Discriminating Multiple Impacts of Biogas Residues Amendment in Selectively Decontaminating Chloroacetanilide Herbicides

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Ⓢ *Supporting Information*

ABSTRACT: There is increasing concern about modifications to pesticide persistence in soil from the application of organic wastes as fertilizers. This study was conducted to discriminate the multiple effects of biogas residues (BR) amendment, including soil nutrients, soil microbial activity and biodiversity, and adsorption and degradation of chloroacetanilide herbicides (acetochlor, metolachlor, and butachlor). Addition of BR to soil increased the release of organic materials (i.e., dissolved organic carbon, dissolved organic nitrogen, and active phosphorus). It not only stimulated soil microorganisms and caused changes to microorganism diversity but also increased herbicide adsorption. Such multiple effects led to selective decontamination of chloroacetanilide herbicides, depending on herbicide structures and BR amendment levels. Stereoselectivity in degradation of acetochlor and metolachlor with biphasic character was magnified by BR amendment, which was well explained by integrating the impacts of BR amendment. Interestingly, BR amendment induced significant accumulation of herbicidally active *aS,CS*-metolachlor, facilitating the utilization of herbicidal activity.

KEYWORDS: Organic amendment, dual effect, chiral pesticide, degradation, soil microorganism

◼ INTRODUCTION

Biosolid wastes such as feedstock manure and sewage sludge are being produced and released into the environment in increasing amounts.[1] The resultant environmental concerns as well as the potential economic merits highlight the need for their recycling. Biogas production is a promising alternative for solid waste treatment, during which methane-rich biogas is produced as a renewable energy source and biogas residues (BR) can be used as a fertilizer.[2] Such recycling of BR improves the fertility and physical structure of soil and often yields higher plant productivity and quality.[3]

Amendment with BR and other organic wastes can alter the soil microbial community,[4−7] which in turn modifies the environmental behavior of pesticides. Organic amendments such as vermicompost and biochar cause a reduction in the microbial degradation or toxicity of acetochlor, atrazine, and chlorpyrifos, which is usually ascribed to the decrease in pesticide availability.[8−10] This is in part a result of increasing the adsorption potential of pesticides in the organic-amended soil. However, addition of compost, vermicompost, mushroom spent compost, and crop-residue-derived char to soil enhances degradation of terbuthylazine,[9] chlorpyrifos,[11] benonitrile,[12] and zaoxystrobin.[13] In another study where seven organic materials were chosen and each material was set at three levels,[14] all organic amendments enhanced atrazine dissipation, whereas the organic materials only at the high level stimulated metolachlor degradation and none of them affected trifuluralin degradation.

However, effects of organic amendments on chiral pesticide degradation are rarely studied, although about 25% of currently used pesticides are chiral. Chiral pesticides have at least one pair of enantiomers, which generally show different biologic effects such as microbial degradation and toxicological impact.[15−19] For example, the fungicide metalaxyl exhibits different levels of stereoselective degradation in soil, depending on soil properties such as soil sources, pH values, and oxygen status.[20,21] The *R*-enantiomer of the fungicide was degraded faster than the inactive *S*-enantiomer in German soil while this result was reversed in Cameroonian soil.[20] The degradation of the *R*-enantiomer was faster than that of the *S*-enantiomer in aerobic soils at pH > 5, whereas both enantiomers were degraded at similar rates in aerobic soils at pH 4−5 and the enantiopreference was reversed in aerobic soils with pH < 4 and in most anaerobic soils.[21] As a result, the general risk assessment, based on standard chemical analysis that designates a chiral compound as a single compound, could give rise to inaccurate classification.[22] To our knowledge, only two studies were conducted to investigate influences of organic amendment on chiral pesticide degradation.[23,24] Both studies revealed some variations in degradation rates of the enantiomers. As organic amendment causes various impacts, the effects of organic amendment on chiral pesticide degradation are poorly understood.

This study was conducted to distinguish the impacts of BR amendment on the degradation of chloroacetanilide herbicides

(i.e., chiral acetochlor and metolachlor and achiral butachlor) in soil. We tested two hypotheses: (1) the changes in soil adsorption potential, soil nutrient status, and soil microorganisms associated with BR amendment would affect degradation of the herbicides and their stereoisomers; (2) application rates of BR would induce variations of relative importance to these changes, thereby leading to an alteration of stereoselectivity in chiral herbicide degradation. Consequently, herbicide adsorption, soil nutrient status, soil dehydrogenase activity, soil microbial community evolution over incubation time, and herbicide and stereoisomer degradation were collectively investigated.

■ MATERIALS AND METHODS

Materials and Reagents. Surface soil (15 cm) was collected from a corn field in Dengta City, Liaoning Province, China. The soil type is brown earth soil with the nature of alluvial plain. The main physico-chemical properties of the soil were as follows: pH (H_2O), 6.56; organic carbon, 18.70 g/kg; cationic exchange capacity, 22.40 cmol/kg; clay, 18.3%; slit, 61.1%; sand, 20.6%. After being air-dried, ground, and sieved (2 mm), the soil was stored in a sealed plastic bag at room temperature (25 °C). Fresh biogas residues (BR) were collected from an anaerobic digestion plant using cattle feedlot manure (Liaoning Academy of Environmental Sciences). The BR was air-dried, sieved (2 mm), and stored at room temperature. The nutrient status of the soil and BR is described in Table S1, Supporting Information.

