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Enantiospecific behaviour of chiral drugs in soil.

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Enantiospecific behaviour of chiral drugs in soil

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Abstract

The importance of stereochemistry on the behaviour and effects of chiral pharmaceutical and illicit drugs in amended agricultural soils has been over looked to date. Therefore, this study was aimed at investigating the enantiospecific behaviour of a chemically diverse range of chiral drugs including naproxen, ibuprofen, salbutamol, bisoprolol, metoprolol, propranolol, acebutolol, atenolol, chlorpheniramine, amphetamine, fluoxetine and citalopram in soil microcosms. Considerable changes of the enantiomeric composition of ibuprofen, naproxen, atenolol, acebutolol and amphetamine were observed within 56 d. This is significant as enantiomer enrichment can favour the pharmacologically active (e.g., *S(-)*-atenolol) or less/non-active forms of the drug (e.g., *R(-)*-amphetamine). Single enantiomer microcosms showed enantiospecific degradation was responsible for enantiomer enrichment of atenolol and amphetamine. However, naproxen and ibuprofen enantiomers were subject to chiral inversion whereby one enantiomer converts to its antipode. Interestingly, chiral inversion was bidirectional and this is the first time it is reported in soil. Therefore, introduction of the less active enantiomer to soil through irrigation with reclaimed wastewater or biosolids as fertiliser can result in the formation of its active enantiomer, or vice versa. This phenomenon needs considered in risk assessment frameworks to avoid underestimating the risk posed by chiral drugs in amended soils.

Capsule

Changes to the enantiomeric composition of chiral drugs in soil due to enantiospecific degradation (e.g., atenolol and amphetamine) or chiral inversion (e.g., ibuprofen and naproxen) could result in the underestimation of environmental risk.

Keywords: pharmaceutical; soil; microcosm; enantiomer; inversion

1. Introduction

Human pharmaceutical and illicit drugs are emerging contaminants as their fate and effects in the environment are not fully understood (Petrie et al., 2015; Noguera-Oviedo and Aga, 2016). It is well established that drugs are incompletely removed during wastewater treatment and are found in both treated wastewater and sludge (or biosolids) (McClellan and Halden, 2010; Gardner et al., 2012). Most research has focussed on the fate and impact of drugs in the aquatic environment (Hughes et al., 2013; Petrie et al., 2015). However, irrigation of farmland with treated wastewater and application of biosolids as fertiliser are growing practices that introduce drugs to the terrestrial environment. Pharmaceutical drugs have been shown to exert toxicity to exposed organisms such as *Eisenia fetida*, which are essential for soil function (Pino et al., 2015). Additionally, bioaccumulation is possible, posing a risk to organisms in higher trophic levels (Kinney et al., 2008). Drugs are also taken up by plants from soils, including those grown for human consumption (Malchi et al., 2014; Wu et al., 2014).

Understanding the behaviour of drugs in amended soils is essential for the development of accurate environmental risk assessment (ERA). Degradation studies have found half-lives ($t_{1/2}$) can range from a few days (e.g., diclofenac) to >200 days (e.g., carbamazepine) (Monteiro and Boxall, 2009; Xu et al., 2009; Lin and Gan, 2011; Grossberger et al., 2014), demonstrating the diverse behaviour of drugs in the environment. An important consideration for assessing both the degradation and toxicity of drugs in the environment is their stereochemistry (Kasprzyk-Hordern, 2010). More than 50 % of pharmaceutical drugs on the market are chiral and exist as two or more enantiomers (Sanganyado et al., 2017). Chiral drugs are usually marketed as racemic mixtures (equimolar concentration of enantiomers), or as single enantiomer preparations. However, chiral drugs are often subject to enantiospecific degradation and toxicity in the environment (Stanley et al., 2006; Stanley et al., 2007; Bagnall et al., 2013; Evans et al., 2017; Petrie et al., 2018). Failing to consider the enantioselectivity of drugs in soils can result in the overestimation or underestimation of risk posed. Current ERA approaches do not require analysis at the enantiomeric level. Consequently, there is a paucity of data on the enantiospecific behaviour of drugs in soil.

Several studies have investigated the degradation of chiral drugs in soil (Monteiro and Boxall, 2009; Xu et al., 2009; Carr et al., 2011; Lin and Gan, 2011; Grossberger et al., 2014). However, most do not consider the role of stereochemistry on drug degradation. Furthermore, they do not report the enantiomeric composition of chiral drugs used in spiking studies (Xu et al., 2009; Lin and Gan, 2011; Grossberger et al., 2014). This is significant considering some analytical standards are available as racemates or in enantiomerically pure forms such as the anti-inflammatory drugs ibuprofen and naproxen. Considering enantiomers of the same drug can behave differently in soil, conclusions drawn from such studies could be misrepresentative. Preliminary studies undertaken at the enantiomeric level found considerable changes to the enantiomeric distribution of the stimulant amphetamine and the beta-blocker atenolol in soil microcosms (Petrie et al., 2018). For example, an initial amphetamine enantiomeric fraction (EF) of 0.5 (racemic) changed to 0.1 after 3 d incubation. The enrichment of *R(-)*-amphetamine was postulated as being the result of the comparatively faster degradation of *S(+)*-amphetamine (Petrie et al., 2018). Nevertheless, there is limited information on drugs that transform enantioselectively, or the processes responsible for these transformations. Both enantioselective degradation and/or chiral inversion can take place under environmental conditions (Sanganyado et al., 2017). Chiral inversion is the conversion of one enantiomer into its antipode without any other structural changes (Hutt and Caldwell, 1983). This process is significant as a non-toxic enantiomer in the environment has potential to convert into the toxic form.

An important factor to consider in the behaviour of chiral drugs in soil is temperature. Previous drug degradation studies have utilised soil temperatures in the range 18-25 ˚C (Monteiro and Boxall, 2009; Xu et al., 2009; Carr et al., 2011; Lin and Gan, 2011; Grossberger et al., 2014; Petrie et al., 2018). In temperate climates such as the United Kingdom (UK), average monthly soil temperatures generally vary from 4 ˚C in winter to 18 ˚C in summer (Busby, 2015), depending on location. Soil temperature had a significant impact on degradation of the herbicide florasulam (Krieger et al., 2000). Florasulam $t_{1/2}$ was found to be 8.5 d at 20 ˚C and 85 d at 5 ˚C in a clay loam soil. Thus, soil temperature is likely to play a considerable role in the degradation of pharmaceuticals, and their enantiomeric composition.

Due to the lack of research undertaken on the enantioselectivity of chiral drugs in soil, the objectives of this study were to: (i) investigate the enantiospecific behaviour of a diverse range of chiral drugs in soil, (ii) establish the influence of temperate summer (18 ºC) and winter (4 ºC) soil temperatures on chiral drug degradation and (iii) determine the processes responsible for enantioselective drug transformation (i.e., selective enantiomer degradation or chiral inversion). To achieve this, the fate of a chemically diverse selection of chiral drugs with one chiral centre (naproxen, ibuprofen, salbutamol, bisoprolol, metoprolol, propranolol, acebutolol, atenolol, chlorpheniramine, amphetamine, fluoxetine and citalopram - Table S1) was investigated in soil microcosms. Data from this work will improve our understanding and prediction of the risks associated with chiral pharmaceutical drugs in amended soils.

2. Materials and methods

2.1. Materials

Methanol, ammonium acetate, acetic acid and ammonium formate were HPLC grade and obtained from Sigma Aldrich. The reference materials *R/S(±)*-naproxen, *R(-)*-naproxen, *S(+)*-naproxen, *R/S(±)*-ibuprofen, *R(-)*-ibuprofen, *S(+)*-ibuprofen, *R/S(±)*-salbutamol, *R/S(±)*-bisoprolol, *R/S(±)*-metoprolol, *R/S(±)*-amphetamine, *S(+)*-amphetamine, *R(-)*-amphetamine, *R/S(±)*-propranolol, *R/S(±)*-acebutolol, *R/S(±)*-fluoxetine, *R/S(±)*-atenolol, *R(+)*-atenolol, *S(-)*-atenolol *R/S(±)*-chlorpheniramine, and *R/S(±)*-citalopram were purchased from Sigma Aldrich (Gillingham, UK) and Toronto Research Chemicals (Toronto, Canada). The corresponding deuterated surrogates were also purchased as racemates: *R/S(±)*-naproxen-d_3, *R/S(±)*-ibuprofen-d_3, *R/S(±)*-salbutamol-d_3, *R/S(±)*-bisoprolol-d_5, *R/S(±)*-metoprolol-d_7, *R/S(±)*-amphetamine-d_{11}, *R/S(±)*-propranolol-d_7, *R/S(±)*-acebutolol-d_5, *R/S(±)*-fluoxetine-d_6, *R/S(±)*-atenolol-d_7, *R/S(±)*-chlorpheniramine-d_6, and *R/S(±)*-citalopram-d_6. All chemicals were purchased as methanolic solutions of 0.1 mg mL^{-1} or 1 mg mL^{-1}, or as powder. Powders were prepared at an appropriate concentration in methanol. All solutions were stored in the dark at -20°C. Oasis HLB cartridges (3cc 60mg) were purchased from Waters (Manchester, UK).

2.2. Soil microcosms

Microcosm studies were performed to investigate drug degradation in soil under biotic and abiotic conditions. Soil (~5 kg) was collected from an arable farm in North-East Scotland during February 2019 (Table S2). The field where soil was collected had not been treated with biosolids or animal manure for the past five years. Consequently, no background levels of any of the studied drugs were found. Sample collection consisted of pooling randomly collected 10 g grab samples from a 20,000 m^2 area. Sub-samples were collected at least 10 m from the field boundary and from the top 10 cm surface layer of the soil. Soil was transferred to the laboratory immediately and sieved to less than 2 mm. To achieve abiotic conditions, 500 g of the sieved soil was autoclaved three times. Sodium azide was then added to soil at a concentration of 200 μg g^{-1} as described by Grossberger et al (2014).

Sacrificial microcosms were utilised in this study and prepared in a laminar flow cabinet. For both biotic and abiotic microcosms, 5 g of the corresponding soil was added to 50 mL sterile polypropylene tubes.

114 24 tubes were prepared for each treatment condition enabling eight different sampling times (triplicate
115 extractions). Soils were left for 12 h at the treatment temperature prior to spiking with drugs. Tubes
116 were spiked with racemic drugs at concentrations of either 100 ng g^{-1} (high spike) or 10 ng g^{-1} (low
117 spike). All spiked and measured concentrations are reported as wet weight (e.g., ng g^{-1} wet weight).
118 Both racemic naproxen and ibuprofen were spiked at 10,000 ng g^{-1} (high) and 1,000 ng g^{-1} (low) to
119 reflect their higher concentration in biosolids and the environment (Radjenović et al., 2009; Albero et
120 al., 2014; Petrie et al., 2015). Spiking was achieved using 500 µL of an aqueous working solution (<2
121 % methanol) of all drugs at their appropriate concentration. Biotic microcosms were incubated at both
122 18 °C and 4 °C for both high and low spike levels. Abiotic microcosms (high and low spike level) were
123 incubated at 18 °C. To ensure abiotic microcosms remained sterile throughout the study, aqueous soil
124 extracts were inoculated on Petri dishes containing 1.5 % agar medium. All microcosms were kept in
125 the dark throughout the study. For biotic microcosms, their weight was adjusted with water every few
126 days to maintain their field moisture content of 26 %. Triplicate samples were collected at times 0, 1,
127 3, 7, 14, 28, 42 and 56 days ready for analysis by accelerated solvent extraction-solid phase extraction-
128 liquid chromatography-tandem mass spectrometry (ASE-SPE-LC-MS/MS).

129 Further biotic microcosms were prepared using single enantiomers to help understand enantioselective
130 transformation processes. These were prepared using the same soil, albeit following storage at 4 °C for
131 60 d (moisture content was adjusted to field conditions prior to initiating the microcosms). Microcosms
132 were spiked at the high level (5,000 ng g^{-1} in the case of naproxen and ibuprofen or 50 ng g^{-1} for
133 amphetamine and atenolol, respectively for individual enantiomers) with either *S(+)*-naproxen, *S(+)*-
134 ibuprofen, *S(+)*-amphetamine and *R(+)*-atenolol, or *R(-)*-naproxen, *R(-)*-ibuprofen, *R(-)*-amphetamine
135 and *S(-)*-atenolol. The same methodology as described for the racemic microcosms was followed. A
136 summary of all microcosms prepared was outlined (Figure S1).

137 **2.3. Soil extraction**

138 Soil samples (5 g) were spiked with a methanolic mixture of all racemic deuterated surrogates to achieve
139 a concentration of 100 ng g^{-1} (10,000 ng g^{-1} in the case of *R/S(±)*-naproxen-d_3 and *R/S(±)*-ibuprofen-d_3).
140 Samples were mixed with Ottawa sand and packed into 10 mL stainless steel ASE cells. Two 2-4 µm

Dionex glass fibre filters were fitted to each end of the cell. The extraction of prepared soil samples was performed using a Dionex ASE-350 system (California, USA). The final method used an extraction solvent of 20:80 water:methanol and an extraction temperature of 80 °C as described in Petrie et al. (2018). Briefly, two extractions cycles were performed for each sample with the following settings: pre-heat for 5 min, heating for 5 min, solvent flush volume of 60% and nitrogen purge time of 150 s. The extraction pressure was 1500 psi.

Solvent extracts obtained from the ASE (~22 mL) were diluted to 250 mL using HPLC water. Oasis HLB cartridges were conditioned with 2 mL methanol followed by 2 mL water under gravity. Samples were then loaded at 5 mL min^{-1} using a vacuum manifold and dried under vacuum. Analytes were eluted under gravity using a 4 mL aliquot of methanol. Extracts were dried at 40 ˚C under nitrogen and reconstituted in 0.5 mL methanol for LC-MS/MS analysis.

