Assessing the effect of organoclays and biochar on the fate of abscisic acid in soil

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ABSTRACT

The potential use of allelopathic and signaling compounds as environmentally friendly agrochemicals is a subject of increasing interest, but the fate of these compounds once they reach the soil environment is poorly understood. In this work, we studied how the sorption, persistence, and leaching of the two enantiomers of the phytohormone abscisic acid (ABA) in agricultural soil was affected by the amendments of two organoclays (SA-HDTMA and Cloi10) and a biochar derived from apple wood (BC). In conventional 24-h batch sorption experiments, higher affinity towards ABA enantiomers was displayed by SA-HDTMA followed by Cloi10 and then BC. Desorption could be ascertained only in BC, where ABA enantiomers presented difficulties to be desorbed. Dissipation of ABA in the soil was enantioselective with *S*-ABA being degraded faster than *R*-ABA, and followed the order: unamended > Cloi10-amended > BC-amended > SA-HDTMA-amended soil for both enantiomers. Sorption determined along the incubation experiment indicated some loss of sorption capacity with time in organoclay-amended soil and increasing sorption in BC-amended soil, suggesting surface sorption mechanisms for organoclays and slow (potentially pore filling) kinetics in BC-amended soil. The leaching of ABA enantiomers was delayed after amending soil to an extent that depended on the amendment sorption capacity, and it was almost completely suppressed by addition of BC due to its irreversible sorption. Organoclays and BC affected differently the final behavior and enantioselectivity of ABA in soil as a consequence of dissimilar sorption capacities and alterations in sorption with time, which will impact the plant and microbial availability of endogenous and exogenous ABA in the rhizosphere.

Keywords: biodegradation; biopesticides; chiral pesticides; signaling compounds; soil amendments; sorption

INTRODUCTION

There is a growing public interest in the use of less harmful alternatives to synthetic pesticides for crop protection.¹ According to the United States Environmental Protection Agency (USEPA),² biopesticides can be defined as naturally occurring substances or compounds that control pests, which are derived from natural materials (e.g., animals, plants, bacteria, and certain minerals). Many of the listed biopesticides contain known signaling compounds involved in the interactions of plants and microorganisms.³ Signaling compounds can be released into the environment and reach the soil by several pathways, for instance: root exudation, decay of plant residues and washing of leaves by precipitation.⁴

S-Absciscic acid (*S*-ABA) has recently been registered by the European Union as a plant protection active substance and by the USEPA as a biopesticide.^{5,6} The agrochemical interest of *S*-ABA is based in being a plant growth regulator.^{3,5,6} ABA is a chiral compound where the *S*-enantiomer is the naturally-occurring enantiomer and its role as a signal molecule for abiotic stress adaptation of plants has been well-recognized.^{7,8} Nevertheless, several physiological functions have been endorsed to the unnatural *R*-ABA enantiomer, related to plant growth and seed germination or plant tissues protection from UV irradiation.^{8,9} To date, very little information regarding the sorption behavior of ABA in soil has been documented,^{10,11} even though *S*-ABA concentration in soil can increase several times as a consequence of its intentional use for crop management.¹² *S*-ABA has been shown to be readily degraded in soils with formation of two main metabolites, phaseic acid and dihydrophaseic acid.^{10,12}

Understanding chirality in pesticides has become a subject of consideration over the last years, since many current pesticides are chiral (30%).¹³ In spite that numerous investigations have been focused on the fate of pesticides in the environment, chirality has often been overlooked and chiral pesticide enantiomers have been treated jointly. Enantiomers of chiral

compounds exhibit practically identical physico-chemical properties, but can differ in their interactions with certain surface moieties and biological receptors.^{14,15} Hence, probing the enantioselective fate of chiral pesticides in various media is an emerging area in agricultural and environmental science.^{15–17}

Several factors can affect the relative distribution or chiral signatures of enantiomers in the environment, but probably the most significant is their biodegradation.^{18,19} Due to the chemical structural arrangement, biodegradation of enantiomers are affected by soil composition, pH, redox conditions, and microbial populations. Abiotic factors, such as sorption, together with some agricultural practices (e.g., addition of organic amendments, repeated pesticide application, or formulation applied) can indirectly influence the enantioselective behavior of pesticides in soils by differentially altering each enantiomer biodegradation rate.^{17,20–22} These differential microbial degradation rates are hypothesized due to enzyme selectivity and are critical when assessing environmental fate and transport.¹⁷ However, differential sorption behavior can also occur.²³

The addition of various amendments to agricultural soils to increase their organic carbon content is a common practice to mitigate pesticide transport. The modification of clay minerals with organic ions, which changes the nature of their surface from hydrophilic to hydrophobic, has been proposed as a strategy to increase their affinity for pesticides²⁴ and, among other applications, organically-modified clays have been proposed as soil amendments.²⁵ Biochar is produced by thermal pyrolysis of organic feedstocks under a very low oxygen atmosphere and has concentrated considerable attention regarding its application as soil amendment.²⁶ Some benefits of using biochar fall on increasing the carbon content of soil provoking soil fertility improvement, enhancement of soil water retention capacity, carbon sequestration potential,²⁷ and augmentation of soil microbial activity.^{26,28} The application of organoclays and biochar to soil has been documented to have various impacts

on the fate of pesticides, including increasing sorption, changing the degradation patterns, and reducing the leaching potential.^{25,29–32} Nevertheless, very little information regarding their effects on the behavior of individual chiral agrochemical enantiomers is available, particularly for these engineered amendments.^{21,33}

