

**Supplementary Material: "Environmental fate and efficiency of bispyribac-sodium in rice soils under conventional and alternative production systems affected by fresh and aged biochar amendment"**

**Text S1.** The conditions used were the following: Nova-Pack C18 column (150 mm length  $\times$  4.6 mm i.d.), mobile phase of acetonitrile/water (55:45, v/v) containing 0.1% phosphoric acid at a flow rate of 1 mL min<sup>-1</sup>, column temperature 30°C, 25  $\mu$ L injection volume, and UV detection at 248 nm. External calibration curves with standard BS solutions between 0.05  $\mu$ M and 10  $\mu$ M were used in the calculations. The repeatability was evaluated by six repeated injections of BS standards. This test was also carried out on a different day and in different media (distilled water, CaCl<sub>2</sub> 0.01 M, and an extracting agent) in order to verify the reproducibility. Adequate linearity and precision ( $R^2 \geq 0.999$ ) and relative standard deviation ( $< 2\%$ ) were obtained. The measurements with control samples (without BS) at the band maxima of UV spectra for BS showed that there were no interfering peaks at the retention times of the herbicide. The detection and quantification limits under these conditions were 0.0068  $\mu$ M and 0.0223  $\mu$ M, respectively.

**Text S2.** Total C, H, and N contents of BHO were determined by combustion (950°C) using a CHNS628 analyser (LECO, United States). The water-soluble organic carbon (WSOC) content was extracted with 0.01 M CaCl<sub>2</sub> de-ionized water at a 20:1 (CaCl<sub>2</sub> to BH) ratio and determined by analysis with a TOC-V analyser (Shimadzu, Japan). The ash content was measured by BHO combustion in a muffle furnace at 750°C for 6 h. Oxygen content was calculated through mass difference based on total C, H, and N determinations. The BET (Brunauer-Emmett-Teller) specific surface area (SSA) was measured by N<sub>2</sub> adsorption at 77 K using a Quadrasorb Evo analyser (Quantachrome Instruments, United States). The surface functional groups of biochar samples were analysed by Fourier transform infrared spectroscopy (FTIR) (Nicolet IS10, Thermo Scientific). The BHO porosity and pore size

distribution (from  $1 \times 10^5$  to 1 nm) were determined using a Poremaster 33GT mercury intrusion porosimeter (Quantachrome Instruments, United States). Electrical conductivity and pH were measured in a 1:5 (w/v) BHO/water suspension using a conductivity meter and a pH meter, respectively.

**Text S3.** According to FTIR results (Figure S1), the peaks appearing between  $704 - 878 \text{ cm}^{-1}$  correspond to aromatic C-H and are presented in both fresh and aged biochar. Peaks at  $1030$  and  $1090 \text{ cm}^{-1}$  were only observed in the aged biochar can be assigned to C-O stretching from polysaccharides groups. Peaks at  $1387$  and  $1615 \text{ cm}^{-1}$  were indicative of C=C stretching from aromatic carbon bonds, being more intensive in aged biochar. The band at  $1714 \text{ cm}^{-1}$  corresponds to C=O stretching from carboxyl group was only found in the aged biochar.

**Text S4.** The BS sorption-desorption experiments were done in triplicate using a batch equilibration method. Equilibrium concentrations in the supernatants were determined by HPLC. The amount of BS sorbed ( $C_s$ ) was calculated from the difference between the initial ( $C_i$ ) and the equilibrium ( $C_e$ ) solution concentrations. Desorption was measured immediately after sorption by successive dilution from the  $20 \mu\text{M}$  initial concentration points. The 5 mL of supernatant removed for the sorption analysis was replaced with 5 mL of  $0.01 \text{ M CaCl}_2$ . The samples were resuspended, shaken for another 24 h, centrifuged, and the equilibrium concentration in the supernatant was determined. This desorption procedure was repeated thrice. The herbicide sorption and desorption experiments were fitted to the empirical Freundlich equation,  $C_s = K_f C_e \exp(1/n_f)$ , where  $C_s$  ( $\mu\text{M kg}^{-1}$ ) is the amount of herbicide sorbed at the equilibrium concentration  $C_e$  ( $\mu\text{M L}^{-1}$ ), and  $K_f$  and  $n_f$  are constants that characterize the relative sorption capacity. The desorption percentage was calculated as  $\%D = ((C_{sa} - C_{sd})/C_{sa}) \times 100$ , where  $C_{sa}$  is the amount of BS adsorbed in adsorption process and  $C_{sd}$  is the amount of BS adsorbed in desorption process.