2.4. Enantioselective liquid chromatography-tandem mass spectrometry

Chromatography was performed using an Agilent 1260 Infinity Series HPLC. Two methods were utilised for the separation of a full suite of analytes. For the separation of naproxen and ibuprofen a CHIRAL ART Amylose-SA column (250 × 4.6 mm; 5 µm) (YMC, Kyoto, Japan) maintained at 25°C was used. The mobile phase consisted of 30:70 water:methanol containing 10 mM ammonium formate adjusted to pH 3.5 using formic acid. The flow rate was 0.8 mL min^{-1} with an injection volume of 20 µL. The run time was 20 min. All remaining drugs were separated using a Chirobiotic V2 column (250 × 2.1 mm; 5 µm) (Supelco, Sigma Aldrich) maintained at 15 ˚C (Ramage et al., 2019). The mobile phase was methanol containing 1 mM ammonium acetate and 0.01% acetic acid at a flow rate of 0.17 mL min^{-1}. The injection volume was 40 µL and the total chromatographic run time was 80 min. The HPLC was coupled to an Agilent 6420 MS/MS triple quadrupole by electro-spray ionisation (ESI) in positive ionization mode. Selective ion monitoring transitions were utilised for naproxen and ibuprofen. All remaining drugs were analysed by multiple reaction monitoring. All monitored transitions can be found in Table S3. Example chromatograms can be found in Figure S2. Method quantitation limits were in the range 18-134 ng g^{-1} for naproxen and ibuprofen, and ≤1.3 ng g^{-1} for all remaining drugs (Table S4).

2.5. Data analysis

169 Enantiomeric fraction (EF) of each drug was calculated according to eq 1:

170 $$EF = \frac{E(+)}{[E(+)+E(-)]} \quad (1)$$

171 *E(+)* and *E(-)* is the concentration of the + and – enantiomers, respectively. Where the enantiomer
172 elution order is unknown the EF was calculated using eq 2:

173 $$EF = \frac{E1}{[E1+E2]} \quad (2)$$

174 Here, *E1* is the first eluting enantiomer and *E2* is the second eluting enantiomer. The EF can vary from
175 0 to 1 and an EF of 0.5 denotes an equimolar or racemic mixture of enantiomers.

176 Drug degradation was fitted to the first-order exponential decay model using eq 3:

177 $$C_t = C_0 \, x \, e^{-kt} \quad (3)$$

178 Here, C_t is the drug concentration at time *t* (d) and C_0 is the drug concentration at the start of the study,
179 and *k* is the degradation rate constant (1/*d*). Furthermore, drug half-life ($t_{1/2}$) was calculated according
180 to eq 4:

181 $$t_{1/2} = \frac{\ln(2)}{k} \quad (4)$$

182

3. Results and discussion

3.1. Enantiospecific behaviour of a diverse range of chiral drugs in soil

The enantiomeric composition of chiral drugs was monitored in biotic and abiotic soil microcosms spiked with racemic drug standards. All drugs were investigated simultaneously. These results are grouped and presented according to therapeutic drug group.

3.1.1. Anti-inflammatories

The anti-inflammatory drugs naproxen and ibuprofen are among the most well studied drugs in soils albeit not at the enantiomeric level (Monteiro and Boxall, 2009; Xu et al., 2009; Carr et al., 2011; Lin and Gan, 2011; Grossberger et al., 2014). In soil microcosms *R/S(±)*-naproxen degraded under biotic conditions at 18 ˚C (Figure 1). In the high spike microcosm (10,000 ng g^{-1}), the starting EF of 0.52±0.01 was increased to 0.67±0.01 after 56 d incubation representing an enrichment of *S(+)*-naproxen. Enantiomer $t_{1/2}$ values were 9.7±0.3 and 11.8±0.4 d for *S(+)*-naproxen and *R(-)*-naproxen, respectively (p-value <0.05) (Table 1). Interestingly, enantiomer degradation was greater at the low spike level (1,000 ng g^{-1}) with $t_{1/2}$ values of 6.9±0.8 and 7.8±0.5 d for *S(+)*-naproxen and *R(-)*-naproxen. For the low spike level, a maximum EF of 0.66±0.04 was observed (Figure 1). Significantly different $t_{1/2}$ values were observed between high and low spike microcosms (p-values <0.05) and is in agreement with Grossberger et al (2014). This observation was apparent for most studied drugs (Table 1). However, the first-order decay model is concentration independent (Alexander, 1999), suggesting the need for a pseudo second-order model (Grossberger et al., 2014). Nevertheless, for comparison between enantiomers of the same drug and published data, the first-order decay model was applied. Literature $t_{1/2}$ values range from 3 d to 69 d under a range of different experimental conditions (Monteiro and Boxall, 2009; Xu et al., 2009; Lin and Gan, 2011; Grossberger et al., 2014).

R/S(±)-ibuprofen degraded rapidly in biotic microcosms at 18 ˚C with enantiomer $t_{1/2}$ values of 1.0-2.3 d (Figure S4). Although previous studies do not report ibuprofen at the enantiomeric level, whole drug studies report $t_{1/2}$ values ranging from <1 d to 15 d (Monteiro and Boxall, 2009; Xu et al., 2009; Lin and Gan, 2011; Grossberger et al., 2014). An enrichment of *R(-)*-ibuprofen was observed resulting in EF

values reaching 0.38-0.39 after 3 d incubation. This is in agreement with Hashim et al (2011) who report racemic ibuprofen becoming enriched with *R(-)*-ibuprofen during wastewater treatment.

Abiotic microcosms were prepared to confirm drug degradation in soil is biologically driven. Sterile conditions were confirmed by the absence of any colony forming units in inoculated agar medium (Figure S3). In abiotic microcosms, no significant degradation of naproxen or ibuprofen, or changes to their EF were observed during the 56 d incubation time at either concentration level (Figure 1, Figure S4), confirming their enantioselective transformation is a result of biological processes. Indeed, no degradation or changes to the enantiomeric composition of any studied drug were found in abiotic conditions. The absence of any drug degradation in abiotic soils is in agreement with previous studies (Lin and Gan, 2011; Grossberger et al., 2014).

In soil incubated at 4 ˚C naproxen enantiomer degradation was reduced significantly, and by 6.2 to 9.2 times at the high spike level (p-value <0.05). To demonstrate, the *S(+)*-naproxen $t_{1/2}$ of 11.8±0.4 d at 18 ˚C was increased to 109±12.1 d at 4 ˚C (Table 1). Here, enantiomeric changes were still observed within the 56 d incubation time. The starting EF of 0.48±0.01 increased to 0.55-0.56 from 28 d onwards (Figure 1). At the low spike level, 4.4 to 8.2 times reduced degradation was found (p-value <0.05). The greatest EF was observed after 56 d where the EF was 0.70±0.03 (Figure 1). Soil incubation temperatures of 4 ˚C saw ibuprofen $t_{1/2}$ values increase by up to 2.7 times (Table 1). However, no change to *R(-)*-ibuprofen $t_{1/2}$ was noted in the low spike microcosms. A minimum EF of 0.43±0.06 was found here after 3 d. EFs of 0.38-0.39 were observed in the high spike microcosms, albeit at days 7 and 14 (Figure S4). Although temperature had a significant effect on naproxen and ibuprofen degradation, less impact was found at the lower concentration level. Previous soil microcosm studies report reduced nitrification kinetics (Tourna et al., 2008) and respiration rates (measured through CO_2 production) (Andrews et al., 2010) in soils incubated at temperatures ≤10 ˚C.

3.1.2. Anti-histamine

Chlorpheniramine is an over the counter antihistamine previously prioritised as a drug for further study in the environment (Boxall, 2004). Research has found it to be incompletely removed during wastewater

235 treatment (Roberts et al., 2016), yet there is still a paucity of information on its environmental fate.
236 Chlorpheniramine is moderately hydrophobic (log K_{OW} 3.67) suggesting it is likely to partition into
237 wastewater sludge. In soil microcosms no notable degradation (>20 %) of *R/S(±)*-chlorpheniramine, or
238 changes to its enantiomeric composition were observed in any of the biotic microcosms (Figure S5).
239 Previous studies prepared under similar conditions using the same drug concentration (albeit with
240 different soil) showed >50 % degradation over 56 d (Petrie et al., 2018). This difference in degradation
241 is attributed to differences in microbial community of the collected soils. However, further work is
242 required to confirm this assumption.

243 **3.1.3.Beta-blockers and beta-agonist**

244 Beta-blockers showed a range of behaviour in soil microcosms. *R/S(±)*-bisoprolol, *R/S(±)*-metoprolol
245 and *R/S(±)*-propranolol all degraded without enantioselective transformation (Figure S6-8). Enantiomer
246 $t_{1/2}$ values at 18 ˚C for the high spike level (100 ng g^{-1}) were 19-20 d, 61-64 d and 91-106 d, respectively
247 (Table 1). *R/S(±)*-propranolol behaviour is similar to that observed previously (Petrie et al., 2018). No
248 previous data exists on the enantiospecific behaviour of bisoprolol and metoprolol in soil. However,
249 metoprolol has shown enantioselective degradation in river waters (Evans et al., 2017).

250 *R/S(±)*-atenolol was found to degrade rapidly with enantiomer $t_{1/2}$ values in the range 3.6-8.0 d at 18 ˚C
251 and 6.0-15.6 d at 4 ˚C (Table 1). An enrichment of *S(-)*-atenolol was observed with EFs reaching a
252 minimum of 0.36±0.10 after 7 d in the low spike microcosm (10 ng g^{-1}) at 18 ˚C (Figure S9). This agrees
253 with previous work in agricultural soil (Petrie et al., 2018), with the same enrichment pathway also
254 found in wastewater (Nikolai et al., 2006; Kasprzyk-Hordern and Baker, 2012; Evans et al., 2017).
255 Enrichment of *S(-)*-atenolol is significant as this enantiomer has greater potency and is found to be about
256 four times more toxic than *R(+)*-atenolol to the environmental toxicity indicator species *Tetrahymena*
257 *thermophila* (de Andrés et al., 2009).

258 The enantiospecific behaviour of *R/S(±)*-acebutolol has not been investigated in the receiving
259 environment despite it being found in wastewater and surface waters (Daneshvar et al., 2010; Gabet-
260 Giraud et al., 2014) as well as having a propensity to adsorb to wastewater sludge and sediments (Ramil

et al., 2010; Sanganyado et al., 2016). In soil, acebutolol-E1 was found to degrade at a comparatively faster rate than acebutolol-E2 (Figure 2). Acebutolol-E1 $t_{1/2}$ values were 36.9±3.9 d and 33.4±3.2 d for the high and low spike levels at 18 ˚C ($t_{1/2}$ values could not be calculated for acebutolol-E2) (Table 1). Minimum EFs were 0.35±0.01 (high spike) and 0.32±0.01 (low spike) after 56 d demonstrating the considerable changes in enantiomeric composition of acebutolol found (Figure 2). Based on the work by Sanganyado et al (2016) using a similar chromatographic column and mobile phase conditions where the order of enantiomer elution is known, it is likely the more persistent enantiomer in soil was *R(+)*-acebutolol. This may be significant in the environment as *R(+)*-acebutolol is the active enantiomer and possesses the beta-blocking activity (Piquette-Miller et al., 1991). Interestingly, at 4 ˚C there was no degradation of either enantiomer and the drug remained unchanged over 56 d (Figure 2).

In contrast, the beta-agonist *R/S(±)*-salbutamol degraded rapidly with enantiomer $t_{1/2}$ values of ≤1.2 d under all biotic conditions, irrespective of temperature (Table 1). A small increase in EF was observed during degradation with an enrichment of salbutamol-E1 (Figure S10). Rapid degradation has been observed previously in soil with complete degradation observed within 14 d (Petrie et al., 2018).

3.1.4. Anti-depressants

Anti-depressants including citalopram and fluoxetine have been determined in biosolids and amended soils previously (Walters et al., 2010; Lajeunesse et al., 2012; Evans et al., 2015). In soil microcosms both *R/S(±)*-citalopram and *R/S(±)*-fluoxetine did not show any considerable degradation over 56 d under biotic conditions (Figure S11 and S12), including any changes to their enantiomeric composition. The persistence of fluoxetine in soils has been previously observed (Monteiro and Boxall, 2009; Walters et al., 2010; Petrie et al., 2018). Walters et al (2010) reported no degradation of fluoxetine in soil over 1,000 d.

3.1.5. Stimulant

The stimulant amphetamine degraded rapidly and enantioselectively in biotic microcosms (Figure S13). Enrichment with *R(-)*-amphetamine was considerable with EFs <0.2 after 3 d. This observation is consistent with previous studies demonstrating greater persistence of *R(-)*-amphetamine in the

environment (Bagnall et al., 2013; Evans et al., 2017), including soil (Petrie et al., 2018). This may be significant as *S(+)*-amphetamine has twice the stimulant activity of *R(-)*-amphetamine (Kasprzyk-Hordern, 2010). However, enantiospecific toxicity towards environmental organisms is yet to be established. Nevertheless, complete enantiomer degradation was observed within 28 d at 18 ˚C and within 42 d at 4 ˚C (Figure S13).

3.2. Transformation processes responsible for enantiospecific drug changes

Individual enantiomer microcosms were prepared to identify the processes responsible for enantiospecific transformations (Figure S1). The drugs studied were naproxen, ibuprofen, atenolol and amphetamine as they were subject to the greatest enantiomeric changes in racemic microcosms (acebutolol could not be obtained as individual enantiomers at the time of the study).

At 18 ˚C the loss of *R(-)*-naproxen coincided with the formation of *S(+)*-naproxen through chiral inversion (Figure 3). The EF changed from an initial value of 0.00 to 0.54±0.02 after 28 d. On the other hand, the loss of *S(+)*-naproxen from its respective microcosm resulted in the formation of *R(-)*-naproxen. In this case an initial EF of 1.00 changed to 0.78±0.01 after 28 d (Figure 3). However, as both inversion and degradation are taking place, it remains unknown which process (or both) is responsible for the overall changes in enantiomeric composition observed in racemic microcosms previously. Nevertheless, this is the first-time chiral inversion of a drug has been reported in soil, and that it can proceed in both directions. Chiral inversion of *S(+)*-naproxen to *R(-)*-naproxen has been observed previously during wastewater treatment (Hashim et al., 2011; Suzuki et al., 2014). Furthermore, Nguyen et al (2017) reported bidirectional inversion of anti-inflammatories (naproxen, ibuprofen and ketoprofen) by an enzymatic membrane bioreactor. The results of the single enantiomer microcosms agree with those of the racemic microcosm whereby an enrichment of *S(+)*-naproxen was found (Figure 1). Incubation of soil at 4 ˚C resulted in little or no inversion of naproxen enantiomers (Figure 3).

