1 **Effect of synthetic clay and biochar addition on dissipation and**

2 **enantioselectivity of tebuconazole and metalaxyl in an agricultural soil:**

3 **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).

75   Tebuconazole and metalaxyl are both chiral compounds. They contain an asymmetrically

76   substituted C-atom in their structure (Fig. S1) and consist of a pair of enantiomers. Chiral

77   pesticide enantiomers exhibit almost the same physico-chemical properties, but they usually

78   differ in their biological efficacy, toxicity to non-target organisms, and biodegradation rates

79   (Celis et al., 2013; Garrison, 2006; Liu et al., 2005; Poiger et al., 2015). In fact, the anti-

80   fungal activity of metalaxyl has been mainly attributed to the R-enantiomer (Buerge et al.,

81   2003; Buser et al., 2002; Marucchini and Zadra, 2002; Monkiedje et al., 2007; Nuninger et

82   al., 1996) and that of tebuconazole has also been shown to be enantiomer-dependent

83   (Stehmann and de Waard, 1995; Yang et al., 2002). In addition, as biologically-mediated

84   processes are important in the degradation of tebuconazole and metalaxyl in soil (Buerge et

85   al., 2003; Potter et al., 2005; Sehnem et al., 2010; Sukul and Spiteller, 2001, 2000; Sukul,

86   2006), the soil degradation rates for the individual R and S enantiomers can differ and be

87   differently affected by agricultural practices such as the application of organic amendments,

88   repeated pesticide treatments, or the type of formulation applied. This is because these

89   agricultural practices can influence the enantiomers availability as well as the nature and

90   activity of the soil microbial population (Celis et al., 2015, 2013; Gámiz et al., 2016, 2013;

91   Lewis et al., 1999).

92   Biochar (BC), i.e. the solid residue remaining after pyrolysis of biomass, has attracted

93   much attention over the last years as a soil amendment, because, among other benefits, it can

94   improve the quality and fertility of soils and contribute to mitigate greenhouse gas emissions

95   (Agegnehu et al., 2015; Genesio et al., 2015; Lehmann et al., 2011; Sohi, 2012). Likewise,

96   the use of BC has been proposed as a strategy to attenuate the mobility of pesticides and

97   mitigate contamination of soils and surface and ground waters (Gámiz et al., 2016; García-

98   Jaramillo et al., 2014; Kookana, 2010; Mesa and Spokas, 2010). Another type of materials

99   suggested as pesticide sorbents are layered double hydroxides (LDHs) or hydrotalcite (HT)-

100   like compounds (anionic clays). These are minerals with high sorption capacity due to anion

101   exchange properties, acid-base buffering capacity, reconstruction from their calcination

102   products, and customization potential (Cavani et al., 1991; Celis et al., 2014, 1999; Cornejo

103   et al., 2008; Forano et al., 2006). For example, Celis et al. (2014) showed that the

104   intercalation of fatty acid anions into a Mg/Al (3:1) LDH resulted in organo-hydrotalcites

105   with very high affinities for neutral (uncharged) pesticides. Amendment with organo-

106   hydrotalcites has also been proposed as a strategy to reduce the mobility of pesticides and

107   other organic pollutants in soils (Bruna et al., 2012; Cornejo et al., 2008).

108   This research was designed as a follow-up study of previous experiments conducted

109   under well-controlled laboratory conditions indicating that olive mill waste (OMW)-derived

110   biochars and organo-hydrotalcites could be useful as soil amendments to mitigate

111   contamination by pesticides. The primary objective was to assess the effect of adding an

112   oleate-modified hydrotalcite (clay) and an OMW-derived biochar (BC) to an agricultural soil

113   on the sorption, persistence, and mobility of two widely used fungicides with contrasting

114   physico-chemical properties (tebuconazole and metalaxyl) under real field conditions.

115   Considering that the studied fungicides were chiral, we also intended to get insight into the

116   effects of the addition of clay and BC on the enantiomeric composition of tebuconazole and

117   metalaxyl soil residues. The information provided in this work should help in the design of

118   real pollution control strategies based on the use of clays and biochars as soil amendments.

119

120   **2. Materials and methods**

121   *2.1. Soil, amendments, and fungicides*

122   The field experiment was conducted on a 4×4 m soil area of an experimental farm

123   located in Sevilla, Spain (37° 17' 02" N, 6° 03' 58" W), devoted to field trials by IRNAS

124   (CSIC). The soil was selected for being a typical low organic carbon content, Mediterranean

125   agricultural soil susceptible to receive the studied fungicides. It was a sandy loam soil with

126   66% sand, 16% silt, 18% clay (16% smectites, 1% illite/mica, 1% kaolinite), 19% $CaCO_3$,

127   0.59% organic carbon, and had a pH of 7.3. It was similar to that used in a previous

128   laboratory study (Gámiz et al., 2016), but with greater carbonate and smectite contents. For

129   the laboratory sorption experiment, a sample of untreated soil was taken (0-20 cm), air dried,

130   sieved to pass a 2 mm-aperture mesh, and used within one week after sampling.

131   The amendments used were oleate-modified hydrotalcite (clay) and biochar (BC). They

132   were prepared under less strictly controlled conditions compared to similar sorbents used in

133   previous laboratory experiments to simulate feasible, larger scale production procedures.

