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Biochar

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Biochar enhances the sorption and degradation of fluridone and its main metabolite in soil: insights into biodegradation potential and remediation of microbial communities

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Abstract Owing to wide application and persistence, fluridone has demonstrated side-effects on non-target plants and aquatic organisms. This study investigated the potential of biochar as remediation in soil using rice hull biochar (BCR) produced at different temperatures and in four types of soil. The results indicated that, with increasing pyrolytic temperature from 300 to 700 °C, biochar properties changed, for example, specific surface area values increased from 38.21 to 126.12 m^2 g^{-1}. Sorption affinity (K_f) of BCR ranged from 409 to 1352 and 1301 to 6666 (μg/g)/(mg/L)^n for fluridone and its metabolite fluridone acid respectively. After amendment with 2% BCR500, fluridone and fluridone acid could easily be adsorbed in different types of soils, and K_f values were 1.30–3.73 times higher than those in pure soil. Half-lives values varied between different soils and fluridone acid (179–306 days) persisted significantly longer than fluridone (39–179 days) in soil. After amendment with 2% BCR500, fluridone and fluridone acid were degraded faster. Experiments under sterilized conditions demonstrated biodegradation to be the dominant process in unamended (61.59%–64.70%) and amended (67.71%–77.67%) soil. Bioinformatic analysis showed that fluridone reduced the diversity of the soil microbial community, but the abundance of microorganisms with degradation function increased and these became dominant species after BCR was added, particularly with higher numbers of degrading bacteria like *Lysobacter, Pseudonocardia* and *Sphingomonas*. Co-occurrences also revealed that BCR tightened bacterial connection and relieved fluridone stress. This work helps us better understand these processes and optimize the application of biochar for reducing pesticide contamination in agricultural soils.

Highlights

- The major metabolite (fluridone acid) indicated longer persistence in soil than fluridone;
- Biochar accelerated degradation of fluridone and fluridone acid, biodegradation was the primary;
- Biochar increase *Lysobacter*, *Pseudonocardia* and *Sphingomonas* related to degradation.

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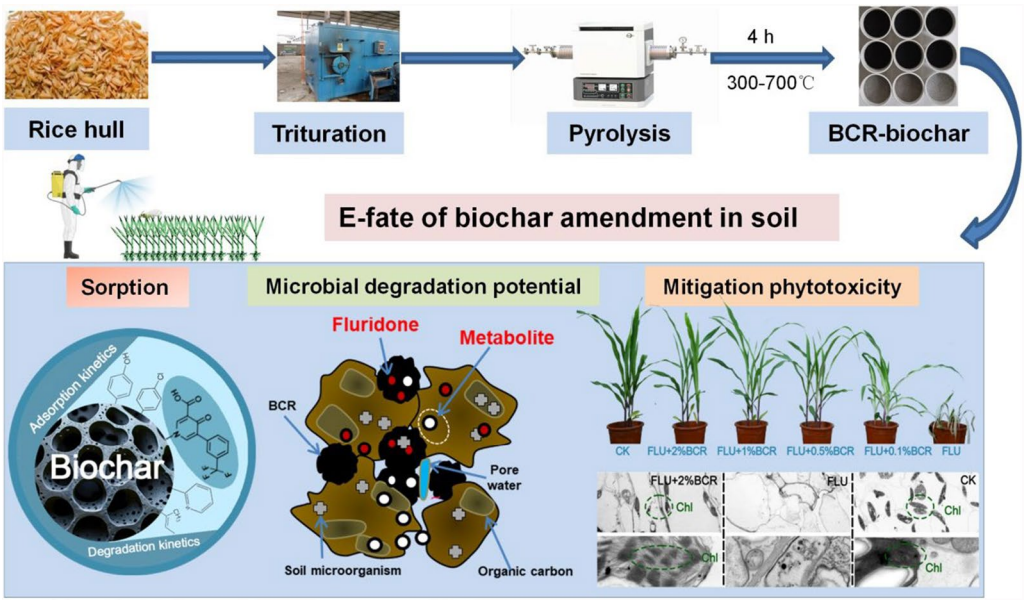
Keywords Fluridone, Biochar, Sorption, Degradation, Microbial community

Graphical Abstract



1 Introduction

Due to changes in farming practices and increasingly intensive agriculture, herbicides have been used extensively worldwide and the use of herbcides has increased dramatically in recent years. The application rates are increasing with time (Parven et al. 2024). As a kind of systemic herbicide, fluridone has been used to effectively control many annual broadleaves and grasses (Waldrep and Taylor 1976). In China, it has been mainly used in cotton fields to control broad-leaved weeds before emergence. The residue concentrations of fluridone in water, soil, and bed sediment have been seen to potentially rise (Netherland et al. 1997; Nance et al. 2022). However, various studies have indicated that fluridone shows side-effects on non-target plants, such as deleterious carryover effects on young seedlings of maize and yield loss of pumpkin (Hamadina and Hamadina 2018; Ferebee et al. 2019). Fluridone significantly suppressed the lycopene buildup in the pericarp of tomato (Góraj-Koniarska et al. 2017). Fluridone injury to a wheat rotational crop was more likely following fluridone application in cotton (Hill et al. 2016). The persistence of fluridone in water has been underestimated, causing high potential risk for aquatic organisms (Van Frost et al. 2024). Previous studies demonstrated evidence of sublethal effects on fish populations (Townsend et al. 2023). Therefore, further studies are required to focus on the control of fluridone pollution, and to devise strategies aiming at minimizing its uptake by crops to ensure crop security.

Biochar is a novel environmental adsorbent with functionality and economic advantages (Peiris et al. 2017). Biochar has significant effects on the sorption of organic pollutants (Wu et al. 2022), having the capacity to alter both the physical and chemical characteristics of soil, as well as influencing soil microbial activity, which in turn affects fluoridone degradation processes (Zhu et al. 2017). It has the ability to influence microbial activity and alter the microbial community structure by changing the levels of nutrients (e.g., N and P), pH, organic matter content, and texture (Shaaban et al. 2018). Moreover, dose optimization and differences in the properties of biochar from different types of feedstocks, have significant impacts on the degradation of pesticides and their transformation into metabolites (Konczak et al. 2023). The effectiveness of biochar to immobilize pesticides in contaminated soil varies, which are affected by environmental conditions and the characteristics of biochar itself (Qiu et al. 2009). The characteristics of biochar are generally influenced by production conditions (i.e., temperature) and properties of the raw biomass, which significantly affect its capacity

to adsorb pesticides (Wang et al. 2016; Zhang et al. 2011). Studies have shown that soil organic matter (SOM), pH value, clay content, and cation exchange capacity (CEC) can also affect the adsorption capacity of the biochar (Daly et al. 2010; Weber et al. 2004). Generally, the biochar with high adsorption capacity normally has larger specific surface area, pore volume, high aromaticity, and abundance of functional groups (Zhang et al. 2011; Zhu et al. 2005). On the other hand, effect of biochar on pesticide degradation varies, which is also affected by several factors. For instance, biochar inhibits the degradation of diuron through adsorption (Yang et al. 2006) but promotes the decomposition of atrazine and methyl isothiocyanate (Fang et al. 2016; Nicolai David et al. 2013). These different and even controversial results from previous studies highlight the necessity of further exploring the role of biochar in eliminating residual pesticides and its influence on soil fate. However, there is limited research on the effect of biochar on the degradation of specific pesticides. Thus, the effectiveness and efficiency of biochar soil amendments targeting degradation of fluridone need to be verified.

After application, pesticides may go through various processes, which can be influenced by different factors including type of soil and environmental conditions (i.e., temperature and humidity) (Chen et al. 2018; de Medeiros et al. 2024). The sorption and degradation of pesticides are key processes after their application to soils, and are governed by several processes such as volatilization, photolysis, microbial degradation, and chemical degradation. They are bound to the soils through redox processes and hydrolysis and affected by factors including organic matter, moisture and soil microbes (Ma et al. 2021; Yogendraiah Matadha et al. 2021). Most pesticides applied to farmland are converted into one or more metabolites via physical, chemical and biological processes. It is noted that metabolites frequently accumulate to a greater extent in organisms and exhibit greater toxicity than parent compounds (Sharma et al. 2014; Huang et al. 2024). For fluridone, fluridone acid has been demonstrated to be a major metabolite (Rodrigues et al. 2013). The sorption and degradation of fluridone and fluridone acid should be investigated under different conditions.