Butachlor (*N*-butoxymethyl-2-chloro-2′,6′-diethylacetanilide; purity = 94.8%), *rac*-metolachlor (2-chloro-6′-ethyl-*N*-((1RS)-2-methoxy-1-methylethyl)acet-*o*-toluidide; purity = 98.9%), and *rac*-acetochlor (2-chloro-*N*-ethoxymethyl-6′-ethylacet-*o*-toluidide; purity = 95.2%) were supplied by Shandong Binnong Technology Co., Ltd. (Binzhou, China). Metolachlor has four stereoisomers, arising from an asymmetrically substituted C-atom in the alkyl moiety and a chiral axis due to hindered rotation around phenyl—nitrogen bond; acetochlor is axially chiral and has a pair of enantiomers (Figure S1, Supporting Information).

Analytical reagent 2,3,5-triphenyltetrazolium chloride was purchased from Shanghai Kefeng Chemical Reagents Co., Ltd. (Shanghai, China). All organic solvents were of HPLC grade (Tedia, Fairfield, OH), and other reagents used were of analytical reagent.

Experimental Design and Procedures. Soil and BR were mixed thoroughly by sieving (2 mm) five times, thereby giving rise to application rates of BR at 0% (control), 1% (low level), 2% (moderate level), and 5% (high level), respectively. The mixed soils were prepared in duplicate. One portion (denoted as dry soil) was used in a follow-up adsorption experiment. The other was homogenized by sieving and adjusted to 60% of the maximum water holding capacity (61.29%) with spray mist. The soils (denoted as preincubated soil) were incubated in an artificial climate incubator in the dark at 25 °C for 18 days to recover soil microbial activities.

Adsorption isotherms of herbicide by soil were obtained using the batch equilibrium technique. Soil of 5 g (dry weight) in triplicate were added to glass tubes containing 25 mL of 10 mmol/L $CaCl_2$ solutions for acetochlor and metolachlor, while 1 g of adsorbent was used for butachlor. The samples were equilibrated in a shaker at 25 °C for 24 h. The suspension was centrifuged at 3000*g*, and the supernatant was purified through a 0.45 μm Millipore glass fiber membrane. Herbicide in the filtrate was measured by RP-HPLC (Table S2, Supporting Information). The adsorption amount of herbicide, Q_s (mg/g), was calculated as loss of herbicide in aqueous solution per adsorbent mass.

The herbicide degradation experiment was performed as follows. Herbicide stock solution dissolved in acetone was spiked into dry soils on aluminum-foil paper. The soils were kept overnight in a fume cupboard to evaporate acetone. After 9-fold equivalent preincubated soil (dry weight) was blended, the mixed soils were homogenized to achieve

an initial concentration of 5.0 mg/kg (dry weight) for each herbicide and adjusted to the prerequisite humidity with spray mist. The soils in triplicate were incubated in the artificial climate incubator. Meanwhile, the soil (i.e., CK) without BR or spiked herbicide served as control. Periodically, the samples were weighed, and water was sprayed to offset loss of humidity. At certain intervals, samples were removed for analysis. Each sample was divided into three portions to measure water content, soil dehydrogenase activities, and herbicide concentrations and stereoisomeric fractions, respectively. At the end of the incubation, the rest of the samples were analyzed to assess soil microbial functional diversity by Biolog tests and to measure soil nutrient status by chemical analysis. In addition, the treatment of soil by sterilization was ignored, on the basis of the fact that choroacetanilide herbicides appear to be degraded primarily by microorganisms in soil.[25−29]

Extraction and Determination of the Herbicide and Its Stereoisomers. Prior to herbicide extraction, one portion of each soil sample was taken to determine the water content by heating in an oven at 110 °C for 7 h. Another portion of the sample was transferred to a glass flask and 15 mL of methanol was added. The sample was ultrasonicated for 30 min, the suspension was centrifuged at 3000*g* for 15 min, and the supernatant was extracted thrice with 30, 20, and 20 mL of dichloromethane in sequence. The combined extracts were passed through a glass column packed with anhydrous sodium sulfate and concentrated to dryness in vacuum at 45 °C. Five milliliters of *n*-hexane was added to dissolve the herbicide residues. The solution was purified through a 0.45 μm Millipore glass fiber membrane and then injected into an RP-HPLC and NP-HPLC (Table S2) to measure herbicide and its stereoisomers, respectively. The extraction recovery was $75.77 \pm 8.10\%$ ($n = 12$), $82.27 \pm 5.89\%$ ($n = 12$), and $76.39 \pm 6.76\%$ ($n = 12$) for acetochlor, metolachlor, and butachlor, respectively. Additionally, major transformation products of the chloroacetanilide herbicides were extracted, concentrated, and purified according to the procedure reported by Aga et al.,[30] and they were identified with HPLC-ESI/MS.[31]

Soil Dehydrogenase Activity Tests. Soil dehydrogenase activity, an indicator of soil microbial activity, was defined as the activation rate of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,3,5-triphenylformazan (TPF).[32] TPF concentration, represented by absorbance at 485 nm, is directly proportional to the vitality level of the soil microbial community. Briefly, the rest of the soil sample was taken in triplicate, to which 5 mL of 0.5% TTC was added. The soil with 5 mL of Tris-HCl buffer (pH 7.6) added served as a control. All samples were incubated at 30 °C for 6 h in the dark. One hundred milliliters of methanol was subsequently added to extract TPF. The extract was filtered with a qualitative filter paper, and the filtrate absorbance at 485 nm was recorded. Relative dehydrogenase activity, defined as differential values of sample and control absorbance per soil, was used to compare changes in soil dehydrogenase activity.

