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Interaction between Biochar and Algae on Problem Soil

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Abstract

An *in-vitro* experiment was conducted to assess the interaction between biochar and algae on a problem soil. Experiments were performed with and without algae to observe the effectiveness of algae for overcoming the challenges posed by problem soils. At the end of incubation periods, the adsorption and desorption of phosphorus (P) on a problem soil *vis-á-vis* algal inoculation were determined. Our results showed that different types of biochars adsorbed different amounts of P suggesting that the source of biochar played a crucial role in determining its behavior towards P. Tannery waste biochar significantly adsorbed 147% and 35% more P compared to that of the chicken litter and orange peel biochars respectively. Significant reductions in adsorption were observed when the biochar was used in combination with the algae which could be due to the beneficial effects of algae leading to the amelioration of the problem soil. Adsorption was reduced to 34%, 24% and 20% for the orange peel biochar + algae, chicken litter biochar + algae and tannery waste biochar + algae, respectively compared to the corresponding biochars present as a single solid. Phosphorus (P) desorption was also reduced significantly in presence of algal inoculation. Overall our findings suggest that the application of algae along with biochar in the problem soil could reduce the adsorption of P which would influence the availability of P.

Keywords

Biochar, Algae, Problem Soil, Interaction, Phosphorus

1. Introduction

Soil is one of the most important resources of the world and fertile soils rich in organic matter are our best insurance against food insecurity and climate vulnerability. About 60% of the soil carbon is in the form of organic matter and the organic matter determines much of the soil's quality [1]. The increase in population has led to a greater demand for food. It was projected that agriculture could supply enough food for the growing population, but at a cost to the environment [2]. We will need to increase crop yield without increasing and ideally decreasing environmental impacts in the near future to maintain sustainable agricultural production. Furthermore, in the future, farmland will need to serve multiple purposes, such as carbon storage in addition to food production. Many of the adverse environmental impacts on agriculture are caused by the use of chemical fertilizers [2] [3].

Recently charred material, biochar, are being used extensively in agricultural soils as an alternative to chemical fertilizers [4]. This would reduce pollution and reduce the need to use energy-intensive chemical fertilizers. Biochar is increasingly central to many of the concerns of society both nationally and globally [5]. The problems of the global environment, the recognition of the need to re-cycle natural resources and the discovery of the high technology of agriculture have placed the biochar in the limelight [4]. Being a stable source of carbon, biochar remains in soil for longer periods imparting long-term soil C sequestration that could mitigate climate change by reducing the emission of greenhouse gases from the soil [6] [7] [8]. Although much is known about the potential advantages of biochar, some important knowledge gaps exist about its drawbacks. Some researchers reported negative impacts of biochar applications, particularly on soil microbes [5], soil pH [9] [10], and leaching of nutrients [11]. Internationally, it has become a questionable fact whether biochar is equally good compared to that of its biomass.

Algae, which are found in almost all terrestrial environments, are the most distinctive organisms with potential agricultural applications [12]. The algal growth performs crucial roles in soil reclamation, stability of soil aggregates, formation of microbiological crust, and bio-controlling of agricultural pests [3]. Being a macronutrient, phosphorus (P) has garnered increasing attention due to its importance in maintaining soil fertility and crop production. Although most plants contain only about 0.2% to 0.4% P by weight [13], P plays a critical role in the development of plant tissue and plant structural compounds. Phosphorus (P) acts as a vital substrate for photosynthesis respiration, signal transduction, energy metabolism and carbohydrate transportation [13] [14]. The focus of the past studies on biochar has mostly been limited to the agronomic status of the soils such as the cation exchange capacity, pH and carbon sequestration potential of the soils. However, there is little published information about the interaction between biochar and algae under extremely poor soil conditions, such as in acid sulphate soil. We hypothesized that biochar in the presence of algae can reduce the negative impacts on acid sulphate soil. Thus, the aim of the present study was to explore the interaction between biochar and algae on a problem soil. The study was carried out with the following objectives in mind:

- To determine the potentiality of biochar to adsorb phosphorus;

- To determine the potentiality of biochar to desorb phosphorus;
- To identify the differences in phosphorus adsorption and desorption between biochar and biochar + algae;
- To investigate the role of algae as ameliorator for the problem soil.

