**Effect of synthetic clay and biochar addition on dissipation and
enantioselectivity of tebuconazole and metalaxyl in an agricultural soil:
laboratory and field experiments**

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ABSTRACT

Laboratory and field experiments were conducted to assess how the addition of oleate-modified hydrotalcite (clay) and biochar (BC) to an agricultural soil affected the sorption, leaching, persistence, and enantiomeric composition of soil residues of two chiral fungicides, tebuconazole and metalaxyl. Laboratory experiments showed that the sorption of both fungicides ranked as follows: unamended soil < BC-amended soil < clay-amended soil. The addition of clay at a rate of 1% increased metalaxyl soil sorption coefficient (K_d) from 0.34 to 3.14 L kg⁻¹ and that of tebuconazole from 2.4 to 47.4 L kg⁻¹. In our experimental set-up, field plots were either unamended or amended with clay (2 t ha⁻¹) or BC (4 t ha⁻¹), and subsequently treated with a mixture of tebuconazole and metalaxyl at 3 and 6 kg ha⁻¹, respectively. The leaching, persistence, and enantiomer composition of fungicides residues was monitored by sampling at different soil depths (0-5, 5-10, 10-20 cm) for 98 days. No significant changes in the scarce mobility and long persistence of tebuconazole upon amending the soil with clay or BC were observed. In contrast, sorption to clay and BC particles reduced the leaching and degradation of metalaxyl and the clay increased its persistence in the topsoil compared to the unamended soil. The enantioselective analysis of tebuconazole and metalaxyl soil residues indicated that tebuconazole remained mostly racemic along the experiment, whereas for metalaxyl the concentration of *S*-enantiomer was greater than the concentration of *R*-enantiomer, more so at longer experimental times and deeper horizons. Nevertheless, for the top 0-5 cm soil layer metalaxyl remained more racemic in clay- and BC-amended soil than in unamended soil. Our results show that addition of amendments with high sorptive capacities can be beneficial in reducing leaching and degradation losses of chiral pesticide enantiomers from the topsoil, and that sorption by the amendments can influence the final enantiomeric composition of pesticide residues.

Keywords: chiral pesticides; degradation; leaching; organic amendments; hydrotalcite

1. Introduction

Modern agriculture relies on the use of pesticides to face the growing global demand for food. It is undeniable that the contribution of pesticides has increased the agricultural production over the past decades, but along with its advantages, there are environmental problems associated with pesticide use (Lefebvre et al., 2015; Pimentel et al., 2005; Waterfield and Zilberman, 2012). These problems are often a consequence of the movement of these chemicals to unwanted zones, which represents a potential risk for non-target organisms including human beings (Rice et al., 2007). In this regard, a number of recent monitoring studies have shown the presence of pesticides in soil, sediments, and surface and ground waters. This is indeed the case of the fungicides tebuconazole and metalaxyl, revealing the importance of controlling their presence in the environment (Li et al., 2015; Masiá et al., 2015; Pose-Juan et al., 2015; Robles-Molina et al., 2014).

Tebuconazole and metalaxyl are both systemic fungicides with protective and curative actions (Tomlin, 2006), but they diverge in their physico-chemical properties, as a result of which they show different behavior in soils. Tebuconazole has a water solubility of 36 mg L⁻¹ and an octanol-water partition coefficient (log P) of 3.7 (Tomlin, 2006). Accordingly, it is strongly sorbed by soil organic matter and slightly mobile in soil (Aldana et al., 2011; Čadková et al., 2013; Herrero-Hernández et al., 2011; Vallée et al., 2013). Metalaxyl has a water solubility of 8400 mg L⁻¹ and a log P of 1.75 (Tomlin, 2006). It is highly polar and mobile in soils and besides organic matter, certain soil clay minerals may play an important role in its sorption (Bermúdez-Couso et al., 2011; Fernandes et al., 2003; Gondar et al., 2013; Sharma and Awasthi, 1997). The addition of organic amendments and modified clay minerals has been shown to enhance the retention of tebuconazole and/or metalaxyl in soils (Fenoll et al., 2011; Fernandes et al., 2006; Herrero-Hernández et al., 2011; Marín-Benito et al., 2012; Rodríguez-Cruz et al., 2007).

Tebuconazole and metalaxyl are both chiral compounds. They contain an asymmetrically substituted C-atom in their structure (Fig. S1) and consist of a pair of enantiomers. Chiral pesticide enantiomers exhibit almost the same physico-chemical properties, but they usually differ in their biological efficacy, toxicity to non-target organisms, and biodegradation rates (Celis et al., 2013; Garrison, 2006; Liu et al., 2005; Poiger et al., 2015). In fact, the anti-fungal activity of metalaxyl has been mainly attributed to the *R*-enantiomer (Buerge et al., 2003; Buser et al., 2002; Marucchini and Zadra, 2002; Monkiedje et al., 2007; Nuninger et al., 1996) and that of tebuconazole has also been shown to be enantiomer-dependent (Stehmann and de Waard, 1995; Yang et al., 2002). In addition, as biologically-mediated processes are important in the degradation of tebuconazole and metalaxyl in soil (Buerge et al., 2003; Potter et al., 2005; Sehnem et al., 2010; Sukul and Spiteller, 2001, 2000; Sukul, 2006), the soil degradation rates for the individual *R* and *S* enantiomers can differ and be differently affected by agricultural practices such as the application of organic amendments, repeated pesticide treatments, or the type of formulation applied. This is because these agricultural practices can influence the enantiomers availability as well as the nature and activity of the soil microbial population (Celis et al., 2015, 2013; Gámiz et al., 2016, 2013; Lewis et al., 1999).

