A.N., 2013. A way to curb phosphorus toxicity in the environment: Use of polyphosphate reservoir of cyanobacteria and microalga as a safe alternative phosphorus biofertilizer for Indian agriculture. Environ. Sci. Technol. 47, 11378–11379. https://doi.org/10.1021/es403057c

Santos, F.M., Pires, J.C.M., 2018. Nutrient recovery from wastewaters by microalgae and its potential application as bio-char. Bioresour. Technol. 267, 725–731. https://doi.org/10.1016/j.biortech.2018.07.119

Schreiber, C., Schiedung, H., Harrison, L., Briese, C., Ackermann, B., Kant, J., Schrey, S.D., Hofmann, D., Singh, D., Ebenhöh, O., Amelung, W., Schurr, U., Mettler-Altmann, T., Huber, G., Jablonowski, N.D., Nedbal, L., 2018. Evaluating potential of green alga Chlorella vulgaris to accumulate phosphorus and to fertilize nutrient-poor soil substrates for crop plants. J. Appl. Phycol. https://doi.org/10.1007/s10811-018-1390-9

Sha, Z., Chen, Z., Feng, Y., Xue, L., Yang, L., Cao, L., Chu, Q., 2020. Minerals loaded with oxygen nanobubbles mitigate arsenic translocation from paddy soils to rice. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2020.122818

Sha, Z., Watanabe, T., Chu, Q., Oka, N., Osaki, M., Shinano, T., 2018. A Reduced Phosphorus Application Rate Using a Mycorrhizal Plant as the Preceding Crop Maintains Soybean Seeds' Nutritional Quality. J. Agric. Food Chem. acs.jafc.8b05288. https://doi.org/10.1021/acs.jafc.8b05288

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011.

Journal Pre-proof

Phosphorus dynamics: From soil to plant. Plant Physiol. 156, 997–1005. https://doi.org/10.1104/pp.111.175232

Shen, Q., Wen, Z., Dong, Y., Li, H., Miao, Y., Shen, J., 2018. The responses of root morphology and phosphorus-mobilizing exudations in wheat to increasing shoot phosphorus concentration. AoB Plants. https://doi.org/10.1093/aobpla/ply054

Shen, Y., Gao, J., Li, L., 2017. Municipal wastewater treatment via co-immobilized microalgal-bacterial symbiosis: Microorganism growth and nutrients removal. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.07.041

Solovchenko, A., Khozin-Goldberg, I., Selyakh, I., Semenova, L., Ismagulova, T., Lukyanov, A., Mamedov, I., Vinogradova, E., Karpova, O., Konyukhov, I., Vasilieva, S., Mojzes, P., Dijkema, C., Vecherskaya, M., Zvyagin, I., Nedbal, L., Gorelova, O., 2019. Phosphorus starvation and luxury uptake in green microalgae revisited. Algal Res. https://doi.org/10.1016/j.algal.2019.101651

Solovchenko, A., Verschoor, A.M., Jablonowski, N.D., Nedbal, L., 2016. Phosphorus from wastewater to crops: An alternative path involving microalgae. Biotechnol. Adv. 34, 550–564. https://doi.org/10.1016/j.biotechadv.2016.01.002

Subramaniyam, V., Subashchandrabose, S.R., Ganeshkumar, V., Thavamani, P., Chen, Z., Naidu, R., Megharaj, M., 2016. Cultivation of Chlorella on brewery wastewater and nano-particle biosynthesis by its biomass. Bioresour. Technol. 211, 698–703. https://doi.org/10.1016/j.biortech.2016.03.154

Tao, R., Kinnunen, V., Praveenkumar, R., Lakaniemi, A.M., Rintala, J.A., 2017. Comparison of Scenedesmus acuminatus and Chlorella vulgaris cultivation in

Journal Pre-proof

liquid digestates from anaerobic digestion of pulp and paper industry and municipal wastewater treatment sludge. J. Appl. Phycol. https://doi.org/10.1007/s10811-017-1175-6

Václavková, Š., Šyc, M., Moško, J., Pohořelý, M., Svoboda, K., 2018. Fertilizer and Soil Solubility of Secondary P Sources - The Estimation of Their Applicability to Agricultural Soils. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.8b02105

Wang, T., Camps-Arbestain, M., Hedley, M., 2014. The fate of phosphorus of ash-rich biochars in a soil-plant system. Plant Soil. https://doi.org/10.1007/s11104-013-1938-z

Wang, Tao, Zhai, Y., Zhu, Y., Peng, C., Wang, Tengfei, Xu, B., Li, C., Zeng, G., 2017. Feedwater pH affects phosphorus transformation during hydrothermal carbonization of sewage sludge. Bioresour. Technol. 245, 182–187. https://doi.org/10.1016/j.biortech.2017.08.114