**Text S5.** For dissipation studies, triplicate soil samples (5 g) for each treatment were weighed into 50 mL glass tubes. Soils were supplemented with distilled water to obtain non-flooded (80% field capacity) and flooded (soil-to-water ratio 1:1.25, w/v) moisture conditions. Prior to the BS addition, the soils were pre-incubated for 7 days in the dark at  $20\pm 1^\circ\text{C}$  to allow the soil microorganisms to adapt to the non-flooded (aerobic) and flooded (anaerobic) incubation conditions, and also to allow the development of reducing conditions in the flooded soils. BS was applied in an amount to correspond to a field dose rate of  $0.5\text{ kg ha}^{-1}$  and the tubes were incubated in the dark at  $20\pm 1^\circ\text{C}$  for 49 days. Moisture was maintained at a constant level throughout the experiment by adding distilled water as necessary. Three replicate tubes were removed (at 2 h, and at 2 and 5 days after herbicide application, and then at 7-day intervals for 49 days) from each treatment to measure the herbicide concentrations. The soils (5 g) were extracted with 10 mL of a 60:40 (v/v) mixture of distilled water/methanol by shaking mechanically on an end-over-end shaker at  $20\pm 1^\circ\text{C}$  for 24 h followed by centrifugation, and the residues of the herbicide in the extracts were determined by HPLC. Recoveries were greater than 95% of the herbicide applied to the soil. BS residues from water samples were also determined by HPLC. The BS dissipation data in soils and water were fitted to a first-order kinetics equation,  $C = C_0 e^{-kt}$ , where  $C$  is the BS concentration at time  $t$  (days),  $C_0$  is the initial herbicide concentration, and  $k$  ( $\text{day}^{-1}$ ) is the degradation constant, and the half-lives ( $t_{1/2}$ ) were calculated.

To measure the dehydrogenase activity, another three replicate soil samples of each treatment were weighed out into glass tubes, and supplemented with sterile distilled water to obtain aerobic (80% field capacity) and anaerobic (1:1.25 w/v soil/water) moisture conditions. From each treatment, the tubes were removed before BS application and at the same times as for the dissipation experiment. The tubes were incubated for 20 h at  $20\pm 1^\circ\text{C}$  in the dark with 1 mL of 0.4% 2-p-iodophenyl-3p-nitrophenyl-5 tetrazolium chloride (INT) as substrate. At the end

of the incubation, the iodonitrotetrazolium formazan (INTF) produced was extracted with methanol, and the absorbance was measured at 490 nm.

**Text S6.** For the leaching studies, PVC was used to prepare disturbed soil columns (5-cm i.d. × 30-cm length). To minimize losses of soil during the experiment, the top 5 cm of the columns was filled with sea sand and the bottom 5 cm with sea sand plus glass wool. The remaining 20 cm was hand-packed with air-dried soil. The soil columns were saturated with 0.01 M CaCl<sub>2</sub> and allowed to drain for 24 h. Then, BS was applied to the top of the soil columns at a rate equivalent to 0.5 kg ha<sup>-1</sup>. Leachates containing the herbicide were collected daily, filtered, and assayed by HPLC. After the leaching experiments, the soil columns were left to drain for 24 h and then sectioned into the following depths: 0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm. In order to determine the residual amounts of BS, the soils (5 g) from different depths were extracted with 10 mL of a 60:40 (v/v) mixture of distilled water/methanol by shaking for 24 h at 20±1°C and assayed by HPLC.

**Table S1**

Effect of different management regimes on selected soil properties (0–20 cm depth).