Determination of Soil Nutrient Status. The contents of dissolved organic carbon and nitrogen pools in soil and BR were investigated using dilute H_2SO_4 extraction.[33] The samples in triplicate were mixed with 2.5 mol/L H_2SO_4 to give suspensions with a solid/liquid ratio of 1/30. The suspensions were heated at 100 °C for 30 min. After being cooled to room temperature, the suspensions were centrifuged. The supernatant was analyzed with a Shimadzu TOC-Vcpn analyzer (Shimadzu, Japan).

Active phosphorus in soil and BR was determined according to the sodium bicarbonate—sulfuric acid resistant Mo—Sb mixed color method.[34] A mixed solution, composed of 1.0 g of soil (dry weight), 1.0 g of phosphorus-free activated carbon, and 20 mL of 0.5 mol/L $NaHCO_3$, was agitated in a mechanical shaker at 25 °C for 30 min. Then the mixture was filtered with phosphorus-free filter paper. Ten milliliters of filtrate was transferred into 50 mL sealed colorimetric tubes, to which 5 mL of sulfuric acid resistant Mo—Sb mixed color reagent had been added. The mixed solutions were homogenized by hand shaking, occasionally allowing CO_2 to escape by uncovering, and deionized water

Table 1. Chloroacetanilide Herbicide Adsorption by Soil[a]

herbicide	biogas residues (BR), %	K_f	$1/n$	R^2
acetochlor	0	1.51 ± 0.15	0.85 ± 0.04	0.99
	1	2.33 ± 0.22	0.85 ± 0.04	1.00
	2	3.92 ± 0.33	0.80 ± 0.04	1.00
	5	5.65 ± 0.35	0.87 ± 0.03	1.00
metolachlor	0	2.41 ± 0.36	0.70 ± 0.06	0.98
	1	3.14 ± 0.22	0.77 ± 0.03	1.00
	2	4.39 ± 0.65	0.77 ± 0.03	0.99
	5	7.89 ± 0.27	0.75 ± 0.02	1.00
butachlor	0	2.91 ± 0.54	1.30 ± 0.12	0.99
	1	4.63 ± 0.46	0.99 ± 0.07	0.99
	2	6.91 ± 1.08	0.89 ± 0.12	0.96
	5	12.71 ± 0.50	0.87 ± 0.04	0.99

[a] K_f, the Freundlich constant that is indicative of relative adsorption affinity; n, the exponent that indicates relative linearity.

was supplemented until the volume was 50 mL. The resultant solutions were allowed to stand for 30 min, and the absorbance at 880 nm was recorded. Replacement of soils with 1 mL of deionized water served as control. Then the active phosphorus levels were obtained according to a prior calibration curve in which potassium phosphate monobasic is used as a standard compound (Figure S2, Supporting Information).

Biolog Assays. Variations in the soil microbial community were assessed by the Biolog Ecoplate technique.[6] In brief, soil microorganisms were extracted from the soil samples (about 5 g dry weight) in triplicate using 45 mL of sterile 0.85% NaCl solution. Each one of 96 wells of the Biolog Ecoplate (Biolog Inc., Hayward, CA) was inoculated with 150 μL of diluted soil suspension. The microplates were subsequently incubated at 25 °C in the dark, and the color development at 590 nm was measured every 24 h for 7 days using a BIO-TEK Elx808 automated microplate reader (Bio-Tek Instruments Inc., Winooski, VT). Average well color development (AWCD) of each plate was calculated as the sum of mean of all 31 response wells relative to the water blank. AWCD versus incubation time reflected development of soil bacterial community, and the metabolic diversity was evaluated using the AWCD values at 168 h.

Three diversity indices (i.e., Shannon–Weaver index, McIntosh index, and Simpson index) were used to highlight the overall effects of BR amendment on soil microbial diversity. The Shannon index (H') can indicate one sample being richer and more even than another (eq 1); it typically ranges from 1.5 (low species richness and evenness) to 3.5 (high species evenness and richness). The Simpson index ($1/D$), hereinto magnifying 1000-fold each absorbance to avoid negative values (eq 2), is heavily weighted toward the most abundant species; the higher the value, the greater the diversity. The dominance index (D_m) (eq 4) can be conversed by the general McIntosh index (eq 3); the greater the value of the index (D_m), the greater the heterogeneity, and the dominant species is more important.

$$H' = -\sum p_i \ln p_i \tag{1}$$

$$D = \sum \frac{n_i(n_i - 1)}{N(N - 1)} \tag{2}$$

$$U = \sqrt{\sum n_i^2} \tag{3}$$

$$D_m = (N - U)/(N - \sqrt{N}) \tag{4}$$

where n_i is the absorbance of well i, N is the sum of 31 well responses relative to the water blank, and p_i is the ratio of n_i to N.

Moreover, soil microbial functional diversity was assessed by comparing the community level physiological profiles (CLPPs) of the soil microbes. The CLPP analysis was performed by subjecting the individual well responses of all treatments to principal component analysis (PCA) using SPSS 15.0 (SPSS Inc., Chicago, IL).