Withers, P.J.A., Forber, K.G., Lyon, C., Rothwell, S., Doody, D.G., Jarvie, H.P., Martin-Ortega, J., Jacobs, B., Cordell, D., Patton, M., Camargo-Valero, M.A., Cassidy, R., 2020. Towards resolving the phosphorus chaos created by food systems. Ambio. https://doi.org/10.1007/s13280-019-01255-1

Xu, G., Zhang, Y., Shao, H., Sun, J., 2016. Pyrolysis temperature affects phosphorus transformation in biochar: Chemical fractionation and 31P NMR analysis. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.06.081

Xu, H.J., Wang, X.H., Li, H., Yao, H.Y., Su, J.Q., Zhu, Y.G., 2014. Biochar impacts

Journal Pre-proof

soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ. Sci. Technol. https://doi.org/10.1021/es5021058

Xu, M., Gao, P., Yang, Z., Su, L., Wu, J., Yang, G., Zhang, X., Ma, J., Peng, H., Xiao, Y., 2019. Biochar impacts on phosphorus cycling in rice ecosystem. Chemosphere 225, 311–319. https://doi.org/10.1016/j.chemosphere.2019.03.069

Yao, Y., Gao, B., Chen, J., Yang, L., 2013. Engineered biochar reclaiming phosphate from aqueous solutions: Mechanisms and potential application as a slow-release fertilizer. Environ. Sci. Technol. 47, 8700–8708. https://doi.org/10.1021/es4012977

Ye, S., Zeng, G., Wu, H., Liang, J., Zhang, C., Dai, J., Xiong, W., Song, B., Wu, S., Yu, J., 2019. The effects of activated biochar addition on remediation efficiency of co-composting with contaminated wetland soil. Resour. Conserv. Recycl. https://doi.org/10.1016/j.resconrec.2018.10.004

Yu, S., Feng, Y., Xue, L., Sun, H., Han, L., Yang, L., Sun, Q., Chu, Q., 2019. Biowaste to treasure: Application of microbial-aged hydrochar in rice paddy could improve nitrogen use efficiency and rice grain free amino acids. J. Clean. Prod. 240, 118180. https://doi.org/10.1016/j.jclepro.2019.118180

Yu, S., Xue, L., Feng, Y., Liu, Y., Song, Z., Mandal, S., Yang, L., Sun, Q., Xing, B., 2020. Hydrochar reduced NH3 volatilization from rice paddy soil: Microbial-aging rather than water-washing is recommended before application. J. Clean. Prod. 122233. https://doi.org/10.1016/j.jclepro.2020.122233

Yuan, T., Cheng, Y., Huang, W., Zhang, Z., Lei, Z., Shimizu, K., Utsumi, M., 2018.

Journal Pre-proof

Fertilizer potential of liquid product from hydrothermal treatment of swine manure. Waste Manag. 77, 166–171. https://doi.org/10.1016/j.wasman.2018.05.018

Zhang, H., Chen, C., Gray, E.M., Boyd, S.E., Yang, H., Zhang, D., 2016. Roles of biochar in improving phosphorus availability in soils: A phosphate adsorbent and a source of available phosphorus. Geoderma. https://doi.org/10.1016/j.geoderma.2016.04.020

Journal Pre-proof

Captions to illustrations

Table 1. Basic physiochemical characteristics of raw microalgae and microalgae-derived hydrochars.

Table 2. Hydrochar yield, total P content and P recovery of the microalgae-derived hydrochars (n=3).

Figure 1. Growth curves of *Chlorella vulgaris* and *Microcystis sp*. and dynamic variation of P concentration in poultry farm wastewater.

Figure 2. Fractionation of P in sequential extracts of raw microalgae and microalgae-derived hydrochars.

Figure 3. The dynamic variation of soil available P with the application of chemical fertilizer, raw microalgae and microalgae-derived hydrochars during 120 days incubation.

Figure 4. P fractionation in the rhizosphere soil grown with wheat, including (A) soluble P fraction, (B) exchangeable P fraction, (C) Fe/Al-bound P fraction, (D) Ca-bound P fraction, and (E) residual P fraction.

Figure 5. Wheat PUE (A) and grain yield (B) in a pot experiment. Each value was the average of results from four replicates.

Journal Pre-proof

Table 1. Basic physiochemical characteristics of raw microalgae and microalgae-derived hydrochars. Values are means ± SE of three independent measurements.