For a better understanding of ABA's role in the soil environment and its proper application as an environmentally friendly chiral agrochemical, we postulated that the behavior and enantioselectivity of ABA in soil could change by the addition of sorbents, such as organoclays and biochar. We aimed in this work i) to establish the sorption of ABA enantiomers to three different sorbents: two organically-modified clays and one biochar and ii) to assess the effect of adding organoclays and biochar on the final enantioselective behavior of ABA enantiomers in soil regarding their sorption, degradation and leaching. A specific methodology (using in-place filtration centrifuge tube) was used to ease the direct determination of sorption and its effect on the persistence of ABA enantiomers in soil with improved efficiency and reduction in experimental sources of error.

MATERIALS AND METHODS

Abscisic Acid. Analytical standard grade racemic (*RS*)-ABA (chemical purity $\geq 98.5\%$) was purchased from Sigma-Aldrich (Spain). ABA is a weak acid with pK_a of 4.61, molecular weight of 264 g mol^{-1} , and water solubility of 3.2 g L^{-1} at 20°C .³⁴ The structure of ABA enantiomers is shown in Fig. 1.

Amendments. Two organically modified montmorillonites (SA-HDTMA and Cloisite® 10A) and one biochar (BC) were used as amendments. SA-HDTMA was synthesized through an ion exchange reaction by treating Ca-rich Arizona montmorillonite (SAz-1) with a solution containing hexadecyltrimethylammonium (HDTMA) equivalent to the cation exchange capacity (CEC) of SAz-1 (120 cmol/kg). More details of the synthesis can be found

elsewhere.²⁵ SAZ-1 and HDTMA were provided by the Clay Minerals Society (Purdue University) and Sigma Aldrich (Spain), respectively. Cloisite® 10A (Cloi10) is a commercial organo-smectite (BYK Additives & Instruments). The CEC of the smectite in Cloi10 is 125 cmol/kg and the interlayer cation is dimethyl, benzyl, hydrogenated alkyl tallow quaternary ammonium. Some characteristics of the organoclays (SA-HDTMA and Cloi10) are given in Table S1. Biochar (BC) was obtained by thermal decomposition of apple wood at 700 °C under oxygen-limited conditions for 2 h with an inert N₂ gas purge. The chemical properties of the BC are: 87% C, 0.43% N, S_{BET} of 381 m² g⁻¹, and pH of 9.8 determined in a 1:2 (w/v) biochar/deionized water slurry.

Soil. An agricultural soil located in Seville (Spain) was collected from a 0-20 cm depth, air-dried, sieved to pass a 2 mm mesh, and stored at 4 °C. It is a sandy loam soil and contains 75% sand, 9% silt, 17% clay, 1.9% CaCO₃ and 0.63% organic carbon. The pH of a 1:2 (w/v) soil/deionized water mixture was 7.4.

Batch Sorption-Desorption Experiments. The batch sorption-desorption technique was used as a conventional method to assess the sorption of ABA enantiomers on the different amendments used. Sorption-desorption isotherms were obtained. Triplicate 40-mg samples of sorbents (SA-HDTMA, Cloi10, or BC) were placed in Pyrex® glass screw-cap centrifuge tubes and were shaken during 24 h at 20 ± 2 °C with 8 mL of rac-ABA solutions prepared in water with initial (*R+S*) concentration (*C_i*) ranging from 1 to 20 mg L⁻¹. An additional set of BC samples was also prepared to determine the sorption of ABA enantiomers on this sorbent at pH levels similar to those displayed by the organoclays (7.0-8.2). For this purpose, previously to the equilibration step, the pH of the BC suspensions was adjusted to a value of about 7.5 by adding 250 µL of 0.1 M HCl to each tube. After 24 h equilibration, the tubes were centrifuged and 4 mL of the supernatant solution were removed and filtered using GHP membrane disk filters (0.45 µm) to determine the *S*-ABA and *R*-ABA equilibrium

concentrations in the aqueous phase (C_e) by chiral high performance liquid chromatography (HPLC). Controls without sorbents were used to identify possible chemical losses during the equilibration. Amount sorbed of ABA enantiomers (C_s) were established from the difference between the initial (C_i) and equilibrium solution concentration (C_e). The desorption branch of the isotherm was obtained immediately after sorption from the highest equilibrium point of the sorption isotherm. The 4 mL of supernatant solution removed for the sorption analysis were replaced with 4 mL of distilled water. The tubes were re-suspended and shaken at 20 ± 2 °C for 24 h, centrifuged, filtered, and analyzed by chiral HPLC. This desorption process was repeated three times. Sorption data were fitted to the log-transformed Freundlich isotherm:

$$\log C_s = \log K_f + N_f \log C_e$$

where K_f ($\text{mg}^{1-N_f} \text{kg}^{-1} \text{L}^{N_f}$) and N_f (unitless) are the empirical Freundlich constants.

