



Dissipation of bentazone, pyrimethanil and boscalid in biochar and digestate based soil mixtures for biopurification systems



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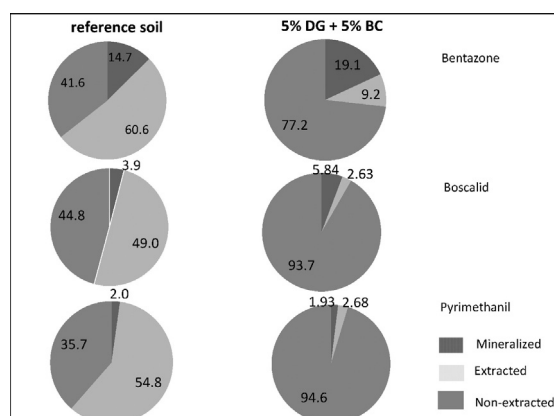
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HIGHLIGHTS

- Biochar and digestate significantly affects the dissipation pattern of pesticides.
- Addition of digestate enhanced mineralization of studied pesticides.
- DT₅₀ values decreased in the order pyrimethanil > boscalid > bentazone.
- Addition of biochar increased non-extractable residues formation for all pesticides.
- 5% biochar and 5% digestate mixture caused the rapid pesticide dissipation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 17 September 2015

Received in revised form 9 November 2015

Accepted 23 November 2015

Available online xxxx

Editor: D. Barcelo

Keywords:

Biobed
Biomixture
Biochar
Digestate
Mineralization
Dissipation

ABSTRACT

Biopurification systems, such as biofilters, are biotechnological tools to prevent point sources of pesticide pollution stemming from on-farm operations. For the purification processes pesticide sorption and mineralization and/or dissipation are essential and both largely depend on the type of filling materials and the pesticide in use. In this paper the mineralization and dissipation of three contrasting ¹⁴C-labeled pesticides (bentazone, boscalid, and pyrimethanil) were investigated in laboratory incubation experiments using sandy soil, biochar produced from Pine woodchips, and/or digestate obtained from anaerobic digestion process using maize silage, chicken manure, beef and pig urine as feedstock.

The results indicate that the addition of digestate increased pesticide mineralization, whereby the mineralization was not proportional to the digestate loads in the mixture, indicating a saturation effect in the turnover rate of pesticides. This effect was in correlation with the amount of water extractable DOC, obtained from the digestate based mixtures. Mixing biochar into the soil generally reduced total mineralization and led to larger sorption/sequestration of the pesticides, resulting in faster decrease of the extractable fraction. Also the addition of biochar to the soil/digestate mixtures reduced mineralization compared to the digestate alone mixture but mineralization rates were still higher as for the biochar/soil alone. In consequence, the addition of biochar to the soil generally decreased pesticide dissipation times and larger amounts of biochar led to high amounts of non-extractable

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residues of pesticide in the substrates. Among the mixtures tested, a mixture of digestate (5%) and biochar (5%) gave optimal results with respect to mineralization and simultaneous sorption for all three pesticides.

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1. Introduction

Inappropriate use of pesticides can cause high concentrations in soils, ground and surface-waters with significant environmental consequences (Kolpin et al., 1995; Kolpin et al., 1998; Acevedo et al., 2011). In general, pesticide pollution of water stems either from diffuse source pollution caused e.g. by pesticide leaching to groundwater or by surface runoff from fields to water bodies (Carter, 2000). Pollution may also originate from point sources caused by the release of pesticide contaminated waters from e.g. washing of the spray equipment, pesticide handling (filling of spray equipment), or e.g. by illegal dumping of post-harvest pesticide treatment waters (Coppola et al., 2011b; Karanasios et al., 2010a). At the catchment scale, studies have elucidated that 40 to 90% of surface water contamination by pesticides can be due to point source pollution (Carter, 2000; Kreuger and Nilsson, 2001).

The fate of pesticides in the environment is closely connected to dissipation, of which mineralization is one key process, and soil sorption, which in combination mainly governs the leaching potential of the substances in soils (Boesten and Van der Linden, 1991). To assess the environmental fate of pesticides, standard laboratory experiments are performed to measure the mineralization (total breakdown of substance to CO₂) and dissipation (sum of mineralization, metabolism, and non-extractable residue formation, which is measured via extractable active ingredient) behavior and to determine appropriate end-points for pesticide registration. These end-points are the half-life values which express the time required for 50% of the initial mass to mineralize (MinT₅₀) or to dissipate (DT₅₀). Hereby the DT₅₀, or dissipation, does not differentiate between transfer processes (e.g., leaching or erosion), sequestration (e.g., non-extractable by organic solvents due to strong sorption), or degradation (biotic or abiotic transformation of the substance) processes (FOCUS, 2006).

Dissipation and mineralization of pesticides are not only influenced by the chemical properties of the substances but they also depend on physico-chemical properties of the soil (such as pH value, soil organic carbon content (SOC), or soil texture), biological properties (activity and distribution of microorganisms), as well as environmental conditions controlling the chemical and biological processes (mainly soil temperature and soil water content). As a consequence, the dissipation (DT₅₀) and mineralization (MinT₅₀) half-life times have to be determined for each pesticide and soil combination individually.

Biopurification systems, like the biobed concept developed in Northern Europe (Castillo et al., 2008), biofilter system in Belgium (De Wilde et al., 2007), biobac or phytobac system in France (Guyot and Chenivresse, 2006), or biomassbed in Italy (Coppola et al., 2007) aim to reduce point pollution from farmyards by collecting all pesticide contaminated waters (e.g., from cleaning spray equipment) and to purify this waste water in a simple treatment system. The basic idea of these biofilter systems is that the pesticides will be degraded or sorbed/sequestered during the passage (drainage) of the water through suitable media (Castillo et al., 2000 and Castillo et al., 2008; Coppola et al., 2011a), whereby systems with a balance between sorption/sequestration, and mineralization/degradation are the most promising purification approach. Typically, different media are in use for such purpose depending on the location of the biopurification system and the availability of substrates such as mixtures of soil, straw, peat, but also residues from agricultural product processing or wastes (e.g., citrus peels, vine branches, coconut byproducts) have been reported (Coppola et al., 2007; De Roffignac et al., 2008; Karanasios et al., 2010a). The addition of fresh organic matter to the biofilter matrix in these setups is

an essential component for pesticide purification because it enhances the microbial activity, and therefore, also the microbial turnover of the pesticides (Perucci et al., 2000; Walker, 1975; Nair and Schnoor, 1994). Not all substrates are locally available or can be sustainably sourced (e.g., peat). On the other hand, byproducts or wastes from bioenergy production (e.g., digestate from biogas production or biochar) become more and more available and might be suitable to substitute more traditional substrates in the biopurification systems.