	CV	CVHW200	CVHW260	CVHCA200	CVHCA260	MS	MSHW200	MSHW260	MSHCA200	MSHCA260
pH[a]	8.2 ± 0.3	7.4 ± 0.2	7.6 ± 0.4	6.1 ± 0.1	6.5 ± 0.1	8.0 ± 0.5	7.9 ± 0.3	8.3 ± 0.2	5.9 ± 0.2	6.4 ± 0.1
C (%)[b]	63.8 ± 2.9	55.6 ± 3.5	56.4 ± 1.8	60.5 ± 5.7	59.3 ± 2.4	65.6 ± 1.8	58.7 ± 3.6	57.2 ± 3.2	60.3 ± 6.4	61.0 ± 3.7
H (%)	4.1 ± 0.2	4.1 ± 0.4	4.4 ± 0.2	3.3 ± 0.1	3.8 ± 0.3	4.0 ± 0.1	3.8 ± 0.1	3.4 ± 0.1	3.0 ± 0.1	3.2 ± 0.2
N (%)	10.8 ± 0.4	8.7 ± 0.7	7.3 ± 0.6	8.1 ± 0.4	6.2 ± 0.5	12.3 ± 0.7	10.2 ± 0.4	9.2 ± 0.6	9.5 ± 1.1	8.8 ± 0.7
S (%)	2.7 ± 0.2	1.5 ± 0.1	0.4 ± 0.03	1.4 ± 0.1	0.8 ± 0.09	2.2 ± 0.2	1.3 ± 0.03	0.6 ± 0.04	1.5 ± 0.1	0.6 ± 0.03
K (%)	1.8 ± 0.1	1.5 ± 0.2	1.1 ± 0.1	1.3 ± 0.07	0.9 ± 0.05	1.6 ± 0.2	1.2 ± 0.1	1.3 ± 0.06	1.1 ± 0.2	0.8 ± 0.04
Al (%)	0.8 ± 0.05	1.1 ± 0.2	1.2 ± 0.2	1.8 ± 0.3	1.9 ± 0.3	1.0 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	2.2 ± 0.3	2.4 ± 0.4
Ca (%)	1.1 ± 0.07	1.3 ± 0.2	1.5 ± 0.1	2.2 ± 0.3	2.4 ± 0.2	0.9 ± 0.08	1.2 ± 0.1	1.3 ± 0.2	1.8 ± 0.08	2.1 ± 0.3
Fe (%)	0.7 ± 0.04	0.9 ± 0.1	1.1 ± 0.06	1.5 ± 0.1	1.6 ± 0.2	0.4 ± 0.03	0.8 ± 0.05	0.8 ± 0.08	1.3 ± 0.1	1.5 ± 0.06
Mg (%)	0.1 ± 0.02	0.3 ± 0.04	0.4 ± 0.02	0.6 ± 0.05	0.6 ± 0.04	0.08 ± 0.01	0.1 ± 0.007	0.2 ± 0.04	0.4 ± 0.03	0.05 ± 0.06
SSA (m^2 g^{-1})[c]	0.6 ± 0.02	5.4 ± 0.05	5.7 ± 0.02	6.6 ± 0.07	6.4 ± 0.06	0.4 ± 0.04	5.2 ± 0.04	5.0 ± 0.06	5.9 ± 0.06	6.1 ± 0.05
Porosity (cm^3 g^{-1})	0.004 ± 0.0005	0.05 ± 0.006	0.03 ± 0.003	0.06 ± 0.008	0.04 ± 0.005	0.002 ± 0.0003	0.03 ± 0.004	0.02 ± 0.003	0.04 ± 0.006	0.03 ± 0.004

a. The pH was determined by a pH meter using a solid/Milli-Q water ratio of 1:2.5 (w/v).
b. The total elemental concentrations of C, H, N, and S were determined by an Elemental Analyzer (EL III; Elementar Analysensysteme GmbH, Germany). The total concentrations of elemental Al, Ca, Fe, and Mg were measured using ICP-OES.
c. SSA and porosity were measured were measured using a NOVA 1200 analyzer (Anton Paar QuantaTec Inc., Graz, Austria), and the parameters were calculated using the Brunauer–Emmett–Teller method.

