



# Effect of biochar on photosynthetic microorganism growth and iron cycling in paddy soil under different phosphate levels



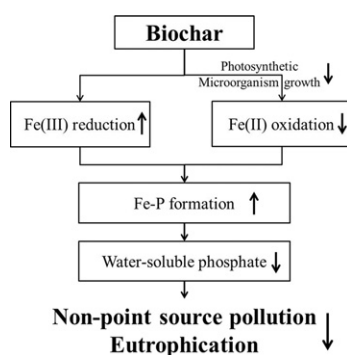
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## HIGHLIGHTS

- Iron cycling was critical for the fate of phosphorous in submerged paddy soil.
- Biochar promoted Fe(III) reduction under anaerobic condition.
- Biochar inhibited Fe(II) oxidation via slowing photosynthetic microorganism growth.
- Promoted Fe(III) reduction and inhibited Fe(II) oxidation decreased P solubility.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The surplus of exogenous and endogenous phosphate in submerged paddy fields could increase the risk of algal blooms, the photosynthesis of which might further influence the redox processes of iron. This work investigated the effects of biochar on photosynthetic microorganism growth and iron redox under different phosphate (P) levels to understand the dynamics of P and thereby control non-point source pollution by biochar addition. Paddy soils were incubated anaerobically with phosphate and biochar addition under controlled illumination conditions to determine the variation in chlorophyll *a* (*Chl a*), ferrous iron [Fe(II)], soil pH and water-soluble phosphate (W-P) with incubation time. Biochar addition significantly inhibited the photosynthetic microorganism growth, with *Chl a* decreased by 4.74–15.78 mg·g<sup>-1</sup> when compared with the control. Fe(III) reduction was significantly stimulated in response to biochar addition, while Fe(II) oxidation was inhibited because of the suppression of photosynthetic microorganism growth. The enhanced Fe(III) reduction and suppressed Fe(II) oxidation decreased the P solubility in the tested soils. These findings provide a cost-effective approach for inhibiting photosynthetic microorganism growth in paddy field and valuable insight into the effect of iron cycling on P retention for further management of eutrophication from exogenous and endogenous P loading.

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**Abbreviations:** Fe(III), iron(III); *a*, Fe(III) reduction potential;  $V_{max}$ , maximum Fe(III) reduction rate;  $T_{Vmax}$ , time to maximum Fe(III) reduction rate; P, phosphate; W-P, water-soluble phosphate; *Chl a*, chlorophyll *a*.

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## 1. Introduction

Owing to the excessive use of fertilizers to improve rice yield, paddy fields are regarded as an important source of non-point pollution (Nagumo et al., 2013; Zhang et al., 2003). The outflow from paddy fields in the processes of drainage and runoff can contain high concentrations of nitrogen and phosphorus, thereby increasing the risk of eutrophication of adjacent lakes and streams (Dupas et al., 2015; Jiang et al., 2007). Hence, it is important to establish a way to minimize nutrient losses from paddy fields.

Phosphate (P) is considered as one of the primary factors in non-point source pollution and eutrophication control. In paddy soils, the dynamics of P, including adsorption-desorption and precipitation-dissolution, are influenced by biogeochemical iron cycling (Cui et al., 2011; Li et al., 2012). When paddy fields were submerged, the concentration of aqueous P increased because of the reductive dissolution of ferric-phosphate to the ferrous phases (Shenker et al., 2005). As ferric iron [Fe(III)] is reduced to ferrous iron [Fe(II)], the Fe(II) became soluble and easily to be bound with P at a molar ratio of Fe(II):P of 1.14 to 2.25 (Li, 2012). The ratio of Fe(II):P was also responsible for the crystalline degree of Fe(II)-P mineral (Li, 2012). Thus, insights into iron cycling in paddy fields contribute to our understanding of P dynamics and the control of non-point source pollution.

Generation of oxygen (O<sub>2</sub>) by photosynthetic microorganisms such as algae and cyanobacteria can increase the redox potential of paddy soil, which might alter the iron redox process (Ponnamperuma, 1972). This means that the iron phase binding P is also regulated by photosynthetic microorganisms via photosynthetic O<sub>2</sub> production. Photosynthetic microorganisms are reported to show beneficial effects on nitrogen-fixation, which is considered as a potential source of N in paddy fields (Kulasooriya and Magana-Arachchi, 2016). Additionally, the uptake of P by photosynthetic microorganisms decreases the available P content in the aqueous phase of submerged paddy fields, which hinders the growth of rice. Blooms of algae and cyanobacteria could also result in the accumulation of microcystins in rice and aquatic animals (Machado et al., 2017). Because of the above mentioned negative effects, it is important to control the excessive growth of photosynthetic microorganisms in paddy fields.