134   Hydrotalcite and sodium oleate were both purchased from Sigma-Aldrich with a purity of

135   99% and 80%, respectively. The preparation of the oleate-intercalated hydrotalcite (clay)

136   was carried out through the reconstruction method, following a procedure similar to that

137   described in Celis et al. (2014). Briefly, 75 g of sodium oleate was stirred in 1.5 L of

138   deionized water for 2 h at 60 ℃ until a clear, yellow solution was obtained. Simultaneously,

139   50 g of hydrotalcite was calcined at 500 ℃ for 2 h, and then added to the sodium oleate

140   solution. The suspension was stirred for 24 h at 60 ℃, filtered (pore size = 0.45μm), and the

141   resultant solid was dried at 60 ℃ to obtain the final oleate-modified hydrotalcite (clay)

142   sample. The properties of the clay were: 17.8% Mg, 7.2% Al, 30.2% C, and a basal spacing

143   value of 3.4 nm, which reflected the successful intercalation of the oleate anions in the

144   interlayer space of the clay (Celis et al., 2014). Biochar (BC) was obtained from the same

145   composted olive-mill waste (OMWc) as that used in Gámiz et al. (2016), but was prepared at

146   higher pyrolysis temperature (550 ℃) and under a less strictly controlled oxygen-restricted

147   atmosphere by pyrolizing 10 kg of OMWc in an experimental, higher capacity pyrolysis

148   furnace for 2 h. This resulted in a BC with a lower carbon content, but slightly greater

149 nitrogen-specific surface area (S_{BET}) compared to that obtained in Gámiz et al. (2016). The

150 properties of BC were: 24.2% C, 2.0 % N, S_{BET} of 2.5 m^2 g^{-1} and pH of 10.2.

151 Technical-grade (racemic) metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-

152 alaninate] (purity 97.7%) and tebuconazole [(*RS*)-1-p-chlorophenyl-4,4-dimethyl-3-(1*H*-

153 1,2,4-triazol-1-ylmethyl)pentan-3-ol)] with a purity > 95% were used in laboratory and field

154 experiments. High-purity (> 99%) standards of (racemic) metalaxyl and tebuconazole

155 purchased from Sigma-Aldrich (Spain) were used to prepare the external calibration curves

156 for the analysis of the fungicides.

157

158 *2.2. Laboratory sorption experiment*

159 A preliminary laboratory batch sorption experiment was conducted in order to determine

160 the effect of the amendments on the sorption capacity of the soil for the fungicides. For this

161 purpose, triplicate 4 g samples of unsterilized soil, either unamended or amended with clay

162 or BC at two different rates (0.5 and 1% w:w), were equilibrated in glass centrifuge tubes

163 with 8 mL of an aqueous solution containing a mixture of racemic metalaxyl at 12 mg L^{-1}

164 and racemic tebuconazole at 6 mg L^{-1}. The amendment rates and fungicide to soil ratios

165 during the sorption experiment were selected to be close to those expected for the upper 2

166 cm of soil during the field experiment (see below). After shaking for 24 h at 20 ± 2ºC, the

167 tubes were centrifuged and the supernatant solutions were filtered (0.45 μm pore size GHP

168 membrane disk filters) and analyzed by HPLC to determine the equilibrium concentration of

169 each fungicide (C_e). The amounts sorbed (C_s) were obtained from the differences between

170 the initial (C_{ini}) and equilibrium (C_e) fungicide concentrations.

171 The percentage of fungicide sorbed by the unamended and amended soil samples (%

172 Ads) was calculated as:

173

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

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

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

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):

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

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 m × 1 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

199 batch sorption experiment. The amendments were manually added and mixed with

200 approximately the top 0-2 cm of soil using a rake until achieving a homogeneous amended

201 soil layer. After that, metalaxyl and tebuconazole were simultaneously applied to all plots at

202 doses of 6 and 3 kg ha^{-1}, respectively. For this purpose, 100 mL of an aqueous solution of

203 metalaxyl (6 g L^{-1}) and 50 mL of an ethanolic solution of tebuconazole (6 g L^{-1}) were diluted

204 with 4 L of water and the resulting solution was applied to the plot using a watering can to

205 uniformly distribute the fungicides on the soil surface. The application rate of metalaxyl was

206 within the range of 0.4-10 kg active ingredient ha^{-1} recommended for the application of this

207 fungicide to crops of the area of study. The application rate of tebuconazole was increased to

208 6 times the maximum recommended application rate (0.5 kg ha^{-1}) to ensure a reliable

209 quantification of the individual enantiomers during the experiment. Application was

210 conducted on October 8, 2014.

211   Weather conditions (rainfall and air temperature) were monitored along the duration of

212 the experiment (Fig. 1) at a weather station close to the study site (4 km). Temperatures were

213 relatively high for the fall season in which the experiment was performed. The maximum

214 daily temperatures ranged between 11.9 and 32.4 ºC with an average of 19.9 ºC, whereas the

215 minimum daily temperatures ranged between 2.7 and 20.7 º C with an average of 10.4 ºC.

216 The total rainfall during the 98 days of experiment was 298 mm, with noticeable

217 precipitation events of 46 mm at day 2, 32 mm at day 34, 39 mm at day 51, and 36 mm at

218 day 66.

219   Sampling was performed 0, 35, 64 and 98 days after treatment (DAT). At these times,

220 triplicate soil subsamples were taken from each plot at three different depths (0-5, 5-10 and

221 10-20 cm) using a 3 cm internal diameter spade for a total of 9 observations per treatment.

222 Soil subsamples were packed in separate plastic bags and immediately frozen at -18 ºC until

223 they were extracted. For extraction, the soil subsamples were homogenized and duplicate

224 aliquots (5 g) were shaken in 10 mL of methanol for 24 h, centrifuged, and the supernatants

225 filtered and analyzed by HPLC to determine the soil fungicide concentration (mg kg^{-1} dry

226 soil). A preliminary experiment showed that this extraction procedure recovered more than

227 95% of the fungicides freshly applied to the soil. To correct for the water content, duplicate 1

228 g-aliquots of each soil subsample were dried at 105 ºC for 24 h and the water loss was

229 calculated. Additionally, 35 DAT soil aliquots rich in sorbent particles (clay and BC) were

230 visually identified and sampled from the 0-5 cm section of the amended soil plots. These soil

231 aliquots were also homogenized and extracted in duplicate (250 mg) with methanol (5 mL)

232 by shaking for 24 h to determine their fungicide content.