2. Materials and Methods

2.1. Sampling Site

The soil samples were collected from the top 20 cm from HorinaPari village of Pekua union which was situated at Pekuaupazila in Cox's Bazar district. Geo-references of the sampling spots were 21˚48'32.1"N and 91˚58'39.3"E. According to USDA soil taxonomy [15], the soil was considered as acid sulphate soil which is one of the problem soils. The area was medium low land and the soil texture was silty clay [15]. The bulk of soil samples were collected by composite soil sampling method and processed.

2.2. Soil Sample Preparation

The soils were air-dried, visible roots and plant debris were discarded and the soils were ground gently to break up larger soil aggregates. After that the soils were sieved at 2 mm, thoroughly homogenized and finally characterized. Some other physico-chemical properties of the soil (pH, cation exchange capacity, moisture percentage, zinc, carbon, total nitrogen, available forms of phosphorous, potassium and sulphur) were determined with the standard method described by Rowell [16].

2.3. Preparation of Biochar

Three different types of waste materials (domestic, poultry, and tannery) were collected to produce biochars. Orange peel was used as a source of domestic waste. Chicken litter was collected from poultry. Tannery waste was collected from the Hazaribagh Tannery area, Dhaka. Wastes were placed in the earthen pots and the pots were covered with lids. The pots were arranged in such a way that the pots were uniformly heated from all sides. Finally, fire was lit and after about two hours, the wastes were turned to biochars [6] [17].

2.4. Collection of Algae

Algae (*Chlorella*) was collected from Virology Laboratory, International Centre for Diarrhoeal Disease Research (ICDDR, B) located at Dhaka, Bangladesh. Two types of nutrient stocks (solution A: trace metals; solution B: phosphorus, nitrogen and vitamins) were used to feed the algae. Two drops of sterile nutrient solution A and B were put in 500 ml of sterile filtered water. The algae were put in the solution to keep them viable [18].

2.5. Incubation Study

An incubation experiment was conducted following a randomized block design to investigate the interaction between biochar and algae on a problem soil. Each glass jar (length = 15 cm, width = 10 cm, height = 8 cm) contained approximately 300 gm soil. Biochar and algae wereapplied separately to the soil at a rate of 5 ton/ha. Regarding the mixture of biochar and algae, equal masses of biochar and algae were mixed and applied to the soil at a rate of 5 ton/ha [17]. All treatments were replicated four times. Thus, the experiment was comprised of 32 glass jars in total including the control treatments. Orange peel biochar, chicken litter biochar, tannery waste biochar and algae were indicated by the symbol OB, CB, TB and AG respectively. The soils were thoroughly mixed to distribute the biochar/algae/biochar + algae as evenly as possible. 100 ml of deionised water was added to each jarto keep the soil moist. The jars were then wrapped with plastic cling film to prevent evaporation and were kept at 30˚C in an incubator for a period of 30 days. After that the soil was collected for further experimental analysis.

2.6. Batch Adsorption Experiments

Kinetic experiment was conducted by the method followed by [19]. 0.1 g solid were placed into the glass vials and 30 ml of 5 mg/L phosphorus (P) solution was added obtained by dissolving analytical grade potassium dihydrogen phosphate in a background electrolyte of 0.1 M sodium nitrate. The vials were then shaken on a shaker at 200 rpm. Control vials were also treated the same way. After 3, 8, 15, 24 and 48 hours solutions were filtered through and the supernatant was analyzed for P content using a spectrophotometer at 420 nm wavelength. We followed the blue colour method using sodium molybdate in acid solution and aqueous hydrazine sulphate [20]. Regarding the mixture of biochar and algae, 0.05 g biochar and 0.05 g algae were mixed in P solution. Although different solids reached steady state at different time, we used 24 hours for further adsorption experiments for the convenience of our study [19].

Adsorption experiments were conducted using 0.1 to 100.0 mg/ L phosphorus (P) solution. Isotherms were constructed using data from the experiments in which 0.1 g solid were shaken at 200 rpm for 24 hours. These suspensions were filtered and analyzed for P. The amount of P adsorbed on solid was calculated from the difference between solid-free control and sample treatments at the end of the adsorption period [21]. Data were fitted to the linearand non-linear isotherms (Equations (1) - (3)).

$$K_d = \frac{C_s}{C_{aq}} \quad (1)$$

$$\frac{C_s}{C_{aq}} = \frac{bC_{SM}}{1 + C_{aq}b} \quad (2)$$

$$C_s = K_f C_{aq}^{1/n} \quad (3)$$

where K_d is the partition coefficient; C_s is the concentration of P adsorbed on solid; C_{aq} is the equilibrium P concentration in solution; b is the binding constant; C_{SM} is the maximum adsorption capacity; K_f and n is the Freundlich constants.