Biochar (BC), i.e. the solid residue remaining after pyrolysis of biomass, has attracted much attention over the last years as a soil amendment, because, among other benefits, it can improve the quality and fertility of soils and contribute to mitigate greenhouse gas emissions (Agegnehu et al., 2015; Genesio et al., 2015; Lehmann et al., 2011; Sohi, 2012). Likewise, the use of BC has been proposed as a strategy to attenuate the mobility of pesticides and mitigate contamination of soils and surface and ground waters (Gámiz et al., 2016; García-Jaramillo et al., 2014; Kookana, 2010; Mesa and Spokas, 2010). Another type of materials suggested as pesticide sorbents are layered double hydroxides (LDHs) or hydrotalcite (HT)-

like compounds (anionic clays). These are minerals with high sorption capacity due to anion exchange properties, acid-base buffering capacity, reconstruction from their calcination products, and customization potential (Cavani et al., 1991; Celis et al., 2014, 1999; Cornejo et al., 2008; Forano et al., 2006). For example, Celis et al. (2014) showed that the intercalation of fatty acid anions into a Mg/Al (3:1) LDH resulted in organo-hydrotalcites with very high affinities for neutral (uncharged) pesticides. Amendment with organo-hydrotalcites has also been proposed as a strategy to reduce the mobility of pesticides and other organic pollutants in soils (Bruna et al., 2012; Cornejo et al., 2008).

This research was designed as a follow-up study of previous experiments conducted under well-controlled laboratory conditions indicating that olive mill waste (OMW)-derived biochars and organo-hydrotalcites could be useful as soil amendments to mitigate contamination by pesticides. The primary objective was to assess the effect of adding an oleate-modified hydrotalcite (clay) and an OMW-derived biochar (BC) to an agricultural soil on the sorption, persistence, and mobility of two widely used fungicides with contrasting physico-chemical properties (tebuconazole and metalaxyl) under real field conditions. Considering that the studied fungicides were chiral, we also intended to get insight into the effects of the addition of clay and BC on the enantiomeric composition of tebuconazole and metalaxyl soil residues. The information provided in this work should help in the design of real pollution control strategies based on the use of clays and biochars as soil amendments.

2. Materials and methods

2.1. Soil, amendments, and fungicides

The field experiment was conducted on a 4 × 4 m soil area of an experimental farm located in Sevilla, Spain (37° 17' 02" N, 6° 03' 58" W), devoted to field trials by IRNAS (CSIC). The soil was selected for being a typical low organic carbon content, Mediterranean

agricultural soil susceptible to receive the studied fungicides. It was a sandy loam soil with 66% sand, 16% silt, 18% clay (16% smectites, 1% illite/mica, 1% kaolinite), 19% CaCO_3 , 0.59% organic carbon, and had a pH of 7.3. It was similar to that used in a previous laboratory study (Gámiz et al., 2016), but with greater carbonate and smectite contents. For the laboratory sorption experiment, a sample of untreated soil was taken (0-20 cm), air dried, sieved to pass a 2 mm-aperture mesh, and used within one week after sampling.

The amendments used were oleate-modified hydrotalcite (clay) and biochar (BC). They were prepared under less strictly controlled conditions compared to similar sorbents used in previous laboratory experiments to simulate feasible, larger scale production procedures. Hydrotalcite and sodium oleate were both purchased from Sigma-Aldrich with a purity of 99% and 80%, respectively. The preparation of the oleate-intercalated hydrotalcite (clay) was carried out through the reconstruction method, following a procedure similar to that described in Celis et al. (2014). Briefly, 75 g of sodium oleate was stirred in 1.5 L of deionized water for 2 h at 60 °C until a clear, yellow solution was obtained. Simultaneously, 50 g of hydrotalcite was calcined at 500 °C for 2 h, and then added to the sodium oleate solution. The suspension was stirred for 24 h at 60 °C, filtered (pore size = 0.45 μm), and the resultant solid was dried at 60 °C to obtain the final oleate-modified hydrotalcite (clay) sample. The properties of the clay were: 17.8% Mg, 7.2% Al, 30.2% C, and a basal spacing value of 3.4 nm, which reflected the successful intercalation of the oleate anions in the interlayer space of the clay (Celis et al., 2014). Biochar (BC) was obtained from the same composted olive-mill waste (OMWc) as that used in Gámiz et al. (2016), but was prepared at higher pyrolysis temperature (550 °C) and under a less strictly controlled oxygen-restricted atmosphere by pyrolyzing 10 kg of OMWc in an experimental, higher capacity pyrolysis furnace for 2 h. This resulted in a BC with a lower carbon content, but slightly greater

nitrogen-specific surface area (S_{BET}) compared to that obtained in Gámiz et al. (2016). The properties of BC were: 24.2% C, 2.0 % N, S_{BET} of $2.5 \text{ m}^2 \text{ g}^{-1}$ and pH of 10.2.

Technical-grade (racemic) metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate] (purity 97.7%) and tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol)] with a purity > 95% were used in laboratory and field experiments. High-purity (> 99%) standards of (racemic) metalaxyl and tebuconazole purchased from Sigma-Aldrich (Spain) were used to prepare the external calibration curves for the analysis of the fungicides.

