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Changes in sorption and bioavailability of herbicides in soil amended with fresh and aged biochar

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Highlights

- Aged biochar removed greater amounts of herbicides from solution than fresh biochar.
- Herbicide sorption by aged biochar was attributed to a dissolvable surface coating.
- Degradation of picloram in soil was accelerated by the presence of aged biochar.
- Slow sorption occurred for terbuthylazine and imazamox in aged biochar amended soil.

ABSTRACT

Knowledge of long-term pesticide behavior in biochar (BC) amended soil is still contradictory. In this work, we compared the sorption of three highly persistent and ionizable pesticides [two anionic (imazamox and picloram) and one weak base (terbuthylazine)] on both fresh and field-aged BC as well as on a soil amended with these biochars. The aging process was performed by burying the biochar at 10 cm in a silt loam soil [Upper Midwest USA (Wisconsin)] for six months. Field aged BC removed the three pesticides from solution to a higher extent ($> 85\%$) than the fresh BC ($< 16\%$). This removal was attributed to water-soluble components on the biochar. Laboratory incubation experiments demonstrated that dissipation in soil and biochar mixtures were both a function of pesticide and biochar type. The amounts of soil extractable (i.e., bioavailable) pesticide were inversely related to the extent of sorption. Similar dissipation and sorption of imazamox was observed in unamended and BC-amended soils. Terbuthylazine dissipated similarly in all treatments, but sorption increased with incubation time in the aged BC-amended soil. Conversely, picloram dissipated to a higher extent in aged BC-amended soil when compared to unamended or fresh BC-amended soil. This work demonstrates temporal variability of biochar sorption capacities due to soil exposure, which alters the efficacy and bioavailability of soil applied pesticides.

Keywords: biodegradation; bound residues; desorption; leaching; organic amendments; soil weathering

1. Introduction

Biochar (BC) is defined as a solid material produced by the thermal decomposition of biomass under limited supply of oxygen. It is more biologically inert as compared to other forms of carbon, due to its highly condensed aromatic structure (Rechberger et al., 2017; Spokas et al., 2014). This material has attracted attention in the field of environmental and agricultural science, because it simultaneously improves soil quality (e.g., nutrient availability, water holding capacity) and acts as agent for mitigating climate change due to its carbon sequestration ability (Lehmann and Joseph, 2015). However, BC is not a homogenous product, showing varying complexity and heterogeneous chemical and physical properties, which are dictated by the feedstock and pyrolysis conditions. This has led to an increasing effort by the scientific community to standardize BC production in order to maximize its benefits as soil amendment (Lehmann and Joseph, 2015; Sohi, 2010; Spokas et al., 2012). However, properties of BC also change with time in soils during weathering or aging processes (Martin et al., 2012; Pignatello et al., 2015). The implications of these modifications are still not yet fully understood (Mia et al., 2017a; Trigo et al., 2014).

Biochar has attracted attention primarily due to its sorption capacity (Cabrera-Mesa and Spokas, 2010; Yavari et al., 2015). The affinity of BC for organic compounds is hypothesized to be determined by its surface area, structural porosity, and surface chemistry. Freshly-applied biochar to soil has been widely documented to affect the fate of pesticides and it has been proposed as a tool to mitigate contamination problems derived from pesticide use. Typically, BC increases the sorption of pesticides in soils (Cabrera-Mesa and Spokas, 2010; Yavari et al., 2015), but their availability is intimately governed by desorption processes. Sorption has been shown to decrease biodegradation and leaching of pesticides (Cabrera-Mesa and Spokas, 2010; Lehmann

et al., 2011; Sopeña et al., 2012), but may also reduce pesticide efficacy (Gámiz et al., 2017a; Yavari et al., 2015).

Once in the soil, BC undergoes a range of biogeochemical processes, in which biochar is believed to react with soil constituents, such as dissolved organic and inorganic matter, gases, nutrients, metals, microorganisms, fauna, and plant roots, all of which will affect BC physical stability (i.e., fragmentation in soils) (Joseph et al., 2010; Spokas et al., 2014), and alter its surface chemistry (Mia et al., 2017a). Thus, BC can be impacted by biotic and/or abiotic reactions (Joseph et al., 2010; Pignatello et al., 2015), modifying its chemical functionality by forming new surface moieties (-OH, -C=O, and -COOH) which change the polarity, acid-basic properties and cationic and anionic exchange capacity properties (Kookana, 2010). In addition, the pore structure and particle size of aged biochar can be altered by their interaction with clay minerals or dissolved organic carbon, which either stabilizes or coats its surface, thus changing the way biochar will interact with the soil environment. These modifications involve changes of differing time scales, which transform BC sorption capacity correspondingly (Gámiz et al., 2017b; Joseph et al., 2010; Mia et al., 2017b). This is particularly critical for biochar, since mechanisms involving the chemical functionality of this material: H-bonding, cation bridging, covalent bonding, hydrophobic and π - π EDA interactions or pore-filling determine its overall sorption capacity (Joseph et al., 2010; Kookana, 2010).