Journal Pre-proof

Table 2. Hydrochar yield, total P content and P recovery of the microalgae-derived hydrochars (n=3). (N/A: not applicable)

Feedstock	HTC reaction medium	Sample label	Solid recovery rate (%)	Total P concentration (wt%)	P recovery (%)
Chlorella vulgaris	Raw	CV	N/A	2.7 ± 0.1	N/A
	Deionized water, 200 °C	CVHW200	40.7 ± 3.9	3.4 ± 0.2	51.3
	Deionized water, 260 °C	CVHW260	37.3 ± 5.0	3.6 ± 0.1	49.7
	1 *wt.%* citric acid, 200 °C	CVHCA200	49.8 ± 3.7	3.8 ± 0.3	70.1
	1 *wt.%* citric acid, 260 °C	CVHCA260	45.4 ± 4.3	4.3 ± 0.4	72.3
Microcystis sp.	Raw	MS	N/A	3.5 ± 0.1	N/A
	Deionized water, 200 °C	MSHW200	44.6 ± 1.7	4.2 ± 0.3	53.5
	Deionized water, 260 °C	MSHW260	42.1 ± 5.4	4.8 ± 0.3	57.4
	1 *wt.%* citric acid, 200 °C	MSHCA200	58.8 ± 3.2	5.2 ± 0.2	87.4
	1 *wt.%* citric acid, 260 °C	MSHCA260	55.2 ± 2.8	5.8 ± 0.4	91.5

Journal Pre-proof

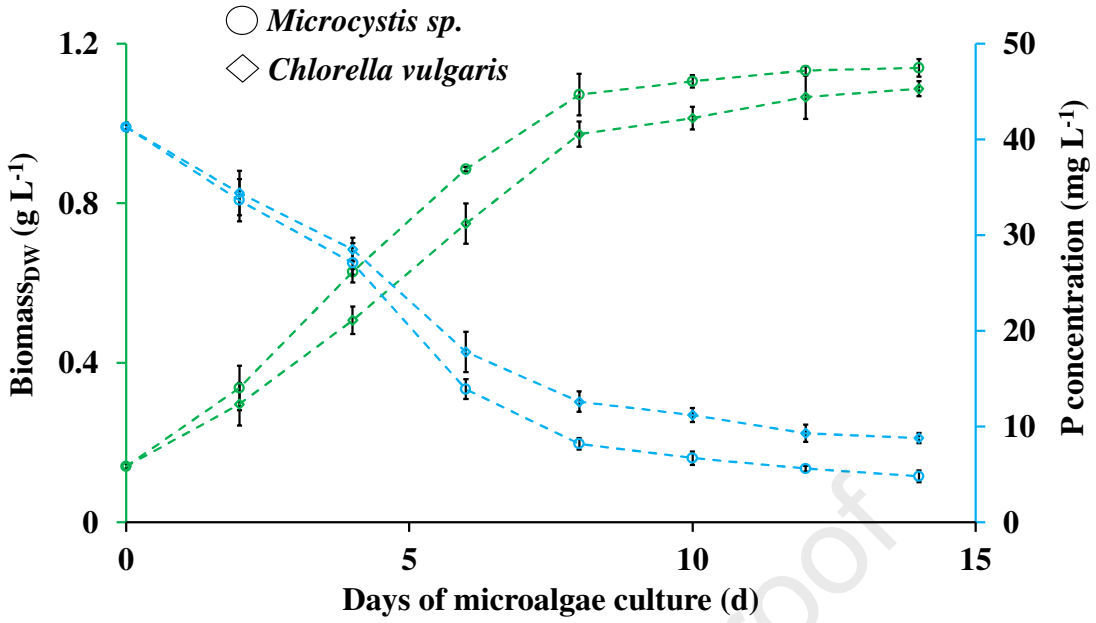


Figure 1. Growth curves of *Chlorella vulgaris* and *Microcystis sp*. and dynamic variation of P concentration in poultry farm wastewater. Green circles and diamonds represent the biomass and blue markers represent the P concentration in the wastewater. Values are means ± SE of three independent measurements. The biomass was based on the dry weight (DW) of microalgae.

