Supplementary Material: "Effects of sustainable rice managements on behavior and bioefficacy of bispyribac-sodium: a medium-term study"

Text S1. The BPS adsorption-desorption experiments were done in triplicate using a batch equilibration method. Equilibrium concentrations in the supernatants were determined by HPLC. The amount of BPS adsorbed (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 μM initial concentration points. The 5 mL of supernatant removed for the adsorption analysis was replaced with 5 mL of 0.01 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 adsorption and desorption experiments were fitted to the empirical Freundlich equation, $C_s = K_f C_e \exp(1/n_f)$, where C_s (μM kg^{-1}) is the amount of herbicide adsorbed at the equilibrium concentration C_e (μM L^{-1}), and K_f and n_f are constants that characterize the relative sorption capacity. The K_d values (partition coefficients) were calculated from the fit of the experimental sorption isotherms ($C_s = K_d C_e$) at 5 μM initial BPS concentration. Hysteresis coefficients (H) for the adsorption-desorption isotherms were calculated as $H = n_a/n_d$, where n_a and n_d are the Freundlich constants obtained from the adsorption and desorption isotherms, respectively.

Text S2. For dissipation experiments, triplicate soil samples (5 g) for each management were weighed into 50 mL glass tubes. Soils were supplemented with distilled water to obtain flooded (soil-to-water ratio 1:1.25, w/v) in TF and TF-CA managements, and non-flooded (80% field capacity) in TS, TS-CA, NTS and NTS-CA managements, moisture conditions. Prior to the BPS addition, the

soils were pre-incubated for 7 days in the dark at 20±1°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 treatments. BPS was applied in an amount to correspond to a field dose rate of 0.5 kg ha^{-1} and the tubes were incubated in the dark at 20±1°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±1°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. BPS residues from water samples were also determined by HPLC. The BPS dissipation data in soils and water were fitted to a first-order kinetics equation, $C = Co\ e^{-kt}$, where C is the BS concentration at time t (days), Co is the initial herbicide concentration, and k (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 anaerobic (1:1.25 w/v soil/water) and aerobic (80% field capacity) moisture conditions. From each treatment, the tubes were removed at the same times as for the dissipation experiment. The tubes were incubated for 20 h at 20±1°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 S3

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. In order to prevent the preferential flow through the wall of the columns, silicone ring was carefully applied in the border of the soil with the column. The soil columns were saturated with 0.01 M $CaCl_2$ and allowed to drain for 24 h. Then, BPS was applied to the top of the soil columns at a rate equivalent to 0.5 kg ha^{-1}. Leachates containing the herbicide were collected daily, filtered, and assayed by HPLC.

