

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

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## 1 **ABSTRACT**

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

23

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

26

## 27 INTRODUCTION

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

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

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

52 compounds exhibit practically identical physico-chemical properties, but can differ in their  
53 interactions with certain surface moieties and biological receptors.<sup>14,15</sup> Hence, probing the  
54 enantioselective fate of chiral pesticides in various media is an emerging area in agricultural  
55 and environmental science.<sup>15-17</sup>

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

66 The addition of various amendments to agricultural soils to increase their organic carbon  
67 content is a common practice to mitigate pesticide transport. The modification of clay  
68 minerals with organic ions, which changes the nature of their surface from hydrophilic to  
69 hydrophobic, has been proposed as a strategy to increase their affinity for pesticides<sup>24</sup> and,  
70 among other applications, organically-modified clays have been proposed as soil  
71 amendments.<sup>25</sup> Biochar is produced by thermal pyrolysis of organic feedstocks under a very  
72 low oxygen atmosphere and has concentrated considerable attention regarding its application  
73 as soil amendment.<sup>26</sup> Some benefits of using biochar fall on increasing the carbon content of  
74 soil provoking soil fertility improvement, enhancement of soil water retention capacity,  
75 carbon sequestration potential,<sup>27</sup> and augmentation of soil microbial activity.<sup>26,28</sup> The  
76 application of organoclays and biochar to soil has been documented to have various impacts

77 on the fate of pesticides, including increasing sorption, changing the degradation patterns, and  
78 reducing the leaching potential.<sup>25,29–32</sup> Nevertheless, very little information regarding their  
79 effects on the behavior of individual chiral agrochemical enantiomers is available, particularly  
80 for these engineered amendments.<sup>21,33</sup>

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

91

## 92 **MATERIALS AND METHODS**

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

97 **Amendments.** Two organically modified montmorillonites (SA-HDTMA and Cloisite®  
98 10A) and one biochar (BC) were used as amendments. SA-HDTMA was synthesized through  
99 an ion exchange reaction by treating Ca-rich Arizona montmorillonite (SAz-1) with a solution  
100 containing hexadecyltrimethylammonium (HDTMA) equivalent to the cation exchange  
101 capacity (CEC) of SAz-1 ( $120 \text{ cmol/kg}$ ). More details of the synthesis can be found

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

111 **Soil.** An agricultural soil located in Seville (Spain) was collected from a 0-20 cm depth,  
112 air-dried, sieved to pass a 2 mm mesh, and stored at 4 °C. It is a sandy loam soil and contains  
113 75% sand, 9% silt, 17% clay, 1.9% CaCO<sub>3</sub> and 0.63% organic carbon. The pH of a 1:2 (w/v)  
114 soil/deionized water mixture was 7.4.

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

127 concentrations in the aqueous phase ( $C_e$ ) by chiral high performance liquid chromatography  
128 (HPLC). Controls without sorbents were used to identify possible chemical losses during the  
129 equilibration. Amount sorbed of ABA enantiomers ( $C_s$ ) were established from the difference  
130 between the initial ( $C_i$ ) and equilibrium solution concentration ( $C_e$ ). The desorption branch of  
131 the isotherm was obtained immediately after sorption from the highest equilibrium point of  
132 the sorption isotherm. The 4 mL of supernatant solution removed for the sorption analysis  
133 were replaced with 4 mL of distilled water. The tubes were re-suspended and shaken at  $20 \pm 2$   
134 °C for 24 h, centrifuged, filtered, and analyzed by chiral HPLC. This desorption process was  
135 repeated three times. Sorption data were fitted to the log-transformed Freundlich isotherm:

$$136 \quad \log C_s = \log K_f + N_f \log C_e$$

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

138 **Dissipation Study.** The enantioselective dissipation of ABA in unamended soil and in  
139 soil amended with SA-HDTMA, Cloi10 or BC under aerobic conditions was studied by  
140 means of an incubation experiment. Portions of 200 g of soil, either unamended or amended  
141 with SA-HDTMA, Cloi10, or BC at a rate of 2% (w/w), were spiked with rac-ABA at a rate  
142 of  $2 \text{ mg kg}^{-1}$  dry soil, and then incubated in glass jars in the dark at  $20 \pm 2$  °C for up to 8 days.  
143 The moisture content was maintained at a constant level ( $\sim 30\%$ ) throughout the experiment  
144 by adding distilled water as necessary. Periodically, at 0, 1, 2, 3, 4 and 8 days after treatment  
145 (DAT), aliquots of 3 g of soil were sampled in triplicate with a sterilized spatula and  
146 immediately frozen until analyzed. *S*-ABA and *R*-ABA residues in the soil samples were  
147 extracted by shaking for 24 h with 8 mL of a mixture (30:70) of acetonitrile:0.01 M  $\text{H}_3\text{PO}_4$   
148 aqueous solution (pH=2.2). Recoveries were always greater than 95% of ABA freshly applied  
149 to unamended or amended soils. The extracts were analyzed by chiral HPLC. *R*-ABA and *S*-  
150 ABA dissipation data in unamended and amended soil were fitted to the linearized form of a  
151 first-order kinetic rate law:

152 
$$\ln C = \ln C_0 - kt$$

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

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

158 
$$\text{EF} = [S\text{-ABA}] / ([S\text{-ABA}] + [R\text{-ABA}])$$

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

162 A separate experiment was set up to determine whether the addition of the amendments to  
163 the soil caused any effect on soil respiration. This parameter was measured following the  
164 alkali trapping–titrimetric procedure described by Anderson,<sup>36</sup> by quantifying the amount of  
165  $\text{CO}_2$  released by samples of unamended soil and of soil amended with SA-HDTMA, Cloi10  
166 and BC at 2% during 8 days under the same conditions as those used in the incubation  
167 experiment.

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



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

181 **Column Leaching Experiment.** In triplicate, glass columns (30 cm long and 3.1 cm  
182 internal diameter) were hand-packed with 160 g of dry soil (unamended soil) to a height of 20  
183 cm soil in each column (bulk density  $\approx 1.1 \text{ g cm}^{-3}$ ). The effect of the amendments on ABA  
184 leaching was studied by amending the upper 5 cm of soil (40 g) at a rate of 2% (w/w) with  
185 SA-HDTMA, Cloi10 or BC. Glass wool was placed at the bottom of the column to avoid soil  
186 losses, and 10 g of sea sand was added at the bottom and top of all soil columns. The columns  
187 were initially saturated with 100 mL of distilled water, and after allowing 24 h drainage, the  
188 maximum water retention capacity of the soil columns or column pore volume ( $V_{\text{pore}}$ ) was  
189 calculated from the gravimetric mass difference. Next, 3 mL of an aqueous solution of 50 mg  
190  $\text{L}^{-1}$  of rac-ABA were added to the columns to give the maximum agronomic application rate  
191 of  $2 \text{ kg ha}^{-1}$  established for ABA (0.15 mg active ingredient). Subsequently, twice a day, 15  
192 mL of distilled water were added to the columns for a total of ten additions (5 days), and the  
193 leachates were collected in vials containing 5 mL of methanol and stored at  $4 \text{ }^\circ\text{C}$  in the dark,  
194 according to Gámiz et al.<sup>11</sup> Then, leachates were filtered and analyzed by chiral HPLC to  
195 determine the *R*-ABA and *S*-ABA concentrations. At the end of the leaching experiment, soil  
196 samples were obtained in 5 cm increments corresponding to different depths (0–5, 5–10, 10–  
197 15, and 15–20 cm) from the columns. Each section was extracted with 100 mL of a mixture  
198 (30:70) of acetonitrile: 0.01 M  $\text{H}_3\text{PO}_4$  aqueous solution (pH=2.2), by shaking for 24 h and the  
199 extracts were subsequently analyzed for ABA residues by chiral HPLC.

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

202 Waters 717 Autosampler injector. The chromatographic conditions used for the analysis are  
203 detailed in Gámiz et al.<sup>11</sup> Briefly, we used a Chiralpak AS-3R column (150 mm length × 4.6  
204 mm i.d., 3 µm particle size), 30:70 of acetonitrile:0.01 M H<sub>3</sub>PO<sub>4</sub> aqueous solution (pH = 2.2)  
205 as mobile phase, flow rate of 1 mL min<sup>-1</sup>, a 50 µL sample injection volume, and UV detection  
206 at 230 nm. The retention times under these conditions were 4.4 and 5.4 min for *R*-ABA and *S*-  
207 ABA, respectively. The limit of quantification (LOQ) calculated as the concentration resulting  
208 in a signal to noise ratio of 10:1 was 0.008 mg L<sup>-1</sup>.

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

216

## 217 RESULTS AND DISCUSSION

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

226 All isotherms of ABA enantiomers on the organoclays showed values of  $N_f$  close to 1 ( $N_f$ =  
227 0.85-0.94, Table S2), whereas BC possessed highly nonlinear isotherms ( $N_f$ = 0.28-0.29). In  
228 addition, both under non-adjusted and similar (adjusted) pH levels, ABA was sorbed to a  
229 greater extent on SA-HDTMA and Cloi10 than on BC, with  $K_f$  values following the rising  
230 trend: BC  $\ll$  Cloi10 < SA-HDTMA (Table S2).

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

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

251 presence of surface functional groups, which can increase the polarity and provide negatively-  
252 charged surface, can also influence the sorption of organic compounds by BCs. Since ABA is  
253 a weak acid ( $pK_a = 4.6$ ) and was present in solution as anionic species at the pH of the sorption  
254 experiments ( $pH > 7$ ), repulsions between ABA anions and negatively charged BC particles<sup>48</sup>  
255 could have occurred and reduced sorption. Interestingly, a remarkable enhancement of ABA  
256 sorption was observed in the isotherms performed at neutral pH (7.5-7.9) compared to those  
257 obtained under non-adjusted (alkaline) conditions (10.3-10.6) (Fig. 2 and Table S1). This  
258 probably resulted from a decrease in the negative surface charge of BC which reduced  
259 repulsions with anionic ABA species. Nevertheless, in spite of greater ABA sorption on BC at  
260 neutral pH level, sorption was still lower than that observed for the organoclays at similar pH  
261 (7.0-8.2) (Fig. 2 and Table S1).