Journal Pre-proof

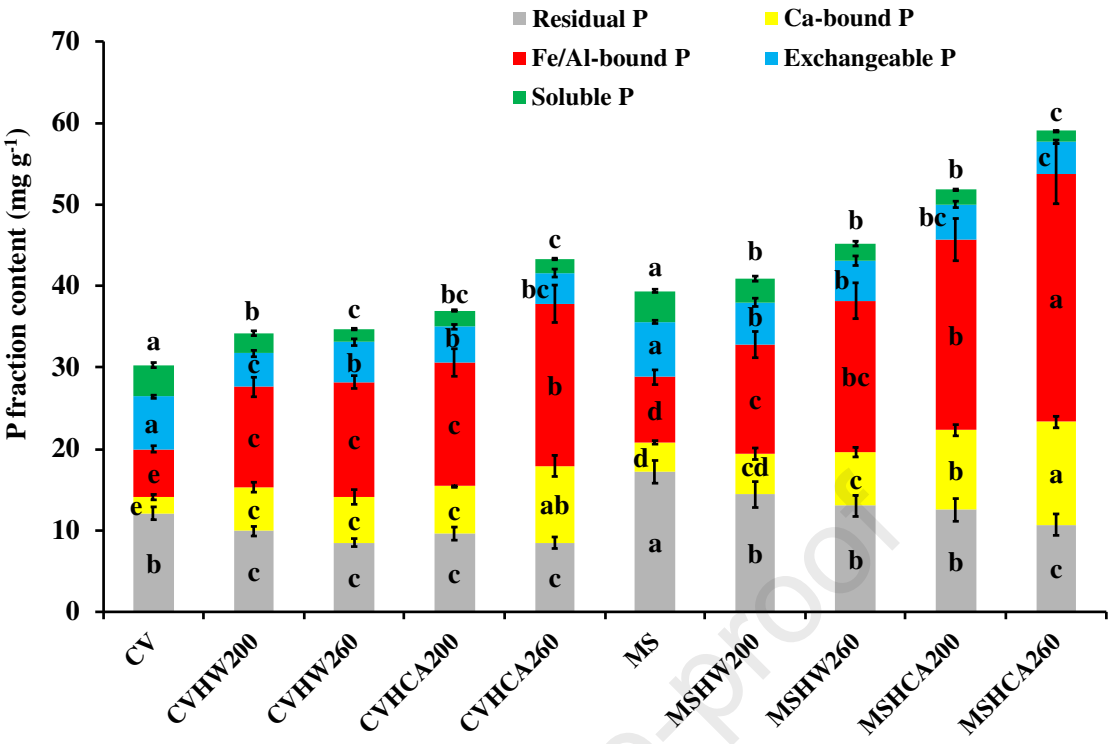


Figure 2. Fractionation of P in sequential extracts of raw microalgae and microalgae-derived hydrochars. Each data was the average of results from three independent extraction experiments. Error bars indicate SE. Means not sharing the same letter (a-e) are significantly different at the $p = 0.05$ level by one-way ANOVA. The abbreviations of materials are the same as those in Table 2. The relative abundance (percentage) of each P fractionation is shown in Table S2.

