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Biopurification of pesticides with novel mixtures

Santanu Mukherjee

Member of the Helmholtz Association

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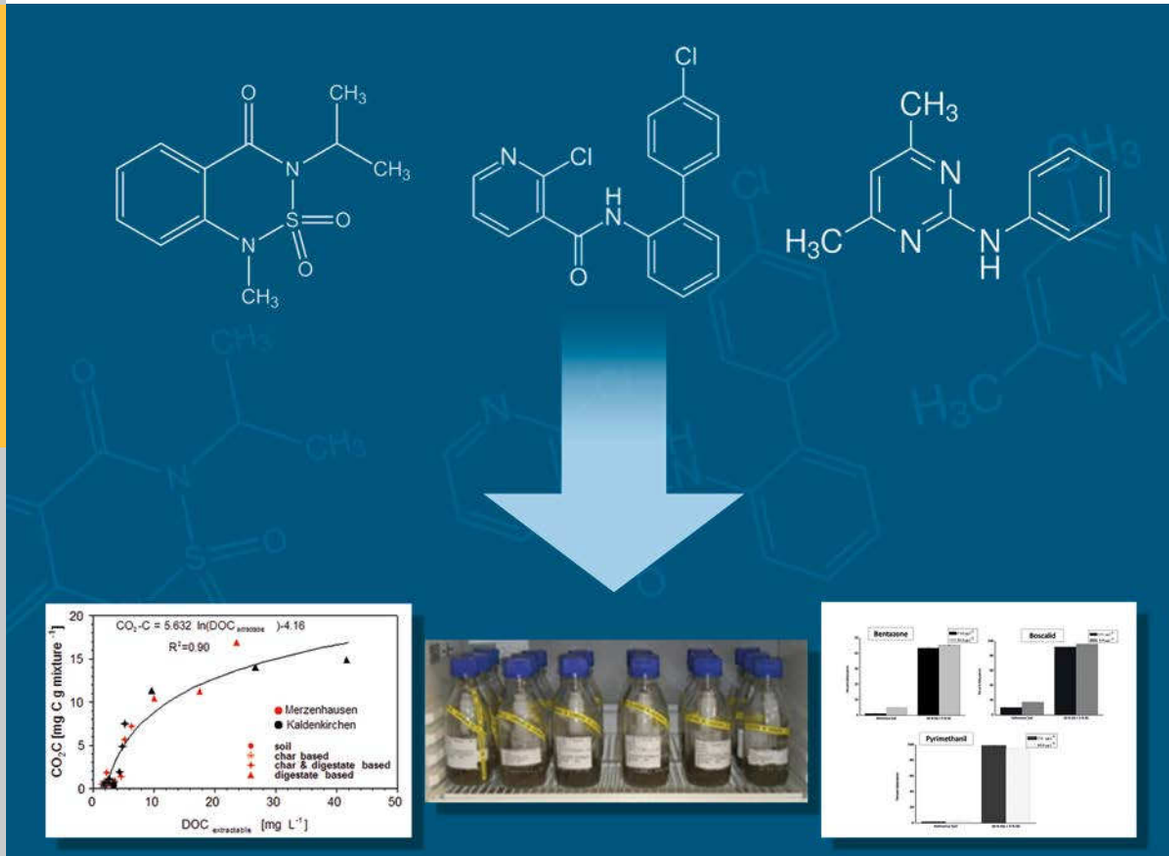

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Dedicated to

my mother

Zusammenfassung

Während der letzten Jahrzehnte wurde der Gewässerverunreinigung durch Anwendung von Pestiziden in der Landwirtschaft zunehmend Beachtung geschenkt. Als Eintragspfade in Gewässer und Grundwasser kann generell zwischen diffusen (indirekten) und punktförmigen (direkten) Einträgen unterschieden werden. Um direkte Verunreinigungen von landwirtschaftlichen Betrieben durch Reinigung von Spritzen und Zubehör auf dem Betriebsgelände zu vermeiden, werden in einigen Regionen „Biobeds“ oder Biofilteranlagen zur Behandlung des Waschwassers betrieben. Das konventionell in diesen Systemen verwendete organische Material ist unter Umweltgesichtspunkten oftmals nicht nachhaltig (wie im Falle von Torf) oder es führt zu heterogenen hydraulischen Flüssen, was sich negative auf den Rückhalt und den Abbau von Pestizide auswirken kann.Das Ziel dieser Arbeit war es deshalb, die üblichen Materialien, Torf und Stroh, durch organische Reste aus der Gewinnung von Bioenergie, wie Gär- und Pyrolyserückstände, zu ersetzen und unterschiedliche Mischungsverhältnisse auf den Verbleib von Pestiziden zu untersuchen.