Statistical Analysis. All the soil samples were weighed on basis of dry weight. Data for dehydrogenase activity, herbicide concentration, stereoisomeric fraction, adsorption amount, AWCD, and diversity index were represented by the mean value ($n = 3$). Statistical analysis for the significance was performed using One-way ANVOA of Origin 8.0 (Microcal Software, Inc., Piscataway, NJ). The values without overlapping 95% confidence intervals were considered to be significantly different.

■ RESULTS AND DISCUSSION

Adsorption of Chloroacetanilide Herbicides by BR-Amended Soil. As shown in Figure S3 (Supporting Information) and Table 1, adsorption isotherms of the herbicides fitted well to the Freundlich model with all R^2 greater than 0.96. Butachlor was adsorbed to the highest extent, followed by metolachlor and acetochlor (Table 1). As expected, addition of BR to soil increased herbicide adsorption at an incremental application level (Table 1), which is in accordance with the previous studies that organic amendments usually increase pesticide adsorption.[35,36] However, BR amendment selectively affected herbicide adsorption potential, e.g., butachlor adsorption by the soil with 5% BR amendment appeared to increase by 3.37-fold relative to the unamended soil, as compared with acetochlor and metolachlor adsorption by 2.74- and 2.27-fold, respectively.

Nutrient Status of BR-Amended Soil. Biogas residues were enriched in organic nutrients (Table S1). They had 32.84-fold dissolved organic carbon (DOC), 30.5-fold dissolved organic nitrogen (DON), and 13.55-fold active phosphorus (AP) higher levels compared to those in soil. After the 18-day preincubation (denoted as before incubation, BI), the nutrient status of the soil without BR amendment did not change. In contrast, BR amendment appeared to drastically improve the levels of soil nutrients (Table 2), further highlighting the quick-acting fertilization from BR.[1,2]

Carbon, nitrogen, and phosphorus nutrients differentially responded to spiked pesticides and BR amendment after the follow-up 49-day incubation (denoted as after incubation, AI). The incubation as well as application of the herbicides did not induce significant changes in the levels of DOC. However, the incubation facilitated release of soil N nutrient, highlighted by an increase in DON. Interestingly, the spiked herbicides caused a reduction of this nutrient in the BR-amended soils relative to the respective controls (referring to the values of CK, shown in Table 2), among which butachlor showed the most pronounced effects. This reduction is generally attributed to cometabolism of chloroacetanilide herbicides by soil microorganisms that can utilize herbicides as nitrogen sources.[28,30] These microorganisms were stimulated and consumed more nitrogen nutrients, which in turn reduced DON levels. As for soil active phosphorus, the levels decreased in the soils with herbicide application and BR amendment. An order of acetochlor > butachlor > metolachlor was observed in 1%- and 2%-BR amended soils, whereas the order was butachlor > acetochlor > metolachlor in the 5%-BR amended soil.

Soil Dehydrogenase Activities. Soil dehydrogenase activities rapidly reached a maximum on day 3 or 7, and thereafter the levels varied depending on BR levels and herbicide structure (Figure 1). In the raw soil without BR amendment or herbicide

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Table 2. Nutrient Status of the Soils with Different Treatments

	biogas residues (BR), %	treatment[a]	CK[b]	acetochlor	metolachlor	butachlor
dissolved organic carbon (DOC, g/kg)	0	BI	3.68 ± 0.04	3.68 ± 0.04	3.68 ± 0.04	3.68 ± 0.04
		AI	3.72 ± 0.08	3.78 ± 0.12	3.54 ± 0.04	3.52 ± 0.03
	1	BI	4.27 ± 0.24	4.27 ± 0.24	4.27 ± 0.24	4.27 ± 0.24
		AI	4.10 ± 0.05	4.34 ± 0.18	4.18 ± 0.11	4.24 ± 0.31
	2	BI	4.99 ± 0.15	4.99 ± 0.15	4.99 ± 0.15	4.99 ± 0.15
		AI	4.93 ± 0.11	5.14 ± 0.27	4.84 ± 0.17	4.77 ± 0.24
	5	BI	6.59 ± 0.30	6.59 ± 0.30	6.59 ± 0.30	6.59 ± 0.30
		AI	7.80 ± 0.24	7.09 ± 0.34	6.88 ± 0.59	6.55 ± 0.18
dissolved organic nitrogen (DON, mg/kg)	0	BI	306.46 ± 14.01	306.46 ± 14.01	306.46 ± 14.01	306.46 ± 14.01
		AI	357.60 ± 10.04	353.70 ± 7.57	299.10 ± 3.24	295.53 ± 8.09
	1	BI	367.40 ± 17.36	367.40 ± 17.36	367.40 ± 17.36	367.40 ± 17.36
		AI	441.90 ± 16.83	379.70 ± 17.25	368.20 ± 7.16	349.70 ± 21.77
	2	BI	460.10 ± 18.77	460.10 ± 18.77	460.10 ± 18.77	460.10 ± 18.77
		AI	512.10 ± 10.70	475.40 ± 19.07	445.30 ± 15.11	379.40 ± 12.32
	5	BI	612.00 ± 20.51	612.00 ± 20.51	612.00 ± 20.51	612.00 ± 20.51
		AI	736.30 ± 20.35	679.50 ± 18.77	663.00 ± 43.76	557.00 ± 7.37
active phosphorus (AP, mg/kg)	0	BI	36.18 ± 1.15	36.18 ± 1.15	36.18 ± 1.15	36.18 ± 1.15
		AI	35.91 ± 1.16	42.03 ± 1.61	36.27 ± 1.59	39.11 ± 1.41
	1	BI	47.97 ± 2.69	47.97 ± 2.69	47.97 ± 2.69	47.97 ± 2.69
		AI	59.95 ± 1.76	45.98 ± 0.96	52.98 ± 0.43	51.88 ± 0.38
	2	BI	64.60 ± 8.43	64.60 ± 8.43	64.60 ± 8.43	64.60 ± 8.43
		AI	79.85 ± 0.76	55.02 ± 0.81	67.84 ± 1.09	60.96 ± 1.86
	5	BI	84.20 ± 4.18	84.20 ± 4.18	84.20 ± 4.18	84.20 ± 4.18
		AI	104.50 ± 2.31	92.89 ± 2.00	98.96 ± 3.83	89.30 ± 0.66