Journal Pre-proof

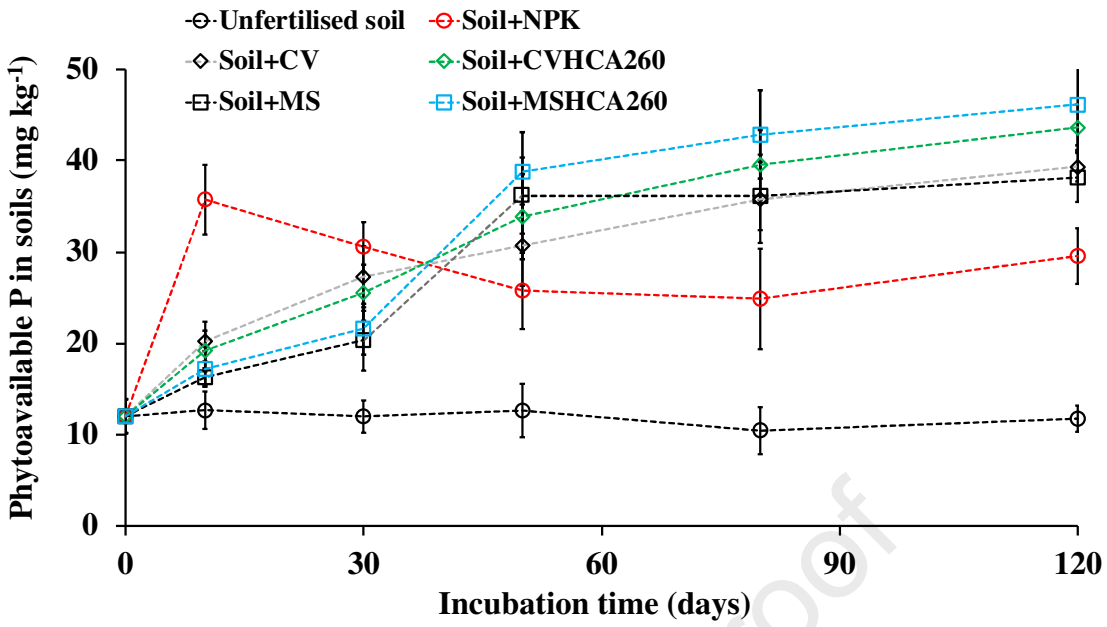
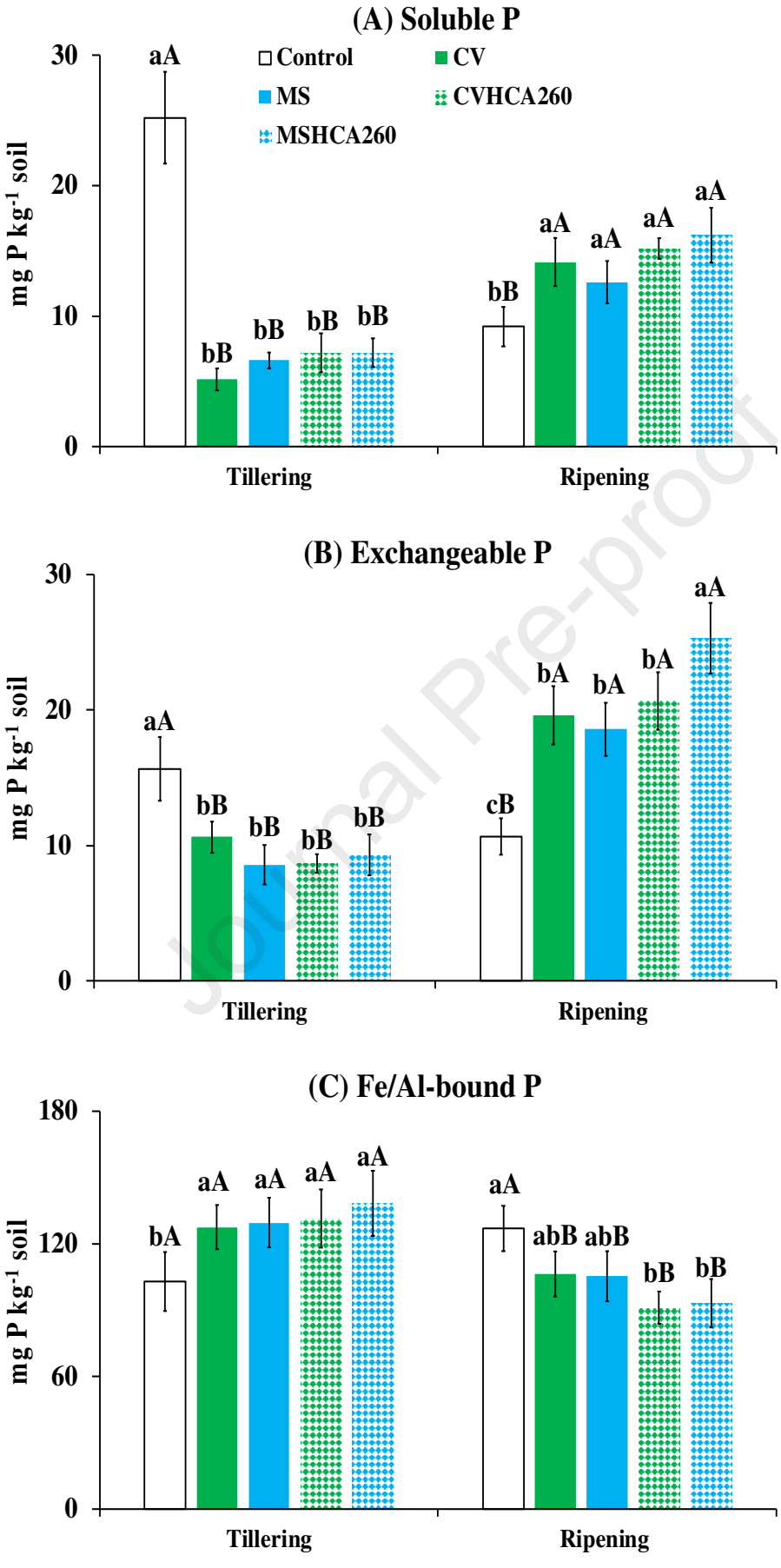


Figure 3. The dynamic variation of soil available P with the application of chemical fertilizer, raw microalgae and microalgae-derived hydrochars during 120 days incubation. Each data was the average of results from four replicates. Error bars indicate SE (n = 4). Significant differences between treatments are indicated with different letters. The abbreviations of materials are the same as those in Table 2. Soil+NPK means the soil applied with chemical N, P, and K fertilizers.