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

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

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

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

285 The soil amendments had different effects on the enantiomer dissipation rates, in spite of  
286 the fact that the preferential degradation of *S*-ABA over *R*-ABA remained unaltered (Fig. 3  
287 and Table 1). The persistence of *R*-ABA, the slowly degraded enantiomer, was not  
288 significantly affected by the addition of Cloi10 ( $t_{1/2}$ = 25 days) or BC ( $t_{1/2}$ = 26 days) to soil ( $p$   
289 > 0.05) (Table 1), while it was further enhanced upon amending soil with SA-HDTMA,  
290 reaching an extrapolated half-life of 139 days ( $p$  < 0.005) (Table 1). The persistence of *S*-  
291 ABA, the rapidly degraded enantiomer, was unaltered by the addition of Cloi10 ( $p$  > 0.05),  
292 but was significantly enhanced by the presence in soil of both SA-HDTMA ( $p$  < 0.05) and BC  
293 ( $p$  < 0.05), reaching half-lives of 12 and 7 days, respectively (Fig. 3 and Table 1). As a result  
294 of these degradation patterns, by the end of the experiment ( $t$ = 8 days) EF reached values of 0  
295 for unamended soil, 0.17 for Cloi10-amended soil, 0.37 for BC-amended soil, and 0.40 for  
296 SA-HDTMA-amended soil (Fig. 4). Consequently, the enantioselectivity of ABA dissipation  
297 contrasted depending on the treatment, decreasing in the following order: unamended >  
298 Cloi10 > BC > SA-HDTMA-amended soil (Fig. 3 and Fig. 4). As enantioselectivity has been  
299 related to biological degradation of chiral compounds, it can be inferred that the amendments

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

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

314 At  $t = 0$ , the soil amended with the two organoclays displayed much greater sorption than  
315 the soil amended with BC. In SA-HDTMA- and Cloi10-amended soil, more than 80% of  
316 ABA residues were present in the sorbed state compared to only 45% in BC-amended soil ( $p$   
317  $< 0.05$ ) (Fig. 5). This result was in good agreement with the observations from the 24 h batch  
318 study (Fig. 2; Table S2).

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

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

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

349 there is evidence that bacteria or extracellular enzymes produced by bacteria are probably able  
350 to access certain specific regions where pesticides are sorbed.<sup>54,55</sup>

351 **Leaching.** The mass balance for *R*-ABA and *S*-ABA at the end of the leaching  
352 experiment for the unamended and SA-HDTMA-, Cloi10- and BC-amended soil columns is  
353 shown in Fig. 6 and the EF value for ABA residues in each fraction is given in Table 2. *R*-  
354 ABA leached to a greater extent than *S*-ABA in all cases (Fig. 6), denoting that *S*-ABA  
355 degraded faster than *R*-ABA under leaching conditions as well. The amendments decreased  
356 the amount of ABA enantiomers detected in the leachates as compared to the unamended soil.  
357 The highest amount of *R*-ABA leached was for unamended soil (96%) followed by Cloi10-  
358 amended soil (63%), SA-HDTMA-amended soil (56%) and BC-amended soil (24%). Same  
359 order was maintained for *S*-ABA: unamended soil (82%) > Cloi10- (24%) > SA-HDTMA-  
360 (22%) > BC-amended soil (12%) (Fig. 6). Conversely to outcomes derived from the  
361 incubation experiment, unamended soil reflected more racemic concentrations of ABA  
362 enantiomers in the leached fraction compared to the amended soil ( $p < 0.05$ ) (Table 2),  
363 presumably because the amendments increased sorption and prolonged the residence time of  
364 ABA enantiomers within the soil columns, which enhanced the impact of biodegradation.  
365 This was supported by the position at which the maximum concentration of ABA enantiomers  
366 ( $C_{\max}$ ) appeared in leachates, which revealed that retardation increased in the order:  
367 unamended- < BC-amended soil < Cloi10-amended soil < SA-HDTMA-amended soil (Table  
368 S3), thus showing a positive correlation with the sorption capacity of the sorbents as  
369 determined by the 24 h batch methodology (Table S1).