The addition of biochar to soils and its influence on pesticide mineralization is currently controversially discussed. Biochar is characterized as a highly recalcitrant pyrolysis product (i.e. charcoal), showing high organic C content and a high specific surface area (Lehmann et al., 2011). Some authors reported an increase of pesticide mineralization as a result of the microbial stimulation in the system, whereas other studies report reduced mineralization, due to a lower pesticide bioavailability to microorganisms because of the increase in sorption/sequestration of pesticides at biochar surfaces. A higher sorption or sequestration on soils amended with biochar (made from wood pellets) has been reported for a range of pesticides (e.g. Cabrera et al., 2014; Si et al., 2011). However, for anionic pesticides or pesticide metabolites, beech wood biochar (fresh and composted) amendments did not show enhanced sorption in soils (Dechene et al., 2014). Regarding biochar influence on pesticide degradation, Loganathan et al. (2009) reported a decrease in atrazine mineralization in soils amended with 1% (w/w) wheat char and they hypothesized that this reduction is associated with the increase in sorption of the herbicide to the char surface. On the other hand, Guo et al. (1991) suggested that atrazine and alachlor degradation could be inhibited in presence of activated carbon, and stimulated by other uncharred amendments, such as municipal sewage sludge and manure. An increase in atrazine mineralization by the addition of organic amendments to a sandy loam soil was also reported by Mukherjee (2009).

In general, there is an increasing trend towards biogas production in most industrial countries because biogas is an important form of renewable energy (Makádi et al., 2008). Digestate is the solid and residual byproduct of the biogas industry following the anaerobic digestion process (Möller et al., 2008; Mukherjee et al., 2015). On the other hand, it is a good source of easily available carbon and lignin rich material which generally enhances microbial activity by increasing the microbial growth and respiration as shown by e.g. Makádi et al. (2008); Odlare et al. (2008), and Kirchmann (1991). To our knowledge, no investigation has been done yet to determine how digestate addition to soil influences the dissipation and mineralization behavior of pesticides.

As mentioned earlier, biobed systems do not only rely on the full mineralization of the pesticides but combine pesticide mineralization, degradation, and sorption/sequestration leading to overall pesticide dissipation, and as a consequence of this, to water purification. Therefore, it is mandatory not only to look at the mineralization (which can be also fairly low for some specific recalcitrant pesticides) but to analyze the overall dissipation potential of the pesticides in the biomatrix, considering also sequestration of pesticide in the soil matrix, which also leads to reduced availability of pesticides for leaching. Additionally, Nowak et al. (2011 and 2013) reported the importance of biogenic non-extractable residues. They stated that microbes utilized carbon from pollutants to build up their own biomass. This microbial biomass containing ¹⁴C from pesticide labeling and full degradation of the pesticides will contribute to the non-extractable fraction, even if it was already turned over completely. However, determining this specific pathways and fraction of microbially immobilized pesticide originated ¹⁴C is out of scope of this paper. As different pesticides react diversely in the soil systems

Table 1
Main physico-chemical properties of the native soil, biochar, and digestate used for incubation (BC = low temperature biochar).

Material	Soil	BC	Digestate
Source/place and texture	Kaldenkirchen (loamy sand)	Woodchips (Pine)	Maize-silage, chicken manure and beef waste
pH (in 1:2 soil/CaCl ₂ solution)	6.12	7.8	8.7
Lignin content (in % w/w dry matter)	NA ^a	NA	17.7 ± 1.75
Organic carbon (%)	0.825 ± 0.006	75.90	40
Total N content (%)	0.082 ± 0.006	0.536 ± 0.046	6.51 ± 0.02
Surface area N ₂ (m ² /g ⁻¹)	2.05	231	3.09
Surface area CO ₂ (m ² /g ⁻¹)	– ^b	634	37.90
DOC (mg L ⁻¹)	3.42 ± 1.10	3.97 ± 0.40	1301.87
SUVA ₂₅₄ (L mg ⁻¹ m ⁻¹)	6.52	1.26	5.92

^a NA: not applicable.

^b –: not determined.

a test of biopurification materials should encompass a range of pesticides with contrasting properties.

The aim of this study was to analyze the pesticide mineralization and dissipation potential of seven different soil-amendment mixtures (biochar and digestate) and the reference soil in a laboratory incubation experiments using ¹⁴C labeled pesticides. In particular, the effects of different biochar and digestate dosages on pesticide fate were evaluated in combination with pesticides of varying chemical properties (bentazone, boscalid, and pyrimethanil). Based on the experimental findings, guidance for appropriate soil/substrate (biochar and/or digestate) mixtures can be provided, helping to design efficient biopurification (biobed) systems for a wide range of pesticides.

2. Material and Methods

2.1. Substrates

For the experiment, loamy sand topsoil (0 to 10 cm depth) from Kaldenkirchen, Germany (51°19'13N and 6°11'47E) (Gleyic Cambisol) was used as basis for the soil biomixtures. The soil was mixed with two different organic amendments, namely low temperature biochar (BC) and digestate (DG), each in different mixing ratios. The BC originates from slow pyrolysis processes (400 °C) using Pine woodchips as feedstock and the DG added was obtained from biogas production using maize silage, chicken manure, as well as beef and pig urine as feedstock (in a ratio of 15:1:5:4). Both amendments were used as received from the production and were not pretreated before the study. A detailed description of both amendments and soil can be found in Mukherjee et al. (2015). The main physico-chemical properties of the raw substances and soil mixtures used for the experiments are listed in Table 1 and Table 2, respectively. It has to be noted that for the experiments already 6 month aged biomixtures were used to ensure that the active microbial population has been already adapted to the biomixture and for being more representative for the long-term use of the biopurification matrix. Therefore, all biomixtures were stored at room temperature for 6 months prior our experiment.

Table 2
Main physico-chemical properties of the soil-mixtures for the Kaldenkirchen (KK) soil (loamy sand), BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios (w/w dry mass) in the mixtures.

Soil substrate composition	pH	ECEC [cmol _c kg ⁻¹ soil]	Organic carbon [%]	Extractable DOC [mg L ⁻¹]	SUVA ₂₅₄ [L mg ⁻¹ m ⁻¹]
Reference soil (KK)	6.12	4.36	0.825	3.42 ± 1.10	6.52
1.0% BC	6.09	6.46	1.57	1.73 ± 0.13	4.43
5.0% BC	6.06	8.26	4.78	3.53 ± 0.46	13.09
5.0% DG	6.16	8.24	2.78	9.69 ± 0.24	17.31
30% DG	6.26	16.96	12.56	41.6 ± 3.10	25.02
5% DG:1% BC	6.13	9.39	5.12	4.06 ± 1.10	6.81
5% DG:5% BC	6.06	9.99	6.74	2.64 ± 0.24	11.05
30% DG:5% BC	6.54	17.84	16.56	4.86 ± 0.29	9.40

2.2. Pesticides

Three different pesticides were used in the experiments, two of them are fungicides (pyrimethanil and boscalid) and one is a herbicide (bentazone). All pesticides were radioactively labeled (¹⁴C labeling, Specific radioactivities for bentazone, boscalid and pyrimethanil were 5.31, 5.34, and 6.42 MBq mg⁻¹, respectively) and provided by BASF SE with >97% chemical and >99% radiochemical purity. Non-radioactive pesticides (>99% purity) for blending the radioactive substance were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The pesticides were selected to span a wide range in their sorption and degradation properties. Their physico-chemical characteristics are given in Table 3.

