1   **An Examination of the Role of Biochar and Biochar Water-Extractable Substances**

2   **on the Sorption of Ionizable Herbicides in Rice Paddy Soils**

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24   **Abbreviations:** AZ, azimsulfuron; PE, penoxsulam; BC, biochar; BCW, washed

25   biochar; BWES, biochar water-extractable substances; DOC, dissolved organic carbon.

26   **ABSTRACT**

27   The application of biochar as a soil amendment can increase concentrations of soil

28   organic matter, especially water-extractable organic substances. Due to their mobility and

29   reactivity, more studies are needed to address the potential impact of biochar water-

30   extractable substances (BWES) on the sorption of herbicides in agricultural soils that are

31   periodically flooded. Two paddy soils (100 and 700 years of paddy soil development),

32   unamended or amended with raw (BC) or washed biochar (BCW), were used to test the

33   influence of BWES on the sorption behavior of the herbicides azimsulfuron (AZ) and

34   penoxsulam (PE). The adsorption of AZ to biochar was much stronger than that to the

35   soils, and it was adsorbed to a much larger extent to BC than to BCW. The depletion of

36   polar groups in the BWES from the washed biochar reduced AZ adsorption but had no

37   effect on PE adsorption. The adsorption of AZ increased when the younger soil (P100)

38   was amended with BC and decreased when it was amended with BCW. In P700, which

39   has lower dissolved organic carbon (DOC) content than P100, the adsorption of AZ

40   increased regardless of whether biochar was raw or washed. The adsorption of PE slightly

41   decreased when P100 was amended with BC or BCW and slightly increased when P700

42   was amended with BC or BCW. In order to evaluate compositional differences in the

43   biochar and BWES before and after the washing treatment, we performed solid-state ^{13}C

44   NMR spectroscopy of BC and BCW, and high resolution mass spectrometry of BWES.

45   Our observations stress the importance of proper consideration of soil and biochar

46   properties before their incorporation into paddy soils, since biochar may reduce or

47   increase the mobility of AZ and PE depending on soil properties and time of application.

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1. INTRODUCTION

Biochars, which are carbon-rich materials used for agricultural and environmental applications, are receiving increased attention for their ability to simultaneously achieve agricultural and environmental goals by sequestering carbon, binding organic compounds, and alleviating soil deficiencies (Spokas et al., 2009; Lehmann et al., 2011; Liu et al., 2018; Xiao at al., 2018). Biochar incorporation into soils has been shown to increase dissolved organic carbon (DOC) content in soil pore water (Beesley and Dickinson, 2011). Biochar water-extractable substances (BWES), defined as the most mobile fraction of biochar-dissolved organic matter, contain a diverse mixture of different organic compounds and inorganic elements containing macro and micro nutrients (Lou et al., 2016). BWES directly contribute to the pool of DOC in the soil (Lin et al., 2012). This has important implications when considering the environmental impact of biochar application in agricultural soils, since DOC greatly affects soil biological activity (Steiner et al., 2009), the fate of metal contaminants in soils (Beesley et al., 2010), and the mobility and bioavailability of pesticides (Cabrera et al., 2014; García-Jaramillo et al., 2014 & 2015). Despite the many studies that have focused on pesticides adsorption by biochar (Yang, et al., 2003; Cornelissen, et al., 2005), to our knowledge, little attention has been paid to the role of BWES on the sorption processes. It is still unclear how the amount and composition of BWES may influence the sorption of ionizable organic compounds in agricultural soils, either reducing or enhancing the mobility of pesticides in amended soils. Likewise, because BWES decrease as biochar ages (Gámiz et al., 2019), an understanding of how ionizable xenobiotic compounds react with raw and aged biochars is needed to predict how biochar amendments will interact with other agronomic inputs.

The limited ability to predict biochar-pesticide interactions is largely due to the variable physiochemical properties of biochars and BWES, which is based on feedstock

76 and production temperature (Zhao et al., 2013). To date, there are few reports that focus

77 on the sorption of ionizable herbicides by biochar-amended soils (Chen et al., 2008;

78 Dechene et al. 2014; Gámiz et al. 2019), and even less are focused on changes in the

79 sorption affinity of biochar-amended soils after the targeted removal of soluble organic

80 matter components from the biochar. These questions are especially salient in cropping

81 systems, like rice, that are seasonally flooded, and as a result, hydrophilic compounds are

82 more likely to enter waterways and applied biochars are expected to age quickly as BWES

83 are solubilized and transported off-site. To better understand how hydrophilic compounds

84 interact with fresh and washed biochar, we examined the sorption behavior of two widely-

85 used ionizable herbicides [azimsulfuron (AZ) and penoxsulam (PE)], in two paddy soils,

86 with biochar that was unmodified or with biochar that was washed to remove most of the

87 BWES (as determined by DOC quantification and high resolution mass spectrometry

88 analyses). This approach was intended to simulate and compare a recently-amended soil

89 with a previously-amended soil where most of the BWES had been removed by frequent

90 flooding.

91   The main objectives of this study were to characterize the molecular composition

92 of biochar and BWES and relate these characteristics to the sorption behavior of

93 azimsulfuron (AZ) and penoxsulam (PE) in soils that were amended with pristine biochar

94 or washed biochar. We hypothesized that the depletion of ion-exchangeable polar groups

95 after the removal of BWES may affect the sorption of ionizable pesticides in amended

96 soils under periodical flooded conditions. We aim to contribute to a better understanding

97 of the adsorption-desorption behavior of AZ and PE in biochar-amended soils dedicated

98 to rice cultivation, in order to prevent and mitigate pesticide dispersion and groundwater

99 contamination.

100

2. MATERIALS AND METHODS

2.1. Soils and Biochar

Two Ap horizons of paddy soils were collected in 2008 from an area located around Cixi, 180 km South of Shanghai and 150 km East of Hangzhou. Each soil had been similarly cultivated (flooded for 3–4 months each year) during the last 100 (P100) or 700 (P700) years. Soils were classified by Vogelsang et al. (2016) as Endogleyic Anthraquic Cambisol (P100; N 30°09.827', E 121°20.971') and Endogleyic Hydragric Anthrosol (P700; N 30°10.408', E 121°09.180'), according to the International Union of Soil Sciences (IUSS) Working Group WRB (2014). The sampling methods were detailed in a previously published chronosequence study that described the development of these soils (Hanke et al., 2013).

Biochar (BC) was produced from composted alperujo (CA), a byproduct of olive oil production, which has low concentrations of heavy metals (Alburquerque et al., 2006). The compost was produced in IFAPA Centro Venta del Llano, Jaén (Spain). Biochar was produced in a biomass pyrolysis unit that consisted of two iron stoves connected with a tube. In the first stove, wood was burned to produce heat, and the hot gases generated without oxygen moved to the other stove to pyrolyze the feedstock for 2h at 500°C. To obtain a washed version of the biochar (BCW), BC was treated with a 0.1 M HCl solution in a glass beaker, and gently stirred for 15 min. Afterwards, to remove HCl traces, biochar was rinsed with distilled water three times. This treatment not only decreased the pH value of biochar by two orders of magnitude, but more importantly removed some nutrients (soluble salts and potassium compounds), carbonates, and dissolved organic matter (DOM) (Azargohar et al., 2006, Sun et al., 2013), which allowed us to test the effects of the biochar surface on the sorption process of AZ and PE with minimal interferences from

125   other organic and inorganics components. Both BC and BCW were oven-dried at 105°C

126   and gently milled to pass a 0.25 mm sieve prior to further analysis.

127   Soils (P100 and P700) were air-dried, sieved through a 2 mm mesh, and

128   subsequently amended at a rate of 5% (w/w) with BC or BCW.

129

130   **2.2. Herbicides**

131   Analytical grade (≥97% purity) AZ (CAS RN 120162-55-2; chemical name: 1-(4,6-

132   dimethoxypyrimidin-2-yl)-3-[1-methyl-4-(2-methyl-2*H*-tetrazol-5-yl)pyrazol-5-

133   ylsulfonyl]urea) and PE (CAS RN 219714-96-2; chemical name: 3-(2,2-difluoroethoxy)-

134   *N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-α,α,α-trifluorotoluene-2-

135   sulfonamide) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The pK_a

136   values for AZ and PE are 3.6 and 5.1, respectively. Molecular structures and log K_{OW} of

137   both compounds are given in Fig. S1. These compounds were used to prepare the stock

138   solution (10 mg L^{-1}) and the solutions (at 0.01, 0.05, 0.1, 0.5, 1.0, 2.0 mg L^{-1}) used as

139   external standards to carry out the experimental assays. All the solutions were prepared

140   in ultra-pure water (HPLC grade). The groundwater ubiquity scores (GUS),

141   experimentally calculated values that relate pesticide half-life and sorption coefficient

142   (K_{oc}) (Gustafson, 1989), are 3.91 and 3.05 for AZ and PE (Tomlin, 2006), respectively.

143   Values obtained for AZ and PE denote the high leachability of both compounds and their

144   high potential to move toward groundwater.