To further complicate matters, studies of pesticide sorption on biochar are inconclusive. Initially, hypotheses focused on the fact that the sorption capacity for BC would decrease with time, due to physical blockage of pores by the absorbed natural organic matter on the aged BC surfaces. Furthermore, this would also reduce the number of sorption sites available on the BC, as has been observed for the antimicrobial sulfamethazine (Teixido et al., 2013). Martin et al. (2012) also observed reduced MCPA

and diuron sorption on aged biochar, indicating the importance of the reduced sorption sites as well as potential chemical alteration on BC's surfaces. Additionally, changes in dissolved organic carbon content (DOC) can attenuate sorption of pesticides and other organic compounds (Luo et al., 2017; Yang and Sheng, 2003). Others have observed greater sorption for indaziflam on aged BC because of the corresponding increases in SSA and porosity, but limited sorption in the case of terbuthylazine and MCPA (Trigo et al., 2014). Ren et al. (2018) found that atrazine was sorbed differently on aged BC, depending on the temperature of pyrolysis at which the biochar was made. Thus, atrazine was sorbed to a greater extent on aged BC produced at 300°C than on aged biochar prepared at 700 °C. These observations illustrate the complexity of the weathering process, hampering our ability to predict the behavior of pesticides on aged biochar, and consequently their bioavailability. Less is known about the impact on bioavailability of pesticide in aged BC-amended soil with time. Trigo et al. (2016) found lower extractable amounts of metolachlor in aged biochar and sorption increased with longer batch equilibration times.

We hypothesized that the behavior of three highly persistent herbicides with different physico-chemical properties can vary according to biochar characteristics (fresh or aged), either limiting or increasing sorption and, in consequence their bioavailability in soil. Hence, the objectives of this work were: i) to characterize an aged biochar and to compare the sorption of three pesticides on fresh and aged biochars, and ii) to assess the bioavailability of three pesticides in unamended and amended soil with fresh and aged biochar and its relationship with sorption. Highly persistent herbicides, such imazamox, terbuthylazine and picloram were selected to avoid problems with rapid biodegradation, which hampers the interpretation of slow sorption kinetics (Gámiz et al., 2017a). Results of this work will guide biochar production according to soil and

pesticide characteristics, which is a critical step in wide-spread use of BC as a soil amendment.

2. Materials and methods

2.1. Biochars, soil and herbicides

The fresh biochar (BC_{fresh}) was prepared from oak wood by slow pyrolysis in a Missouri-type concrete kiln at 550 °C (maximum pyrolysis temperature). Some of this biochar was initially placed into a mesh bag (Mesh Cord Bags, Vaultz®, Idea Stream Consumer Products LLC, Cleveland, OH; 1.5 mm openings) and buried in a silt loam soil located in Columbia County, Wisconsin (USA) for 6 months (April–October). The soil at the site had 14% sand, 71% silt, and 15% clay, 2.0% organic carbon (OC), cation exchange capacity (CEC) of 14 cmol_c kg⁻¹ and a pH of 7.0. After the aging period, pieces of biochar (BC_{aged}) were rinsed with large quantities of deionized water (10 L per 100 g of char), while the biochar was still in the mesh bag, to dislodge soil that was visibly present on the biochar surfaces. Following this rinsing, the biochar was removed from the mesh bag and oven dried at 105 °C. Both fresh and aged BC were ground to <2 mm prior use, to aid in homogenization. The elemental composition of the biochar samples was analyzed using thermal combustion (LECO analyzer Model CHN 932). Specific surface area (S_{BET}) of the two BCs was measured by physisorption of CO₂ (ASAP; Model 2420). The pH was determined in a 1/2.5 (w/v) biochar:deionized water slurry. Dissolved organic carbon (DOC) was extracted from biochar suspensions prepared in 0.01 M of CaCl₂ (1:2, w/v) after shaking for 15 min, then the samples were centrifuged and the supernatant was removed to measure the DOC (Shimadzu TOC-5050A analyzer). The extracts of DOC were also characterized by fluorescence spectroscopy by recording the spectra from 300 to 480 nm under excitation at 254 nm

(HITACHI F-2500 FL-Spectrophotometer). Zeta potential values were measured with a Zetasizer Nano ZS equipment (Malvern Instrument) in BCs suspensions of 1 mg mL⁻¹. Fourier transform infrared (FT-IR) spectra of 1% w/w samples in KBr pellets were recorded using a Jasco FT/IR 6300 spectrometer (Jasco Europe s.r.l.) to evaluate surface functional groups. The X-Ray Photoelectron Spectra (XPS) were gathered using a Leybold-Herens mod. LHS-10/20 instrument with a mono-chromated Al-K α X-ray XPS with photon energy of 1486.61 eV, a power of 100 eV, and a 500- μ m spot size.