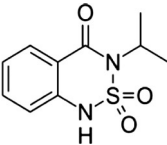
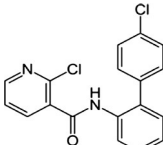
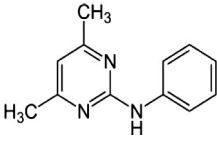
2.3. Characterization of used soil-mixtures

Extractable dissolved organic carbon (DOC) from mixtures was characterized according to Cox et al. (2004). To this aim, 10 g dry mass equivalents of soil (– mixture) and 20 ml 10 mM CaCl₂ were mixed in a jar and placed on a horizontal shaker at 225 rpm (SM25, Edmund Bühler) for 10 min at 20 ± 2 °C. Subsequently, the soil-water slurry was centrifuged (Allegra 6 KR, Beckman Coulter Inc. CA, USA, GH-3.8 Swinging-bucket Rotor) for 15 min at 2910 × g and the supernatant was filtered sterile through a 0.45-µm cellulose acetate membrane filter. DOC was measured with a TOC analyzer 5050A equipped with an autosampler ASI-5000A from Shimadzu (Kyoto, Japan) after acidification and sparging the samples for 1 min.

UV absorbance at 254 nm (UVA₂₅₄) in water-based soil extracts was measured with a Uvikon 860 UV/Vis spectrophotometer (Tegimenta AG, Rotkreuz, Switzerland). DOC-specific UV-absorbances at 254 nm (SUVA₂₅₄) (Leenheer and Croue, 2003; Cox et al., 2004) of the extracts were obtained by dividing the UVA₂₅₄ values by the respective DOC concentrations. The pH of the soil/soil-mixtures was determined by equilibrating soil/soil-mixture with 10 mM CaCl₂ (soil/solution ratio 1:2 (w/v)) with a portable pH-meter (Orion 3-star, Thermo Electron Co., USA) using a glass electrode.

Effective cation exchange capacity (ECEC) of soil (– mixtures) was determined according to Lürer and Böhmer (2000): In a first step 2.5 g

Table 3
Physico-chemical and degradation properties of used pesticides (PPDB, 2015).

	Bentazone	Boscalid	Pyrimethanil
Structure			
Type	Herbicide	Fungicide	Fungicide
Molecular formula	C ₁₀ H ₁₂ N ₂ O ₃ S	C ₁₈ H ₁₂ Cl ₂ N ₂ O	C ₁₂ H ₁₃ N ₃
Molecular weight (g mol ⁻¹)	240.3	343.21	199.11
Melting point (°C)	140	143.3	96.3
Vapor pressure (25 °C, mPa)	0.17	72 × 10 ⁻⁵	1.1
Water solubility at 20 °C (mg L ⁻¹)	570	4.6	121
Log K _{ow} (at pH 7 and 20 °C)	-0.46	2.96	2.84
pKa (25 °C)	3.28	Not applicable	3.52
Soil laboratory DT ₅₀ (days)	13 (8–102)	200 (108–284)	55 (28–72)
Soil field DT ₅₀ (days)	14 (4–21)	118 (28–208)	30 (23–54)
Soil sorption coefficient (K _{oc} L Kg ⁻¹)	13–176	507–1110	75–500

soil was equilibrated with 10 mL 1 M NH₄Cl for 24 h. Subsequently, a folded paper filter (640d, Macherey-Nagel, Düren, Germany) was wetted with 1 M NH₄Cl and placed in a filter funnel. The wet soil was completely transferred to the filter and percolated with 1 M NH₄Cl until a volume of 100 mL percolate was collected. Exchangeable cations (Al³⁺, Ca²⁺, K, Mg²⁺, Na) were determined in the filtrate using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Ciros CCD, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

2.4. Mineralization/Dissipation experiments

All mineralization/dissipation experiments were performed in accordance to the OECD guideline 307 (OECD, 2002) for the duration of 120 d. Overall eight different soil/– mixtures were investigated for each pesticide in triplicate, resulting in 24 incubation flasks for each pesticide. With respect to the three pesticides analyzed, a total of 72 Schott Duran flasks were used and filled with 150 g (dry mass) soil/biomixture each. An overview of all soil/– mixtures is listed in Table 2. All incubation flask were covered by aluminum foil to minimize light exposure and the incubation flasks were stored in dark over the entire incubation time. The soil water content was adjusted to 50% WHC_{max} using demineralized water (OECD, 2002). Applied pesticide mass added to each incubation system was based on recommended field application rates (960 g ha⁻¹ for bentazone, 100 g ha⁻¹ for boscalid, and 800 g ha⁻¹ for pyrimethanil), assuming full distribution in the soil with a mixing depth of 5 cm (assumed soil bulk density of 1.5 g cm⁻³). To simulate much higher concentrations in biopurification matrices, as expected for biobed systems, these loads were multiplied by 10. The resulting pesticide concentrations in the experiments were therefore 12.80 mg kg⁻¹ soil/biomixture for bentazone, 1.33 mg kg⁻¹ for boscalid, and 10.67 mg kg⁻¹ for pyrimethanil.

¹⁴C labeled pesticides were applied in organic solvent to inert quartz sand, which served after evaporation of solvent as a carrier to achieve a homogeneous mixing with the soil and biomixtures. This procedure avoids the addition of any potentially toxic solvents/solution directly to the soils. Therefore, approx. 5 g of the quartz sand was mixed with

the calculated loads of pesticides solved in corresponding solvents (bentazone & boscalid in acetonitrile and pyrimethanil in toluene) in a smooth porcelain container. Afterwards, the solvent was allowed to evaporate under a fume hood for 5 h and the quartz sand was well homogenized. Finally, the pesticide-loaded quartz sand was well homogenized with the biomixtures using a spatula. The flasks were equipped with a carbon-dioxide trap, consisting of 1.5 ml 2 M NaOH (maximum entrapment capacity of one filling: 18.03 mg CO₂-C) solution and then closed air-tight. The water content of incubation flasks was controlled once a week via weighing of the flasks and water losses > 5 g were compensated by adding the respective amounts of deionized water.

To determine any pesticide losses over the course of preparation of the incubation system, soil subsamples were taken immediately from each incubation flask and combusted via an biological oxidizer (OX 500, R.J. Harvey Instrument Corp., Tappan, NY, USA). Evolving ¹⁴CO₂ was trapped in Oxysolve C-400 oxidizer scintillation cocktail (Zinsser Analytic, Germany), and analyzed using liquid scintillation counting (LSC) (LSC; 2500 TR, Tri-Carb, Packard). Based on the results (recoveries of pesticides in the sand after spiking ranged from 99.5 to 99.7% based on the radioactivity measurement), the initial pesticide concentrations per flask were calculated. Analytical quality control tests have shown that the recovery of pesticides (based on active ingredient) after mixing the spiked sand to the soils ranged from 87.7 to 108.6% for soil and 82.0 to 88.7% for mixtures. The low recovery from BC-amended soil is explained by instantaneous sequestration on biochar. The increased concentration of biochars categorically enhanced (irreversible) adsorption/sequestration due to increased micropore quantity in amended soils.

Pesticide mineralization from the incubation flasks was measured by trapping evolved ¹⁴CO₂ in 2 M NaOH solution, whereby the NaOH traps were replaced after 0, 3, 8, 14, 23, and 30 days after application, and thereafter twice a month until day 135. Quantification of trapped ¹⁴CO₂ was done via LSC. Based on a preliminary study (Mukherjee et al., 2015) and calculations, it was ensured that all evolved CO₂ could be trapped in the NaOH and that the traps were exchanged much earlier as maximum saturation capacity would be reached for all biomixtures. In the worst case (30% digestate based mixture), less than 50% of the entrapment capacity was used.

