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## Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice

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## Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice

### Abstract

2020 The Authors Biochar-based compound fertilizers (BCF) and amendments have proven to enhance crop yields and modify soil properties (pH, nutrients, organic matter, structure etc.) and are now in commercial production in China. While there is a good understanding of the changes in soil properties following biochar addition, the interactions within the rhizosphere remain largely unstudied, with benefits to yield observed beyond the changes in soil properties alone. We investigated the rhizosphere interactions following the addition of an activated wheat straw BCF at an application rates of 0.25% (g·g<sup>-1</sup> soil), which could potentially explain the increase of plant biomass (by 67%), herbage N (by 40%) and P (by 46%) uptake in the rice plants grown in the BCF-treated soil, compared to the rice plants grown in the soil with conventional fertilizer alone. Examination of the roots revealed that micron and submicron-sized biochar were embedded in the plaque layer. BCF increased soil Eh by 85 mV and increased the potential difference between the rhizosphere soil and the root membrane by 65 mV. This increased potential difference lowered the free energy required for root nutrient accumulation, potentially explaining greater plant nutrient content and biomass. We also demonstrate an increased abundance of plant-growth promoting bacteria and fungi in the rhizosphere. We suggest that the redox properties of the biochar cause major changes in electron status of rhizosphere soils that drive the observed agronomic benefits.

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## Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice

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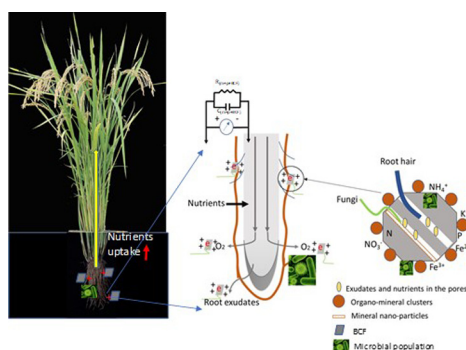
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### HIGHLIGHTS

- Biochar-based fertilizer (BCF) is known to enhance crop yields and soil properties.
- Wheat straw BCF was applied to the soil to investigate rhizosphere interactions.
- There was an increase in rice yield, and N (40%), P (46%), Mg, K and Na uptakes.
- Micron and submicron-sized biochar were embedded in the plaque layer.
- Biochar increased soil Eh, which resulted in greater plant nutrient content.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Biochar-based compound fertilizers (BCF) and amendments have proven to enhance crop yields and modify soil properties (pH, nutrients, organic matter, structure etc.) and are now in commercial production in China. While there is a good understanding of the changes in soil properties following biochar addition, the interactions within

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## 1. Introduction

Adding pyrogenic carbon (referred to as biochar; BC) to soil is often reported to alter various soil physical and chemical properties (e.g. pH, water and nutrient retention, particle aggregation). These alterations are widely viewed to have positive knock-on effects on plant performance, including crop yield and plant health (Liu et al., 2013; Jeffery et al., 2015; Nguyen et al., 2017). Yet, in many situations, it has been noted that biochar-induced plant growth and health stimulation go beyond obvious contributions to plant nutrition and improved soil physicochemical properties, a phenomenon termed “The Biochar Effect” (Elad et al., 2011). The Biochar Effect appears to be linked to biochar-induced changes in microbial community structure, taxon-functional diversity and microbial activity, which are a function of complex interactions between many physical, chemical and biological components of the intricate soil/plant/biochar system (Jaiswal et al., 2017).

Despite the often-positive impacts of biochar in modern agricultural systems, it is commonly too expensive to apply at application rates  $>5$  t/ha. This is due to the high cost of collecting available biomass residues as well as the high capital, operating and maintenance costs of pyrolysis plants (Clare et al., 2014). To overcome the economic barriers to utilize biochar in agriculture, efforts have been made over the past decade to develop compound biochar-chemical fertilizers (BCF) that can capitalize on the Biochar Effect. BCFs are generally made up of 20–80% biochar and 5–8% clay, other minerals, organic binders and chemical compounds containing nitrogen (N), phosphorus (P) and potassium (K). BCFs are commonly applied at  $\sim 500$  kg/ha, at a cost similar to that of conventional chemical fertilizers. Relative to conventional chemical fertilizers, BCFs have been shown to increase crop yields, N and P use efficiency, vegetable quality (e.g. increase vitamins and sugars content), abundance of beneficial microorganisms, and farm profitability, and to reduce pesticide inputs and lower soil greenhouse gas emissions (Joseph et al., 2013; Qian et al., 2014; Zheng et al., 2017; Yao et al., 2015; Blackwell et al., 2015).

The means by which BCF application results in observed positive changes relative to chemical fertilizers are however still poorly understood. Ye et al. (2016) found that microorganisms, which can fix carbon dioxide and oxidise Fe and thiosulphate, grow on iron-rich minerals embedded in the biochar portion of a BCF. (Joseph et al., 2015a, 2015b) noted that when mineral enhanced magnetic biochars were applied at low application rates, there was an increase in mycorrhizal fungal root colonisation, which led to an increase in plant nutrient uptake. Chen et al. (2018) found that a rice husk/urea BCF not only released N at a slower rate than urea, but also immobilized cadmium and prevented its uptake into plants. However, the mechanism(s) behind the beneficial impact of BCFs remains an enigma.

One mechanism that has not yet been explored is that BCFs change the ion potential across the root membrane. Ion potential is important, because it governs the uptake of nutrient cations and anions, especially nitrates (Yan et al., 2011). The energy required to transport an ion

against a potential gradient (e.g. a root cell gradient) is derived from ATP (adenosine triphosphate). The greater the energy spent on ion absorption, the lower the plant growth (Schachtman et al., 1998). Increasing the potential difference between the root membrane and the soil (which we will refer to as the root membrane potential) can increase the free energy for transportation of nutrients. (Joseph et al., 2015a, 2015b) noted that the Eh and pH of the soil changes when biochar is added and the magnitude of change is a function of biochar type, the application rate and soil properties. Biochar can embed into the plaque layer formed on rice roots and root hairs can also enter the pores of the biochar (Joseph et al., 2013). When biochar interacts with the roots of a plant, both Eh and pH can theoretically change and thus lead to changes in both the root membrane potential and the microbial population structure (Husson, 2013).

In support of such a process, Sun et al. (2017) found that biochar can directly transfer electrons more than three times faster than the charge/discharge cycles of surface functional groups and has a 1.5 V potential range for biogeochemical reactions that invoke electron transfer processes. Root membrane potential can also be increased when there are changes in the abundance of particular microorganisms that promote changes in the surrounding soil Eh through complex redox reactions involving electron shuttling (Zhou et al., 2016). BCFs have been reported to induce shifts in rhizosphere microbial populations. We hypothesized that deposition of biochar in the rhizosphere and in the plaque layer that surrounds the roots of rice leads to changes in soil Eh that might result in some changes of plant growth and microbial population. This, in turn, leads to changes in the ion potential across the root, and these changes are, in part, responsible for observed improvements in plant growth.