In einem ersten Schritt wurde die mikrobielle Respiration über drei Monate bestimmt, um Kenntnisse über die Umsatzrate der Boden/Organik-Mischungen zu erhalten. Diesekann als erster Hinweis auf das Abbaupotential der unterschiedlichen Mischungen auf Pestizide genutzt werden und Informationen über die Langzeitstabilität der Materiale liefern. Mischungen aus Boden mit Gär- und Pyrolyserückständen ergaben eine mittlere CO_2-Freisetzungsrate verglichen mit Mischungen aus Boden und den jeweils einzelnen Komponenten. Die Respiration in Bodenmischungen mit Gärrückständen lag generell niedriger, wenn zusätzlich Pyrolyserückstände eingearbeitet wurden.Desweiteren wurde in einer Laborstudie über eine Inkubationszeit von 135 Tagen mit drei unterschiedlichen Pestiziden (Bentazon, Boscalid und Pyrimethanil) die Korrelation zwischen mikrobieller Respiration und dem Abbaupotential der Mischungen für Pestizide untersucht.Mischungen, welche Pyrolyserückstände enthielten erhöhten generell die Festlegung der untersuchten Pestizide bei einer entsprechend schlechteren Extrahierbarkeit. Andererseits wurde die Mineralisierung der Pestizide durch Einmischung von 5% und 30% Gärrückständen in Boden erhöht und mit zusätzlich 5% Pyrolyserückständen wurde eine gewünschte Balance zwischen verstärkter Festlegung und Mineralisierung der Pestizide erreicht.Sorptions-

Desorptionsversuche ergaben für alle Gemische stärkere Sorptionseigenschaften im Vergleich zu reinem Boden. Die K_d- und K_{oc}-Werte der Pestizide waren entsprechend ihrer physiko-chemischen Eigenschaften und der Art des beigemischten organischen Materials unterschiedlich. Die Desorption aller Pestizide verhielt sich hysteretisch zur Sorption.

Diese Arbeit erweitert und ergänzt das derzeitige Wissen bezüglich des Mechanismus` des Kohlenstoffumsatzes in den neuartigen Bodenmischungen für Biofilteranlagen und das Langzeitverhalten dreier unterschiedlicher Pestizide und ihrer Wechselwirkungen mit diesen Bodenmischungen. Dennoch bedarf es weiterer Forschung zur Bestätigung der Eignung dieser Bodenmischungen in technischen Biofilteranlagen über noch längere Zeiträume (> 3 Jahre) unter Freilandbedingungen und unter wechselnden hydraulischen Bedingungen und Wirkstoffbelastungen.

Abstract

Worldwide, water contamination from agricultural use of pesticides has received increasing attention within the last decades. In general, sources of pesticide water pollution are categorized into diffuse (indirect) and point sources (direct). To reduce point pollution from farm yards, where the spray equipment is washed, biobed or biofilter systems are conventionally used to treat the washing water. The organic material usually used in these systems is often not environmentally sustainable (e.g. peat) and incorporated organic material such as straw leads to a highly heterogeneous water flow, with negative effects on the retention and degradation behavior of the pesticides. Therefore, the objective of this present study was to substitute the classical materials (peat and straw) with bioenergy residues namely biochar and digestate to investigate their effects on fate of pesticides in soil at different mixing ratios.

Prior to study the pesticides fate, the microbial respiration was measured over 3 months to gain information about the turnover rate of the organic biomixtures, which can be used as an indirect indicator of the soils/biomixture degradation potential for pesticides and provides information about the long-term stability of the material. Mixtures of biochar and digestate showed an intermediate CO_2 flux compared to the single addition of biochar or digestate, whereby the oxygen consumption in presence of biochar was generally significantly lower compared to the consumption after addition of digestate only. Additionally, to correlate the microbial respiration with the dissipation (or degradation) potential of pesticides a laboratory incubation study was performed over 135 days with three contrasting pesticides (bentazone, boscalid, and pyrimethanil). In general, biochar based mixtures resulted in stronger binding of all studied pesticides, and therefore, ensued higher dissipation. On the other hand, 5 % and 30 % digestate based mixtures enhanced mineralization and addition of 5 % biochar to these mixtures showed a desired balance between stronger sequestration and mineralization for all pesticides. A sorption-desorption study revealed that biochar and digestate based mixtures caused stronger sorption for all compounds compared to bare soil. K_d and K_{oc} values of the pesticides were different according to their physico-chemical properties and quality (nature) of organic matter. Desorption was hysteretic for all pesticides.

Overall, this thesis elucidated and updated the knowledge of the mechanisms for C-turnover rates of novel biomixtures for biopurification (or biobed) systems along with

the long term behavior of three different pesticides and their interaction with these biomixtures. However, future work is required to qualify these mixtures for long-term (>3 yrs) outdoor biofilter constructions under varying hydraulic and chemical conditions.

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List of abbreviations

χ^2 Chi-square test

$\overline{x}_{obs}$ Observed cumulative CO_2-C fluxes(gCO_2-C g^{-1} mixture)

$\overline{x}_{sim}$ SimulatedcumulativeCO$_2$-C fluxes(gCO_2-C g^{-1} mixture)

1/n $_{ads}$ Freundlich adsorption exponent

1/n $_{des}$ Freundlich *desorption exponent*

^{14}C Carbon-14

Ads Adsorption

BC Biochar

BET Brunauer, Emmett and Teller

C Carbon

C Constant partitioning

C(t) Instantaneous CO_2-C flux at time t (min)