2.2. Laboratory sorption experiment

A preliminary laboratory batch sorption experiment was conducted in order to determine the effect of the amendments on the sorption capacity of the soil for the fungicides. For this purpose, triplicate 4 g samples of unsterilized soil, either unamended or amended with clay or BC at two different rates (0.5 and 1% w:w), were equilibrated in glass centrifuge tubes with 8 mL of an aqueous solution containing a mixture of racemic metalaxyl at 12 mg L^{-1} and racemic tebuconazole at 6 mg L^{-1} . The amendment rates and fungicide to soil ratios during the sorption experiment were selected to be close to those expected for the upper 2 cm of soil during the field experiment (see below). After shaking for 24 h at $20 \pm 2^\circ\text{C}$, the tubes were centrifuged and the supernatant solutions were filtered (0.45 μm pore size GHP membrane disk filters) and analyzed by HPLC to determine the equilibrium concentration of each fungicide (C_e). The amounts sorbed (C_s) were obtained from the differences between the initial (C_{ini}) and equilibrium (C_e) fungicide concentrations.

The percentage of fungicide sorbed by the unamended and amended soil samples (% Ads) was calculated as:

$$\% \text{ Ads} = [(C_{\text{ini}} - C_e)/C_{\text{ini}}] \times 100 \quad (1)$$

whereas distribution coefficients, K_d (L kg^{-1}), were calculated as:

$$K_d = C_s/C_e \quad (2)$$

In addition, an estimate of the contribution of the sorbents ($K_{d\text{-sorbent}}$) to the sorption of the fungicides by the amended soil samples was made, assuming linear sorption, using the equation (Gámiz et al., 2010):

$$K_{d\text{-mixture}} = K_{d\text{-soil}} f_{\text{soil}} + K_{d\text{-sorbent}} f_{\text{sorbent}} \quad (3)$$

where $K_{d\text{-mixture}}$ and $K_{d\text{-soil}}$ were the experimentally measured distribution coefficients for the amended and unamended soil, respectively, and f_{soil} and f_{sorbent} were the fractions of soil and sorbent (clay or BC) in the mixtures. The values of $K_{d\text{-mixture}}$, $K_{d\text{-soil}}$, f_{soil} and f_{sorbent} were used to calculate $K_{d\text{-sorbent}}$.

2.3. Field experiment

The experimental design consisted of nine $1 \text{ m} \times 1 \text{ m}$ plots confined by 15 cm-high woody frames and separated by a distance of 0.5 m from each other. Three treatments with 3 replicates for each treatment were established. The plots were randomly selected to be unamended or amended with clay (2 t ha^{-1}) or BC (4 t ha^{-1}). The application rate of BC was selected to be close to the minimum rates at which biochars have been shown to be beneficial for growing crops (Jeffery et al., 2015; Major, 2010). We used a lower application rate for clay on the basis of the higher sorption performance it displayed in the preliminary

batch sorption experiment. The amendments were manually added and mixed with approximately the top 0-2 cm of soil using a rake until achieving a homogeneous amended soil layer. After that, metalaxyl and tebuconazole were simultaneously applied to all plots at doses of 6 and 3 kg ha⁻¹, respectively. For this purpose, 100 mL of an aqueous solution of metalaxyl (6 g L⁻¹) and 50 mL of an ethanolic solution of tebuconazole (6 g L⁻¹) were diluted with 4 L of water and the resulting solution was applied to the plot using a watering can to uniformly distribute the fungicides on the soil surface. The application rate of metalaxyl was within the range of 0.4-10 kg active ingredient ha⁻¹ recommended for the application of this fungicide to crops of the area of study. The application rate of tebuconazole was increased to 6 times the maximum recommended application rate (0.5 kg ha⁻¹) to ensure a reliable quantification of the individual enantiomers during the experiment. Application was conducted on October 8, 2014.

Weather conditions (rainfall and air temperature) were monitored along the duration of the experiment (Fig. 1) at a weather station close to the study site (4 km). Temperatures were relatively high for the fall season in which the experiment was performed. The maximum daily temperatures ranged between 11.9 and 32.4 °C with an average of 19.9 °C, whereas the minimum daily temperatures ranged between 2.7 and 20.7 °C with an average of 10.4 °C. The total rainfall during the 98 days of experiment was 298 mm, with noticeable precipitation events of 46 mm at day 2, 32 mm at day 34, 39 mm at day 51, and 36 mm at day 66.

Sampling was performed 0, 35, 64 and 98 days after treatment (DAT). At these times, triplicate soil subsamples were taken from each plot at three different depths (0-5, 5-10 and 10-20 cm) using a 3 cm internal diameter spade for a total of 9 observations per treatment. Soil subsamples were packed in separate plastic bags and immediately frozen at -18 °C until they were extracted. For extraction, the soil subsamples were homogenized and duplicate

aliquots (5 g) were shaken in 10 mL of methanol for 24 h, centrifuged, and the supernatants filtered and analyzed by HPLC to determine the soil fungicide concentration (mg kg^{-1} dry soil). A preliminary experiment showed that this extraction procedure recovered more than 95% of the fungicides freshly applied to the soil. To correct for the water content, duplicate 1 g-aliquots of each soil subsample were dried at 105 °C for 24 h and the water loss was calculated. Additionally, 35 DAT soil aliquots rich in sorbent particles (clay and BC) were visually identified and sampled from the 0-5 cm section of the amended soil plots. These soil aliquots were also homogenized and extracted in duplicate (250 mg) with methanol (5 mL) by shaking for 24 h to determine their fungicide content.