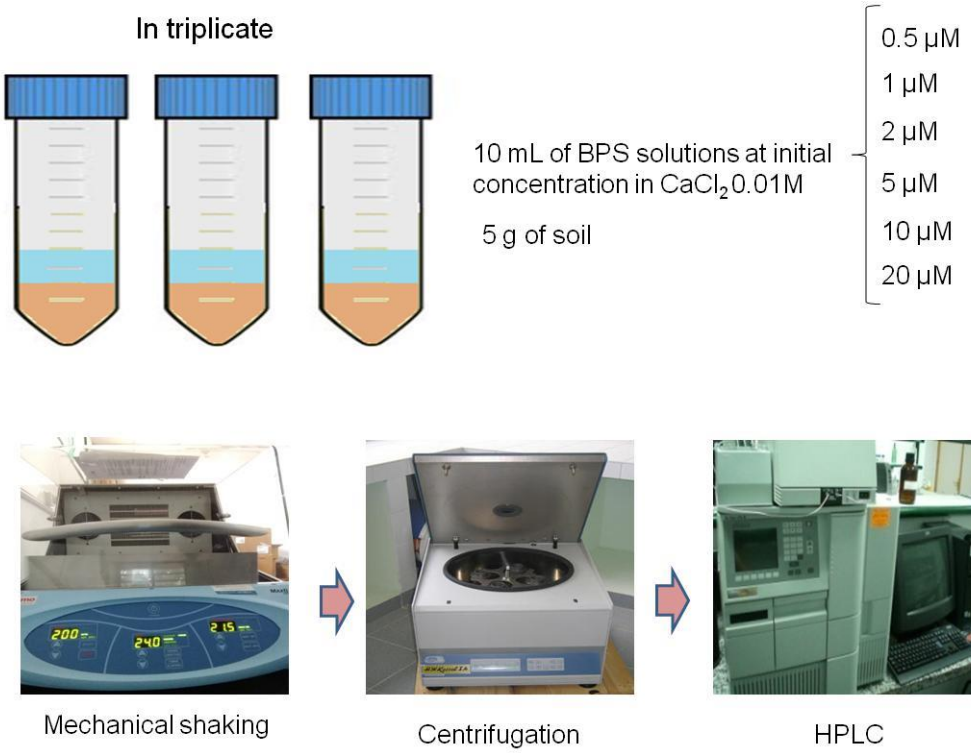


Figure S1. Process of BPS adsorption-desorption experiments

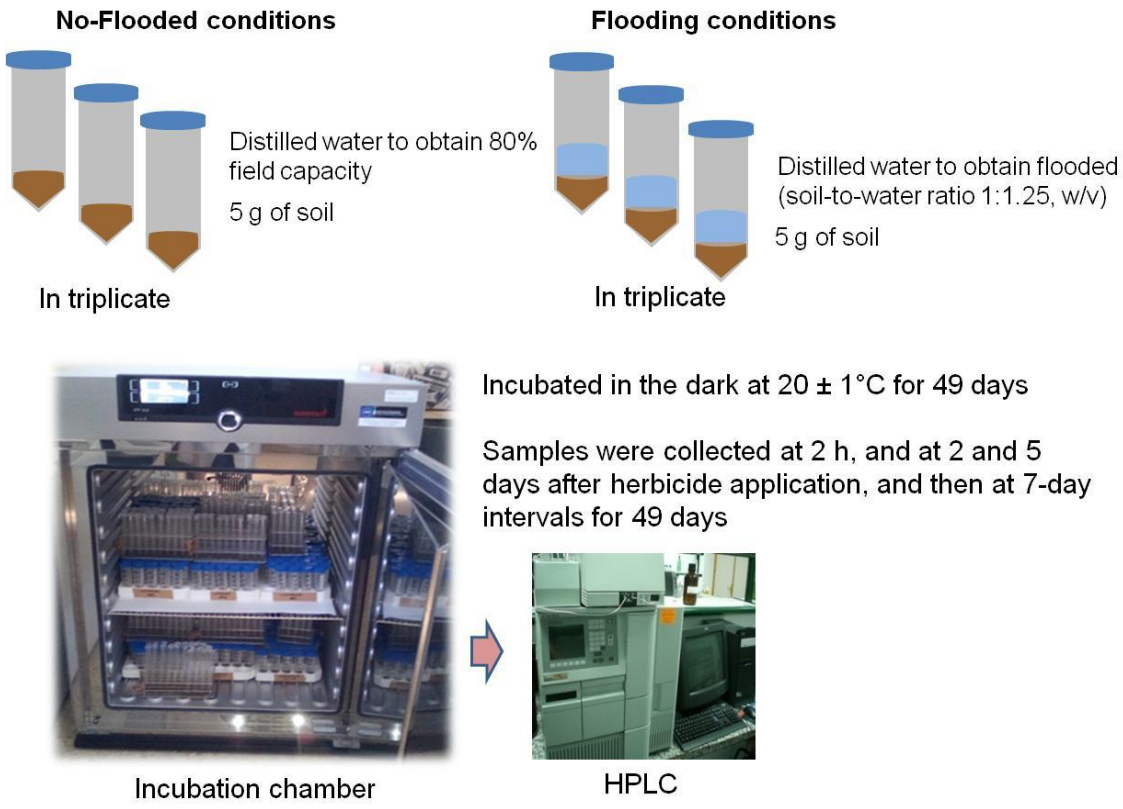


Figure S2. Process of BPS dissipation experiments



Figure S3. Process of BPS leaching experiments

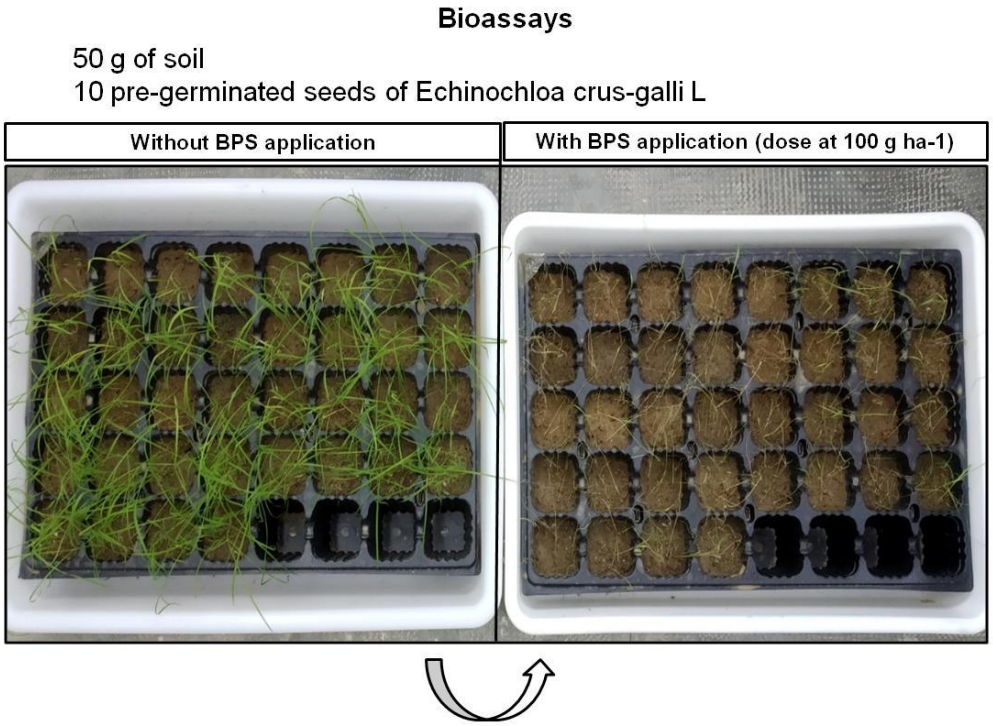


Figure S4. Process of BPS bioassays experiments