145        $$GUS = \log10 \text{ (half-life) x } [4 - \log10 (K_{oc})]$$

146   **2.3. Physical and Chemical Analysis of Soils and Biochar**

147   Sub-samples of soils were air-dried and sieved to <2 mm. Bulk density was

148   calculated by dividing the mass of oven-dried soil (at 105ºC) by the core volume (100

149   cm^3). Iron-oxalate content, texture and soil organic carbon (OC) content were previously

6

150   reported (Hanke et al., 2013). The elemental composition (C, H, and N) of the BC and

151   BCW was analyzed by a high-temperature combustion method (Elementar Vario EL,

152   Hanau, Germany). The pH of the biochar was measured in a 1:5 (w/v) biochar/deionized

153   water mixture. Dissolved organic matter (DOM) from each biochar, soils and amended

154   soils was obtained by aqueous extraction with deionized water and DOC concentrations

155   were determined with a total organic carbon analyzer (TOC-V$_{CPH}$, Shimadzu, Kyoto,

156   Japan). The extraction ratio was 1:5 (soil:solution), by adding 30 ml of H$_2$O to 6 g of

157   amended and unamended soils, and 1:20 (biochar:solution), by adding 20 mL of

158   Nanopure H$_2$O to 1g of biochar. Suspensions were shaken at 20 ± 1°C for 24 h,

159   centrifuged at 3,500 rpm for 20 min, and then filtered through pre-rinsed 0.45 µm

160   cellulose-acetate filters (Zhao et al., 2008). Samples were kept at 4°C until analysis.

161   Specific UV absorbance (SUVA$_{280}$) of the DOM extracts from soils and amended soils

162   was quantified by dividing the absorption at 280 nm (using a Spectroquant Pharo 300

163   spectrometer, Merck KGaA, Darmstadt, Germany) by the total organic carbon content

164   (TOC).

165

166   **2.4. Adsorption – Desorption Experiments**

167   Adsorption of AZ and PE to BC and BCW was studied using a batch equilibration

168   technique. Based on our preliminary experiments, the sorbent's dosages were adjusted to

169   allow for 20–80% of the added pesticide to be adsorbed at equilibrium. We studied

170   concentrations from 0.05 to 2 mg L^{-1}, in a ratio 0.1:20 (w/v) for BC and 0.5:20 (w/v) for

171   BCW. The amount of herbicide adsorbed to the biochars was calculated from the

172   difference between the initial (C$_i$) and the equilibrium (C$_e$) solution concentrations.

173   Pesticide solutions were prepared in 0.01 M CaCl$_2$. Suspensions were shaken at 20 ± 2°C

174   for 24 h and centrifuged at 3,500 rpm for 15 min. According to our preliminary sorption

175   rate and equilibrium studies, it was determined that equilibrium was reached after 16–18

176   h of biochar-solution contact, and that no measurable degradation occurred during this

177   period. The uptake of pesticides by the walls of centrifuge tubes was negligible.

178   Supernatants were filtered, and equilibrium concentrations C_e determined by HPLC as

179   described below.

180   Adsorption of AZ and PE to amended and unamended soils were measured using

181   the above detailed batch equilibration method. Duplicate samples (1.0 g) of unamended

182   and amended soils (with 5% w/w BC or BCW) were treated with 2 mL of AZ and PE

183   solutions with initial concentrations (C_i) ranging from 0.05 to 1 mg L^{-1}. In preliminary

184   studies we made sure that soils were adequately saturated with that volume of pesticide

185   solution. Desorption was carried out after adsorption using the highest C_i (1 mg L^{-1}) by

186   replacing half of the supernatant with 0.01 M CaCl$_2$. This cycle was repeated 3 times (24

187   h each one) for each sample.

188   Adsorption and desorption isotherms were fitted to the Freundlich equation (Eq. 2),

189   expressed by the following equation:

190   $$C_s = K_f \, C_e^{\, \eta f}$$

191   where the constants K_f and η_f indicate the adsorption affinity and the adsorption linearity,

192   respectively. The apparent sorption coefficient (aK_d) was calculated as C_s / C_e at C_i = 0.5

193   mg L^{-1}.

194

195   **2.5. Herbicides Analysis**

196   The concentrations of AZ and PE were analyzed using a Prominence 20 HPLC

197   System coupled to a diode-array detector (Shimadzu, 's-Hertogenbosch, the Netherlands).

198   The separation was achieved with a Phenomenex LiChrospher® 5µm RP-18, 125x4 mm,

199   equipped with a C18 guard-column. The injection volume was 25 µl. The mobile phase

200   was a mixture of acetic acid (0.05%) and acetonitrile (65:35) at a flow rate of 1 mL min^{-1}

201   under isocratic conditions and the wavelengths monitored for UV detection were 242

202   nm for AZ and 230 nm for PE. External calibration curves were obtained analyzing

203   standard solutions in ultrapure water at concentrations ranging from 0.01 mg L^{-1} to 2 mg

204   L^{-1}. A very high linearity (R^2<0.9999) was always obtained. LOD values were 0.001 mg

205   L^{-1} for both pesticides. The values of LOQ were approximately 0.005 mg L^{-1}.

206

207   **2.6.   Solid-state ^{13}C Nuclear Magnetic Resonance (NMR) of biochars**

208   Solid-state cross-polarization magic angle spinning ^{13}C NMR spectra of all

209   biochar samples were obtained with a Bruker Advance III NMR spectrometer operating

210   at a ^{13}C frequency of 150.91 MHz and a magic-angle-spinning rate of 15 kHz. Between

211   1600 and 14000 single scans were accumulated with a pulse delay of 300 ms. A ramped

212   ^{1}H pulse was used during the contact time of 1 ms to circumvent spin modulation during

213   the Hartmann–Hahn contact. The ^{13}C chemical shifts were calibrated relative to

214   tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). Using MestreNova 9.0

215   (Mestrelab Research S.L., Santiago de Compostela, Spain) the contributions of the

216   various C groups to the total C were determined by integration of their signal intensity in

217   the respective chemical shift regions under consideration of spinning side band

218   disturbance according to Knicker et al. (2005). Based on the chemical shift differences,

219   the relative intensities were used to calculate the polarity (R) index (Eq. 1), which

220   represents the ratio of polar and non-polar groups (Abelmann et al., 2005):

221            $R = (I_{carboxyl\ C} + I_{O\text{-alkyl}\ C} + I_{O\text{-aryl}\ C}) / (I_{C/H\text{-aryl}\ C} + I_{alkyl\ C})$

222

223   **2.7. High Resolution Mass Spectrometry and van Krevelen Analysis of BWES**

224   BWES from each biochar was obtained by aqueous extraction with deionized water.

225   The extraction ratio 1:20 (biochar:solution) was achieved by adding 20 mL of Nanopure

226   H_2O to 1 g of biochar. The BWES of BC and BCW were further diluted with methanol

227   (1:100 and 1:10, respectively) for analysis by high resolution mass spectrometry using a

228   Q-TOF instrument (MaXis 4G, Bruker Daltonics, Leiderdorp, The Netherlands). A

229   validation of this novel approach can be found in Brock et al. (2019). Samples were

230   infused directly into and measured with an ESI source using a spray voltage of 3.5 kV in

231   negative ionization mode. Data were collected in the mass range of 50 – 1000 *m/z* at a

232   rate of 0.5 Hz, and 75 scans were averaged together using Bruker Compass 1.7

233   DataAnalysis software. The infusion analyses were split into three segments. During the

234   first segment (0 – 1 min) a mass calibration solution (2 mM sodium acetate in 1:1

235   H_2O:isopropanol solution) was infused for later re-calibration of the data. The second

236   segment (1 – 6 min) was specifically optimized to measure compounds with low *m/z*,

237   while the third segment (6 – 11 min) was used to measure compounds with high *m/z*. A

238   chemical formula for each mass spectrum was predicted using the SmartFormula feature

239   and exported to Microsoft Excel for further processing.

240   Van Krevelen diagrams are plots of the H/C versus O/C ratios of the molecular

241   formulas, which assigns the components into chemical classes in the van Krevelen space

242   (Kim et al., 2003; Sleighter et al., 2007). The van Krevelen space for this study was

243   divided into six discrete regions, modified from the diagram proposed by Hockaday et al.

244   (2009): lipids (H/C = 1.5-2.0, O/C = 0-0.3); proteins (H/C = 1.5-2.2, O/C = 0.3-0.67);

245   lignins (H/C = 0.7-1.5, O/C = 0.1-0.67); carbohydrates (H/C = 1.5-2.4, O/C = 0.67-1.2);

246   unsaturated hydrocarbons (H/C = 0.7-1.5, O/C = 0-0.1); and condensed aromatic

247   structures (H/C = 0.2-0.7, O/C = 0-0.67). Comparison of the van Krevelen plots was used

248 to track changes in BWES before and after the removal of acid hydrolysable biochar

249 components.