Journal Pre-proof



Journal Pre-proof

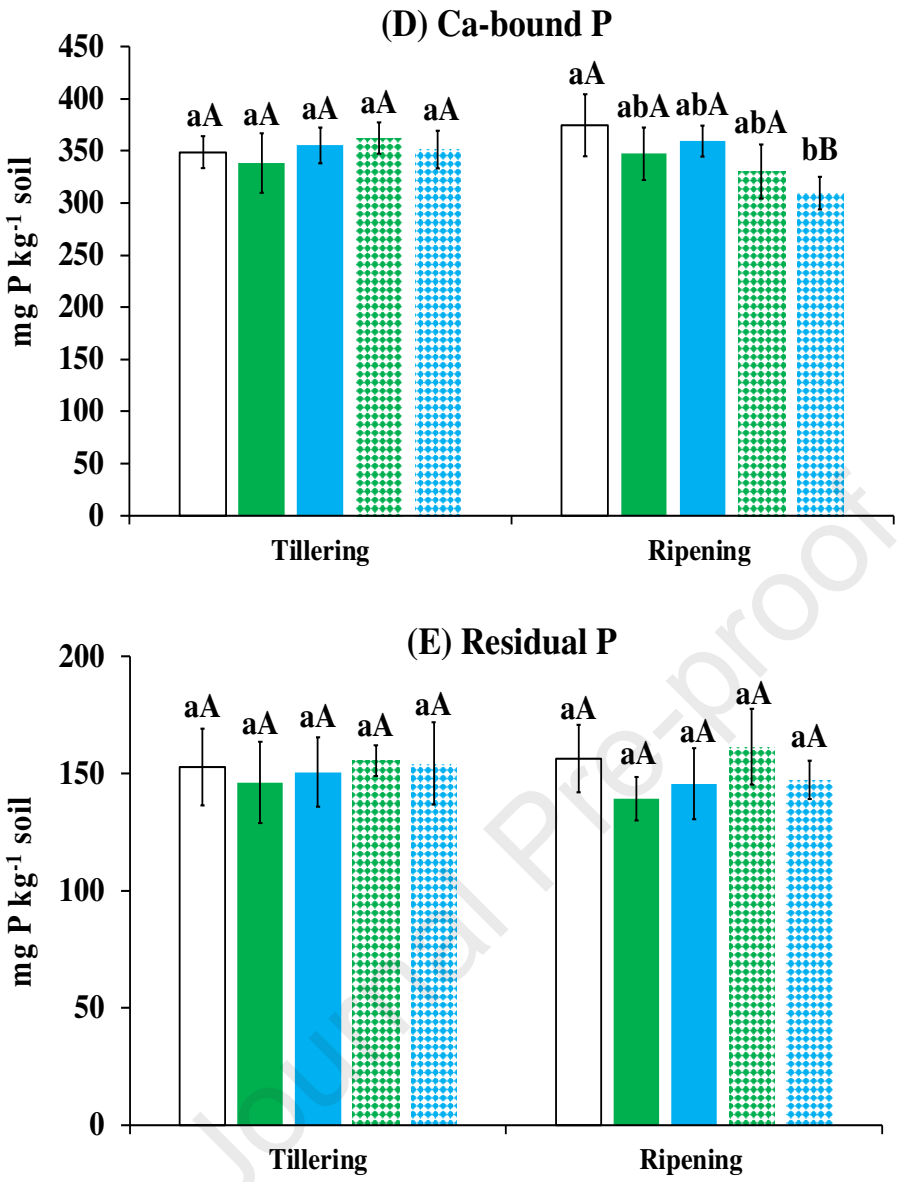
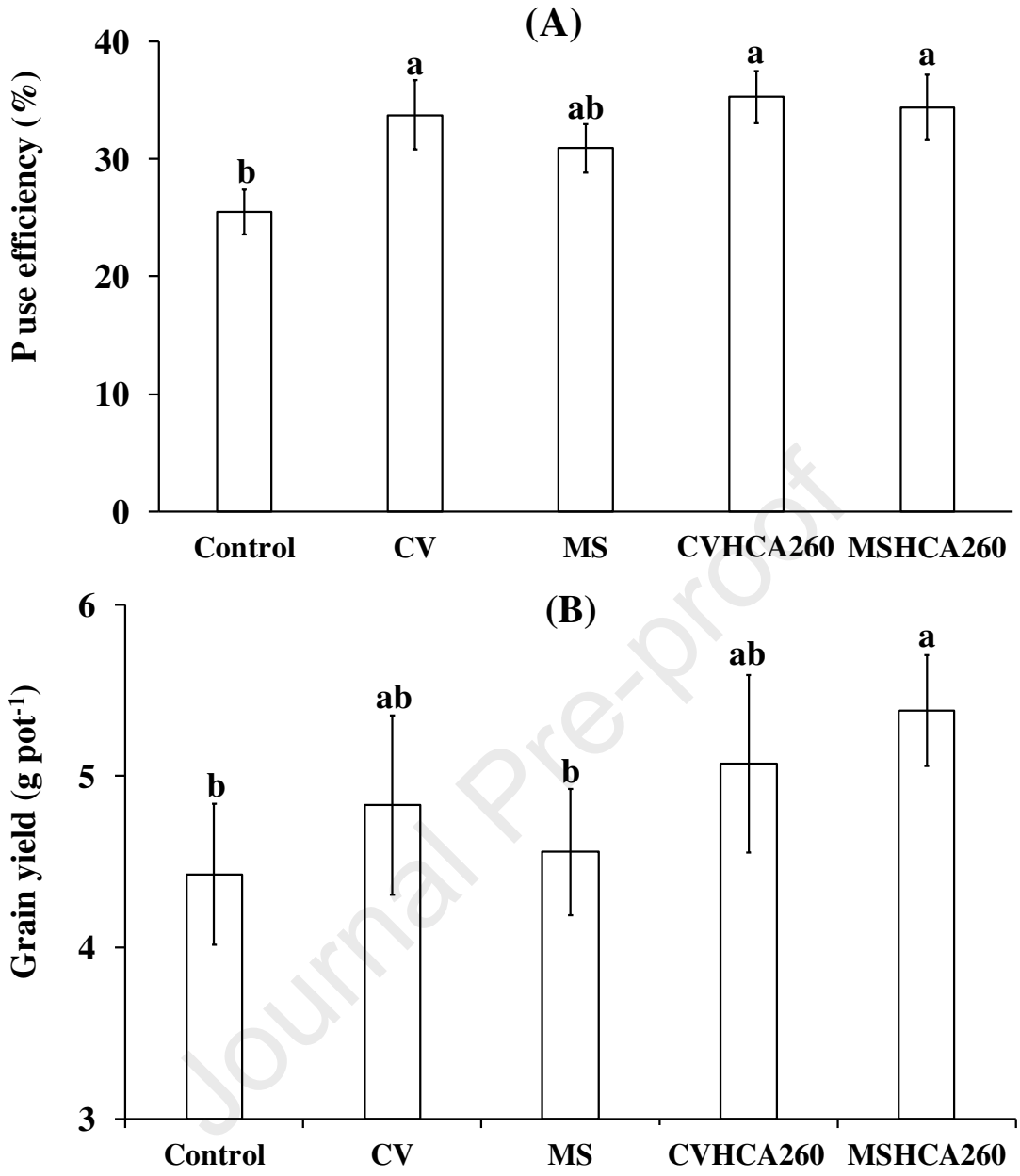