Soil/biomixture samples were taken at day 0, 8, 30, 60, 90, and 120. To this aim, 5 times 1 g were randomly sampled to give an aliquot of approx. 5 g (dry mass) of each flask. Each subsample was shaken with 50 ml of methanol (MeOH, Merck Lichrosolv, ≥99.9% purity) and Milli-Q ultrapure water (50:50 (v/v)) on a horizontal shaker (225 rpm, 25 h) at room temperature in the dark (by covering the flasks with aluminum foil). Analytical quality assurance data have shown that recoveries of pesticide extraction using above solvent mixture varies

Table 4
Mathematical expressions for different kinetic models used in the incubation study and estimation of DegT₅₀ and DT₅₀.

Model	Mathematical equation	DegT ₅₀ /DT ₅₀ determination
Simple first order (SFO)	M _t = M ₀ e ^{-kt}	DegT ₅₀ /DT ₅₀ = ln 2/k
Bi-exponential (DFOP)	M _t = M ₁ e ^{-k₁t} + M ₂ e ^{-k₂t} Where, M ₂ = 100 - M ₁	Iterative method

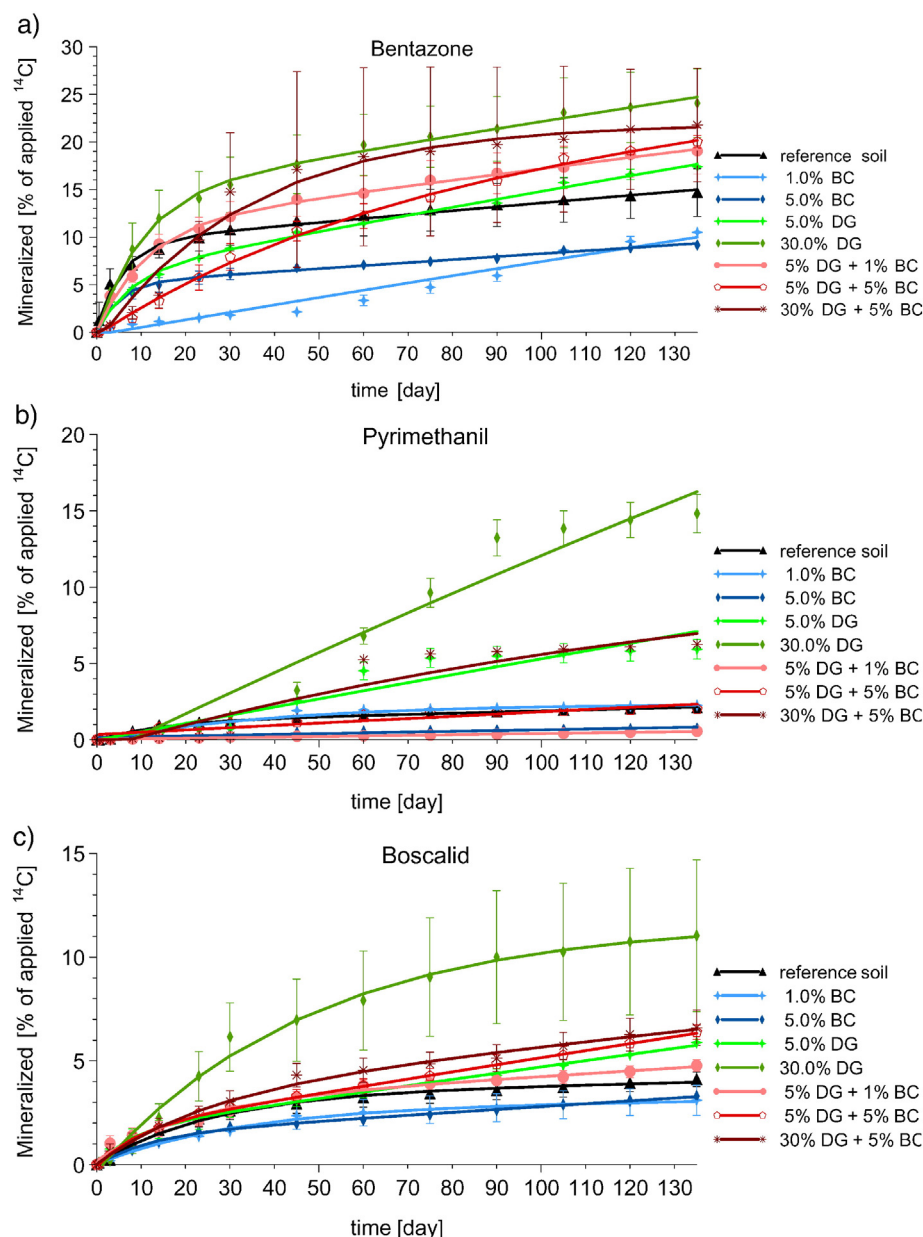


Fig. 1. a–c. Mineralization of ^{14}C -bentazone, pyrimethanil, and boscalid in % of initial concentrations for the different soil/amendment mixtures. Error bar represents standard deviation ($n = 3$). Reference soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Note that the y-axis do not have the same scale for better visualization. Points indicate measurements and line the best fitting model as listed in Table S1.

from 67.8 to 82.7% for reference soil and 4.0 to 88.7% for biomixtures. [Marinozzi et al. \(2013\)](#) and [Marín-Benito et al. \(2012 and 2014\)](#), also reported >65% recoveries by using methanol as an extraction solvent for different pesticides and biobed substrates. The low recovery from biomixtures in our study, can be explained by different physico-chemical properties (poor water solubility and hydrophobicity) of the pesticides and strong instantaneous sequestrations/sorption of pesticides on biochar as already described above. The final activities and pesticide concentrations were determined after centrifugation from the supernatants by LSC and HPLC. Total residual ^{14}C activity was determined by incineration-oxidation to $^{14}\text{CO}_2$ and quantified via LSC.

2.5. Analytical procedures

Pesticide concentrations in the liquid phase were measured using HPLC equipped with a UV and radioactivity detector. A reversed phase

C-18 column (HPLC column Agilent Technologies, Zorbax eclipse XDB-C18, $150 \times 4.6 \text{ mm} \times 5 \mu\text{m}$ particle size) was used and a 0.25 ml aliquot of each sample was injected into the combined UV/Radio-HPLC. Solvent A was Millipore water with 0.1% conc. H_3PO_4 (pH 3.0) for all studied pesticides. As a solvent B methanol (Merck Lichrosolv, $\geq 99.9\%$ purity) was used for bentazone and pyrimethanil and acetonitrile (Merck Lichrosolv, $\geq 99.9\%$ purity) for boscalid. The flow rate was 0.80 ml min^{-1} and the column temperature was kept constant at 25°C . A linear gradient was used: 0 to 5 min: 70% solvent A, then to 100% solvent B for 11 min. Hold 100% B for 16 min, switch back to 70% A and hold for 25 min. The UV detector was adjusted to 219, 243, and 270 nm for bentazone, boscalid, and pyrimethanil, respectively. Quantification of active ingredients via radio-HPLC was performed by calculating the measured radioactivity for each substance peak. The limits of quantification (LOQs) and limits of detection (LODs) of the method were 10 and 3 Bq ml^{-1} , respectively, for all of the studied pesticides