Pollution of agricultural soils with pesticides can result in fluctuations in soil physicochemical properties, and can affect growth of plants and eventually human health (Karpouzas et al. 2016). Owing to their xenobiotic characteristics, the presence of pesticides in soil can affect the soil microbial activity (Arora et al. 2019), which is considered as a vital indicator of soil tolerance to pollutants (Arora et al. 2019). Microorganisms engage in interactions with one another (Freilich et al. 2010), which leads to the formation of intricate systems. The interactions are involved in shaping the ecological community structure and, as a result, the functioning of ecosystems (Fuhrman 2009; Freilich et al. 2010). However, prior research on effects of pesticide on soil microbial communities has mostly examined the quantity and variety of species (Du et al. 2018a, 2018b). It is vital to obtain better understandings of the interrelationships between various species in a community and how they react to changes in their surrounding conditions.

Considering possible environmental hazards, it is necessary to ascertain the impact on the soil microbiome of fluridone and its metabolite in soils with and without biochar amendment. The aims of this study were as follows: (1) evaluate the sorption capacity for fluridone and fluridone acid in four types of soils and these soils amended with addition of five biochars; (2) study the degradation behavior of fluridone and fluridone acid in soil and the effects of biochar amendment under sterilized and unsterilized conditions; (3) evaluate the effect of fluridone on soil microbial ecology, and the further effect of amendment with biochar. The results are important for revealing interactions of biochar with soil and pesticide, knowledge of which is crucial for using biochar to mitigate negative effects of pesticides on the soil ecosystem.

2 Materials and methods

2.1 Reagents

Fluridone (99%, analytical standard) and fluridone acid (99%, analytical standard) were acquired from the China Reference Material Center (Beijing, China). More details are given in the Supplementary Information Part 1 Sect. 1.1.

2.2 Biochar and soils

Biochar of rice hull (BCR) was obtained from the Zhejiang Biochar Engineering Technology Research Center. The air-dried rice hull was pyrolyzed in a muffle furnace. The rice hull was pyrolyzed in N_2 atmosphere at a rate of 15 °C per minute to final temperature of 300, 400, 500, 600, and 700 °C with residence time at the highest heating temperature for 4 h. The produced biochar samples are labeled as BCRN, with N referring to 300, 400, 500, 600, and 700. The produced biochar samples were pulverized with a grinder and sieved with a 2-mm mesh.

Soil samples studied in this work were collected from four different provinces in China: Jiangsu, Hunan, Shanxi, and Heilongjiang, and named as JSS, HNS, SXS, and DBS, respectively. The soil samples were without measurable amounts of fluridone or fluridone acid when taken. The soil was collected at a depth ranging from 0 to 10 cm for the purpose of conducting sorption and degradation

studies. It was kept at 4 °C for a duration of 1 month prior to its intended use.

Physicochemical properties, including the elemental composition, ash, pH, specific surface area (SSA) of biochar (Table S1), total organic carbon (TOC), cation exchange capacity (CEC), and texture of soil (Table S2) were determined using standard procedures, which are given in the Supplementary Information.

2.3 Sorption experiments

The sorption studies were performed utilizing a batch equilibration approach (Tang et al. 2015). The experiments were conducted in the following series: (1) Control group; (2) five types of BCR with just fluridone and fluridone acid; (3) four types of soil samples treated with fluridone and fluridone acid, with and without addition of 2% BCR500. We weighed 4.00 g soil and added 5 mL of a 0.01 M solution of $CaCl_2$ (which included 200 mg L^{-1} of NaN_3 to hinder microbial activity). Subsequently, the ratio of soil to water was modified to 1:100. The final spiked concentration of fluridone and fluridone acid was varied between 0.04 and 4 mg L^{-1}, and 0.05 and 5 mg L^{-1}, respectively. The suspensions were agitated on a rotary shaker at a room temperature of 25 ± 1 °C for a duration of 72 h while being kept in the dark. Subsequently, each suspension was centrifuged at 6000 rpm for a duration of 10 min. The supernatant was diluted by three times and examined using UPLC-MS/MS. Each treatment was conducted three times.

2.4 Degradation of fluridone and fluridone acid in soil

The degradation of fluridone and its metabolite was studied under sterilized and unsterilized conditions in different types of soils with or without biochar. In order to investigate the potential roles of soil microbes in the degradation, DBS was sterilized at 121 °C for 30 min using an autoclave (Shanghai Shenan Medical Machinery Company, China) prior to the experiment (Xu et al. 2009). The soil or 2% BCR500 amended soil (10.0 g dry weight of mixed soil) was weighed and transferred into 40-mL glass vials with cotton plugs (Shanghai Anpel, China). Deionized water was applied to modify the the moisture content of soil to 60% maximal water-holding capacity. Fluridone and fluridone acid were dissolved in an organic solvent and transferred to soil to obtain 10 mg kg^{-1}. A 200 mg L^{-1} concentration of $HgCl_2$ solution was prepared and thereafter introduced to the sterilized group (Xu et al. 2009; Ren et al. 2016). The test soil was transferred to an incubator and cultured under 25 ± 1 °C in the dark for a maximum duration of 300 days. Samples were taken on days 1, 7, 15, 30, 45, 60, 90, and 120 for fluridone, and on days 1, 7, 15, 30, 60, 90, 120, 180, 240, and 300 for fluridone acid. The samples were freeze-dried and stored at a temperature of − 20 °C until they were analyzed. There were three replications for each test group. More details of pretreatment and analysis method are given in the Supplementary Information Part 3, and the results were given in Tables S6 and S7.

2.5 Soil microbial analysis

The influence of biochar on microorganisms in fluridone-contaminated DBS soil was evaluated. Three types of experiment were conducted, including: control, soil without addition of fluridone and biochar; T1, DBS with addition of fluridone (10 times predicted environmental concentration based on standard application rate for cotton cultivation in China, 2 mg kg^{-1}); T2, 2% BCR amended DBS with addition of with 2 mg kg^{-1} fluridone. The incubation process was conducted in sterilized brown wide-mouth bottles (15 cm × 8 cm). Each bottle was coated with a permeable plastic film and incubated under degradation test conditions. Three bottles were selected and terminated at certain time intervals (1, 7, 15, 30, 60, and 90 days) for determination. 20 g soil sample was sampled and kept at − 20 °C for further testing. The residue of fluridone was detected during the sampling time. The whole genomic DNA of soil was isolated from the specimens with the Power soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA). The validation of DNA isolation for all specimens was performed with 1% agarose gel electrophoresis, while the final DNA concentration was assessed with a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Hudson, NH, USA). The PCR amplification was performed using a TGreat PCR System (TIANGEN Biotech (Beijing) Co. Ltd, China). More details of the analysis method are given in the Supplementary Information Part 1 Sect. 1.2.

2.6 Bioinformatic and statistical analysis

For microbial alpha diversity, the Shannon index was calculated to assess the abundance and evenness of the community across various treatments using QIIME v1.91 pipeline (Caporaso et al. 2010). Using vegan package (version 2.6.4) in R (version 4.1.0), the beta diversity represented by Principal coordinates analysis (PCoA) was implemented to show the differences in microbial community structures of the genus level based on the Bray–Curtis distance algorithm. Additionally, the PERMANOVA (Permutational Multivariate Analysis of Variance) was also tested to evaluate the significance of differences. Linear discriminant analysis effect size (LEfSe, https://usegalaxy.org/) was used in combination with the Kruskal–Wallis Sum Rank test to determine the significant differences in the number of bacterial genera present in the fluridone treatments and 2% BCR500

amended soil compared to the control groups (Segata et al. 2011).

Co-occurrence analysis was conducted using the taxa with relative abundances greater than 0.05% via the Hmisc package (version 5.1.1) in R. We applied Spearman's correlation to examine all pairwise correlations between absolute variables, specifically focusing on those with a correlation coefficient (r) more than 0.7 and a p-value less than 0.05. The resultant correlations were graphically represented with the Gephi platform (version 0.9.0) using the Frucherman–Reingold method (Bastian et al. 2009). And the heatmap figure was created using the pheatmap package (version 1.0.12) in R. Furthermore, the Tax4Fun program (version 0.3.1) integrated the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Silva database was utilized to perform functional prediction based on the 16S rDNA sequencing data in the study (Aßhauer et al. 2015). More details of the analysis method for sorption and degradation are given in the Supplementary Information Part 1 Sect. 1.3. All data were processed using GraphPad Prism 9. To compare intergroup differences, a two-way analysis of variance (ANOVA) was performed using SPSS (29.0 PREMIUM). For outputs where p-value were derived, statistical significance was considered as $p < 0.05$.