[a] BI is before 49-day incubation, and AI is after 49-day incubation. [b] CK means without spiked herbicide.

application, the levels rapidly reached peak values around 7th day, remained roughly constant until the 28th day, and thereafter began to decline. Herbicide application showed insignificant effects on dehydrogenase activities in the soils without BR amendment, except for metolachlor, which inhibited the activities by ca. 50% at the end of the incubation.

Addition of BR drastically stimulated soil dehydrogenase activities but did not affect their evolution over incubation time (Figure 1). This trend was composed of initial stimulation and subsequent decline, irrespective of spiked herbicide and BR level. As the incubation expired, a recovery of microbial activities was observed in 1% and 2% BR-amended soils with spiked acetochlor and metolachlor, whereas the activities were inhibited by 22.17% and 30.84% for the BR amendment at the high level, respectively. In contrast, butachlor inhibited microbial activities in all BR-amended soils. The variances in soil dehydrogenase activities, together with the previous findings,[5,37] highlight that soil microbial activities are governed by the nature of organic amendment and pesticide.

Soil Microbial Function Diversity. Addition of BR caused an observable increase in AWCD values at 168 h (Figure S4, Supporting Information), while herbicide application exhibited insignificant impacts. Such variations commonly displayed changes in the extents of substrate utilization by soil microorganisms. The substrates of Biolog EcoPlate comprise 10 carbohydrates, 6 amino acids, 9 carboxylic acids, 2 amines, and 4 polymers (Table S3, Supporting Information). The utilization extents of five types of substrates varied with BR amendment and herbicide application (Table S4, Supporting Information), among which carbohydrates, carboxylic acids, and amino acids were utilized in higher degrees. Addition of BR at moderate and high levels enhanced utilization of all substrates, particularly amines and polymers. The herbicide application affected the relative importance of five types of substrate in terms of respective AWCD values (Table S4).

The variations in microbial community were highlighted by the Shannon index (H'), Simpson index ($1/D$), and McIntosh index (D_m) (Figure 2). Increases in H' and $1/D$ values were observed for BR-amended soils, further evidencing enhancement of species evenness and richness of soil microorganisms with organic amendment.[4,6] The reduction of the D_m value indicated suppression of the dominance of microorganisms, which could be explained by the fact that organic amendment alleviates species competition by releasing sufficient nutrients.

The community level physiological profiles of microorganisms were assessed using principal component analysis (PCA). Three principal components were extracted (Figure S5, Supporting Information), and they accounted for 53.1%, 8.9%, and 5.7% of the total variance, respectively. BR amendment and herbicide application caused observable changes in the microbial community, illustrated by variations of the substrate utilization pattern by microorganisms (Figure 3). Points closer together mean a higher similarity among individual treatments. BR amendment to soil induced a higher degree of dispersion relative to herbicide application. In detail, high loadings onto principal components 1 and 2 appeared in the BR-amended soils without herbicide application and with spiked metolachlor. For the BR-amended soils with spiked acetochlor and butachlor, principal components 1 and 3 accounted for most of the total variances.

Herbicide Degradation in BR-Amended Soil. Figure S6 (Supporting Information) shows that a rapid degradation process in the absence of a lag phase occurred during the initial
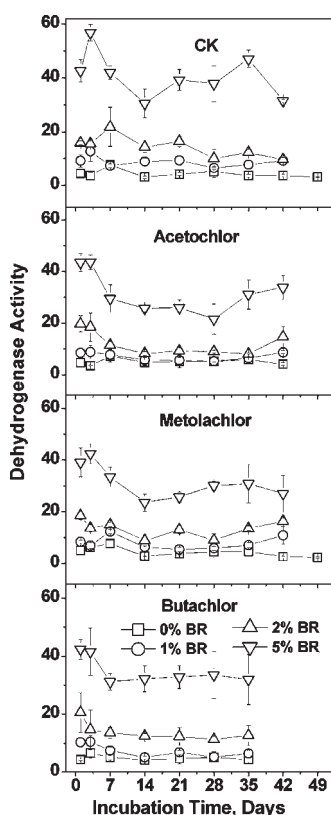
Figure 1. Effects of biogas residues (BR) amendment and herbicide application on soil dehydrogenase activities.