## 2. Materials and methods

### 2.1. Soil characteristics

A clay-loam soil (Fimi-Orthic Anthrosol) was collected from a vegetable plot in Nanjing ( $31^{\circ}58' \text{N}$ ,  $118^{\circ}48' \text{E}$ ). The soil pH ( $\text{H}_2\text{O}$ ) was 5.4, EC  $13.5 \mu\text{S}/\text{cm}$ , organic matter was  $13.53 \text{ mg}/\text{g}$ . N, P, K concentrations are presented in Table 1. The analytical method followed those described by Lu (2000).

### 2.2. Production and characterisation of a nutrient enhanced biochar (BCF)

In the production of BCF, 200 g of dry wheat straw was mixed with 15 g urea, 15 g bentonite clay, 15 g rock phosphate, 5 g  $\text{Fe}_2\text{O}_3$  and 5 g  $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$  (Yao et al., 2015). The non-biomass ingredients were dissolved and dispersed in 100 g demineralized water at  $80^{\circ}\text{C}$  and then the straw was added. The mixture was left to stand for 24 h, then dried for 3 h at  $110^{\circ}\text{C}$  in a laboratory pyrolysis system as described by Rawal et al. (2016). The temperature was increased at a heating rate of  $5^{\circ}\text{C}/\text{min}$  and held at  $400^{\circ}\text{C}$  for 30 min. It was then cooled to room

**Table 1**

NPK concentration of soil; CK = control and BCF.

	Initial unamended soil mg/kg (dry weight)	Available NPK added to soil from addition of fertilizer mg/kg	Available NPK added to soil from addition of BCF mg/kg	CK nutrient content mg/kg soil	Total nutrient content of BCF treatment mg/kg soil
N	850	100	0.027	950	950
P	700	50	25	750	775
K	38,100	70	50	38,170	38,220

temperature over a period of 24 h and stored at 4 °C in sealed containers. The pH, EC, total C, N and P, water-soluble and citrate-soluble P and exchangeable cations of the BCF were measured using the procedures detailed in Van Zwieten et al. (2010) and Slavich et al. (2012) in an ISO17025 accredited facility. The equipment and further methods are detailed in the supplementary information. During pyrolysis, some of the nitrogen will be liberated and part of the nitrogen will be incorporated into the carbon lattice. Analysis of the BCF using XPS (Table S1) and liquid chromatography with organic carbon detection (LC-OCD) (Table S2) indicated that the total dissolved organic nitrogen (DON) was 0.026 mg/g of carbon.

### 2.3. Plant growth trials

Rice seedlings were cultivated in growth bags (10 cm wide and 15 cm deep) into 540 g soil (dry weight equivalent), which are chemical-fertilized soil without BCF (CK treatment) or with BCF (BCF treatment, Table 1). The CK treatment contains total N 0.95 g/kg soil, total P 0.75 g/kg soil and total K 38.17 g/kg soil. The BCF treatment was added 0.25% BCF (w/w, 0.25 g per 100 g soil) to chemical-fertilized soil. In chemical-fertilized soil, the additional available nutrient levels brought by 0.25% BCF was very low (0.027 mg N/kg soil, 25 mg P/kg soil, and 50 mg K/kg soil).

Rice plants (*Oryza sativa* L., cv. Japonica) were grown from seeds and the soil was initially wetted to 95% of water holding capacity (WHC) and maintained at this level. There were 5 replicates for each of the treatments and the grow bag experiment was repeated three times. The randomly placed plants were grown in a greenhouse with a temperature that ranged between 22 and 27 °C and at natural lighting.

### 2.4. Measurement of electrophysiology/potential across the root membrane

Sixty days after germination, all bags with and without BCF were opened to expose the roots. The open bags were placed into a Faraday Cage using Ag/AgCl microelectrodes to measure the electric potential of root cells, the potential of the soil (Eh), and the soil pH. All the measurements were taken on three roots per plant. The Ag/AgCl electrode was placed approximately 2 cm from the root and the working electrode was placed in the epidermal cells at distances 1, 3 and 5 cm along the root tip (Fig. S1). Five readings were taken at each point along each root on four roots per plant. Readings of voltage were converted to the standard hydrogen electrode (SHE). The microelectrodes were constructed using filamented single-barrelled borosilicate glass (1 mm outer diameter and 0.8 mm inner diameter, Hilgenberg, Germany) constructed on a Narashige micromanipulator (model NMN-21, Narashige, Japan). The voltage was amplified by an Axon 900A amplifier and analysed and displayed using Clampex software. After the electrophysiology measurements, the roots were separated from the above-ground biomass and weighed both wet and dry.

### 2.5. Microbial analysis

Total DNA was extracted in triplicate from 0.25 g aliquots of both bulk and rhizosphere samples using a PowerSoil™ DNA isolation kit (Mo Bio Laboratories Inc., CA) following the manufacturer's protocols. The quantity and purity of DNA extracts were checked using

spectrophotometry (Nanodrop 1000) before sequencing of bacterial 16S rRNA genes and fungal ITS regions. The primers 515F and 807R targeting the V4 hypervariable region of the 16S rRNA gene (REF), and the primers ITSF1 and ITS4 targeting the ITS region 2 (REF) were used. Paired-end sequencing was conducted using the Illumina MiSeq platform (Kit v2, 2 × 250 bp). PCR and sequencing were conducted at the Ramaciotti Centre for Genomics (UNSW, Sydney Australia) following the centre's protocols (<https://www.ramaciotti.unsw.edu.au/>). Processing of sequences, clustering into operational taxonomic units (OTUs) and taxonomic assignment and is described in the supporting information.

### 2.6. Data analysis

Fresh and dried shoot biomass, soil pH, soil Eh, and the electric potential across the root cell membranes were examined using linear models and analysis of variance (ANOVA). Treatments and distance from root tip were treated as fixed factors, and an alpha level of 5% ( $P < .05$ ) was used for hypothesis testing. Means and 95% confidence intervals were obtained from the linear models and used for the figures. Microbial communities in the samples were compared using distance-based and per-taxon level approaches. For distance-based comparisons, each sample was subsampled ('rarefied') to the lowest total number of read counts observed and square root transformed before comparing every sample using the Bray-Curtis similarity coefficient. The resulting distance matrix was visualised using non-metric multidimensional scaling (NMDS). Permutational ANOVA (PERMANOVA) was used to examine the effect of BCF addition (absent vs present) and environment (bulk soil vs rhizosphere soil) and the biochar by environment interaction. P values were calculated by 999 permutations of the data. Models and P values were generated using the R package vegan. Further details are given in the supporting information.