233   The ability of the amendments to extend the presence of the fungicides in the upper 0-5

234 cm soil section was assessed by fitting the soil concentration data to the linearized form of a

235 first-order kinetic rate law:

236

237 $$ln\ C = ln\ C_0 - kt \qquad (4)$$

238

239 where C (mg kg^{-1}) and C_0 (mg kg^{-1}) are the concentration of the fungicide in the top 0–5 cm

240 of soil at time t (days) and t= 0, respectively, and k (days^{-1}) is the first-order dissipation rate

241 constant. The time for the dissipation of 50% and 90% of the fungicide from the top 0-5 cm

242 of soil was calculated as DT_{50}=0.693/k and DT_{90}= 2.303/k, respectively.

243

244 *2.4. Enantiomeric fraction*

245   The possible enantiomer-selective behavior of tebuconazole and metalaxyl in the

246 laboratory and field experiments was assessed by monitoring the individual concentration of

247 each of the two enantiomers of the fungicides, and then calculating the enantiomer fraction

248 (EF) using the formula proposed by Harner et al. (2000):

249

250   EF= [1]/([1]+[2])   (5)

251

252   where [1] and [2] are, respectively, the concentration of the first and last eluting enantiomer

253   during the chiral chromatographic analysis, which is described in the next section.

254

255   *2.5. Analysis of the fungicides*

256   The analysis of tebuconazole and metalaxyl was conducted by achiral and chiral high-

257   performance liquid chromatography (HPLC), in both cases using a Waters chromatograph

258   consisting of a Waters 600E System Controller, a Waters 717 Autosampler injector, and a

259   Waters 998 Photodiode Array Detector (PDAD). For the achiral analysis, both fungicides

260   were determined simultaneously according to the following chromatographic conditions:

261   Kinetex C18 chromatographic column of 150 mm length $\times$ 4.6 mm internal diameter (i.d.)

262   and 5 μm particle size (Phenomenex), 25 μL injection volume, 50:50 acetonitrile:water

263   isocratic eluent mixture at a flow rate of 1 mL min^{-1}, and detection wavelength at 226 nm for

264   tebuconazole and 213 nm for metalaxyl. Under these conditions metalaxyl (*R*+*S*) and

265   tebuconazole (*R*+*S*) eluted at 3.3 and 6.7 min, respectively.

266   The enantioselective analysis of metalaxyl was performed using a Chiralpak IB column

267   of 150 mm length $\times$ 4.6 mm i.d. and 5 μm particle size (Chiral Technologies Europe), a

268   60:40 (v/v) water:acetonitrile eluent mixture at a flow rate of 1 mL min^{-1}, and an injection

269   volume of 50 μL. According to these analytical conditions, *S*-metalaxyl and *R*-metalaxyl

270   eluted at 4.9 and 6.1 min, respectively (Celis et al., 2013). For the enantioselective analysis

271   of tebuconazole, we used a Chiralpak AY-RH column of 150 mm length $\times$ 4.6 mm i.d. and 5

272   μm particle size (Chiral Technologies Europe), a 60:40 (v/v) water:acetonitrile eluent

273   mixture at a flow rate of 1 mL min^{-1}, and an injection volume of 50 μL. The elution order

274 was 5.6 and 18.4 min for the first and last eluted enantiomers, respectively. Under nearly

275 identical chromatographic conditions, Wang et al. (2012) assigned the first eluted

276 enantiomer to *R*-tebuconazole and the last eluted enantiomer to *S*-tebuconazole. The

277 detection wavelengths for the chiral analyses were the same as those used for the achiral

278 determinations. For both the achiral and chiral analyses, external calibration was carried out

279 by injection of five standard solutions containing a mixture of tebuconazole and metalaxyl at

280 concentrations ranging between 0.1 and 6 mg L^{-1} (R^2=0.999). The experimental limit of

281 detection (LOD), calculated as the concentration resulting in a signal to noise ratio (S/N) of

282 3:1, was 0.03 mg L^{-1} for both fungicides.

283

284 *2.6. Statistical analysis*

285 Statistical analysis was carried out using IBM SPSS Statistics 22. Standard error was used

286 to indicate variability among triplicates. In the case of the field experiment, each triplicate

287 value came from averaging three subsample observations. Distribution coefficients (K$_d$),

288 fungicide concentrations in field soil samples, and enantiomer fractions (EF) were compared

289 using ANOVA followed by Tukey´s test to establish differences between treatments. An

290 analysis of covariance (ANCOVA) was performed to compare pairwise the slopes of the

291 regression lines (*k*) of the first-order dissipation data. All statistical analyses were performed

292 at the 95 % significance level ($p < 0.05$).

293

294 **3. Results and discussion**

295 *3.1. Laboratory sorption experiment*

296 Metalaxyl and tebuconazole sorption data on unamended soil and on soil amended with

297 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 L kg^{-1}) and the sorption of tebuconazole from 55% (K_d= 2.4 L

310   kg^{-1}) up to 96% (K_d= 47.4 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

323   metalaxyl and tebuconazole could reflect the small S_{BET} value registered for this BC (2.5 m^2

324   g^{-1}). Nevertheless, the contribution of BC to the sorption of metalaxyl in BC-amended soil

325   ($K_{d-sorbent}$= 25-36 L kg^{-1}, Table 1) was even lower than that (~ 100 L kg^{-1}) we observed in our

326   earlier study after amending a sandy loam soil with a OMWc-derived BC having a S_{BET}= 0.3

327   m^2 g^{-1} (Gámiz et al., 2016). Compared with the BC used in the present study, the BC used in

328   Gámiz et al. (2016) came from the same feedstock but was prepared at lower temperature

329   (400 ºC) and under a more strictly controlled anoxic atmosphere. It appears that these

330   variables are important in determining the performance of BCs as pesticide sorbents. It

331   should also be pointed out that N_2 is not considered a suitable adsorbate to analyze materials

332   with pores of size < 0.5 nm (de Jonge and Mittelmeijer-Hazeleger, 1996), which is the case

333   of biochars, and that additional properties related to the preparation procedure can influence

334   the sorption of organic compounds by char materials (Lattao et al., 2014).