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



374 remained in unamended soil and in soil amended with organoclays, SA-HDTMA and Cloi10  
375 (Fig. 6). A potential explanation in the case of organoclays is that the weak sorption  
376 prolonged the presence of the enantiomers in the soil columns, retarding leaching but  
377 allowing ABA molecules to be degraded once they surpassed the 0-5 cm amended soil layer.  
378 The rapid degradation of *R*-ABA observed in the leaching in organoclay-amended soil  
379 contrasted with the result obtained in the incubation experiments where *R* enantiomer was  
380 scarcely degraded (Fig. 3). This divergence could result from the dynamic and saturated  
381 conditions of the leaching experiment compared to the static and aerated conditions associated  
382 with the incubation experiment. On the contrary, 52% of *R*-ABA and 40% of *S*-ABA were  
383 extracted from the BC-amended soil columns at the end of the leaching experiment, verifying  
384 higher irreversibility in sorption of ABA on BC. This result was also supported by the  
385 smallest amounts leached and high sorption registered for both enantiomers in BC-amended  
386 soil at longer incubation times (Fig. 5 and Fig. 6), since these amounts were only obtained  
387 from the upper 5 cm of soil columns, which was the portion of the column which was  
388 amended with BC. Additionally, the residues extracted were almost racemic with EF of 0.44  
389 (Table 2), which suggests either sorption (which does not generally alter enantiomer  
390 distribution in soils)<sup>11,21</sup> or pore-filling<sup>56</sup> were important mechanisms in this retention of ABA  
391 on biochar.

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

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

416

#### 417 **ABBREVIATIONS USED**

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

421

#### 422 **ASSOCIATED CONTENT**

##### 423 **Supporting Information**

424 The Supporting Information Available:

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

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

430

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440 The authors declare no competing financial interest. The use of trade, firm, or corporation  
441 names in this manuscript is for the information and convenience of the reader. Such use does  
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446

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**FIGURE CAPTIONS**

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**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 <sup>a,b</sup> (mg CO <sub>2</sub> kg <sup>-1</sup> dry soil week <sup>-1</sup> )
	k <sup>a</sup>	t <sub>1/2</sub>	R <sup>2</sup>	k <sup>a</sup>	t <sub>1/2</sub>	R <sup>2</sup>	
	(d <sup>-1</sup> )	(d)		(d <sup>-1</sup> )	(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

<sup>a</sup> Value ± standard error

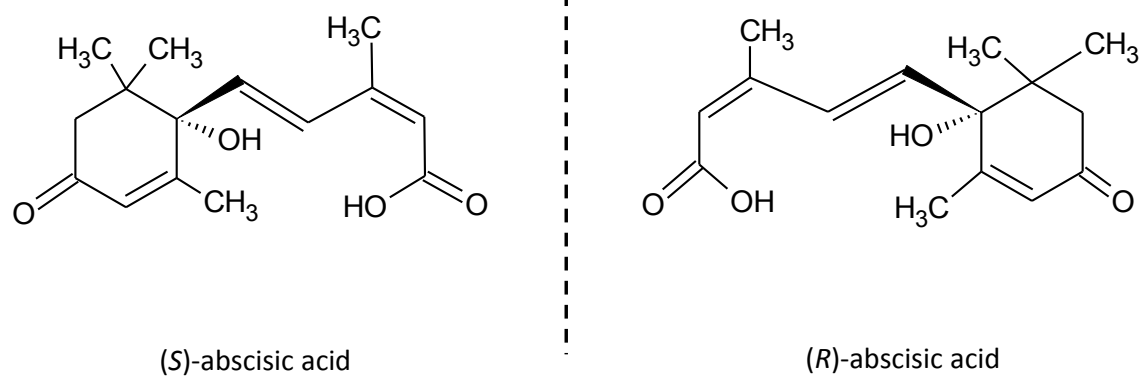
<sup>b</sup> Measured under incubation experiment conditions