The free energy for uptake of specific nutrients was calculated using the following equation (Chesworth, 2008):

$$\Delta\mu = RT \ln C_i/C_o + zF\Delta E \quad (1)$$

$C_i$  = concentration inside the cell

$C_o$  = concentration outside the cell

$E$  = the electrical potential difference across the cell

$F$  = Faraday constant

$z$  = valence on the ion

## 3. Results

### 3.1. Basic properties of the BCF

It was important to first establish the profile of the BCF before addition of the chemical NPK fertilizer. Detailed characterisation of the BCF aimed at elemental, micron and submicron scale features are summarised here, with full details in the SI:

1. The BCF exhibited the following elemental analysis: C, 43%; N, 2.7%; K, 2%; P (as citrate extractable), 1.1%, slightly lower than total P, 1.4%. Its pH was near neutral, with an acid neutralising capacity of 5.8% in calcium carbonate equivalents. Its exchangeable cations were dominated by K, with low levels of Al and Na. The BCF had a

- relatively high content of soluble K, S and Ca and a lower content of Na and Br (Table S3) compared to woody biochars (Van Zwieten et al., 2010).
- Water extraction tests of BCF showed detectable amounts of water-soluble organic compounds, metals and non-metals (Table S2). Total dissolved C constituted approximately 1% of the total C in the BCF at an extraction temperature of 20 °C. The greatest percentage of organic compounds constituted low molecular weight acids. Low molecular weight neutrals and high molecular weight compounds, which had similar characteristics to humic substances, were also detected.
  - Gas chromatography/mass-spectrometry analysis of compounds using the NIST98 library indicated that there were 20 predominant organic acids and five neutral compounds, such as alkanes, sugars and aldehydes (Table S4).
  - The surface of the biochar was heterogeneous with a range of minerals and inorganic compounds many with a diameter of <100 nm (Fig. 1 and S2). Most of these mineral particles were surrounded by organic molecules. There was a relatively high concentration (compared to woody biochars) of C functional groups (phenols, esters, ketones and carboxylic acids) and N groups including NH<sub>3</sub><sup>+</sup>, amines, amino groups (Table S1, Figs. S3 and S4) on the surface of biochar.
  - Assessment of the radical concentration in the biochar by Electron Spin Resonance (ESR) spectroscopy is presented in Table S6 reveals that the BCF has a hundred-fold greater concentration of radicals than the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) and there is a slight shift in the g band. The high concentration of radicals is indicative of p-type aromatic free radicals that are stabilized by delocalization in the aromatic ring systems. The value of the g band at 2.003 is indicative of the presence of a C structure similar to that observed in activated C with a significant concentration of quinone/semi-quinone functional groups (Xu et al., 2016) known to facilitate redox reactions with soil organic matter.
  - Solid state NMR spectroscopy revealed that the detectable carbon content has a high concentration (65%) of both protonated and non-protonated aromatic C (Fig. S3). Hydrogen pyrolysis indicated that the aromatic carbon was stable on a long timescale (Bird et al., 2015). Details of the methods used are given the supplementary

information. Furthermore, the biochar is redox active (Fig. S5) and is ferromagnetic (Fig. S6) with a relatively high concentration of both hematite and magnetite particles having diameters ranging from 5 nm to >100 nm (Fig. S3). The smaller <10 nm particles are likely to be superparamagnetic and the >10 nm particles paramagnetic.

### 3.2. Growth experiments and plant properties

The addition of the BCF significantly increased the wet and dry shoot biomass of rice plants compared to the control treatment without BCF. The average increase of the wet biomass was 82%, from 1.60 g to 3.02 g per plant and the average increase of dry biomass was 67%, from 0.3 g to 0.5 g/plant ( $F_{1,4} = 177, P < 0.001$ , Fig. 2a and b). Nutrient content (N, P, K, Mg, and Na) was significantly higher ( $P < 0.05$ ) in the plants from the BCF treatment than the plants in control (Table 2). Herbage N increased by 40%, P by 46% and K by 26%. However, there was no difference between the total N and P content in the soil amended by the BCF and the control (Tables 1 and 2).

There was a significantly higher concentration of K, Ca, Mg, Na in the BCF soil than in control and a significantly higher concentration of K, Mg and Na in the BCF plant compared to the control (Table 2). To determine if the higher concentration of K Ca, Mg and Na was responsible for a large proportion of the plant growth the total free energy and the free energy component related to the concentration difference between plant and soil was calculated using the Nernst equation (Chesworth, 2008). This showed that in all cases the free energy for cation uptake was greater for the BCF treatment and this was mainly due to the difference in root membrane potential (Table 3).

### 3.3. Electrophysiology, soil Eh and pH, examination of the plaque on the root and microbial analysis

The root cell membrane potential compared with NPK was significantly increased as a result of BCF addition (Fig. 3a and Table S7). The more negative the root membrane potential, the greater is the difference between the root membrane and the soil. There was a significant decrease in the potential difference ( $P < 0.001$ ) as measurements were