A typical Mediterranean soil characterized by low organic carbon content was selected for the laboratory incubations. The soil was collected from an experimental farm of IRNAS (Coria del Río, Sevilla, Spain), by sampling the top 0-20 cm of soil, then mixed, air-dried and sieved (2 mm mesh) and finally stored at 4°C. The soil was a sandy loam soil with 86% sand, 4% silt, and 11% clay, 5.9% of CaCO₃, organic carbon (OC) of 1.3%, CEC of 15 cmol_c kg⁻¹ and pH of 7.9.

Three ionizable herbicides were selected based on their specific structures as model molecules. Two anionic herbicides, picloram (pyridine moieties) and imazamox (imidazolinone) and the weak basic herbicide terbuthylazine (triazine). All three herbicides, supplied by Sigma-Aldrich, were analytical grade (> 97%). The main characteristics of the compounds are compiled in Table S1 and their structures are shown in Fig. 1.

2.2. Standard sorption experiments on biochar

The sorption of pesticides on the biochars was determined at a single initial concentration by the standard batch equilibration method. Triplicate 40 mg of BC samples were equilibrated separately in Pyrex® glass tubes with 8 mL of an aqueous solution of each herbicide with initial concentration, C_i, of 1 mg L⁻¹. The solution pH of

BC suspensions ranged between 8.2 and 9.2. Samples were shaken for 24 h at 20 ± 2 °C in a rotating (end-over-end) shaker at 30 r.p.m. Then, the suspensions were centrifuged (7000 x g) for 15 min and 4 mL of the supernatant was removed and filtered using GHP membrane filters (pore size 0.45 µm) prior to analysis. No herbicide sorption to Pyrex® tubes, syringes or syringe filters was observed from control samples (data not shown). Aqueous concentration at 24 h equilibrium time of the herbicides (C_e) was measured by high-performance liquid chromatography (HPLC). The amount of each herbicide sorbed, C_s (mg kg⁻¹), was determined from the difference between the initial (C_i) and aqueous (C_e) herbicide concentrations. Percentages of herbicide sorbed (% Ads) were calculated by using the formula:

$$\%Ads = [(C_i - C_e)/C_i] \times 100.$$

Distribution coefficients, K_d (L kg⁻¹), were also calculated as:

$$K_d = C_s / C_e.$$

2.3. Soil incubation experiment

The dissipation of the herbicides in unamended soil and in soil amended with fresh and aged BC was assessed by a laboratory soil incubation experiment under aerobic conditions. In addition to this, the extent of herbicide sorption during the experiment was simultaneously evaluated. Glass bottles (250 mL) with polypropylene screw caps were filled with either 200 g of unamended soil or soil amended with BC_{fresh} or BC_{aged} samples. The amendment of the soil consisted of the addition of the biochars at 2% (w/w). In separate samples, each herbicide was spiked to reach a final application rate of 2 mg kg⁻¹ dry soil and then the samples were incubated in darkness at 20 ± 2 °C for 60 days. The moisture content was maintained at a constant level, nearly to the water holding capacity of the soil (~30%), throughout the experiment by adding deionized

water as necessary. All soil samples were periodically sampled in aliquots of 3 g-soil (triplicate), and placed into 50 mL centrifuge tubes and frozen until analysis (-20°C). All herbicide extractions were performed by adding 8 mL of the corresponding solvent. Tubes were shaken for 24 h, centrifuged and the extracts were analyzed by HPLC to determine the total amount of herbicide in soil residues. Methanol was the solvent used for the extraction of terbuthylazine, whereas picloram and imazamox were extracted from soil using a mixture of 30:70 (v/v) CH₃OH/0.01 M H₃PO₄ (pH 2.2) and 20:80 (v/v) CH₃CN/0.01 M H₃PO₄ (pH 2.2), respectively. Recoveries were greater than 94% for the three herbicides. The amount extractable with organic solvent was considered as a measurement of the amount of herbicide potentially available for biological processes, whereas the non-extractable fractions were considered non-bioavailable (Cox and Walker, 1999).

Dissipation data in unamended and amended soil with biochars were fitted to the linearized form of a first-order kinetic rate law:

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

where C (mg kg⁻¹) and C₀ (mg kg⁻¹) are the concentration of each herbicide in the soil at time t (days) and t = 0, respectively, and k (day⁻¹) is the first-order dissipation constant.