Ibuprofen enantiomers were also inverted in soil microcosms (Figure S14). Enrichment was preferential towards *R(-)*-ibuprofen (pharmacologically inactive enantiomer) resulting in those EFs <0.5 found in racemic microcosms previously (Figure S4). For example, incubation at 4 ˚C resulted in EF changes from 0.00 to 0.28±0.02 in the *R(-)*-ibuprofen spiked microcosm and from 1.00 to 0.48±0.03 in the *S(+)*-ibuprofen spiked microcosm over 56 d (Figure S14). *In vivo* mammalian studies have reported unidirectional conversion of *R(-)*-ibuprofen to *S(+)*-ibuprofen (Hao et al., 2005). Similarly, fungi such as *Verticillium lecanii* have been found to preferentially convert *R(-)*-ibuprofen to *S(+)*-ibuprofen by an enzymatic process related to mammalian studies (Thomason et al., 1998). However, evidence reporting the inversion of *S(+)*-ibuprofen to *R(-)*-ibuprofen by *Nocardia* bacteria exists (Mitsukura et al., 2002), as well as in lake water microcosms (Buser et al., 1999) and during wastewater treatment (Nguyen et al., 2017). The mechanism of chiral inversion remains poorly understood, particularly in the environment, but it is believed several enzymes play a role (Kato et al., 2003; Kato et al., 2004; Khan et al., 2014). It is thought that S-enantiomers form an activated coenzyme A derivative followed by epimerization to the *R*-enantiomer and hydrolysis of the *R*-acyl-coenzyme (Khan et al., 2014). Essentially, following an enzyme mediated deprotonation from the stereogenic centre an intermediate compound with a c=c double bond is formed. A subsequent (re)protonation can then take place either side of the c=c resulting in the formation of the antipode.

Degradation of atenolol enantiomers showed enantioselective degradation with $t_{1/2}$ values of 5.0±0.4 and 3.4±0.1 d for *S(-)*-atenolol and *R(+)*-atenolol at 18 ˚C, respectively (p-value <0.05) (Figure S15). No evidence of chiral inversion (or changes to EF) was observed. The comparatively faster degradation of *R(+)*-atenolol confirms the enrichment of *S(-)*-atenolol (EF <0.5) observed in racemic microcosms previously (Figure S9). However, the degradation rates were significantly different between the single enantiomer and racemic microcosms. Greatest differences were observed for soils incubated at 4 ˚C. For example, in racemic microcosms the $t_{1/2}$ value of *S(-)*-atenolol was 15.6±0.6 d and in single enantiomer microcosms it was 35.5±3.7 d (p-value <0.05). Although the same soil was used in both studies, soil was stored for 60 d at 4 ˚C prior to initiation of the single enantiomer microcosms. While this satisfies accepted guidelines (OECD, 2002), the differences in post-sampling storage is likely to

account for this. Stenberg et al (1998) reported effects on microbial biomass and activities in soils under cold storage. Nevertheless, the transformation processes identified and changes to EF observed correspond to those enantiospecific changes found in racemic microcosms.

Amphetamine enantiomers also showed enantioselective degradation without evidence of inversion (Figure S16). The $t_{1/2}$ values were 1.0±0.2 d for *S(+)*-amphetamine and 2.3±0.0 d for *R(-)*-amphetamine (p-value <0.05). To the best of our knowledge this is the first study to confirm the enantioselective degradation of amphetamine and atenolol in the environment (over other enantioselective processes such as chiral inversion) using single enantiomer microcosms.

3.3. Future perspective on the environmental risk assessment of chiral drugs in soil

Irrigation of agricultural land with reclaimed wastewater and recycling of biosolids as fertiliser are growing practices. Current environmental risk assessment guidelines for pharmaceuticals drugs in soils do not require enantiospecific toxicity or fate assessments for chiral compounds (European Medicines Agency, 2018). The main reasons for this are (i) there is a lack of information on the enantiomeric composition of drugs in biosolids and irrigation water, as well as their fate in amended soils, and (ii) there are no studies on the enantiospecific toxicity of chiral drugs to terrestrial organisms. Nevertheless, the limited data available for biosolids demonstrating non-racemic composition of drugs being applied to land (Evans et al., 2015), and the extent of enantiomer enrichment in amended soils observed in our study demonstrate studies on enantiospecific toxicity are now needed. Establishing the extent of enantiospecific toxicity towards terrestrial organisms will be a driver for further enantioselective studies of drugs in amended soils.

Care is needed if the analysis of biosolids or irrigation water is used to estimate soil enantiomer concentrations for risk assessment purposes (an approach taken for other trace pollutants (Stasinakis et al., 2008; Petrie et al., 2019). The inversion of pharmacologically less active enantiomers to more active enantiomers in soil or vice versa (e.g., naproxen and ibuprofen - Figure 3, Figure S14) could result in the underestimation or overestimation of risk, respectively (assuming pharmacological activity in

humans is reflected in environmental toxicity studies). Nevertheless, further fate studies are needed on chiral drugs in amended soils that were out with the scope of this study (different soils, exposure conditions etc) to ensure robust data for risk assessments is obtained. Such investigations need to study the microbial community of studied soils to improve our understanding of chiral drug degradation. It is recommended that laboratory fate studies utilise freshly collected soils to avoid any storage induced effects to the soil microbial community. Risk assessments must also account for soil temperature in fate assessments as it had a considerable impact on chiral drug transformation. For example, application of biosolids as fertiliser in temperate climates is typically done in preparation for spring or winter crop sowing where soil temperatures are notably different.

4. Conclusions

This study is the first to evaluate the enantiospecific behaviour of a diverse range of chiral drugs in soil. It found that five of the 12 studied drugs were subject to enantioselective transformation. Both enantioselective degradation (amphetamine and atenolol) and chiral inversion (naproxen and ibuprofen) were identified as transformation processes. Significantly, chiral inversion was found to be bidirectional. Thus, the introduction of the inactive enantiomer to soil can lead to the formation of the active antipode, or vice versa. This observation needs considered in future environmental risk assessments to avoid overestimating or underestimating the associated risks of irrigating agricultural land with reclaimed wastewater, or applying biosolids as fertiliser. However, further studies are now needed on the enantiospecific toxicity of chiral drugs in the terrestrial environment.

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390

391 **Supplementary material**

392 Additional information includes the experimental set up (Figure S1), example chromatograms (Figure
393 S2) comparison of biotic and abiotic soils inoculated on agar plates (Figure S3), degradation of various
394 chiral drugs in soils as racemates (Figure S4-13) and single enantiomers (Figure S14-16), chemical
395 properties of studied drugs (Table S1), properties of collected soil (Table S2), mass spectrometry
396 information (Table S3) and method performance data (Table S4).

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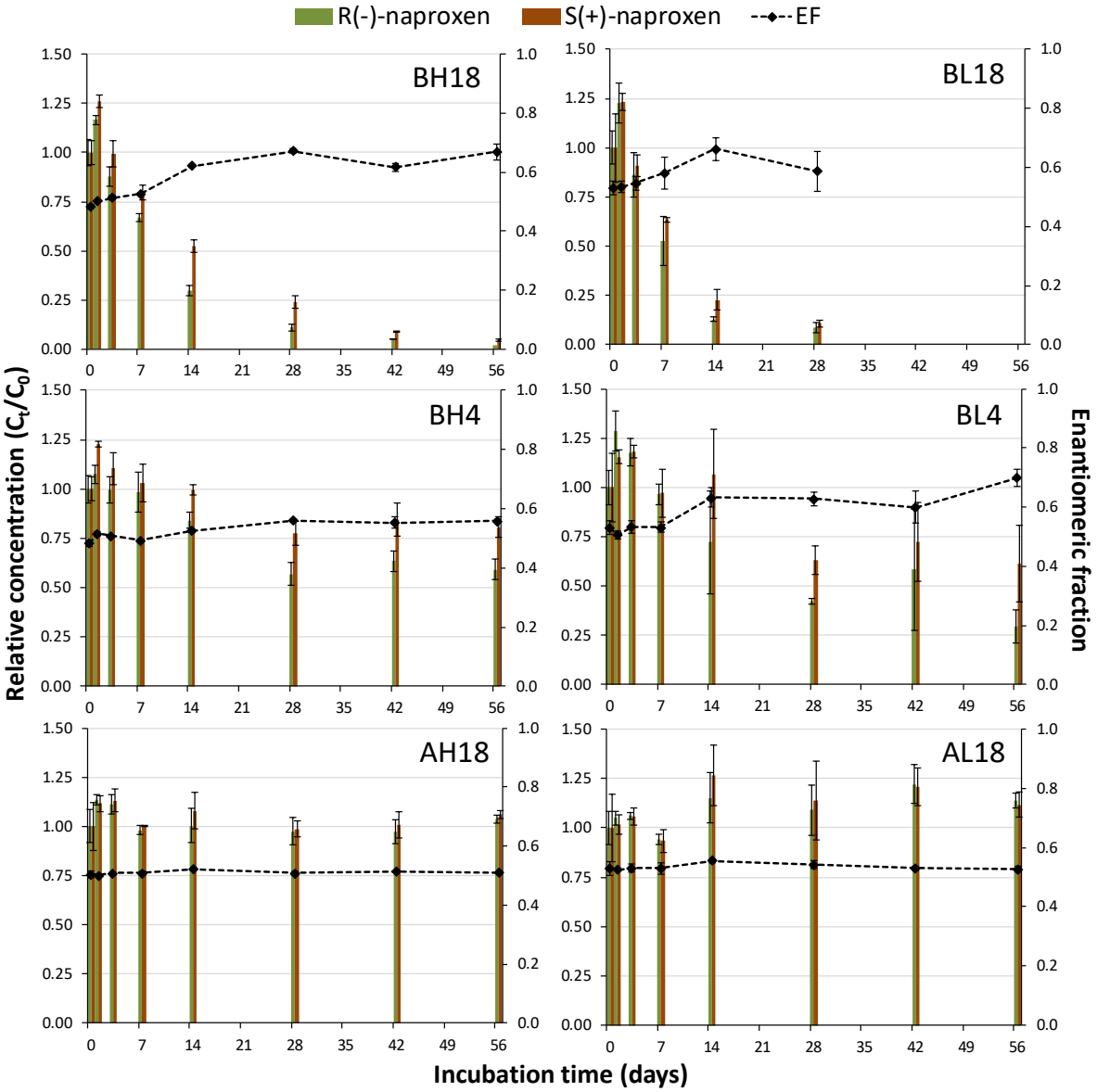

Figure 1. Relative concentration of *R(-)*-naproxen and *S(+)*-naproxen and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-naproxen

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

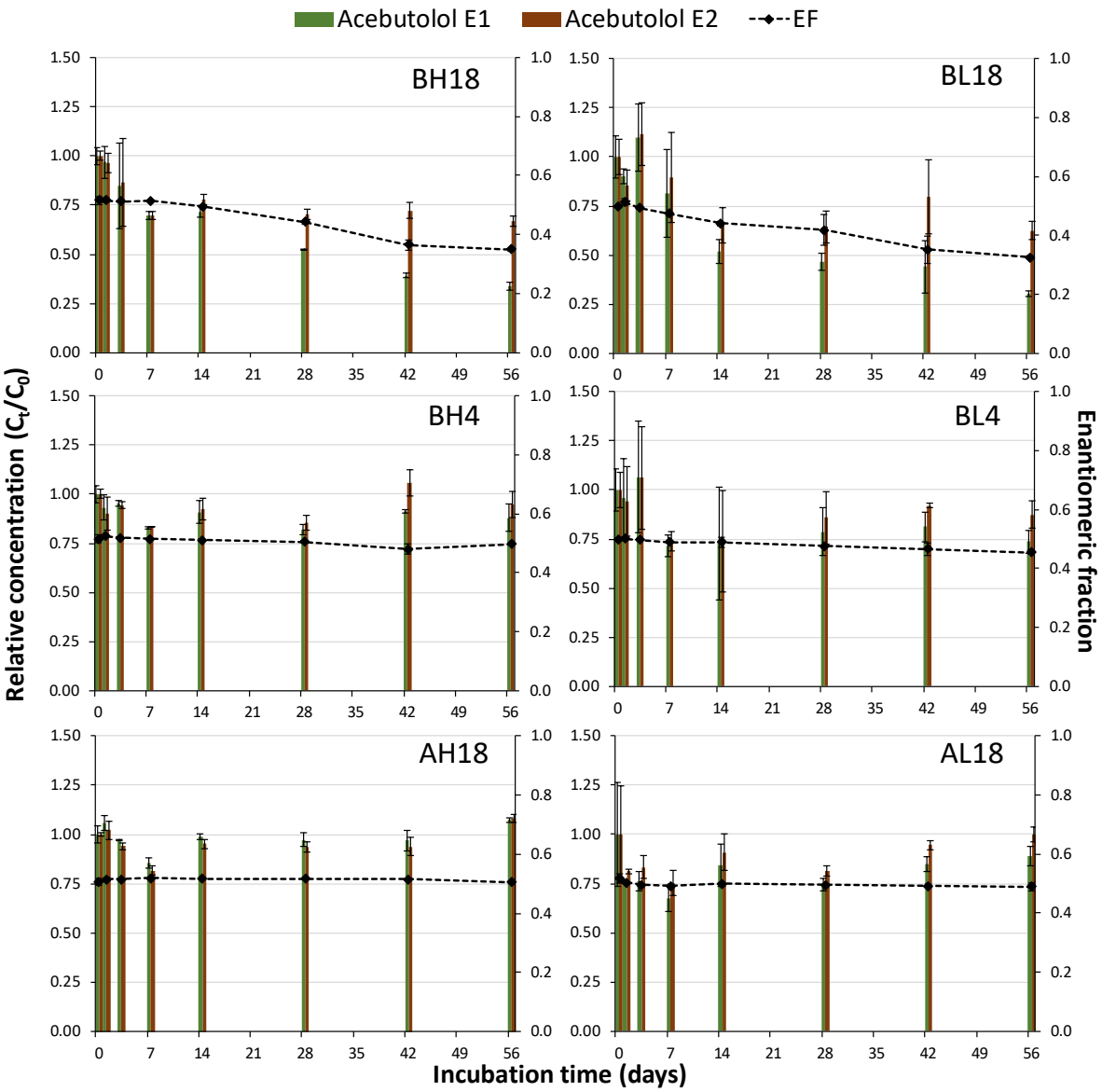

Figure 2. Relative concentration of acebutolol-E1 and acebutolol-E2 and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-acebutolol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