**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 ( $p < 0.05$ ).**

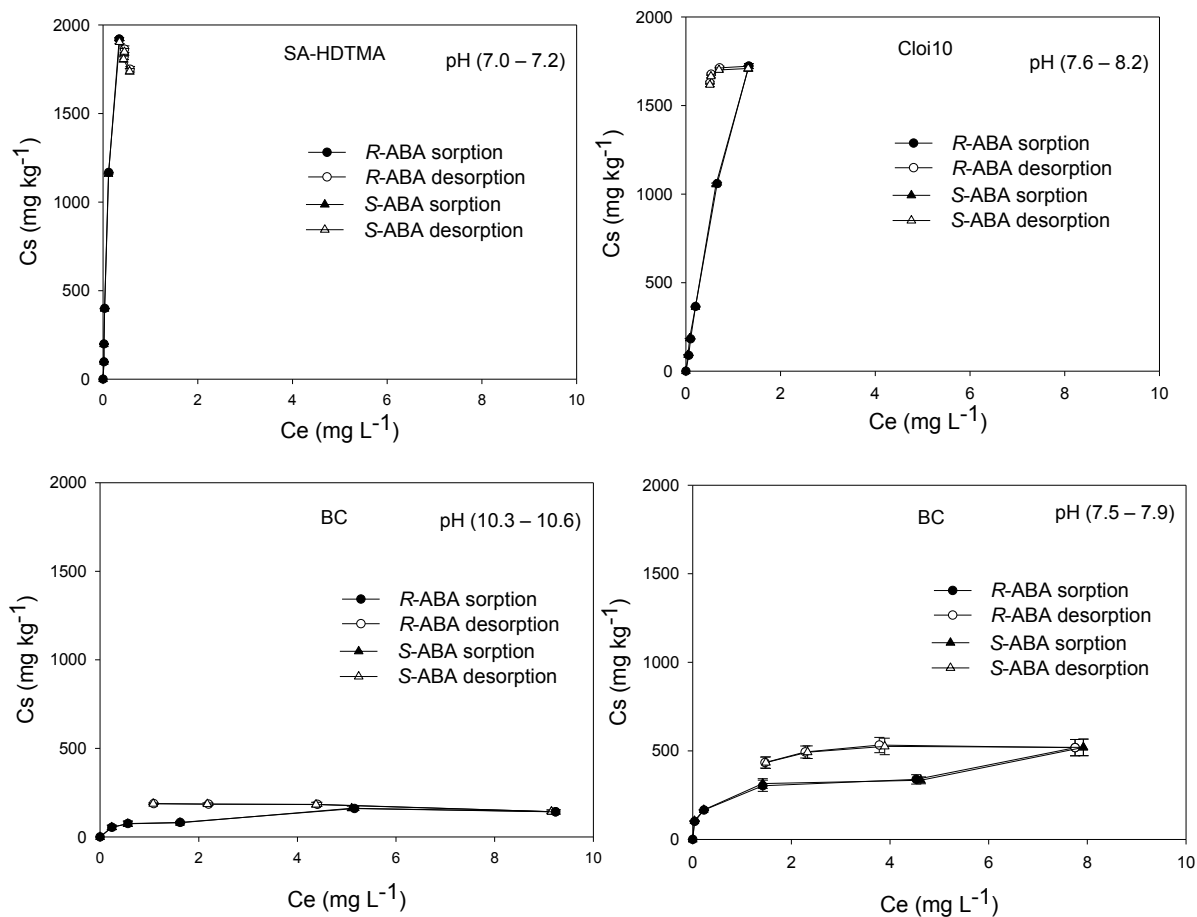
Fraction	EF <sup>a</sup>			
	Unamended soil	SA-HDTMA-amended soil	Cloi10-amended soil	BC-amended soil
Leached	0.46 ± 0.01 a	0.28 ± 0.03 b	0.26 ± 0.03 b	0.32 ± 0.02 b
Extracted	n.d. <sup>b</sup>	n.d.	n.d.	0.44 ± 0.01
Not-recovered	0.82	0.64	0.67	0.67

<sup>a</sup> EF = [S]/([S]+[R])