		TC (g kg <sup>-1</sup> )	WSOC (mg kg <sup>-1</sup> )	EC (dS m <sup>-1</sup> )	pH	TN (g kg <sup>-1</sup> )
<b>2018</b>						
	SNT	9.30aA	101bcA	1.45aA	6.73dB	1.16abA
	SNT-BHO	15.8cA	107cA	1.99cA	7.10eB	1.16abA
	ST	10.0bA	94.6bcA	1.87bcA	6.27bA	1.10abA
	ST-BHO	17.5dA	81.7abA	1.72bA	7.09eA	1.02aA
	FT	10.2bA	63.3aA	1.93bcA	5.53aA	1.23abA
	FT-BHO	22.2eB	69.5aA	2.70dA	6.40cA	1.32bB
<b>2019</b>						
	SNT	9.70aA	105bA	5.98dB	6.46cA	1.36aB
	SNT-BHO	17.0dB	126cA	3.44cB	7.03dA	1.28aB
	ST	10.7cA	83.5aA	2.12aB	6.29bA	1.32aA
	ST-BHO	17.9eA	83.0aA	1.88aB	7.15dB	1.35aA
	FT	10.1bA	74.0aB	2.74bB	5.65aB	1.35aA
	FT-BHO	20.4fA	73.5aA	2.77bA	6.50cB	1.22aA
Y	F-values	7.39*	4.25*	391***	4.85*	17.1**
M	F-values	6690***	15.4***	102***	1214***	0.965NS
Y x M	F-values	74.7***	1.78NS	129***	28.9***	2.54NS

The data for total carbon (TC), water-soluble organic carbon (WSOC), electrical conductivity (EC), pH and total nitrogen (TN) are mean values (n=3). ANOVA factors are: Y, year; M, management regime; Y × M, interaction year × management regime; F-values indicate the significance levels \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ , respectively, and NS: not significant. Different letters indicate significant differences ( $p < 0.05$ ) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters).

**Table S2**

Pore volumes ( $\text{mm}^3 \text{g}^{-1}$ ) size on the fresh and aged biochar.

Pore range ( $\mu\text{m}$ )	Fresh	Aged SNT-BHO	Aged ST-BHO	Aged FT-BHO
0-0.1	310a	325ab	347b	355b
0.1-1.0	222a	194a	192a	231a
1.0-10	63.4a	60.7a	56.8a	58.1a
10-100	121c	96.8b	85.8ab	73.7a
Total pore volume	716b	676a	682a	718b
Micropore volume/total pore volume	0.743a	0.768ab	0.790bc	0.816c