335   An enantioselective analysis of the supernatants during the laboratory sorption

336   experiment was also conducted and revealed that neither metalaxyl nor tebuconazole showed

337   enantioselectivity in their sorption on unamended or amended soil, as indicated by the

338   supernatant enantiomer fractions EF ~ 0.50. Values of solution EF values equal or close to

339   0.5 indicated no preferential sorption of one enantiomer over the other. This agreed with

340   previous observations indicating lack of enantioselectivity during the sorption of different

341   chiral pesticides on soils from racemic initial pesticide solutions (Celis et al., 2013; Gámiz et

342   al., 2013; Qi et al., 2015; Sukul et al., 2013).

343

344   *3.2. Field experiment*

345   *3.2.1. Fungicide persistence and leaching*

346   The soil concentrations of (*R*+*S*)-tebuconazole and (*R*+*S*)-metalaxyl (mg kg^{-1} dry soil)

347   present at different depths (0-5, 5-10, and 10-20 cm) of the unamended and clay- and BC-

348   amended soil plots as a function of time are compiled in Fig. S2 and Fig. S3 of the

349   Supplementary material. It was noticeable that the concentrations of metalaxyl (20-25 mg

350   kg^{-1}) and tebuconazole (10-12 mg kg^{-1}) in the top 0-5 cm soil layer at the beginning of the

351   experiment (0 DAT) were relatively high, since they corresponded to those expected after

352   assuming a soil density of about 0.6 g cm^{-3}. This low density was attributed to some soil

353   disturbance upon sampling, as the 0-5 cm soil section in the sampling spade was visually less

354   compacted than deeper soil sections. The slightly higher initial fungicide concentrations

355   observed for clay- and BC-amended soil compared to unamended soil (significant for

356   metalaxyl) could also reflect slight differences in soil density after amendment, as previously

357   suggested for organically-amended field soils (Gámiz et al., 2012). On the other hand, since

358   soil concentration data were simultaneously influenced by fungicide degradation and

359   transport losses, to evaluate leaching we calculated the fraction of residues at different soil

360   depths relative to the total amount extracted from the 0-20 cm soil profile. The results are

361   summarized in Fig. 2 and Fig. 3 for tebuconazole and metalaxyl, respectively.

362   *Tebuconazole.* For all treatments and sampling times, the amount of tebuconazole

363   residues present in the top 0-5 cm soil layer represented more than 75% of the total extracted

364   residues (Fig. 2). This fraction was significantly greater ($p< 0.05$) than that present at deeper

365   depths (5-10 and 10-20 cm). The low mobility of tebuconazole observed in the field trial was

366   in accordance with the results of the laboratory sorption experiment indicating high sorption

367   of the fungicide on the unamended and clay- and BC-amended soil (Table 1). It also agreed

368   with previous works reporting low leaching of tebuconazole in unamended soils and in soils

369   treated with different organic amendments, in line with the high affinity of this fungicide for

370   endogenous and exogenous soil organic matter (Aldana et al., 2011; Fenoll et al., 2011;

371   Kalbe et al., 2014). Herrero-Hernández et al. (2011), however, observed higher mobility of

372   tebuconazole to deeper soil layers in a sandy clay loam soil amended with spent mushroom

373   substrate compared to the unamended soil, and attributed this effect to the presence of

374   dissolved organic matter released by the amendment. This mechanism did not appear to play

375   a major role in our study.

376   The results of fitting tebuconazole concentration data for the top 0-5 cm soil section to

377   first order dissipation kinetics are given in Table 2. Differences in the dissipation rate

378   constants (k) for tebuconazole under the different treatments were found to be not

379   statistically significant. DT_{50} values ranged between 50 and 58 days, whereas DT_{90} values

380   ranged between 165 and 192 days (Table 2). These values agree with the average values of

381    $DT_{50}= 47$ days and $DT_{90}= 177$ days reported by the Pesticide Properties Database (PPDB,

382   2016) for the field dissipation of tebuconazole. Under laboratory conditions, the degradation

383   of tebuconazole has generally been observed to proceed slowly (Fenoll et al., 2010; Li et al.,

384   2015; Mosquera et al., 2010; Muñoz-Leoz et al., 2011; Wang et al., 2012). Given that it is

385   well-known that sorption influences the bioavailability of pesticides in soils (Koskinen et al.,

386   2001), the high sorption of tebuconazole on unamended soil (Table 1) could have

387   contributed to limit its transport and degradation losses from the topsoil and also to reduce

388   the impact of clay and BC addition on the DT_{50} and DT_{90} values.

389   *Metalaxyl.* Metalaxyl displayed greater downward mobility than tebuconazole and this

390   resulted in greater differences between treatments in the leaching of this fungicide compared

391   to tebuconazole. Differences were particularly noticeable at later sampling times (64 and 98

392   DAT), at which the relative distribution patterns of metalaxyl residues showed that the

393   fungicide displayed higher leaching in unamended soil than in clay- and BC-amended soil

394   (Fig. 3). For example, at 98 days, 43% of the metalaxyl residues extracted from unamended

395   soil came from the 10-20 cm soil layer (Fig. 3). This value was significantly higher than the

396   fraction that came from the 0-5 cm (14%, $p < 0.001$) and 5-10 cm (35%, $p < 0.05$) soil

397   layers, that is, metalaxyl residues accumulated in the deepest (10-20 cm) layer of the

398   unamended soil. In contrast, for the clay- and BC-amended soil the fraction metalaxyl

399   residues present at the 10-20 cm soil layer was not significantly different from those present

400   at the 0-5 and 5-10 cm soil depths (Fig. 3).

401   The effect of the amendments on metalaxyl leaching was less evident at earlier sampling

402   times, even though several noticeable rainfall events occurred during this period (Fig. 1). We

403   attributed this result to the high temperatures reached at the beginning of the experiment

404   (Fig. 1), which should have favored water evaporation and reduced water percolation. It is

405   interesting to note, however, that 35 DAT the percentage of soil subsamples collected from

406   the deepest horizon (10-20 cm) that contained detectable metalaxyl residues (LOD= 0.07 mg

407   kg^{-1}) was reduced from 78% for the unamended soil to 22% for the clay-amended soil. This

408   showed the immobilizing capacity of the amendment that would become more evident at

409   later sampling dates.