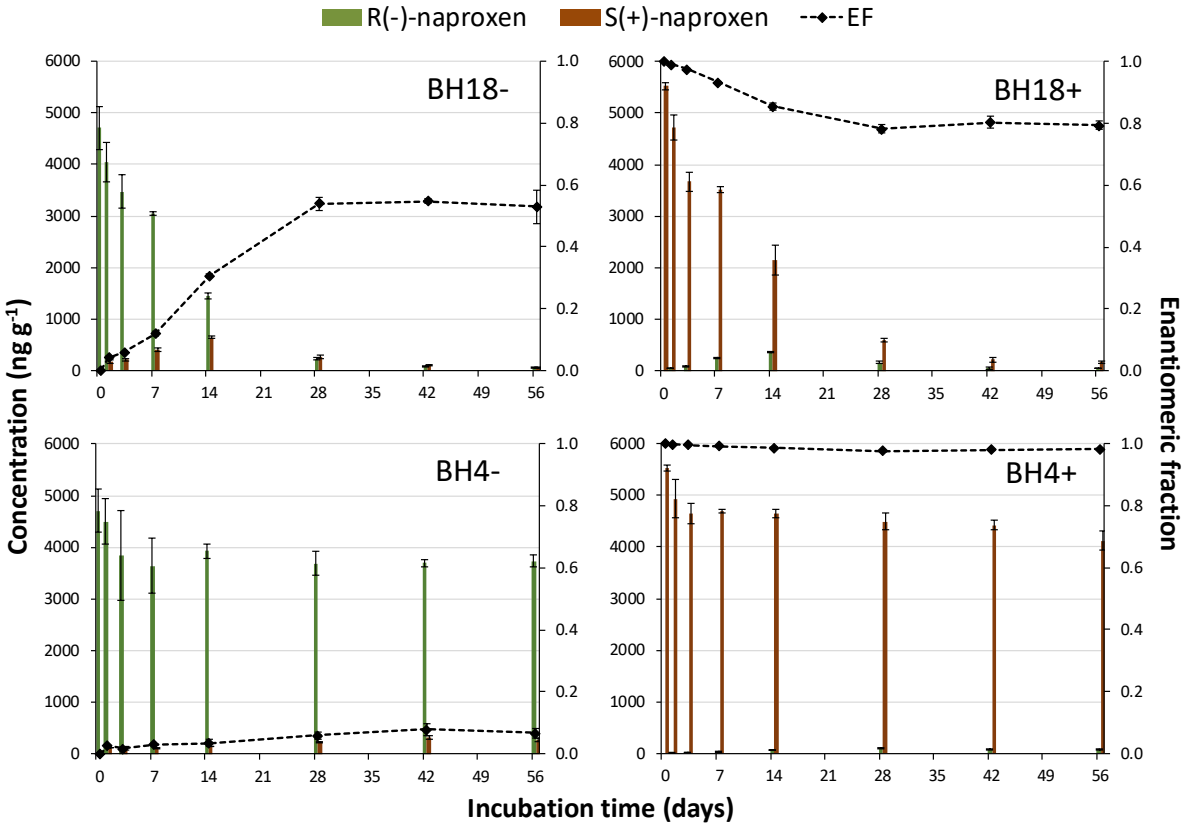

Figure 3. Concentration of *R(-)*-naproxen and *S(+)*-naproxen and the corresponding enantiomeric fraction in soil microcosms spiked with individual naproxen enantiomers

Key: BH18-, biotic high spike level of *(-)*-enantiomer 18 ˚C microcosm; BH18+, biotic high spike level of *(+)*-enantiomer 18 ˚C microcosm; BH4-, biotic high spike level of *(-)*-enantiomer 4 ˚C microcosm; BH4+, biotic high spike level of *(+)*-enantiomer 4 ˚C microcosm

Table 1. Degradation rate constants and half-lives of drug enantiomers spiked in racemic microcosms

Drug class	**Enantiomer**	**Microcosm**	***k* (d^{-1})**	**r^2**	**$t_{1/2}$ (d)**
Anti-inflammatory	*R(-)*-naproxen	BH18	0.071±0.002	0.987	9.7±0.3
		BL18	0.101±0.011	0.890	6.9±0.8
		BH4	0.011±0.001	0.779	60.6±4.0
		BL4	0.024±0.007	0.780	30.5±9.2
	S(+)-naproxen	BH18	0.059±0.002	0.991	11.8±0.4
		BL18	0.089±0.006	0.947	7.8±0.5
		BH4	0.006±0.001	0.619	109±12.1
		BL4	0.012±0.004	0.638	63.8±22.0
	R(-)-ibuprofen	BH18	0.320±0.050	0.989	2.2±0.4
		BL18	0.592±0.268	0.852	1.4±0.9
		BH4	0.116±0.011	0.964	6.0±0.6
		BL4	0.533±0.141	0.971	1.4±0.4
	S(+)-ibuprofen	BH18	0.302±0.041	0.993	2.3±0.3
		BL18	0.790±0.319	0.904	1.0±0.4
		BH4	0.123±0.005	0.993	5.6±0.2
		BL4	0.407±0.075	0.978	1.7±0.4
Anti-histamine	*S(+)*-chlorpheniramine	BH18[a]	-	-	-
		BL18[a]	-	-	-
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	R(-)-chlorpheniramine	BH18[a]	-	-	-
		BL18[a]	-	-	-
		BH4[a]	-	-	-
		BL4[a]	-	-	-
Beta-blocker	Bisoprolol E1	BH18	0.034±0.002	0.969	20.4±1.1
		BL18	0.093±0.006	0.948	7.5±0.5
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	Bisoprolol E2	BH18	0.036±0.002	0.969	19.4±1.0
		BL18	0.083±0.005	0.968	8.4±0.5
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	Metoprolol E1	BH18	0.011±0.001	0.846	63.7±8.1
		BL18	0.014±0.003	0.786	49.7±9.9
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	Metoprolol E2	BH18	0.012±0.002	0.848	60.6±7.9
		BL18	0.014±0.002	0.857	50.3±6.8
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	S(-)-propranolol	BH18	0.007±0.001	0.596	106±18.1
		BL18[b]	-	-	-
		BH4[b]	-	-	-
		BL4[b]	-	-	-
	R(+)-propranolol	BH18	0.008±0.001	0.619	91.4±11.9
		BL18[b]	-	-	-
		BH4	0.006±0.002	0.600	129±31.4
		BL4[b]	-	-	-
	Acebutolol E1	BH18	0.019±0.002	0.919	36.9±3.9
		BL18	0.021±0.002	0.817	33.4±3.2
		BH4[a]	-	-	-
		BL4[a]	-	-	-

	Acebutolol E2	BH18[b]	-	-	-
		BL18[b]	-	-	-
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	S(-)-atenolol	BH18	0.159±0.011	0.982	4.4±0.3
		BL18	0.133±0.110	0.753	8.0±5.5
		BH4	0.045±0.002	0.946	15.6±0.6
		BL4	0.094±0.057	0.859	9.0±4.1
	R(+)-atenolol	BH18	0.195±0.012	0.980	3.6±0.2
		BL18	0.195±0.172	0.662	7.4±7.6
		BH4	0.051±0.001	0.935	13.6±0.1
		BL4	0.138±0.074	0.941	6.0±2.6
Beta-agonist	Salbutamol E1	BH18	1.44±0.123	0.961	0.5±0.0
		BL18[c]	-	-	-
		BH4	0.641±0.221	0.881	1.2±0.3
		BL4[c]	-	-	-
	Salbutamol E2	BH18	1.57±0.199	0.972	0.4±0.1
		BL18[c]	-	-	-
		BH4	0.684±0.220	0.896	1.1±0.3
		BL4[c]	-	-	-
Anti-depressant	*S(+)*-fluoxetine	BH18[a]	-	-	-
		BL18[a]	-	-	-
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	R(-)-fluoxetine	BH18[a]	-	-	-
		BL18[a]	-	-	-
		BH4[a]	-	-	-
		BL4[a]	-	-	-
	S(+)-citalopram	BH18[b]	-	-	-
		BL18[b]	-	-	-
		BH4[b]	-	-	-
		BL4[b]	-	-	-
	R(-)-citalopram	BH18[b]	-	-	-
		BL18[b]	-	-	-
		BH4[b]	-	-	-
		BL4[b]	-	-	-
Stimulant	*S(+)*-amphetamine	BH18[c]	-	-	-
		BL18[c]	-	-	-
		BH4	0.378±0.091	0.849	1.9±0.4
		BL4[c]	-	-	-
	R(-)-amphetamine	BH18	0.244±0.014	0.879	2.8±0.2
		BL18[c]	-	-	-
		BH4	0.125±0.008	0.937	5.6±0.4
		BL4	0.399±0.092	0.988	1.8±0.5

[a]degradation was <20 % over 56 d; [b]r^2 <0.5 therefore *k* not reported [c]insufficient data points to report *k*
Key: *k*, degradation rate constant; $t_{1/2}$, half-life; BH18, biotic high spike level 18 ˚C; BL18, biotic low spike level 18 ˚C; BH4, biotic high spike level 4 ˚C; BL4, biotic low spike level 4 ˚C

Supplementary material

Enantiospecific behaviour of chiral drugs in soil

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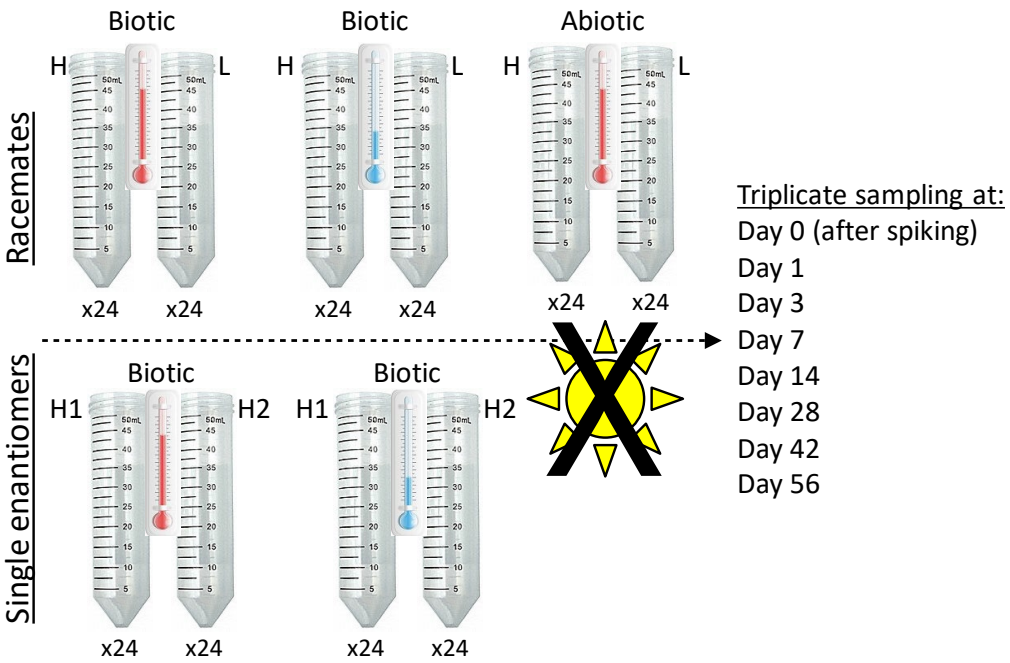
The supplementary material (23 pages) contains 16 figures and four tables. This includes the experimental setup, example chromatograms, comparison of biotic and abiotic soils inoculated on agar plates, degradation of various chiral drugs in soils as racemates and single enantiomers, chemical properties of studied drugs, properties of collected soil, mass spectrometry information and method performance data.



H = high spike level of racemate (100 ng g^{-1})[a]
L = low spike level of racemate (10 ng g^{-1})[b]
H1 = high spike level of selected *(+)*-enantiomers (50 ng g^{-1})[c]
H2 = high spike level of selected *(-)*-enantiomers (50 ng g^{-1})[c]

Figure S1. Experimental set-up and incubation conditions for soil microcosms
Key: [a]10,000 ng g^{-1} for naproxen and ibuprofen [b]1,000 ng g^{-1} for naproxen and ibuprofen [c]5,000 ng g^{-1} for naproxen and ibuprofen

Figure S2. MRM chromatograms of chiral drugs spiked in soil at 100 ng g^{-1} (10,000 ng g^{-1} for ibuprofen and naproxen).

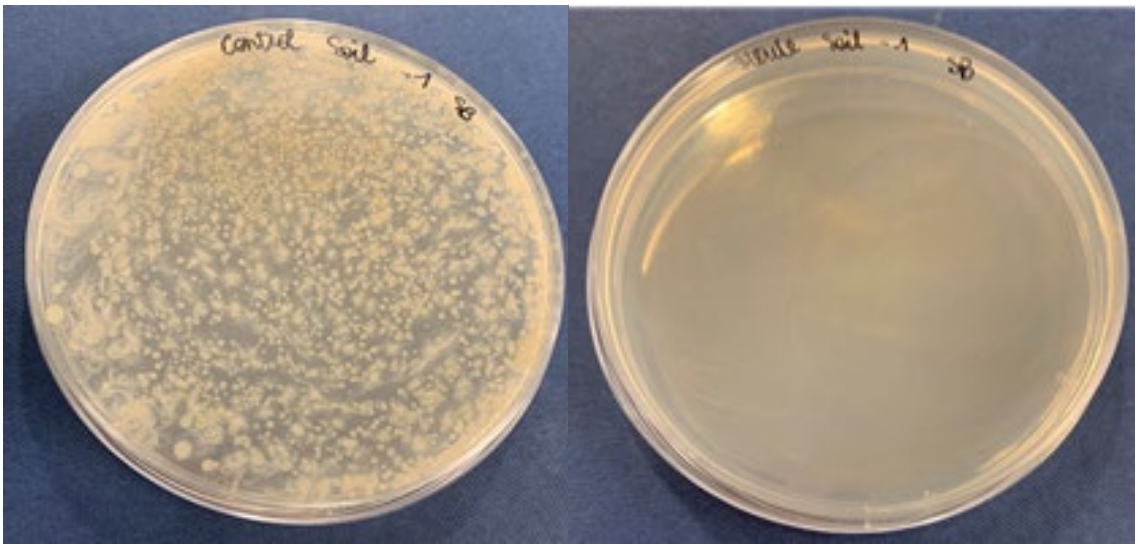


Figure S3. Comparison of 56 d biotic (left) and abiotic microcosm soil (right) inoculated Petri dishes incubated at 25 ˚C for 72 h.