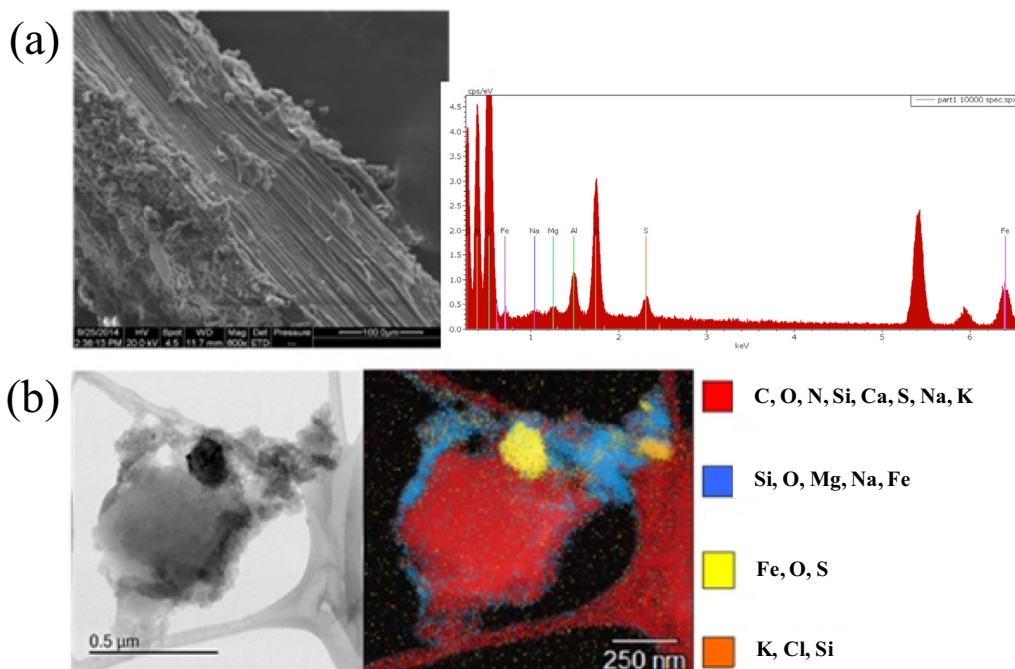
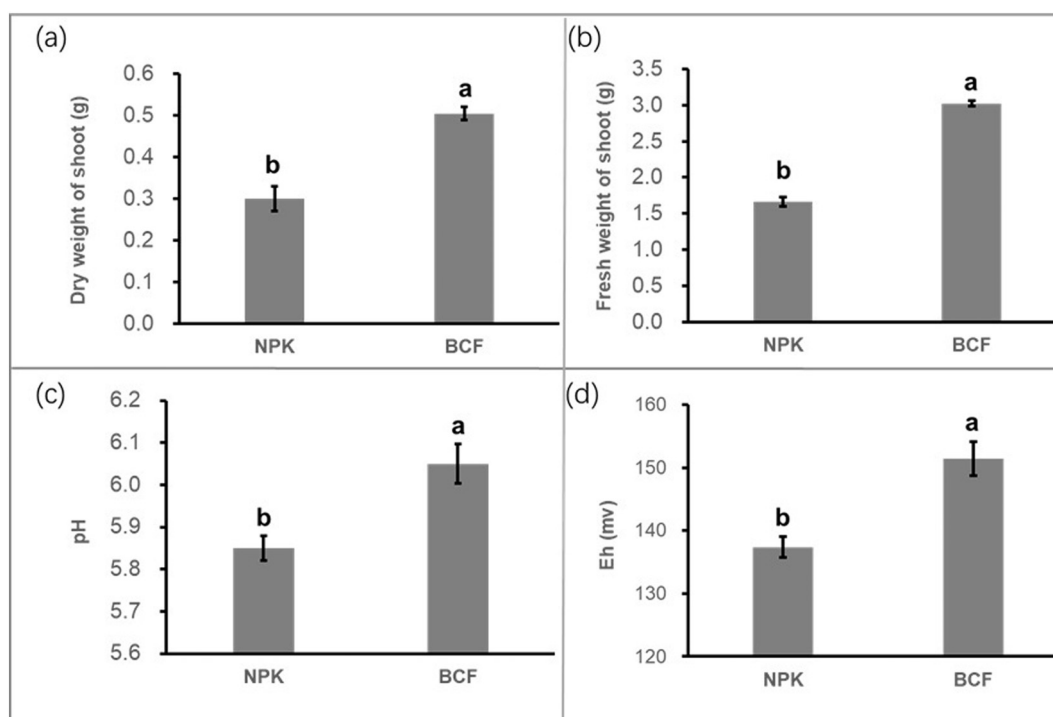


Fig. 1. Secondary electron SEM image of fresh BCF. (a) EDS spectrum of the external and internal surfaces of the carbon. (b) STEM Bright field image and EDS phase maps.



**Fig. 2.** Dry weight, root cell membrane potential, soil Eh and pH near root tip. (a) Dry weight of shoot. (b) Fresh weight of shoot. (c) Bulk soil pH and (d) soil Eh (SHE) adjusted for pH after 60 days in response to BCF under 95% WHC conditions. Error bars: SE ( $n = 3$  experimental replicates). Significant differences between NPK and BCF are indicated by different letters ( $P < 0.05$ , one-way ANOVA).

taken upwards from the root tip regardless of whether BCF was present or not (root membrane potential became less negative). The potential was approximately 65 mV more negative than the control as a result of BCF addition at all measurement points along the root. The bulk soil Eh for the BCF increased over the time of the experiment by approximately 80 mV, whereas the control was only 35 mV (Fig. 3d). The soil Eh in the rhizosphere was significantly ( $P = 0.002$ ) greater (by 85 mV) as a result of BCF addition. There was a significant increase in Eh as measurements were taken upwards from the root tip in both the control and BCF amended soil (Fig. 3b and Table S7). The pH was significantly ( $P = 0.002$ ) greater (by ca. 0.5 units) as a result of BCF addition and pH increased upwards from the root tip in both control and the BCF amended soil.

SEM analysis of the root surface was carried out to determine if BCF particles had imbedded in the plaque layer and whether there was a detected concentration of macro and micronutrients remaining in these particles. Fig. 4 represents an image and an X-ray elemental analysis (EDS) of a piece of BCF that was embedded in the plaque layer of the root. Fig. 4b is a higher magnification image of the biochar structure that is derived from the phloem and xylem of the wheat straw. There is a range of submicron particles on the BCF surface with a relatively high concentration of Si, Ca, Fe, K, Al and smaller quantities of Mg, Na, P, S, Ti and Cl (Fig. 4). There was no detectable N or P on the surface of the BCF fixed with 2% glutaraldehyde in phosphate-buffered saline (PBS, pH 6.2) for 1 hr.

Changes in concentration of total P and N in the plant could also be due to changes in microbial community. Multivariate analysis of bacterial community composition using distance-based methods showed clustering of samples with respect to BCF addition, high variability among samples without BCF addition and little difference between samples from bulk soil and rhizosphere soil environments (Figs. 5 and S7). Permutational analysis of variance (PERMANOVA) confirmed an effect of the BCF addition ( $F = 3.19$ ,  $P = 0.014$ ) and no detectable effects elsewhere (Table S8). Similarly, the sum of likelihood ratios (sum of LR) from individual generalised linear models (GLMs) of each bacterial operational taxonomic unit (OTU) revealed a sole effect of BCF addition on bacterial communities ( $LR_{\text{sum}} = 3983$ ,  $P = 0.047$ ). Fungal communities within the rhizosphere environment also showed clustering of samples in the BCF amended soils (Figs. 5 and S8). PERMANOVA suggested a possible small effect of BCF addition (BCF,  $F = 2.07$ ,  $P = 0.1$ , with the acceptable P value possible by permutation equal to 0.1) given the small sample size (Table S8). The sum of LR from individual GLMs models of each fungal OTU revealed no strong effect of BCF addition ( $LR_{\text{sum}} = 609$ ,  $P = 0.141$ ).

There were 121 bacterial OTUs that responded strongly to the addition of BCF ( $P < 0.01$ ), including 75 and 46 that responded positively and negatively, respectively, in terms of abundance changes (Fig. 6a and Table S9). These OTUs were associated with a diverse range of taxonomic ranks, which included related taxa showing opposite responses to the BCF (Fig. S7). The most abundant OTUs responding positively to

**Table 2**

Analysis of specific element concentration (Mean  $\pm$  SD,  $n = 3$ ) in plants and rhizosphere soil. Different letters in a single row indicate a significant difference between the CT and BCF treatments ( $P < 0.05$ ).