The ability of the amendments to extend the presence of the fungicides in the upper 0-5 cm soil section was assessed by fitting the soil concentration data to the linearized form of a first-order kinetic rate law:

$$\ln C = \ln C_0 - kt \quad (4)$$

where C (mg kg^{-1}) and C_0 (mg kg^{-1}) are the concentration of the fungicide in the top 0–5 cm of soil at time t (days) and $t = 0$, respectively, and k (days^{-1}) is the first-order dissipation rate constant. The time for the dissipation of 50% and 90% of the fungicide from the top 0-5 cm of soil was calculated as $DT_{50} = 0.693/k$ and $DT_{90} = 2.303/k$, respectively.

2.4. Enantiomeric fraction

The possible enantiomer-selective behavior of tebuconazole and metalaxyl in the laboratory and field experiments was assessed by monitoring the individual concentration of each of the two enantiomers of the fungicides, and then calculating the enantiomer fraction (EF) using the formula proposed by Harner et al. (2000):

$$EF = [1]/([1]+[2]) \quad (5)$$

where [1] and [2] are, respectively, the concentration of the first and last eluting enantiomer during the chiral chromatographic analysis, which is described in the next section.

2.5. Analysis of the fungicides

The analysis of tebuconazole and metalaxyl was conducted by achiral and chiral high-performance liquid chromatography (HPLC), in both cases using a Waters chromatograph consisting of a Waters 600E System Controller, a Waters 717 Autosampler injector, and a Waters 998 Photodiode Array Detector (PDAD). For the achiral analysis, both fungicides were determined simultaneously according to the following chromatographic conditions: Kinetex C18 chromatographic column of 150 mm length $\times$ 4.6 mm internal diameter (i.d.) and 5 μ m particle size (Phenomenex), 25 μ L injection volume, 50:50 acetonitrile:water isocratic eluent mixture at a flow rate of 1 mL min⁻¹, and detection wavelength at 226 nm for tebuconazole and 213 nm for metalaxyl. Under these conditions metalaxyl (*R+S*) and tebuconazole (*R+S*) eluted at 3.3 and 6.7 min, respectively.

The enantioselective analysis of metalaxyl was performed using a Chiralpak IB column of 150 mm length $\times$ 4.6 mm i.d. and 5 μ m particle size (Chiral Technologies Europe), a 60:40 (v/v) water:acetonitrile eluent mixture at a flow rate of 1 mL min⁻¹, and an injection volume of 50 μ L. According to these analytical conditions, *S*-metalaxyl and *R*-metalaxyl eluted at 4.9 and 6.1 min, respectively (Celis et al., 2013). For the enantioselective analysis of tebuconazole, we used a Chiralpak AY-RH column of 150 mm length $\times$ 4.6 mm i.d. and 5 μ m particle size (Chiral Technologies Europe), a 60:40 (v/v) water:acetonitrile eluent mixture at a flow rate of 1 mL min⁻¹, and an injection volume of 50 μ L. The elution order

was 5.6 and 18.4 min for the first and last eluted enantiomers, respectively. Under nearly identical chromatographic conditions, Wang et al. (2012) assigned the first eluted enantiomer to *R*-tebuconazole and the last eluted enantiomer to *S*-tebuconazole. The detection wavelengths for the chiral analyses were the same as those used for the achiral determinations. For both the achiral and chiral analyses, external calibration was carried out by injection of five standard solutions containing a mixture of tebuconazole and metalaxyl at concentrations ranging between 0.1 and 6 mg L⁻¹ ($R^2=0.999$). The experimental limit of detection (LOD), calculated as the concentration resulting in a signal to noise ratio (S/N) of 3:1, was 0.03 mg L⁻¹ for both fungicides.

2.6. Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 22. Standard error was used to indicate variability among triplicates. In the case of the field experiment, each triplicate value came from averaging three subsample observations. Distribution coefficients (K_d), fungicide concentrations in field soil samples, and enantiomer fractions (EF) were compared using ANOVA followed by Tukey's test to establish differences between treatments. An analysis of covariance (ANCOVA) was performed to compare pairwise the slopes of the regression lines (k) of the first-order dissipation data. All statistical analyses were performed at the 95 % significance level ($p < 0.05$).

3. Results and discussion

3.1. Laboratory sorption experiment

Metalaxyl and tebuconazole sorption data on unamended soil and on soil amended with clay and BC at 0.5% and 1% (w/w) are summarized in Table 1. The contribution of the

298 sorbent (clay or BC) to the K_d value of the amended soil was calculated using Eq. 3 and is
 299 also included in Table 1.

