Postprint of: Journal of Agricultural and Food Chemistry 67(2): 589–596 (2019)**Nanoengineered Sorbents to Increase the Persistence of the Allelochemical Carvone in the Rhizosphere**

Beatriz Gámiz,* Gracia Facenda and Rafael Celis

Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina Mercedes 10, 41012 Sevilla, Spain

***Corresponding Author:** Dr. Beatriz Gámiz

Address: Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC

Avenida Reina Mercedes 10

41012 Sevilla, Spain

Phone: +34 954624711

E-mail: bgamiz@irnase.csic.es

ABSTRACT

This study investigates the changes in sorption/desorption, dissipation and leaching of the two enantiomeric forms of the allelochemical carvone, R-carvone and S-carvone, after amending an agricultural soil sample with two nanoengineered sorbents: biochar (BC) and organoclay (OCl). The sorption of carvone enantiomers was non-enantioselective and similarly improved by the addition of OCl and BC to the soil. However, OCl-amended soil showed reversible sorption, whereas BC-amended soil displayed sorption-desorption hysteresis. Dissipation of carvone enantiomers was enantioselective. Both amendments increased the half-life of the enantiomers in soil compared to unamended soil. This effect was more pronounced for BC-amended soil and for S-carvone. Leaching of R- and S-carvone through soil columns was scarce in unamended soil (< 7%), due to their rapid degradation during leaching, and null for OCl- and BC-amended soil, for which much of the applied R- and S-carvone remained in the top 0-5 cm amended soil layer. Addition of biochars and organoclays could help increase the persistence of carvone enantiomers in the rhizosphere, which may favor their use as residual pest management substances.

KEYWORDS: biochar, biopesticides, chiral pesticides, monoterpenes, organoclays, soil amendments, sorption

INTRODUCTION

The unbridled use of synthetic pesticides is still a matter of concern due to the environmental risk, pest resistance problems, and loss of soil biodiversity associated with pesticide applications.¹ This has led to a more restrictive legislation aiming at phasing out the application of these xenobiotics² and implementing eco-friendly alternatives, compelling scientists to seek innovative approaches.³ In this regard, the use of natural products as a source of new pesticides is receiving increasing attention.²

Many natural compounds are allelochemicals, that is, chemicals which are released by living organisms (plants, insects, microbes, etc.) and are capable of stimulating or inhibiting the growth, health, behavior, or population biology of neighboring organisms.⁴ One important group of allelochemicals that has received the interest of scientists in the field of sustainable crop protection are the monoterpenes.^{5,6} Monoterpenes operate as a chemical defense against insects, bacterial and fungal pathogens, and herbivores; fragrances attractive to pollinators;⁷ and phytotoxins to other plants.⁸ Nevertheless, in contrast to conventional pesticides, the direct use of monoterpenes for crop protection is not that simple. Once in the soil, monoterpenes rapidly undergo a series of transformation (degradation) and transport (e.g., leaching, volatilization) processes that decrease their biological efficacy, and this is possibly one of their weaknesses or “Achilles’ heel”.^{9,10} To control these processes, the sorption-desorption of these compounds in soil plays a crucial role, since it dictates their bioavailability and consequently their final fate in soil.^{3,11}

Nanotechnology seems to be a promising tool in the direction of designing improved formulations based on nanostructured sorbents for monoterpenes and other allelochemicals, since innovative nanoformulations can be used to enhance the stability and effectiveness of natural compounds in the environment.^{12,13} An alternative approach of using nanostructured

materials is their direct application to soils to modulate the bioavailability and activity of allelochemicals either after these are released directly by plants or exogenously applied to soil. In this way, their residual activity in the rhizosphere, where they should exert their action, may be prolonged.¹⁴

Biochar and clay minerals are nanostructured materials which have been proposed to control the behavior of synthetic pesticides in soils. Biochar (BC) is defined as the solid material produced as a result of the pyrolysis of biomass, used to achieve agricultural and/or environmental benefits, such as reducing greenhouse gas (GHG) emissions and improving crop yield, plant growth, nutrient and water retention capacity and biological activity of soils.^{15,16} Simultaneously, due to the peculiarities of their surfaces, BC is a good sorbent for organic compounds. It has been reported that lower pyrolysis temperature biochars (350–500°C) could be the more suitable for minimizing environmental impacts of applied pesticides while maintaining their efficacy, because the main sorption mechanism implies reversible, partitioning and specific interactions on biochar surfaces.^{17,18} On the other hand, based on their nano-sized layers and the versatility of their structures, expandable clay minerals can easily be modified in order to obtain a new type of material which shares properties of both natures (organic/inorganic), known as nano-hybrid or organoclay (OCI).¹⁹ OCIs usually exhibit excellent sorbent properties towards organic compounds. In agricultural and environmental sciences, they have been proposed as supports in slow release pesticide formulations, immobilizing materials, and soil amendments.^{20,21}

Carvone is a chiral monoterpene existing in nature as two enantiomers, S-carvone and R-carvone (Figure S1), which are constituents of the essential oils from different seeds, such as caraway, dill, and spearmint seeds.²² Carvone enantiomers can be targeted as new active ingredients in commercial biopesticide formulations due to their reported fungistatic, bacteriostatic, insecticidal, and phytotoxic features.^{6,23} In a previous work, we found that

organic carbon is a key factor in the sorption of carvone enantiomers in soil, although the mineral fraction can also be important in soils with very low organic carbon contents.²⁴ The dissipation of the enantiomers in soil was fast, microbial-mediated, and enantioselective.²⁴ Given that the addition of BC and OCIs to soil has been reported to affect the fate of soil-applied pesticides by modifying their sorption-desorption behavior, changing their degradation patterns and altering their leaching in soils,^{20,21,25} here we hypothesized that these nanostructured sorbents could be used to modulate the soil behavior of carvone enantiomers as well, in order to prolong their presence in the rhizosphere and increase their residual activity. Hence, the objective of this work was to assess the effect of adding OCI and BC to an agricultural soil on the sorption-desorption, degradation and leaching patterns of carvone enantiomers, and, to provide data for establishing new scientific-based management options for the potential application of carvone as a residual crop protection product.