410   The immobilizing capacity of clay and BC can reasonably be attributed to the presence of

411   particles of the sorbent which, as shown in Fig. S4, accumulated in the top 0-5 cm of soil and

412   increased the sorption capacity of the topsoil layer. To confirm this hypothesis, soil aliquots

413   rich in sorbent particles (clay and BC) were sampled from the amended soil plots 35 DAT

414   and subsequently extracted. The results indicated they indeed contained greater fungicide

415   concentrations than bulk soil samples (Table 3).

416   Metalaxyl dissipated more rapidly from the top 0-5 cm of soil than tebuconazole.

417 Differences in the dissipation rate constants (k) for the different treatments increased,

418 becoming statistically significant between the clay-amended soil and the unamended soil

419 (Table 2). DT_{50} values ranged between 16 and 23 days, whereas DT_{90} values ranged between

420 52 and 76 days (Table 2). Our DT_{50} values are somewhat lower than the average value of 46

421 days reported by the PPDB (2016) for the field dissipation of metalaxyl, but within the range

422 of 9 and 70 days observed in different field studies where metalaxyl was applied to

423   unamended and organically-amended soils (Kookana et al., 1995; Liu et al., 2012;

424   Triantafyllidis et al., 2013). The enhanced persistence of metalaxyl in the top 0-5 cm soil

425   layer after amending the soil with clay was most likely a consequence of the higher sorption

426   exerted by the amendment (Table 1), which reduced not only the leaching of the fungicide to

427   deeper soil layers, but also its bioavailability to be degraded, as suggested by other authors

428   for organically-amended soils (Fernandes et al., 2006; Rodríguez-Cruz et al., 2012) and

429   supported by the results of the enantioselective study presented in the next section.

430

431   *3.2.2. Enantioselective study*

432   The enantiomer fractions (EFs) determined for tebuconazole and metalaxyl residues

433   present at different soil depths along the field experiment are shown in Fig. S5 and Fig. 4,

434   respectively. Individual enantiomer concentration data for the top 0-5 cm soil section were

435   also fitted to first order dissipation kinetics, and the corresponding parameters are compiled

436   in Table S1 of the Supplementary material.

437   For tebuconazole, EFs were only calculated for the top 0-5 cm, because the fungicide

438   concentrations at deeper profiles were very low (Fig. S2). There were no significant changes

439   ($p > 0.05$) in the original (racemic) tebuconazole EF value of 0.5 along the experiment either

440   for unamended or amended soil (Fig. S5), i.e. tebuconazole remained mainly as a racemate

441   during the whole experiment. Accordingly, the dissipation rate constant (k) for the *R*-

442   enantiomer of tebuconazole was not significantly different from that of the *S*-enantiomer

443   (Table S1). Enantiomer-selective studies on tebuconazole degradation in soil under field

444   conditions are very scarce (Ye et al., 2013). Under laboratory conditions, recent studies have

445   indicated limited enantioselectivity for the degradation of this fungicide in soils. For

446   example, Li et al. (2015) reported tebuconazole residues had EF values in the range 0.452-

447   0.475 at the end of a 180-d incubation study conducted on seven soils under aerobic

448   conditions, and Wang et al. (2012) reported EF values between 0.474 and 0.481 after a 160

449   d-incubation study with three soils. Even though it is well-known that microbially-mediated

450   processes play an important role in the degradation of tebuconazole in soil (Potter et al.,

451   2005; Sehnem et al., 2010), it seems that degradation occurred too slow to develop a marked

452   enantioselectivity within the time-scale of the experiments (Wang et al., 2012).

453   In contrast to tebuconazole, the behavior of metalaxyl was prominently enantioselective

454   and the EF of its soil residues differed with soil depth and with soil treatment (Fig. 4). EF

455   values of 0.50 ± 0.01 were only obtained at the beginning of the experiment; shortly after

456   fungicide application metalaxyl residues became non-racemic (EF> 0.5) for all treatments

457   (Fig. 4). EF values greater than 0.5 indicate metalaxyl residues were richer in the first eluting

458   enantiomer (*S*-metalaxyl) than in the second eluting enantiomer (*R*-metalaxyl), which is

459   congruent with previous observation indicating that *R*-metalaxyl degrades faster than *S*-

460   metalaxyl in soils with pH > 5 under aerobic conditions (Buerge et al., 2003; Celis et al.,

461   2013; Monkiedje et al., 2003). This was confirmed by the greater dissipation rate constant

462   obtained for *R*-metalaxyl compared to *S*-metalaxyl, particularly in unamended soil (Table

463   S1). In general, the DT_{50} values reported in Table S1 for *R*- and *S*-metalaxyl are similar or

464   lower compared to previously reported values obtained under controlled laboratory

465   conditions for similar unamended and organically-amended Mediterranean soils (Gámiz et

466   al., 2016, 2013). In part, this is due to the fact that transport losses contributed to the

467   dissipation of metalaxyl in the field, but not in laboratory incubations. An exception was the

468   short laboratory half-life reported by Gámiz et al. (2016) for *R*-metalaxyl in unamended

469   sandy loam soil ($DT_{50}= 3$ days). It was inferred that the microbial population of the soil

470   sample was particularly prone to the degradation of *R*-metalaxyl (Gámiz et al., 2016).

471   With regard to the degree of enantioselectivity, EF values were generally higher in

472   unamended soil compared to clay- and BC-amended soil (Fig. 4), particularly for the top 0-5

473   cm of soil. Thus, at the end of the experiment (98 DAT), EF of residues in the 0-5 cm soil

474   layer followed the order: unamended soil (0.88) > BC-amended soil (0.78) > clay-amended

475   soil (0.70) with differences being statistically significant at the $p < 0.05$ level. For non-

476   amended soil at t= 98 days, differences in EF values for the different soil depths were

477   statistically significant ($p < 0.05$) only between the 0-5 and 10-20 cm soil layers, whereas for

478   clay- and BC-amended soil EF values significantly increased with soil depth (p< 0.05) (Fig.