Changes in sorption with the aging of pesticides in soil was determined concomitantly throughout the incubation experiment. In this case, conditions are more realistic to estimate sorption compared to the standard batch experiment. For the simultaneous determination of herbicide sorption, minor modifications of a published method were made (Cox and Walker, 1999; Gámiz et al., 2017b). After the spiking of imazamox and picloram, at selected times (t= 0, 1, 21 and 60 days), duplicate aliquots of 5 g of soil were weighed in a Macrosep®Advance Centrifugal tubes (Pall

Corporation) containing a polyethersulfone membrane of 0.45 μm . Then, the tubes were centrifuged (7000 $\times$ g for 15 min) to obtain an aliquot of soil solution which was directly analyzed by HPLC to determine the aqueous concentration (C_e) of imazamox and picloram. In the case of terbuthylazine, a sequential extraction was carried out. At each sampling time, in triplicate, 3 g of soil was transferred to Pyrex® tubes with 8 mL of distilled water and shaken for 24 h. Then, suspensions were centrifuged and 6 mL of the supernatant was removed and filtered (0.45 μm) to quantify terbuthylazine aqueous concentration (C_e) by HPLC. Next, the 6 mL of supernatant solution removed was replaced with 6 mL of methanol. The tubes were resuspended and shaken at 20 ± 2 °C for 24 h, centrifuged, filtered, and analyzed by HPLC to measure the amount of extractable terbuthylazine in soil residues. Amounts of pesticide in aqueous solution were considered as readily bioavailable fraction. Amounts of herbicide sorbed were calculated from the difference between the total residues extracted of each compound and their concentration in the aqueous phase, to evaluate the fraction of pesticide potentially bioavailable.

In parallel, soil respiration of the unamended soil and soil amended with BC_{fresh} - and BC_{aged} -amended soil was determined following the alkali trapping–titrimetric method (Anderson, 1982), following the same procedure that is detailed in Gámiz et al. (2016a).

2.4. Herbicide analysis

The three herbicides were analyzed by HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector. For the analysis, a Kinetex C18 chromatographic column of 150 mm length $\times$ 4.6 mm internal diameter (i.d.) and 5 μm particle size (Phenomenex) was used, as well as flow rate of 1 mL min^{-1}

and 25 μL injection volume. The specific analytical conditions were: 50:50 (v/v) acetonitrile/water for terbuthylazine, 30:70 (v/v) $\text{CH}_3\text{OH}/0.01 \text{ M H}_3\text{PO}_4$ (pH 2.2) for picloram and 20:80 (v/v) $\text{CH}_3\text{CN}/0.01 \text{ M H}_3\text{PO}_4$ (pH 2.2) for imazamox, and UV detection at 220 nm for terbuthylazine, 225 nm for picloram and 255 nm for imazamox. External calibration curves with four concentrations between 0.1 and 2 mg L^{-1} were constructed. Specific details of each analysis are compiled in Table S2 of the supplementary information.

2.5. Data analysis

The statistical analysis and fitting of the dissipation curves were performed by using SigmaPlot (v. 13) software. The first-order dissipation constant (k) was calculated as the slopes of the linear regressions of $\ln C$ vs t . The variability among triplicate samples was evaluated using the standard error. Sorption percentage, amount of pesticides extracted, and first-order dissipation constants were compared using an ANOVA followed by a post-hoc Tukey's test to establish if statistically significant differences existed between treatments. Differences between results were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Characterization of the biochars

According to the data compiled in Table 1, both biochars showed similar physicochemical characteristics. Slightly lower C percentage, SSA value and dissolved organic carbon (DOC) were observed for BC_{aged} . This trend is in consonance with previous studies. For instance, the SSA decreases with aging in soil owing to the blocking of BC surfaces by soil organic matter or destruction of their physical structures

(Martin et al., 2012; Mia et al., 2017a). The slight increase in O/C ratio suggests a small increase in the polarity of BC_{aged} when compared with BC_{fresh} , which has been associated with the oxidation of BC surface due to the aging process (Mia et al., 2017a). Additionally, less negative zeta potential values were measured for BC_{aged} (Table 1), suggesting that the anionic exchange capacity for this biochar increased with aging as others authors have pointed out (Mukherjee et al., 2014).

The total content of DOC was lower for BC_{aged} (Table 1), which indicates partial removal of the natural organic covering on the fresh biochar surface either by weathering or the rinsing of the field aged biochar (Trigo et al., 2014). Likewise, the quality of DOC differed as a function of aging. It is remarkable that the DOC from BC_{fresh} presented great intensity of fluorescence towards wavelengths >380 nm (Fig. 2), indicating that the water-soluble organic fraction in this biochar was highly condensed, similar to that in humified organic matter (Cox et al., 2004). On the contrary, the water soluble fraction of BC_{aged} fluoresced more intensively at lower wavelengths (300-380 nm) (Fig. 2), which is related with the presence of less-condensed and polar organic material (Cox et al., 2004, 2000). With weathering, the more condensed molecules in BC_{fresh} can be lost, since hydrophobic DOC fractions can be immobilized by the presence of polyvalent metal ions in soils (Lin et al., 2012; Luo et al., 2010; Pignatello et al., 2015; Ren et al., 2018).