MATERIALS AND METHODS

Carvone enantiomers. R-carvone and S-carvone were purchased as individual enantiomers from Sigma-Aldrich with a purity of 99.9% and 98.5%, respectively. As a typical characteristic of enantiomers, R- and S-carvone present the same physicochemical properties, e.g. molecular weight (150.2 g/mol), water solubility (27 mg/L) and vapor pressure (21.3 Pa at 25 °C).²⁶ The aqueous solutions of carvone used for the sorption-desorption experiments were all racemic and were prepared by diluting a racemic methanolic stock solution of 200 mg L⁻¹ of (R+S) carvone, which was obtained by dissolving 10 mg of each enantiomer in 100 mL of methanol.²⁴

Sorbents and Soil. The two sorbents used in this work as soil amendments were a low-temperature biochar (BC) and an organophilic organoclay (OCI). The BC was prepared by pyrolyzing composted olive mill waste at 400 °C under a flow of N₂ at 1.5 L/min for 4 h. The

chemical properties of the BC were: 30% C, 1.8% N, 58% ash, $N_2-S_{BET} < 1 \text{ m}^2/\text{g}$, and a pH of 9.8 determined in a 1:2 (w/v) BC/deionized water suspension. The OCl was cetyltrimethylammonium (CTMA)-saturated Arizona montmorillonite and was prepared by treating Ca-rich Arizona montmorillonite (SAz-1) from The Clay Minerals Society with a solution containing an amount of CTMA equivalent to the cation exchange capacity (CEC) of SAz-1 (1200 mmol/kg). Specific details of the synthesis can be found elsewhere.²¹ This OCl had a basal spacing value of 2.25 nm, 22% C, 1.3% N, and a N_2-S_{BET} of $11 \text{ m}^2/\text{g}$.

The soil used was a sandy loam soil devoted to olive crop from an experimental farm located in Seville (Spain). It was collected from a 0-20 cm depth, air-dried, sieved through a 2 mm mesh, and stored at 4 °C prior to use. The soil contained 75% sand, 9% silt, 17% clay, 1.9% CaCO_3 and 0.63% organic carbon. The pH of a 1:2 (w/v) soil/deionized water mixture was 7.3.

Sorption-Desorption Isotherms. The sorption-desorption isotherms of R- and S-carvone in unamended soil and in soil amended with BC and OCl were obtained by the batch equilibration method. In triplicate, 4 g of soil samples, either unamended or amended with BC or OCl at 2% (w/w), were weighed in Pyrex[®] glass screw-cap centrifuge tubes. To avoid the degradation of carvone enantiomers, the tubes containing the soil samples were subjected to three sequential autoclaving cycles for 20 min at 121 °C and 200 kPa on three consecutive days, with 24 h-incubation periods at 25 °C between successive cycles.²⁴ Subsequently, 8 mL of racemic aqueous solutions of carvone were added to each tube in concentrations (R+S) ranging from 0.1 to 2 mg/L, and the suspensions were equilibrated for 24 h by shaking at 25 ± 2 °C in an end-over-end shaker. Then, the tubes were centrifuged at $5000 \times g$ for 15 minutes and 4 mL of the supernatant solutions were removed and stabilized with 4 mL of methanol to prevent losses of carvone prior to the analysis.²⁴ The stabilized supernatants were filtered (0.45 μm) and analyzed by chiral-HPLC to quantify the aqueous solution concentration of

each enantiomer of carvone (C_e). The amount of R- and S-carvone sorbed, C_s (mg/kg), was calculated from the difference between their initial, C_i (mg/L), and final, C_e (mg/L), aqueous solution concentrations. Controls without soil were also prepared, and indicated negligible chemical losses by processes other than sorption by the soil.

Desorption was measured by the successive dilution method, from the highest equilibrium point of the sorption isotherm, replacing the 4 mL of supernatant solution removed for the sorption analysis with 4 mL of deionized water. The tubes were re-suspended, shaken, and re-equilibrated for 24 h at 25 ± 2 °C. Subsequently, the suspensions were centrifuged and 4 mL of the supernatant solutions were removed, stabilized with methanol, filtered, and analyzed by chiral HPLC. This procedure was repeated three times to complete the desorption isotherm.

Data from both sorption and desorption isotherms were fitted using the log-linear form of the empirical Freundlich model:

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

where K_f ((mg/kg)/(mg/L) ^{N_f}) and N_f (unitless) are the Freundlich constants.

Additionally, to evaluate the irreversibility of the sorption-desorption process, the Thermodynamic Index of Irreversibility (TII) was used:

$$TII = 1 - (N_{fd}/N_f)$$

where N_f and N_{fd} are the Freundlich constants obtained from the sorption and desorption isotherm, respectively.²⁷ TII ranges from 0 to 1, where TII= 0 denotes completely reversible sorption and TII= 1 indicates irreversible sorption.

Incubation Experiment. An incubation experiment in darkness and under aerobic conditions was designed to evaluate the dissipation of carvone enantiomers under three treatments, unamended soil and soil amended with BC or OC1 at 2% (w/w). In duplicate, 50 g of soil, either unamended or amended, were placed in glass jars and spiked with 0.5 mL of the methanolic stock solution (200 mg/L) of (R+S)-carvone to reach a final rate of 2 mg of (R+S)-carvone/kg dry soil. The moisture content was adjusted to a value close to the water holding capacity of the soil (~ 30%) and was regularly checked and gravimetrically re-adjusted by adding distilled water as necessary. Separately and prior to carvone spiking, another set of soil samples (unamended and BC- and OC1-amended soil) was autoclaved as detailed above, to estimate the biodegradation of carvone in sterilized soil samples. All glass jars were incubated at 25 ± 2 °C for one week. At selected times, duplicate subsamples of 3 g of soil were sampled from each jar and immediately frozen until analyzed. Residues of R- and S-carvone were determined by extraction with 8 mL of methanol by shaking for 24 h. Recoveries were greater than 95% of the R- and S-carvone freshly applied to the unamended or amended soil. The extracts were analyzed by chiral HPLC.