479   4). Interestingly, the difference in k values between the R- and S-metalaxyl enantiomers for

480   the top 0-5 cm soil layer was significant ($p < 0.05$) for unamended soil, but became non-

481   significant for the amended soil (Table S1).

482   Several important consequences can be derived from the enantioselective analysis of

483   metalaxyl residues. Bearing in mind the biologically-mediated degradation of metalaxyl in

484   soil (Kalathoor et al., 2015; Sukul and Spiteller, 2001; Sukul, 2006) and the non-

485   enantioselective sorption displayed by the amendments used (Table 1), the fact that for the

486   top 0-5 cm soil layer metalaxyl remained more racemic in clay- and BC-amended soil than

487   in unamended soil (Fig. 4) strongly indicated that clay and BC protected metalaxyl from its

488   enantioselective biodegradation. This result is in agreement with the results of laboratory

489   experiments suggesting that sorption to biochar protected metalaxyl enantiomers from

490   biodegradation in soil, prolonging their presence in a racemic form (Gámiz et al., 2016). In

491   fact, the enantioselective analysis of metalaxyl residues in soil aliquots rich in sorbent

492   particles confirmed they were more racemic than residues extracted from bulk soil samples

493   (Table 3). On the other hand, the fact that EF values increased with soil depth, particularly

494   for clay- and BC-amended soil, further indicated that sorption protected the fungicide from

495   biodegradation, and that there was a relationship between the biodegradable fraction and the

496   leachable fraction, as would be expected.

497

498 **4. Conclusions**

499   Oleate-modified hydrotalcite (clay) and olive mill waste-derived biochar (BC) behaved as

500 efficient sorbents for the chiral fungicides tebuconazole and metalaxyl both under laboratory

501 and field conditions. Batch sorption laboratory experiments showed that the clay was much

502 more efficient in increasing the sorption capacity of the soil for the studied fungicides than

503 the biochar, and that the pyrolysis conditions influenced the performance of the BC as a

504 pesticide sorbent. In the field experiment, tebuconazole showed scarce leaching and long

505 persistence both in unamended and in clay- and BC-amended soil plots. Metalaxyl showed

506 greater mobility and shorter persistence than tebuconazole, but addition of clay and BC to

507 the top 0-5 cm soil layer reduced transport and degradation losses of the fungicide. One of

508 the main results of this work was to illustrate how the soil amendments affected the

509 enantiomeric composition of tebuconazole and metalaxyl soil residues. While tebuconazole

510 remained mostly racemic for all treatments during the 98-day field study, the *R*-enantiomer

511 of metalaxyl degraded faster than the *S*-enantiomer, and the addition of clay and BC affected

512 the changes in enantiomer fraction of metalaxyl residues with time, as predicted by earlier

513 laboratory incubation studies. In clay- and BC-amended soil, owing to the sorption exerted

514 by the sorbents, residues monitored in the top 0-5 cm of soil remained more racemic than in

515 unamended soil, and the fraction of *S*-enantiomer increased sharply with depth. The

516 outcomes from this study support the use of clays and biochars as sorbents to prolong the

517 presence of mobile and/or scarcely persistent chiral pesticide enantiomers in the topsoil by

518 reducing enantiomer leaching and degradation processes. Finally, this work has also

519 corroborated under real field conditions that sorption can indirectly influence the final

520 enantiomeric composition of chiral pesticide residues by affecting the enantiomers

521 biodegradation rate.

522

523

Acknowledgments

531

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764

765 **FIGURE CAPTIONS**

766 **Fig. 1.** Rainfall (bars) and temperatures (lines) during the field experiment. Vertical dotted

767 lines indicate sampling dates.

768

769 **Fig. 2.** Distribution of tebuconazole residues at different soil depths for unamended, clay-

770 amended, and BC-amended soil as a function of time after fungicide application. Error bars

771 correspond to the standard errors of triplicate soil plots.

772

773 **Fig. 3.** Distribution of metalaxyl residues at different soil depths for unamended, clay-

774 amended, and BC-amended soil as a function of time after fungicide application. Error bars

775 correspond to the standard errors of triplicate soil plots.

776

777 **Fig. 4.** Enantiomeric fractions of metalaxyl at different soil depths for unamended soil, clay-

778 amended and BC-amended soil as a function of time after fungicide application. Error bars

779 correspond to the standard errors of triplicate soil samples.

780

Table 1

Summary of metalaxyl (C_{ini}= 12 mg L^{-1}) and tebuconazole (C_{ini}= 6 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 ± 0.04 a	14 ± 1	-	2.4 ± 0.3 a	55 ± 3	-
Soil + Clay (0.5%)	1.55 ± 0.07 b	44 ± 1	242	22.7 ± 2.6 b	92 ± 1	4062
Soil + Clay (1%)	3.14 ± 0.09 c	61 ± 1	280	47.4 ± 2.4 c	96 ± 1	4502
Soil + BC (0.5%)	0.52 ± 0.03 d	21 ± 1	36	5.4 ± 0.7 a,d	73 ± 2	602
Soil + BC (1%)	0.59 ± 0.02 d	23 ± 1	25	9.0 ± 2.0 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 ($p < 0.05$).