More details of the statistical analysis method for sorption and degradation are given in the Supplementary Information Part 1 Sect. 1.3.

2.7 Soil remediation using waste generated biochar

Pot experiments were conducted to verify biochar mitigation of soil residual fluridone following previous literature methods (Wu et al. 2019). The test soil (DBS) was weighed (400 ± 2 g) in a 17 × 24 cm plastic Ziplock bag. Maize was selected as test organisms, with 10 seed for each treatment. Then the fluridone solution was added to make up the initial content of fluridone in the soil. After solvent volatilization, biochar was added in soil samples to make up the proportion of biochar in the soil. Three treatments were set up in blank, fluridone and fluridone/biochar, and each treatment was conducted in quintuplicate. At the end of this experiment (28 days), root and stem length of maize as well as weight were measured. The residue in root and stem was determined at 7, 14 and 28 days. At end of experiment, organelle structure of leaves was observed using biological transmission electron microscopy (Hitachi-HT7800).

3 Results and discussion

3.1 Biochar physicochemical characteristics

The elemental compositions of biochars are shown in Table S1. As the pyrolytic temperature increased, the C content rose from 27.50 to 37.20%. In contrast, the contents of H and O declined from 3.33 to 1.06% and 16.21–11.35%, respectively. At a pyrolytic temperature of 700 °C, the H/C ratio considerably decreased to 0.34, indicating a significant hindrance in the original organic contents due to more intensive degradation and carbonization at high pyrolysis temperature (Liu et al. 2019). The O/C and (O + N)/C ratios showed a significant reduction at a temperature of 500 °C which indicated an augmentation in hydrophobicity and a decrease in polar groups (Zhang et al. 2017). The SSA values increased as the pyrolytic temperature increased. For BCR300, the SSA value was 38.21 $m^2\ g^{-1}$, while for BCR700, it was 126.12 m^2/g (Table S1); the increase of surface area is mainly due to decomposition of the organic fraction of the BCR. Similar SSA analysis results of BCR have been reported by Priya et al. (2020). By raising the pyrolytic temperature, the amount of aromatic C and the elimination of aromatic C=O and phenolic − OH connected to the aromatic core enlarge SSA (Konczak et al. 2023). Meanwhile, the large SSAs suggest the presence of a refined pore structure inside the biochar (Parlavecchia et al. 2019; Kamali et al. 2022). With the temperature rising from 300 to 700 °C, the ash content of biochar also increased. In summary, biochar properties changed with the temperature.

3.2 Sorption of fluridone and fluridone acid in soil and amended soil

3.2.1 Sorption of fluridone with different biochars

The sorption affinity (K_f) of the produced biochar toward Fluridone differed. The K_f of the biochar increased with increasing temperature (Table S3), with values in the range 409–1352 $(\mu g/g)/(mg/L)^n$ (Table S3). A strong negative correlation ($r^2 = 0.9774$) was seen between K_f and (O + N)/C (Fig. S1), in agreement with the decrease in the (O + N)/C ratios with increasing pyrolysis temperature (Table S1). The decrease of (O + N)/C ration suggested increasing hydrophobicity and reduced number of polar groups (Zhang et al. 2011), indicating surface polarity mechanism involved in adsorbed by biochar. In addition, the carbonization levels of biochars increased as the H/C ratios decreased from 1.45 to 0.34 (Table S1), leading to a negative correlation between K_f values and H/C ratios, with an observed $r^2 = 0.9472$. This indicates that biochar produced at lower temperatures exhibits a stronger ability to bind fluridone via π-π electron donor–acceptor (EDA) interactions (Liu et al. 2018; Sun et al. 2012a). The rise in concentrated aromatic carbon suggests enhanced carbonization, which is typically associated with increased specific surface areas (SSAs) and a higher presence of micropores within the biochar (Sun

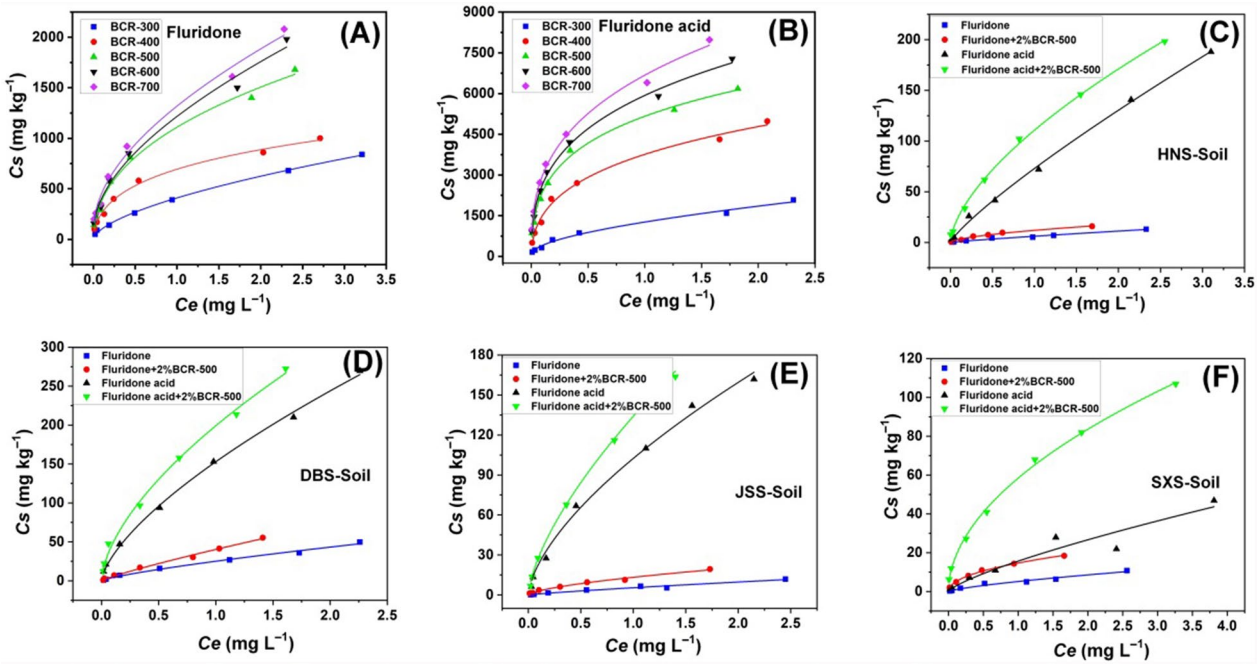


Fig. 1 Sorption kinetic curves of fluridone and its metabolite fluridone acid on biochar (**A** and **B**) and soil with and without biochar amendment (**C** for HNS, **D** for DBS, **E** for JSS, **F** for SXS).

et al. 2012b), consistent with our findings. Conversely, K_f values exhibited a positive correlation with SSA values (Figure S1), suggesting a pore-filling mechanism between fluridone and biochar. This relationship shows a strong positive correlation between K_f values and SSA ($r^2 = 0.9554$). Additionally, there is a robust positive association between K_f values and ash content ($r^2 = 0.9940$), consistent with published article (Sun et al. 2011).

3.2.2 Sorption of fluridone acid with different biochars

The change in K_f value of fluridone acid was the same as for fluridone, which indicates a similar sorption mechanism, but the K_f was 1300–6665, which was higher than for fluridone. Under the same conditions, fluridone acid showed higher potential for sorption in soil than fluridone. It could be concluded that fluridone acid will persist for a longer time in soil, causing a high exposure risk. However, biochar could enlarge the sorption capacity, causing less residue to be exposed to the environment. The effect of addition of 2% of BCR500 was not more evident than that of BCR600 or BCR700, no matter for fluridone or fluridone acid. Therefore, 2% BCR500 was selected in further studies.

3.2.3 Sorption of fluridone and fluridone acid with different soils and amended soils

Figure 1 illustrates the adsorption kinetics of fluridone and fluridone acid in the four agricultural soils. The adsorption of fluridone varied significantly across the different soils. The order for log K_f followed an order of SXS ≈JSS < HNS < DBS from 0.71 to 1.40 for fluridone. and SXS < HNS < JSS < DBS from 1.20 to 2.18 for fluridone acid (Table S3). DBS exhibited significantly greater adsorption capacity compared to the other soils ($p < 0.05$), which appeared to be related to pH and cation exchange capacity (CEC). Positive correlations were observed between Kf values and both pH ($r^2 = 0.9247$) and CEC ($r^2 = 0.9230$) (Figure S1A and S2B). Upon amendment with 2% BCR500, ability to sorb fluridone improved by an average of 60.16%–183.50%, while the sorption capacity for fluridone acid increased by 30.92%–274.36%. In pure biochar, the logKf values for fluridone acid were 1.43–2.74 times higher than those for fluridone across four soil types, both with and without the addition of 2% BCR500.