(mg/g)	Treatment	N	P	Mg	Ca	Na	K
Plant	CT	3.25 $\pm$ 0.45b	0.88 $\pm$ 0.12b	0.78 $\pm$ 0.13b	1.31 $\pm$ 0.19a	0.43 $\pm$ 0.03b	15.7 $\pm$ 0.80b
Plant	BCF	5.24 $\pm$ 0.17a	1.63 $\pm$ 0.43a	1.28 $\pm$ 0.36a	2.13 $\pm$ 0.63a	0.58 $\pm$ 0.09a	26.9 $\pm$ 4.28a
Soil	CT	0.75 $\pm$ 0.03a	0.36 $\pm$ 0.18a	1.81 $\pm$ 0.03b	3.95 $\pm$ 0.008b	0.36 $\pm$ 0.02b	19.6 $\pm$ 0.18b
Soil	BCF	0.77 $\pm$ 0.01a	0.38 $\pm$ 0.01a	2.58 $\pm$ 0.05a	4.97 $\pm$ 0.07a	0.52 $\pm$ 0.03a	23.7 $\pm$ 0.08a

**Table 3**  
Free energy for selected cations in the rhizosphere in kJ/mol.

Element	Ca/kj/mol.	Mg/kj/mol.	Na kj/mol.	K kj/mol.
Total Free Energy Control	-22.85b	-23.16b	-12.17b	-3.84b
$\Delta\mu = RT\ln Ci/Co + zF\Delta E$	sd = 0.36	sd = 0.26	sd = 0.74	sd = 0.23
Total Free Energy BCF	-34.7a	-34.27a	-18.74a	-9.21a
$\Delta\mu = RT\ln Ci/Co + zF\Delta E$	sd = 0.2	sd = 0.45	sd = 0.24	sd = 0.1
Free Energy Control due to concentration difference = $RT\ln Ci/Co$	9.24a	8.94a	3.88a	12.21a
	sd = 0.36	sd = 0.46	sd = 0.74	sd = 0.23
Free Energy BCF due to concentration difference = $RT\ln Ci/Co$	8.72a	9.16a	2.98a	12.51a
	sd = 0.35	sd = 0.77	sd = 0.24	sd = 0.1

Note: Calculation based on the cell membrane potential at 2 cm from the root tip.

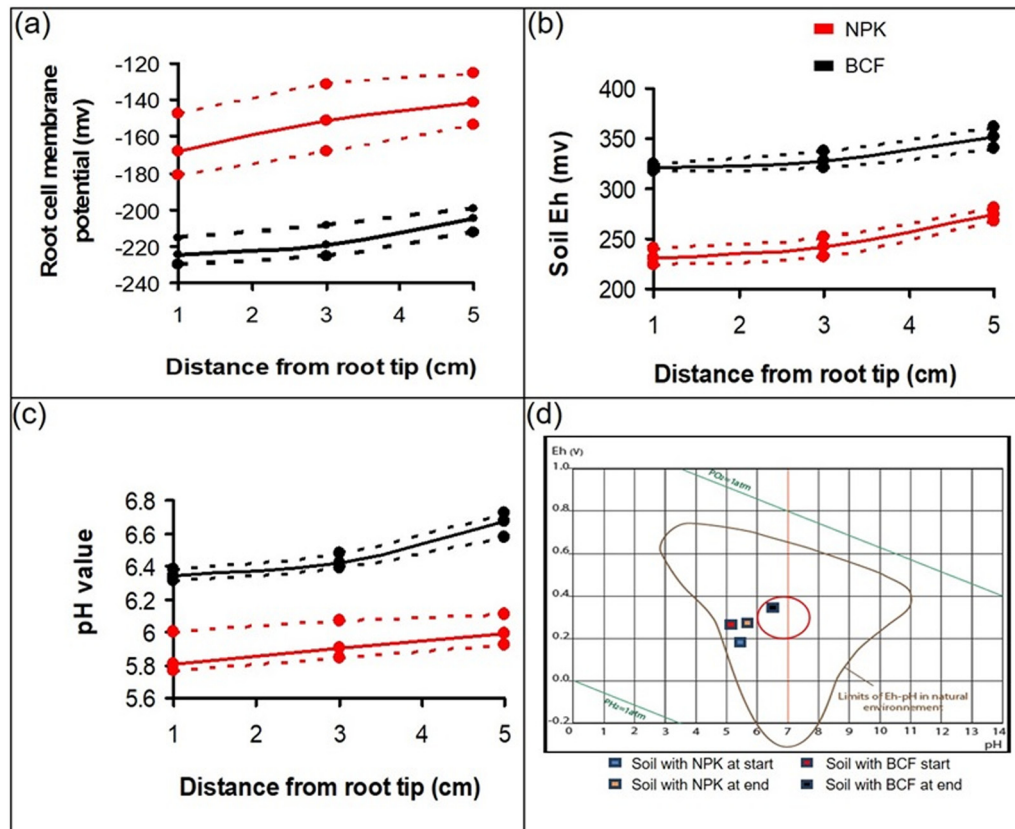
the BCF were associated with the family *Comamonadaceae* (Phylum Proteobacteria) and the genus *Phormidium* (Phylum Cyanobacteria), while the most abundant OTU responding negatively to the BCF addition was associated with the family *Oscillatoriophycidae* (Cyanobacteria), (Figs. 6a and S7, Table S9). The number of different genera from the order Rhizobiales (Phylum Proteobacteria) increased in abundance in the presence of BCF, including *Afifella*, *Rhodoplans* and *Devosia*. Some bacteria from the order Rhizobiales are known for their phosphorus-solubilizing activity and others for nitrogen fixation (Souza et al., 2013).

Investigation of fungal OTUs responding to biochar addition showed 19 and 25 to respond positively and negatively, respectively, to BCF addition (Figs. 5, 6b and S8, Table S9). Some of these OTUs were the most abundant observed in the communities, including the fungal species *Mortierella alpina*, *Cyberlindnera saturnus*, and *Phaeosphaeria microscopica* that responded positively to BCF. The fungal species *Fusarium pseudonygami*, *Alternaria tenuissima*, *Paraphaeosphaeria sporulosa* and members of the genera *Cladosporium* and *Aureobasidium* were observed to decrease in abundance with the addition of BCF. Some of

these latter fungal taxa have members that are pathogenic (Logrieco et al., 1990). A large number of fungal OTUs, however, were only classified to kingdom level suggesting changes to a number of uncharacterized fungal groups. Furthermore, it was found that fungi grew on the BCF embedded in the plaque layer (Fig. S9), where Al, Si, Fe, K, S, Na, Mg and Ca have been detected.