300 In unamended soil, the K_d value measured for tebuconazole (2.4 L kg^{-1}) was seven-fold
 301 higher than that measured for metalaxyl (0.34 L kg^{-1}) (Table 1). The higher sorption of
 302 tebuconazole can be attributed to its greater hydrophobicity and affinity for soil organic
 303 matter (Čadková et al., 2013) compared to metalaxyl. It is known that other factors, such as
 304 the amount and nature of mineral constituents, can dictate the sorption of metalaxyl in low
 305 organic carbon content soils (Bermúdez-Couso et al., 2011; Celis et al., 2013; Fernandes et
 306 al., 2003). In amended soil, sorption of tebuconazole and metalaxyl increased with the
 307 amount of clay and BC added, but the effect of adding clay was much more pronounced than
 308 that of adding BC. The organo-clay increased the sorption of metalaxyl from 14% ($K_d = 0.34$
 309 L kg^{-1}) up to 61 % ($K_d = 3.14 \text{ L kg}^{-1}$) and the sorption of tebuconazole from 55% ($K_d = 2.4 \text{ L}$
 310 kg^{-1}) up to 96% ($K_d = 47.4 \text{ L kg}^{-1}$) (Table 1). The enhancement in sorption upon amendment
 311 can be attributed to the inherent sorption capacity of the sorbents, as reflected by the $K_{d-\text{sorbent}}$
 312 values calculated for clay and BC in the mixtures (Table 1). For clay, $K_{d-\text{sorbent}}$ ranged
 313 between 242 and 307 L kg^{-1} for metalaxyl and between 4062 and 4502 L kg^{-1} for
 314 tebuconazole. For BC, $K_{d-\text{sorbent}}$ ranged between 25 and 36 L kg^{-1} for metalaxyl and between
 315 602 and 662 L kg^{-1} for tebuconazole.

316 The affinity of neutral pesticides for anionic clays intercalated with unsaturated fatty
 317 acid anions has been related to the hydrophobicity of the pesticide, which can be expressed
 318 by its octanol-water partition coefficient (Celis et al., 2014). The high octanol-water partition
 319 coefficient of tebuconazole ($\log P = 3.7$) compared to metalaxyl ($\log P = 1.75$) would thus
 320 explain its very high sorption in the organoclay-amended soil (Table 1). On the other hand,
 321 the sorption capacity of biochars is often attributed to their high specific surface area
 322 (Cabrera et al., 2011), so that the relatively low sorption displayed by BC-amended soil for

metalaxyl and tebuconazole could reflect the small S_{BET} value registered for this BC ($2.5 \text{ m}^2 \text{ g}^{-1}$). Nevertheless, the contribution of BC to the sorption of metalaxyl in BC-amended soil ($K_{\text{d-sorbent}} = 25\text{-}36 \text{ L kg}^{-1}$, Table 1) was even lower than that ($\sim 100 \text{ L kg}^{-1}$) we observed in our earlier study after amending a sandy loam soil with a OMWc-derived BC having a $S_{\text{BET}} = 0.3 \text{ m}^2 \text{ g}^{-1}$ (Gámiz et al., 2016). Compared with the BC used in the present study, the BC used in Gámiz et al. (2016) came from the same feedstock but was prepared at lower temperature ($400 \text{ }^\circ\text{C}$) and under a more strictly controlled anoxic atmosphere. It appears that these variables are important in determining the performance of BCs as pesticide sorbents. It should also be pointed out that N_2 is not considered a suitable adsorbate to analyze materials with pores of size $< 0.5 \text{ nm}$ (de Jonge and Mittelmeijer-Hazeleger, 1996), which is the case of biochars, and that additional properties related to the preparation procedure can influence the sorption of organic compounds by char materials (Lattao et al., 2014).

An enantioselective analysis of the supernatants during the laboratory sorption experiment was also conducted and revealed that neither metalaxyl nor tebuconazole showed enantioselectivity in their sorption on unamended or amended soil, as indicated by the supernatant enantiomer fractions $\text{EF} \sim 0.50$. Values of solution EF values equal or close to 0.5 indicated no preferential sorption of one enantiomer over the other. This agreed with previous observations indicating lack of enantioselectivity during the sorption of different chiral pesticides on soils from racemic initial pesticide solutions (Celis et al., 2013; Gámiz et al., 2013; Qi et al., 2015; Sukul et al., 2013).

3.2. Field experiment

3.2.1. Fungicide persistence and leaching

The soil concentrations of (*R+S*)-tebuconazole and (*R+S*)-metalaxyl (mg kg^{-1} dry soil) present at different depths (0-5, 5-10, and 10-20 cm) of the unamended and clay- and BC-

amended soil plots as a function of time are compiled in Fig. S2 and Fig. S3 of the Supplementary material. It was noticeable that the concentrations of metalaxyl (20-25 mg kg⁻¹) and tebuconazole (10-12 mg kg⁻¹) in the top 0-5 cm soil layer at the beginning of the experiment (0 DAT) were relatively high, since they corresponded to those expected after assuming a soil density of about 0.6 g cm⁻³. This low density was attributed to some soil disturbance upon sampling, as the 0-5 cm soil section in the sampling spade was visually less compacted than deeper soil sections. The slightly higher initial fungicide concentrations observed for clay- and BC-amended soil compared to unamended soil (significant for metalaxyl) could also reflect slight differences in soil density after amendment, as previously suggested for organically-amended field soils (Gámiz et al., 2012). On the other hand, since soil concentration data were simultaneously influenced by fungicide degradation and transport losses, to evaluate leaching we calculated the fraction of residues at different soil depths relative to the total amount extracted from the 0-20 cm soil profile. The results are summarized in Fig. 2 and Fig. 3 for tebuconazole and metalaxyl, respectively.