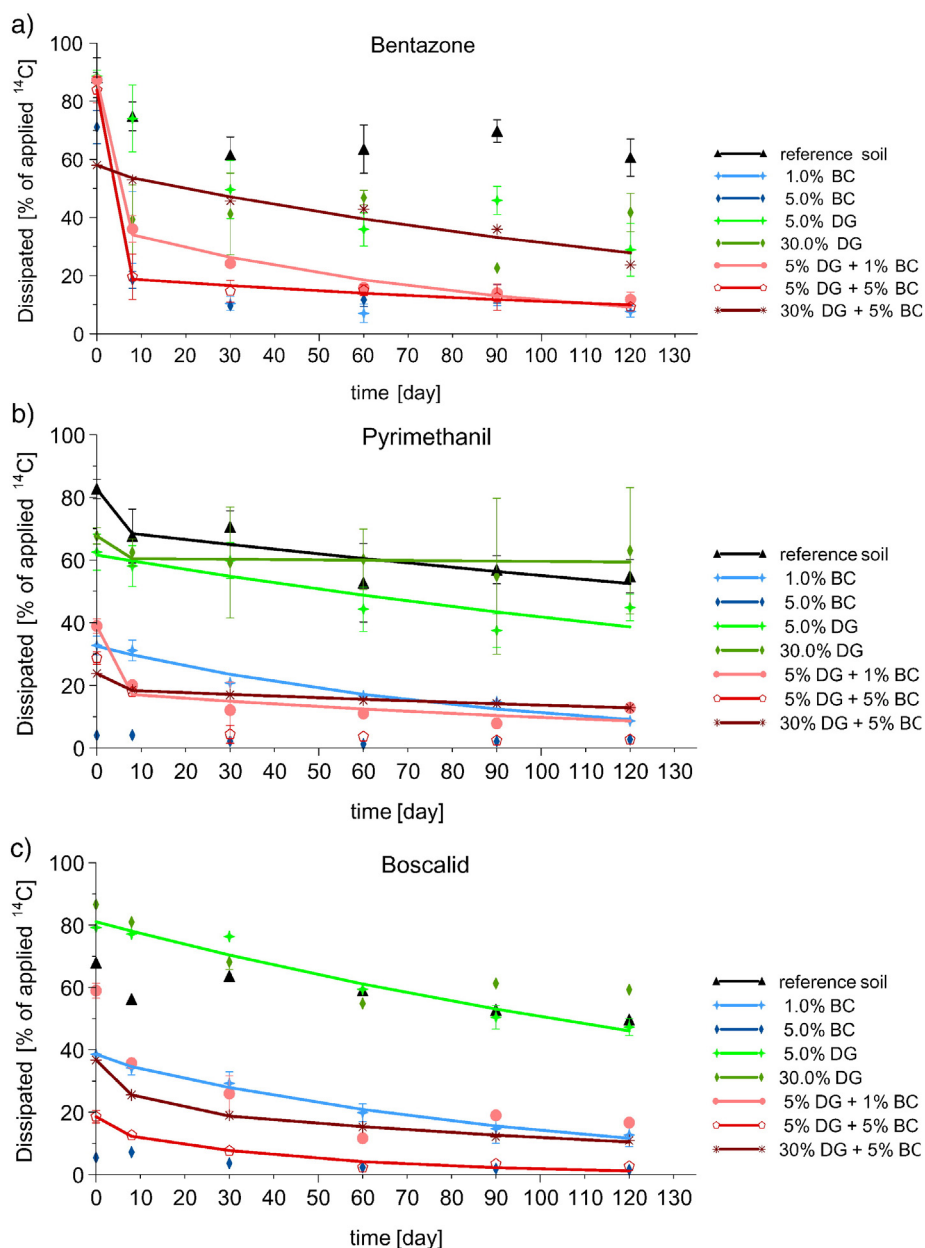


Fig. 2. a–c. Extractable pesticide residues of ^{14}C -bentazone, pyrimethanil, and boscalid in % for the different soil/amendment mixtures. Error bar represents standard deviation ($n = 3$). Reference soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Points indicate measurements and line the best fitting model as listed in Table 5.

based on an injection volume of 0.25 ml. Therefore, LOQs for the labeled pesticide concentrations were 2.00 ng ml^{-1} for bentazone, 2.24 ng ml^{-1} for boscalid, and 1.66 ng ml^{-1} for pyrimethanil, respectively. No metabolites were detected and quantified in these concentration ranges (which corresponds to 0.002 to 0.021% of applied radioactivity) which are in line with the observations of Coppola et al. (2011a) and Marín-Benito et al. (2012).

2.6. Pesticide mineralization/dissipation kinetics

Different kinetic models were fitted to the data of the incubation experiment in order to derive mineralization and dissipation parameters (MinT_{50} and DT_{50}). For each data set, the single first order (SFO) model and the bi-exponential or double first-order in parallel (DFOP) model as proposed by the FOCUS Kinetics guidance document (FOCUS,

2006) were tested in order to derive best-fit endpoints. The respective model descriptions and corresponding equations for calculating endpoints (MinT_{50} and DT_{50}) are shown in Table 4. MinT_{50} was determined directly from fitting of the $^{14}\text{CO}_2$ evolution curves (Fig. 1a–c).

2.6.1. Goodness-of-fit statistics

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by FOCUS (2006). The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting (Schäfer et al., 2007; Schmitt et al., 2011). The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively. For visual inspection both the observed and modeled decline curves over time as well as the distribution of the residuals over time were used. As a statistical measure of the goodness-of-fit a χ^2 test was performed. Moreover, the sum of squared

Table 5
Kinetic parameters for the dissipation (derived from extractable pesticide residues) of the different pesticides (bentazone, pyrimethanil, boscalid) for the KK = loamy sand soil, 1% BC, 5% BC, 5% DG, 30% DG, KK + 5% DG + 1% BC, KK + 5% DG + 5% BC, KK + 30% DG + 5% BC (BC = low temperature biochar, and DG = digestate) obtained from fitting kinetics to a single first order (SFO) and bi-exponential (DFOP) model (bold data indicate fairly good fit and italics indicate no good fit to the described models).