2.7. Desorption Experiments

Desorption experiments were carried out using 0.01 M calcium chloride ($CaCl_2$) after the sorption experiment [22]. Following centrifugation, 15 mL of the supernatant was removed and filtered for Panalysis. Then, 15 mL of 0.01 M $CaCl_2$ was added to each tube and the tubes were shaken for 24 hours. Removing the supernatant and addition of $CaCl_2$ was conducted repeatedly for five times. Finally the desorbed Pwas measured using spectrophotometer (Equation (4)).

$$P_{des}(\%) = \left[(W \times Q) - (C \times V)\right] * \left[100/(W \times Q)\right] \quad (4)$$

where, C = Concentration of phosphorus in equilibrium solution.

V = Solution volume;

W = Sample weight;

Q = Amount of phosphorus adsorbed.

2.8. Quality Control

Detection limits were calculated as the mean plus six times the standard deviation of ten repeated measurements of the blank standard. Accuracy of calibration was determined by analysis of an in-house certified reference material (CRM). Analytical precision was calculated from the coefficient of variation determined from the duplicate analysis of 10% of the samples that were at least 100 times higher than the detection limit and determining the median of the difference between the duplicate measurements expressed as a percentage of their mean value [23]. All data were analyzed using SigmaPlot (version 14) software. Data were tested for normal distribution using the Shapiro-Wilk and Kolmogorov-Smirnov test and equal variance using Levene's mean test [23].

3. Results and Discussion

The selected soil sample was analyzed to ascertain the physico-chemical properties of soil (**Table 1**).

The soil pH was found to be 5.04 indicating strong acidic condition. The amount of iron was higher than other nutrients which could be due to the acidic characteristics of the soil (**Table 1**).

3.1. Changes in Soil pH

We observed changes in soil pH before and after the adsorption of phosphorus (**Table 2**). There was no definite pattern in the changes of soil pH. Soil pH decreased significantly ($p \leq 0.05$) after adsorption ofphosphorus (P) for the soils treated with chicken litter biochar (CB), and mixture of tannery waste biochar + algae (TB + AG). There was significant ($p \leq 0.05$) increases in pH of the soil for the orange peel biochar (OB) when present as a single solid as well as mixture of

Table 1. Physicochemical properties of the soil.

Soil parameter	Value
pH	5.04
CEC (me/100g)	25.56
Moisture content (%)	35.71
Organic carbon (%)	1.61
Total nitrogen (%)	0.18
Available phosphorous (ppm)	9.62
Available potassium (ppm)	14.77
Available sulphur (ppm)	127.61
Iron (ppm)	80.32
Manganese (ppm)	45.32
Zinc (ppm)	12.85

orange peel biochar and algae (OB + AG). Non-significant ($p > 0.05$) variation was found for the tannery waste biochar (TB), chicken litter biochar + algae (CB + AG) (**Table 2**).

3.2. Adsorption Experiments

Concentration of phosphorus (P) in solution was decreased over time for all solids bearing suspensions and then reached a steady state which was confirmed by Tukey test. Different solids reached steady state at different times; biochar and algae reached the state within 24 hours whereas the mixture of biochar and algae took 48 hours to reach the steady state. The extent of P adsorption by solids was found to increase and then gradually approach a more or less constant value (steady state) with the increase in time as observed in kinetic experiments. Rate of increase in adsorption was higher within the first 10 hours of the experiment but there was a little increase in adsorption between 10 to 24 hours as in other experiments [24] [25]. Authors reported relatively rapid adsorption from 3 to 15 hours, followed by a slow adsorption after 15 hours, although the adsorption was likely to be highly dependent on the characteristics of adsorbate. As the time proceeds, some sites of the solid experience adsorption and there remain fewer charged adsorption sites that have yet to adsorb.