Experimental data of R- and S-carvone dissipation in unamended and amended soil were fitted to a first-order kinetic rate law:

$$C = C_0 e^{-kt}$$

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

The enantioselectivity of the dissipation process for carvone under the different treatments was evaluated using the enantioselective factor, ES, which considers the excess of

the rate of the fastest over the slowest degraded enantiomers using the formula proposed by Müller and Buser:²⁸

$$ES = (k_1 - k_2) / (k_1 + k_2)$$

where k_1 and k_2 are the first-order rate constants for the fastest and slowest degraded enantiomer, respectively. The ES value ranges between 0 (for a non-enantioselective process) and 1 (for a fully enantioselective process: $k_2 = 0$ or $k_2 \ll k_1$).

Enantiomer fractions (EF) along the dissipation experiment were calculated according to Harner et al.²⁹ criteria as:

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

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

Soil Respiration Experiment. The effect of the amendments (BC and OCl) as well as the effect of the autoclaving process on soil respiration was also assessed. This parameter was measured following the alkali trapping–titrimetric procedure described by Anderson.³⁰ Soil samples (20 g) were placed in volumetric flasks joined to a trap containing 20 mL of 0.2 M NaOH to capture the released CO₂. The flasks were then closed with Teflon-lined stoppers, and incubated at 25 ± 2 °C. After 7 days, an excess of BaCl₂ was added to the alkali traps to precipitate carbonate, and then the unreacted NaOH was back-titrated with 0.1 M HCl using phenolphthalein as an indicator. In this way, the amount of CO₂ released by samples of

unamended soil and of soil amended with BC and OCl, either autoclaved or non-autoclaved, was quantified. The experiment was performed in duplicate.

Column Leaching Experiment. Leaching was studied in 30 cm length $\times$ 3.1 cm internal diameter (i.d.) glass columns filled with 160 g of dry soil (unamended soil) to a height of 20 cm of soil in each column (bulk density $\approx$ 1.1 g/cm³). Amendment consisted of mixing the upper 5 cm of soil in the column (40 g) with BC or OCl at a rate of 2% (0.8 g), which was equivalent to about 10 t/ha. Glass wool was placed at the bottom of the columns to avoid soil losses, and 10 g of sea sand was added at the bottom and top of all soil columns. Next, the columns were saturated by adding 100 mL of distilled water and allowed to drain overnight. The pore volume calculated for the soil columns was 60 ± 1 mL. Subsequently, (R+S)-carvone was applied to the top of the columns in amount equivalent to a dose of 2 kg/ha (0.75 mL of the 200 mg/L methanolic stock solution of R+S carvone). Next, three times a day, 15 mL of distilled water was added to complete 12 additions in 4 days. Three hours after each water addition, the leachates were collected in vials containing 5 mL of methanol and stored at 4 °C in the dark prior to analysis. Then, the leachates were filtered and analyzed by chiral HPLC to determine the R- and S-carvone concentrations. At the end of the leaching experiment, the residual amounts of carvone enantiomers remaining in the soil columns at different depths (0–5, 5–10, 10–15, and 15–20 cm), were extracted with 100 mL of methanol and analyzed by chiral HPLC.

Analysis of Carvone Enantiomers. Carvone enantiomers were determined by chiral HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector. The enantiomers were resolved with a Chiralpak IG column (150 mm length $\times$ 4.6 mm i.d. and 3 μ m of particle size from Chiral Technologies Europe) using a mobile phase of acetonitrile:water (50:50) at a flow rate of 1 mL/min, with a sample injection volume of 50 μ L and UV detection at 236 nm. The retention times under these conditions were 8.0 and 8.9

min for R-carvone and S-carvone, respectively. The limit of quantification (LOQ), calculated as the concentration resulting in a signal to noise ratio of 10:1, was 0.01 mg/L. More details regarding the analytical conditions can be found elsewhere.²⁴

Data Analysis. Statistical analysis was performed using Sigmaplot v.13.0. Standard error was used to specify variability among independent replicates. Soil respirations, enantiomer fractions (EF), distribution coefficients (K_d), first-order dissipation rate constants, and column leached and extracted fractions were compared using paired t-test to classify significant differences between treatments. Significant statistical differences were considered at $p < 0.05$.

RESULTS AND DISCUSSION

Sorption-desorption Isotherms of Carvone Enantiomers on Unamended and Amended Soil. Sorption of R- and S-carvone by unamended soil and by soil amended with OCl and BC was non-enantioselective; both enantiomers were sorbed equally at all tested concentrations (Figure 1). The same behavior was observed in a previous work, where the sorption of carvone enantiomers in six different soils, was assessed,²⁴ and also for the sorption of the chiral phytohormone abscisic acid by unamended and amended soil.¹⁴ It is known that when sorption of chiral solutes on soil is measured using racemic initial solutions, the process is usually apparently non-enantioselective.^{31–33} The fact that for all treatments the sorption-desorption isotherms of R- and S-carvone overlapped (Figure 1) also confirmed that little, if any, biodegradation occurred during the experiment. This is because, given that the biodegradation of carvone is an enantioselective process, biodegradation during the sorption-desorption experiment would have resulted in the pathway of sorption or desorption of R-carvone being different from that of S-carvone.²⁴

The sorption isotherms fitted well to the Freundlich equation ($R^2 > 0.989$). The uptake of carvone enantiomers was non-linear with N_f values ranging from 0.75 to 0.83 (Table S1). This

indicated that sorption was concentration-dependent and sorption sites became limited at high carvone solution concentrations.³⁴ The addition of the amendments to the soil enhanced the sorption of carvone enantiomers. According to the K_f values, sorption followed the order: unamended soil < OCl-amended soil ~ BC-amended soil (Table S1).