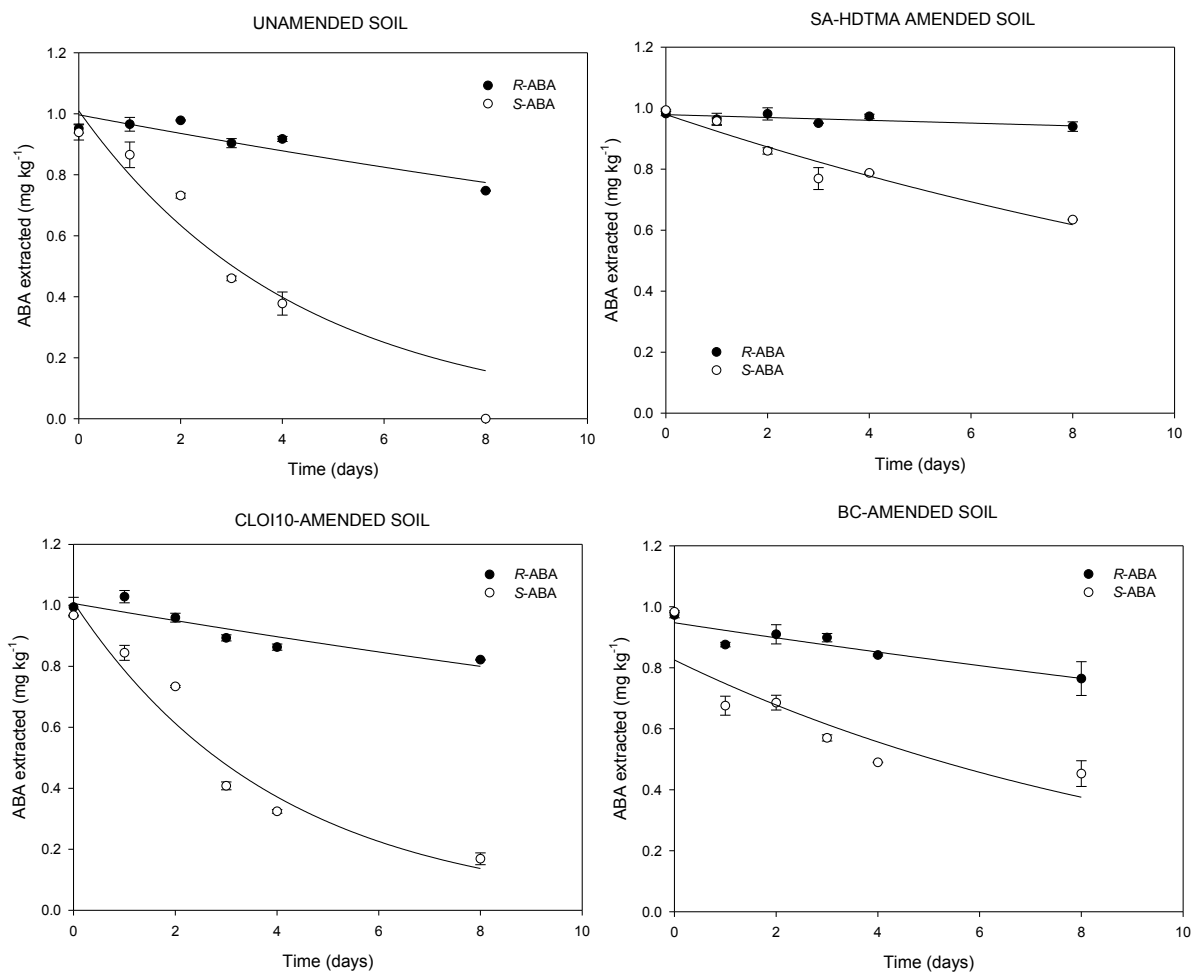
<sup>b</sup> n.d.: not detected



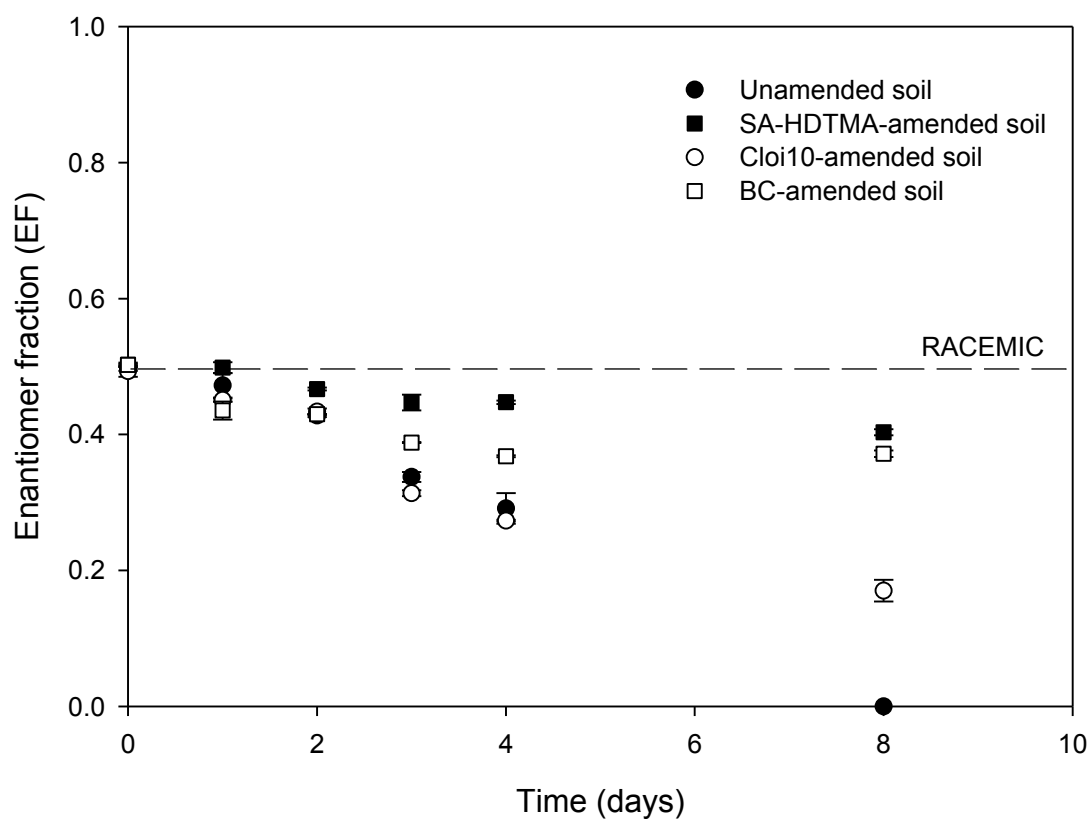
**Figure 1.** Structures of *S*-abscisic acid and *R*-abscisic acid.