Dissipation Study. The enantioselective dissipation of ABA in unamended soil and in soil amended with SA-HDTMA, Cloi10 or BC under aerobic conditions was studied by means of an incubation experiment. Portions of 200 g of soil, either unamended or amended with SA-HDTMA, Cloi10, or BC at a rate of 2% (w/w), were spiked with rac-ABA at a rate of 2 mg kg⁻¹ dry soil, and then incubated in glass jars in the dark at 20 ± 2 °C for up to 8 days. The moisture content was maintained at a constant level (~ 30%) throughout the experiment by adding distilled water as necessary. Periodically, at 0, 1, 2, 3, 4 and 8 days after treatment (DAT), aliquots of 3 g of soil were sampled in triplicate with a sterilized spatula and immediately frozen until analyzed. *S*-ABA and *R*-ABA residues in the soil samples were extracted by shaking for 24 h with 8 mL of a mixture (30:70) of acetonitrile:0.01 M H₃PO₄ aqueous solution (pH=2.2). Recoveries were always greater than 95% of ABA freshly applied to unamended or amended soils. The extracts were analyzed by chiral HPLC. *R*-ABA and *S*-ABA dissipation data in unamended and amended soil were fitted to the linearized form of a first-order kinetic rate law:

$$\ln C = \ln C_0 - kt$$

where C (mg kg^{-1}) and C_0 (mg kg^{-1}) are the concentration of each enantiomer in the soil at time t (d) and $t = 0$, respectively, and k (d^{-1}) is the first-order dissipation constant. The half-lives ($t_{1/2}$) of *S*-ABA and *R*-ABA enantiomers were calculated as $t_{1/2} = 0.693/k$.

Chiral signatures of ABA were represented as enantiomer fractions (EF) along the experiment, calculated according to Harner et al.³⁵ criteria as:

$$EF = [S\text{-ABA}] / ([S\text{-ABA}] + [R\text{-ABA}])$$

where $[S\text{-ABA}]$ and $[R\text{-ABA}]$ are the individual concentration of each ABA enantiomer. EF equal to 0.5 denotes racemic residues and EF higher or lower than 0.5 indicates non-racemic residues.

A separate experiment was set up to determine whether the addition of the amendments to the soil caused any effect on soil respiration. This parameter was measured following the alkali trapping–titrimetric procedure described by Anderson,³⁶ by quantifying the amount of CO_2 released by samples of unamended soil and of soil amended with SA-HDTMA, Cloi10 and BC at 2% during 8 days under the same conditions as those used in the incubation experiment.

A novel aspect of this work was to establish the synergetic effect between the dissipation of ABA enantiomers and their sorption by improving similar methodologies.^{37,38} To this aim, sorption of ABA enantiomers was determined during the incubation experiment, i.e. under more realistic conditions compared to those obtained by the 24 h batch equilibration method. In duplicate, 10 g of soil were sampled at selected times coinciding with sampling times established in the incubation (0, 4 and 8 days). An aliquot of the aqueous phase (C_e) was removed by centrifugation using specialized centrifuge tubes [Macrosep® Advance Centrifugal Devices (Pall Corporation) with $0.45 \mu\text{m}$ polyethersulfone membranes] (Fig. S1). The solution obtained after centrifugation was immediately analyzed by chiral HPLC to

quantify the individual aqueous concentration of ABA enantiomers. The percentage of ABA sorbed and the distribution coefficients of the enantiomers at different times during the incubation experiment were calculated from the difference between the total residues extracted of each enantiomer and their concentration in the aqueous phase.

Column Leaching Experiment. In triplicate, glass columns (30 cm long and 3.1 cm internal diameter) were hand-packed with 160 g of dry soil (unamended soil) to a height of 20 cm soil in each column (bulk density $\approx 1.1 \text{ g cm}^{-3}$). The effect of the amendments on ABA leaching was studied by amending the upper 5 cm of soil (40 g) at a rate of 2% (w/w) with SA-HDTMA, Cloi10 or BC. Glass wool was placed at the bottom of the column to avoid soil losses, and 10 g of sea sand was added at the bottom and top of all soil columns. The columns were initially saturated with 100 mL of distilled water, and after allowing 24 h drainage, the maximum water retention capacity of the soil columns or column pore volume (V_{pore}) was calculated from the gravimetric mass difference. Next, 3 mL of an aqueous solution of 50 mg L^{-1} of rac-ABA were added to the columns to give the maximum agronomic application rate of 2 kg ha^{-1} established for ABA (0.15 mg active ingredient). Subsequently, twice a day, 15 mL of distilled water were added to the columns for a total of ten additions (5 days), and the leachates were collected in vials containing 5 mL of methanol and stored at 4°C in the dark, according to Gámiz et al.¹¹ Then, leachates were filtered and analyzed by chiral HPLC to determine the *R*-ABA and *S*-ABA concentrations. At the end of the leaching experiment, soil samples were obtained in 5 cm increments corresponding to different depths (0–5, 5–10, 10–15, and 15–20 cm) from the columns. Each section was extracted with 100 mL of a mixture (30:70) of acetonitrile: 0.01 M H_3PO_4 aqueous solution ($\text{pH}=2.2$), by shaking for 24 h and the extracts were subsequently analyzed for ABA residues by chiral HPLC.