Tebuconazole. For all treatments and sampling times, the amount of tebuconazole residues present in the top 0-5 cm soil layer represented more than 75% of the total extracted residues (Fig. 2). This fraction was significantly greater ($p < 0.05$) than that present at deeper depths (5-10 and 10-20 cm). The low mobility of tebuconazole observed in the field trial was in accordance with the results of the laboratory sorption experiment indicating high sorption of the fungicide on the unamended and clay- and BC-amended soil (Table 1). It also agreed with previous works reporting low leaching of tebuconazole in unamended soils and in soils treated with different organic amendments, in line with the high affinity of this fungicide for endogenous and exogenous soil organic matter (Aldana et al., 2011; Fenoll et al., 2011; Kalbe et al., 2014). Herrero-Hernández et al. (2011), however, observed higher mobility of tebuconazole to deeper soil layers in a sandy clay loam soil amended with spent mushroom

substrate compared to the unamended soil, and attributed this effect to the presence of dissolved organic matter released by the amendment. This mechanism did not appear to play a major role in our study.

The results of fitting tebuconazole concentration data for the top 0-5 cm soil section to first order dissipation kinetics are given in Table 2. Differences in the dissipation rate constants (k) for tebuconazole under the different treatments were found to be not statistically significant. DT_{50} values ranged between 50 and 58 days, whereas DT_{90} values ranged between 165 and 192 days (Table 2). These values agree with the average values of $DT_{50}= 47$ days and $DT_{90}= 177$ days reported by the Pesticide Properties Database (PPDB, 2016) for the field dissipation of tebuconazole. Under laboratory conditions, the degradation of tebuconazole has generally been observed to proceed slowly (Fenoll et al., 2010; Li et al., 2015; Mosquera et al., 2010; Muñoz-Leoz et al., 2011; Wang et al., 2012). Given that it is well-known that sorption influences the bioavailability of pesticides in soils (Koskinen et al., 2001), the high sorption of tebuconazole on unamended soil (Table 1) could have contributed to limit its transport and degradation losses from the topsoil and also to reduce the impact of clay and BC addition on the DT_{50} and DT_{90} values.

Metalaxyl. Metalaxyl displayed greater downward mobility than tebuconazole and this resulted in greater differences between treatments in the leaching of this fungicide compared to tebuconazole. Differences were particularly noticeable at later sampling times (64 and 98 DAT), at which the relative distribution patterns of metalaxyl residues showed that the fungicide displayed higher leaching in unamended soil than in clay- and BC-amended soil (Fig. 3). For example, at 98 days, 43% of the metalaxyl residues extracted from unamended soil came from the 10-20 cm soil layer (Fig. 3). This value was significantly higher than the fraction that came from the 0-5 cm (14%, $p < 0.001$) and 5-10 cm (35%, $p < 0.05$) soil layers, that is, metalaxyl residues accumulated in the deepest (10-20 cm) layer of the

unamended soil. In contrast, for the clay- and BC-amended soil the fraction metalaxyl residues present at the 10-20 cm soil layer was not significantly different from those present at the 0-5 and 5-10 cm soil depths (Fig. 3).

The effect of the amendments on metalaxyl leaching was less evident at earlier sampling times, even though several noticeable rainfall events occurred during this period (Fig. 1). We attributed this result to the high temperatures reached at the beginning of the experiment (Fig. 1), which should have favored water evaporation and reduced water percolation. It is interesting to note, however, that 35 DAT the percentage of soil subsamples collected from the deepest horizon (10-20 cm) that contained detectable metalaxyl residues (LOD= 0.07 mg kg⁻¹) was reduced from 78% for the unamended soil to 22% for the clay-amended soil. This showed the immobilizing capacity of the amendment that would become more evident at later sampling dates.

The immobilizing capacity of clay and BC can reasonably be attributed to the presence of particles of the sorbent which, as shown in Fig. S4, accumulated in the top 0-5 cm of soil and increased the sorption capacity of the topsoil layer. To confirm this hypothesis, soil aliquots rich in sorbent particles (clay and BC) were sampled from the amended soil plots 35 DAT and subsequently extracted. The results indicated they indeed contained greater fungicide concentrations than bulk soil samples (Table 3).

Metalaxyl dissipated more rapidly from the top 0-5 cm of soil than tebuconazole. Differences in the dissipation rate constants (k) for the different treatments increased, becoming statistically significant between the clay-amended soil and the unamended soil (Table 2). DT₅₀ values ranged between 16 and 23 days, whereas DT₉₀ values ranged between 52 and 76 days (Table 2). Our DT₅₀ values are somewhat lower than the average value of 46 days reported by the PPDB (2016) for the field dissipation of metalaxyl, but within the range of 9 and 70 days observed in different field studies where metalaxyl was applied to

unamended and organically-amended soils (Kookana et al., 1995; Liu et al., 2012; Triantafyllidis et al., 2013). The enhanced persistence of metalaxyl in the top 0-5 cm soil layer after amending the soil with clay was most likely a consequence of the higher sorption exerted by the amendment (Table 1), which reduced not only the leaching of the fungicide to deeper soil layers, but also its bioavailability to be degraded, as suggested by other authors for organically-amended soils (Fernandes et al., 2006; Rodríguez-Cruz et al., 2012) and supported by the results of the enantioselective study presented in the next section.

3.2.2. *Enantioselective study*

The enantiomer fractions (EFs) determined for tebuconazole and metalaxyl residues present at different soil depths along the field experiment are shown in Fig. S5 and Fig. 4, respectively. Individual enantiomer concentration data for the top 0-5 cm soil section were also fitted to first order dissipation kinetics, and the corresponding parameters are compiled in Table S1 of the Supplementary material.