C_1 Total percentage of the labile pool from total carbon stock

C_2 Total percentage of the refractory pool from total carbon

C_e Equilibrium solute concentration in water phase

C_i Initial solute concentration in water phase

CO_2-C Carbon-dioxide carbon

C_{org} Organic carbon content

C_s Amount of sorbed pesticides

C_{smax} Langmuir constant implies maximum sorption capacity

d Day

Da Dalton

DFOP Double first-order in parallel

DG Digestate

DOC Dissolved organic carbon

dof Degrees of freedom

dt Time interval

DT_{50} Dissipation half-life time-50

ECEC Effective cation exchange capacity

ESI Electrospray ionization

FOCU Forum for the Co-ordination of pesticide fate models and their Use

g The ratio between the slow and fast pool

h Hours

H Hysteresis coefficient

H_3PO_4 Phosphoric acid

HPLC High-performance liquid chromatography

HTB High temperature biochar

i Time step

ICP-OES Inductively coupled plasma optical emission spectrometer

IR Infrared

k Rate constant

K Kelvin

k_1 first- order mineralization rate of the labile C pool

k_2 first- order mineralization rate of the refractory C pool

K_d Distribution coefficient

K_f	Adsorption coefficient
KK	Kaldenkirchen soil
K_L	Langmuir sorption coefficient
K_{oc}	Carbon normalized partitioning coefficient
K_{ow}	Octanol-water partition coefficient
kV	Kilovolt
L	Liter
L	Langmuir type
LODs	Limits of detection
LOQs	Limits of quantification
LSC	Liquid scintillation counter
LTB	Low temperature biochar
M	Mass of the substances
MBq	Mega Becquerel
$MinT_{50}$	Mineralization half-life time-50
M_o	Total amount of chemical present at time=0
MRT	Mean residence time
MRZ	Merzenhausen soil
MS	Mass spectrometry
M_t	Total amount of chemical present at time t
N	Nitrogen
N	Total number of observations
NA	Not applicable

NaOH Sodium-hydroxide

NH_3 Ammonia

NH_4Cl Ammonium chloride

O Oxygen

OC Organic carbon

OECD Organization for Economic Co-operation and Development

Pka Logarithmic value of the acid dissociation constant,

Ppm Parts per million

R^2 Coefficient of determination

Rpm Revolutions per minute

S Side-by-side association

SD Standard deviation

SFO Single first order model

SOC Soil organic carbon

SOM Soil organic matter

SSA Specific surface area

SSR Sum of squared residuals

STD Standard deviation

$SUVA_{254}$ Specific ultraviolet absorbance

T Time of sampling

T Time

TOC Total organic carbon

TQ-S Triple Quadrupole mass spectrometry

UPLC Ultra performance liquid chromatography

UVA_{254} Ultraviolet absorbance

V Volume of the solution

v/v Volume/volume

w/v Weight/volume

w/w Weight/weight

WHC_{max} Maximum water holding capacity

I. General introduction

I.1Theory

Indiscriminate usages of pesticides by farmers in agriculture increase the risk of environmental contamination due to widespread non-target specific dispersions of pesticides. A good agricultural practice provides the reduction of application doses and number of treatments in an integrated pest management strategy. This concept is strictly applied, when pest damage reaches below the economic injury level with a purpose to minimize risks to human and environment. However, the agricultural sector continues to be one of the most prominent sources for delivering contaminants into the environment. In order to decrease pollution of the environment, and more specifically of water bodies, it is important to know the extent of environmental contamination and its origins. Therefore, it is necessary to adopt a good prevention strategy because especially groundwater has a low self-purification capacity.

The term pesticide will be used throughout this thesis and refers to synthetic organic plant protection products, which can be subdivided into insecticides, fungicides, herbicides, rodenticides, etc. When pesticides are applied under appropriate ecological conditions in recommended dosages using specified practices, they can be effective in pest control with little adverse effects on the surrounding environment.

A vulnerable and important compartment of the environment is water. The contamination of water by pesticides is a major environmental issue in Europe (Kolpin et al., 1995;Kreuger and Nilsson, 2001). Water covers about two-thirds of the earth's surface, and this is predominantly salt water. Only 2.5% is fresh water, and thereof, two-thirds are locked up in the icecaps and glaciers. Drinking water for human purposes is therefore limited to only 0.08% of the entire water inventory on earth. Therefore, contamination of these limited resources could be catastrophic and fatal to the human race and other species living on this planet.

Rivers, lakes and other water bodies are vital natural resources of drinking water. They are the important habitats for many different types of wildlife, and are necessary resources for industry and recreation. A significant proportion of them are under risk partly due to indiscriminant use of toxicants. Drinking water companies across the EU have taken initiative to spend large sums on water treatment every year. An annual investment of €24.4 million in the Netherlands, €130 million in Germany, and €170 million in the UK is made for water purification purposes (PAN Europe, 2016). Actually, these huge amounts are passed on to the consumer. Quality standards for pesticide concentrations in drinking water are specified by the EU Directive and allow

a maximum residue of 0.1 µg L^{-1} for a single active ingredient and of 0.5 µg L^{-1} for the total pesticide load at the tap (98/83/EEC) which is not a toxicity based measurement (O'Shea, 2002; De Wilde et al., 2007).

A schematic overview of groundwater contamination in 2012 in Europe is given in Figure I.1.The red marks indicate that the contamination of groundwater is significant and that mitigation measurements should be taken whereas green marks imply non-significant pollution.