The most important bands recorded in the FT-IR spectra for BC_{fresh} and BC_{aged} are highlighted in Fig. 3. Both biochars exhibited similar absorption spectra, which suggests that, under the conditions studied, aging did not produce significant differences in surface functionality (Fig. 3a). The peaks appearing between $746-872\text{ cm}^{-1}$ were assigned to aromatic C-H out of plane deformation (Keiluweit et al., 2010; Mia et al., 2017b). The broad band centered at 1188 cm^{-1} was indicative of C-O stretching

vibration, distinctive of cellulose-derived products (Cheng et al., 2006; Keiluweit et al., 2010). Furthermore, in this range bands can overlap with those belonging to the –OH stretching of phenolic compounds (Rechberger et al., 2017). Peaks at 1430 and 1600 cm^{-1} were attributed to C=C stretching of aromatic carbon bonds, which were more intense for BC_{aged} . Finally, the band appearing at 1700 cm^{-1} is assigned to –C=O stretching from carboxyl groups (Keiluweit et al., 2010) in their molecular form (–COOH) (Cox et al., 2004). XPS analysis confirmed the presence of these functional groups on the surfaces of BC_{fresh} and BC_{aged} , surprisingly without significant difference between them (Fig. 3b). The maximum binding energy found around 286.5 eV was assigned to hydroxyl (–CO) and carboxyl (–COOH) groups, with contribution of carbonyl (–C=O) near 288 eV.

3.2. Sorption of the herbicides on fresh and aged BC

The affinity of the three pesticides towards the biochars was determined by a batch sorption experiment at a single initial herbicide concentration of 1 mg L^{-1} . The results of this experiment are presented in Table 2. Overall, despite the lack of differences observed in the compositional chemistry, there were significant differences observed in the sorption as a function of aging. It is worth mentioning that BC_{fresh} did not possess a great sorption affinity for the three herbicides (Table 3). In the best case, for terbutylazine, sorption only reached 16%, being almost non-detectable for picloram and imazamox (Table 3). It is known that the sorption capacity of biochars towards weak acids, which is the case of picloram and imazamox, is pH-dependent. The latter are mainly dissociated at solution pH (8.6 - 8.8) at the end of the experiment (Table 2), which would explain the limited sorption due to the repulsion between negatively charged herbicides and the negatively charged biochar surfaces (Cabrera et al., 2011;

Gámiz et al., 2017a; Hall et al., 2018; Trigo et al., 2014). This is also supported by the zeta potential values as well as the FTIR and XPS spectra. The sorption of triazines on carbonaceous materials is expected to occur through different mechanisms: entrapment into micropores together with π - π EDA interactions and solvophobic effect (Kah et al., 2017; Trigo et al., 2014; Xiao and Pignatello, 2015). However, the intensity of these phenomena between the two biochar samples is not sufficient to explain the enhancement in the sorption of terbuthylazine. The presence of more condensed and less polar DOC on BC_{fresh} could compete with terbuthylazine for sorption sites (Celis et al., 1998), as well as physically block pores (Pignatello et al., 2006). Steric hindrance has been linked to decreased sorption of triazines in microporous biochars (Xiao and Pignatello, 2015).

BC_{aged} exhibited completely different sorption behavior than that displayed by BC_{fresh}, even though both biochars possessed similar total C content, SSA and pH (Table 1). BC_{aged} removed more than 80% of the herbicides from solution (Table 2). A relationship correlating sorption with herbicide characteristics was not found, due to similar adsorption percentages on BC_{aged} ($P > 0.05$). Clearly, aging altered the sorption properties of BC. An additional experiment was performed to corroborate the results obtained. The experiment consisted in measuring the amount of each pesticide desorbed after sorption. Details about the procedure are described in the SI. It is known that pesticide degradation will bias sorption estimations when the sorbed concentration is calculated solely by the difference between mass initially added and mass in solution (Gámiz et al., 2016b). Accordingly, 97 % of terbuthylazine and 70% of imazamox sorbed on BC_{aged} was recovered, whereas picloram was not recovered. This may indicate that BC_{aged} could have catalyzed the degradation of picloram (Zhang et al., 2013). A complex coating on surfaces of aged biochar has been identified, which alters

hydrophilicity and surface active redox-active moieties (Hagemann et al., 2017). We also performed a similar sorption experiment of picloram on different aged BC-soil combination and similar results were obtained (data not shown). The specific process occurring during the weathering of the biochar needs to be further investigated, due to the drastic effect it may have on agrochemical sorption characteristics.

The implications of the water-soluble components of BC_{aged} was additionally evaluated. The BC_{aged} was washed by three successive cycles using CaCl₂ (0.01 M), following a procedure similar to that described for the extraction of DOC in BCs (Section 2.1). Next, samples were washed with distilled water to eliminate remaining dissolved ions (Ca²⁺ or Cl⁻) and air dried. Batch sorption experiments were then conducted on the washed biochar. Interestingly, the sorption of the three herbicides was greatly reduced: picloram and imazamox sorption percentages were reduced to 6%, whereas the percentage of terbuthylazine sorbed decreased to 16 %. This clearly shows the contribution of this extractable fraction present in the biochar to pesticide sorption, which is contrary to the formerly reported mechanism in which DOC blocks available sorption sites (Luo et al., 2017) or alteration in surface moities. These results support the formation of a surface coating (which possesses a soluble fraction) during the aging process, suggesting a coating rather than actual chemical alteration of the biochar surface.