250

251 **2.8. Statistical Analysis**

252 Pearson correlation coefficients (two-tailed and with 95% confidence interval) and

253 linear regression analysis between the adsorption affinity, the adsorption linearity, DOC

254 content, pH, and $SUVA_{280}$ of the DOC, were obtained by using GraphPad Prism v.7 (La

255 Jolla, CA).

256

257 **3. RESULTS AND DISCUSSION**

258 **3.1. Characterization of soils and biochars**

259 The paddy soils used in this work have been previously characterized in studies that

260 examined redox control of carbon mineralization and dissolved organic matter (Hanke et

261 al., 2013); the link between soil formation and paddy management (Kölbl et al., 2014);

262 and the transformation of clay-sized minerals due to prolonged regular alteration of redox

263 conditions (Vogelsang et al., 2016). From the above mentioned chronosequence, we used

264 two paddy soils that had been used for submerged rice production for either 100 (P100)

265 or 700 (P700) years. Both soils are characterized by a low amount of organic matter, a

266 high clay content, and an average oxalate extractable iron content (Table 1) based on

267 previously published data by Shahandeh et al. (1994). The amount of extractable iron (Fe

268 oxalate) in these soils can also serve as an indicator of the stage and degree of soil

269 development (Seal et al., 2006). Vogelsang et al. (2016) found that prolonged paddy

270 cultivation caused the loss of silicate- and oxide-bound Fe in the topsoil. The contribution

271 of smectite clay minerals and iron oxides to the sorption of sulfonylureas has been

272 previously reported (Calamai et al., 1997; Pusino et al., 2000), as well as their important

role on the adsorption of other weak acidic pesticides (Hermosin y Cornejo, 1991; Cox et al., 1995).

We found that the amount of DOC in P700 was significantly lower than in P100 (Table 1), as expected by the differences in the time that these soils were under periodically flooded conditions. The washing treatment removed approximately 80% of the DOC in the biochar (Table 2). The incorporation of BC or BCW (5% w/w) increased DOC by approximately 2.7 and 1.2 times, respectively, in P100; and 2.5 and 1.5 times, respectively, in P700 (data not shown). The pH of the 1:5 (biochar:water) extract of BCW was reduced by 2 units when compared with the BC extract (Table 2). The total C and N content (%) were also slightly, but similarly reduced after washing, as was the ash content.

Absorption around 280 nm is a measure of the aromatic character of dissolved organic matter (Chin et al., 1994, McKnight et al., 1997, Kalbitz et al., 1999). The aromatic character of the DOM-extracts of P100 and P700, based on the specific UV absorbance at 280 nm ($SUVA_{280}$), was very similar, and increased with the amendment in all the conditions tested (Fig. 1). The pH values of the unamended and amended soils are also shown in Fig. 1.

3.2. Adsorption and Desorption Experiments

Azimsulfuron (AZ)

To determine if BC or BWES influences the adsorption of AZ in soils, we examined the adsorption kinetics of the herbicide in biochars, unamended and amended soils. The adsorption of AZ to biochar was much stronger than that to the soils (Table S1), and it was adsorbed to a much larger extent to BC than to BCW (Table S1, Fig. 2a). The apparent sorption coefficient (aK_d) values at 0.5 mg L^{-1} after the removal of BWES from biochar decreased by ~56% for AZ (Table S1). In P100, the adsorption of AZ

298   increased when the soil was amended with BC and reduced when the soil was amended

299   with BCW. This result suggests that BWES play an important role in the sorption of AZ.

300   In contrast, the low sorption capacity of P700, which may be explained by its lower DOC

301   content when compared with P100 (Table 1), was enhanced by the addition of either BC

302   or BCW (Table S1, Fig. 3).

303   ***Penoxsulam (PE)***

304   To determine if the trends we observed with AZ were consistent with other

305   herbicides, we examined the adsorption of a similar herbicide, penoxsulam (PE). The

306   adsorption of PE to BC and BCW (Table S2, Fig. 2b) was much lower than the one

307   observed for AZ (Table S1, Fig. 2a), and small differences were found between the

308   adsorption to BC and BCW. The apparent sorption coefficient values at 0.5 mg L^{-1} after

309   the removal of BWES from biochar decreased by ~13% for PE (Table S2). HCl treatment

310   hydrolyzed and dissolved a fraction of the organic matrix. The subsequent rinsing of the

311   sample with distilled water removed this dissolved fraction, bringing the surface to a

312   neutral pH. During the batch sorption experiments with fresh and washed biochar, the pH

313   of the solution was higher than the pK of the sorbate. Therefore, PE was predominantly

314   deprotonated. As expected, the adsorption of a deprotonated molecule on a neutral surface

315   was low.

316   Amendment of P100 with BC and BCW reduced the sorption of PE (Table S2, Fig.

317   4). In contrast, PE exhibited a completely different behavior in P700, showing a much

318   weaker adsorption than the one measured for P100. The slightly higher adsorption of PE

319   in the younger soil (P100) when compared with the older soil (P700) may be due to the

320   higher DOC content of P100, which could facilitate the adsorption of PE. The same

321   behavior was observed by Jabusch and Tjeerdema (2005), in soils amended with manure

322   compost, where the sorption of PE was mainly regulated by the soil organic matter content

323 and clay mineral sorption sites. The very low sorptive capacity of P700 for PE, however,

324 was enhanced when raw or washed biochar was added to that soil (Table S2, Fig. 4).

325

326 **3.3. Correlation Analysis**

327 We found a strong correlation (p=0.001) between the adsorption affinity (K_f) of AZ on

328 P100 and the DOC content (which is proportional to the BWES content) of the biochar

329 and amended soil. We also observed a significant correlation (p=0.020) between the

330 desorption linearity (η_f) of AZ on P100 and the DOC content. Additionally, a significant

331 correlation (p=0.028) was revealed between the desorption linearity (η_f) of PE on P100

332 and the specific UV absorbance (SUVA) at 280 nm. No significant correlations were

333 identified for the affinity and linearity adsorption/desorption constants of AZ or PE in

334 P700. These constants did not correlate with the DOC content nor with SUVA when

335 testing PE adsorption/desorption. Pusino et al. (2004) studied the sorption behavior of AZ

336 on five different soils. They observed the highest K_f value in the soil with the greatest

337 amount of organic matter, and found a significant negative correlation between $K_{f\,ads}$ and

338 pH, indicating that the extent of adsorption increases as the pH decreases (because

339 increased hydrogen ions are available in the soil solution), following the general trend

340 observed for sulfonylureas and other weakly acidic herbicides (Hay et al., 1990; Azcarate

341 et al., 2015). We also observed a significant correlation between the adsorption and

342 desorption linearity of PE and the pH values (p=0.048 and p=0.025, respectively) of P700

343 un-amended and amended with BC and BCW. Other studies have reported the influence

344 of pH and OM on the sorption of ionizable pesticides (Pinna et al., 2007; Földényi et al.,

345 2013). With pK$_a$ values of 3.6 and 5.1, both herbicides are expected to be predominantly

346 in the anionic form at the pH of the un-amended and amended soils (Kah and Brown,

347   2016), which lead to the enhancement of their mobility in the soil profile (Földényi et al.,

348   2013).

349

3.4. Solid-state ^{13}C NMR Spectroscopy of Biochars

351   In order to evaluate compositional differences in the biochar before and after the

352   washing treatment, we performed solid-state ^{13}C NMR spectroscopy of BC and BCW

353   (Fig. 5). The solid-state ^{13}C NMR spectra of BC shows a pattern typical of pyrolyzed N-

354   rich material (Knicker, 2010). No significant differences derived from treating the biochar

355   could be detected by NMR. The dominant signal in the chemical shift region between 140

356   and 90 ppm (Table 3) is caused by aromatic C formed by dehydration and cyclization of

357   carbohydrates, peptides and some lipids.

358   The *N*- and O-substituted aryl C contributed to the shoulder in the chemical shift

359   region between 160 and 140 ppm. The strong signal between 45 and 0 ppm is assigned to

360   alkyl C that is likely derived from lipids that survived the pyrolysis process and from non-

361   aromatized degradation products of carbohydrates and peptides. Considering the high N

362   content of the samples (Zhao et al., 2013) and the low lignin content of the feedstock

363   material used to produce the biochar (García-Jaramillo et at., 2015), the clear signal at 54

364   ppm is best explained by the presence of *N*-alkyl C, possibly from partially decomposed

365   peptides. This suggests that the pyrolysis process, which sequesters C by formation of

366   aromatic molecules, can also sequester some N by incorporating it into condensed

367   aromatic OM components, in good agreement with previous observations (Knicker et al.,

368   2010; Ohno et al., 2010). The respective amide C adds to the signal at 172 ppm. Ketones

369   and aldehydes formed during the combustion of carbohydrates can explain the intensity

370   around 200 ppm. The signal at 72 ppm in the region of *O*-alkyl C (90 to 60 ppm) indicates

371   that not all carbohydrates were decomposed during pyrolysis.

372   The NMR spectra reveal a considerable contribution of O/N-substituted C (carboxyl

373   C, O/N-aryl C, O/N-alkyl C). A high O contribution in a biochar material may indicate

374   the presence of hydroxyl, carboxylate, and carbonyl groups that can contribute to a high

375   cation exchange capacity (CEC) of biochar (Lee et al., 2010), with important

376   consequences when considering the adsorption of ionizable pesticides.

377

378   **3.5.   High Resolution Mass Spectrometry and van Krevelen Analysis of BWES**

379   To further explain the sorption behavior of AZ and PE as affected by BWES from

380   BC and BCW, we assessed the molecular composition of their BWES by performing high

381   resolution mass spectrometry and van Krevelen analysis. The molecular formulas

382   assigned to mass spectral peaks in the BWES from BC and BCW are plotted in the van

383   Krevelen diagrams shown in Fig. 6, which are separated into CHO and CHON elemental

384   formula classes. The main difference between the BWES from BC and BCW is in the

385   amount of CHON molecules (Fig. 6, c and d). The loss of N-containing molecules in

386   BCW, and the subsequent loss of hydrogen bond accepting character, may be responsible

387   for the differences observed in the sorption processes of both pesticides to BC and BCW.