To accurately assess the influence of interactions between biochar and soil on sorption, we conducted a comparison between the estimated K_d values of biochar-soil mixtures and the anticipated values, using the equation $K_d = f_{soil} K_d + f_{biochar} K_d$, where f are the percentages of soil and biochar in the mixture. In all instances (Table S3), the experimental K_d values were consistently 38%–66% lower than the expected K_d values, possibly due

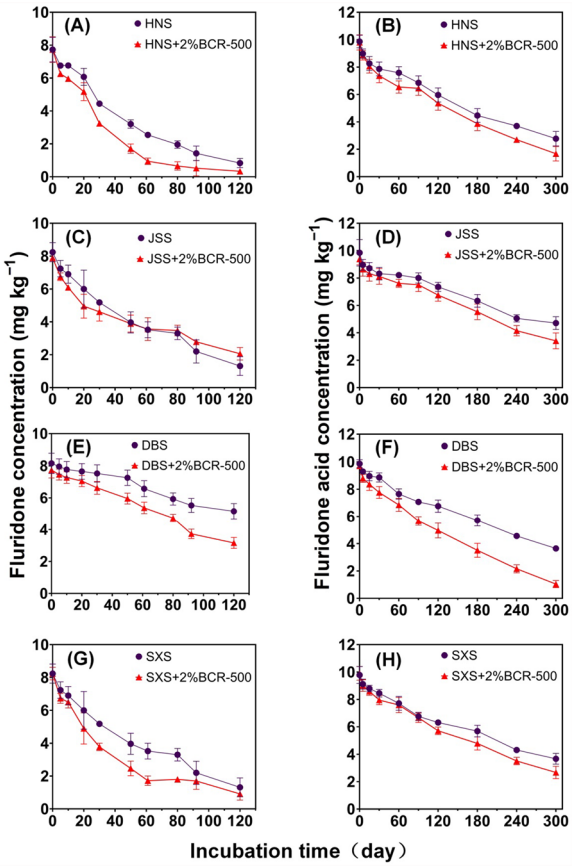


Fig. 2 Degradation trends for fluridone and fluridone acid in different soils (**A** and **B** for HNS, **C** and **D** for JSS, **E** and **F** for DBS, and **G** and **H** for SXS) under unsterilized conditions

to the presence of soil fine mineral particles which might reduce the accessibility of micropore and inner sorption sites (Ren et al. 2016).

The change in ΔG may be used to quantitatively ascertain the adsorption processes and the driving factors behind them throughout the adsorption process (Cea et al. 2010). Furthermore, the change in ΔG may serve as an indicator to quantify the strength of chemical adsorption. The absolute values of ΔG for fluridone and fluridone acid in both pure soils and amended soils were below 40 kJ mol^{-1} (−10.60 to −2.89 kJ mol^{-1} at 298 K; Table S3). This suggests that the adsorption of fluridone and fluridone acid in these soils mostly happened by physical adsorption (Cea et al. 2010). The partial molar free energy of fluridone and fluridone acid (F) ranged from 3041.42 to 843.66 in the soils that were examined. The F value of fluridone and fluridone acid was negatively correlated with K_f in both pure soils and 2% BCR500-amended soil. This indicates that, with application with 2% BCR500, fluridone and fluridone acid have a higher tendency to be adsorbed in different types of soils. Sorption capacity of biochar was diminished as a result of its interactions with soil. However, the addition of biochar improved sorption in the soil. Therefore, the biochar amendment might improve the process of immobilizing pesticides in soil.

3.3 Degradation of fluridone and fluridone acid in different conditions

3.3.1 Degradation under unsterilized conditions

The fluridone and fluridone acid residues in the soil decreased along the incubation time, and varied with different types of soil with or without biochar. A faster degradation rate was observed initially, becoming slower in HNS, JSS, and SXS with fluridone, which is consistent with a previous study (Wu et al. 2021). Other treated soils showed a slow and stable decrease in fluridone and fluridone acid (Fig. 2). The degradation of fluridone and fluridone acid followed a first-order kinetic equation. Table S4 shows the degradation kinetic parameters and half-life data.

The degradation half-life time (DT_{50}) of fluridone in four types of soil was from 39 to 179 days under unsterilized conditions (Table S4), in the order of HNS > SXS > JSS > DBS. The DT_{50} of fluridone acid (from 179 to 634 days) was significantly greater than for fluridone ($p < 0.01$, $F = 6.78$). The DT_{50} of fluridone in soil amended with 2% BCR500 biochar, was shorter than that in unamended soil, decreasing by 29% to 45%. The differences in degradation between fluridone and fluridone acid in amended soils were the same as in unamended soils, increasing in the order of HNS > SXS > JSS > DBS. The change in fluridone acid after the 2% BCR500 amendment was the same as in fluridone; the DT_{50} decreased by 21.79% to 46.88% for fluridone acid. After amendment with biochar, fluridone and fluridone acid were degraded faster. The discrepancy degradation of fluridone and fluridone in different soils may be related to the different physicochemical properties of the soil, showing the positive correlations between the DT_{50} of fluridone and soil organic matter (SOM) ($r^2 = 0.8340$) and CEC ($r^2 = 0.8745$) (Fig. S2A). Furthermore, the incorporation of biochar in soil could alter the soil properties, limit the adsorption capacity, and positively impact the microbial activity in the soil (Clivot et al. 2020).

3.3.2 Degradation under sterilized conditions

The degradation tests under sterilized conditions were conducted using the DBS with and without biochar to confirm whether biodegradation is dominant. The DT_{50} values for fluridone were 179 days under unsterilized conditions and 466 days under sterilized conditions (Table S4), indicating a significantly higher degradation rate in unsterilized environments. This difference is likely

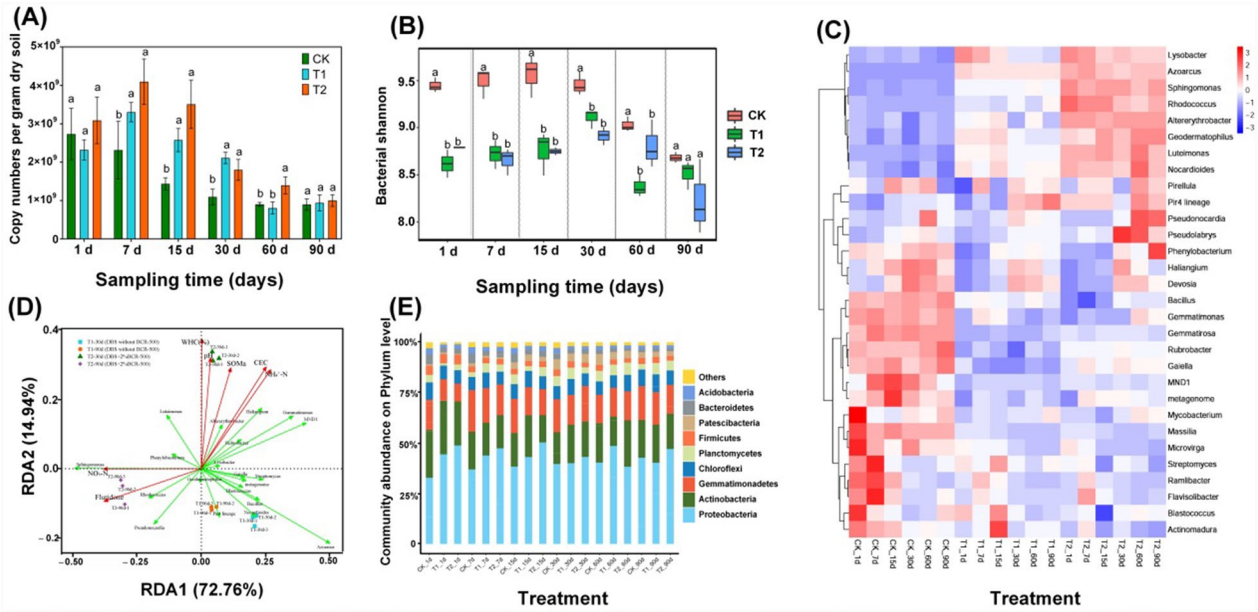


Fig. 3 Effects of control (CK), fluridone treatment (T1) and BCR500 amendment (T2) on the bacterial abundance (**A**), diversity (**B**), heat map of the top bacteria at genus level (**C**) and relative abundance of bacteria at phylum level (**E**) at 1, 7, 15, 30, 60 and 90 days in DBS. Redundancy analysis (RDA) of the bacterial community (at the genus level) and environmental variables (**D**) for all the soils treated with fluridone without and with BCR500 amendment. The letters 'a' and 'b' above the columns indicate significant differences ($p < 0.05$)