Different types of surface interactions could have controlled carvone sorption in OCl- and BC-amended soil. The improved affinity of R- and S-carvone towards OCl-amended soil can be ascribed to hydrophobic-type interactions with the interlayer organic phase of the OCl,³⁵ facilitated by the large basal spacing value ($d_{001} = 2.25$ nm) that OCl possesses.^{36,37} Polar interactions between the ammonium group of the alkylammonium cations (CTMA) of OCl and the O-containing functionality present in the structure of carvone (Figure S1) could also have contributed to the sorption mechanism.^{21,37,38} Regarding BC-amended soil, several works have reported that sorption of organic compounds in BC-amended soils is influenced by physical-chemical properties of the BC such as its carbon content and specific surface area.^{39,40} For the BC used in this study, neither the carbon content nor the SSA was particularly high, which suggests that other BC properties could have contributed to the sorption of carvone. Biochars produced at lower pyrolysis temperatures (e.g., 400 °C) contain a considerable amount of amorphous organic matter, which provides a partitioning medium for the sorption of organic compounds.^{41,42} Furthermore, the polarity of carvone could have played an important role, given that low temperature BCs maintain surface functional groups from feedstock which favor polar interactions, as reported for other polar organic compounds.¹⁷

The bioavailability and bioactivity of carvone in soil will be controlled, not only by the extent of sorption, but also by the degree of sorption reversibility. The shape of desorption isotherms was consistent with the sorption mechanism proposed above. Only in the case of OCl-amended soil, carvone enantiomers exhibited a fully reversible behavior with the

desorption branches completely coinciding with the sorption ones (Figure 1). This indicated that sorption was dominated by weak, hydrophobic-type interactions, as proposed for the interaction of different organic compounds with organophilic organoclays.^{20,35} On the contrary, both unamended soil and BC-amended soil showed certain resistance to carvone desorption (Figure 1). The effect was more pronounced in the case of BC-amended soil, with TII values of 0.76 and 0.77 for R- and S-carvone, respectively, in comparison to the TII values of 0.49 and 0.50 for R- and S-carvone in unamended soil (Table S1). The hysteresis observed indicated that there was a fraction of carvone molecules in BC-amended soil, which was strongly or irreversibly bound to soil or BC particles. It has been reported that polar interactions contributed to hysteresis more than the partitioning mechanism in the sorption of organic compounds on biochar.⁴³ Furthermore, the L-type isotherms indicated sorption sites with different energy levels which could have resulted in rate-limited sorption or desorption.⁴³ Additional causes of irreversibility of organic compounds in BC-amended soil are linked to intraparticle diffusion, pore deformation during sorption, or entrapment into micropores,⁴⁴ together with some experimental artefacts.⁴⁵

Dissipation. The dissipation curves of carvone enantiomers in unamended soil and in soil amended with OCl and BC are shown in Figure 2. The first-order dissipation rate constants and half-lives for R- and S-carvone together with the enantioselectivity factor (ES) are given in Table 1. The degradation of carvone enantiomers followed the same pattern as that observed by Gámiz et al.²⁴ for unamended soils, that is, dissipation was enantioselective and the S-enantiomer was degraded faster than the R-enantiomer (Figure 2) in all treatments. Specifically, in unamended soil, the dissipation of both enantiomers was faster than to that in BC- and OCl-amended soil ($p < 0.05$), with half-lives of 1.2 days for R-carvone and 0.7 days for S-carvone (Table 1). These half-life values are in agreement with data formerly reported for the dissipation of carvone in a similar sandy loam soil.²⁴ The short half-lives revealed the

need to increase the persistence of this type of compound if it is intended to be used as a residual (soil-applied) pest management product.^{46,47}

Amendment of the soil affected the dissipation rates of the enantiomers, although the preferential degradation of S-carvone over R-carvone remained (Figure 2). The addition of OCl or BC to the soil resulted in an increase in the persistence of both R- and S-carvone ($p > 0.05$) (Table 1). When comparing treatments, the persistence of R-carvone in soil ($t_{1/2} = 1.2$ days) significantly changed upon the addition of OCl ($t_{1/2} = 2.6$ days) or BC ($t_{1/2} = 5.6$ days) ($p < 0.05$) (Table 1). Similarly, the addition of OCl and BC to the soil increased the half-life of S-carvone from 0.7 days to 1.6 and 3.4 days, respectively ($p < 0.05$) (Figure 2 and Table 1).

Regarding the enantioselectivity, when ES was used to describe the overall enantioselectivity in the dissipation process, both unamended and OCl-amended soil showed an ES value of 0.24 (Table 1). This indicated that the dissipation process, as described by ES, was scarcely enantioselective, most likely, due to the short half-lives observed, which were not long enough to develop a marked enantioselectivity. Nevertheless, the low persistence of S-carvone in unamended soil rendered an EF= 0 at only 3 days after the spiking with the enantiomers (Figure S2), contrasting with an EF= 0.31 ($p < 0.05$) for OCl-amended soil at the same sampling time. This result confirmed that for compounds which are rapidly degraded in soil, the interpretation of enantioselectivity by ES becomes complicated. For BC-amended soil, ES reached a value of only 0.18 (Table 1), which indicated on even lower enantioselectivity than in unamended soil, also supported by the EF value of 0.44 at the end of the experiment (Figure S2).

Enantioselectivity in the dissipation of chiral compounds has been related to biological degradation processes.³² By autoclaving the soil, we confirmed that carvone dissipation in soil mainly proceeded via biodegradation.²⁴ More than 80% of the initial carvone added to the

sterilized (unamended or amended) soil samples remained after 8 days of incubation, and the remaining residues were racemic ($EF = 0.5$) (Figures 2 and S2).