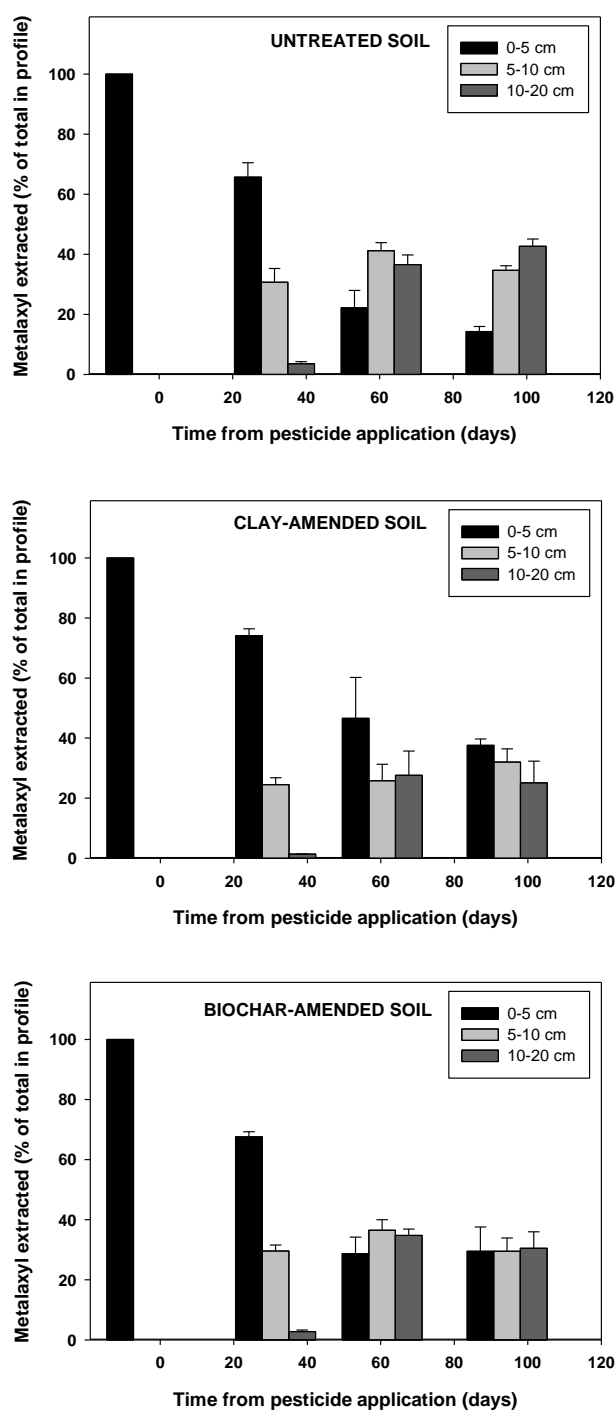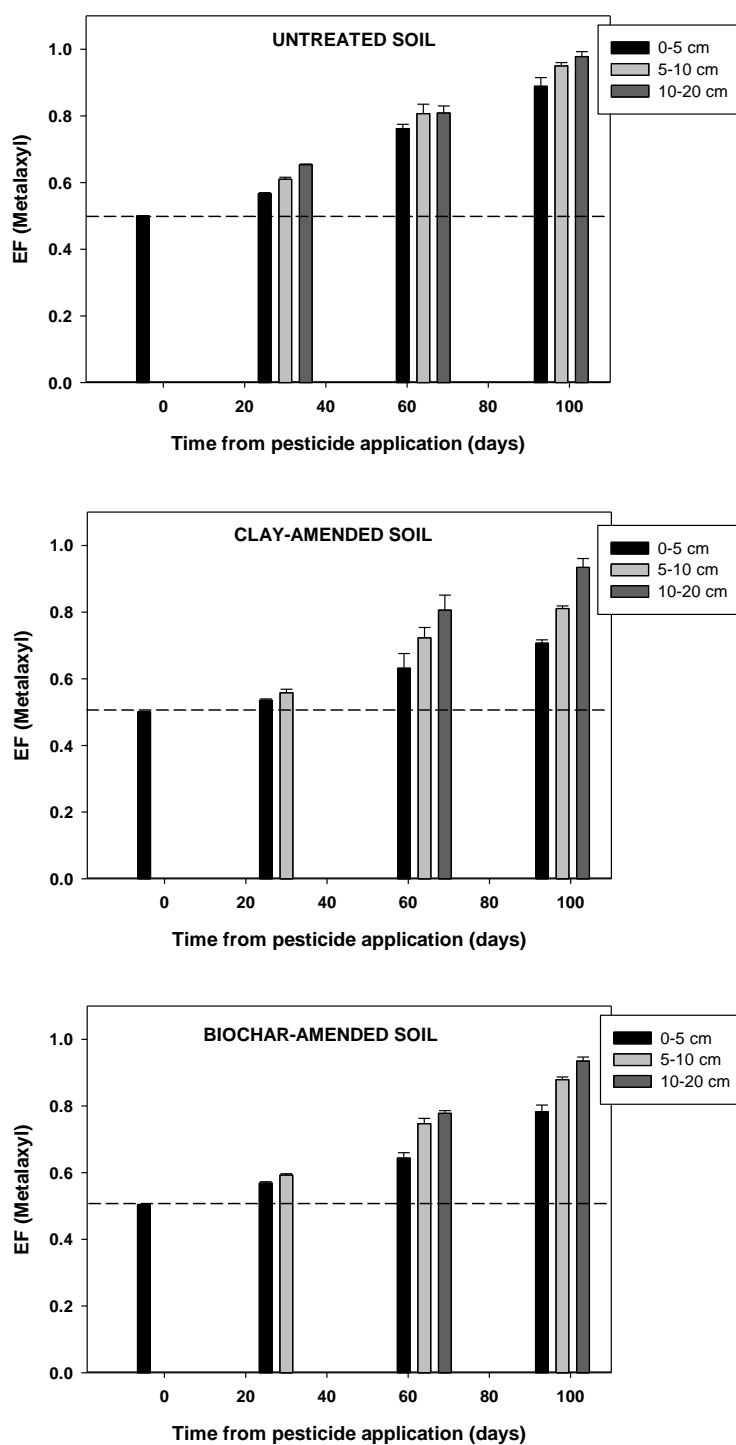|	Clay-amended soil		BC-amended soil	
	Metalaxyl concentration (mg kg^{-1})	EF	Metalaxyl concentration (mg kg^{-1})	EF
Bulk amended soil	8.5 ± 3.4 a	0.536 ± 0.004 a	9.1 ± 1.7 a	0.569 ± 0.004 a
Soil aliquots rich in sorbent particles	72 ± 27 b	0.497 ± 0.008 b	106 ± 16 b	0.512 ± 0.001 b
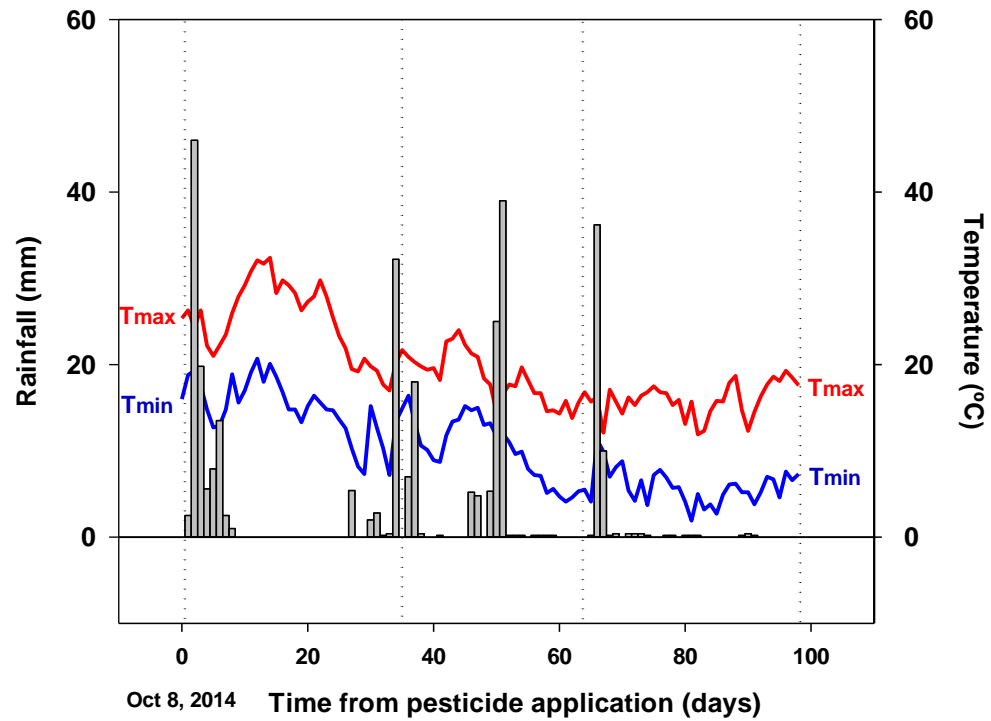