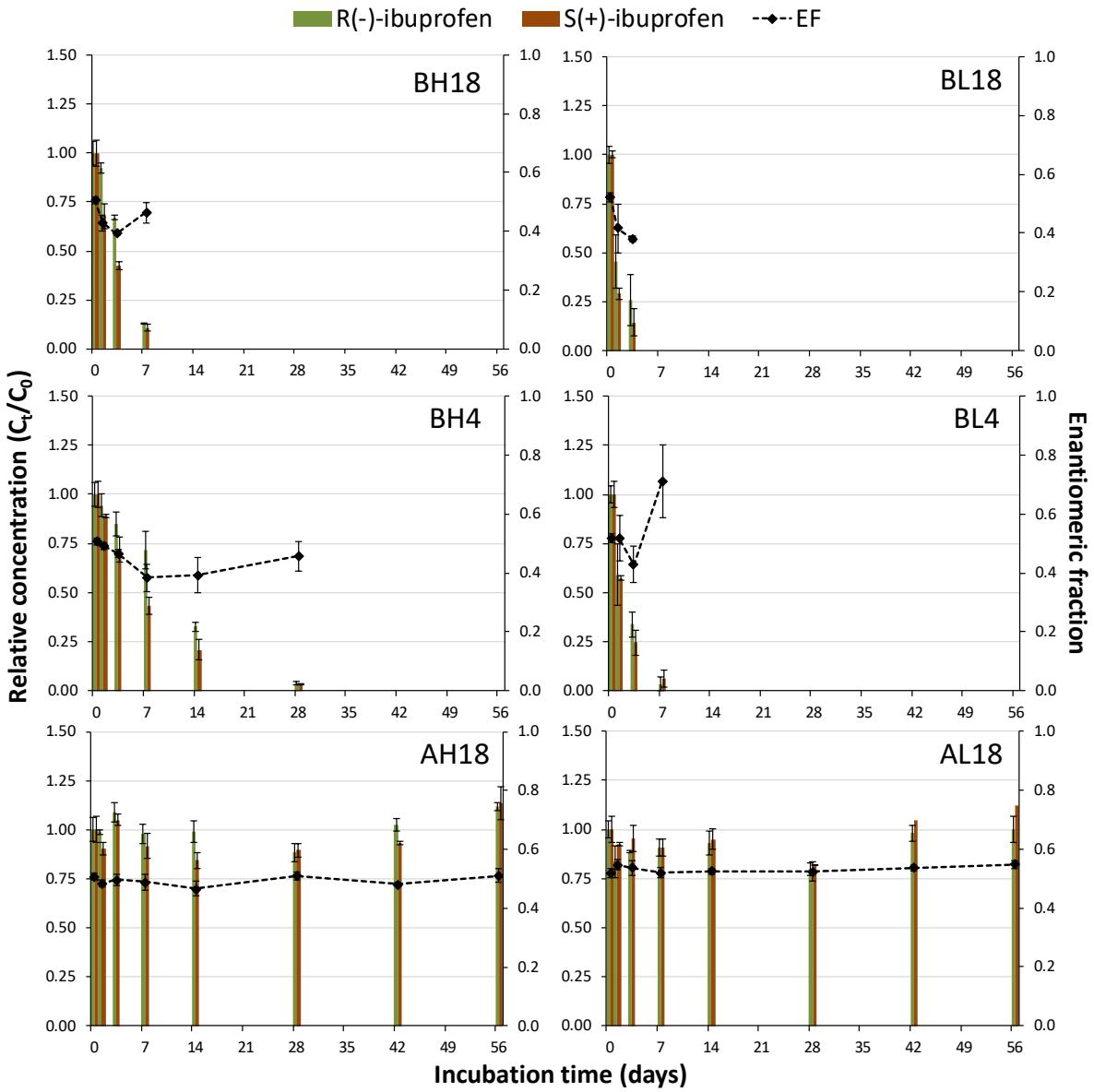


Figure S4. Relative concentration of *R(-)*-ibuprofen and *S(+)*-ibuprofen and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-ibuprofen

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

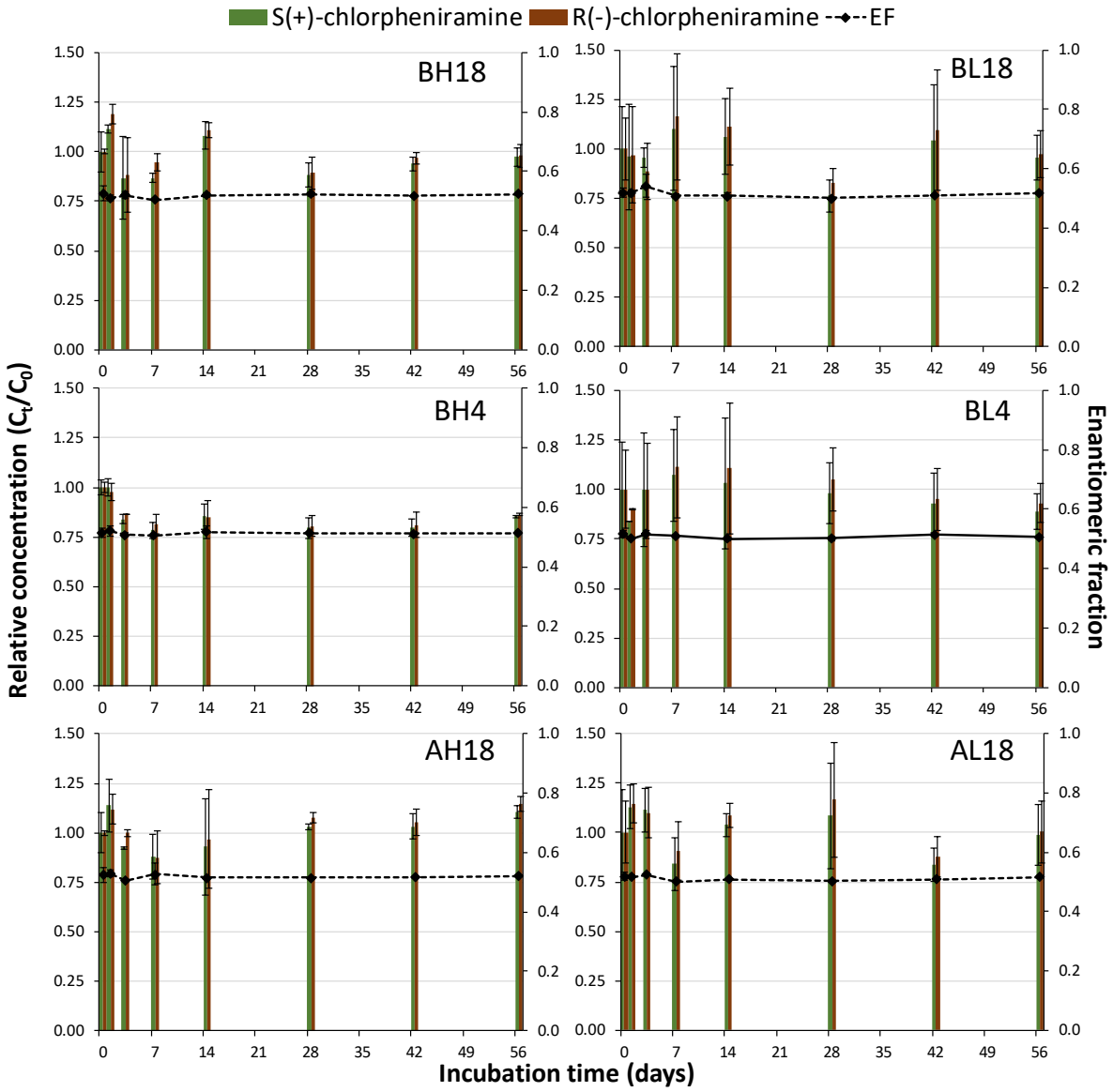


Figure S5. Relative concentration of *S(+)*-chlorpheniramine and *S(-)*-chlorpheniramine and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-chlorpheniramine

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

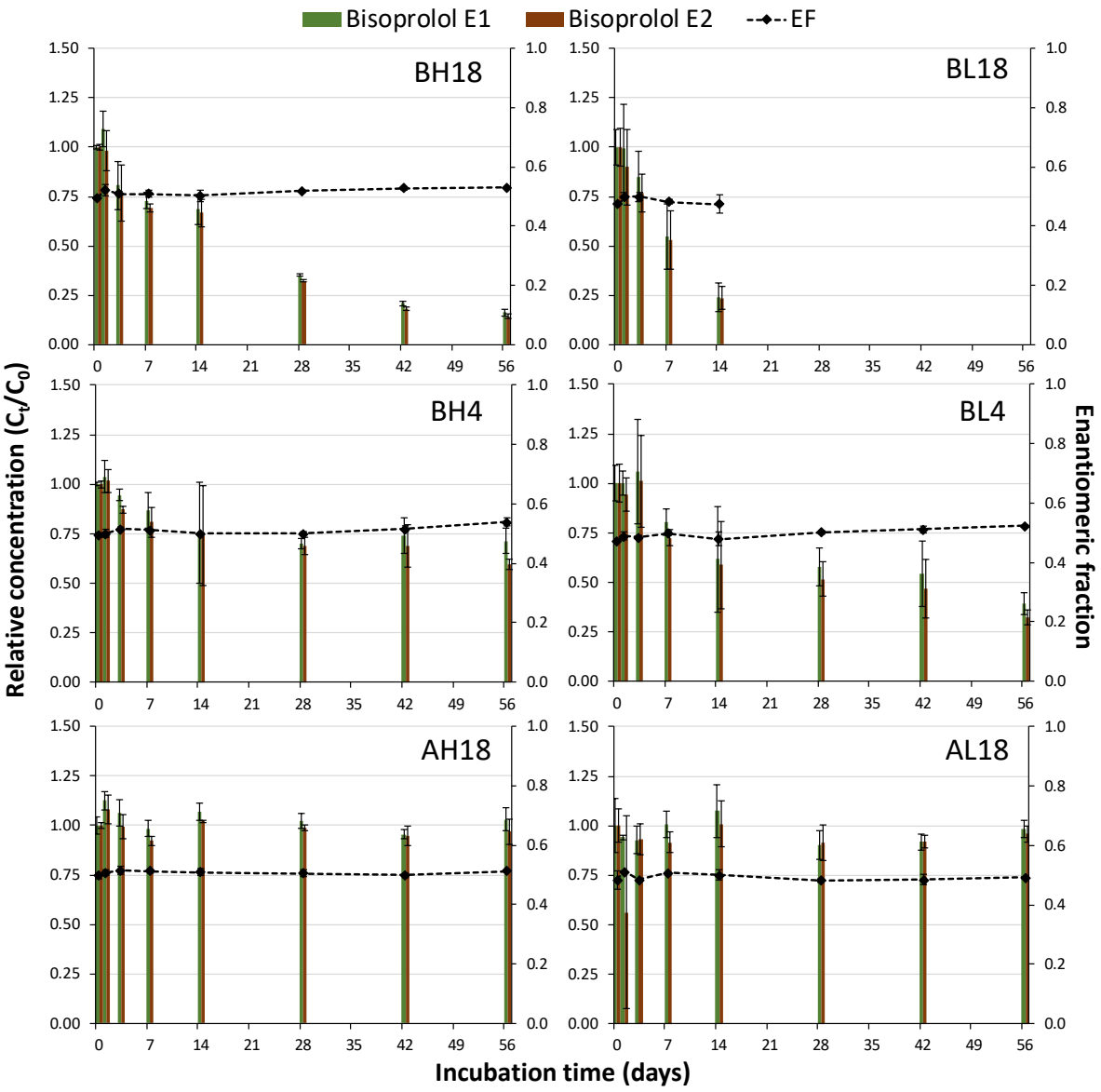


Figure S6. Relative concentration of bisoprolol E1 and bisoprolol E2 and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-bisoprolol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

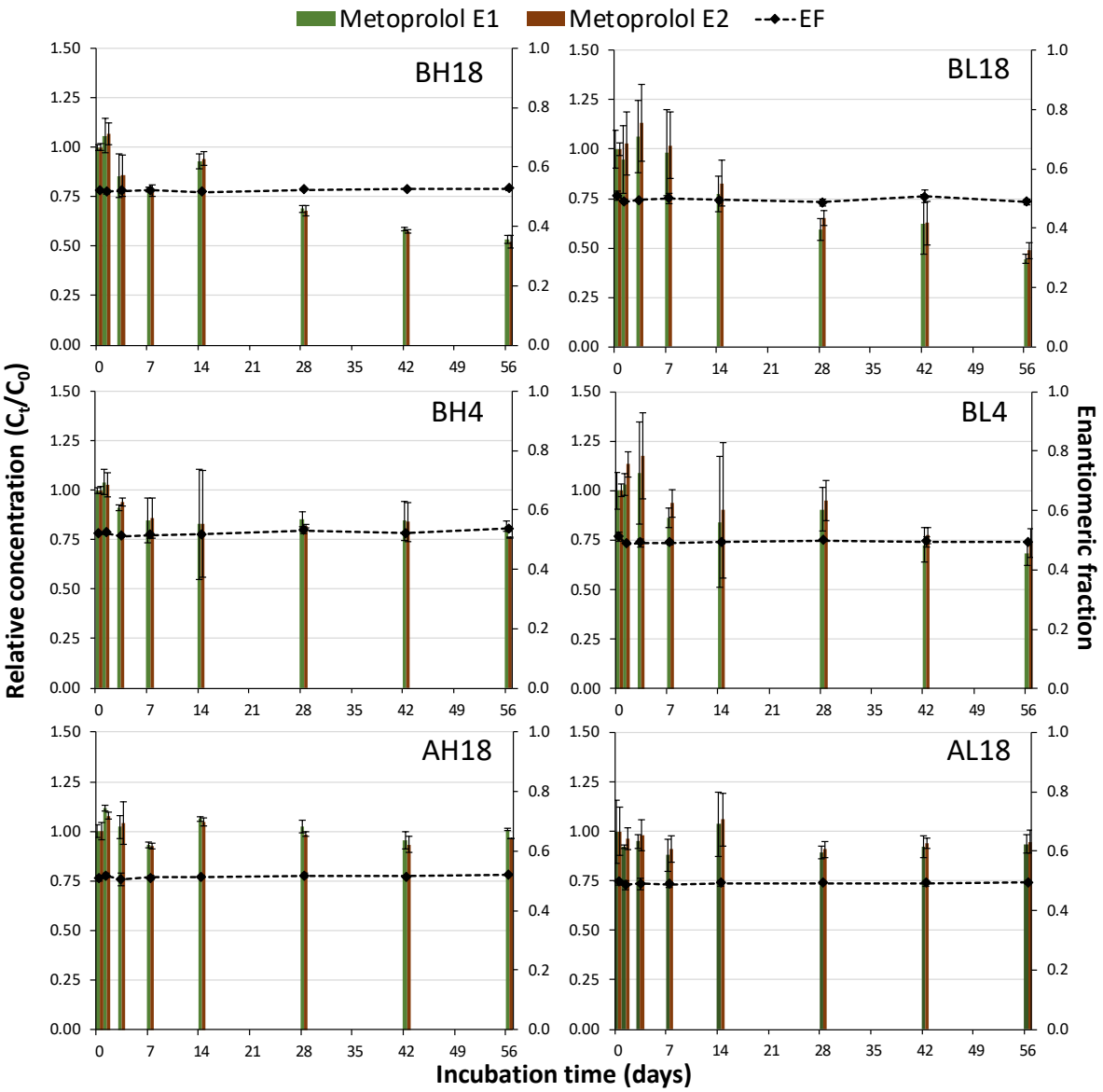


Figure S7. Relative concentration of metoprolol E1 and metoprolol E2 and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-metoprolol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