Kinetic model																		
SFO									DFOP									
Pesticide	Substrate	M ₀ (% of initial)	k (day ⁻¹)	DT ₅₀ (days)	R ²	χ ²	SSR	χ ² passed	M ₀ (% of initial)	g	k ₁ (day ⁻¹)	k ₂ (day ⁻¹)	DT ₅₀ (days)	R ²	χ ²	SSR	χ ² passed	
Bentazone	KK	77.90	0.003	305.70	0.75	8.00	293.20	x	88.09	0.80	0.00102	1.90360	457.3	0.70	6.20	110.50		
	1% BC	86.98	0.099	7.00	0.98	21.50	324.10		87.96	0.45	0.02509	1.92520	1.2	0.96	21.50	204.14		
	5% BC	70.88	0.161	4.30	0.97	32.20	489.95		71.09	0.22	0.00376	1.92370	0.5	0.98	10.70	34.26		
	5% DG	80.56	0.010	72.50	0.84	12.40	433.20		88.65	0.80	0.00768	1.92910	62.3	0.89	12.80	282.80		
	30% DG	62.71	0.007	101.80	0.38	26.40	1414.80		86.36	0.48	0.00133	1.93010	1.7	0.86	15.80	320.00		
	5% DG + 1% BC	76.38	0.043	16.10	0.99	29.90	841.40		87.26	0.42	0.01172	1.93380	1.0	0.99	6.30	23.83		
	5% DG + 5% BC	83.67	0.174	4.00	0.97	32.50	667.40		83.88	0.23	0.00572	1.93330	0.5	0.99	4.00	6.45	x	
	30% DG + 5% BC	57.02	0.006	114.30	0.95	4.80	40.20	x	57.98	0.96	0.00586	1.94190	112.90	0.95	5.90	38.42	x	
	Pyrimethanil	KK	75.49	0.0034	206.20	0.71	7.00	191.70	x	82.67	0.84	0.00237	1.90429	220.4	0.85	6.30	98.20	
		1% BC	32.42	0.0107	65.00	0.96	6.10	15.20	x	32.47	0.99	0.01061	1.90120	64.90	0.97	7.70	15.10	
5% BC		3.68	0.0069	99.90	0.42	23.40	3.82		4.03	0.81	0.00509	1.73870	95.00	0.46	28.40	3.54		
5% DG		61.58	0.0039	178.00	0.77	7.00	122.00	x	62.49	0.97	0.00370	1.73660	180	0.77	8.80	120.50		
30% DG		63.47	0.0007	965.50	0.22	4.50	71.80	x	67.67	0.89	0.00016	1.73660	3632.6	0.87	4.30	41.60	x	
5% DG + 1% BC		30.66	0.0171	40.50	0.67	28.90	231.50		38.89	0.46	0.00610	1.47550	1.7	0.93	15.50	42.20		
5% DG + 5% BC		28.39	0.0557	12.50	0.98	14.60	19.90		28.45	0.97	0.05370	1.33490	12.5	0.98	18.20	19.50		
30% DG + 5% BC		21.08	0.0047	147.50	0.82	7.10	13.50	x	23.76	0.79	0.00320	1.15000	143.6	0.99	0.90	0.13	x	
Boscalid		KK	63.86	0.0021	337.00	0.65	5.20	83.93	x	67.76	0.10	1.70390	0.00152	387.5	0.76	5.40	57.20	x
		1% BC	38.02	0.0100	69.30	0.99	3.00	5.41	x	38.58	0.03	1.70440	0.00975	67.90	0.99	3.60	4.80	x
	5% BC	6.34	0.0143	48.40	0.85	17.20	3.69		6.34	0.00	1.70438	0.01431	48.40	0.85	21.60	3.70	x	
	5% DG	81.07	0.0047	147.00	0.95	3.60	50.73	x	81.07	0.00	1.70441	0.00471	147.20	0.95	4.50	50.73		
	30% DG	81.21	0.0036	195.00	0.74	7.00	216.87	x	86.58	0.11	1.70090	0.00283	202.20	0.79	7.60	163.64		
	5% DG + 1% BC	49.28	0.0158	44.00	0.77	22.20	366.34		58.93	0.40	1.71740	0.00876	19.10	0.92	16.10	121.00		
	5% DG + 5% BC	17.39	0.0272	25.50	0.95	14.40	12.05		18.55	0.21	1.71330	0.02110	21.20	0.97	13.90	7.10		
	30% DG + 5% BC	31.85	0.0116	59.60	0.88	58.89	12.50		36.76	0.38	0.15594	0.00646	32.80	0.99	1.40	0.48	x	

residuals (SSR) was evaluated (FOCUS, 2006) and the endpoints MinT₅₀ for the mineralization and (DT₅₀) for dissipation were reported. For all fittings a χ² error threshold was set to 15%, which corresponds to a probability level of p = 0.05. That means that a calculated χ² error less than 15% indicates a good fit. For those pesticide/soil (-mixture) combination where a model did not show good results, based on the χ² error and SSR, no kinetic parameters and end-points are reported. For the χ² test Eq. (1) was used:

$$\text{error} = 100 \sqrt{\frac{1}{\chi^2_{\text{tabulated}}} \cdot \sum \frac{(C-O)^2}{\bar{O}^2}} \quad (1)$$

where, the error is model error at which the χ² test is passed, χ²_{tabulated} is tabulated value of χ² distribution (m = degree of freedom and α chosen probability), C is the calculated value and O is the observed value and \bar{O} is the average of all observed values.

For the reliability of individual parameters Eqs. (2 and 3), a single-sided t-test was used:

$$t = \frac{\text{parameter-value}}{\text{SD(parameter-value)}} \quad (2)$$

$$\text{type-I error rate} = t\text{-distribution}(t, \text{dof}, 1). \quad (3)$$

Hereby, t is the empirical t-value, SD is the standard deviation of parameter value and dof is the degrees of freedom. Significance level was considered at p < 0.05. The goodness-of-fit statistics, i.e. χ² error level

and type-I error rate, were calculated within the KinGUI runs and documented in the respective output files. The fit passed the χ² test if the calculated χ² is lower than the tabulated χ² for a given degree of freedom and significance level (here 5% significance level). The parameters of the kinetic models were optimized according to the recommendation of the FOCUS working group using the least-squares method.

3. Results and discussion

3.1. Pesticide mineralization and kinetics

Overall seven different biomixtures plus the native soil for comparison were analyzed with respect to their pesticide mineralization capabilities. Fig. 1a–c shows the ¹⁴C₂O evolution curves in percentage of total applied ¹⁴C bentazone, pyrimethanil, and boscalid as a function of incubation time. As can be seen, the different mixtures behave differently in the mineralization pattern but also the physico-chemical characteristics of the three compounds influence the complete mineralization of pesticides substantially. After 135 days, the lowest mineralization of bentazone was found in the biochar amended soils (1 and 5% biochar) with <11%, followed by the reference soil (~15%) and the digestate–soil mixtures (18 to 25%). Addition of biochar to the digestate–soil mixtures resulted in more complex effects, whereby the addition of 1 and 5% biochar to 5% digestate showed an increase of mineralization compared to the addition of the same amount of digestate only. On the other hand, addition 5% biochar to the higher load of digestate (30%) reduced the total mineralization slightly (Fig. 1a).