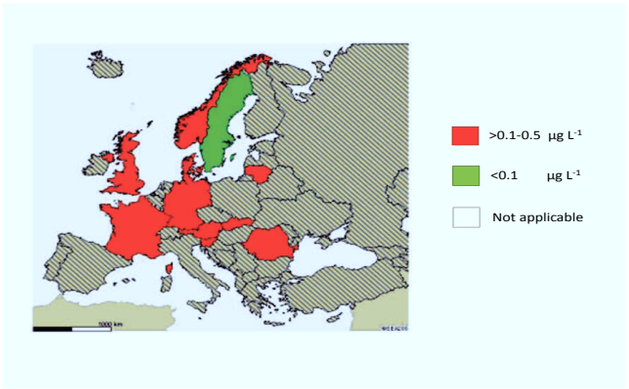

Figure I.1: Distribution of pesticides in the groundwater in different countries of Europe (Source: Modified after European Commission, 2010).

I.2Rationale

Registration of new pesticides and the mitigation of environmental contaminations require the testing of fate of pesticides in and between different environmental compartments. In fact, sometimes, plant protection products are spread over areas far from the point of application depending on the characteristics of the molecules and different agro-climatic conditions (Carter, 2000;Trevisan et al., 1996). Pesticide pollution caused by point (direct) or diffuse (indirect) sources may lead to the contamination of ground and surface water (Carter, 2000). Diffuse contamination via percolation, runoff, drainage and drift contributes only to a part of the pesticides pollution of surface and groundwater (Acevedo et al., 2011).Previous researchers have documented that a small percentage of the applied pesticides reach the surface water and groundwater from diffuse contaminations. Several studies on the catchment scale have demonstrated that 40 to 90% of surface water contamination by pesticides is attributed to direct sources (Carter, 2000; Kreuger and Nilsson, 2001). The direct losses stem from spillages resulting from e.g., the filling operation,

leakages of the spray equipment, spray leftovers and rest volumes in the tank and rinsing water from cleaning the internal tank to avoid carry over effects (damage and residues) onto the following crop(Coppola et al., 2011b; Karanasios et al., 2010).Point sources bear a high risk for direct contamination of water bodies: In fact, a few drops of a pesticide concentrate from a container can easily contain 1 g of active substance. 10,000 m³ of water is needed to dissolve this amount to the acceptable concentration of 0.1 µg L^{-1} water (Torstensson and Castillo, 1997). The pesticide, once distributed to the crop or soil, can contaminate water bodies via processes depending on environmental (soils, climate and organic carbon) and pesticide characteristics. Capri et al. (1999) estimated the percentage distributions of the sources of contamination of water bodies (Figure I.2).

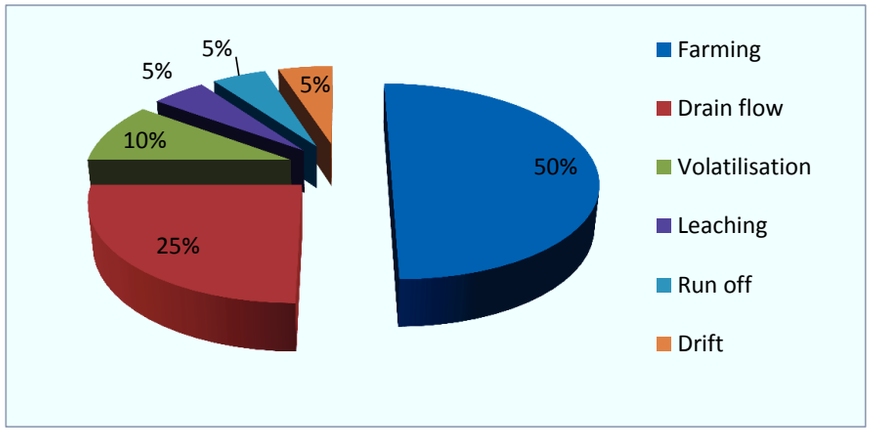

Figure I.2: Causes of water contamination (modified after Capri et al., 1999).

In fact as showed in Figure I.2, the point contamination (arising from farming activities) is more relevant to environmental impact. Usually cleaning, filling, and spraying operations are done at the same place on the farm each time, often near a water source where the topsoil has been removed and replaced with a layer of gravel and sand, or on hard surfaces with connection to sewers. As a consequence wash off of remnants via surface runoff to the next sewage collector or stream may occur. Besides, the poor degradation and sorption capacity of pesticides on gravel or concrete materials increase the risk for leaching of the pesticides to the ground water via vadose zone (Karanasios et al., 2010). The modern management of a farm cannot ignore the issues of protection of environmental resources, because they are closely related to the quantity and quality of agricultural production. Mitigation or prevention of point sources can on the one hand be achieved by implementing best

management practices, on the other hand by using advanced depurification systems based on sophisticated physical, chemical, and/or biological methods to treat any remnants of pesticides on farm (De Wilde et al., 2009; Ramwell et al., 2004). Unfortunately, many methods for remnant treatment are cost and labor intensive. To overcome these obstacles the “biobed” concept was developed in Sweden in the early 1990s to establish an environmentally sustainable low cost technology; easy to install and maintained by the farmer (De Wilde et al., 2007).