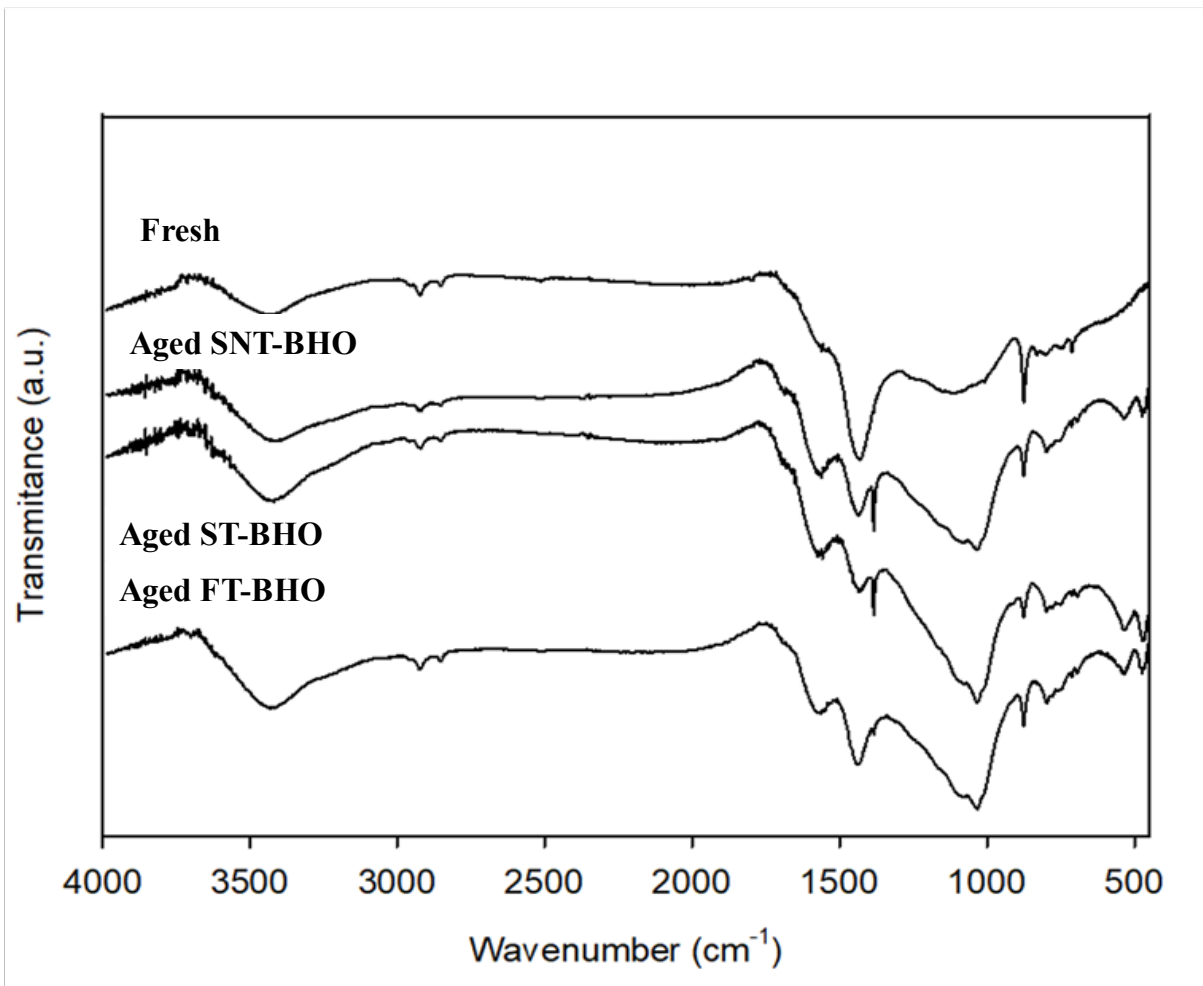
The data are mean values. Values with the same letter within a row are not significantly different at the  $p < 0.05$  level of probability.

**Table S3**

Effect of different management regimes on bispyribac-sodium efficiency against *Echinochloa crus-galli* L. Beauv.