3.3. Dissipation of herbicides in soil amended with fresh and aged biochar

Two different behaviors were observed in the dissipation of the herbicides according to data in Fig. 4. Data followed first-order dissipation kinetics with the exception of terbuthylazine in unamended soil ($R^2= 0.754$). For imazamox the addition of BC_{fresh} or BC_{aged} to soil did not alter its dissipation and the values for the first-order

dissipation constants (k) were similar for all treatments, ranging between 0.011 and 0.013 d⁻¹ ($P > 0.05$) (Table 3). These values are in accordance with data reported by the USEPA (1997) for this herbicide. Accordingly, at 60 days after treatment (DAT), imazamox residues in unamended soil were not significantly different from those in BC_{fresh}- or BC_{aged}-amended soil ($P > 0.05$) (Fig. 4). The experiments were performed in absence of light, so that, photodegradation can be discarded here and biodegradation can be considered the main degradation pathway (Milan et al., 2017). Finally, as will be discussed below, the high affinity of BC_{aged} for imazamox observed in the standard batch experiment did not translate into greater soil persistence of the herbicide under incubation conditions.

Regarding terbuthylazine, the first-order dissipation constants for unamended and amended soil were not significantly different ($P > 0.05$) (Table 3). The remaining amount of this herbicide at the end of the experiment was almost identical for all treatments ($P > 0.05$). After 24 d, however, terbuthylazine residues were greater in unamended soil and in soil amended with BC_{fresh} than in the BC_{aged}-amended soil ($P < 0.05$). This was associated to initially more rapid biodegradation of terbuthylazine in soil with BC_{aged}, even though the greater sorption in BC_{aged}-amended soil (Fig. 5) reduced the amount of terbuthylazine in the soil solution. Consequently, the sorbed fraction of terbuthylazine could have been more available for microbial decomposition or have reduced the toxic effect of the chemical on the degrading microflora, since high concentration of pesticides in soil solution usually has a toxic effect (Delgado-Moreno and Peña, 2009; FOCUS, 2006). Biochar had no toxic effect to soil since differences in soil respiration were not significantly different between treatments ($P > 0.05$) (Table 3).

Picloram was the herbicide that displayed marked differences among aging treatments. This herbicide has been cited as highly persistent (PPDB, 2018) which is in

agreement with the very low dissipation constant value of $0.005 \pm 0.001 \text{ d}^{-1}$ obtained for unamended soil (Table 3). With the addition of both BC_{fresh} and BC_{aged} the dissipation rates increased (Table 3). At 60 DAT, picloram residues were significantly different among treatments ($P < 0.05$): the extractable amounts of picloram were 20% and 50% lower for BC_{fresh} - and BC_{aged} -amended soils, respectively, than for unamended soil. Although picloram, as imazamox, is photosensitive, the action of light was minimized in the experiment. The reduced potentially bioavailable amounts of picloram can be explained if its degradation was triggered by the presence of BC_{aged} in soil, as indicating by the desorption batch experiment results, where this herbicide was almost completely removed from the solution, but not recovered by subsequent extraction. Hence, aged biochar additions can become a way to reduce the presence of specific pesticides in soil with time.

3.4. Sorption of herbicides in soil amended with fresh and aged biochar

The sorption percentages of each herbicide during the incubation experiment at selected times, calculated for the total amount extracted, are shown in Fig. 5. No changes in sorption of imazamox were observed within the 60 days of the experiment in unamended soil. When soil was treated with BC_{fresh} and BC_{aged} , a slight and significant increase in the sorption of imazamox was measured compared to untreated soil (Fig. 5). From the total imazamox extracted, approximately 10% of the herbicide was sorbed for both BC_{fresh} or BC_{aged} -amended soil (Fig. 5). The increase in sorption of imazamox with time in BC-amended soil could be reasonably attributed to slow sorption kinetics on BC particles, as it has previously been observed (Gámiz et al., 2017b).