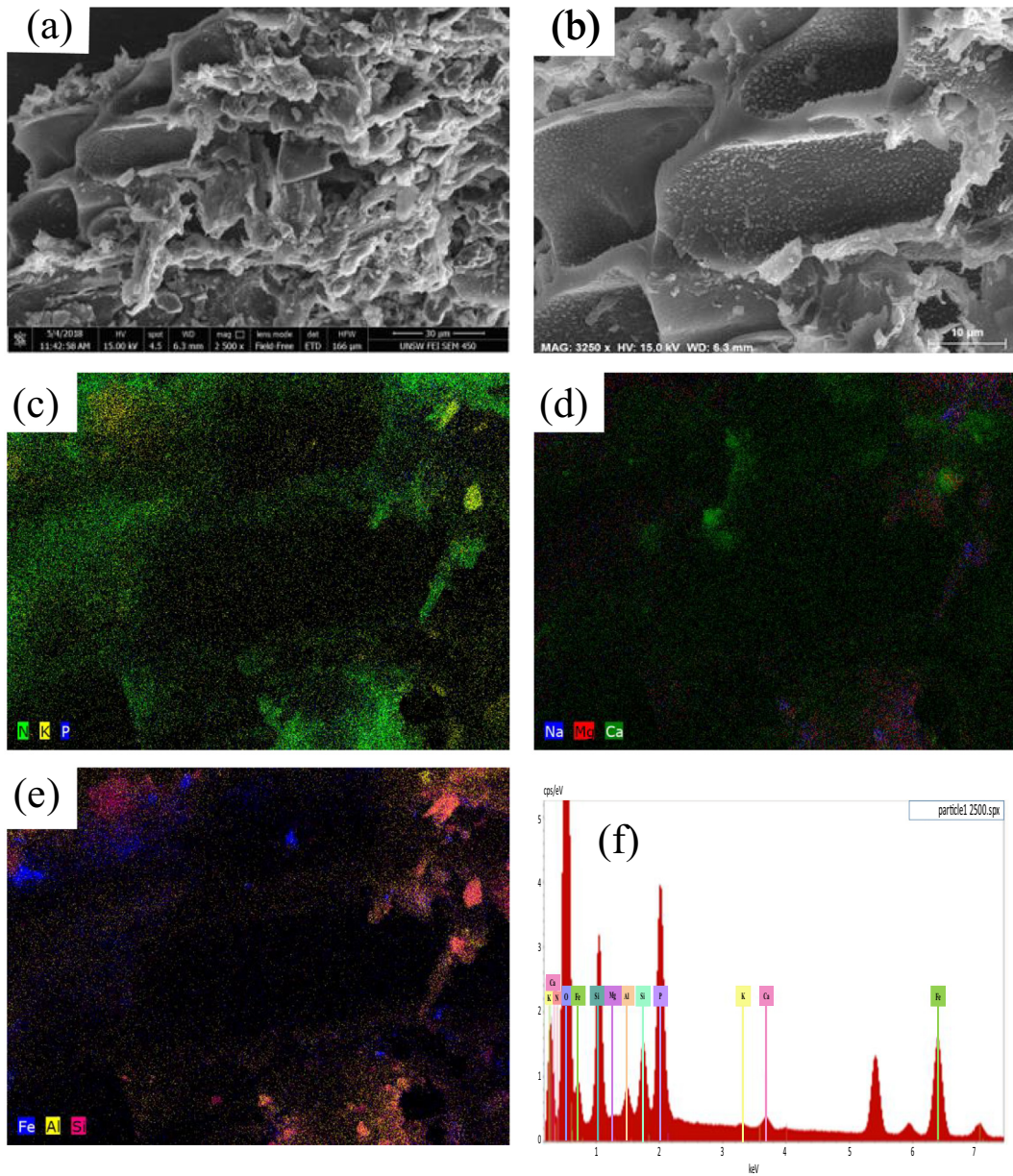
#### 4. Discussion

Adding BCF to the soil resulted in increased dry plant biomass by 67% over the control and increased plant N, P and K by 40%, 46% and 26% for, respectively, despite there being no significant differences in the soil N and P content between the BCF and chemical fertilizer treatments. BCF treated plants had substantially higher root membrane potentials than plants from the CF treatment, and microbial communities in the rhizosphere and bulk soil were different in the two treatments. These observations, together with the presence of BCF particles in the root plaque layer, support the hypothesis that improved rice plant performance under BCF treatment is connected to biochar interactions in the

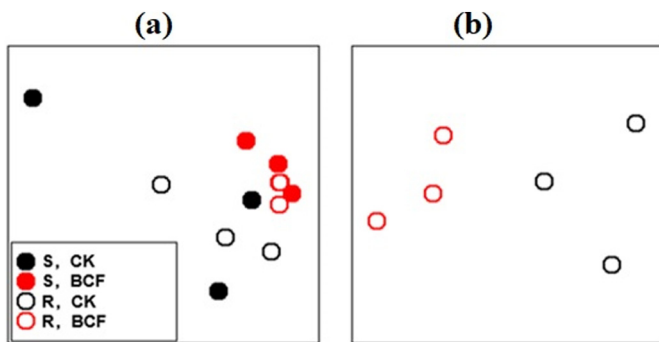


**Fig. 3.** The root membrane potential, soil Eh and pH. (a) Potential difference between the inside of the root membrane and the soil. (b) Soil Eh and (c) pH as a function of distance from root tip for moisture content under 95% WHC conditions ( $n = 5$  for each mean). (d): Eh and pH of soil at the beginning and end of the experiment plotted on a modified Pourbaix.





**Fig. 4.** The X-ray elemental analysis of BCF. (a); (b) Secondary electron image of biochar embedded in the plaque layer and (c); (d); (e) EDS spectrum and map of an area where BCF has embedded in the plaque layer. (f) The unidentified peak is Cr that was used to coat the specimen to facilitate high magnification imaging.



**Fig. 5.** NMDS ordination of (a) bacterial, (b) fungal communities in soil (S, filled circles) and rhizosphere (R, open circles) in rice plant grow bags after 90 days with (Red) and without (Black) BCF compared using the Bray-Curtis similarity coefficient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

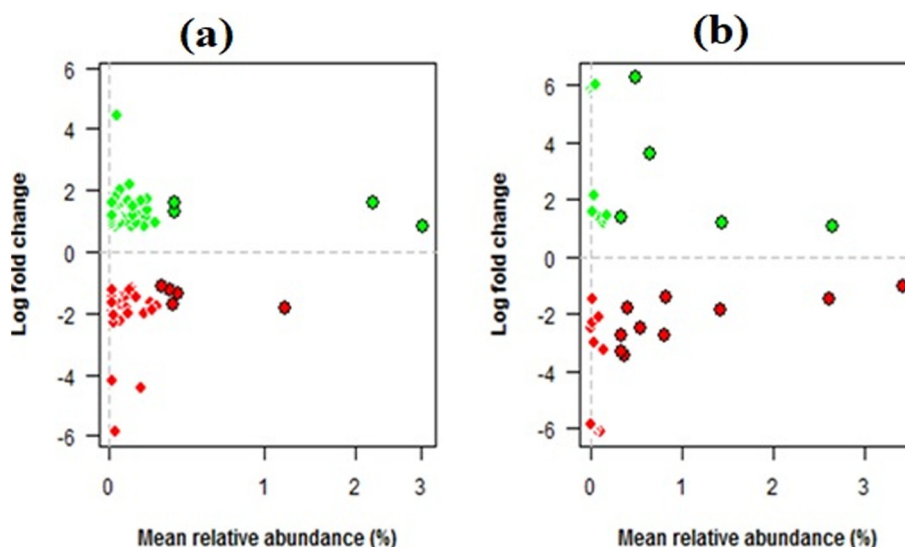
rhizoplane that contribute to increases in the ion potential across the root and improved macronutrient uptake.