388   The van Krevelen diagrams indicates that lignins and proteins (with and without N

389   atoms) are the dominant molecule in both BC and BCW extracts (Fig. 6). This is

390   consistent with other studies of DOM derived from plant biomass (Ohno et al., 2010).

391   Interestingly, the condensed aromatic components of BC and BCW water extractable

392   substances are dominated by N-containing components. The only polar groups in

393   aromatic structures are carboxyl groups, O and N in furans and pyrroles, or OH groups

394   (which are not easily substituted). Polar compounds, such as AZ and PE, will probably

395   adsorb on the O or N atoms in the BWES or of biochar's surface. Since these bonds are

396   relatively weak, they can be exchanged. The high abundance of acidic groups present in

16

397   BWES (Fig. 6) may be responsible for the increase in the acidity of the biochar surface,

398   enhancing the influence of pH on the adsorption of dissociable pesticides (Qiu et al.,

399   2009). However, this influence is not likely to be important in this study based on the

400   major ionic species for azimsulfuron and penoxsulam found at the pH of the amended

401   and unamended soils, based on protonation calculations performed with Marvin Sketch

402   software v19.21 (ChemAxon) (see Supplementary Materials, Figures S2, S3 and S4).

403   The majority of peaks appear at odd m/z values, which indicates a predominance of

404   zero or an even number of nitrogen atoms in the molecules (Chen et al., 2014). Across

405   the entire mass range, peaks at even m/z values are generally 1.003 m/z units (the mass of

406   a neutron) higher than the parent peak at odd m/z values. These compounds are likely

407   isotopologues that contain one ^{13}C atom in place of a ^{12}C atom (Chen et al., 2014). This

408   result indicates that most peaks are singly charged, which is consistent with other studies

409   of DOM (Stenson et al., 2002; Kim et al., 2003; Sleighter and Hatcher, 2008). Repeated

410   patterns every 14.015 mass units were also noticed, which indicates the incremental

411   addition of a CH_2 group (Kim et al., 2003; Sleighter and Hatcher, 2008). The replacement

412   of one oxygen atom by a CH_4, for example by substitution of an aldehyde group (CHO)

413   for an ethyl group (C_2H_5) was also detected in peaks differing by 0.036 m/z units within

414   each nominal mass, as previously described by Stenson et al. (2003).

415

416   **3.6. Potential mechanisms of adsorption**

417   The sorption isotherms for AZ and PE to the soils (Figures 3 and 4) were nonlinear

418   in all cases, with slopes ($ŋ_f$) resembling L-type isotherms (Giles et al., 1960). An L-type

419   curve indicates a relatively high herbicide-soil affinity at low concentration; that is, the

420   extent of sorption decreases as the herbicide concentration increases, because the

421   adsorption sites were gradually saturated with the herbicide compounds. As expected for

422   these weakly acidic, polar compounds, the sorption of AZ and PE to the unamended paddy

423   soils was fairly weak as indicated by the Freundlich adsorption coefficients (Tables S1

424   and S1). In soils with pH values greater than their pK_a, as the soils we used, weakly acidic

425   pesticides exist predominantly in the neutral or anionic form (Figures S2, S3 and S4),

426   which minimizes potential interactions with primarily negative binding sites (Hyun et al.,

427   2003).

428   Sorption properties of biochars mostly depend on their own physical and chemical

429   properties, which are strongly influenced by the pyrolysis conditions, specifically the

430   charring temperature (Keiluweit et al., 2010). Biochars, like any soil organic matter,

431   consists of volatile (Ghidotti et al., 2016), stable (Leng et al., 2019), and mineral fractions

432   (such as alkali or alkaline earth metals in the form of carbonates, phosphates, or oxides)

433   (Xu et al., 2017), and are quite heterogeneous. Therefore, the mechanisms responsible for

434   the immobilization of pesticides will depend on the adsorption to all these fractions (Chen

435   et al., 2008). In addition, polar organic compounds containing N, O and F atoms in their

436   structure, such as AZ and PE, can form hydrogen bonds with H donor groups on the

437   surface of the biochar (Sun et al., 2012). These mechanisms are relevant for the interaction

438   of pollutants with the DOM from the soil or from the amendments (Kah and Brown, 2007;

439   García-Jaramillo et al., 2016). Teixidó et al. (2011) identified new details in the sorption

440   behavior of the ionizable antibiotic sulfamethazine to biochar. These include the

441   formation of relatively strong H-bonds with surface hydroxyl and carboxyl groups, and

442   the contribution of proton exchange phenomena to facilitate these interactions, leading to

443   release of hydroxide or hydrogen ions to solution. Ni et al. (2011) suggested that proton

444   exchange-assisted adsorption on natural particles rich in carboxylic and phenolic groups

445   is characteristic of any weak acid able to form a strong H-bond as a result of pK_a

446   equivalency. Proton exchange-assisted sorption to soil organic matter may be difficult to

447   observe, however, because of the high buffering capacity of soil organic matter (Ni et al.,

448   2011). The ligand-exchange mechanism has been also proposed as a mechanism of

449   retention of AZ on different geosorbents (Pinna et al., 2004, Kah & Brown, 2006). Pinna

450   et al. (2004) also found an unusual infra-red spectrum of the azimsulfuron-iron oxide

451   complex, due to the Fe^{3+} coordination to the azimsulfuron sulfonylurea group, acting as

452   a bridge ligand between sulfonyl and carbonyl oxygen atoms. The loss of N-containing

453   molecules in BCW, as shown in Fig. 6, and the subsequent loss of hydrogen bond

454   accepting character, may be responsible for the lower adsorption of AZ to BCW than to

455   BC. The adsorption of PE to BC and BCW was much lower than the one observed for AZ

456   (Table S2, Fig. 2), and no differences were found between the adsorption to BC and BCW,

457   suggesting that hydrogen-bonding to oxygen-containing functional groups within

458   biochars is not a dominant mechanism for the adsorption of this herbicide.

459   While the behavior of AZ and PE towards BC and BCW appears to be different

460   than towards biochar amended soils, further work may reveal there is a lot in common

461   between their adsorption to biochar and soil organic carbon.

462

463   **4.   CONCLUSIONS**

464   Dissolved OC dynamics in paddy topsoil, affected by organic amendment

465   additions, can modify the behavior of ionizable pesticides in flooded paddy soils. Biochar

466   has been shown to be a significant source of highly mobile organic matter (defined here

467   as BWES), and especially when it is applied to soils subjected to periodically flooded

468   conditions. The speciation and sorption behavior of ionizable compounds has been well

469   described in the literature for nonfunctionalized and very low variable-charge materials

470   (i.e. graphite), and also for biochars. Our results show that both AZ and PE adsorption to

471   the studied paddy soils were affected by biochar incorporation and, in the case of AZ, it

472 also depends on the amount and composition of BWES. The depletion of ion-
473 exchangeable polar groups in the BWES from the washed biochar, especially O- and N-
474 containing components as shown by high-resolution mass spectrometry, had an effect on
475 the adsorption of AZ to the amended paddy soils, but not on PE. The results of our study
476 propose that BWES characterization is an important contribution to the study of the fate
477 of AZ, but not PE, in biochar amended soils. However, soil clay content, pH of the soil
478 solution, and soil organic matter content need to be integrated with biochar and BWES
479 properties, to get a more complete picture of the fate of these herbicides in flooded paddy
480 soils. Further work may be needed to better understand the complex mechanisms leading
481 the sorption behavior of AZ and PE in biochar-amended paddy soils as affected by
482 periodically changing redox conditions.

483

497   the U.S. Department of Agriculture. USDA is an equal opportunity provider and

498   employer.

CONFLICT OF INTEREST STATEMENT

500   We have no conflict of interest to declare.

SUPPLEMENTAL MATERIALS

502   The Supplemental Material section includes AZ and PE molecular structures

503   (Figure S1) and adsorption-desorption coefficients (Tables S1 and S2).