At $t=0$, terbutylazine sorption was variable depending on the treatment. The sorption percentages follow the order: unamended (25%) < BC_{fresh} - (39%) < BC_{aged} -

397 amended soil (73%) (Fig. 5), with $P < 0.05$ for all treatments. BC_{aged}-amended soil
398 showed much greater sorption, which is consistent with sorption determined in the batch
399 experiment (Table 2). At longer incubation times, the percentage of terbutylazine
400 sorbed progressively increased for all treatments. After 60 days of experiment,
401 terbutylazine sorption increased by a factor of 1.56 for unamended soil and by 1.82
402 and 1.18 for BC_{fresh}- and BC_{aged}-amended soil, respectively (Fig. 5). These results agree
403 with those reported by other authors who also observed greater sorption of pesticides in
404 soil at longer times in incubation experiments (Cox et al., 1998; Cox and Walker, 1999;
405 Gámiz et al., 2017b; Trigo et al., 2016). Other authors, however, have observed limited
406 sorption of organic compounds on carbonaceous materials subjected to aging process.
407 Attenuated sorption was attributed to blockage of the aromatic domains and micropores
408 on biochar surfaces (Luo et al., 2017; Martin et al., 2012; Teixido et al., 2013). Until
409 now, it has been reported that higher sorption of pesticides in BC-amended soil results
410 in retarded pesticide dissipation (Gámiz et al., 2016a; Semple et al., 2013). Slow
411 sorption kinetics (Gámiz et al., 2017b) would explain the progressive increase in
412 sorption as the terbutylazine concentration decreased in the soil which was the case of
413 unamended and BC_{fresh}-amended soil (Gámiz et al., 2017b). Regarding the behavior of
414 terbutylazine in BC_{aged}-amended soil, faster dissipation and higher sorption were
415 observed at the beginning of the experiment (Fig. 4 and 5). As it has been explained
416 above, the highest sorption in BC_{aged}-amended soil resulted in very low terbutylazine
417 concentration in soil solution, which could facilitate its biodegradation since,
418 sometimes, pesticide inhibits degrading microflora at high concentrations (FOCUS,
419 2006). Furthermore, some authors have proven that bacteria are able to access certain
420 specific regions where pesticide molecules are sorbed (Park et al., 2003), which could
421 help explain our results. In any case, there was an increase in the percentage of

terbuthylazine sorbed, which may have diffused into less accessible or stronger sorption sites while a faster degradation (in solution and on labile sites) than desorption occurred, or even a combination of both in BC-amended soils (Trigo et al., 2016).

The behavior of picloram was intermediate between imazamox and terbuthylazine. Neither unamended nor BC_{fresh}-amended soil showed affinity for the herbicide until 60 DAT, when its sorption barely reached 7-8%, i.e. very low sorption kinetics succeeded for these treatments (Fig. 5). Thus, picloram and imazamox performed similarly in unamended and BC_{fresh}-amended soil, which can be attributed to their anionic character. On the contrary in BC_{aged}-amended soil, apparently there was a progressive sorption of picloram, which reached the 90% of the total residues at the end of the experiment (Fig. 5). Nevertheless, it should be noted that, at this time, 75% of the picloram applied was not extracted, either as a consequence of its degradation or formation of strongly bound residues with time (Trigo et al., 2016). Undoubtedly, the alteration of biochar surface after aging which generated the disappearance of picloram in solution during the sorption experiment had a specific role in the dissipation of the herbicide in soil. Although the mechanism was not explicitly deduced, it may be envisaged that, as it has been found, there was a coating on the biochar particles (Archanjo et al., 2017; Hagemann et al., 2017), which could have also improved associations of biochar with mineral phases (Burrell et al., 2016). Further investigation is needed to get insight into the fundamental mechanisms to clarify the specific pesticide characteristics that determine each behavior.

4. Conclusion

Outcomes from this study have demonstrated that the aging of BC in soil has direct impact on the bioavailability of highly persistence herbicides. Soluble-water

fraction of BC_{aged} contributed to the removal efficiency for the three herbicides on this aged biochar in batch experiments. Under more realistic conditions and after biochar aging, sorption and bioavailability, as characterized by the extractable amount of the pesticide, were pesticide-dependent. Indirect effects on sorption, either due to the slow sorption or enhanced degradation, were partly behind the differing sorption kinetics. This may have repercussions for successive applications of these xenobiotics to agricultural soils (Potter et al., 2005). Biochar aging effects must be considered to properly predict the impact of biochar amendment on the bioavailability of pesticides. This necessitates the matching of biochars to specific conditions, soils and organic compound characteristics. Our work demonstrates that the potential sorption capacity of this material changes with time and this is a key factor to consider when appraising the impacts of biochar amendment on the dissipation of organic contaminants. Further experiments are needed to prepare customized biochars with beneficial properties in long-term scales, to increase the bioavailability of pesticides, but reduce their environmental impact.

Acknowledgments

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Figure captions

Fig. 1. Chemical structure of the three pesticides used in this work.

Fig. 2. Emission fluorescence spectra of the DOC of the fresh and aged biochars at excitation wavelength of 254 nm.

Fig. 3. FT-IR (A) and XPS (C 1s)(B) spectra of the BC_{fresh} and BC_{aged} samples.

Fig. 4. Dissipation curves of the three herbicides after 60 days of incubation. Symbols correspond with experimental data points and bars indicate standard error of three replicates. Lines indicate the fitting to first order kinetic degradation.

Fig. 5. Percentage of herbicide sorbed from the total amount extracted at different times of the incubation experiment.

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Table 1

Some properties of the biochars used in this work.