Figure 4. P fractionation in the rhizosphere soil grown with wheat, including (A) soluble P fraction, (B) exchangeable P fraction, (C) Fe/Al-bound P fraction, (D) Ca-bound P fraction, and (E) residual P fraction. Each data was the average of results from four replicates. Error bars indicate SE (n = 4). Columns denoted by different lowercase letters indicate statistically significant differences affected by different microalgae and microalgae-derived hydrochar treatments, and those labelled with different uppercase letters indicate significant differences for the same treatment between tillering and ripening stages. The abbreviations of materials are the same as those in **Table 2**. The sequential extraction method and explanation of different P fractions are detailed in **Fig S2**. The results of labile P pool and stable P pool are shown in **Fig S5**.

Journal Pre-proof



Journal Pre-proof

Figure 5. Wheat PUE (A) and grain yield (B) in a pot experiment. Each value was the average of results from four replicates. Error bars indicate SE (n = 4). Statistically significant differences between treatments are indicated with different letters. The abbreviations of materials are those detailed in **Table 2**.

Highlights

- *Chlorella vulgaris* and *Microcystis sp.* (MS) removed 78.7% and 88.4% phosphorous (P)
- 91.5% P was recovered from MS to MS-derived hydrochar produced at 260 °C (MSHCA260)
- Microalgal hydrochars slowly released P and improved soil P availability
- MSHCA260 increased P use efficiency and wheat yield compared to chemical fertilizer
- Microalgae and hydrochar technology recycled P from wastewater to crop food

Journal Pre-proof

Credit author statement

QC designed the experiments; GP and LX acquired the funding and supervised the research; QC, TL, BY, and MC performed the experiments; QC analyzed the data; YF and LY visualized the work; QC wrote the manuscript; TL, RM, LY, and MC reviewed and edited the manuscript; QC, GP, and LX finalized the manuscript.

Journal Pre-proof

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof

https://dspace.org/

Hydrothermal carbonization of microalgae for phosphorus recycling from wastewater to crop-soil systems as slow-release fertilizers

Chu, Qingnan; Lyu, Tao; Xue, Lihong; Yang, Linzhang; Feng, Yanfang ...

2020-10-12
Elsevier
https://doi.org/10.1016/j.jclepro.2020.124627
Chu Q, Lyu T, Xue L, et al., (2021) Hydrothermal carbonization of microalgae for phosphorus recycling from wastewater to crop-soil systems as slow-release fertilizers. Journal of Cleaner Production, Volume 283, February 2021, Article number 124627

Downloaded from a DSpace repository. https://dspace.org/