504

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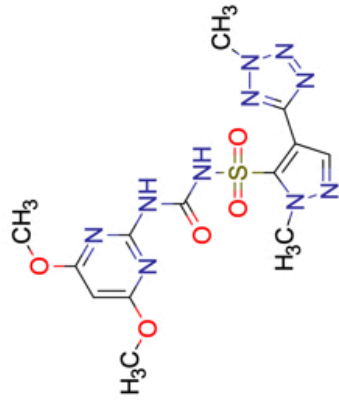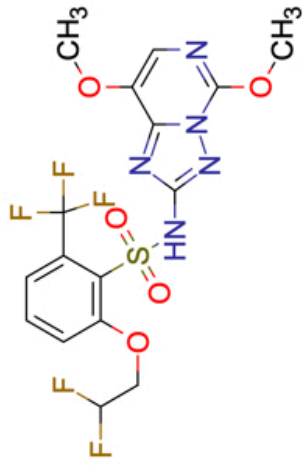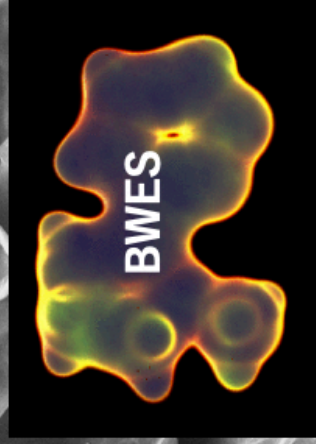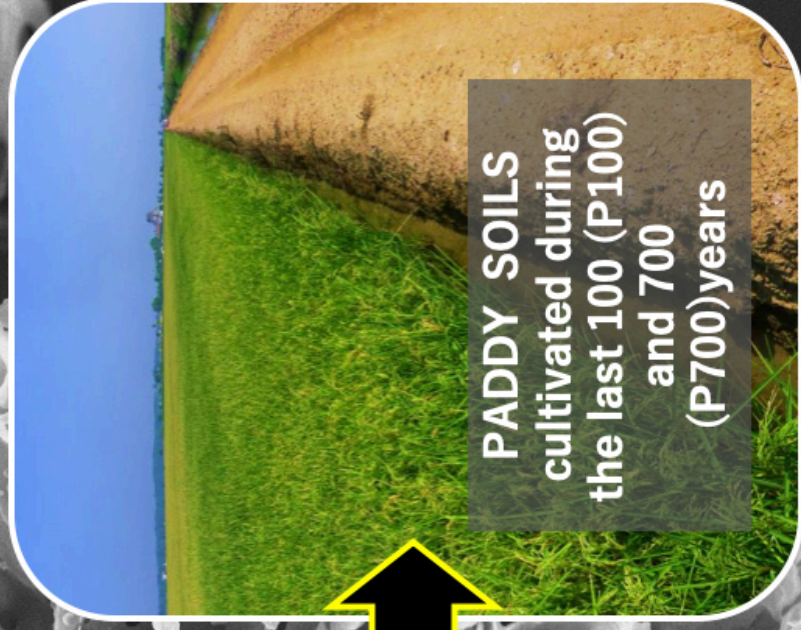
BC: Biochar; BCW: Washed biochar; BWES: Biochar water-extractable substances
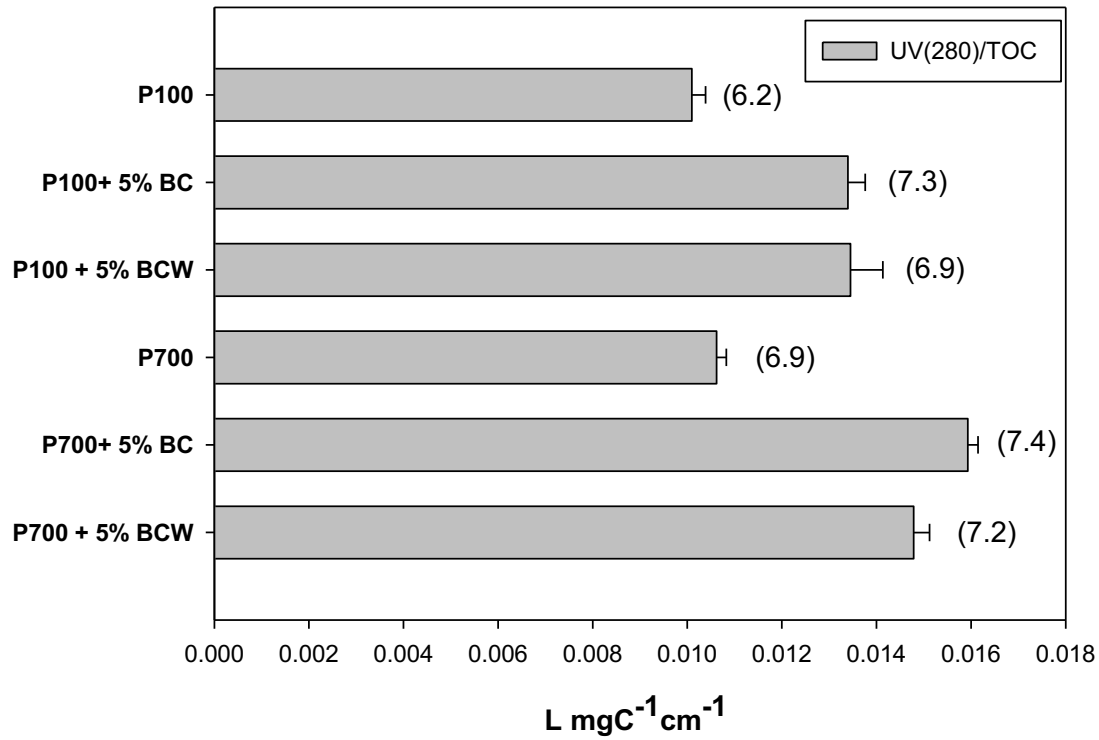
Figure 1. Specific UV absorbance (L mg C^{-1} m^{-1}) at 280nm ($SUVA_{280}$) and pH values (in brackets) of the amended and unamended soil extracts used for DOC quantification. Bars represent the mean and standard deviation of two replicates.
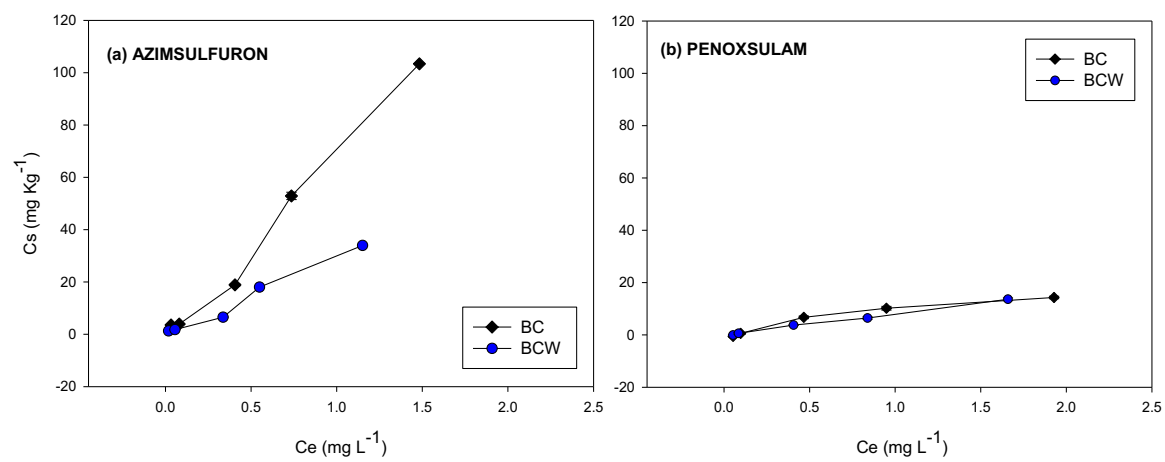
Figure 2. Isotherms of azimsulfuron (a) and penoxsulam (b) adsorption to BC and BCW. Mean and standard deviation of two replicates.
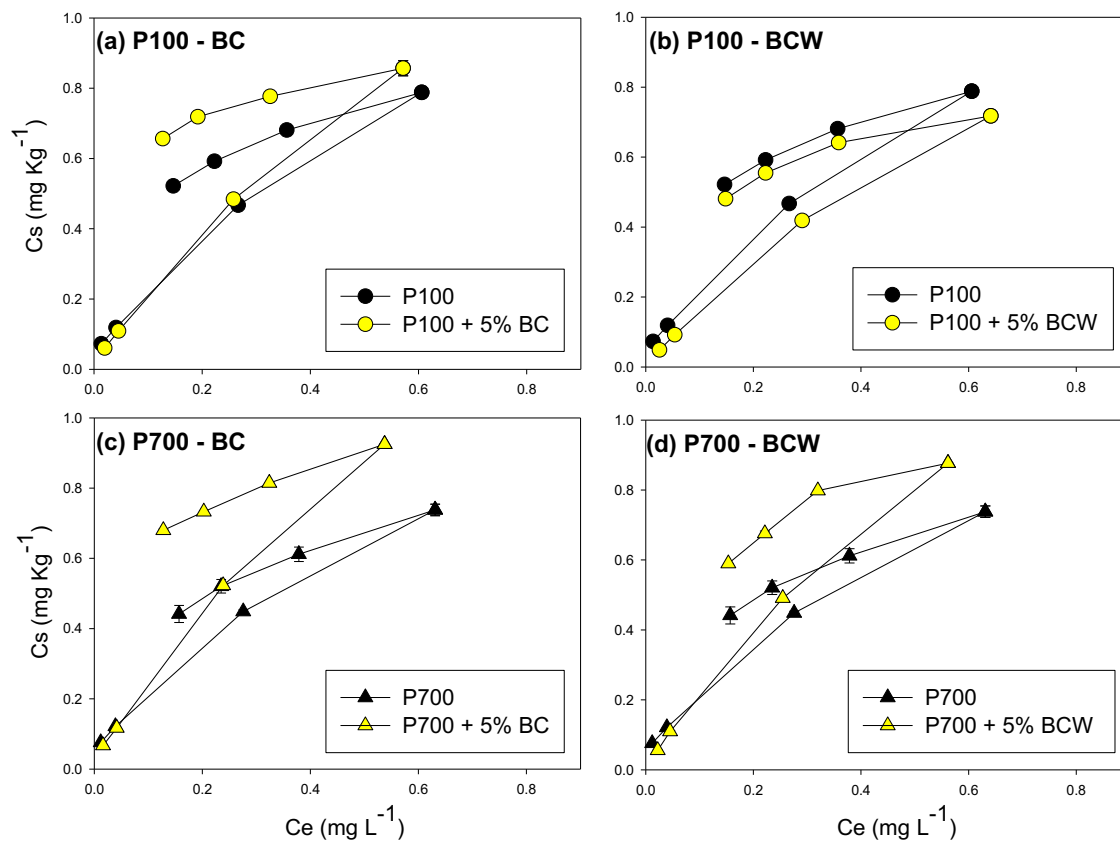
Figure 3. Isotherms of azimsulfuron adsorption - desorption to (a) P100 amended or not with BC, (b) P100 amended or not with BCW, (c) P700 amended or not with BC, (d) P700 amended or not with BCW. Mean and standard deviation of two replicates.
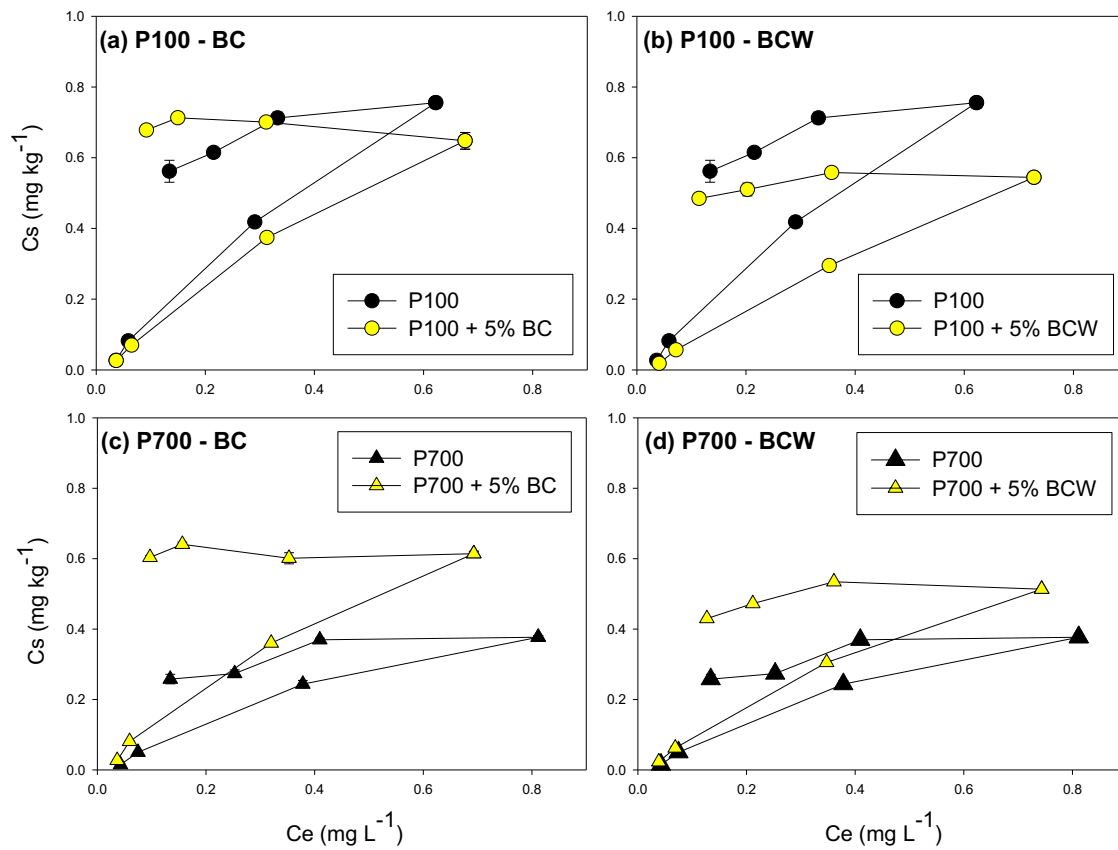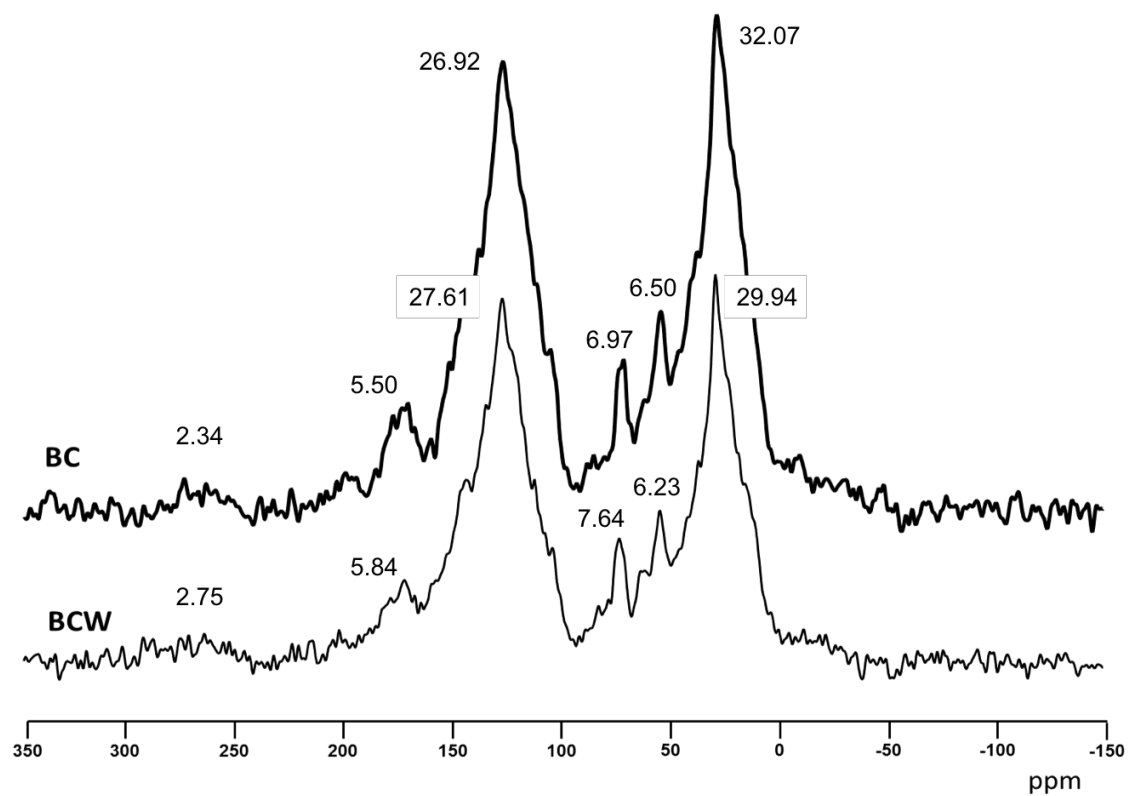
Figure 4. Isotherms of penoxsulam adsorption - desorption to (a) P100 amended or not with BC, (b) P100 amended or not with BCW, (c) P700 amended or not with BC, (d) P700 amended or not with BCW. Mean and standard deviation of two replicates.