Adsorption isotherms were constructed for all solid types using the data from adsorption isotherm experiments. Phosphorus (P) adsorption to different solid types was best described by linear isotherms (**Figure 1**, **Figure 2**), indicating the relationship between the equilibrium concentration of phosphorus solution and the concentration adsorbed to the solid surface. Although Langmuir and Freundlich isotherms were not a good fit for the biochar/algae/biochar + algae, the fit to the Langmuir and Freundlich isotherms were used to determine the maximum adsorption capacities (C_{SM}) and heterogeneity factor(1/n) (**Table 3**).

Table 2. Changes in soil pH before and after phosphorus adsorption. 95% confidence intervals are shown in brackets. Upper and lower limit of confidence intervals are separated by commas (,).

Solid type	Initial pH	pH after adsorption
Control (CT)	5.04 (5.01, 5.33)	4.88 (4.75, 4.92)
Orange peel biochar (OB)	4.87 (4.73, 4.91)	5.32 (5.11, 5.64)
Chicken litter biochar (CB)	6.21 (6.03, 6.43)	5.11 (5.02, 5.36)
Tannery waste biochar (TB)	4.96 (4.75, 5.14)	4.84 (4.61, 4.98)
Orange peel biochar + algae (OB + AG)	6.24 (6.17, 6.58)	7.36 (7.27, 7.45)
Chicken litter biochar + algae (CB + AG)	6.35 (6.15, 6.64)	6.42 (6.21, 6.53)
Tannery waste biochar + algae (TB + AG)	7.24 (7.17, 7.35)	5.83 (5.52, 5.95)

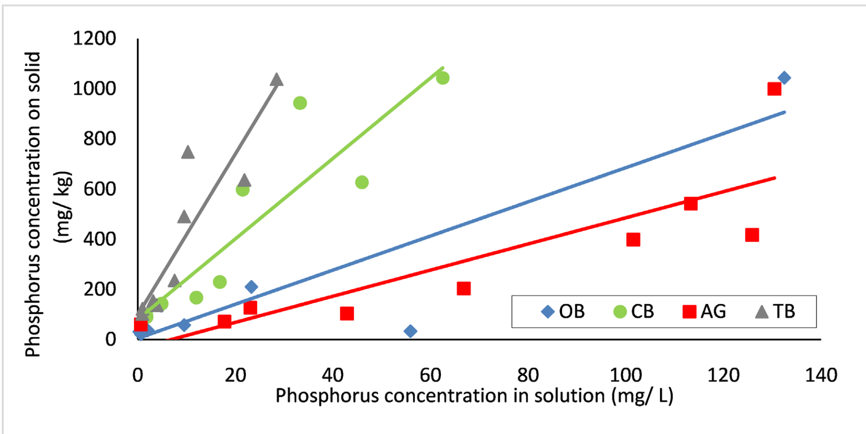

Figure 1. Adsorption of phosphorus by biochar and algae. OB, CB, TB and AG indicated orange peel biochar, chicken litter biochar, tannery waste biochar and algae respectively.

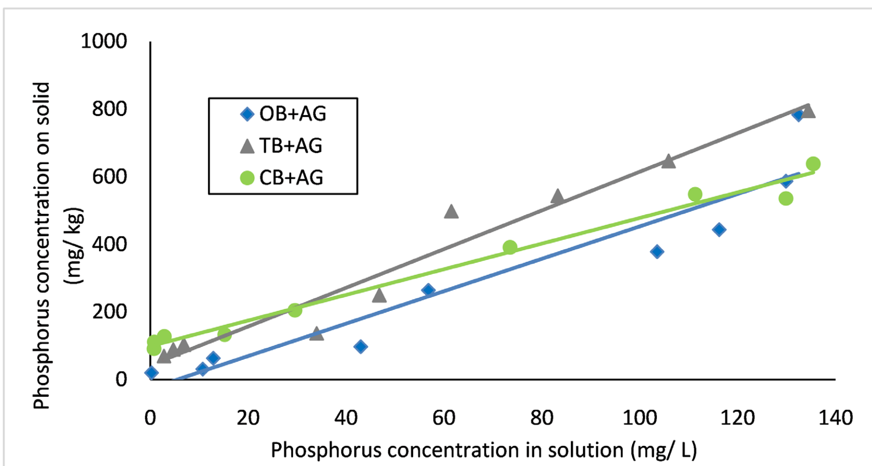

Figure 2. Adsorption of phosphorus by the mixture of biochar and algae. OB + AG, CB + AG and TB + AG indicated mixture of orange peel biochar and algae, chicken litter biochar and algae, tannery waste biochar and algae respectively.