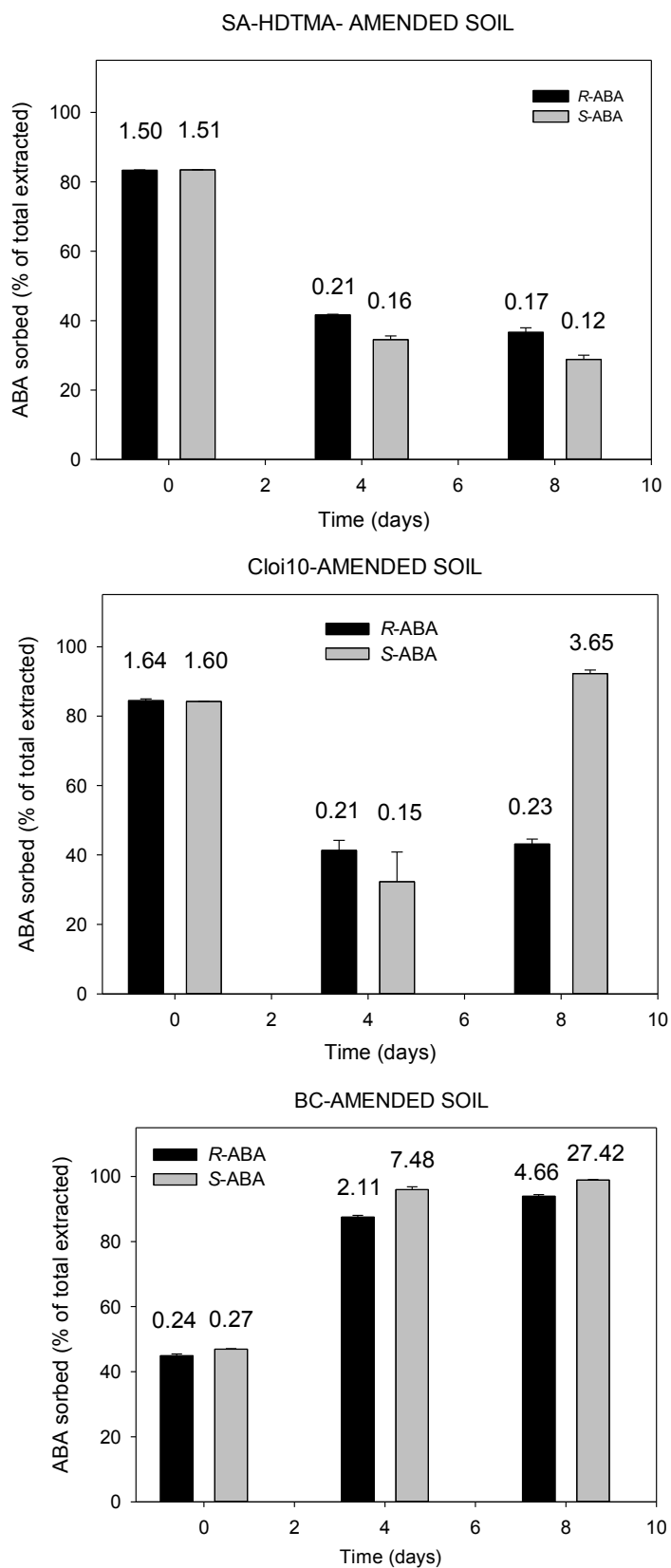
**Figure 2.** Sorption–desorption isotherms of *R*-ABA and *S*-ABA on SA-HDTMA, Cloi10 and BC. The pH range of the equilibrated suspensions is indicated in the graphs.



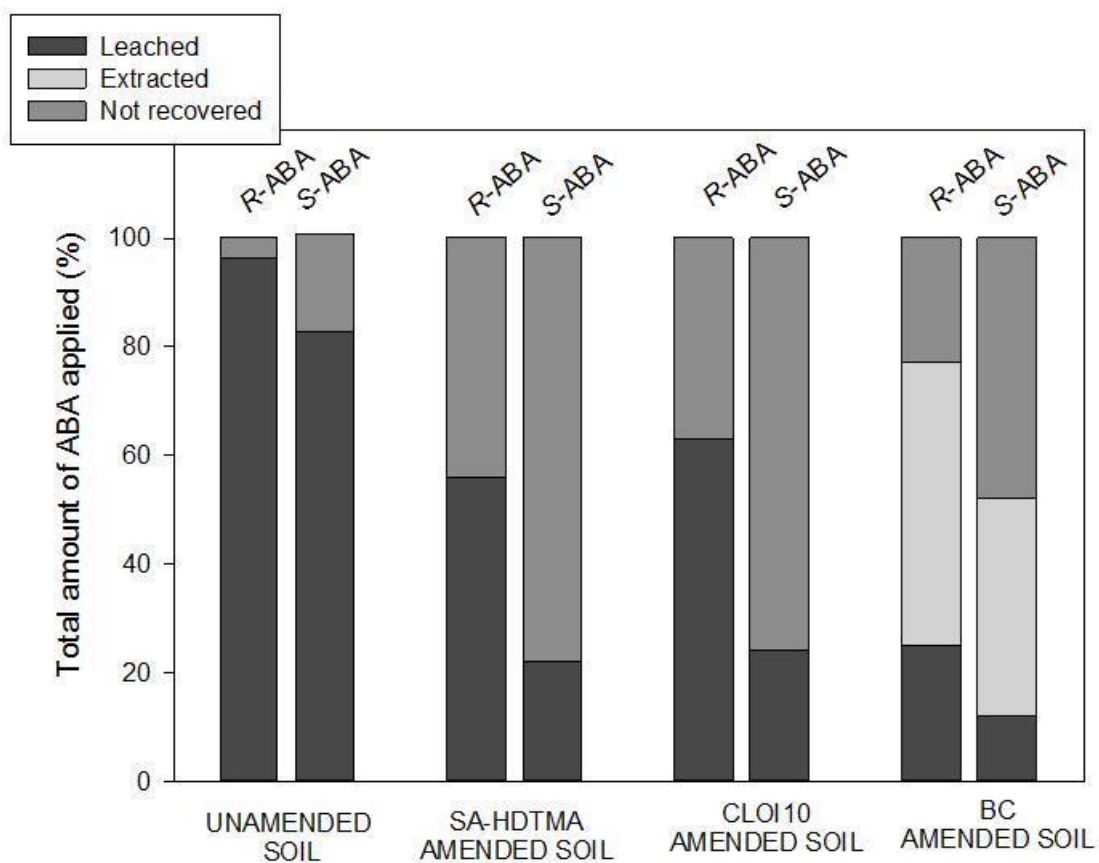
**Figure 3.** Dissipation curves for *R*-ABA and *S*-ABA in unamended soil and in soil amended with SA-HDTMA, Cloi10 and BC at 2% (w/w). Symbols correspond to experimental data points, whereas solid lines represent their fitting to the linearized form of the first order kinetics. Error bars correspond 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 for soil amended with SA-HDTMA, Cloi10 and BC during the incubation experiment. Values on bars indicate the distribution coefficients (L kg<sup>-1</sup>) 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.