Figure 5. ^{13}C solid-state NMR-spectra of BC and BCW and chemical shift of the signals at 270, 172, 128, 72, 54, and 30 ppm. No significant differences were found between both chars.
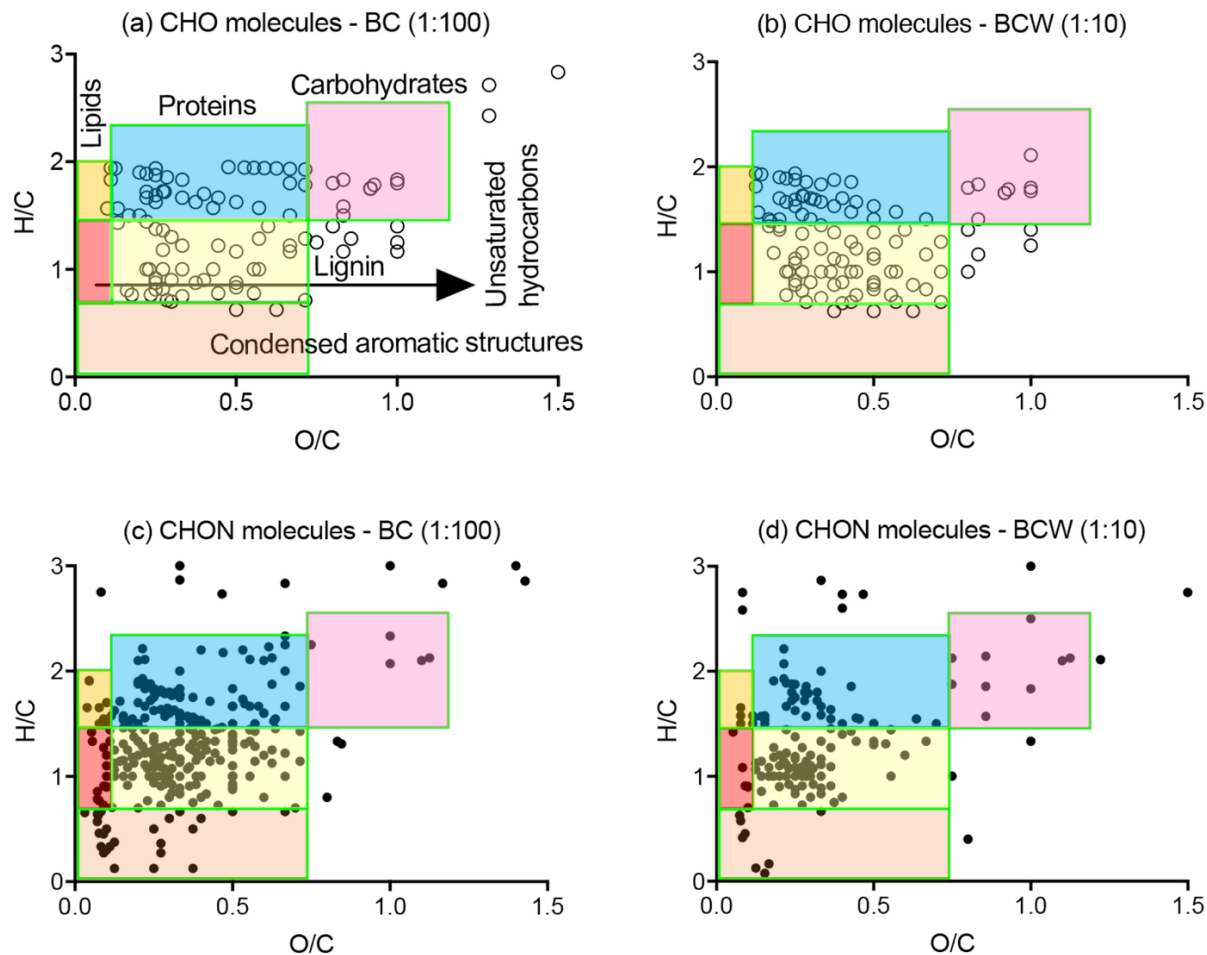
Figure 6. Van Krevelen diagrams for the molecular formulas assigned to the mass spectral peaks of BWES derived from BC. BWES from BC was diluted 1:100 in methanol, and BWES from BCW was diluted 1:10 in methanol. (a) CHO elemental formula classes from BC, (b) CHO elemental formula classes from BCW, (c) CHON elemental formula classes from BC, (d) CHON elemental formula classes from BCW. Boxes overlain on the plots indicate major biomolecular compound classes: lipids, proteins, carbohydrates, unsaturated hydrocarbons, lignins, and condensed aromatic structures.