Enantioselective Analysis of ABA. ABA enantiomers were determined by chiral HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector and a

Waters 717 Autosampler injector. The chromatographic conditions used for the analysis are detailed in Gámiz et al.¹¹ Briefly, we used a Chiralpak AS-3R column (150 mm length $\times$ 4.6 mm i.d., 3 μ m particle size), 30:70 of acetonitrile:0.01 M H₃PO₄ aqueous solution (pH = 2.2) as mobile phase, flow rate of 1 mL min⁻¹, a 50 μ L sample injection volume, and UV detection at 230 nm. The retention times under these conditions were 4.4 and 5.4 min for *R*-ABA and *S*-ABA, respectively. The limit of quantification (LOQ) calculated as the concentration resulting in a signal to noise ratio of 10:1 was 0.008 mg L⁻¹.

Data analysis. Statistical analysis was performed using IBM SPSS Statistics 22. Standard error was used to specify variability among triplicates. Soil respirations, enantiomer fractions (EF), distribution coefficients (K_d) and column leached fractions were compared using ANOVA followed by Tukey's test to establish differences between treatments. An analysis of covariance (ANCOVA) was performed to compare pairwise the slopes of the regression lines (k) of the first-order dissipation data. Differences between results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Sorption-Desorption Isotherms on SA-HDTMA, Cloi10 and BC. Sorption-desorption isotherms are shown in Fig. 2 and the corresponding Freundlich coefficients for the sorption isotherms are compiled in Table S2. *R*-ABA and *S*-ABA were equally sorbed on each sorbent, as reflected by the fact that sorption-desorption isotherms for both enantiomers overlapped (Fig. 2). Consequently, sorption for ABA was a non-enantioselective process. The lack of enantioselectivity in sorption measured from racemic initial solutions has previously been observed for ABA in soils,¹¹ as well as for other chiral agrochemicals in unamended and amended soil systems.^{21,33,39}

All isotherms of ABA enantiomers on the organoclays showed values of N_f close to 1 ($N_f = 0.85-0.94$, Table S2), whereas BC possessed highly nonlinear isotherms ($N_f = 0.28-0.29$). In addition, both under non-adjusted and similar (adjusted) pH levels, ABA was sorbed to a greater extent on SA-HDTMA and Cloi10 than on BC, with K_f values following the rising trend: $BC \ll \text{Cloi10} < \text{SA-HDTMA}$ (Table S2).

The greater affinity of ABA enantiomers for the organoclays compared to BC could be ascribed to the type of surface interactions controlling sorption. N_f values close to unity are in agreement with partitioning of ABA enantiomers through hydrophobic-type interactions into the bulk state of the interlayer organic phase of the organoclays,⁴⁰ as it has been formerly demonstrated for the sorption of another anionic agrochemical, mecoprop, on SA-HDTMA.⁴¹ In looking for the causes explaining the slightly higher affinity of ABA for SA-HDTMA in comparison to Cloi10, it is known that SAz-1, due to its high negative surface charge density, promotes the vertical arrangement of large organic cations (HDTMA) forming a paraffin-like structure.⁴² This resulted in a basal spacing value (d_{001}) of 2.4 nm for SA-HDTMA, which is higher than that of 1.9 nm for Cloi10 (Table S1). This larger spacing could expose additional surfaces to sorb ABA favoring hydrophobic interactions. Furthermore, some polar interactions between ammonium groups of the alkylammonium cations and carboxylic groups of ABA could have also increased sorption capacities.⁴¹

The sorption of ABA on BC was concentration-dependent, according to the $N_f < 1$ obtained from the sorption isotherms (Fig. 2 and Table S2).⁴³ Non-linearity has also been reported for the sorption of acidic pesticides on biochars produced at high temperatures.⁴⁴⁻⁴⁶ Despite higher carbon content of BC (87%) compared to the organoclays (~ 30%) and higher S_{BET} of BC, BC possessed the lowest sorption capacity for ABA of the sorbents evaluated here. Commonly, the sorption capacity of biochars has been attributed to their S_{BET} , aromaticity (hydrophobicity), or microporosity.^{44,47} However, other factors, such as the

presence of surface functional groups, which can increase the polarity and provide negatively-charged surface, can also influence the sorption of organic compounds by BCs. Since ABA is a weak acid ($pK_a = 4.6$) and was present in solution as anionic species at the pH of the sorption experiments ($pH > 7$), repulsions between ABA anions and negatively charged BC particles⁴⁸ could have occurred and reduced sorption. Interestingly, a remarkable enhancement of ABA sorption was observed in the isotherms performed at neutral pH (7.5-7.9) compared to those obtained under non-adjusted (alkaline) conditions (10.3-10.6) (Fig. 2 and Table S1). This probably resulted from a decrease in the negative surface charge of BC which reduced repulsions with anionic ABA species. Nevertheless, in spite of greater ABA sorption on BC at neutral pH level, sorption was still lower than that observed for the organoclays at similar pH (7.0-8.2) (Fig. 2 and Table S1).