The greater sorption registered for carvone enantiomers in the amended soil (Figure 1, Table S1) would explain their longer persistence compared to the unamended soil (Figure 2, Table 1). Greater sorption typically implies greater persistence in soil, because the sorbed compound is protected from biodegradation.⁴⁸ Enantioselective dissipation patterns can also change due to an indirect effect of sorption,⁴⁹ because sorption will preserve originally racemic residues which are not as degraded and therefore, more racemic in soils.^{14,32} It should be noted that the bioavailability and bioaccessibility of organic compounds in soil will depend not only on sorption, but also on the desorption process.⁴⁸ In spite of the fact that BC- and OCl-amended soil displayed similar affinity for carvone enantiomers, the latter did not extend the presence of S-carvone as much as BC did. This finding could be related to the higher reversibility of carvone in OCl-amended soil compared to the resistance to desorption manifested by R- and S-carvone in BC-amended soil (Figure 1). In addition, Gámiz et al.¹⁴ recently reported that organoclays can lose their sorption performance with incubation time in soils, whereas the opposite appears to happen with BC, which progressively increased its affinity for organic compounds.

It could be argued that the increase in persistence of carvone enantiomers in soil upon amendment with OCl and BC, could have been a consequence of some toxic effect of the amendments on the soil microbial activity.⁵⁰ To address this possibility, soil respiration measurements were performed on non-autoclaved and autoclaved soil samples, and the result is shown in Figure 3. First, it was confirmed that sterilization, through three consecutive autoclaving steps, significantly reduced the soil respiration in all treatments ($p < 0.05$). This further supported biodegradation as the main degradation pathway of carvone enantiomers in the soil. Additionally, regarding a possible toxic effect of the amendments, data from the non-

sterilized treatment indicated that the addition of BC increased the soil respiration ($p < 0.05$), whereas the addition of OCl did not have any significant effect ($p > 0.05$) (Figure 3). This supported that the greater $t_{1/2}$ values and variations in EF values for carvone upon soil amendment, were most likely to be a consequence of the indirect effect of sorption rather than of a direct (negative) effect of the amendments on microbial degraders.

Leaching. Very low amounts of R- and S-carvone leached from the unamended soil columns. These amounts scarcely reached 5% and 1% for R- and S-carvone, respectively (Table 2). For OCl- and BC-amended soil, neither R-carvone nor S-carvone was detected in leachates (Table 2). This behavior can be attributed to: i) the rapid degradation of the enantiomers during leaching through the soil column and ii) the greater sorption registered after amending the soil with OCl or BC, which could have retained the enantiomers in the first 0-5 cm of soil, the portion of the soil where the amendment was added. These two mechanisms were confirmed by the extraction of the soil columns at the end of the leaching experiment (Figure 4, Table 2). The presence of the amendments in the soil increased the amounts of R-carvone extracted ($p < 0.05$) in respect to unamended soil and followed the order: unamended (7%) < OCl-amended (22%) < BC-amended soil (39%) (Table 2). For S-carvone, differences between the three treatments were also significant ($p < 0.05$) and the trend was identical: unamended (3%) < OCl-amended (12%) < BC-amended soil (39%) (Table 2). These results were in agreement with the tendency observed in the dissipation experiment where the amendments enhanced the persistence of the enantiomers in the soil (Figure 2), so that sorption delayed the dissipation and allowed the enantiomers to remain for longer in the soil. The effect of sorption was more noticeable for BC-amended soil, where the entire amount extracted corresponded to the upper 5 cm of soil (where BC was added) and carvone was not detected further down (Figure 4). Likewise, this probably reflected the difficulty that R- and S-carvone encountered to desorb from BC-amended soil compared to

unamended and OCl-amended soil (Figure 1). In the latter cases, both R- and S-carvone surpassed the 0-5 cm of soil and reached the next 15 cm of soil (Figure 4). This indicated weaker interaction and easier desorption of the enantiomers in unamended soil and OCl-amended soil assisted by rapid degradation, bearing in mind that the non-sorbed fraction is expected to be more susceptible to microbial degradation than the sorbed fraction.¹⁴ As a result, the amount of carvone not recovered can reasonably be endorsed to the biotic transformation rather than to the formation of bound residues.

Differences in the enantioselectivity between treatments were evaluated according to the amounts extracted from the soil columns. The calculated EF values of extracted residues of carvone were similar for unamended soil ($EF=0.32 \pm 0.02$) and for OCl-amended soil ($EF=0.32 \pm 0.03$). In contrast, carvone residues in BC-amended soil remained completely racemic with $EF= 0.5 \pm 0.03$, which suggested sorption, which normally does not modify the enantiomer distribution in soils, keeping carvone residues more racemic in soil.¹⁴

In summary, OCl and BC were appropriate amendments in increasing the affinity of soil towards carvone enantiomers and modifying their dissipation and leaching patterns. Carvone was sorbed similarly on both OCl- and BC-amended soil, but divergences were found in the desorption process. BC-amended soil manifested hysteretic sorption, whereas a fully reversible process for OCl-amended soil was observed. There was no enantioselectivity in carvone sorption. Dissipation of carvone was microbially mediated and enantioselective, with preferential degradation of S-carvone over R-carvone in both unamended and amended soil. The degradation and leaching of carvone enantiomers was influenced by the addition of OCl and BC to soil; both amendments increased the persistence and reduced the leaching of the enantiomers in soil, but the effect was more pronounced and resulted in more racemic residues for BC-amended soil. Results from this study suggest that the addition of organoclays and biochars to soil could be a good strategy to prolong the presence of carvone

enantiomers in the topsoil by reducing their biodegradation and transport losses. In this way, their bioactivity in the rhizosphere could be enhanced, which may be useful in taking advantage of their allelopathic activity.