Table 1. Basic soil parameters of sampled paddy soils.

Soil	Cultivation Time	Horizon*	Depth	Bulk density	Fe oxalate*	Clay[#]	Texture*	OC[#]	DOC	pH[#]
	(years)		(cm)	(g cm^{-3})	(mg g^{-1})	(%)		(mg g^{-1})	(mg L^{-1})	(KCl)
P100	100	Alp	0 - 15	1.0 ± 0.05	3.1 ± 0.3	28.0	Silt loam	16.5 ± 1.2	86.2 ± 1.9	5.4 ± 0.1
P700	700	Alp	0 - 16	1.1 ± 0.02	4.6 ± 0.1	28.5	Silt loam	20.6 ± 1.8	63.5 ± 0.2	6.7 ± 0.1

Horizon description suffixes: Alp (A=mineral surface horizon, l=capillary fringe mottling, p=plowing).
*Data from Hanke et al., 2013. [#]Data from Kölbl et al., 2014.

Table 2. Physical and chemical properties of BC and BCW. Ash content was evaluated at 950 °C.

Mean and standard deviation of two replicates.

Biochar	pH (1:5)	Ash (mg g^{-1})	C (%)	N (%)	C/N	C/H	DOC (mg L^{-1})	BWES (%)
BC	9.4 ± 0.1	446.7 ± 9.1	27.9	2.1	12.6 ± 0.2	12.3 ± 0.2	$2,820.2 \pm 50.9$	5.6
BCW	7.3 ± 0.1	431.9 ± 2.2	26.3	1.9	13.3 ± 0.3	12.5 ± 0.7	544.8 ± 15.3	1.1

Table 3. NMR data under consideration of spinning side bands. Polarity index is also given. BC (biochar made from alperujo compost at 500 °C) and BCW (biochar of alperujo compost yield to a washing treatment, as it is described in the materials and methods section).

Sample	Carboxyl C (225-160 ppm)	O/N-Aryl C (160-140 ppm)	H/C-Aryl C (140-90 ppm)	O-alkyl C (90-60 ppm)	N-alkyl/methoxyl C (60 -45 ppm)	alkyl C (45-0 ppm)	Polarity R
BC	7.8	9.2	37.4	7.0	6.5	32.1	0.44
BCW	8.6	9.9	37.7	7.6	6.2	29.9	0.48

1 <p style="text-align:center">**SUPPLEMENTARY MATERIAL**</p>

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4 **An examination of the role of biochar and biochar water-extractable substances on**

5 **the sorption of ionizable herbicides in rice paddy soils**

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8

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Fig. S1. Molecular structures and chemical properties of azimsulfuron and penoxsulam. Herbicides are presented at neutral pH.
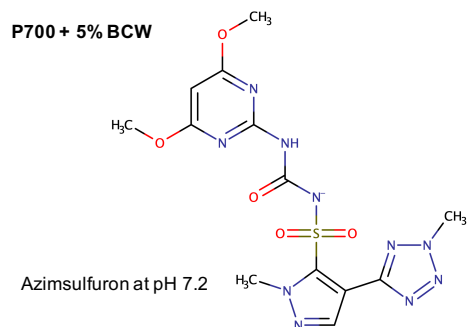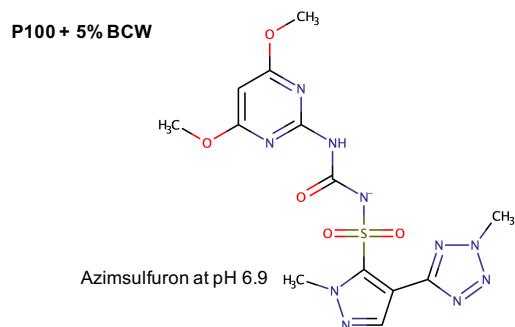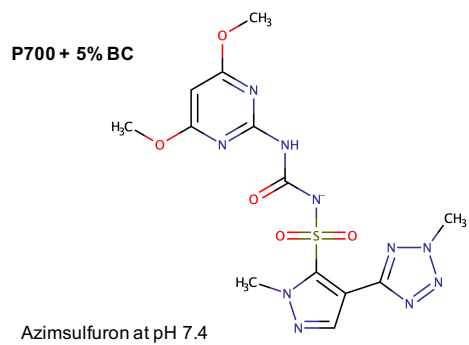
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Azimsulfuron
pKa = 3.6
Log Kow = 0.65 at 25°C and pH5
Log Kow = -1.37 at 25°C and pH7
Solubility (water) = 1,050 mg L^{-1}

Penoxsulam
pKa = 5.1
Log Kow = 1.14 at 25°C and pH5
Log Kow = -0.60 at 25°C and pH7
Solubility (water) = 408 mg L^{-1}
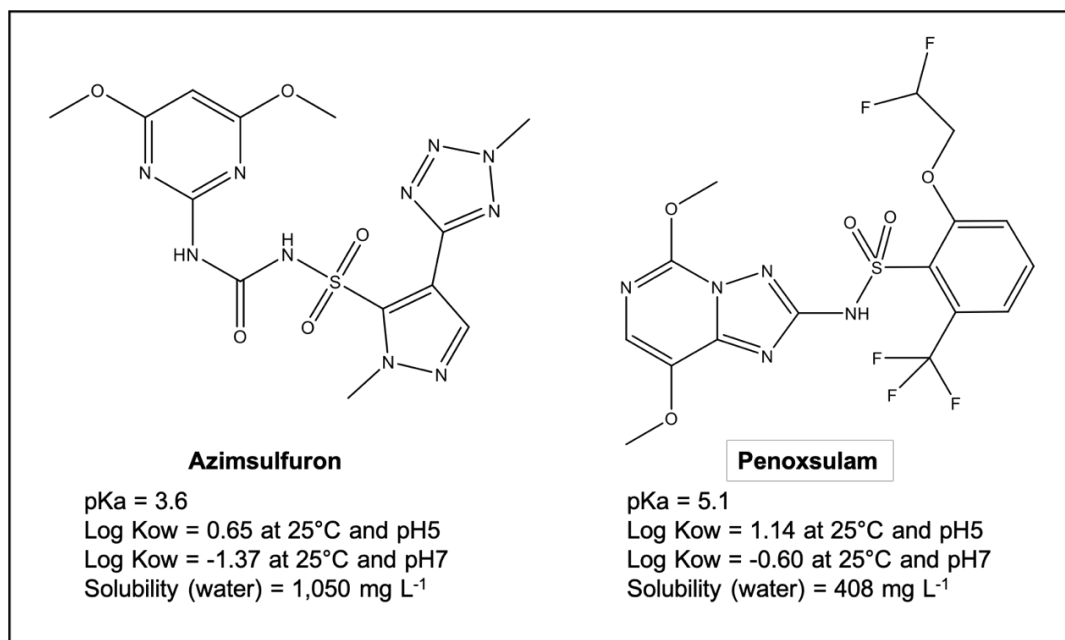
26   **Fig. S1.** Molecular structures and chemical properties of azimsulfuron and penoxsulam.