attributable to biodegradation processes, with biodegradation percentages reaching 61.59% in unamended soil and 67.71% in amended soil. Following the addition of 2% BCR500, the biodegradation percentage increased further, from 64.70% to 77.67% for fluridone acid. Therefore, biodegradation was considered as the dominant factor in the degradation process. Previous studies showed biochar addition gave considerable impacts on degradation of pesticides in soil (Zhou et al. 2019; Twagirayezu et al. 2024). The primary factor is the biochar contains nutrients, which provides a favorable environment for microorganisms andenhances soil microbial activity (Sandhu et al. 2019). Previous investigations reported enhanced degradation of studied pesticides by biochar amendment (Liu et al. 2018). Thus, the BCR500 can be considered an excellent soil remediation with the capability of enhanced degradation of pesticides in contaminated soils.

3.4 Bacterial profiles in response to fluridone and biochar amendment

The bacterial profiles are displayed in Fig. 3A. The total number of bacterial 16S rRNA genes increased significantly in fluridone-treated soils and 2% BCR500 amended soils compared with pure soils from days 7–30. Moreover, the bacterial abundance in soil with biochar was significantly greater than in the soil without remediation contaminated with fluridone at day 60. The abundance of 16S rRNA profiles in treated soils with and without BCR500 corresponded to the findings of the ANOVA ($p > 0.05$) except at 60 days, which indicated that DBS significantly impacted bacterial abundance from the beginning. Upon thorough analysis of all 54 samples, the alteration in bacterial diversity was assessed by examining the operational taxonomic unit levels with the Shannon indices, which showed that bacterial diversity significantly decreased in two treated groups except at day 90 ($p < 0.05$) (Fig. 3B). After 90 days of incubation, the Shannon index showed no significant difference between treatment and control, indicating bacterial diversity recovery.

3.5 Effect of fluridone on the biochar amended soil bacterial community

3.5.1 Diversity and composition of soil microbiota under the phylum level

The population and activity of soil microbes have a considerable impact on the functioning of the soil (Twagirayezu et al. 2024). After treatment with fluridone and 2% BCR500, the bacterial community composition in the DBS soil was changed. The dominant phyla found in both the control and treatment groups were Proteobacteria (32.8%–48.9% relative abundance), Actinobacteria (13.5%–26.5%), Gemmatimonadetes (8.8%–19.46%), Chloroflexi (6.2%–8.9%), Planctomycetes (1.1%–6.9%), Firmicutes (1.9%–4.8%), Patescibacteria (0.6%–5.4%), Bacteroidetes (1.3%–5.1%), and Acidobacteria (1.3%–3.3%). However, these effects were not consistent

(Fig. 3E), with varying changes in the amounts of different bacterial phyla. Other studies have also shown that amendment with biochar in the soil enhances the reproduction rate and abundance of the microbial population (Qiu et al. 2019; Ge et al. 2019). Meanwhile, the change of abundance of the phyla related to degradation. For example, Proteobacteria and Chloroflexi increased with the 2% BCR500 amendment over the exposure period, perhaps leading to an accelerated degradation. The phylum Proteobacteria has a significant impact on the global carbon, nitrogen, and sulfur cycles because of its extensive range of metabolic abilities (Spain et al. 2009; Carrerofigueroa 2011). The majority of denitrifiers belong to the Proteobacteria group, and some of the groups are capable of degrading carbonaceous substances (Cebron et al. 2008). Chloroflexi has the ability to metabolize organohalides and is important in the chlorine cycle (Krzmarzick et al. 2012; Temme 2018).

3.5.2 Soil microbial alteration and beta diversity at the genus level

A hierarchical clustering heatmap was generated using the top 30 bacteria at the genus level (Fig. 3C). The two treatments elevated the abundances of *Lysobacter*, *Azoarcus*, *Sphingomonas*, and *Rhodococcus.* This is consistent with previous studies, in which soils polluted with epoxiconazole, trifluralin, and thiamethoxam also showed a comparable rise in bacterial abundance (Wu et al. 2020; Alexandrino et al. 2020).

Fluridone exposure altered bacterial diversity, with greater differences observed in fluridone-treated soils compared to BCR500 amended soils. Biochar stimulates specific genera abundance and mitigates declines in others, potentially enhancing degradation rates (You et al. 2020). Twelve genera showed increased levels, particularly in BCR500 treatments. *Sphingomonas* catalyzes monohydroxylation of fluoranthene (van Herwijnen et al. 2003), likely applying the same mechanism for fluridone. *Rhodococcus* facilitates defluorination via dioxygenase-catalyzed oxidation (Yano et al. 2015; Matsui et al. 2016), suggesting similar pathways in fluridone transformation (Fig. S4). *Lysobacter* contributes to pollutant-degrading capabilities and enzyme activity tolerance (Yu et al. 2012) through ring-opening reactions via hydrolytic enzymes or oxidative cleavage (Xu et al. 2022; Wei et al. 2020; Guengerich and Yoshimoto 2018). Meanwhile, *Sphingomonas* shows resilience to pollutants under low-nutrient and stress conditions (Yang et al. 2024). More details are in supplemental information about Microbial functions and pathways.

Redundancy analysis (RDA) established connections between environmental conditions, the levels of fluridone in the soils, and the bacterial communities (Fig. 3D). NO_3^-N values showed positive correlations between Luteimonas, Sphingomonas, Rhodococcus, Pesudonocardia, Geodermatophilus. Conversely, the WHC (water hold capacity) showed strongest effects on the bacterial community among above parameters. Additionally, the PCoA analysis revealed that the fluridone and BCR500 treatment had a significant impact on the bacterial community composition structure (Fig. S3). Meanwhile, the PERMANOVA test based on the Bray-Curits distance measures showed the bacterial community structure was significantly ($P<0.05$, $R^2=0.65$) different among these clusters grouped by objective elevations. The soil community experienced considerable changes in reaction to the presence of fluridone. This indicates that when the soil was amended with BCR500, it effectively expanded the variety of microorganisms in the soil. This is probably due to biochar, with its porous structure and high surface area, facilitating the provision of refuges and homes for soil bacteria, as reported in other studies (Quilliam et al.

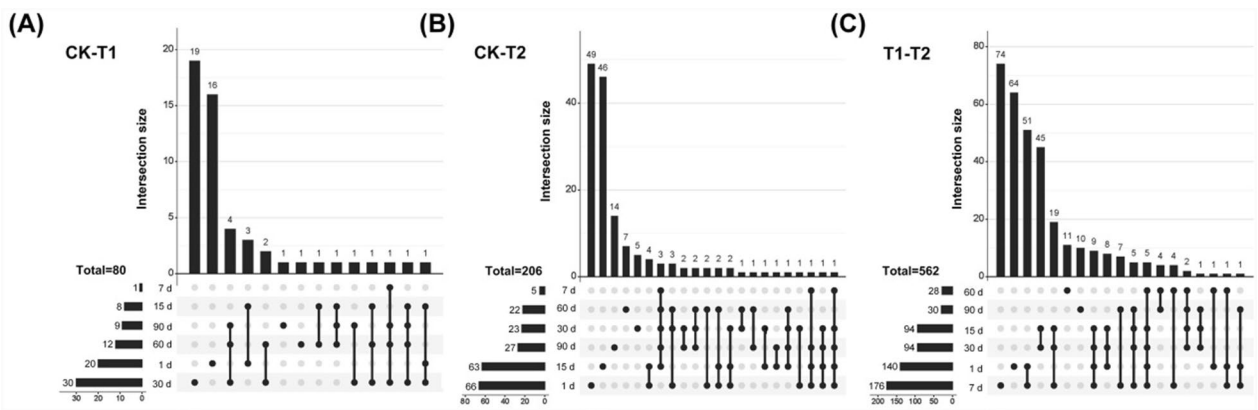


Fig. 4 UpSet plots of the significantly increased bacterial species in fluridone (T1) and BCR500 amended (T2) treatments compared with the control: (**A**) CK-T1, (**B**) CK-T2, and (**C**) T1 compared with T2 (T1-T2)