ABBREVIATIONS USED

BC, biochar; EF, enantiomer fraction; ES, enantioselectivity factor; HPLC, high performance liquid chromatography; K_d , distribution coefficient; OCl, cetyltrimethylammonium-modified Arizona montmorillonite.

ASSOCIATED CONTENT

Supporting Information

Supporting Information available:

Table S1, Freundlich Coefficients for R- and S-carvone on unamended and amended soil with OCl or BC; Figure S1, structures of R-carvone and S-carvone; Figure S2, profiles of enantiomer fraction (EF) during the incubation experiment.

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

AUTHOR INFORMATION

Corresponding Author

*(B.G.): Phone: +34 954624711,

E-mail: bgamiz@irnase.csic.es.

ORCID:

Beatriz Gámiz: 0000-0002-7244-778X

Rafael Celis: 0000-0002-0548-0774

417

418 **Funding**

419 This work was financed by the Spanish Ministry of Science, Innovation and Universities
420 (MICINN Project AGL2017-82141-R). B.G. would like to thank MICINN for her Juan de la
421 Cierva-Incorporación postdoctoral contract (IJCI-2015-23309).

422

423 **Notes**

424 The authors declare no competing financial interest.

425

REFERENCES

- (1) Cordeau, S.; Triolet, M.; Wayman, S.; Steinberg, C.; Guillemin, J.-P. Bioherbicides: Dead in the water? a review of the existing products for integrated weed management. *Crop Prot.* **2016**, *87*, 44–49.
- (2) Sparks, T. C.; Hahn, D. R.; Garizi, N. V. Natural products, their derivatives, mimics and synthetic equivalents: role in agrochemical discovery. *Pest Manag. Sci.* **2017**, *73*, 700–715.
- (3) Soltys, D.; Krasuska, U.; Bogatek, R.; Gniazdowska, A. Allelochemicals as Bioherbicides - Present and Perspectives. In *Herbicides - Current Research and Case Studies in Use*; Price, A. J., Kelton, J. A., Eds.; Intech, 2013; pp 517–542.
- (4) Zeng, R.; Mallik, A.; Luo, S. *Allelopathy in Sustainable Agriculture and Forestry*; Zeng, R., Mallik, A., Luo, S., Eds.; Springer: New York, 2008.
- (5) Chaimovitsh, D.; Shachter, A.; Abu-Abied, M.; Rubin, B.; Sadot, E.; Dudai, N. Herbicidal activity of monoterpenes is associated with disruption of microtubule functionality and membrane integrity. *Weed Sci.* **2017**, *65*, 19–30.
- (6) Vokou, D.; Douvli, P.; Blionis, G. J.; Halley, J. M. Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. *J. Chem. Ecol.* **2003**, *29*, 2281–2301.
- (7) Abraham, D.; Braguini, W. L.; Kelmer-Bracht, A. M.; Ishii-Iwamoto, E. L. Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *J. Chem. Ecol.* **2000**, *26*, 611–624.
- (8) Weidenhamer, J. D.; Macias, F. A.; Fischer, N. H.; Williamson, G. B. Just how insoluble are monoterpenes? *J. Chem. Ecol.* **1993**, *19*, 1799–1807.
- (9) Li, B.; Liu, H.; Amin, M.; Wegiel, L. A.; Taylor, L. S.; Edgar, K. J. Enhancement of naringenin solution concentration by solid dispersion in cellulose derivative matrices. *Cellulose* **2013**, *20*, 2137–2149.
- (10) Xiao, Z.; Le, C.; Xu, Z.; Gu, Z.; Lv, J.; Shamsi, I. H. Vertical leaching of allelochemicals affecting their bioactivity and the microbial community of soil. *J. Agric. Food Chem.* **2017**, *65*, 7847–7853.
- (11) Tharayil, N.; Bhowmik, P. C.; Xing, B. Preferential sorption of phenolic phytotoxins to soil: implications for altering the availability of allelochemicals. *J. Agric. Food Chem.* **2006**, *54*, 3033–3040.
- (12) Oliveira, J. L. D.; Campos, E. V. R.; Pereira, A. E. S.; Pasquoto, T.; Lima, R.; Grillo, R.; Andrade, D. J. De; Santos, F. A. Dos; Fraceto, L. F. Zein nanoparticles as eco-friendly carrier systems for botanical repellents aiming sustainable agriculture. *J. Agric. Food Chem.* **2018**, *66*, 1330–1340.
- (13) Campos, E. V. R.; Proenç, P. L. F.; Oliveira, J. L.; Perei, A. E. S.; Pasquoto-stigliani, T.; Lima, R.; Melville, C. C.; Jaqueline, F. Carvacrol and linalool co-loaded in β -cyclodextrin-grafted chitosan nanoparticles as sustainable biopesticide aiming pest control. *Sci. Rep.* **2018**, *8*, 1–14.
- (14) Gámiz, B.; Cox, L.; Hermosín, M. C.; Spokas, K.; Celis, R. Assessing the effect of organoclays and biochar on the fate of abscisic acid in soil. *J. Agric. Food Chem.* **2017**, *65*, 29–38.
- (15) Lehmann, J.; Rillig, M. C.; Thies, J.; Masiello, C. A.; Hockaday, W. C.; Crowley, D.