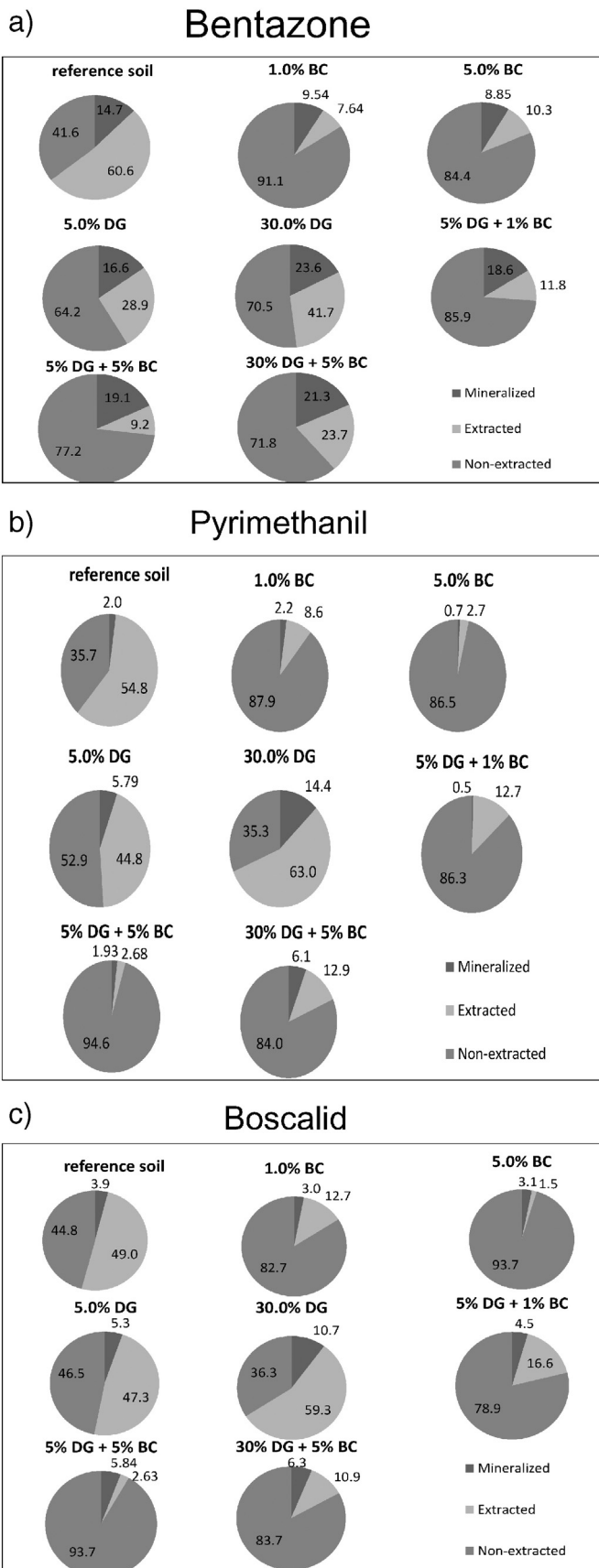


Fig. 3. a–c. Cumulated $^{14}\text{CO}_2$ and (extractable + non-extractable) pesticide residues (at day 120) of ^{14}C -bentazone, pyrimethanil, and boscalid in % of applied radioactivity for the different soil/amendment mixtures ($n = 3$). Reference soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures.

Pyrimethanil (see Fig. 1b) is less mineralized compared to bentazone as it can be expected from its known properties (Table 3). It was mineralized to less than 6.5% except for the 30% digestate mixture where about 15% of pyrimethanil was mineralized until 135 d after application. Similarly to bentazone, biochar-only mixtures showed the lowest mineralization while the digestate–biochar mixtures again showed an increased mineralization of these two pesticides.

The same trend was found for boscalid with a mineralization of <7.0% for all substrates except for the 30% digestate based mixture, where mineralization was ~11% (Fig. 1c) until 135 days after application. Mineralization is clearly increased in mixtures with digestate contents $\geq 5\%$, but the additional application of 5% biochar to soil–digestate mixtures reduces boscalid mineralization significantly.

The observed findings of reduced pesticide mineralization in biochar-containing soils has been already reported by e.g. Yang and Sheng (2003a); Yang et al., 2006); Cornelissen et al. (2005); Sobek et al. (2009), and Yu et al. (2006). In those studies, lower mineralization of pesticides was attributed to the stronger (in terms of quality) and larger (in terms of quantity) pesticide sorption onto biochar surfaces, and as a consequence, a reduction of bioavailable pesticides in the soil liquid phase (Fernandes et al., 2006; Cabrera et al., 2007).

Digestate alone increased the mineralization of the studied pesticides compared to the native soil and all other mixtures, which can be attributed to the high ligno-cellulosic compounds found in digestate (see Table 1). The positive effect of ligno-cellulosic compounds in different maturity stages has been already observed by Tortella et al. (2012) and Marinozzi et al. (2013), and the mechanisms for the higher mineralization may be ascribed to the higher activity of white-rot fungi, which co-metabolize pesticides by extracellular enzymes, targeting ligno-cellulosic structures (Coppola et al., 2011a; Castillo et al., 2000; Castillo et al., 2008).

It has to be pointed out that the increase in pesticide mineralization was not proportional to the amount of added digestate (5 or 30%). Mineralization was increased only ~1.4 fold (bentazone), ~2 fold (boscalid), and 2.5 fold (pyrimethanil) when digestate was added in six-fold amounts. A kind of saturation effect occurred, leading to non-proportional turnover of pesticides for higher digestate based C contents, which may relate to higher N content of the pure digestate (see Table 1). This is supported by the observations of Cayuela et al. (2009) and Tenuta and Lazarovits (2004), who illustrated that the higher percentage of amendment lead to NH_3 toxicity to different microbial species in soils. Additionally, the water extractable DOC quantity is not proportional to the digestate content (see Table 2) and it is widely accepted that DOC provides the most important carbon and energy source for heterotrophic bacteria. Moreover, DOC quality and quantity have been shown to affect microbial community composition and functionality which has direct or indirect effects on pesticide mineralization behavior (Metting, 1993; Findlay et al., 2003; Docherty et al., 2006).

In biomixtures of digestate and biochar a positive effect on the mineralization rates for all pesticides was observed (least for pyrimethanil) in comparison with soils amended only with biochar. This finding can be explained by the priming effect of the digestate addition and the observation that biochars can act as a good habitat for soil microbes (Lehmann et al., 2011) and that soil microbial communities changed in biochar-amended soils, thereby enhancing mineralization (Anderson et al., 2011).

The mineralization of pyrimethanil solely in the digestate based mixtures as well as in the 30% DG and 1% BC amended soil shows a lag phase of up to 40 days (Fig. 1b), with an initially slow mineralization, followed by a phase of more rapid mineralization. The existence of a lag phase has already been observed for some pesticides, and it can be attributed to the adaptation time needed for the microbial community to mineralize the pesticide (e.g., Rodríguez-Cruz et al., 2006). On the other hand, it is not clear yet why only the digestate-based mixtures exhibit such behavior and why it is only detectable for the pyrimethanil mineralization.

To describe the mineralization kinetics of the pesticides added to the different substrates two different kinetic models, namely the single first-order, and the double first-order in parallel (Table 4) were tested to identify which best describes the mineralization (based on cumulative $^{14}\text{CO}_2$ fluxes) kinetics.

The fitted MinT_{50} , the ratio between the slow and fast pool (g -parameter) for the DFOP model, as well as the χ^2 error and the SSR for the mineralization are provided in the Supplementary information (Table S1). As can be seen, the single first-order model (SFO) is not appropriate to describe the bentazone and pyrimethanil mineralization, whereas mineralization of boscalid could be described by this model. The double first-order in parallel (DFOP) model could describe all pesticide mineralization and despite the fact that boscalid is a stable compound and SFO model is sufficient to describe the kinetics, the mineralization could be even better described using the DFOP model compared to the SFO model based on statistical measures such as SSR and also visual inspection. It has to be noted that the MinT_{50} values are not of primary interest in this study and lie well beyond any valid extrapolation range from our observation period (see Supplementary information, Table S1). For our study, the main interest is on the different mineralization dynamics among the tested substrates for one pesticide, which is discussed.

As already described in the mineralization plots over time (Fig. 1a–c) the impact of the different soil amendments becomes clear. Biochar addition to the soil generally increases mineralization and larger amounts of biochar inhibited the mineralization of pesticides in the substrates. In contrast, the addition of digestate accelerates pesticide mineralization. Unfortunately, the DFOP fit for pyrimethanil in the 30% soil/digestate mixture was not able to describe the lag-phase appropriate, but nevertheless passed the statistical test. For example, the addition of 30% DG led to a mineralization of 14.4% of applied radioactivity until 135 days after application, for pyrimethanil, compared with 5.8% for the addition of 5% DG.

Finally, simultaneous addition of biochar and digestate lead to slower mineralization compared to the digestate based mixtures but faster as compared to the biochar based ones. The general mechanisms and processes for this accelerated or decelerated mineralization have been already discussed before.