I.3State of the art

I.3.1 Biopurification system and Biofilter

Bioremediation or biopurification is defined as the process in which organic substances are degraded under controlled conditions by microorganisms or their enzymes to an innocuous state, or to levels below concentration limits established by regulatory authorities (Braschi et al., 2000). The concept of biopurification of pesticide remnants on farm has generated interest in various countries all over the world. The concept of biobed originated in Sweden, but several other systems, based on the principles of the biobed, have now been developed and implemented in many countries, where they have often been renamed, for example as biofilter, biomassbed, phytobac, and biobac (Torstensson and Castillo, 1997). Actually, these are often the more effective systems to reduce environmental pollution compared to other cost and labour intensive methods like chemical coagulation, sedimentation, oxidation and photo catalysis. As a low-cost operating system, the biofilter concept can minimize the risks of pollution when filling and storing the sprayer at the places near the farm. The concept of all of these systems is similar: They are basically composed of different mixtures of topsoil with organic matter (e.g. lignocellulosic material like straw) through which pesticides containing waste water is percolated. While the waste water passes through the biofilter, the pesticides are retained (sorbed) and/or degraded and the water is released with reduced concentrations of pesticides to surface waters or it is percolated into the surrounding soils. Depending on climate, hydraulic load, and mode of operation, a substantial part of the treated water volume might be reduced by evaporation.

Biofilter (in Figure I.3) is constructed of 2 to 3 containers or Intermediate Bulk Carriers (IBCs) of 1m^3 vertically stacked onto each other and filled with the same organic materials as the biobeds (De Wilde et al., 2007). These systems are in general much smaller and have lower amounts of active filter substrate or biomix (2-5

m³) than biobeds where 10-30 m^3 filter substances can be used. The principle of the biopurification system relies on degradation under aerobic conditions, which means it is necessary to maintain proper moisture conditions throughout the experiment.

Figure I.3: Model Biofilter System (Source: Modified after Pussemier et al., 1998).

I.3.2The role of biomixtures in biopurification system

Basically, biobed systems are filled with locally available organic carbon-rich materials mixed with topsoil. Typically, a mixture of soil, peat, and straw in the volumetric ratio of 2:1:1 is used. The addition of organic rich substances is essential to retain the pesticides in the biofilter matrix and to stimulate and sustain microbial growth, which promotes pesticide degradation. Therefore, the choice of the biomixture material plays a crucial role for its effectiveness. Additionally, the choice of material also determines the hydraulic regime, and therefore, the residence time of the pesticides in the soil, which directly influences the sorption and degradation processes (Castillo et al., 2008). Besides the hydraulic load, the chemical load is also an important factor that influences the elimination effectiveness of the system, whereby this role is less well studied (Karanasios et al., 2010).

It is well known that the presence of ligno-cellulosic material, like straw, promotes the activity of white rot fungi which accelerate the co-metabolic degradation of pesticides by ligninolytic enzymes (phenoloxidases) (Castillo et al., 2008). Peat on the other hand is essential to maintain optimum moisture conditions, to improve aeration, and to keep acidic pH conditions, which are favorable for microbial (mainly fungi) activity (Torstensson and Castillo, 1997). The addition of soil is recommended as a source of native microorganisms, nutrients and carbon (C) source for the microbes (Mukherjee et al., 2016a), whereby the choice of soil material (e.g. different soil textures) was

reported to have only little or no effect on the biopurification of the contaminants in the system (De Wilde et al., 2007).

I.3.3 Biochar and Digestate as novel biomixtures

In the present study/dissertation, the biomixtures were prepared using two bioenergy residues, namely biochar and digestate. Biochar is a man-made product of incomplete combustion, i.e. thermal conversion of C-rich biomass under limited oxygen supply at temperatures ranging from 500- >1000°C (Glaser et al., 2002;Sun et al., 2014). This process of thermal conversation is called dry pyrolysis (Smith et al., 2010). Biochar contains ash, labile and recalcitrant C (Lehmann et al., 2011). The compounds of recalcitrant C refer to black carbon (BC), describing the aromatic microstructures of biochars (Accardi-Dey and Gschwend, 2003; Keiluweit et al., 2012).

In general, there is an increasing trend towards biogas production in most industrial countries because biogas is an important form of renewable energy (Möller et al., 2008; Gunnarsson et al., 2010). It is also documented that by the year 2050 most of the world's energy demand (approx. 77%) will be fulfilled by renewable energy (IPCC, 2011). Digestate is the solid by product of the biogas industry following the anaerobic digestion process (Arthurson, 2009). Digestate, as a source of easily available carbon can enhance the microbial activity by increasing the microbial growth and respiration as shown by e.g Mukherjee et al. (2016a), Makádi et al. (2008).

To our knowledge, no investigation is reported on turnover rate and stability of recalcitrant carbon source like biochar under different soil conditions, and in the presence of easily available sources of organic C like digestate.

I.3.4 Stabilization mechanisms of natural, pyro (biochar) - and bio (digestate) - genic organic matter

The processes responsible for the stabilization of soil organic matter (SOM) constitute an essential component of global biogeochemical cycles (Lehmann et al., 2011). Overall, the chemical composition of the organic matter (OM) and the interactions with other soil components such as the mineral phase largely drive the mechanisms for SOM stabilization (Rasmussen and Rohde, 1988), which can be summarized as: (1) biochemical stabilization, (2) physical stabilization and (3) chemical stabilization (Six et al., 1998; Tryon, 1948). The extent of protection offered by each mechanism depends on the chemical and physical properties of the mineral

matrix and the morphology and chemical structure of the organic matter (Six et al., 1998). Thus, each mineral matrix presents a unique and finite capacity to stabilize organic matter (Rasmussen and Rohde, 1988). According to Kögel-Knabner et al. (2008), the protection of organic matter (OM) against decomposition by the following mechanisms decreases in the order: chemically protected > physically protected > biochemically protected > non-protected.