- Biochar effects on soil biota – a review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836.
- (16) Lehmann, J.; Kleber, M. The Contentious Nature of Soil Organic Matter. *Nature* **2015**, *528*, 60–68.
- (17) Gámiz, B.; Velarde, P.; Spokas, K. A.; Hermosín, M. C.; Cox, L. Biochar soil additions affect herbicide fate: importance of application timing and feedstock species. *J. Agric. Food Chem.* **2017**, *65*, 3109–3117.
- (18) Li, J.; Li, Y.; Wu, M.; Zhang, Z.; Lü, J. Effectiveness of low-temperature biochar in controlling the release and leaching of herbicides in soil. *Plant Soil* **2013**, *370*, 333–344.
- (19) Ruiz-Hitzky, E.; Aranda, P.; Darder, M.; Ogawa, M. Hybrid and biohybrid silicate based materials: molecular vs. block-assembling bottom-up processes. *Chem. Soc. Rev.* **2011**, *40*, 801–828.
- (20) Cornejo, J.; Celis, R.; Pavlovic, I.; Ulbarri, M. A. Interactions of pesticides with clays and layered double hydroxides: a review. *Clay Miner.* **2008**, *43*, 155–175.
- (21) Gámiz, B.; Celis, R.; Hermosín, M. C.; Cornejo, J. Organoclays as soil amendments to increase the efficacy and reduce the environmental impact of the herbicide fluometuron in agricultural soils. *J. Agric. Food Chem.* **2010**, *58*, 7893–7901.
- (22) European Food Safety Authority. A Statement on the Assessment of the Pesticide Active Substance Carvone (d-/l-Carvone at a Ratio of at Least 100:1) for Inclusion in Annex IV of Regulation (EC) No 396/2005. *EFSA J.* **2016**, *14*, 4405.
- (23) De Carvalho, C. C. C. R.; Da Fonseca, M. M. R. Carvone: Why and how should one bother to produce this terpene. *Food Chem.* **2006**, *95*, 413–422.
- (24) Gámiz, B.; Hermosín, M. C.; Celis, R. Appraising factors governing sorption and dissipation of the monoterpene carvone in agricultural soils. *Geoderma* **2018**, *321*, 61–68.
- (25) Cabrera-Mesa, A.; Spokas, K. A. Impacts of Biochar (Black Carbon) Additions on the Sorption and Efficacy of Herbicides. In *Herbicides and Environment*; Kortenkamp, A., Ed.; In Tech: Rieja, Croatia, 2010; pp 315–340.
- (26) PPDB. Pesticides Properties Database. *University of Hertfordshire*. 2018.
- (27) Sander, M.; Lu, Y.; Pignatello, J. J. A thermodynamically based method to quantify true sorption hysteresis. *J. Environ. Qual.* **2005**, *34*, 1063–1072.
- (28) Müller, M. D.; Buser, H. Environmental behavior of acetamide pesticide stereoisomers. 2. stereo- and enantioselective degradation in sewage sludge and soil. *Environ. Sci. Technol.* **1995**, *29*, 2031–2037.
- (29) Harner, T.; Wiberg, K.; Norstrom, R. Enantiomer fractions are preferred to enantiomer ratios for describing chiral signatures in environmental analysis. *Environ. Sci. Technol.* **2000**, *34*, 218–220.
- (30) Anderson, J. P. E. Soil Respiration. In *Methods of soil analysis, Part 2*; Page, A. L., Page, R. H., Miller, R. H., Eds.; ASA: Madison, WI, 1982; pp 831–871.
- (31) Matallo, M.; Romero, E.; Sanchez-Rasero, F.; Pena, A.; Dios, G. Adsorption of mecoprop and dichlorprop on calcareous and organic matter amended soils: comparative adsorption of racemic and pure enantiomeric forms. *J. Environ. Sci. Heal. Part B* **1998**, *33*, 51–66.
- (32) Gámiz, B.; Pignatello, J. J.; Cox, L.; Hermosín, M. C.; Celis, R. Environmental fate of

- the fungicide metalaxyl in soil amended with composted olive-mill waste and its biochar: an enantioselective study. *Sci. Total Environ.* **2016**, *541*, 776–783.
- (33) Liang, C.; Huang, J.; Zhang, X. Effects of engineered nanoparticles on the enantioselective transformation of metalaxyl agent and commercial metalaxyl in agricultural soils. *J. Agric. Food Chem.* **2016**, *64*, 7688–7695.
- (34) Giles, C. H.; MacEwan, T. H.; Nakhwa, S. N.; Smith, D. Studies in adsorption. part xi. a system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface area of solids. *J. Chem. Soc.* **1960**, 3973–3993.
- (35) Zhao, H.; Jaynes, W. F.; George F. Vance. Sorption of the ionizable organic compound, dicamba (3,6- dichloro-2-methoxybenzoic acid), by organo-clays. *Chemosphere* **1996**, *33*, 2089–2100.
- (36) Jaynes, W. F.; Boyd, S. A. Clay mineral type and organic compound sorption by hexadecyltrimethylammonium-exchanged clays. *Soil Sci. Soc. Am. J.* **1991**, *55*, 43–48.
- (37) Gámiz, B.; Hermosín, M. C.; Cornejo, J.; Celis, R. Hexadimethrine-montmorillonite nanocomposite: characterization and application as a pesticide adsorbent. *Appl. Surf. Sci.* **2015**, *332*, 606–613.
- (38) Cabrera, A.; Cox, L.; Spokas, K. A.; Celis, R.; Hermosín, M. C.; Cornejo, J.; Koskinen, W. C. Comparative sorption and leaching study of the herbicides fluometuron and 4-chloro-2-methylphenoxyacetic acid (MCPA) in a soil amended with biochars and other sorbents. *J. Agric. Food Chem.* **2011**, *59*, 12550–12560.
- (39) Yu, X.; Pan, L.; Ying, G.; Kookana, R. S. Enhanced and irreversible sorption of pesticide pyrimethanil by soil amended with biochars. *J. Environ. Sci.* **2010**, *22*, 615–620.
- (40) Khorram, M. S.; Zhang, Q.; Lin, D.; Zheng, Y.; Fang, H.; Yu, Y. Biochar: a review of its impact on pesticide behavior in soil environments and its potential applications. *J. Environ. Sci.* **2016**, *44*, 269–279.
- (41) Yavari, S.; Malakahmad, A.; Sapari, N. B. Biochar efficiency in pesticides sorption as a function of production variables—a review. *Environ. Sci. Pollut. Res.* **2015**, *22*, 13824–13841.
- (42) Kearns, J. P.; Wellborn, L. S.; Summers, R. S.; Knappe, D. R. U. 2,4-D adsorption to biochars: effect of preparation conditions on equilibrium adsorption capacity and comparison with commercial activated carbon literature data. *Water Res.* **2014**, *62*, 29–28.
- (43) Liping, L.; Guanghuan, C.; Jingyou, D.; Mingyang, S.; Huanyu, C.; Qiang, Y.; Xinhua, X. Mechanism of and relation between the sorption and desorption of nonylphenol on black carbon-inclusive sediment. *Environ. Pollut.* **2014**, *190*, 101–108.
- (44) Sander, M.; Pignatello, J. J. Sorption irreversibility of 1,4-dichlorobenzene in two natural organic matter-rich geosorbents. *Environ. Toxicol. Chem.* **2009**, *28*, 447–457.
- (45) Koskinen, W. C.; O'Connor, G.; Cheng, H. Characterization of hysteresis in the desorption of 2,4,5-t from soils. *Soil Sci. Soc. Am. J.* **1979**, *43*, 871–874.
- (46) Duke, S. O.; Romagni, J. G.; Dayan, F. E. Natural products as sources for new mechanisms of herbicidal action. *Crop Prot.* **2000**, *19*, 583–589.
- (47) Gámiz, B.; Hermosín, M. C.; Celis, R. Sorption, persistence and leaching of abscisic acid in agricultural soils: an enantiomer-selective study. *Geoderma* **2016**, *269*, 112–