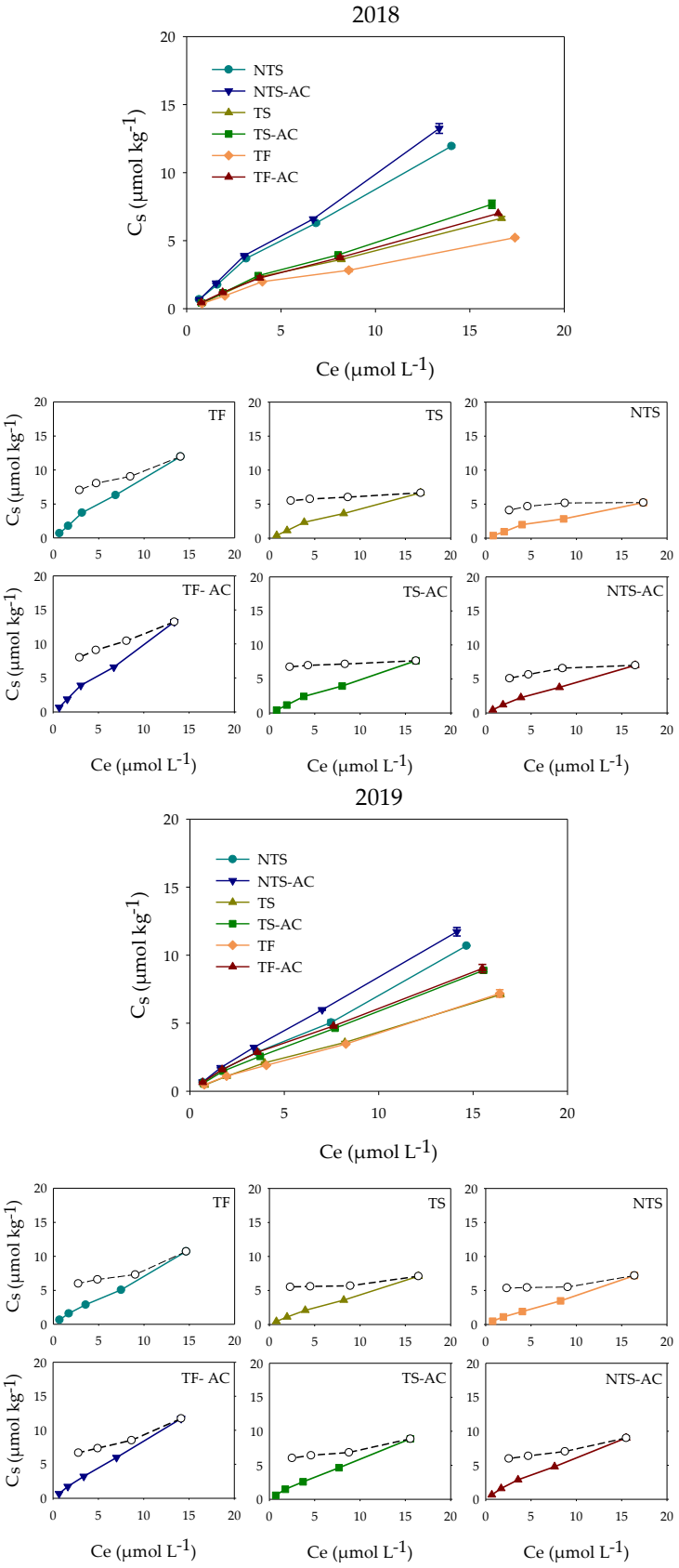


Figura S5. Bispyribac-sodium adsorption and desortion isotherms. Vertical bars representing one standard error of the mean were smaller than the symbols in all cases. Ce: equilibrium bispyribac-sodium concentration; Cs: amount of bispyribac-sodium adsorbed