3.2. Pesticide dissipation and kinetics

To assess pesticide dissipation in the soil/– mixtures, the active ingredient contents were quantified in methanol/water soil extracts (Fig. 2a–c). The extraction of soil/–mixtures with methanol/water can be assumed to exhaustively extract the potentially water-desorbable and thus also bioavailable pesticide residues (e.g. Laabs and Amelung, 2005; Cabrera et al., 2008). In general, pesticides dissipated over time in all substrates, whereby significant difference ($p < 0.05$; t -test) in dissipation was observed for all pesticides among the tested soil treatments. The slowest dissipation was always observed for the control soil and the digestate based mixtures. In comparison, fastest dissipation was measured for the biochar-based mixtures (biochar/soil and biochar/digestate/soil). For the reference soil and the solely digestate-based mixtures, only bentazone showed a priming effect on dissipation, while for boscalid and pyrimethanil no clear effect of digestate addition could be observed. For the biochar-amended soils, pesticide dissipation increased substantially with increasing biochar content for boscalid and pyrimethanil, while for bentazone biochar addition also increased dissipation, but no clear difference between the two biochar treatments was detectable.

An observed low extractability of pesticides (and thus faster dissipation) for the biochar-amended soils was also reported by Sopena et al. (2012) and Spokas et al. (2009). The faster pesticide dissipation in biochar-amended soils is thus mainly caused by the higher sequestration (and hence lower extractability), which is caused by the strong or irreversible sorption of the tested pesticide onto biochar with its high

surface area, hydrophobic surface properties, as well as their nanoporous structure. Because the biobed systems are designed to purify pesticide containing waters irrespectively of the processes involved (mineralization or sorption) a better comparison of the suitability of the soil/–mixtures can be drawn from the dissipation (here derived from extractable residues) kinetics. The fitted end-points DT_{50} , the ratio between the slow and fast pool (g -parameter) for the DFOP model, as well as the χ^2 error and the SSR for the dissipation are listed in Table 5. Unfortunately, the picture is less clear as for the mineralization, where full pesticide sets could be either described by one model or not. As can be seen in Table 5, only 5 combinations could be best described using the SFO model, whereas 12 combinations could be well described using the DFOP model, respectively. Additionally, some combinations could not be described using any model such as for bentazone mixed into 30% digestate, pyrimethanil mixed into 5% biochar, and boscalid mixed into the reference soil, 5% BC, 30% DG, and 5% DG + 1% BC, respectively.

Nevertheless, even from these sparse data it can be seen that the addition of biochar accelerated dissipation of the pesticides, which is mainly driven by the sequestrations of pesticides onto the biochars and corresponding low extractability. The influence of sequestration/strong sorption on the dissipation kinetics of pesticides in soils has been observed in many studies (e.g., Laabs et al., 2000), due to a decrease in the bioavailability and biodegradation of compounds sequestered in soil (Cabrera et al., 2007; Alexander, 2000).

3.3. Formation of non-extractable pesticide residues

As discussed, dissipation for the three pesticides is mainly controlled by a fast formation of non-extractable residues rather than full mineralization to CO_2 . The intention of biochar and digestate additions to the test soil was two-fold. The primary aim was to increase mineralization, which would be the preferred dissipation pathway regarding any environmental long-term effects of residues. Since a full mineralization of any pesticide in soil is hardly achievable, the second objective was to immobilize (i.e. sequester) as much pesticides as possible to minimize the pesticide concentrations in water percolating through and potentially exiting the biopurification system. In the long-term view, also the leaching potential to groundwater needs to be minimized, based on the assumption that used biopurification material might be returned to the agricultural fields after its use period (usually 3 to 5 years) (Castillo et al., 2008). The maximization of sequestration of pesticide residues, while mineralization rates are kept high, were achieved with the combination of digestate/biochar additions, as shown in Fig. 3a–c. The positive effect of biochar on the sequestration of pesticides is one of the desired effects in biobed systems, especially for pesticides with low mineralization potential or high mobility in soil. This will ensure minimal export of pesticides via percolate (in case the total amount of water added to the system cannot be evapo-transpired to a sufficient degree), and therefore, a high overall water purification rate.

For all studied pesticides the amounts of non-extractable residues increased for bentazone from 0 to 120 d after application from 4.38 to 91.1%, for pyrimethanil from 8.73 to 94.6%, and for boscalid from 10.5 to 93.7% (detailed data not shown) (Fig. 3a–c), as reported previously for other compounds (Fenlon et al., 2011 and Marín-Benito et al., 2012). The percentages of non-extractable residues of bentazone formed at the incubation time of 120 days were ~42% of the applied radioactivity for the reference soil and ~85%, ~64% and 77% for 5% BC, 5% DG, and 5% BC + 5% DG mixtures, respectively. For boscalid and pyrimethanil, these percentages for non-extractable residues were 36 to 45% of applied radioactivity for the reference soil and 87 to 94%, 47 to 53%, and 94 to 95% for 5% BC, 5% DG, and 5% BC + 5% DG mixtures, respectively.

The formation of non-extractable residues in the biochar and digestate amended mixtures was in general higher for boscalid and pyrimethanil than for bentazone, possibly due to the higher sorption

of these pesticides by the biomixtures than bentazone (Table 3). The formation of non-extractable residues for all pesticides was always higher after the addition of biochar (1 and 5%) and digestate (5%) than reference soil. Moorman et al. (2001) and Mamy et al. (2005) reported that organic carbon content is the key factor involved in the formation of non-extractable residues of pesticides in soil. An exception to this was the 30% DG mixture, which led to a decrease of non-extractable residues formation for boscalid and pyrimethanil (~36 and ~35% of applied radioactivity, respectively), presumably due to its high content of DOC, which may co-solubilize these moderately non-polar pesticides or compete for available strong sorption sites in soil.

4. Summary and conclusion

Before proposing new materials for use in biopurification systems for pesticide remnants, the materials need to be tested for their purification potential. The optimal biopurification system setup should find a balance between high mineralization and sufficient sorption/sequestration of pesticides for long-term effectiveness of the system and for reducing potential export of pesticides via percolate from these systems.

In our experiments, total mineralization varied among the pesticides with generally lower mineralization for boscalid and pyrimethanil (0.7 to 15% of applied radioactivity) and slightly larger one for bentazone (9 to 24%). The results indicated that the addition of digestate as an easily available carbon source increased pesticide mineralization mainly by the stimulation of the soil microbial activity. However, the mineralization did not increase proportionally with increasing digestate content in the mixture. Biochar addition decreased the mineralization for all pesticides and led to larger formation of non-extractable residues, resulting in increased dissipation of pesticides via sequestration in soil for all tested mixtures. Using mixtures of 5% biochar and 5% digestate in soil showed intermediate mineralization and high sorption, resulting in largest pesticide dissipation of all tested mixtures.

However, more work is required to analyze also the hydraulic response and the resulting contact times of the biopurification mixtures and the pesticide-containing drainage water, which are fundamental for the setup of an optimal biobed system. Additional research is also required to study the long term fate (> 1 year) and effects of aged pesticide residues in biomixtures, which might be returned to and distributed on agricultural fields.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2015.11.111>.

Acknowledgments

S. Mukherjee was funded by the Indian Council of Agricultural Research (ICAR) (Grant No. 29-1/2009-EQR/Edn). Thanks are given to Mr. Ulrich Disko for analytical measurements.

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