With regard to desorption, it is necessary to highlight that for SA-HDTMA and Cloi10, owing to their high affinity towards ABA, very low equilibrium concentrations of the compound were analyzed, and this hampered the accurate assessment of their limited desorption. For BC, hysteretic desorption isotherm was observed (Fig. 2) regardless of the solution pH, which suggests restriction of ABA enantiomers to be desorbed, as typically described for other ionizable organic compounds on biochars.^{48,49} Furthermore, the upward slope observed in the desorption branch at basic pH (10.3-10.6) could be indicative of experimental artifacts during the desorption measurement,⁵⁰ such as insufficient equilibration time to reach the sorption equilibrium, as observed in other studies for nano- and microporous materials.⁵¹

Incubation Experiment. Fig. 3 depicts the dissipation curves for *R*-ABA and *S*-ABA in unamended soil and in soil amended with SA-HDTMA, Cloi10 and BC, and Fig. 4 shows the time progression of the ABA residue enantiomer fraction (EF). The first-order dissipation constants and half-lives for the individual enantiomers are given in Table 1. In all cases,

degradation of ABA was enantiomer-selective; the natural enantiomer *S*-ABA degraded faster than the unnatural *R* enantiomer (Fig. 3). This is in agreement with the enantioselective degradation pattern of ABA in three different soils observed by Gámiz et al.¹¹

The greatest enantioselectivity in the degradation of ABA was observed in unamended soil, with complete disappearance of *S*-enantiomer by the end of the incubation experiment (EF= 0, Fig. 4). The half-life ($t_{1/2}$) of *S*-ABA was 3 days whereas that of *R*-ABA was 21 days (Table 1). These values were consistent with those reported by Gámiz et al.¹¹ for ABA enantiomers in a loamy sand soil and with that reported for ABA in a non-enantioselective study conducted by Hartung et al.¹⁰

The soil amendments had different effects on the enantiomer dissipation rates, in spite of the fact that the preferential degradation of *S*-ABA over *R*-ABA remained unaltered (Fig. 3 and Table 1). The persistence of *R*-ABA, the slowly degraded enantiomer, was not significantly affected by the addition of Cloi10 ($t_{1/2}$ = 25 days) or BC ($t_{1/2}$ = 26 days) to soil ($p > 0.05$) (Table 1), while it was further enhanced upon amending soil with SA-HDTMA, reaching an extrapolated half-life of 139 days ($p < 0.005$) (Table 1). The persistence of *S*-ABA, the rapidly degraded enantiomer, was unaltered by the addition of Cloi10 ($p > 0.05$), but was significantly enhanced by the presence in soil of both SA-HDTMA ($p < 0.05$) and BC ($p < 0.05$), reaching half-lives of 12 and 7 days, respectively (Fig. 3 and Table 1). As a result of these degradation patterns, by the end of the experiment (t = 8 days) EF reached values of 0 for unamended soil, 0.17 for Cloi10-amended soil, 0.37 for BC-amended soil, and 0.40 for SA-HDTMA-amended soil (Fig. 4). Consequently, the enantioselectivity of ABA dissipation contrasted depending on the treatment, decreasing in the following order: unamended > Cloi10 > BC > SA-HDTMA-amended soil (Fig. 3 and Fig. 4). As enantioselectivity has been related to biological degradation of chiral compounds, it can be inferred that the amendments

protected ABA enantiomers from biodegradation to different extents (Cloi10 < BC < SA-HDTMA).

The notable enhancement of *S*-ABA persistence in soil upon amendment with SA-HDTMA and BC could be plausibly due to lower bioavailability of the enantiomers as a consequence of their sorption and/or even to some possible toxic effect of these amendments on the soil microbial community. The latter did not appear to be particularly important, since changes in soil respiration after amending soil with the sorbents were found to be insignificant ($p > 0.05$) (Table 1). Consequently, a specific methodological approach was used to get insight into the role of sorption in the degradation of ABA enantiomers. The results are summarized in Fig. 5, where percentages of sorbed residues and distribution coefficients for *R*-ABA and *S*-ABA at selected times during the incubation experiment with the amended soil samples are reported. For the unamended soil, we did not observe any indications of sorption of the ABA enantiomers ($K_d < 0.01 \text{ L kg}^{-1}$), in other words all *R*-ABA and *S*-ABA remained in the aqueous phase for the soil only treatment (Fig. 5).

At $t = 0$, the soil amended with the two organoclays displayed much greater sorption than the soil amended with BC. In SA-HDTMA- and Cloi10-amended soil, more than 80% of ABA residues were present in the sorbed state compared to only 45% in BC-amended soil ($p < 0.05$) (Fig. 5). This result was in good agreement with the observations from the 24 h batch study (Fig. 2; Table S2).