Due to the physical and chemical diversity of biochar, unknown environmental effects on biochar decomposition rates, the mean residence time of biochar in soil is still unknown (Prayogo et al., 2014). Some studies indicate that biochar may persist in soil for millennia (e.g. Kuzyakov et al., 2009; Liang et al., 2008); others reported from laboratory incubations that significant parts of biochar may be decomposed within weeks (Smith et al., 2010; Cross and Sohi, 2011). On the contrary, charcoal is not totally stable and several authors (e.g. Kuzyakov et al., 2009; Steinbeiss et al., 2009) have drawn attention to the need for long-term experiments under a diverse range of environmental conditions, soil types and biochar to better understand their fate in soil. Biochar made from hard wood is mechanically and biologically more stable than biochar from soft wood and herbaceous plants (Zimmerman et al., 2011). The mechanical stability and hardness of biochar made from plant feedstock relates to their higher lignin contents (Marchetti and Castelli, 2013). However, Keith et al. (2011) found for tropical soils that during the first 30 years after deposition there was a rapid decrease of biochar content in soil, though apparently after 30 years decomposition and/or loss declined to very slow rates and a steady state evolved.

However, there is little information available for the stability of digestate based mixtures. On amending soil with digestate, an instant flush of high CO_2 (response in respiration) production has been reported, unlike with other organic residues (Mukherjee et al., 2016a). This instant response in respiration is most likely an effect of a comparatively higher fraction of easily degradable carbon in the digestate becoming immediately accessible to the soil microorganisms (Möller et al., 2008), compared with e.g. non-digested animal manure (Arthurson, 2009) and compost (Odlare et al., 2008).The origin of organic residues (digestate, animal manure, compost) also causes different responses in soil respiration (Walsh et al., 2012).

I.3.5Soil respiration as an indicator of pesticides degradation

Soil respiration is a general process performed by most microorganisms and methods for measuring this activity are probably the most common tool for

investigating soil microbial activity (Stenstrom et al., 2001). Several methods exist for determination of soil respiration based on either oxygen consumption or release of carbon dioxide. The background respiration activity of a soil microbial community, also called basal respiration, can simply be measured as CO_2 produced without any addition of substrate. Instead of adding glucose or a set of carbon sources, the respiratory response of the active microbial biomass can also be measured after addition of different organic fertilizers (Alburquerque et al., 2012). This assesses the capacity of the soil community to utilize a complex mixture of organic substances under more natural conditions where the microorganisms in the soil sample have to compete for the substrates. Adding organic residues to soil generally increases soil respiration, since carbon serves as an energy source for most soil microorganisms, is termed as substrate induced respiration (Marchetti and Castelli, 2013).

The combination of the basal and substrate induced respiration represent carbon availability index (Cheng et al. 1996). Therefore, soil respiration can be used as an indirect indicator of a soils pesticide degradation potential (Torstensson and Castillo, 1997).Like other metabolic activities, it depends on the physiological state of the microbial cells and is influenced by several soil factors. De Wilde et al. (2008) also found a good correlation between basal respiration and degradation of pesticides for conventional biobed materials. It supports the findings of Karanasios et al. (2010) and Mukherjee et al. (2016b) who demonstrated that microbial respiration is a strong or good indicator for co-metabolic degradation or dissipation of pesticides. There is no information available how microbial respiration will change, if biochar and digestate mixtures with soils will be used in such setup. This information is vital for using novel biofilter material in replacement with conventional mixtures, especially to analyze and interpret further pesticide degradation studies using such biomixtures in the biopurification process.

I.3.6 Biochar and Digestate as adsorbents

One possibility to characterize biochar and/or digestate surface properties is to investigate their role in adsorption processes. In general, their chemical and physical properties (e.g. aromaticity, porosity, surface area and surface chemistry) determine their abilities to adsorb organic or inorganic substances. Applications of both of them for remediation or restoration of contaminated soils are thus considered as environmentally beneficial (Kookana, 2010).

Nevertheless, positive effects on biochar amendments for contaminant retention have not always been observed (Keiluweit et al., 2012). Additionally, application of biochar containing high amounts of labile C may reduce adsorption of contaminants due to competing adsorption sites. Biochar with high ash contents elevate soil pH, and thus the mobility of organic contaminants (Kookana, 2010). And, if dissolved organic matter (DOM) is released, there might be a co-transport of contaminants (e.g. Uchimiya et al., 2012; Mukherjeeet al., 2016b). However, the influence of biochar amendments on sorption/desorption of contaminants in soils was hardly explored. Usually, previous research only focused on the sorption properties (Yang and Sheng, 2003; Yu et al., 2010).