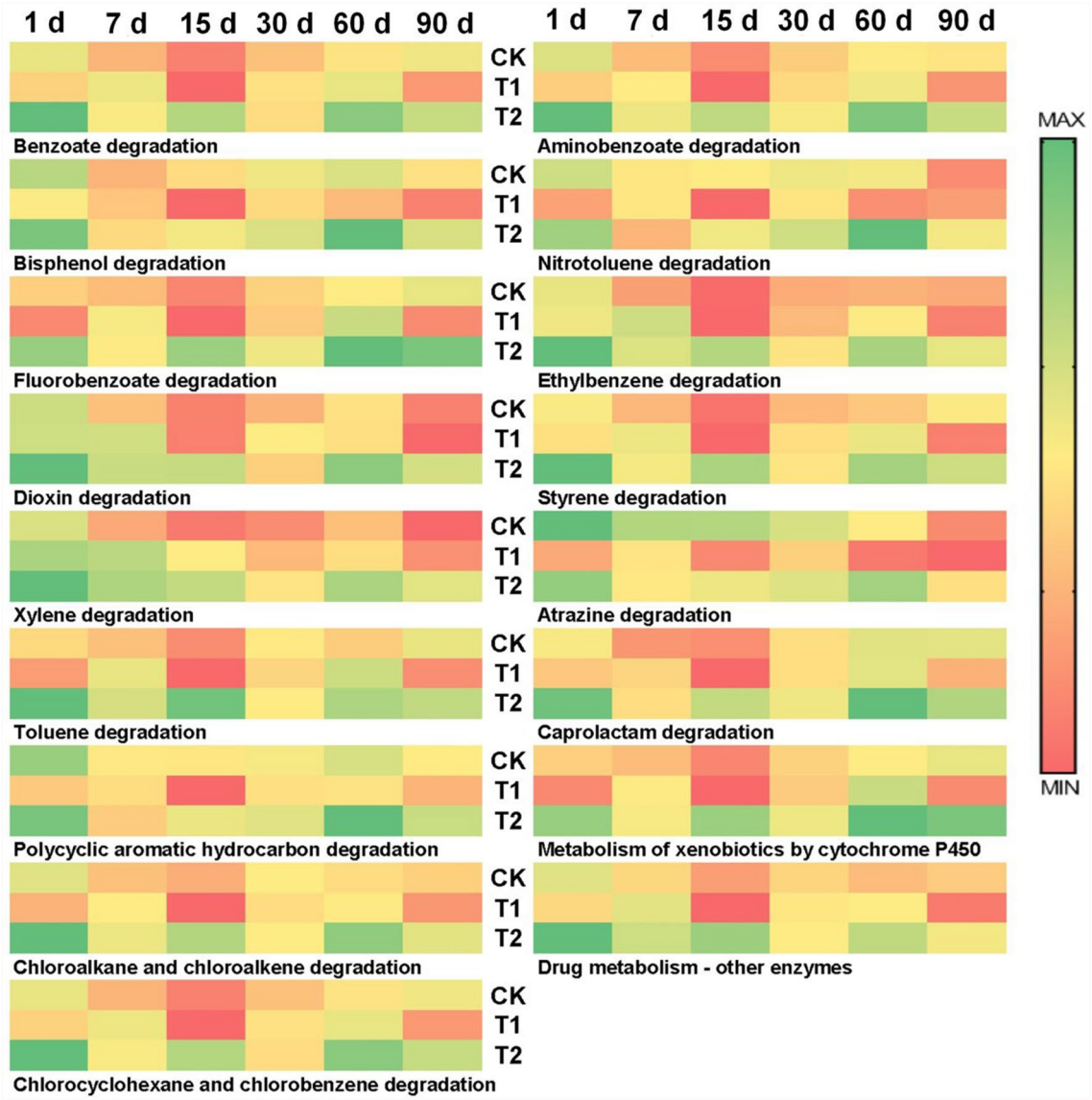


Fig. 5 Heat map of degradation pathways impacted by control (CK), fluridone (T1) and BCR500 amended (T2) treatments

2013; Kuryntseva et al. 2023). Furthermore, the nutrients in the biochar have the potential to enhance the growth of soil microorganisms (Joseph et al. 2013; Haider et al. 2022). After amendment with BCR500, there was a tendency to cluster closely together and distinct from the control group between days 7 and 60. The microbial community structure at the final sample point (90 days) showed an elevated degree of similarity between the BCR treatment and the control group. The observed changes in the soil bacterial community could be attributed to the resistance (lack of sensitivity to disturbance) and resilience (speed of recovery following disturbance) of the microbial community (Griffiths and Philippot 2013). These alterations align with findings from prior investigations that examined various external pressures (Du et al. 2018a; Lourenco et al. 2018; Suleiman et al. 2016). This showed that biochar has the ability to expedite the restoration of the bacterial community.

3.5.3 Identification of soil microbial biomarkers by LEfSe analysis

Linear discriminant analysis Effect Size (LEfSe) was applied to conduct biomarker analysis (Fig. 4). Compared with the control, detailed results for the fluridone

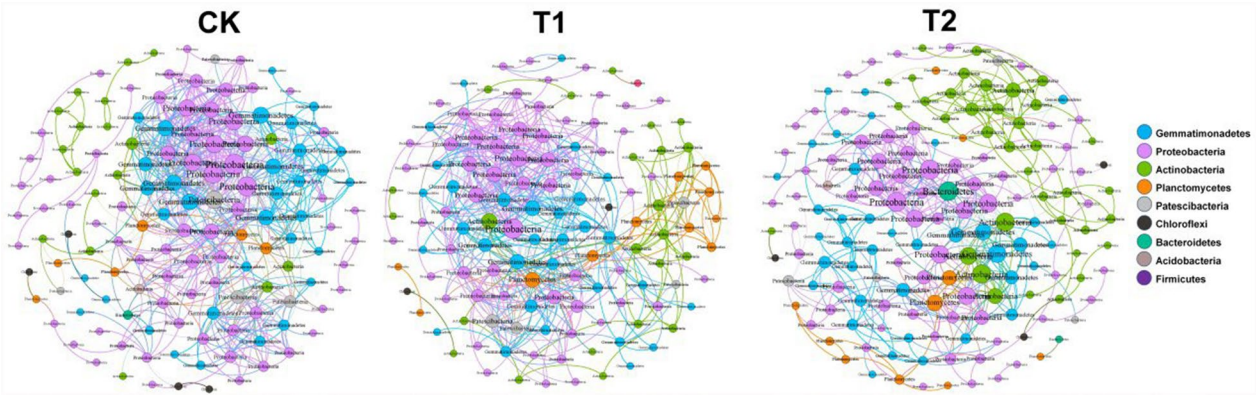


Fig. 6 Network co-occurrence analysis of bacterial communities in treated soil. A connection stands for a strong (Spearman's r > 0.8) and significant (*p* value < 0.01) correlation for the control (CK), fluridone-treated (T1) and BCR500amended (T2) soils. Colors of nodes in network represent the bacterial phyla. The size of each node is proportional to the number of connections (degree). Each node was colored according to the bacterial phylum

treatment and BCR500 amendment groups are shown in the supplementary Excel file "excel 1 LEfSe information.xlsx". These results provided information on dominant and suppressed populations, as well as biomarkers (i.e., microbial species), at six distinct sampling times. Figure 4A ~ C displays the quantities of the notably increased species in various treatments, as indicated by the UpSets. There were 80 and 206 persistently increased species (PISs) in abundance in the fluridone treated and BCR500 amended treatments, respectively, in contrast with the control group. Additionally, there were 7 biomarkers that exhibited the same effect for the fluridone treatment when comparing the control group at days 60 and 90 (Fig. 4A). However, after the 2% BCR500 amendment, there were 67, 14, and 5 biomarkers that revealed the same influence of biochar in the first week, first month, and second month, respectively (Fig. 4C). In addition, the LEfSe analysis demonstrated that 19 biomarkers at the genus level exhibited sensitivity to fluridone after BCR500 amended treatment at day 1 and 7 ($p < 0.05$, LDA > 2.0), including *Bacillus*, *Geodermatophilus*, *Agromyces*, *Haliangium*, *Hyphomicrobium* (the table cells were filled with green in supplementary excel 1). These demonstrate important role in degradation of pesticides (Abraham and Silambarasan 2016; Bisht et al. 2019; Zaborowska et al. 2020; Du et al. 2021).