At $t = 4$ days, a considerable decrease occurred in the percentage of ABA residues sorbed and associated K_d values for SA-HDTMA- and Cloi10-amended soil, whereas an opposite behavior was observed for BC-amended soil. Sorption of ABA enantiomers in BC-amended soil even significantly exceeded ($p < 0.05$) that in the soil amended with the organoclays (Fig. 5). While the increase in sorption of ABA enantiomers with time in BC-amended soil can reasonably be attributed to slow sorption kinetics on BC particles,^{38,52,53} the behavior of the

organoclays was more intriguing. A possible explanation is that the effectiveness of the organoclays to sorb ABA enantiomers decreased with time during the first days of experiment. Non-linear sorption with S-type isotherms would reduce sorption as the concentration of ABA enantiomers was depleted by microbial degradation; however, sorption isotherms of ABA enantiomers on the organoclays did not display S-character (Fig. 2). Furthermore, the decrease in K_d occurred not only for *S*-ABA but also for *R*-ABA, for which degradation was very low during the first 4 days of experiment (Fig. 3). Consequently, our results strongly indicate that the interaction of the organoclays with soil constituents (e.g., dissolved organic matter or salts) probably resulted in competition with ABA enantiomers for sorption sites on the organoclay surface and/or blockage of access to such sorption sites, thus reducing the sorption of ABA enantiomers. This competitive mechanism has been previously proposed for the sorption of the herbicide fluometuron in organoclay-amended soil.²⁵

At $t = 8$ days, the sorption of ABA enantiomers in the organoclay-amended soil remained similar to that observed at $t = 4$ days, while the sorption in BC-amended soil further increased (Fig. 5). The exceptionally high K_d value of *S*-ABA in Cloi10-amended soil, with 92% of ABA residues present in the sorbed state (Fig. 5), can be attributed to the extensive degradation of *S*-ABA in this soil, where the small residual amount of *S*-ABA present could have been particularly resistant to desorption. It is also interesting to note that the degradation of *S*-ABA in SA-HDTMA-amended soil occurred slower than in Cloi10-amended soil (Fig. 3), and that this could not be related to the stronger sorption in SA-HDTMA-amended soil (Fig. 5). It is possible that sorption of ABA on Cloi10 particles, with smaller basal spacing (d_{001}) value and greater external specific surface area (S_{BET}) compared to SA-HDTMA (Table S1), could have occurred on more accessible sites compared to sorption on SA-HDTMA particles, making the sorbed compound more available to soil microorganisms. In this regard,

there is evidence that bacteria or extracellular enzymes produced by bacteria are probably able to access certain specific regions where pesticides are sorbed.^{54,55}

Leaching. The mass balance for *R*-ABA and *S*-ABA at the end of the leaching experiment for the unamended and SA-HDTMA-, Cloi10- and BC-amended soil columns is shown in Fig. 6 and the EF value for ABA residues in each fraction is given in Table 2. *R*-ABA leached to a greater extent than *S*-ABA in all cases (Fig. 6), denoting that *S*-ABA degraded faster than *R*-ABA under leaching conditions as well. The amendments decreased the amount of ABA enantiomers detected in the leachates as compared to the unamended soil. The highest amount of *R*-ABA leached was for unamended soil (96%) followed by Cloi10-amended soil (63%), SA-HDTMA-amended soil (56%) and BC-amended soil (24%). Same order was maintained for *S*-ABA: unamended soil (82%) > Cloi10- (24%) > SA-HDTMA- (22%) > BC-amended soil (12%) (Fig. 6). Conversely to outcomes derived from the incubation experiment, unamended soil reflected more racemic concentrations of ABA enantiomers in the leached fraction compared to the amended soil ($p < 0.05$) (Table 2), presumably because the amendments increased sorption and prolonged the residence time of ABA enantiomers within the soil columns, which enhanced the impact of biodegradation. This was supported by the position at which the maximum concentration of ABA enantiomers ($C_{\max}$) appeared in leachates, which revealed that retardation increased in the order: unamended- < BC-amended soil < Cloi10-amended soil < SA-HDTMA-amended soil (Table S3), thus showing a positive correlation with the sorption capacity of the sorbents as determined by the 24 h batch methodology (Table S1).

Extraction of soil columns at the end of the leaching experiment was set out to address the questions of whether: i) longer residence time of the enantiomers inside the columns coupled with weaker interactions with sorbent surfaces favored biodegradation and ii) sorption hindered the ABA leaching. The extraction illustrated that neither *R*-ABA nor *S*-ABA