Biochar	% C	% H	% O ^a	% N	%Ash	SSA (m ² /g) ^b	pH ^c	H/C	O/C	Zeta potential (mV)	DOC ^d (mg/g _{BC})
BC _{fresh}	80.1	2.0	17.5	0.4	3.4	226.0	7.8	0.02	0.22	-34.5	7.6
BC _{aged}	78.8	2.5	18.3	0.4	4.7	210.9	7.8	0.03	0.23	-26.4	6.8

^a calculated assuming less than 1 % of S without ash content^b measured by physisorption of CO₂^c measured in a 1:2.5 water:biochar slurries^d dissolved organic carbon

Table 2. Distribution coefficients and percentages of sorption for the herbicides in BC_{fresh} and BC_{aged}^a

Sample	Picloram			Imazamox			Terbuthylazine		
	Kd	Ads	pH ^b	Kd	Ads	pH	Kd	Ads	pH
	(L kg ⁻¹)	(%)		(L kg ⁻¹)	(%)		(L kg ⁻¹)	(%)	
BC _{fresh}	6 ± 1	3 ± 0	8.8	< 1	< 1	8.6	39 ± 20	16 ± 7	9.2
BC _{aged}	1109 ± 353	83.5 ± 6.3	8.3	999 ± 290	80.0 ± 8.2	8.2	6576 ± 2904	96 ± 2	8.9

^a Calculated for 40 mgBC: 8 mL of pesticide solution (1 mg/L)^b final pH after 24 h equilibration

Table 3. Single first-order dissipation constants for picloram, imazamox and terbuthylazine and soil respiration in unamended and amended soils

Soil	Picloram		Imazamox		Terbuthylazine		Soil respiration ^a
							(mg CO ₂ kg ⁻¹ dry soil week ⁻¹)
	k	R ²	k	R ²	k	R ²	
	(d ⁻¹)		(d ⁻¹)		(d ⁻¹)		
Unamended soil	0.005 ± 0.001 ^b	0.857	0.011 ± 0.001	0.950	0.018 ± 0.004	0.754	341 ± 49
BC _{fresh} -amended soil	0.008 ± 0.001	0.899	0.013 ± 0.001	0.979	0.016 ± 0.003	0.804	314 ± 18
BC _{aged} -amended soil	0.014 ± 0.001	0.929	0.013 ± 0.001	0.942	0.013 ± 0.001	0.950	259 ± 9

^a Measured under incubation experiment conditions^b Value ± standard error.

Supplementary Information

Changes in sorption and bioavailability of herbicides in soil amended with fresh and aged biochar

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Table S1. Selected physicochemical properties of the pesticides used in this work.

	Molecular weight (g/mol)	Solubility	pKa	Log P
Imazamox	305.3	626 g/L (pH 7)	2.3,3.3,10.8	-1.03
Picloram	241.5	560 mg/L	2.3	-1.92
Terbuthylazine	229.7	6.6 mg/L	1.9	3.4

Table S2

Chromatographic conditions used for the analysis of the herbicides by HPLC^a.

Pesticide	Mobile phase ^b	Flow rate (mL/min)	Injection	Detection
			volume (μ L)	wavelength (nm)
Picloram	MeOH:H ₃ PO ₄ (pH=2) (30:70)	1	25	225
Imazamox	ACN:H ₃ PO ₄ (pH=2) (20:80)	1	25	255
Terbuthylazine	ACN:H ₂ O (50:50)	1	25	220

^a In all cases, the chromatographic column was a Kinetex C18 column of 150 mm length $\times$ 4.6 mm internal diameter.

^b ACN: acetonitrile; MeOH: methanol; H₃PO₄: 0.01M (pH= 2.2).

Extraction of pesticide after sorption in batch experiment

Additional experiment was performed to measure, directly, the sorption of the pesticides on BC samples. Triplicate 40 mg of BC samples were equilibrated separately in Pyrex® glass tubes with 8 mL of an aqueous solution of each herbicide with initial concentration of 1 mg L⁻¹. Samples were shaken for 24 h at 20 ± 2 °C. Then, the suspensions were centrifuged for 15 min and 6 mL of the supernatant was removed and filtered using GHP membrane filters (pore size 0.45 µm) prior to analysis by HPLC. After that, the 6 mL of supernatant removed were replaced by 6 mL of corresponding solvent, depending of the pesticide, was added. Samples were resuspended and shaken for 24 h at 20 ± 2 °C, centrifuged, filtered and analyzed by HPLC. In this way, the amount of pesticide initially sorbed was directly determined from their desorbed amounts determined during the desorption step.

Methanol was the solvent used for the extraction of terbuthylazine, whereas picloram and imazamox were extracted from BC using a mixture of 30:70 (v/v) CH₃OH/0.01M H₃PO₄ (pH 2.2) and 20:80 (v/v) CH₃CN/0.01M H₃PO₄ (pH 2.2).