Figure 2. Variations in the indices of soil microorganism diversity. BR, biogas residues; BI is before 49-day incubation, and AI is after 49-day incubation.

Figure 3. Treatment loadings on three principal components. BR, biogas residues; BI is before 49-day incubation, and AI is after 49-day incubation.

14-day period of incubation, and thereafter herbicides gradually degraded. Herbicide degradation followed the first-order kinetics equation (Table 3). In the soil without BR amendment, acetochlor was most easily degraded with a half-life ($t_{1/2}$) of 7.0 days, followed by butachlor ($t_{1/2}$ = 8.3 days) and metolachlor ($t_{1/2}$ = 12.4 days). This order corresponded well to the order of decrease in the molecular electrophilicity index (acetochlor (1.52 eV) > butachlor (1.45 eV) > metolachlor (1.29 eV)) calculated using the Gaussian 03 program system.[31] The electrophilicity index, an indicator of the ability to accept electrons,[38] illustrates the relative reaction activities of chloroacetanilide herbicides in various reaction systems.[31] As the herbicides degrade,[26,30,39] the chlorine atom in the molecule is commonly eliminated to form new degradation products such as ethanesulfonic acids (ESAs), oxanilic acids (OXAs), and substituted anilines, during which the herbicide molecule would accept electrons. These transformation products were also identified in this study (Figure S7, Supporting Information). As the herbicides share common metabolic pathways in animals, plants, and soil microorganisms,[26] it is acceptable that the electrophilicity index indicates their relative degradation potential in soil.

Addition of BR accelerated herbicide degradation to varying extents (Table 3). Degradation rates of acetochlor and butachlor positively correlated with BR amendment level, except that butachlor degraded at identical rates with 2% and 5% BR amendment. In contrast, for metolachlor, BR amendments only at a 5% level caused a minor but significant increase in degradation rates. Such variances were due to the dual but opposite effects of the organic amendment. One effect was the increase in herbicide adsorption potential (Table 1), which in turn would
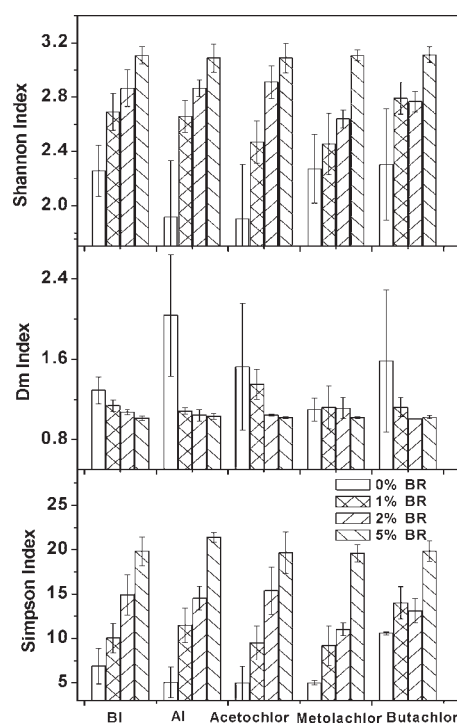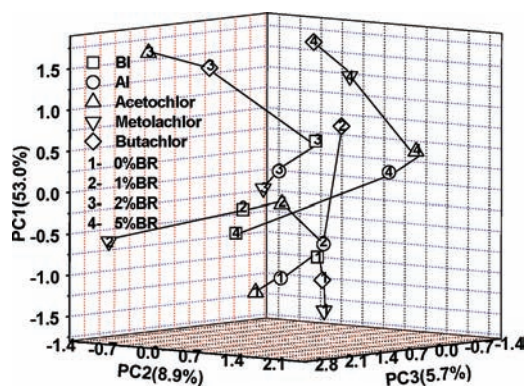
inhibit its degradation. The other effect was the stimulation of soil microorganisms (Figures 1–3), commonly facilitating herbicide degradation. The effects depended on both the nature of the herbicide and the levels of BR amendment, which canceled out each other.

Stereoselective Degradation of Acetochlor and Metolachlor. Actually, insufficient enantiomeric resolution has limited the understanding of the environmental fate of chiral herbicides acetochlor and metolachlor.[23,40,41] In this study, baseline separation of acetochlor was achieved with a resolution of 2.52, and the four stereoisomers of metolachlor were completely resolved with resolutions of 1.69, 1.26, and 1.12, respectively; thus, both herbicides could be accurately quantified (Table S2).