#### 4.1. BCF interacts physically with roots

A plaque layer slowly forms on rice roots within the first few weeks of growth (Chen et al., 1980), and as new roots elongate, they will intersect the BCF particles (Prendergast - Miller et al., 2014). We demonstrate the presence of BCF embedded in the plaque layer (Fig. 4), a phenomenon that may underlie several of the interactions we report here including being partly responsible for the substantially higher potential difference between the inside of the root and the soil (ca 65 mV) in the BCF treatment compared to the control.

#### 4.2. BCF interacts with roots to modify local redox conditions

As noted by Joseph et al., 2015a, 2015b, the introduction of high mineral ash biochar results in significant changes in both soil Eh and pH.



**Fig. 6.** Mean relative abundance and log fold changes of (a) bacterial and (b) fungal OTUs detected to significantly differ ( $P < 0.01$ ) between BCF treatments. OTUs responding positive or negative to biochar are coloured green or red, respectively. Circled points refer to OTUs shown in Table S9. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

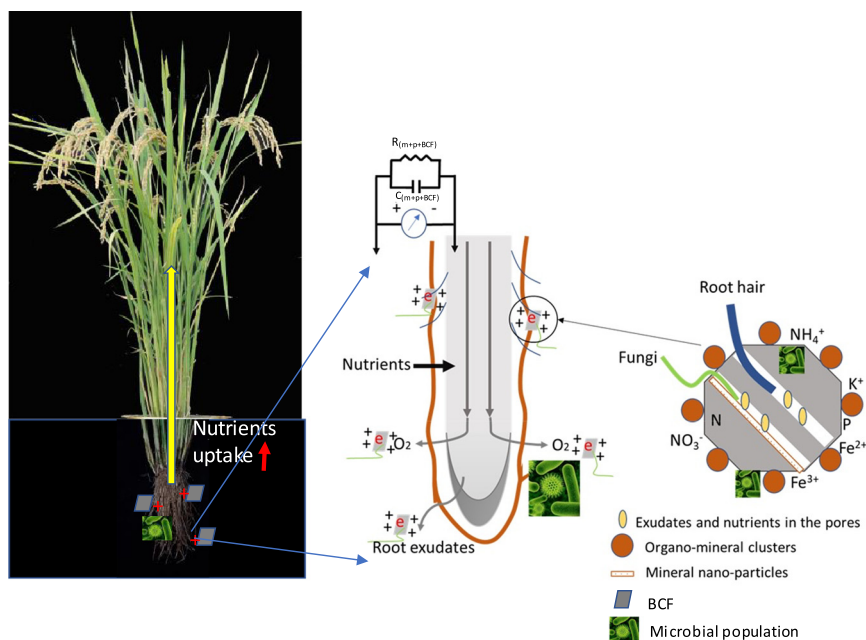
This increase is in part related to the release of both cations and anions, inorganic oxide particles (especially Fe/O and Mn/O) and organic molecules that can be redox active, as well as changes in microbial population that may also affect changes in soil redox state (Ye et al., 2016). It is also in part due to the interaction of the redox active species in the BCF with the oxygen both in soil and from the plant. In the simulated rice paddy trials reported here, we have demonstrated the role of the BCF in influencing soil redox condition, and how this increased Eh as well as increased root membrane potential difference improves nutrient accumulation (Quin et al., 2015; Sun et al., 2017) and thereby leads to higher biomass.

Ultimately the increase in root membrane potential results in a reduction in the energy the plant expends to translocate specific cations from the soil through the cell membrane. Our results also indicate a

complex series of biological, chemical and physical changes taking place on the plaque layer and surrounding rhizosphere in the presence of BCF. The processes that are taking place are summarised in Fig. 7.

Higher root membrane potential is known to drive the uptake of nutrients, particularly nitrate, by roots, because energy requirements to move nutrient ions from the soil into the plant is lower (Yan et al., 2011). Environment factors that influence root membrane potential are  $O_2$  (or Eh),  $K^+$  and pH (Zeng et al., 2014; Haruta et al., 2018), so that roots with higher membrane potential will take up more nutrients, especially nitrogen, than roots with lower membrane potential.

The results presented here show that the addition of BCF substantially increased plant biomass and macronutrient uptake. The characteristics of BCF particles attached to the roots and the measured changes in soil Eh,  $K^+$  and pH, microbial communities and root membrane provide



**Fig. 7.** The model of possible processes with the incorporation of BCF in plaque layer of rice roots. There is a potential difference between the membrane and the soil. The biochar, membrane and plaque layer have a capacitance and a resistance. The biochar under reducing conditions can store electrons and donate electrons when there are electron acceptors ( $O_2$  and  $NO_3^-$ ). Root hairs and micro-organisms can reside in the pores of the biochar increasing the availability and reducing the energy plants have to expend to uptake nutrients. Root exudates (organic and  $O_2$ ) can diffuse into the biochar resulting in increases of beneficial microbes and release of certain nutrients.

an understanding of the possible mechanisms behind the enhanced growth and increased nutrient uptake. Plaque layers typically and slowly form on the roots within the first few weeks of growth (Chen et al., 1980) and as new roots elongate, they will interact with biochar particles (Prendergast - Miller et al., 2014). We demonstrate such a presence of biochar in the form of a BCF embedded in the plaque layer (Fig. 4) and demonstrate here several interactions that are responsible for a substantially greater root membrane potential (ca 65 mV) in the BCF treatment compared to the control (Fig. 7).

It has been reported the difference of Eh value markedly increased redox potential close to the root tips where aerobic conditions were reached (Flessa and Fischer, 1992). Furthermore, both the extension of the oxidation zone around the root tips and the maximum redox potential reached were influenced by the reducing capacity of the soil (Zeng et al., 2014). Our results showed that the Eh difference between the bulk soil at the beginning of the experiment and the soil Eh in the rice rhizosphere at the end of the experiment can be 85 mV which also should attribute to fluctuating conditions around rice roots (Fig. 3d).

Yan et al. (2011) have previously shown that roots with higher membrane potential difference will take up more nutrients, especially nitrogen, than those roots with lower membrane potential difference. We also determined that the cells in the root tip and the differentiation zone have a slightly different membrane potential. Previous studies have found that a greater nutrient influx and efflux occur in the differentiation zone than in the root tip (Fan et al., 2007; Barberon and Geldner, 2014). Both these phenomena are likely to have contributed to the observed increased in nutrient uptake in the BCF treatment compared to the control.

O<sub>2</sub> content increase around the root cell activated the inward K<sup>+</sup> influx and inhibited the outward K<sup>+</sup> efflux (Zeng et al., 2014; Lew and Nasserifar, 2009; Pang et al., 2006). Our results also showed that the both external and internal K<sup>+</sup> of plants were significantly increased by BCF compared with NPK (Table 2). The increased Eh should contribute partially for the internal K<sup>+</sup> increase in plants and the increase in available K on the surface of the BCF that had embedded in the root plaque (Fig. 4, Table S3) would also contribute the uptake of K and higher root membrane potential (Lew and Nasserifar, 2009).