Table 3. Parameters for Langmuir and Freundlich adsorption isotherms. 95 % confidence intervals are shown in brackets. Upper and lower limit of confidence intervals are separated by commas (,).

Solid type	Maximum adsorption capacity, C_{SM} (mg/kg)	Heterogeneity factor, 1/n
Control (CT)	120.45 (118.12, 123.54)	0.35 (0.32, 0.38)
Orange peel biochar (OB)	165.98 (164.78, 167.95)	0.58 (0.57, 0.60)
Chicken litter biochar (CB)	211.75 (209.52, 213.88)	0.79 (0.78, 0.82)
Tannery waste biochar (TB)	278.12 (275.82, 280.22)	0.90 (0.88, 0.91)
Algae (AG)	95.25 (94.12, 97.43)	0.32 (0.28, 0.33)
Orange peel biochar + algae (OB + AG)	110.58 (109.11, 112.07)	0.42 (0.41, 0.44)
Chicken litter biochar + algae (CB + AG)	160.37 (158.32, 162.59)	0.65 (0.64, 0.68)
Tannery waste biochar + algae (TB + AG)	220.54 (218.48, 223.71)	0.75 (0.74, 0.77)

When biochar/algae was present as a single solid in the adsorption experiment, phosphorus (P) adsorbed on the biochar was significantly ($p < 0.05$) greater than that of the algae (**Figure 1**) indicated by higher values of partition coefficient (K_d), maximum adsorption capacity (C_{SM}) and heterogeneity factor (1/n) (**Table 3**, **Table 4**). Adsorption of P was significantly ($p < 0.05$) lower on the algae compared to the control soils. Large differences were found in the values of K_d, C_{SM} and 1/n for the biochar and algae. However, very little variation was found between the values of 1/n (**Table 4**). Among the three biochars, tannery waste biochar (TB) adsorbed more P than the orange peel biochar (OB) and chicken litter biochar (CB). We observed approximately 147% and 35% decreases in P adsorption for the OB and CB respectively compared to the TB. Similar trend was followed by the mixture of biochar and algae. The TB + AG adsorbed about 145% and 60% higher P compared to that of the OB + AG and CB + AG respectively (**Figure 2**; **Table 3**, **Table 4**). The mixture of biochar and algae tended to show adsorption that was more similar to the soil than the algae. Regression analysis between the P adsorption and the treatments applied was carried out to determine the R^2 value; analysis of variance (ANOVA) of the data was carried out to determine the F value. The test of significance of treatments was calculated by LSD. No marked differences were observed in the 1/n for the biochar treatments.

Our adsorption data were in agreement with our findings of soil pH. The pH was found to be lower in the soil treated with TB compared to the OB and CB. When the biochar was mixed with algae, the pH was lower on the TB + AG than the OB + AG and CB + AG indicating higher adsorption of phosphorus on the TB + AG. Our findings were in agreement with [26] who found that P adsorption was lower at high soil pH which was attributed to the increased negative charge on the surface causing electrostatic repulsion of the P.

Table 4. Parameters for linear adsorption isotherms. 95 % confidence intervals are shown in brackets. Upper and lower limit of confidence intervals are separated by commas (,).

Solid type	Partition coefficient, K_d(L/ kg)
Control (CT)	45.87 (43.12, 47.44)
Orange peel biochar (OB)	98.39 (97.67, 99.84)
Chicken litter biochar (CB)	132.45 (131.12, 134.65)
Tannery waste biochar (TB)	242.46 (240.12, 243.44)
Algae	30.81 (29.12, 32.75)
Orange peel biochar + algae (OB + AG)	60.85 (58.02, 63.04)
Chicken litter biochar + algae (CB + AG)	92.46 (90.56, 93.74)
Tannery waste biochar + algae (TB + AG)	148.24 (147.37, 149.94)