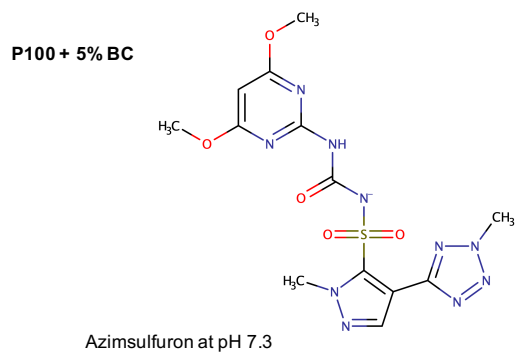27   Herbicides are presented at neutral pH.

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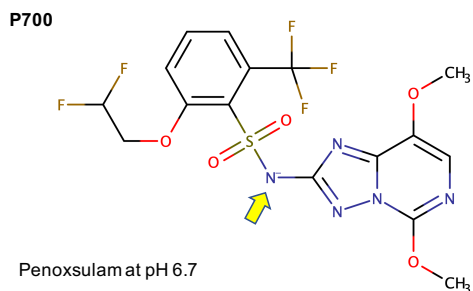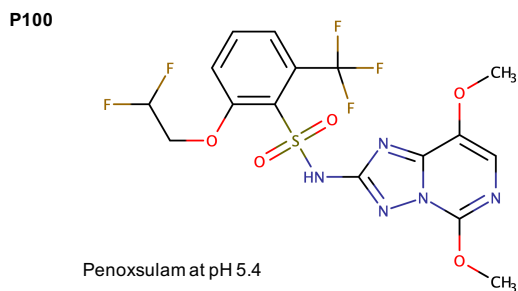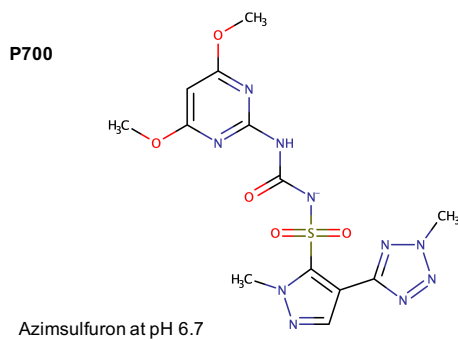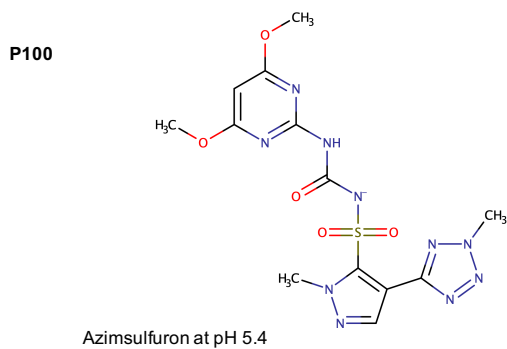
Fig. S2. Predicted major ionic species for azimsulfuron and penoxsulam at pH 5.4 and 6.7 based on protonation calculations performed with Marvin Sketch software v19.21 (ChemAxon). The yellow arrow points out the only difference between penoxsulam at pH 5.4 and 6.7 as a result of the pH differences.

P100 + 5% BC

Azimsulfuron at pH 7.3

P700 + 5% BC

Azimsulfuron at pH 7.4

P100 + 5% BCW

Azimsulfuron at pH 6.9

P700 + 5% BCW

Azimsulfuron at pH 7.2

Fig. S3. Predicted major ionic species for azimsulfuron at the pH of the amended soils based on protonation calculations performed with Marvin Sketch software v19.21 (ChemAxon). No differences were found among the different pH values.
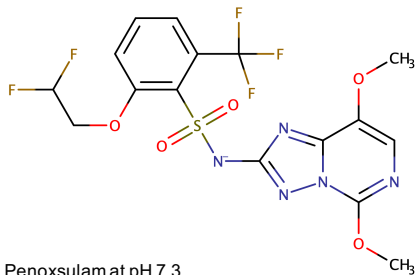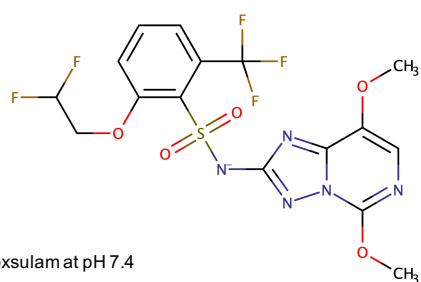
P100 + 5% BC

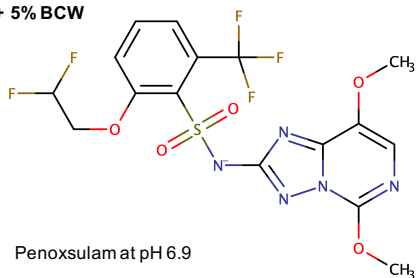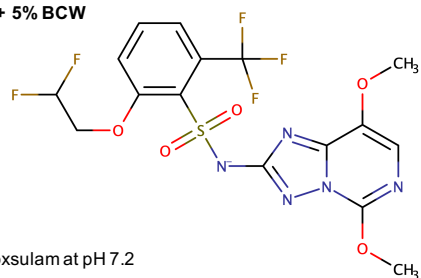Penoxsulam at pH 7.3

P700 + 5% BC

Penoxsulam at pH 7.4

P100 + 5% BCW

Penoxsulam at pH 6.9

P700 + 5% BCW

Penoxsulam at pH 7.2

Fig. S4. Predicted major ionic species for penoxsulam at the pH of the amended soils based on protonation calculations performed with Marvin Sketch software v19.21 (ChemAxon). No differences were found among the different pH values.

64 **Table S1.** Azimsulfuron adsorption-desorption coefficients: K_f ($mg^{1-nf} L^{nf} kg^{-1}$) and η_f, and R^2 values;

65 apparent sorption coefficient, aK_d ($L\ kg^{-1}$) at the concentration of 0.5 ppm; and the hysteresis

66 coefficient (H= η_f des / η_f ads). Values not measured (n.m.). Mean and standard deviation of two

67 replicates.

Samples	K_f ads	R^2	η_f ads	aK_d	K_f des	R^2	η_f des	H
BC	60.20 ± 12.68	0.95	0.93 ± 0.12	63.15	n.m.	n.m.	n.m.	n.m.
BCW	24.17 ± 5.47	0.95	0.80 ± 0.11	27.75	n.m.	n.m.	n.m.	n.m.
P100	1.07 ± 0.1	0.99	0.65 ± 0.04	1.37	0.92 ± 0.01	0.99	0.29 ± 0.00	0.45
P100 + BC (5%)	1.37 ± 0.07	0.99	0.80 ± 0.02	1.57	0.95 ± 0.02	0.99	0.17 ± 0.01	0.22
P100 + BCW (5%)	1.11 ± 0.07	0.99	0.85 ± 0.03	1.23	0.83 ± 0.03	0.98	0.27 ± 0.03	0.32
P700	0.95 ± 0.1	0.99	0.59 ± 0.04	1.25	0.87 ± 0.01	0.99	0.37 ± 0.01	0.62
P700 + BC (5%)	1.51 ± 0.13	0.99	0.77 ± 0.03	1.77	1.05 ± 0.02	0.99	0.22 ± 0.01	0.28
P700 + BCW (5%)	1.51 ± 0.09	0.99	0.86 ± 0.02	1.66	1.08 ± 0.07	0.96	0.31 ± 0.05	0.36

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71 **Table S2.** Penoxsulam adsorption-desorption coefficients: K_f ($mg^{1-nf} L^{nf} kg^{-1}$) and η_f, and R^2 values;

72 apparent sorption coefficient, aK_d ($L\ kg^{-1}$) at the concentration of 0.5 ppm; and the hysteresis

73 coefficient (H= η_f des / η_f ads). Values not measured (n.m.). Mean and standard deviation of two

74 replicates.

Samples	K_f ads	R^2	η_f ads	aK_d	K_f des	R^2	η_f des	H
BC	9.56 ± 2.26	0.92	0.92 ± 0.16	10.09	n.m.	n.m.	n.m.	n.m.
BCW	8.01 ± 1.43	0.95	0.87 ± 0.11	8.79	n.m.	n.m.	n.m.	n.m.
P100	1.49 ± 0.4	0.97	1.13 ± 0.14	1.36	0.85 ± 0.04	0.95	0.20 ± 0.03	0.18
P100 + BC (5%)	1.14 ± 0.22	0.98	1.09 ± 0.10	1.08	0.66 ± 0.03	0.31	0.03 ± 0.02	0.03
P100 + BCW (5%)	0.88 ± 0.19	0.98	1.14 ± 0.12	0.8	0.57 ± 0.02	0.74	0.07 ± 0.03	0.06
P700	0.57 ± 0.15	0.96	1.07 ± 0.15	0.55	0.41 ± 0.04	0.84	0.24 ± 0.08	0.22
P700 + BC (5%)	1.02 ± 0.27	0.96	1.01 ± 0.14	1.01	0.61 ± 0.02	0.03	0.01 ± 0.02	0.01
P700 + BCW (5%)	0.80 ± 0.16	0.98	1.03 ± 0.10	0.78	0.55 ± 0.03	0.70	0.11 ± 0.05	0.10

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