3.6 Xenobiotic biodegradation pathways with addition of fluridone and biochar

Typically, 17 KEGG pathways were associated with the biodegradation of xenobiotics (Fig. 5). After fluridone treatment, the pathway controlling the degradation of atrazine was significantly decreased on day 1 compared to the control. However, 9 pathways were increased after BCR500 addition compared to fluridone treatment group, and these pathways controlled the degradation of bisphenol, dioxin, xylene, toluene, chloroalkene, aminobenzoate, caprolactam and metabolism of xenobiotics by cytochrome P450 and drug metabolism. In addition, a pathway related to the degradation of xylene was significantly increased in both treatments on day 7. On day 15, the overwhelming majority of the pathways were increased by biochar amendment compared to the control, except for the pathways governing the degradation of polycyclic aromatic hydrocarbon, and atrazine. On day 60 and 90, all pathways were significantly elevated in 2% BCR500 amendment compared to without biochar. Consequently, biochar appears to lead to an increased metabolism (Bass and Field 2011). The identification of additional xenobiotic biodegradation pathways in the fluridone treatment group implies that these pathways contribute to the enhancement of degradation. The increase in the activity of the biodegradation pathways in the soil after 90 days likely had a role in the shorter amount of time it took for BCR500 to be amended, compared to the soil treated with fluridone.

3.7 Effect of fluridone and biochar amendment on the soil community network

Co-occurrence network analysis was applied to investigate the relationship intricacy in the soil microbiomes (Fig. 6). Spearman's rank coefficients were computed between microbial taxa at the OUT level, namely those with relative abundances over 0.01%, and their presence in all samples. The computations were derived from 16S rRNA data. Table S5 displays the topological indices for each bacterial network. Compared with the untreated control soil, the clustering coefficient and network

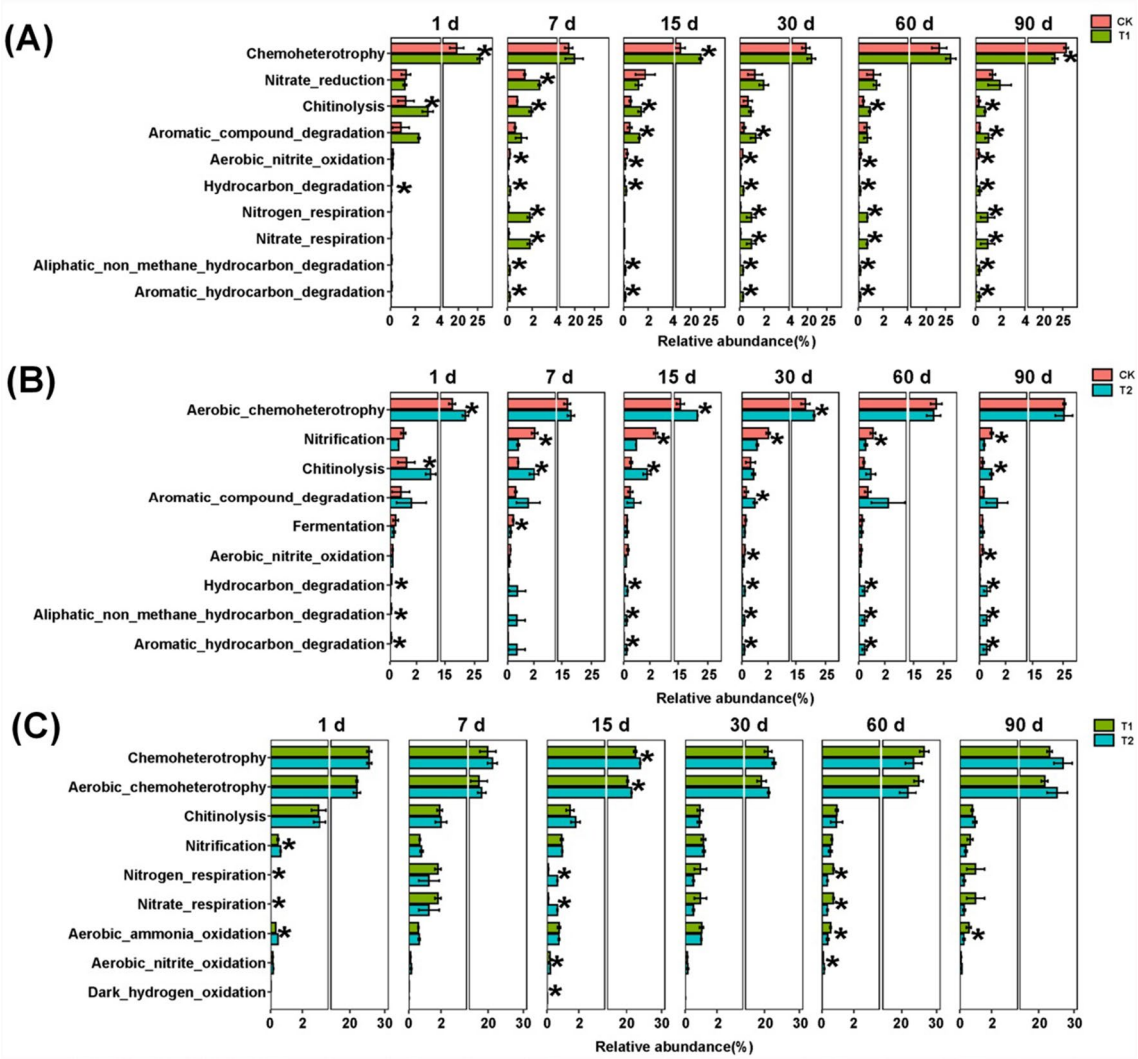


Fig. 7 Predicted functions of the bacteria by FAPROTAX in both soils. The letter '*' on the right of the columns means significant difference ($p < 0.05$)

densities were increased by 0.578 and 0.081 respectively (Table S5) in the fluridone treatment, showing that the soil microorganisms formed more tightened under the influence of fluoridone (Du et al. 2022), more bacterial species cooperated with each other to resist the pollutant (Hoek et al. 2016). However, compared with the fluridone treated group, the clustering coefficient and network densities were clearly decreased by 0.499 and 0.036 respectively in BCR500 amended group, which indicated that the soil microbiome experienced relieved stress from fluridone. Bacterial communication could increase pesticide degradation, while more competition might decrease pesticide degradation efficiency after BCR amendment (Fuentes et al. 2013; Abraham et al. 2002). Also, we performed a Spearman correlation analysis between microbial relative abundances (at the genus level) and degradation rates under fluridone and BCR amendment treatment groups at different time points (Fig. S5). Notably, some well-documented degradation-associated genera, such as *Sphingomonas*, *Rhodococcus*, *Lysobacter*, and *Pseudonocardia,* exhibited strong correlations with degradation rates ($|r| \geq 0.7$). These results clearly demonstrate how changes in microbial diversity correlate with the faster degradation rates observed with biochar amendments.

3.8 Predicting the influence of fluridone and biochar amendment on bacterial function

We conducted further investigations into the presumed metabolic and ecologically significant roles of bacterial OTUs with the FAPROTAX database (Fig. 7). Fluridone has an effect on 10 functions in DBS soil (Fig. 7A). The ANOVA analysis showed that the chemoheterotrophy was significantly impacted by fluridone at days 1, 15, and