Table 3. Degradation Dynamics of Chloroacetanilide Herbicides

biogas residues, %	herbicide	rate constant (k, d^{-1})	half-life ($t_{1/2}$, d)	R^2	biogas residues, %	herbicide	rate constant (k, d^{-1})	half-life ($t_{1/2}$, d)	R^2
0	rac-acetochlor	0.10 ± 0.05	7.0	0.98	1	rac-acetochlor	0.15 ± 0.01	4.6	0.99
	aR-acetochlor	0.14 ± 0.01	4.8	0.97		aR-acetochlor	0.18 ± 0.01	3.9	1.00
	aS-acetochlor	0.15 ± 0.01	4.7	0.96		aS-acetochlor	0.17 ± 0.00	4.1	1.00
	rac-metolachlor	0.06 ± 0.00	12.4	0.99		rac-metolachlor	0.05 ± 0.01	13.3	0.99
	aR,CR-metolachlor	0.02 ± 0.01	34.6	0.99		aR,CR-metolachlor	0.05 ± 0.01	12.9	0.96
	aR,CS-metolachlor	0.06 ± 0.02	11.7	0.96		aR,CS-metolachlor	0.04 ± 0.01	17.8	0.98
	aS,CR-metolachlor	0.06 ± 0.01	12.1	0.94		aS,CR-metolachlor	0.06 ± 0.01	12.0	0.99
	aS,CS-metolachlor	0.03 ± 0.01	24.1	0.91		aS,CS-metolachlor	0.04 ± 0.01	18.5	0.99
	butachlor	0.08 ± 0.01	8.3	0.99		butachlor	0.13 ± 0.00	5.5	0.99
2	rac-acetochlor	0.18 ± 0.00	4.0	0.99	5	rac-acetochlor	0.22 ± 0.00	3.1	0.99
	aR-acetochlor	0.23 ± 0.01	3.0	1.00		aR-acetochlor	0.23 ± 0.01	3.1	0.97
	aS-acetochlor	0.18 ± 0.00	3.8	0.93		aS-acetochlor	0.25 ± 0.00	2.8	0.99
	rac-metolachlor	0.06 ± 0.00	11.9	0.99		rac-metolachlor	0.08 ± 0.01	8.5	0.99
	aR,CR-metolachlor	0.09 ± 0.01	7.4	1.00		aR,CR-metolachlor	0.11 ± 0.01	6.4	0.99
	aR,CS-metolachlor	0.05 ± 0.01	13.8	0.99		aR,CS-metolachlor	0.05 ± 0.01	13.2	0.96
	aS,CR-metolachlor	0.10 ± 0.01	6.7	0.97		aS,CR-metolachlor	0.11 ± 0.03	6.2	0.98
	aS,CS-metolachlor	0.04 ± 0.01	15.7	0.98		aS,CS-metolachlor	0.11 ± 0.01	6.6	1.00
	butachlor	0.17 ± 0.08	4.0	0.95		butachlor	0.17 ± 0.01	4.1	0.93
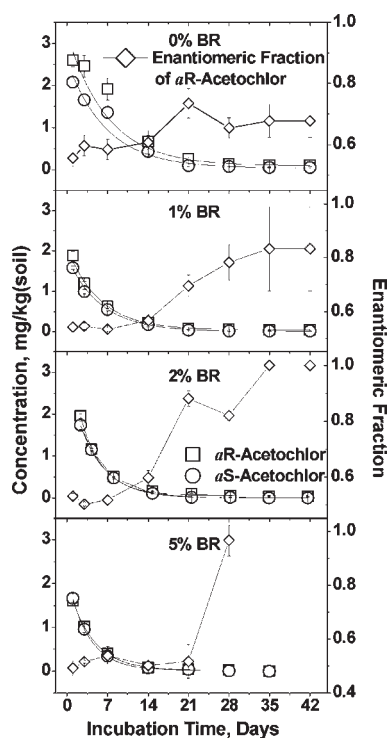
Figure 4. Effects of biogas residues (BR) amendment on degradation of acetochlor and its enantiomers.

Figure 5. Effects of biogas residues (BR) on the degradaton of the four diastereoisomers of metolachlor.

A pair of enantiomers of acetochlor exhibited almost identical rate constants in the unamended soil (Table 3). By contrast, organic amendment selectively stimulated degradation of the enantiomers to a lesser extent (Figure 4). Chiral preference for the aR-isomer was observed in 1% and 2% BR-amended soils, while an inverse of chiral preference appeared in 5% BR-amended soil. This chiral preference has been greatly indicated
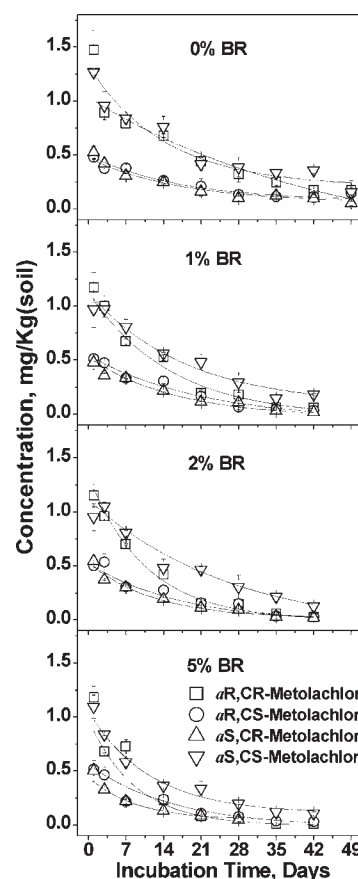
by the enantiomeric fractions (EFs) of the herbicide, defined as the fraction of aR-isomer in the mixture of aR- and aS-isomers.
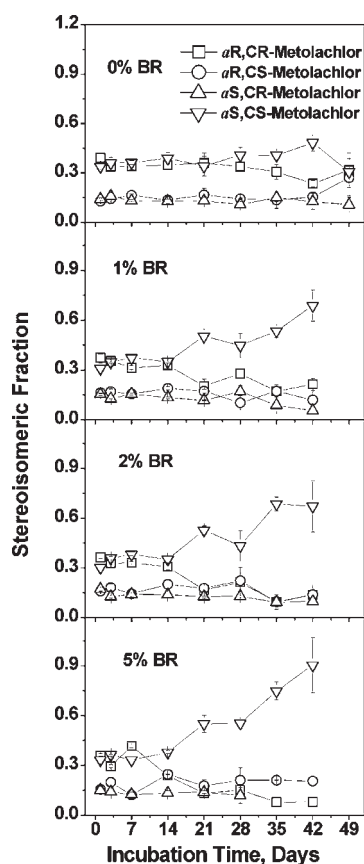
Figure 6. Effects of biogas residues (BR) on the evolution of stereo-isomeric fractions of metolachlor over incubation time.