Retention of cationic nutrients and contaminants is primarily affected through Cation Exchange Capacity (CEC) (De Wilde et al., 2009; El Bakouri et al., 2007). To elevate CEC in soils, applications of soil conditioners with higher CEC are required! However, the CEC of fresh and/or ash-free biochar is low (Accardi-Dey and Gschwend, 2003, Tatarkova et al., 2013). Considerable increase of CEC of biochar in soils (via surface oxidation with enrichment of carboxylic groups) requires long time (e.g.Jin, 2010; Martin et al., 2012) which diminishes the potential use of biochars assoil conditioner. Hence, biochar surface properties should be improved prior application using well established technologies, e.g. physical activation and/or composting. Yet, so far to our knowledge no research has been done to elucidate effects of digestate in single or combined application (with biochar) on sorption-desorption properties and/or nutrient retention.

I.4 Objectives and outline of the thesis

The overall aim of the present study was to examine the processes and factors that influence the fate of three contrasting pesticides (bentazone, boscalid, and pyrimethanil) in novel biomixtures (biochar and digestate based) for biopurification systems. The aim of the first study (chapter II) was to analyze the effect of novel biofilter materials on the microbial respiration to gain information about the optimal composition with respect to heterotrophic respiration as an indirect measure for pesticide degradation. In the second study (chapter III), pesticides dissipation (DT_{50}) and mineralization ($MinT_{50}$) potential was analyzed by using different soil/amendment mixtures in laboratory degradation studies. While the first and second study were focused either on the fundamental biological processes, in a third study (chapter IV), the basic physico-chemical properties (sorption-desorption) of selected

soil/biochar/digestate mixtures were examined. Based on these experiments, guidance for an appropriate soil / substrate (biochar and/or digestate) combinations for a novel biofilter setup can be derived.

In the above studies, the following questions/hypotheses were addressed:

i) **How resistant are biochar- and digestate- based mixtures in soil to degradation and how do they affect biological and chemical soil properties?**

To assess the stability of biochars and digestate, their impact on soil properties under laboratory conditions, a short term respiration experiment (90 days) was conducted. This experiment was performed with two different biochars (produced at 400°C and 800°C) as well as digestate from biogas production. They were added in different combinations to two soils (loamy sand and silt loam texture).Additionally, both amendments were mixed together into the soils to study interactions between biochar and digestate and to investigate the interactions of both amendments with clay minerals resulting in a total of 13 mixtures (plus control soils) per soil type.

ii) **How doesthe biomixtures affect the fate (dissipation and degradation) of three different pesticides (bentazone, boscalid and pyrimethanil) use for biopurification systems?**

In order to elucidate the dissipation and degradation behavior of three pesticides with varying properties (ranging from low sorption and fast degradation to high sorption and slow degradation), a short term lab incubation study (135 days) was conducted using different configurations of mixtures. Seven different biomixtures comprised of two bioenergy residues (low temperature biochar and digestate) in combination with a loamy sand soil were used to investigate the pesticide degradation potential. The mineralization and dissipation kinetics were fitted to a single first order (SFO), the modified Gustafson-Holden (FOMC), and the bi-exponential or double first-order in parallel (DFOP) model.

iii) **How do these novel mixtures affect the adsorption-desorption of studied pesticides used for biopurification systems?**

To assess sorption/desorption properties of three contrasting pesticides to novel biomixtures (biochar and digestate based) and loamy sand soil, a laboratory batch equilibrium experiments were investigated. Attempts were

made to correlate sorption-desorption properties of the studied pesticides with the organic carbon content of the biomixtures and their surface areas. Interaction of soil minerals with biomixtures and their effects on sorption-desorption properties of pesticides were also discussed. To describe adsorption and desorption properties, Henry (linear), Freundlich and Langmuir isotherms were used and hysteresis was calculated using the Index of irreversibility.

II. Microbial respiration of biochar- and digestate-based mixtures

Modified on the basis of

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II.1Introduction

Soil organic C (SOC) or soil organic matter (SOM) plays an important role with respect to soil fertility and agricultural productivity, mainly yield (Möller et al., 2008; Feller et al., 2010). There are different ways to add external organic C to the soil or to increase soil organic C stocks, namely by N fertilization with organic manure (Rasmussen and Rohde,1988), reduced or zero-tillage (e.g., Ismail et al., 1994; Lal, 2009), application of larger amounts of plant residues (e.g., cover crops) manure or compost (e.g., Buyanowski and Wagner, 1998; Lal, 2009), or by introducing black carbon or biochar to the soil (e.gTryon, 1948; Glaser et al., 2002). It is generally known that the C added to the soil will be turned over and CO_2 will be released (heterotrophic respiration), whereby the heterotrophic respiration is a function of C quantity (size of the carbon stocks), environmental drivers (soil water content, soil temperature, and aeration), C availability or accessibility for microbial degradation, and C quality (Skopp et al., 1990; Six et al., 1998; Bauer et al., 2012).