118.

- (48) Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J.; Mejuto, J. C.; García-Río, L. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.* **2008**, *123*, 247–260.
- (49) Celis, R.; Gámiz, B.; Adelino, M. A.; Hermosín, M. C.; Cornejo, J. Environmental behavior of the enantiomers of the chiral fungicide metalaxyl in mediterranean agricultural soils. *Sci. Total Environ.* **2013**, *444*, 288–297.
- (50) Sarkar, B.; Megharaj, M.; Xi, Y.; Krishnamurti, G. S. R.; Naidu, R. Sorption of quaternary ammonium compounds in soils: implications to the soil microbial activities. *J. Hazard. Mater.* **2010**, *184*, 448–456.

FIGURE CAPTIONS

Figure 1. Sorption–desorption isotherms of R-carvone and S-carvone on unamended soil and soil amended with OCl or BC.

Figure 2. Dissipation curves for R- and S-carvone during the incubation experiment with non-autoclaved (circles) and autoclaved (squares) soils. In the dissipation curves, symbols indicate experimental data points obtained from the mean of two independent replicates and four observations per treatment. Lines correspond to the fits to first-order dissipation kinetics for non-autoclaved samples. Error bars denote standard errors of independent duplicates.

Figure 3. Soil respiration values of unamended soil (Soil) and soil treated with organoclay (Soil + OCl) or biochar (Soil + BC). Error bars indicate $\pm$ standard errors of independent duplicate measurements.

Figure 4. Percentage of carvone extracted from different depths of soil columns at the end of the leaching experiment. Error bars indicate $\pm$ standard errors of triplicate columns.

Table 1. Single First-Order Dissipation Rate Constants and Half-Lives for R- and S-Carvone and the Enantioselectivity Factor (ES) in Unamended or Amended Soils

		<i>R</i> -carvone			<i>S</i> -carvone			ES ^a
treatment		k	t _{1/2}	R ²	k	t _{1/2}	R ²	
		(d ⁻¹)	(d)		(d ⁻¹)	(d)		
unamended soil	non-autoclaved	0.598 ± 0.074 ^b	1.2	0.969	0.989 ± 0.043	0.7	0.984	0.24
	autoclaved	n.f. ^c			n.f.			
OCl-amended soil	non-autoclaved	0.267 ± 0.025	2.6	0.975	0.435 ± 0.018	1.6	0.996	0.24
	autoclaved	n.f.			n.f.			
BC-amended soil	non-autoclaved	0.125 ± 0.008	5.6	0.984	0.181 ± 0.006	3.4	0.996	0.18
	autoclaved	n.f.			n.f.			

^a Enantioselectivity factor: ES=(k_S-k_R)/(k_S+k_R)

^b Value ± standard error.

^c Not fitted due to very low dissipation

597

598 **Table 2. R- and S-carvone Leached, Extracted from Soil Columns, and not Recovered after the Leaching Experiment with Unamended**
599 **and OCl- and BC-amended Soil**

600
601

Fraction		unamended soil	OCl-amended soil	BC-amended soil
leached (%)	<i>R</i> -carvone	5 ± 0 ^a	< 1	< 1
	<i>S</i> -carvone	1 ± 1	< 1	< 1
extracted (%)	<i>R</i> -carvone	7 ± 3	22 ± 7	39 ± 1
	<i>S</i> -carvone	3 ± 1	12 ± 3	39 ± 1
not recovered (%)	<i>R</i> -carvone	88 ± 3	78 ± 7	61 ± 1
	<i>S</i> -carvone	96 ± 2	88 ± 3	61 ± 1

602
603

^a Mean ± standard error of triplicate columns.

Figure 1. Sorption–desorption isotherms of R-carvone and S-carvone on unamended soil and soil amended with OCI or BC.

Figure 2. Dissipation curves for R- and S-carvone during the incubation experiment with non-autoclaved (circles) and autoclaved (squares) soils. In the dissipation curves, symbols indicate experimental data points obtained from the mean of two independent replicates and four observations per treatment. Lines correspond to the fits to first-order dissipation kinetics for non-autoclaved samples. Error bars denote standard errors of independent duplicates.

Figure 3. Soil respiration values of unamended soil (Soil) and soil treated with organoclay (Soil + OCl) or biochar (Soil + BC). Error bars indicate $\pm$ standard errors of independent duplicate measurements.

Figure 4. Percentage of carvone extracted from different depths of soil columns at the end of the leaching experiment. Error bars indicate + standard errors of triplicate columns.