Moreover, the time course of EFs of the herbicide showed biphasic character (Figure 4). In the unamended soil, EFs ranged from 0.56 to 0.61 in the initial 14 days and rapidly rose to a maximum on day 21, and thereafter the level remained unchanged. BR amendments, especially at a high level, induced a more pronounced increase in EFs during the latter incubation.

Figure 5 and Table 3 were highly illustrative of stereoselective degradation of metolachlor in soil. Interestingly, enantioselectivity in degradation of either carbon-chiral or axial-chiral enantiomers was negligible in the unamended soil, although significant stereoselectivity appeared among the diastereoisomers (e.g., *aR,CR*- and *aR,CS*-forms). Addition of BR differentially increased depletion of *aR,CR*-, *aS,CR*-, and *aS,CS*-metolachlor but did not affect *aR,CS*-form degradation. In detail, *aR,CR*-form dissipation was correlated positively with BR level, *aS,CR*-form degradation was accelerated by BR amendments only at moderate and high levels, and *aS,CS*-form degradation was accelerated only by 5% BR amendment. Accordingly, stereoisomeric fractions of metolachlor, corresponding to the fraction of each isomer in the mixture of four isomers, varied with BR level (Figure 6), indicating the accumulation of the *aS,CS*-form. It should be noted that *aS,CS*- and *aR,CS*-forms, accounting for ca. 95% of herbicidal activity, are less sensitive to BR amendment, suggesting persistant activity associated with *rac*-metolachlor application.

Several studies have been carried out to determine the stereoselectivity involved in the environmental fate of metolachlor, which depends on soil properties. Low to moderate stereospecific

degradation was obtained in sewage sludge and soil resulting in the accumulation of the *aS,CS*-form,[23] compared to wetland systems with the absence of stereoselectivity.[42] As Polcaro et al.[40] reported, SFs of *aS,CS*-metolachlor increased in the soil spiked with *rac*-metolachlor, SFs of the *aR,CS*-form declined with the involvement of conversion from the *aR,CS*- to *aR,CR*-form, and SFs of the rest remained unchanged. In another study,[29] *S*-metolachlor, a mixture of the *aS,CS*- and *aR,CS*-forms, was preferably degraded in the soil relative to *rac*-metolachlor.

Discrimination of Multiple Roles of BR Amendment. The enantiomeric or stereoisomeric fractions of acetochlor and metolachlor remained nearly constant in the initial 14-day period of incubation (Figures 4−6), which corresponded to the initial rapid degradation process of the herbicides (Figure S6, Supporting Information). A lack of variation in EFs or SFs was the common consequence of identical degradation for the enantiomers or stereoisomers. This was probably due to the fact that sufficient nutrients stimulated soil microorganisms to utilize the herbicide as a substrate to higher extents but in less specific ways (Figure 1 and Table 2). During the follow-up incubation period, low levels of the herbicides existed in soil, due to the initial degradation (Figure S6), and soil microbial activities began to decline, due to the consumption of nutrients (Figure 1 and Table 2), thereby limiting the availability of the isomers by soil microorganisms. Certain species could compete for utilization of the herbicides as substrates (Figures 1 and 2) and led to the presence of a chiral preference (Figures 4−6). This interpretation was highlighted by the fact that higher stereoselectivity was accompanied by larger variations in the soil microbial community (Figures 2 and 3). Additionally, an increase in adsorption of herbicide usually reduced their availability and facilitated the selective utilization of stereoisomers by soil microorganisms (Figure 3), thus affecting the stereoisomeric preference for the herbicides. Particularly, high levels of BR amendment induced drastic differences in degradation of the stereoisomers (Figures 4 and 6).

Environmental Significance. More types of chiral pesticides are being introduced into use. The standard analysis that designates chiral pesticides as single compounds usually is invalid to indicate dissipation of the enantiomers, thus leading to misunderstanding of the environmental fate and ecological risks of chiral pesticides. Application of organic wastes to soil probably adds complexity to soil microorganisms but can reduce the environmental burden from pesticides. This impact generally complicates the environmental fate of pesticides, particularly, chiral pesticides. Therefore, it is important to consider stereochemistry when determining the environmental fate of chiral pesticides in organic-amended soils.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Additional information on the nutrient levels of soil and biogas residues (Table S1), the HPLC analysis for herbicides and stereoisomers (Table S2), the substrates of Biolog EcoPlate (Table S3), the AWCD of five types of substrates (Table S4), the chemical structures of the herbicides (Figure S1), the standard curve for active phosphorus (Figure S2), adsorption of the herbicides (Figure S3), the AWCD of the soil samples (Figure S4), the substrate loadings on three principal components (Figure S5), the degradation of the herbicides (Figure S6), and the major degradation products of the herbicides (Figure S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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