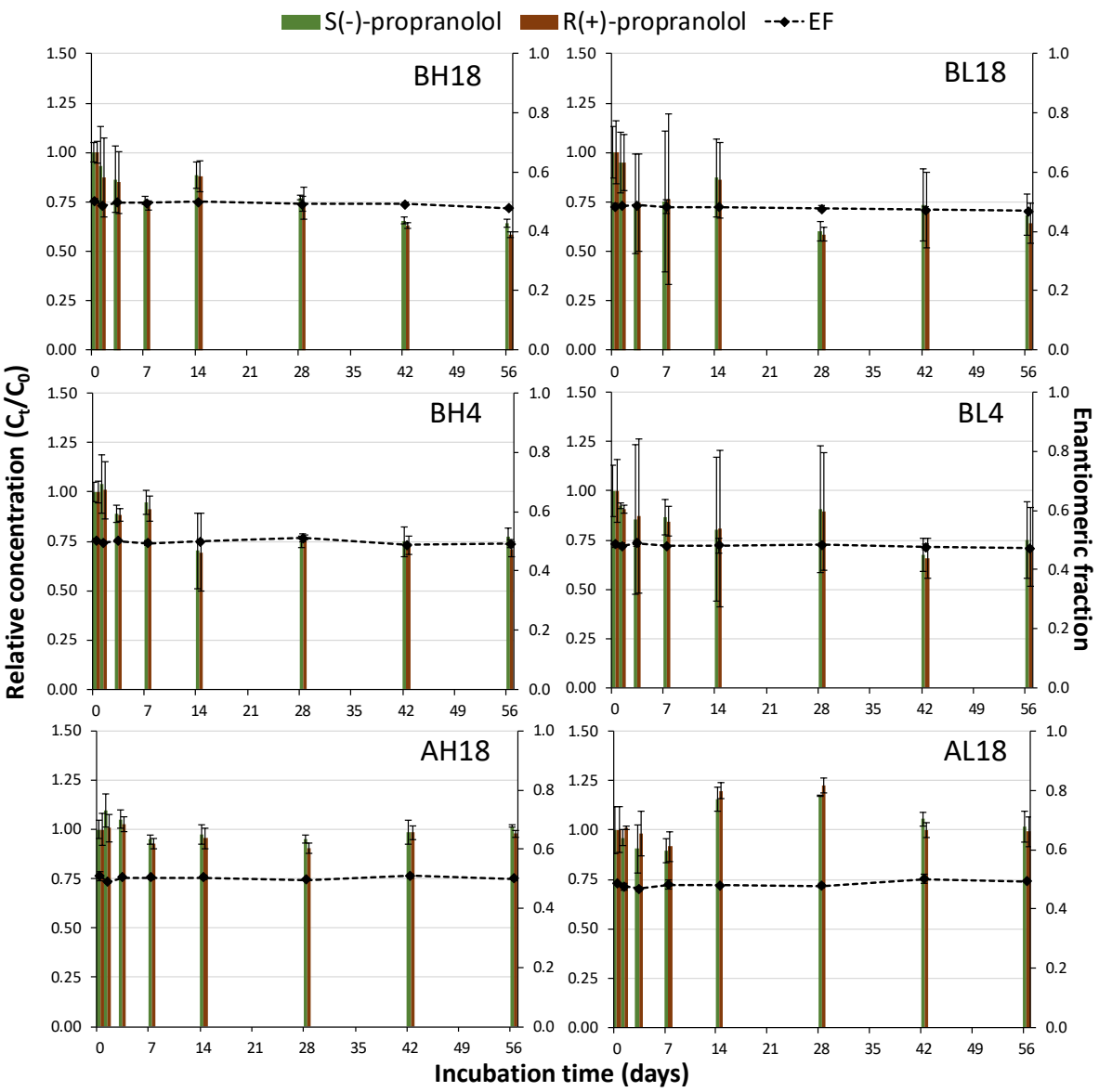


Figure S8. Relative concentration of *S(-)*-propranolol *R(+)*-propranolol and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-propranolol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

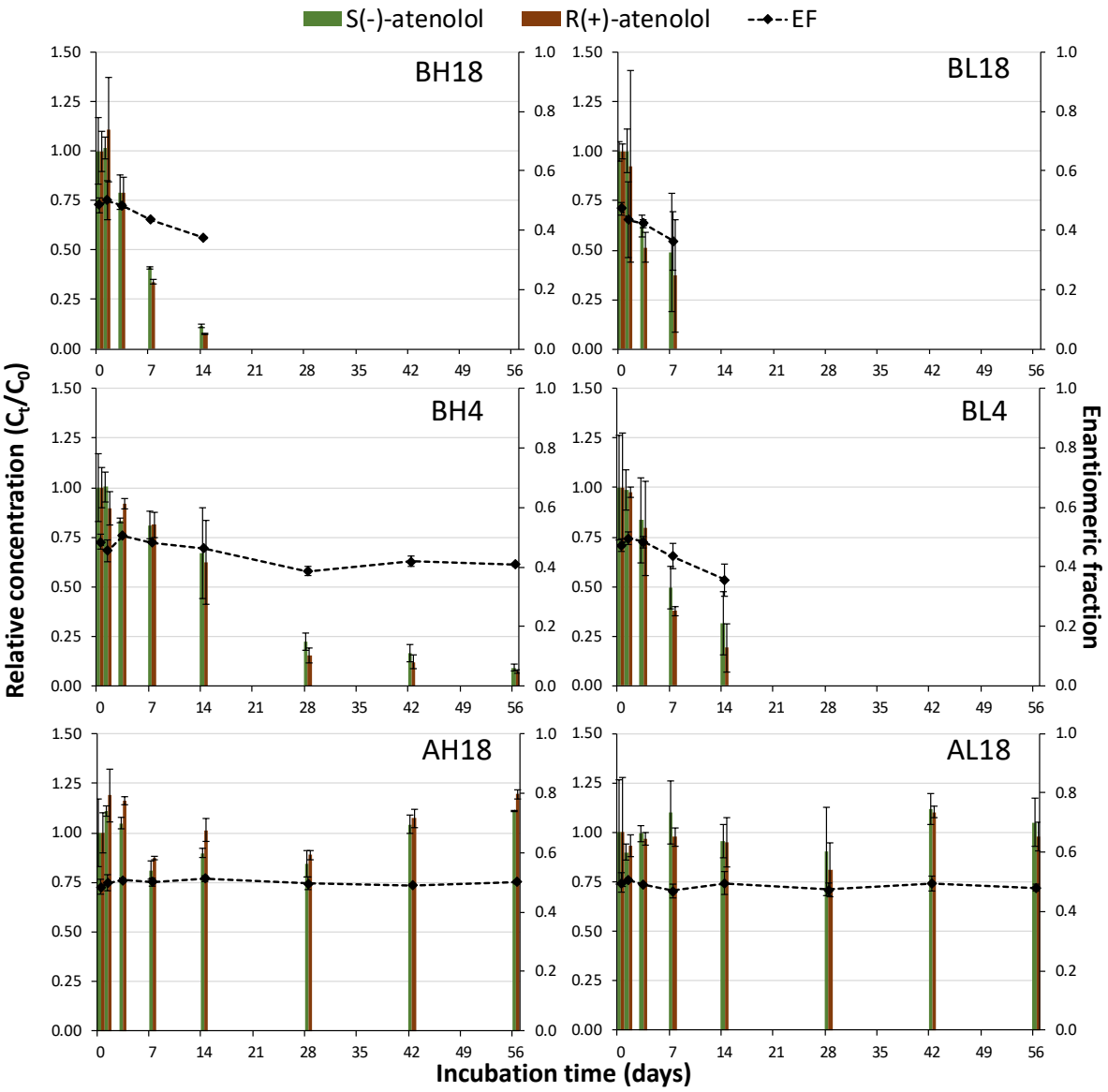


Figure S9. Relative concentration of *S(-)*-atenolol and *R(+)*-atenolol and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-atenolol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

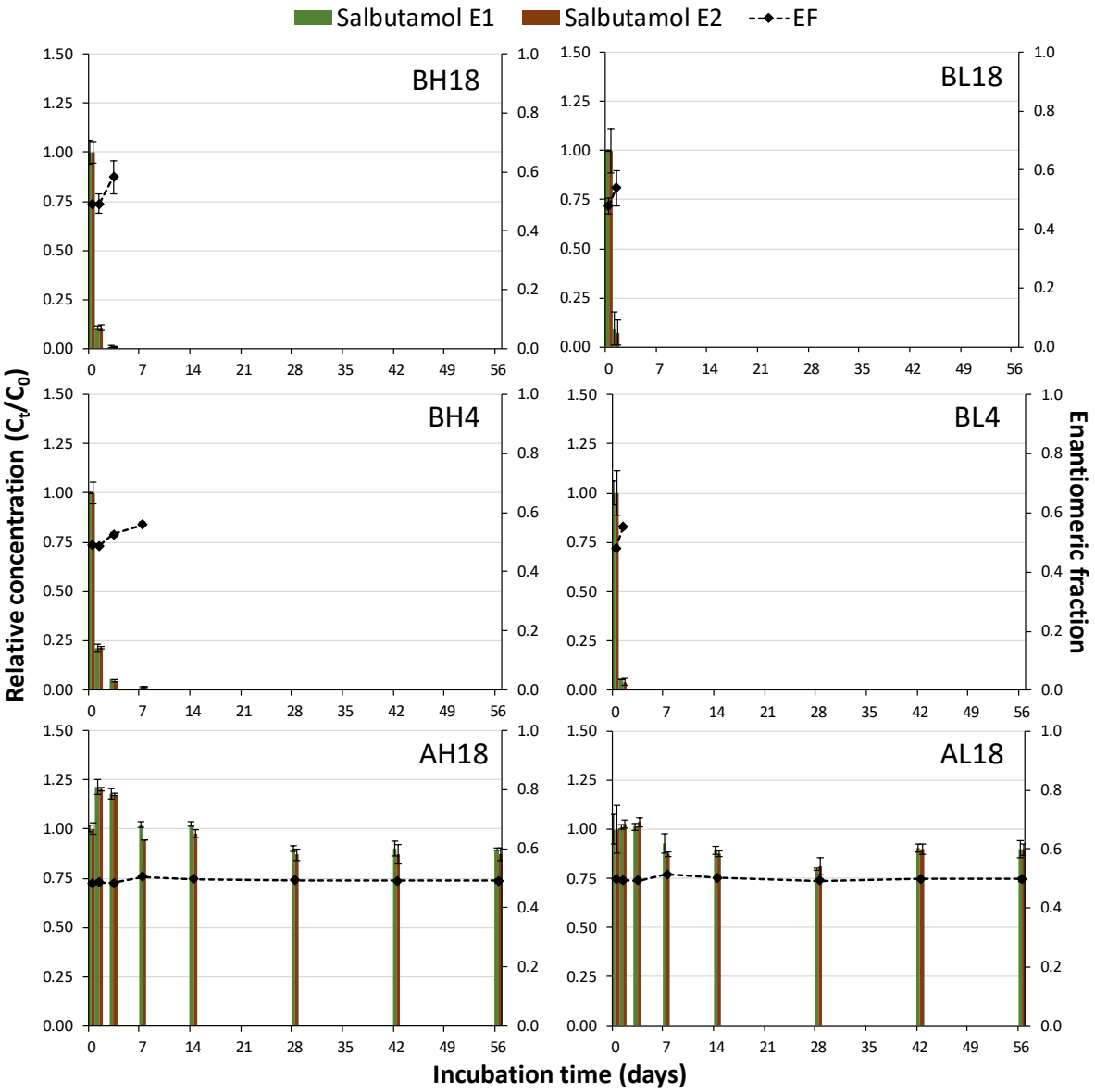


Figure S10. Relative concentration of salbutamol E1 and salbutamol E2 and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-salbutamol