The biochar adsorbed phosphorus which could be due to the negative charges present on its surface. Negative charge of biochar is due to the presence of more -OH functional group and is closely related to the soil pH [26]. When soil pH increases, the magnitude of negative charge also increases because of increased deprotonation of the functional group at high pH [27]. Specific adsorption (adsorbed ions are typically bound by chemical attraction through the formation of inner sphere complexes) could happen between the biochar and P as both elements possess negative charges [19]. Negative charge of biochar would repel the negatively charged P and possibly there was cation bridging. Comparatively lower adsorption on the algae than the biochar could be attributed to the greater surface area of the biochar. Higher adsorption on TB could be due to the presence of heavy metals in tannery wastes as heavy metals tend to have higher adsorption capacities [28]. Our findings were supported by previous studies. Studies by [7] [9] [10] observed that biochar produced from industrial waste had higher adsorption capacity compared to that of the biochar produced from burning of the household waste.

Due to the porous structure, surface area, and surface functional groups, biochar could act as a promising adsorbent for P [29]. Biochar was used to recover P from water [9] [29] but till today no evidence of P adsorption was found in the soil in presence of biochar. The application of biochar resulted in a reduction in nitrogen leaching indicating a higher nitrogen retention in biochar treated soils [30] [31]. Experimental results showed that the biochar differing in their molecular composition and pore diameter had significant effects on metal (Cd, Cu, Pb) adsorption. Biochar is comprised of numerous functional groups that would form complexes with metal ions leading to ion exchange reactions [32] [33].

3.3. Desorption Experiments

Desorption experiments indicated that there was no significant ($p < 0.05$) variation found between the desorption of phosphorus (P) from the OB and CB. Significantly ($p \geq 0.05$) lower (compared to the OB and CB) desorption was observed from the TB. The TB + AG desorbed significantly lesser amounts of P compared to that of the CB + AG and OB + AG. All biochars whether present as a single solid or in combination with the algae contributed to higher percentage of P desorption, suggesting that the adsorption was dominated by weak van der waal forces [19]. Data (**Tables 3-5**) showed that the TB and TB + AG samples that adsorbed greater amounts of P, released less to the equilibrium solution during the desorption process. However, our findings were contradictory to the studies of Khan *et al.* [7] and Gaskin *et al.* [22] who showed that the sugarcane biochar adsorbed and desorbed higher percentages of P compared to the straw and wood biochars. It could be due to the differences in the source of biochar and pyrolysis temperature [34].

Desorption of P was significantly higher when the biochar was applied to the soil in combination with the algae. It might be due to the fact that the algae could provide nutrients and organic matter to the soil leading to increased microbial activities that would influence P desorption process.

4. Conclusion

The present study revealed that the biochar had the potentiality to adsorb phosphorus (P).Different types of biochar adsorbed different amounts of P in the following order: tannery waste biochar > chicken litter biochar > orange peel biochar. When the biochar was mixed with the algae adsorption of P was significantly reduced which could be due to the dilution effects. Algae could provide organic matter to the soil leading to a reduction in P adsorption. Desorption data showed that biochar could release significant amounts of P that was previously sorbed. The tannery waste biochar desorbed least amounts of P compared to the chicken litter and orange peel biochars. Desorption was comparatively higher when the biochar was mixed with the algae. Our study highlights the need for a wider range of biochars, differing in surface chemistries and sorption characteristics, together with a wide range of soil nutrients (macro and micro nutrients), to be investigated. Smaller particles than those investigated here will have a

Table 5. Phosphorus desorption from the solids. 95% confidence intervals are shown in brackets. Upper and lower limit of confidence intervals are separated by commas (,).

Solid type	Phosphorus desorbed from solid (%)
Control (CT)	25.22 (23.10, 27.01)
Orange peel biochar (OB)	16.67 (14.11, 18.24)
Chicken litter biochar (CB)	14.56 (12.27, 16.56)
Tannery waste biochar (TB)	9.82 (9.01, 10.02)
Algae (AG)	32.26 (30.26, 34.18)
Orange peel biochar + algae (OB + AG)	22.96 (21.45, 23.64)
Chicken litter biochar + algae (CB + AG)	19.15 (18.34, 20.22)
Tannery waste biochar + algae (TB + AG)	15.23 (13.21, 17.44)

higher surface area to mass ratio which may impact on relative adsorption between soil and biochar such that the impact of biochar particle size also warrants further investigation. Although not done in our experiments, further experiments should focus on the combination of biochar, algae, soil and plant in a single experiment to better mimic the field conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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