For tebuconazole, EFs were only calculated for the top 0-5 cm, because the fungicide concentrations at deeper profiles were very low (Fig. S2). There were no significant changes ($p > 0.05$) in the original (racemic) tebuconazole EF value of 0.5 along the experiment either for unamended or amended soil (Fig. S5), i.e. tebuconazole remained mainly as a racemate during the whole experiment. Accordingly, the dissipation rate constant (k) for the *R*-enantiomer of tebuconazole was not significantly different from that of the *S*-enantiomer (Table S1). Enantiomer-selective studies on tebuconazole degradation in soil under field conditions are very scarce (Ye et al., 2013). Under laboratory conditions, recent studies have indicated limited enantioselectivity for the degradation of this fungicide in soils. For example, Li et al. (2015) reported tebuconazole residues had EF values in the range 0.452-0.475 at the end of a 180-d incubation study conducted on seven soils under aerobic

conditions, and Wang et al. (2012) reported EF values between 0.474 and 0.481 after a 160 d-incubation study with three soils. Even though it is well-known that microbially-mediated processes play an important role in the degradation of tebuconazole in soil (Potter et al., 2005; Sehnem et al., 2010), it seems that degradation occurred too slow to develop a marked enantioselectivity within the time-scale of the experiments (Wang et al., 2012).

In contrast to tebuconazole, the behavior of metalaxyl was prominently enantioselective and the EF of its soil residues differed with soil depth and with soil treatment (Fig. 4). EF values of 0.50 ± 0.01 were only obtained at the beginning of the experiment; shortly after fungicide application metalaxyl residues became non-racemic ($EF > 0.5$) for all treatments (Fig. 4). EF values greater than 0.5 indicate metalaxyl residues were richer in the first eluting enantiomer (*S*-metalaxyl) than in the second eluting enantiomer (*R*-metalaxyl), which is congruent with previous observation indicating that *R*-metalaxyl degrades faster than *S*-metalaxyl in soils with $pH > 5$ under aerobic conditions (Buerge et al., 2003; Celis et al., 2013; Monkiedje et al., 2003). This was confirmed by the greater dissipation rate constant obtained for *R*-metalaxyl compared to *S*-metalaxyl, particularly in unamended soil (Table S1). In general, the DT_{50} values reported in Table S1 for *R*- and *S*-metalaxyl are similar or lower compared to previously reported values obtained under controlled laboratory conditions for similar unamended and organically-amended Mediterranean soils (Gámiz et al., 2016, 2013). In part, this is due to the fact that transport losses contributed to the dissipation of metalaxyl in the field, but not in laboratory incubations. An exception was the short laboratory half-life reported by Gámiz et al. (2016) for *R*-metalaxyl in unamended sandy loam soil ($DT_{50} = 3$ days). It was inferred that the microbial population of the soil sample was particularly prone to the degradation of *R*-metalaxyl (Gámiz et al., 2016).

With regard to the degree of enantioselectivity, EF values were generally higher in unamended soil compared to clay- and BC-amended soil (Fig. 4), particularly for the top 0-5

cm of soil. Thus, at the end of the experiment (98 DAT), EF of residues in the 0-5 cm soil layer followed the order: unamended soil (0.88) > BC-amended soil (0.78) > clay-amended soil (0.70) with differences being statistically significant at the $p < 0.05$ level. For non-amended soil at $t = 98$ days, differences in EF values for the different soil depths were statistically significant ($p < 0.05$) only between the 0-5 and 10-20 cm soil layers, whereas for clay- and BC-amended soil EF values significantly increased with soil depth ($p < 0.05$) (Fig. 4). Interestingly, the difference in k values between the *R*- and *S*-metalaxyl enantiomers for the top 0-5 cm soil layer was significant ($p < 0.05$) for unamended soil, but became non-significant for the amended soil (Table S1).

Several important consequences can be derived from the enantioselective analysis of metalaxyl residues. Bearing in mind the biologically-mediated degradation of metalaxyl in soil (Kalathoor et al., 2015; Sukul and Spiteller, 2001; Sukul, 2006) and the non-enantioselective sorption displayed by the amendments used (Table 1), the fact that for the top 0-5 cm soil layer metalaxyl remained more racemic in clay- and BC-amended soil than in unamended soil (Fig. 4) strongly indicated that clay and BC protected metalaxyl from its enantioselective biodegradation. This result is in agreement with the results of laboratory experiments suggesting that sorption to biochar protected metalaxyl enantiomers from biodegradation in soil, prolonging their presence in a racemic form (Gámiz et al., 2016). In fact, the enantioselective analysis of metalaxyl residues in soil aliquots rich in sorbent particles confirmed they were more racemic than residues extracted from bulk soil samples (Table 3). On the other hand, the fact that EF values increased with soil depth, particularly for clay- and BC-amended soil, further indicated that sorption protected the fungicide from biodegradation, and that there was a relationship between the biodegradable fraction and the leachable fraction, as would be expected.