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

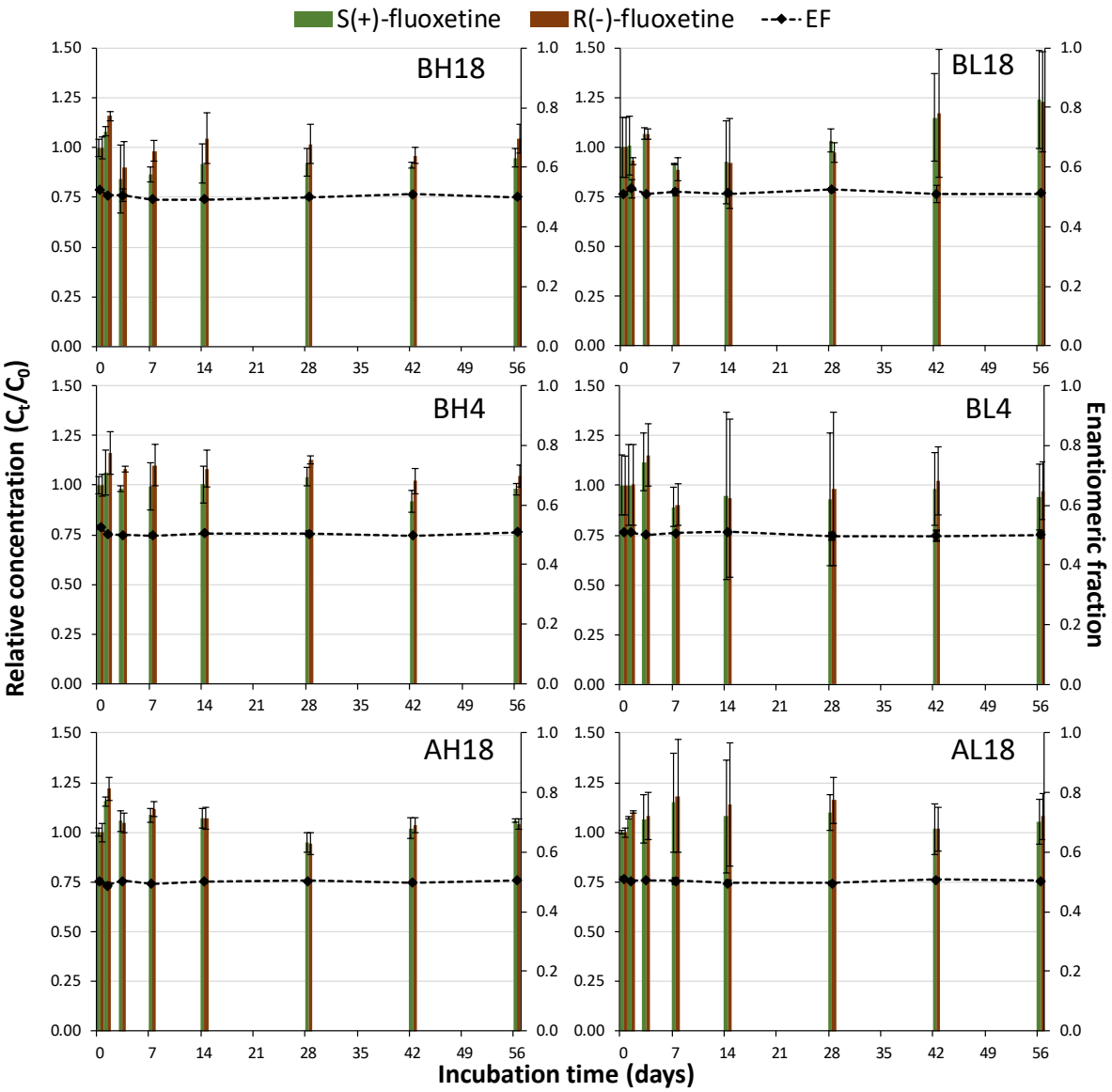


Figure S11. Relative concentration of *S(+)*-fluoxetine and *R(-)*-fluoxetine and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-fluoxetine

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

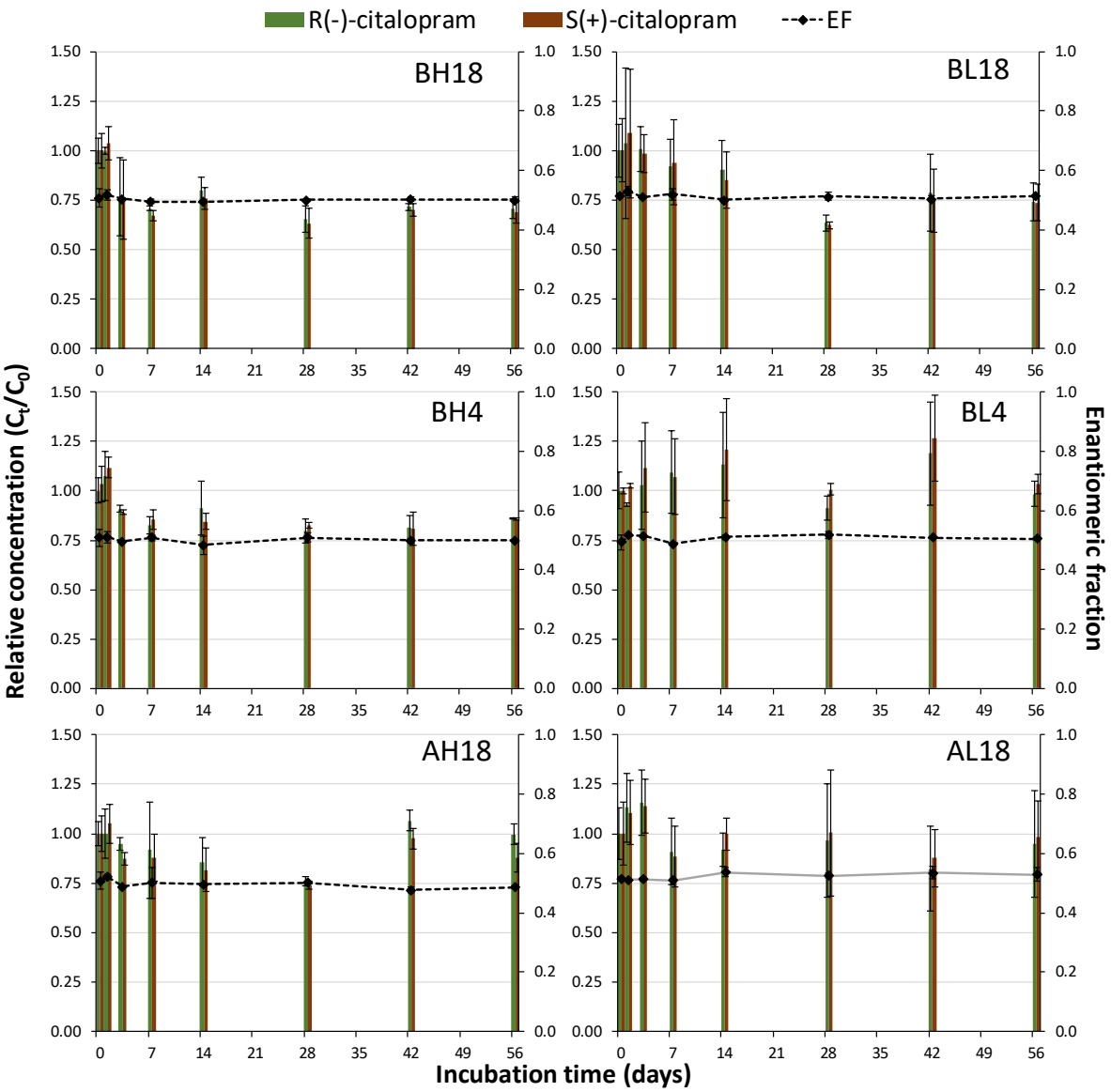


Figure S12. Relative concentration of *R(-)*-citalopram and *S(+)*-citalopram and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-citalopram

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

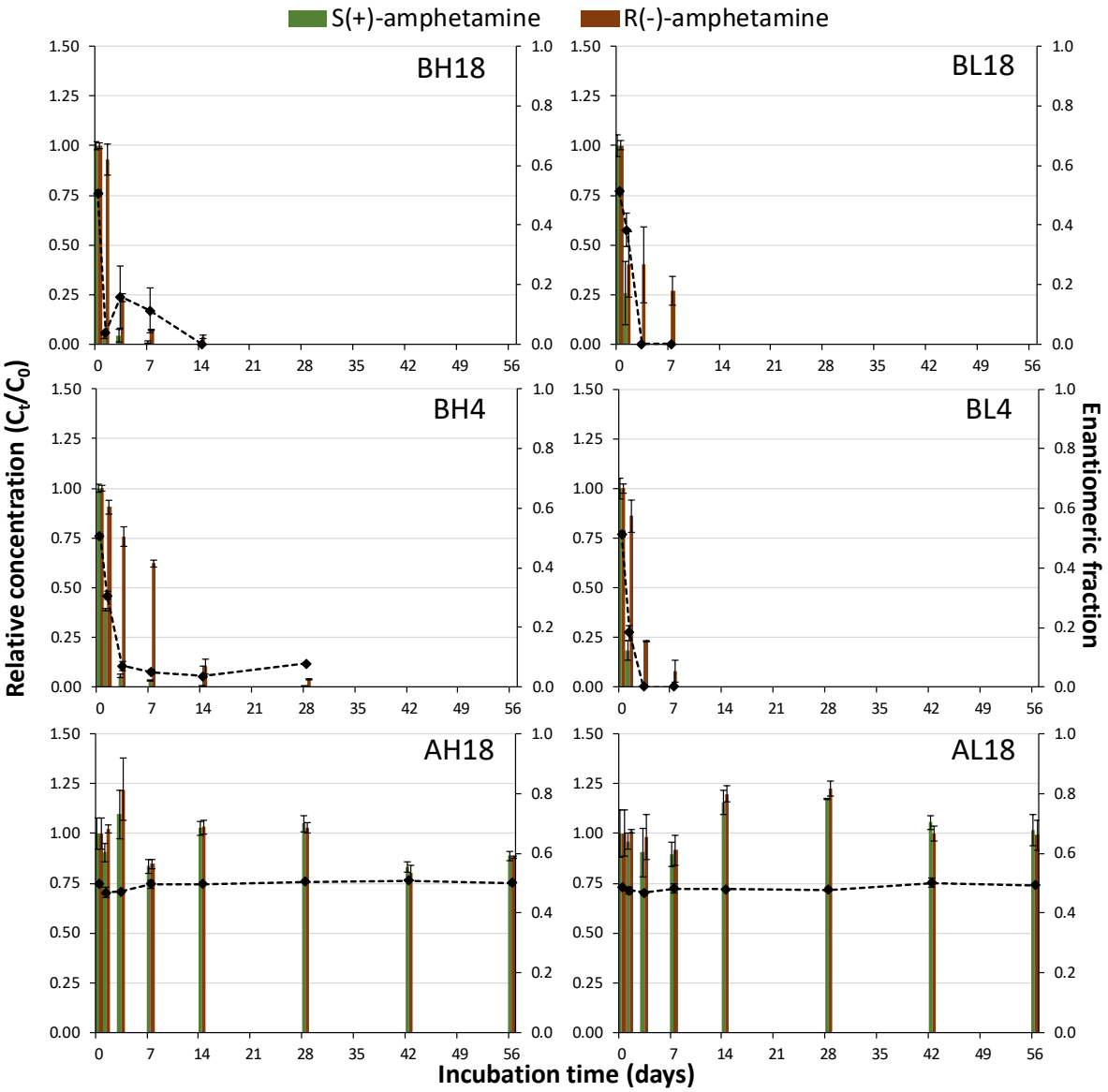


Figure S13. Relative concentration of *S(+)*-amphetamine *R(-)*-amphetamine and the corresponding enantiomeric fraction in soil microcosms spiked with racemic *R/S(±)*-amphetamine

Key: BH18, biotic high spike level 18 ˚C microcosm; BL18, biotic low spike level 18 ˚C microcosm; BH4, biotic high spike level 4 ˚C microcosm; BL4 biotic low spike level 4 ˚C microcosm; AH18, abiotic high spike level 18 ˚C microcosm; AL18, abiotic low spike level 18 ˚C microcosm.

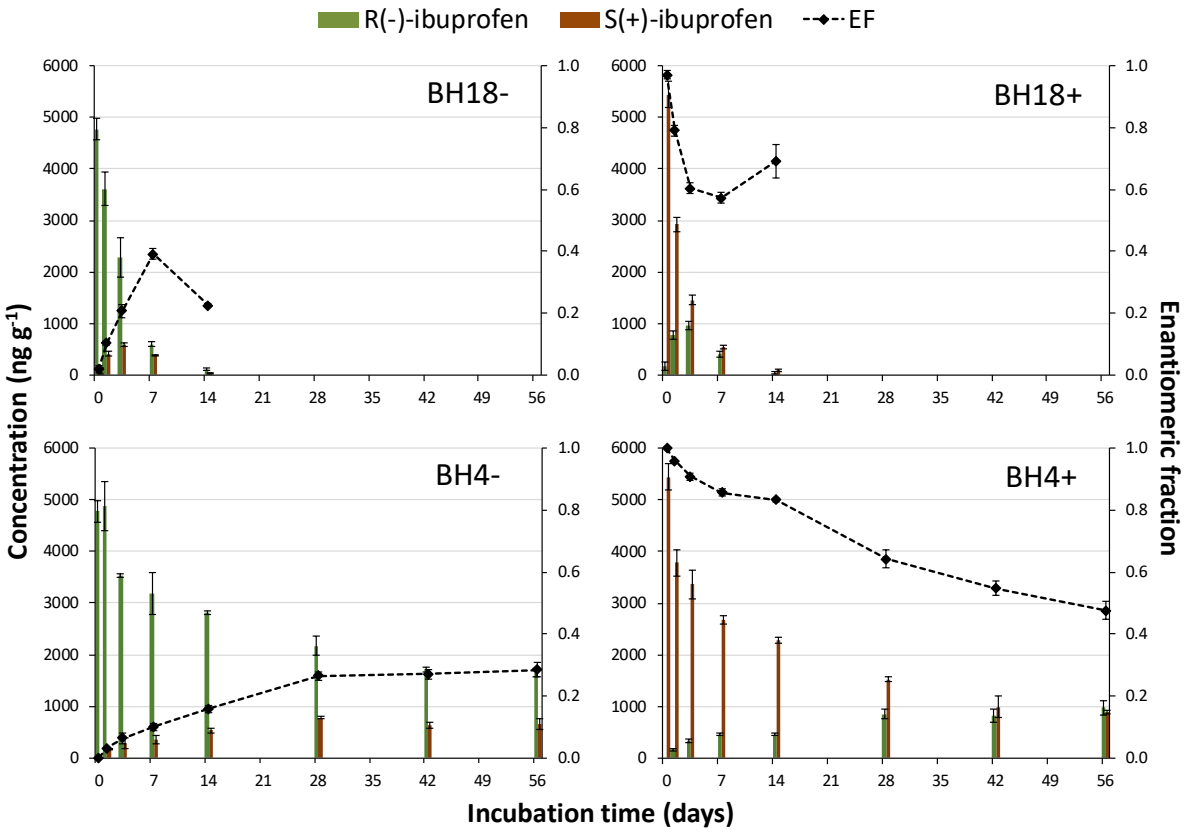


Figure S14. Concentration of *R(-)*-ibuprofen and *S(+)*-ibuprofen and the corresponding enantiomeric fraction in soil microcosms spiked with individual ibuprofen enantiomers