Biochar has been widely applied to paddy fields as a soil amendment. It has benefits in soil fertility, crop yield, climate change mitigation, and inorganic/organic pollutants sorption and degradation (Beesley et al., 2011; Kammann et al., 2012; Ren et al., 2016). It can also enhance Fe(III) reduction by providing an electron shuttle and increasing the contribution of crystalline iron oxides in anoxic paddy soil without illumination (Jia et al., 2016; Kappler et al., 2014). A previous study also showed that a combined application of biochar and phosphorus could alleviate heat-induced effects on the physiological, agronomical and quality attributes of rice (Fahad et al., 2016). However, few studies have focused on the effect of biochar on growth of photosynthetic microorganisms and iron cycling in paddy fields under different P levels.

In this study, we investigated both the response of photosynthetic microorganism growth and iron cycling to biochar addition in paddy soils, and discussed the relationship between P retention, photosynthesis and iron cycling under different P levels. To accomplish our objectives, paddy soils were incubated anaerobically with biochar and

phosphate addition under dark and light conditions. Results of this work demonstrated the interaction of important biochemical processes with biochar application and suggest an effective method for control of algal blooms and P retention in paddy fields.

## 2. Materials and methods

### 2.1. Soil sampling and analyses

Paddy soils (0–20 cm depth) were collected from two drained post-harvest paddy fields located in Baodi District, Tianjin Municipality (BD; 28°49'N, 115°20'E) and Zhongwei, Ningxia Hui Autonomous Region (ZW; 37°29'N, 105°08'E). After removing the visible stone fragments and plant residues, the air-dried soil samples were passed through a 1 mm sieve. The chemical properties and iron oxide contents of the BD and ZW paddy soils were listed in Table 1.

### 2.2. Biochar preparation

The biochar was prepared using the abandoned branch of an apple tree as feedstock and pyrolyzed at 500 °C for 6–8 h. The composition of carbon, oxygen, hydrogen and nitrogen contents in biochar as measured by elemental analyzer (ECS 4010, Costech Inc., Valencia, CA) were 72%, 24%, 2.6% and 1.2%, respectively. The surface area of biochar was 87 m<sup>2</sup> g<sup>-1</sup>. The biochar had an ash content of 14% and a pH of 10.43.

### 2.3. Experimental design and anaerobic incubation

Soils without P input were conducted as the control treatment (P1). A low P level was set by adding 6.6 mmol P kg<sup>-1</sup> (P2) to soil samples, which was two times higher than the average dosage of P application into agro-ecosystems in consideration of the relatively larger demand of P input in the tested calcareous or alkaline soils. A higher P level of 20 mmol P kg<sup>-1</sup> (P3) was amended according to our results of pre-experiment to achieve a higher water-soluble phosphate (W-P) concentration, for a better understanding of the biogeochemical coupling between Fe and P. Following the ratio of soil weight to liquid volume of 1:1, exactly 5.0 g soil were submerged with 5 mL monopotassium phosphate solution or sterilized water in a 10-mL sterilized serum bottle. Treatments P1, P2 and P3 with 0.2 g biochar addition were also prepared (treatments P1 + B, P2 + B and P3 + B, respectively).

There was a total of 108 serum bottles for each treatment. The serum bottles were flushed with pure nitrogen gas (N<sub>2</sub>) for 5 min and closed with rubber septa and aluminum caps. Thirty-six serum bottles for each treatment were incubated in a controlled-environment incubator at 30 °C for 40 days under dark condition and 72 bottles were incubated under light condition. The dark-light cycle of the light condition was 12 h:12 h with a light intensity of 3000 lx.

### 2.4. Sample analysis

During anaerobic incubation, three bottles were randomly selected from each treatment on days 0, 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, and 40 for determination of Fe(II) concentration, soil pH and W-P. Treatments incubated under light condition were also sampled in triplicate at the same time interval for chlorophyll *a* (*Chl a*) determination.

**Table 1**  
The chemical properties and iron oxides content of the tested paddy soil (means ± SD, n = 3).

Soils	pH	Organic matter (mg g <sup>-1</sup> )	Available phosphorus (mg kg <sup>-1</sup> )	Available potassium (mg kg <sup>-1</sup> )	Nitrate nitrogen (mg kg <sup>-1</sup> )	Ammonium nitrogen (mg kg <sup>-1</sup> )	Amorphous iron oxides (mg g <sup>-1</sup> )	Free iron oxides (mg g <sup>-1</sup> )
BD	8.09 ± 0.05	33.48 ± 2.23	13.89 ± 1.44	302.41 ± 3.14	5.92 ± 0.14	12.13 ± 0.15	3.55 ± 0.02	14.49 ± 0.05
ZW	8.24 ± 0.02	21.74 ± 0.95	16.76 ± 2.82	73.93 ± 4.91	7.85 ± 0.39	6.10 ± 0.01	1.89 ± 0.19	9.70 ± 0.14

Note: BD, paddy soil collected from Baodi District, Tianjin Municipality (BD; 28°49'N, 115°20'E); ZW, paddy soil collected from Zhongwei, Ningxia Hui Autonomous Region (ZW; 37°29'N, 105°08'E), the same below.

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