Fig. 1. Rainfall (bars) and temperatures (lines) during the field experiment. Vertical dotted lines indicate sampling dates.
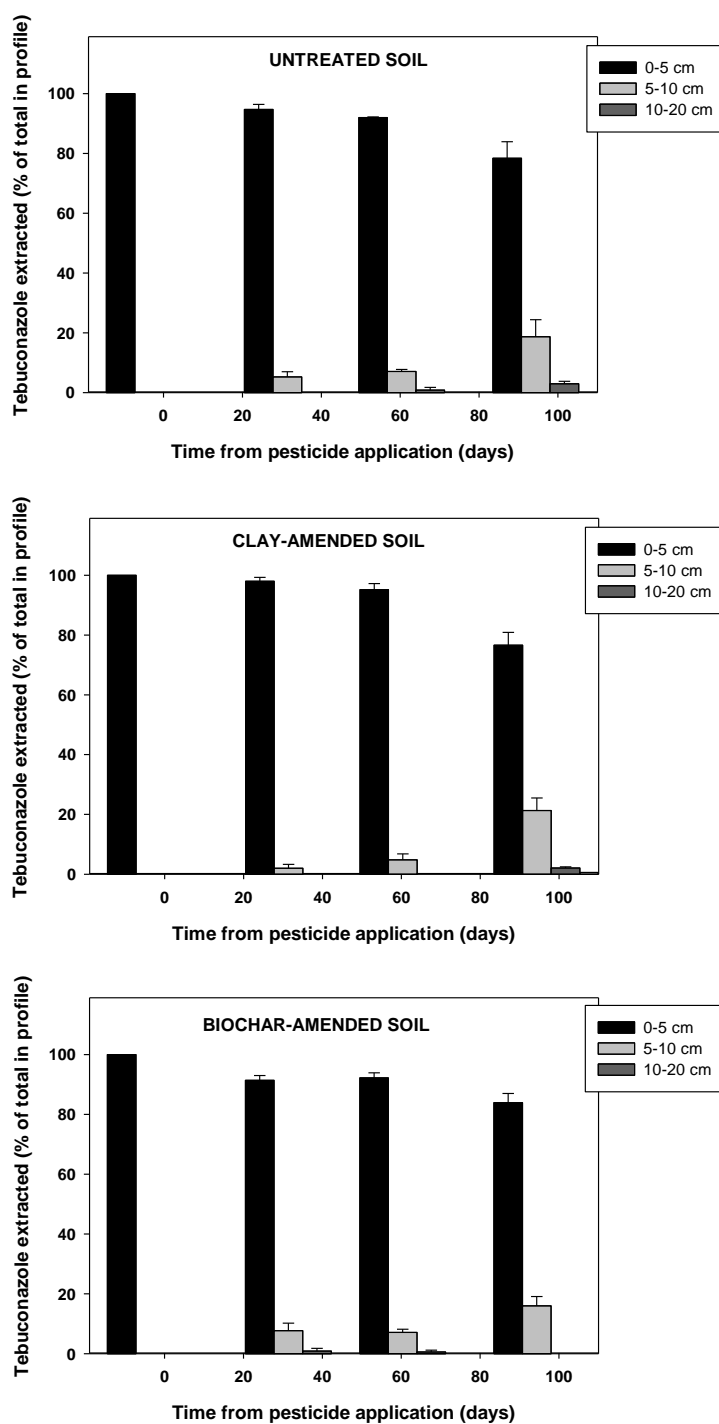
Fig. 2. Distribution of tebuconazole residues at different soil depths for unamended, clay-amended, and BC-amended soil as a function of time after funficide application. Error bars correspond to the standard errors of triplicate soil plots.

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.