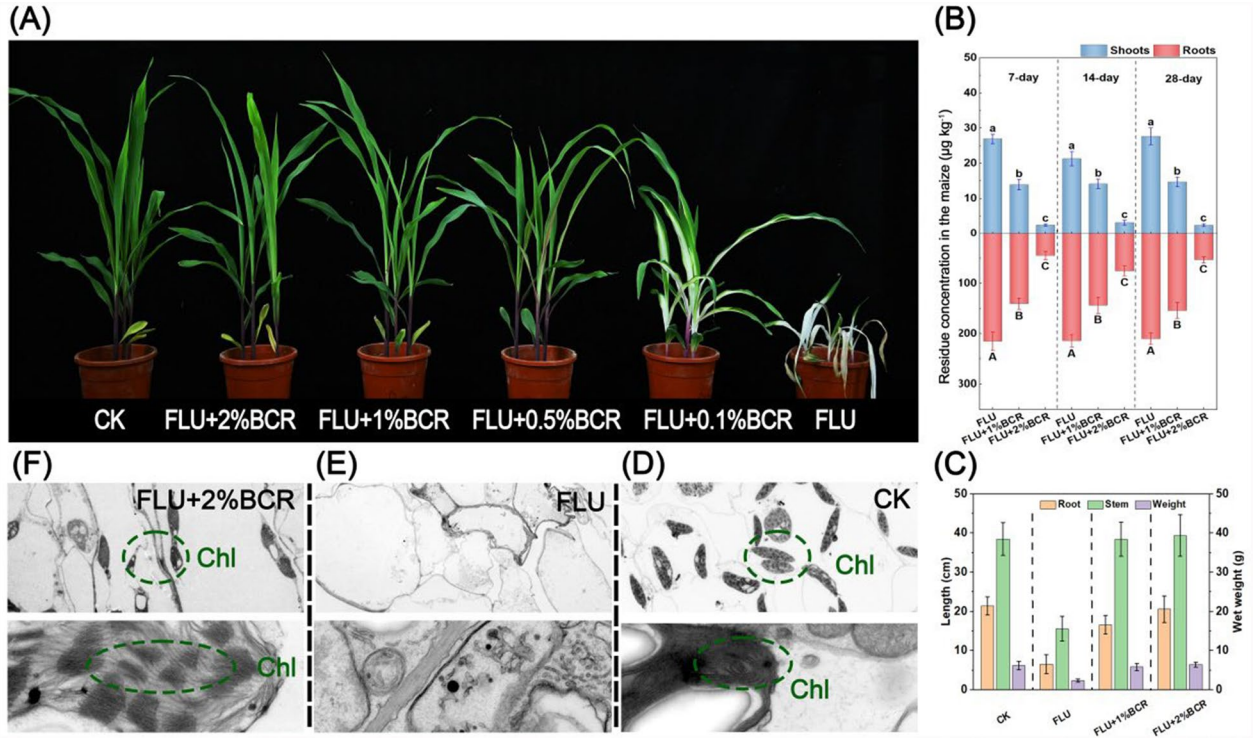


Fig. 8 **A** Growth of maize seedlings in fluridone-treated soil with varying amounts of BCR. **B** Residue of fluridone in roots and stems of maize plants after fluridone treatment and BCR addition at different collection points. **C** Stem height, root length and fresh weight of maize plants grown after fluridone treatment and BCR addition. Values (**A**–**C**, **a**–**c**) with different letters are significantly different ($p < 0.05$). Transmission electron microscopy (TEM) photos of mesophyll cells in maize seedlings. The ultrastructure of mesophyll cells in maize leaves: control **D**, fluridone treatment **E** and fluridone treatment and BCR addition **F**; Chl: chlorophyll

90. Nitrate reduction increased on day 7, and chitinolysis was significantly elevated by fluridone except on day 30. Previous studies have also shown that the specific microbial taxa associated with denitrification could be inhibited by organic pollutants, such as metam sodium (Li et al. 2017), 1,3-dichloropropene (Fang et al. 2019), dazomet (Fang et al. 2018), and multi-walled carbon nanotubes (Ge et al. 2018), although the identified sensitive taxa differed across studies and soils. The degradation of aromatic compounds was also increased at three sampling points (day 15, 30, and 90). Hydrocarbon degradation significantly increased during days 1 to 90 compared with the control, whereas aerobic nitrite oxidation decreased from day 7 to 90 in the fluridone treatment. In addition, nitrogen respiration, nitrate respiration during days 30 to 90, aliphatic non methane hydrocarbon degradation, and aromatic hydrocarbon degradation during the whole incubation period except for day 1 were all significantly increased after fluridone treatment in comparison with the control. In the BCR500 treatment, there were 9 functions that were significantly altered at more than three sample points (Fig. 7B), including nitrification (except for day 1) significantly decreased by the BCR500 amendment. In contrast, the degradations of hydrocarbons, aliphatic non methane hydrocarbons, and aromatic hydrocarbons (except for day 7) were all significantly increased by biochar amendment in the BCR500 treatment compared with the control, indicating that a community that is able to degrade the herbicide increased in response to the presence of the chemical compound. Compared with the fluridone treatment (Fig. 7C), the functions of chemoheterotrophy and aerobic chemoheterotrophy, which are responsible for in situ remediation and organic material recycling in ecosystems, significantly increased on day 15 (Kämpfer et al. 1993).

As indicated by the test results, the addition of biochar potentially enhanced the activity of fluridone-degrading bacteria in the soil and altered the process of biodegradation. Hence, biochar has the capacity to mitigate the residual hazard related to the presence of fluridone in soil.

3.9 Effects of biochar on soil remediation

As shown in Fig. 8A, the growth of maize in DBS contaminated with fluridone was significantly impaired. Symptoms included shorter plants, shorter root lengths,

reduced fresh weights, some leaves curling and turning yellow, and even plant death. The effects of BCR500 on fluridone uptake by the roots and shoots of maize in DBS are shown in Fig. 8B. Adding BCR500 to the soil significantly reduced fluridone concentrations in the plants ($p < 0.05$). Due to strong adsorption and digestion-promoting abilities of BCR500, bioavailability of fluridone in plants was reduced. Additionally, fluridone concentrations were higher in roots than stems, consistent with previous findings that roots are the primary site of accumulation (Wang et al. 2013; Li et al. 2018; Wu et al. 2019) However, the addition of BCR500 at 2% to the soil effectively mitigated the stress caused by fluridone ($p < 0.05$). With the addition of BCR500, maize plant height increased by 146–153%, root length increased by 154–215%, and the fresh weight increased by 142–167%, with the most pronounced improvement observed in root length (Fig. 8C). This significant improvement is primarily attributed to the adsorption and isolation of fluridone by BCR500, which reduces the amount of fluridone absorbed by plant roots from the soil, allowing the plants to recover faster. Moreover, the organelle structure of their leaves was observed using biological transmission electron microscopy (Hitachi-HT7800) (Fig. 8D–F), indicating chlorophyll decrease and leaves turning pale. BCR amendment could effectively protect plant leaf structures from damage.

4 Conclusions

The present study provides a comprehensive assessment of the environmental fate of fluridone and its primary metabolite, fluridone acid, in biochar-amended soils. Our findings demonstrate that the addition of 2% BCR500 significantly enhanced the sorption and degradation of both compounds, reducing their persistence in soil. Notably, biochar amendments not only increased the biotic degradation rate but also influenced the soil microbial community composition, particularly those capable of degrading fluridone such as *Lysobacter*, *Pseudonocardia*, and *Sphingomonas*, which became dominant following the addition of BCR. These shifts suggest that biochar amendment can mitigate the negative impacts of fluridone on soil microbial diversity while simultaneously enhancing its degradation potential.

From an applied perspective, this study highlights the potential of biochar as a sustainable remediation strategy for pesticide-contaminated soils. The dual function of biochar—adsorbing pesticides and stimulating microbial degradation—offers a promising approach to reducing residual pesticide burdens while maintaining soil health. However, further research is needed to refine biochar application strategies, particularly in optimizing the balance between pesticide immobilization and bioavailability for microbial degradation.

Moreover, the findings have significant implications for agricultural sustainability and pesticide residue management. Future studies should focus on field-scale validation, exploring the long-term effects of biochar on pesticide dynamics, soil microbial ecology, and potential trade-offs in crop productivity. By integrating biochar amendments with complementary agronomic practices, such as crop rotation and soil conditioning, a more resilient and environmentally sustainable approach to pesticide management can be achieved.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00469-9.

Additional file 1

Additional file 2

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Author contributions

All authors contributed to the study conception and design. Chi Wu performed the main experiments, analyzed the data, produced the illustrations and wrote the paper, provided financial support; Yuzhu Wang performed the experiments, analyzed the data, produced the illustrations and checked the paper. Jihong Liu Clarke response for project administration and provided financial support; Hang Su conducted formal analysis and data curation; Liang Wang response for writing –original draft; Olga A. Glazunova and Konstantin V. Moiseenko conducted the writing-review & editing and visualization; Lan Zhang detected the samples by instrument, and helped with the data analysis; Liangang Mao conceived the idea and helped to design the experiments; Lizhen Zhu response for writing –review &editing; Xingang Liu responsed for project administration and supervision and provided financial support; All authors read and approved the final manuscript.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest

The authors have no conflicts of interest to declare.

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