remained in unamended soil and in soil amended with organoclays, SA-HDTMA and Cloi10 (Fig. 6). A potential explanation in the case of organoclays is that the weak sorption prolonged the presence of the enantiomers in the soil columns, retarding leaching but allowing ABA molecules to be degraded once they surpassed the 0-5 cm amended soil layer. The rapid degradation of *R*-ABA observed in the leaching in organoclay-amended soil contrasted with the result obtained in the incubation experiments where *R* enantiomer was scarcely degraded (Fig. 3). This divergence could result from the dynamic and saturated conditions of the leaching experiment compared to the static and aerated conditions associated with the incubation experiment. On the contrary, 52% of *R*-ABA and 40% of *S*-ABA were extracted from the BC-amended soil columns at the end of the leaching experiment, verifying higher irreversibility in sorption of ABA on BC. This result was also supported by the smallest amounts leached and high sorption registered for both enantiomers in BC-amended soil at longer incubation times (Fig. 5 and Fig. 6), since these amounts were only obtained from the upper 5 cm of soil columns, which was the portion of the column which was amended with BC. Additionally, the residues extracted were almost racemic with EF of 0.44 (Table 2), which suggests either sorption (which does not generally alter enantiomer distribution in soils)^{11,21} or pore-filling⁵⁶ were important mechanisms in this retention of ABA on biochar.

In summary, we obtained that addition of organoclays and biochar as agricultural soil amendments had distinct effects on the behavior of ABA enantiomers in soil. SA-HDTMA and Cloi10 displayed higher affinity for ABA enantiomers compared to BC in 24 h batch experiment. The degradation of ABA enantiomers was influenced by addition of organoclays and BC to soil, with the natural enantiomer, *S*-ABA, being degraded faster than the unnatural (*R*-ABA). Enantioselectivity of ABA degradation was greater in unamended soil compared to amended soils without direct relationship between higher 24 h sorption coefficients and more

racemic ABA residues in amended soils. Improvements in the methodology performed in the incubation experiments revealed that organoclay-amended soils rapidly lost some of their sorption capacity, whereas sorption progressively increased with time in BC-amended soil. Contradictory behaviors of ABA were associated to different sorption mechanisms, more superficial for the case of organoclays and time-dependent (suggesting pore diffusion) in BC-amended soil. The leaching experiment also confirmed different behavior of ABA after addition of the amendments and was related to the type of sorption. Organoclays were capable of retarding leaching, to a greater extent for SA-HDTMA which sorbed ABA in greater amounts in the 24 h batch sorption study. The amounts not leached of the enantiomers were attributed to biodegradation in organoclay-amended soil and irreversible sorption or entrapment in BC-amended soil. Higher immobilizing capacity for ABA was observed in BC-amended soil, which was the sorbent with greater irreversible sorption of ABA. We also established that the non-sorbed fraction of ABA (leachable) was more susceptible to microbial degradation. These results indicate that the type of soil amendment impacts both the short and long-term bioavailability of ABA enantiomers. This factor needs to be considered in order to understand the bioavailability and functions of both endogenous and exogenous ABA, and probably other chiral allelopathic and signaling compounds in the rhizosphere.

ABBREVIATIONS USED

ABA, abscisic acid; BC, biochar; Cloi10, Cloisite®10A; EF, enantiomer fraction; HPLC, high performance liquid chromatography; SA-HDTMA, hexadecyltrimethylammonium modified-Arizona montmorillonite; V_{pore} , pore volume.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information Available:

Figure S1, example of the Macrosep® Advance Centrifugal Devices (Pall Corporation); Table S1, properties of the organoclays; Table S2, Freundlich coefficients for *R*-ABA and *S*-ABA on SA-HDTMA, Cloi10 and BC; Table S3, summary of *R*-ABA and *S*-ABA column leaching data.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest. The use of trade, firm, or corporation names in this manuscript is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture, the Agricultural Research Service, or the Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC) of any product or service to the exclusion of others that may be suitable.

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FIGURE CAPTIONS

Figure 1. Structures of *S*-abscisic acid and *R*-abscisic acid.

Figure 2. Sorption-desorption isotherms of *R*-ABA and *S*-ABA on SA-HDTMA, Cloi10 and BC. The pH range of the equilibrated suspensions is indicated in the graphs.

Figure 3. Dissipation curves for *R*-ABA and *S*-ABA in unamended soil and in soil amended with SA-HDTMA, Cloi10 and BC at 2% (w/w). Symbols correspond to experimental data points, whereas solid lines represent their fitting to the linearized form of the first order kinetics. Error bars correspond to standard errors of triplicate measurements.

Figure 4. Enantiomer fraction of ABA residues during the incubation experiment for unamended soil and for soil amended with SA-HDTMA, Cloi10 and BC at 2%.

Figure 5. Percentage of *R*-ABA and *S*-ABA sorbed in soil amended with SA-HDTMA, Cloi10 and BC during the incubation experiment. Values on bars indicate the distribution coefficients ($L\ kg^{-1}$) of the enantiomers at selected times.

Figure 6. Mass balance for *R*-ABA and *S*-ABA after the leaching experiment with unamended soil and with soil amended with SA-HDTMA, Cloi10 and BC.

Table 1. Single First-Order Dissipation Constants and Half-Lives for *R*-ABA and *S*-ABA and Soil Respiration in Unamended Soil and in Soil Amended with SA-HDTMA, Cloi10 and BC at 2%. Different Letters in Each Column Indicate Significant Differences in Values ($p < 0.05$).