Key: BH18-, biotic high spike level of *(-)*-enantiomer 18 ˚C microcosm; BH18+, biotic high spike level of *(+)*-enantiomer 18 ˚C microcosm; BH4-, biotic high spike level of *(-)*-enantiomer 4 ˚C microcosm; BH4+, biotic high spike level of *(+)*-enantiomer 4 ˚C microcosm

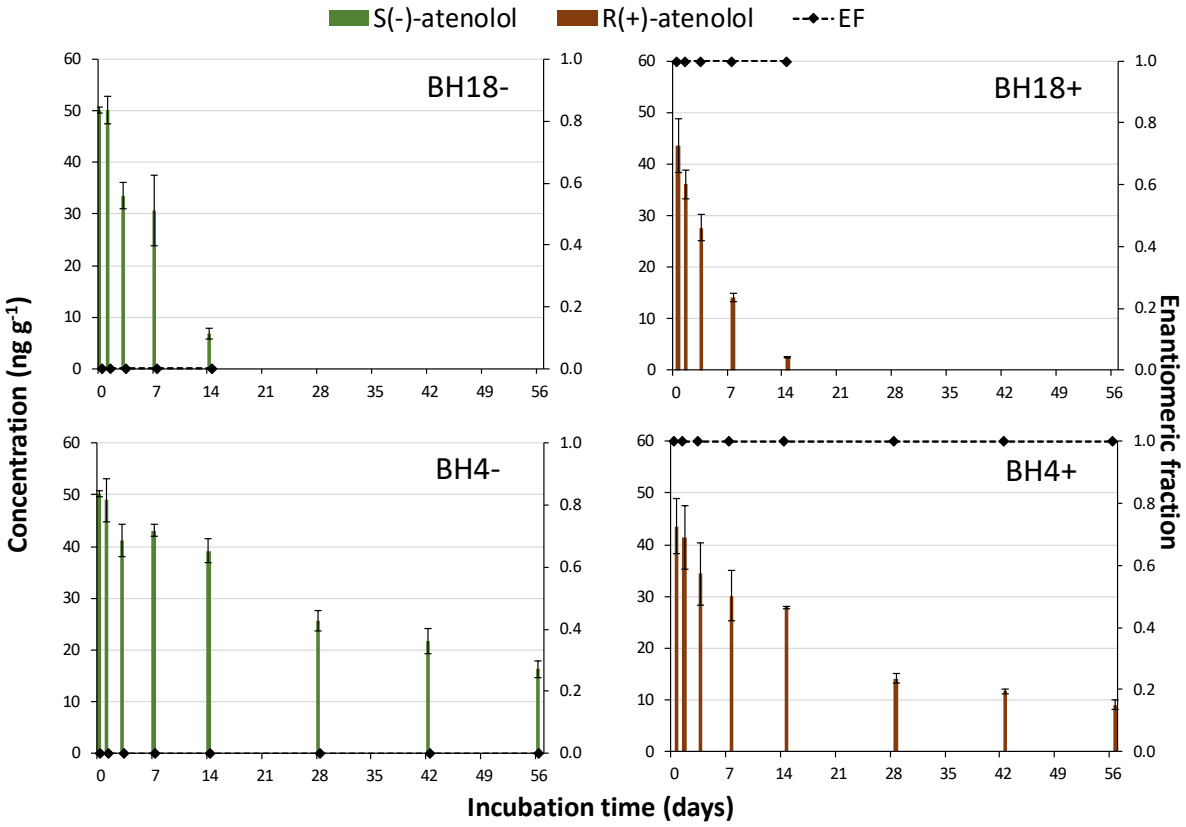


Figure S15. Concentration of *S(-)*-atenolol and *R(+)*-atenolol and the corresponding enantiomeric fraction in soil microcosms spiked with individual atenolol enantiomers

Key: BH18-, biotic high spike level of *(-)*-enantiomer 18 ˚C microcosm; BH18+, biotic high spike level of *(+)*-enantiomer 18 ˚C microcosm; BH4-, biotic high spike level of *(-)*-enantiomer 4 ˚C microcosm; BH4+, biotic high spike level of *(+)*-enantiomer 4 ˚C microcosm

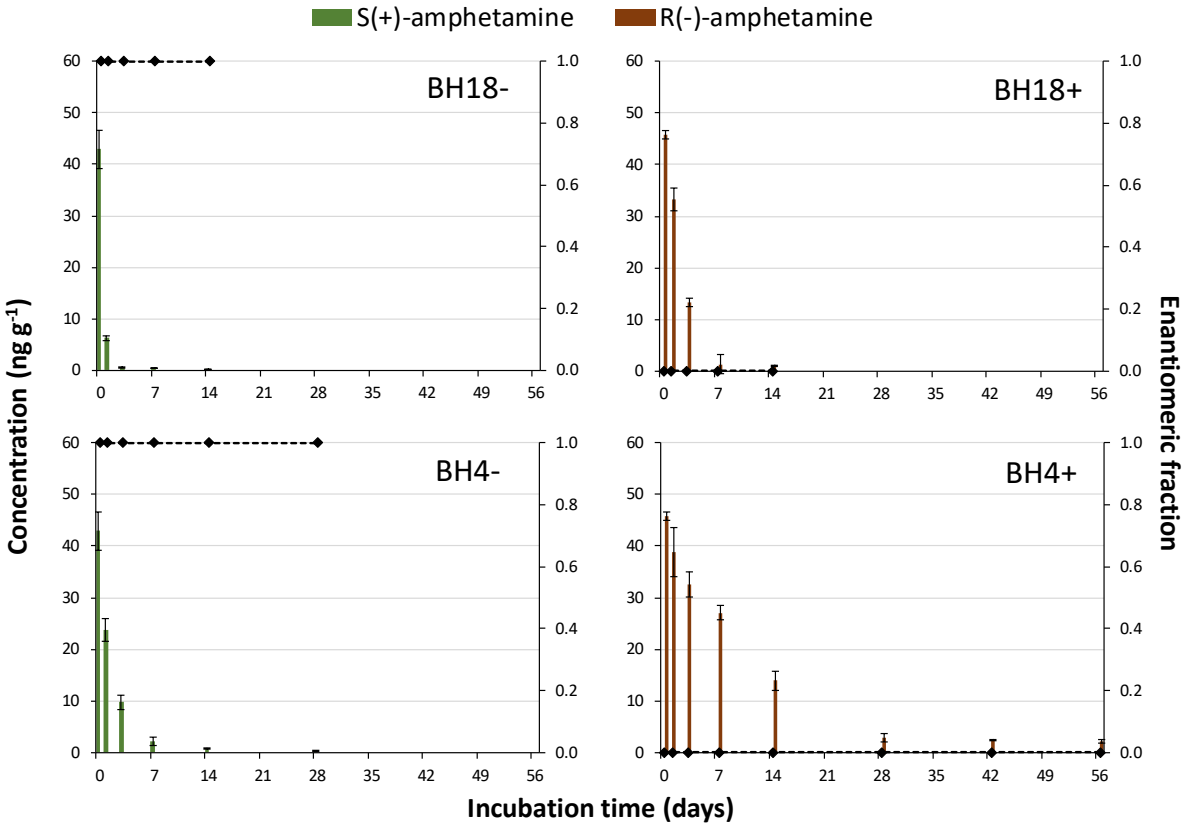
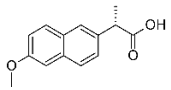
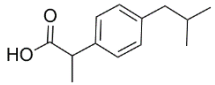
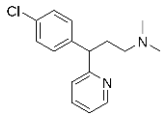
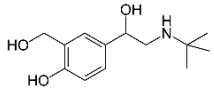
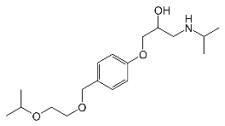
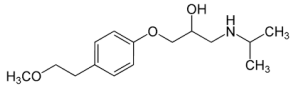
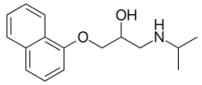


Figure S16. Concentration of *S(+)*-amphetamine and *R(-)*-amphetamine and the corresponding enantiomeric fraction in soil microcosms spiked with individual amphetamine enantiomers

Key: BH18-, biotic high spike level of *(-)*-enantiomer 18 ˚C microcosm; BH18+, biotic high spike level of *(+)*-enantiomer 18 ˚C microcosm; BH4-, biotic high spike level of *(-)*-enantiomer 4 ˚C microcosm; BH4+, biotic high spike level of *(+)*-enantiomer 4 ˚C microcosm

Table S1. Chemical properties of studied chiral drugs (US EPA, 2015)

Drug	Chemical structure	Molecular weight (g mol^{-1})	Water solubility (mg L^{-1})	Log Kow	p*Ka*
Naproxen		230.26	15.9	3.18	4.15
Ibuprofen		206.28	21.0	3.97	4.91
Chlorpheniramine		274.79	5.5E3	3.67	9.47 (basic)
Salbutamol		239.31	1.4E4	0.40	10.12 (acidic) 9.40 (basic)
Bisoprolol		325.44	2.2E3	1.87	14.09 (acidic) 9.27 (basic)
Metoprolol		267.36	>1.0E4	1.88	14.09 (acidic) 9.67 (basic)
Propranolol		259.35	228.0	2.60	13.84 (acidic) 9.50 (basic)

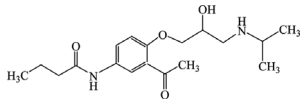
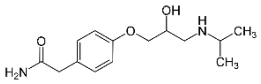
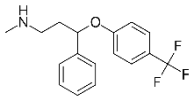
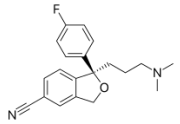
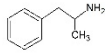
Acebutolol		336.40	259.0	1.71	13.91 (acidic) 9.57 (basic)
Atenolol		266.34	685.2	-0.03	13.88 (acidic) 9.43 (basic)
Fluoxetine		309.33	60.3	4.65	10.05 (basic)
Citalopram		324.40	31.1	3.76	9.78
Amphetamine		135.21	2.8E4	1.76	9.94 (basic)

Table S2. Properties of collected soil

Soil property	**Result**
pH	6.6±0.1
Moisture content (%)	26.3±0.6
Specific surface area (m^2/kg)	667
Cation exchange capacity (meq/100g)	17.5
d_{10} (μm)	3.68
d_{50} (μm)	35.6
d_{90} (μm)	277
Loss on ignition @ 450°C (%)	6.2±0.2
Loss on ignition @ 900°C (%)	8.4±0.1

Table S3. Mass spectrometry information for studied drugs

Drug	Precursor (m/z)	Fragmentor (V)	Product 1 (m/z)	Collision energy (eV)	Product 2 (m/z)	Collision energy (eV)
R/S(±)-naproxen	231.1	90	-	-	-	-
R/S(±)-ibuprofen	224.2	50	-	-	-	-
R/S(±)-chlorpheniramine	274.9	90	229.9	10	166.8	40
R/S(±)-salbutamol	239.9	90	165.9	10	147.9	10
R/S(±)-bisoprolol	326.2	120	116.0	10	74.1	30
R/S(±)-metoprolol	268.1	110	191.1	10	116.0	12
R/S(±)-propranolol	259.9	110	182.9	10	115.9	10
R/S(±)-acebutolol	337.2	90	319.3	10	116.1	20
R/S(±)-atenolol	266.9	100	189.9	20	145.0	30
R/S(±)-fluoxetine	309.8	90	147.7	2	44.0	10
R/S(±)-citalopram	325.0	130	262.0	20	108.9	30
R/S(±)-amphetamine	135.8	70	90.9	20	65.0	40
R/S(±)-naproxen-d_3	234.1	90	-	-	-	-
R/S(±)-ibuprofen-d_3	227.2	50	-	-	-	-
R/S(±)-chlorpheniramine-d_6	281.0	100	229.9	10	-	-
R/S(±)-salbutamol-d_3	243.0	90	150.9	10	-	-
R/S(±)-bisoprolol-d_5	331.2	120	121.0	10	-	-
R/S(±)-metoprolol-d_7	275.2	110	123.0	15	-	-
R/S(±)-propranolol-d_7	267.0	110	115.9	20	-	-
R/S(±)-acebutolol-d_5	342.2	90	121.0	20	-	-
R/S(±)-atenolol-d_7	274.1	100	145.0	30	-	-
R/S(±)-fluoxetine-d_6	316.0	90	44.1	10	-	-
R/S(±)-citalopram-d_6	331.0	130	109.0	30	-	-
R/S(±)-amphetamine-d_{11}	147.0	70	98.0	20	-	-

Table S4. Method performance data for studied drugs

Enantiomer	**Linear range (μg mL^{-1})**	**Method trueness (%)**	**MQL (ng g^{-1})**
R(-)-naproxen	0-100	89±8	17.9
S(+)-naproxen	0-100	92±11	20.4
R(-)-ibuprofen	0-100	100±6	98.0
S(+)-ibuprofen	0-100	103±7	134
S(+)-chlorpheniramine	0-1	86±9	0.07
R(-)-chlorpheniramine	0-1	77±1	0.07
Salbutamol E1	0-1	94±6	0.26
Salbutamol E2	0-1	98±5	0.30
Bisoprolol E1	0-1	102±1	0.12
Bisoprolol E2	0-1	104±2	0.12
Metoprolol E1	0-1	102±1	0.71
Metoprolol E2	0-1	104±2	0.74
S(-)-propranolol	0-1	101±5	0.08
R(+)-propranolol	0-1	102±6	0.07
Acebutolol E1	0-1	90±4	0.10
Acebutolol E2	0-1	85±2	0.11
S(-)-atenolol	0-1	92±3	0.81
R(+)-atenolol	0-1	98±6	0.69
S(+)-fluoxetine	0-1	72±3	0.10
R(-)-fluoxetine	0-1	72±4	0.07
S(+)-citalopram	0-1	101±6	1.31
R(-)-citalopram	0-1	104±9	1.21
S(+)-amphetamine	0-1	113±2	0.17
R(-)-amphetamine	0-1	110±2	0.15

Key: MQL, method quantitation limit

References

US EPA (2015) Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11]. United States Environmental Protection Agency, Washington, DC, USA.