#### 4.3. BCF changes microbial communities and impacts biogeochemical processes

Microorganisms play an important role in soil by altering the bioavailability of nutrients, which can then be used by plants as well as changing root membrane potential. Biochar and BCF have also been previously shown to influence the community composition in soils and the rhizosphere (Nguyen et al., 2018). We also observed a change in the relative abundances of a number of taxa from the presence of BCF in soil and below we provide factors that drive the changes on microbial communities. Here we briefly discuss the consequence of the changes. The *Comamonadaceae*, which were found to be enriched with BCF addition, play a role in the mineralization of C bound sulphur in cereal rhizospheres, thus likely allowing for cycling of soil sulphur between organic and inorganic forms (Zak et al., 1994).

There was also a relative increase in various species of cyanobacteria and Rhizobiales with the addition of BCF, and this group of bacteria has been associated with increasing the soil fertility through biological N<sub>2</sub> fixation, with Rhizobiales also being shown to assist in solubilizing soil P (Rodríguez and Fraga, 1999; Allouti et al., 2006). The fungal species we observed to respond positively to the BCF, including *Cyberlindnera saturnus* and *Mortierella alpina*, are important in the degradation of decaying leaves and organic matter, and also interact with plant roots via the production of a number of fatty acids, including arachidonic acid, IAA and indole-3-pyruvic acid (IPYA) (Nassar et al., 2005). Thus, the microorganisms found in the treatment BCF could stimulate improve soil health increasing nutrient cycling and nutrient bioavailability.

Interestingly, an abundant OTU assigned to the family *Oscillatoriothycideae* was negatively impacted by the BCF addition. *Oscillatoriothycideae* are filamentous, photoautotrophic bacteria that contribute to the formation of soil organic carbon, particularly in nutrient-poor biocrusts and can be inhibited by liming. The reduction of this bacterial group might therefore be a consequence of the increase pH caused by the BCF amendment (see above).

#### 4.4. BCF provides soluble organic components that can influence root processes

As the soils were maintained under wet conditions, it is likely that dissolution of organic molecules from BCF modified redox reactions (Graber et al., 2014), which would have contributed to the alteration in the abundance of specific microorganisms (see above). GC-MS of the liquid extracts (Table S4) indicated the presence of organic compounds similar to those reported for other biochar-mineral complexes (e.g. succinic acid, hexadecanoic acid, octadecenoic acid, hydroxybenzoic acid) (Chia et al., 2014). In addition, some of these compounds, for example succinic acid, have also been reported to act directly as plant growth promoters (Yoshikawa et al., 1993; Pizzeghello et al., 2006).

LC-OCD of BCF also showed that there was a significant concentration of large macromolecules that have similar properties to humic-like substances and polyphenols. These macromolecules have been associated with increased absorbance of heavy metals, changes in microbial communities (Lovley et al., 1996), increased cation exchange and availability of essential micronutrients, especially iron (Hättenschwiler and Vitousek, 2000; Chantigny, 2003; Chen et al., 2004; Schmidt et al., 2013). Humic-like macromolecules from biochar have also been reported to have hormone-like effects resulting in an increase in rice calli and rice cells (Wang et al., 1999).

Organic root exudates (especially acids) can be adsorbed by the BCF. This can result in an increase in certain microorganisms growing on the biochar (Masiello et al., 2013) and the dissolution of nutrients (especially P) on the surfaces of the biochar, in turn altering the Eh as and pH around the root. Since BCF is redox active and has a high reducing capacity it may play a role in abiotic formation of humic structures in soil, in solubilizing Mn and Fe, in microbial electron shuttling between bacterial cells and Fe-bearing minerals, in scavenging radicals, and in contaminant immobilization (Li et al., 2012; Nassar et al., 2005). Similarly, high concentrations of stabilized free radicals (Table S6) have been associated with increased resistance to plant stresses (Edreva et al., 1998).

#### 4.5. BCF may act as a 'micro-geobattery'

BCF can be represented by an equivalent circuit that has a capacitance and an impedance (Fig. 7). Sun et al. (2017) has previously noted that biochar can act as a geobattery enabling rapid transfer of electrons within the biochar and to the rhizosphere where there are electron acceptors. The BCF in soil has a complex water-filled porous carbon structure where there are areas that have a net positive charge or a net negative charge. Recent work by Huggins et al. (2014), has shown that biochar can be used as electrodes in a microbial fuel cell where organic compounds can be converted to CO<sub>2</sub> and electrons by specific microorganisms in an anoxic environment and these electrons can be transferred to a biochar electrode in an anoxic environment when oxygen can be reduced to water. BCF particles in the rhizosphere is therefore potentially analogous to a microbial fuel cell where anoxic areas within the pores are anodic and surface areas near the roots are that are exuding oxygen are cathodic (Xia et al., 2018). Further work is required to confirm this hypothesis.

## 5. Conclusion

This study shows that a BCF made from nutrient-enhanced wheat straw biochar and NPK chemical fertilizers has dramatically increased nutrient uptake and biomass in rice. The observed increases are likely due to a number of linked BCF-rhizosphere interactions that increase the membrane potential difference between the soil and the root. The BCF that is both in direct contact with the roots and present within the rhizosphere can both store and donate electrons, root and microbial exudates, cations and anions (Sun et al., 2017). Exudation of oxygen from the roots also can result in an increase in both biotic and abiotic redox reactions on the surface of the BCF that will alter both the Eh and pH in the plaque layer and rhizosphere (Zeng et al., 2014; Haruta et al., 2018.). This and the increased concentration of organic molecules dissolving from the BCF likely causes changes in the relative abundance of certain microbial groups, that contributed to nutrient cycling and availability (Fig. 7). Our data also indicates BCF particles embedded in the plaque layer of the roots may increase root membrane potential resulting in an increase in nutrient uptake and plant biomass. The interactions between BCF, plant, soil, soil solubles and microbiota ultimately underpin the agronomically beneficial outcomes for rice growth and have great potential to be engineered in the future for other agricultural systems.

## Declaration of competing interest

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.

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## Author contributions

J.C., X.F. and L.Z. conducted experiment designing, the bag experiment, discussion and manuscript writing; S.J. did discussion and manuscript writing and characterisation. L. Z. B. R. and ML conducted the soil analysis; S.N. and T.T. undertook the microbial analysis interpretation and writing. ZS assisted in fungal analysis and paper review; DM, JH, EG, LZ, SD, PM, ST, BP, AR, JH, CM, DT, AB, MB, were involved in characterisation of the BCF and review of the document. OH, GP and LL reviewed the document and provided valuable advice.

## Materials & correspondence

S.J and G. P are corresponding for or the BCF; X.F is corresponding for the plant materials.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.136431>.

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