### **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 (%)	$S_{\text{BET}}$ ( $\text{m}^2/\text{g}$ )
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)	$0.91 \pm 0.17$	0.910
Cloi10	1466 (1334 - 1610)	$0.94 \pm 0.05$	0.991
BC (original pH)	82 (76 - 89)	$0.29 \pm 0.05$	0.911
BC (neutral pH)	249 (236-263)	$0.29 \pm 0.03$	0.975
Sorbent	<i>S</i> -ABA		
	$K_f$	$N_f$	$R^2$
SA-HDTMA	6107 (3488 - 10691)	$0.89 \pm 0.18$	0.894
Cloi10	1446 (1342 - 1558)	$0.85 \pm 0.04$	0.994
BC (original pH)	83 (76 - 89)	$0.29 \pm 0.05$	0.903
BC (neutral pH)	253 (238-269)	$0.28 \pm 0.03$	0.965

<sup>a</sup>Values in parentheses correspond to the standard error range about the Freundlich coefficients

<sup>b</sup> 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.**

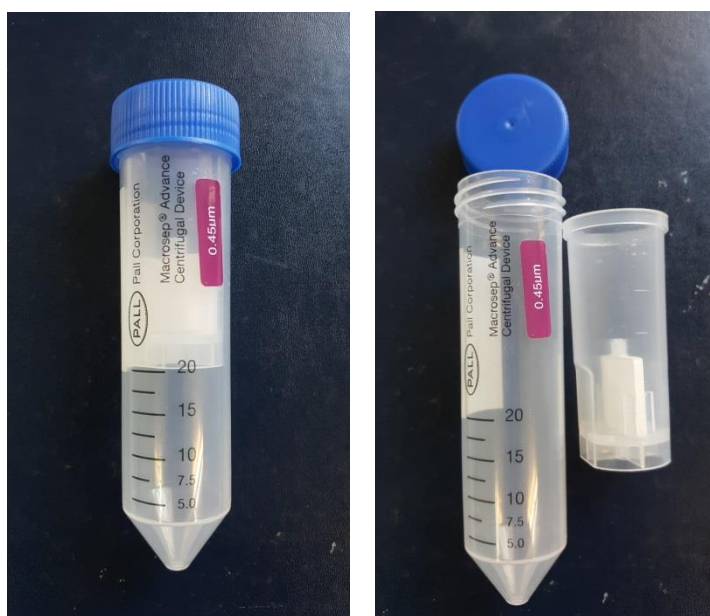
		$C_{\max}^{a,b}$ (mg L <sup>-1</sup> )	EF <sup>b,c</sup>	Position of $C_{\max}$ (x Vpore) <sup>d</sup>
Unamended soil	<i>R</i> -ABA	2.02 ± 0.15	0.48 ± 0.01	1.00
	<i>S</i> -ABA	1.87 ± 0.14		1.00
SA-HDTMA- amended soil	<i>R</i> -ABA	0.52 ± 0.03	0.32 ± 0.05	1.32
	<i>S</i> -ABA	0.24 ± 0.09		1.32
Cloi10-amended 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

<sup>a</sup>  $C_{\max}$ : maximum concentration of *R*-ABA and *S*-ABA in leachates

<sup>b</sup> Value ± standard error

<sup>c</sup> Enantiomeric fraction in leachates containing the highest concentration of *R*-ABA and *S*-ABA

<sup>d</sup> 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.