Treatment	<i>R</i> -ABA			<i>S</i> -ABA			Soil Respiration ^{a,b} (mg CO ₂ kg ⁻¹ dry soil week ⁻¹)
	k ^a	t _{1/2}	R ²	k ^a	t _{1/2}	R ²	
	(d ⁻¹)	(d)		(d ⁻¹)	(d)		
Unamended soil	0.033 ± 0.007 a	21	0.854	0.245 ± 0.037 a	3	0.937	1030 ± 50 a
SA-HDTMA-amended soil	0.005 ± 0.002 b	139	0.575	0.056 ± 0.007 b	12	0.947	910 ± 44 a
Cloi10-amended soil	0.028 ± 0.006 a	25	0.839	0.232 ± 0.026 a,c	3	0.950	938 ± 11 a
BC-amended soil	0.027 ± 0.005 a	26	0.867	0.087 ± 0.024 b,c	7	0.766	938 ± 77 a

^a Value ± standard error

^b Measured under incubation experiment conditions

Table 2. EF Values for ABA Residues in Different Fractions of Unamended Soil and SA-HDTMA-, Cloi10- and BC-amended soil at the End of the Leaching Experiment. Different Letters in Each Row Indicate Significant Differences in Values ($p < 0.05$).

Fraction	EF ^a			
	Unamended soil	SA-HDTMA-amended soil	Cloi10-amended soil	BC-amended soil
Leached	0.46 ± 0.01 a	0.28 ± 0.03 b	0.26 ± 0.03 b	0.32 ± 0.02 b
Extracted	n.d. ^b	n.d.	n.d.	0.44 ± 0.01
Not-recovered	0.82	0.64	0.67	0.67

^a EF= $[S]/([S]+[R])$

^b n.d.: not detected

Figure 1. Structures of *S*-abscisic acid and *R*-abscisic acid.

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Figure 6. Mass balance for *R*-ABA and *S*-ABA after the leaching experiment with unamended soil and with soil amended with SA-HDTMA, Cloi10 and BC.

Supporting Information

Assessing the effect of organoclays and biochar on the fate of abscisic acid in soil

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Table S1. Characteristics of the Organoclays Used in this Work.

Organoclay	Gallery d-spacing d_{001} (nm)	Carbon content (%)	S_{BET} (m^2/g)
SA-HDTMA	2.40	29.6	11
Cloi10	1.92	27.2	20

Table S2. Freundlich Coefficients for *R*-ABA and *S*-ABA Sorption Isotherms on SA-HDTMA, Cloi10 or BC.

Sorbent	<i>R</i> -ABA		
	K_f^a	N_f^b	R^2
SA-HDTMA	6437 (3827 - 10827)	0.91 ± 0.17	0.910
Cloi10	1466 (1334 - 1610)	0.94 ± 0.05	0.991
BC (original pH)	82 (76 - 89)	0.29 ± 0.05	0.911
BC (neutral pH)	249 (236-263)	0.29 ± 0.03	0.975
	<i>S</i> -ABA		
	K_f	N_f	R^2
SA-HDTMA	6107 (3488 - 10691)	0.89 ± 0.18	0.894
Cloi10	1446 (1342 - 1558)	0.85 ± 0.04	0.994
BC (original pH)	83 (76 - 89)	0.29 ± 0.05	0.903
BC (neutral pH)	253 (238-269)	0.28 ± 0.03	0.965

^aValues in parentheses correspond to the standard error range about the Freundlich coefficients

^b Value $\pm$ standard error

Table S3. Summary Data of *R*-ABA and *S*-ABA Column Leaching from the Relative Breakthrough Curves (BTCs) in the Unamended Soil and in Soil Amended with SA-HDTMA, Cloi10 or BC.

		$C_{\max}^{a,b}$ (mg L ⁻¹)	EF ^{b,c}	Position of $C_{\max}$ (x Vpore) ^d
Unamended soil	<i>R</i> -ABA	2.02 ± 0.15	0.48 ± 0.01	1.00
	<i>S</i> -ABA	1.87 ± 0.14		1.00
SA-HDTMA- amended soil	<i>R</i> -ABA	0.52 ± 0.03	0.32 ± 0.05	1.32
	<i>S</i> -ABA	0.24 ± 0.09		1.32
Cloi10-amended soil	<i>R</i> -ABA	1.28 ± 0.15	0.33 ± 0.03	1.20
	<i>S</i> -ABA	0.64 ± 0.16		1.20
BC-amended soil	<i>R</i> -ABA	0.40 ± 0.02	0.37 ± 0.01	1.10
	<i>S</i> -ABA	0.23 ± 0.03		1.10

^a $C_{\max}$: maximum concentration of *R*-ABA and *S*-ABA in leachates

^b Value ± standard error

^c Enantiomeric fraction in leachates containing the highest concentration of *R*-ABA and *S*-ABA

^d Number of pore volumes (Vpore) of water added at which $C_{\max}$ appeared in leachates

Figure S1. Macrosep® Advance Centrifugal Devices (Pall Corporation) used to measure the aqueous ABA concentrations during the incubation experiment and detail of the membrane which is placed into the tube.