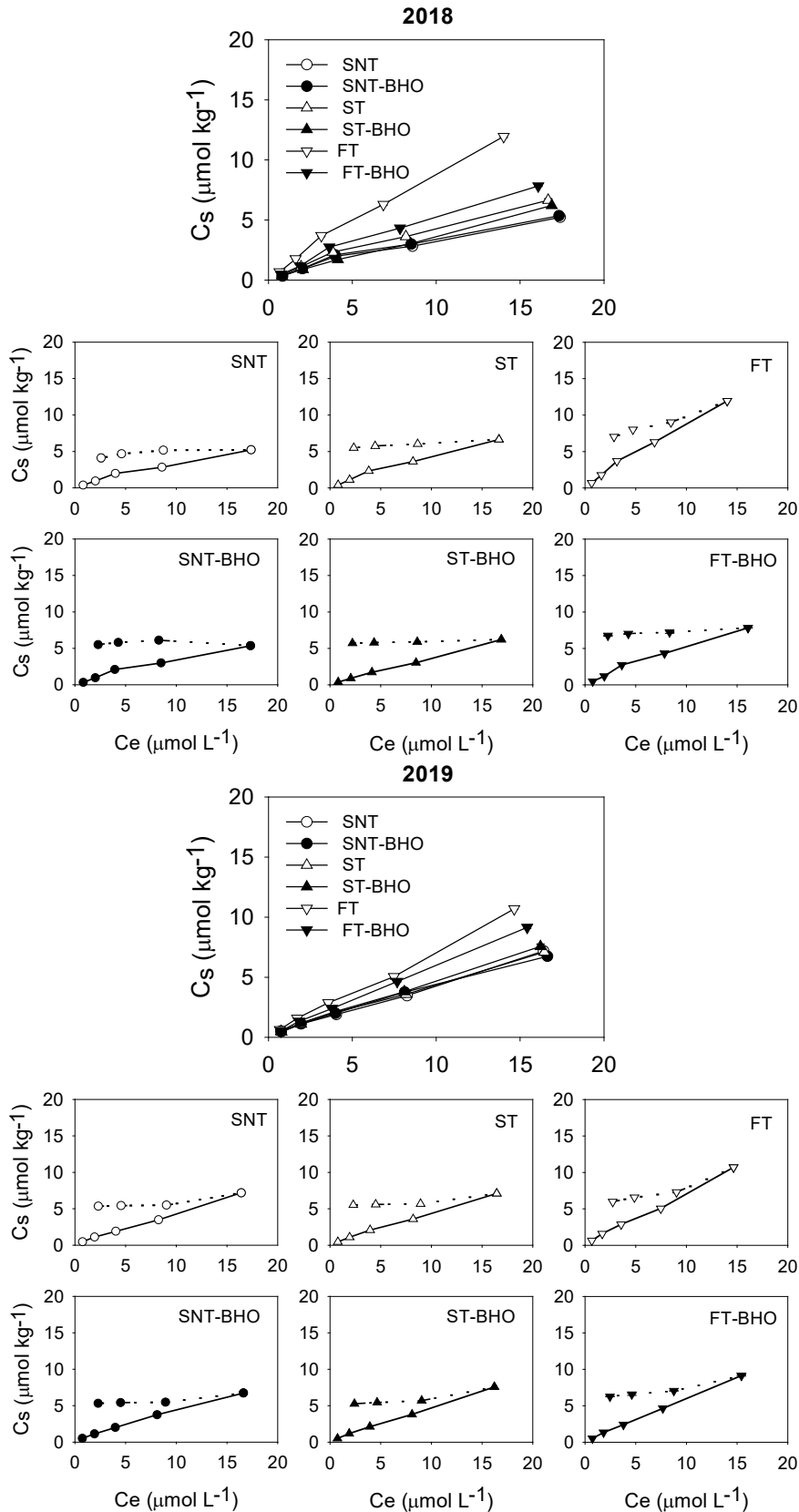
		WCE (%)
<b>2018</b>		
SNT		80.4 cdA
SNT-BHO		50.1 bA
ST		83.8 dB
ST-BHO		71.8 cA
FT		56.4 bA
FT-BHO		33.9 aA
<b>2019</b>		
SNT		69.8 bcA
SNT-BHO		84.4 cB
ST		72.3 bcA
ST-BHO		81.6 cB
FT		63.9 bA
FT-BHO		46.5 aB
Y	F-values	11.0**
M	F-values	27.1***
Y*M	F-values	10.8***

The ANOVA factors are Y: year; M: management regime; Y\*M: interaction year\*management regime. F-values indicate the significance levels \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$  respectively. Different letters indicate significant differences ( $p < 0.05$ ) between management systems in the same year (lowercase letters) and between years within the same management system (uppercase letters). WCE: weed control efficiency.



**Figure S1.** FTIR spectra of the fresh and aged biochar samples under different management regimens.





**Fig. S2.** Effect of different management regimes on bispyribac-sodium sorption–desorption parameters. Vertical bars representing one standard error of the mean were smaller than the symbols in all cases.  $C_e$ : equilibrium bispyribac-sodium concentration;  $C_s$ : amount of bispyribac-sodium sorbed. Treatments are: sprinkler irrigation and no-tillage without (SNT) or with biochar application (SNT-BHO); sprinkler irrigation and conventional tillage without (ST) or with biochar application (ST-BHO); traditional flooding irrigation and tillage without (FT) or with biochar application (FT-BHO).