4. Conclusions

Oleate-modified hydrotalcite (clay) and olive mill waste-derived biochar (BC) behaved as efficient sorbents for the chiral fungicides tebuconazole and metalaxyl both under laboratory and field conditions. Batch sorption laboratory experiments showed that the clay was much more efficient in increasing the sorption capacity of the soil for the studied fungicides than the biochar, and that the pyrolysis conditions influenced the performance of the BC as a pesticide sorbent. In the field experiment, tebuconazole showed scarce leaching and long persistence both in unamended and in clay- and BC-amended soil plots. Metalaxyl showed greater mobility and shorter persistence than tebuconazole, but addition of clay and BC to the top 0-5 cm soil layer reduced transport and degradation losses of the fungicide. One of the main results of this work was to illustrate how the soil amendments affected the enantiomeric composition of tebuconazole and metalaxyl soil residues. While tebuconazole remained mostly racemic for all treatments during the 98-day field study, the *R*-enantiomer of metalaxyl degraded faster than the *S*-enantiomer, and the addition of clay and BC affected the changes in enantiomer fraction of metalaxyl residues with time, as predicted by earlier laboratory incubation studies. In clay- and BC-amended soil, owing to the sorption exerted by the sorbents, residues monitored in the top 0-5 cm of soil remained more racemic than in unamended soil, and the fraction of *S*-enantiomer increased sharply with depth. The outcomes from this study support the use of clays and biochars as sorbents to prolong the presence of mobile and/or scarcely persistent chiral pesticide enantiomers in the topsoil by reducing enantiomer leaching and degradation processes. Finally, this work has also corroborated under real field conditions that sorption can indirectly influence the final enantiomeric composition of chiral pesticide residues by affecting the enantiomers biodegradation rate.

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FIGURE CAPTIONS

Fig. 1. Rainfall (bars) and temperatures (lines) during the field experiment. Vertical dotted lines indicate sampling dates.

Fig. 2. Distribution of tebuconazole residues at different soil depths for unamended, clay-amended, and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil plots.

Fig. 3. Distribution of metalaxyl residues at different soil depths for unamended, clay-amended, and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil plots.

Fig. 4. Enantiomeric fractions of metalaxyl at different soil depths for unamended soil, clay-amended and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil samples.

Table 1

Summary of metalaxyl ($C_{ini}= 12 \text{ mg L}^{-1}$) and tebuconazole ($C_{ini}= 6 \text{ mg L}^{-1}$) sorption data on unamended and clay- and biochar (BC)-amended soil. Measured distribution coefficients (K_d) and percentage of fungicide sorbed (%Ads), and calculated K_d for sorbents ($K_{d-sorbent}$). Values within the same column followed by different letters are significantly different from each other ($p < 0.05$).

	Metalaxyl			Tebuconazole		
	K_d	%Ads	$K_{d-sorbent}$	K_d	%Ads	$K_{d-sorbent}$
	(L kg^{-1})		(L kg^{-1})	(L kg^{-1})		(L kg^{-1})
Unamended soil	$0.34 \pm 0.04 \text{ a}$	14 ± 1	-	$2.4 \pm 0.3 \text{ a}$	55 ± 3	-
Soil + Clay (0.5%)	$1.55 \pm 0.07 \text{ b}$	44 ± 1	242	$22.7 \pm 2.6 \text{ b}$	92 ± 1	4062
Soil + Clay (1%)	$3.14 \pm 0.09 \text{ c}$	61 ± 1	280	$47.4 \pm 2.4 \text{ c}$	96 ± 1	4502
Soil + BC (0.5%)	$0.52 \pm 0.03 \text{ d}$	21 ± 1	36	$5.4 \pm 0.7 \text{ a,d}$	73 ± 2	602
Soil + BC (1%)	$0.59 \pm 0.02 \text{ d}$	23 ± 1	25	$9.0 \pm 2.0 \text{ d}$	81 ± 3	662

Table 2

Single first-order dissipation parameters for metalaxyl and tebuconazole for the top 0-5 cm of unamended soil and soil amended with clay and biochar. Values within the same column followed by different letters are significantly different from each other ($p < 0.05$).

	Metalaxyl				Tebuconazole			
	R^2	k (days ⁻¹)	DT ₅₀	DT ₉₀	R^2	k (days ⁻¹)	DT ₅₀	DT ₉₀
Unamended soil	0.981	0.044 ± 0.004 a	16	52	0.996	0.014 ± 0.001 a	50	165
Clay-amended Soil	0.985	0.032 ± 0.003 b	22	72	0.917	0.013 ± 0.003 a	51	168
BC-amended Soil	0.855	0.030 ± 0.009 a,b	23	76	0.895	0.012 ± 0.003 a	58	192

Table 3

Concentration and EF values of metalaxyl residues in soil aliquots rich in sorbent particles compared to those in bulk soil samples taken from the amended plots at $t = 35$ days. Values within the same column followed by different letters are significantly different from each other ($p < 0.05$).

	Clay-amended soil		BC-amended soil	
	Metalaxyl concentration (mg kg ⁻¹)	EF	Metalaxyl concentration (mg kg ⁻¹)	EF
Bulk amended soil	8.5 ± 3.4 a	0.536 ± 0.004 a	9.1 ± 1.7 a	0.569 ± 0.004 a
Soil aliquots rich in sorbent particles	72 ± 27 b	0.497 ± 0.008 b	106 ± 16 b	0.512 ± 0.001 b

Fig. 1. Rainfall (bars) and temperatures (lines) during the field experiment. Vertical dotted lines indicate sampling dates.

Fig. 2. Distribution of tebuconazole residues at different soil depths for unamended, clay-amended, and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil plots.

Fig. 3. Distribution of metalaxyl residues at different soil depths for unamended, clay-amended, and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil plots.

Fig. 4. Enantiomeric fractions of metalaxyl at different soil depths for unamended soil, clay-amended and BC-amended soil as a function of time after fungicide application. Error bars correspond to the standard errors of triplicate soil samples.