Over the last 20 years the application of C-rich pyrogenic biomass (e.g., biochar or charcoal) has been suggested to increase soil C stocks and to improve soil fertility especially of C-poor soils (Sun et al., 2014; Prayogo et al., 2014; Smith et al., 2010). Unfortunately, the impact of biochar addition to soils on heterotrophic respiration is not fully understood and inconsistent findings are reported. Despite the recalcitrant nature of biochar, several studies have reported increased soil respiration rates when biochar was added to soils (e.g., Pietikainen et al., 2000; Zimmerman et al., 2011). Basically, pure biochar is comprised of a small labile C-pool with short turnover times (days to months) and a large recalcitrant C-pool with long turnover times from years to decades (Smith et al., 2010). The application of biochar to the soil can impact (increase or decrease) the mineralization of native SOM and fresh inputs of labile organic matter, which is classically described by a double exponential models to account for the mineralization of the active and slow carbon pools, respectively (Liang et al., 2008; Zimmerman et al., 2011). Often, C mineralization after biochar addition shows an initial flush, after which CO_2 evolution continues at much lower rates, similar to the biphasic mineralization observed after addition of non-pyrolyzed organic materials to soils. Das et al. (2008) observed this phenomenon in soils amended by biochar made from poultry litter, and explained the observed phenomena by the presence of labile compounds in the poultry litter biochar. These labile compounds of the biochar can be easily and rapidly degraded followed by slow

to negligible degradation of the condensed aromatic ring structures of the biochar (Smith et al., 2010; Cross and Sohi, 2011). The initial stage of fast mineralization has been reported to last between 6 (Smith et al., 2010) to 60 days (Kuzyakov et al., 2009; Steinbeiss et al., 2009), whereby 2 to 20% of the biochar-C can be mineralized. On the other hand, biochar addition has also been reported to affect freshly added organic residues as well as soil organic matter turnover. For example, sugarcane residues were stabilized into soil aggregates more rapidly in biochar-rich than in biochar-poor Brazilian soils resulting in lower heterotrophic respiration and long-term C-enrichment for the biochar-rich soils as reported by Liang et al. (2010). Keith et al. (2011) studied different biochars (high and low temperature biochar) added to sugarcane mulch. Their results indicated an increased mineralization of the biochar in presence of mulch, which acts as labile organic matter, but also a decrease of mulch turnover in presence of biochar. The authors speculated that the reactive surfaces of the aged biochar particles in soils may protect the labile organic matter of the mulch much better than freshly added biochars. In another study Zimmerman et al. (2011) compared the addition of different high temperature biochars to soils with different SOM contents and observed that C mineralization decreased in the soils amended with biochars.

Although biochar is very stable there are several mechanisms by which biochar can also interact with soil minerals particularly with clay. Joseph et al. (2010) hypothesized that the process of intercalation within clay minerals surfaces by hydrophobic-hydrophilic interactions are the main mechanisms behind this interaction. Additionally, biochar can be protected in soil micro-aggregates and by other types of physical protection (Liang et al., 2008; Kuzyakov et al., 2009). Therefore, the soil type especially clay content, is an important driving factor affecting the stability of biochar in soils. However, there are only few data available regarding the effects of soil characteristics on biochar stability.

Anaerobic digestion of different feedstocks (e.g., manure, organic wastes, or energy plants (e.g., maize) allows the production of biogas as a renewable energy, but at the same time it enables the conservation of practically all plant nutrients contained in the initial feedstock material, which can then be applied to soils as fertilizer (Möller et al., 2008; Gunnarsson et al., 2010; Walsh et al., 2012). In comparison to the direct application of the feedstock to the agricultural fields, digestate contained less amount of total C and highly enriched in N (Möller et al., 2008), and therefore, less organic C

is available for growth and activity of the soil microbial community, which might lead to a gradual depletion of the soil organic matter stocks with time (Arthurson, 2009). It has been also observed that heterotrophic respiration will increase directly after digestate amendment due to the easily available C as shown by Marchetti and Castelli (2013).

In some cases both biochar and digestate might be applied to the soil at the same time or at different years. Both amendments seem to influence each other by co-metabolism or suppression and their overall turnover is not well studied. To our knowledge, there is scarcity of data regarding interaction of digestate with clay minerals and the stabilization effect by the clay. Similarly, only few studies are available describing the soil respiration response with respect to simultaneous biochar and digestate amendment. As already mentioned, Marchetti and Castelli (2013) showed that digestate addition to the soil increased CO_2 evolution, whereby a suppression of CO_2 flux was observed when biochar was added to the system. Because the findings for biochar as well as digestate addition to soils are controversially discussed further systematical studies are urgently needed. To our knowledge the influence of different biochars (high and low temperature), contrasting soils (light to heavy), and amounts of biochar and digestate addition (low to high), and their response if added simultaneous are not studied yet within one experiment.

In the present study we therefore investigated the effects of the addition of biochar and digestate on microbial respiration in two contrasting soils at different mixing ratios. Additionally, the two amendments were mixed together into the soils to investigate any interactions with soil organic matter and potentially also with soil texture, particularly with clay. For interpretation of the respiration data physico-chemical characteristics of the mixtures in terms of dissolved organic C (DOC) content, and aromaticity were also measured and correlated with observed CO_2 fluxes.

II.2 Materials and Methods

II.2.1 Soils and Organic Amendments

Two contrasting soil types, a loamy sand (Gleyic Cambisol) from Kaldenkirchen, Germany (51°19’13 N and 6°11’47E) and a silt loam (Orthic Luvisol) from Merzenhausen, Germany (50°55’48 N and 6°17’51 E) were used in this study (see TableII.1). A detailed description of both soils can be found in Kasteel et al. (2010). These soils were mixed with three different organic amendments at different mixing

ratios, namely low temperature (400°C) biochar (LTB) (Carbon Terra GmbH, Augsburg, Germany), high temperature (800°C) biochar (HTB) (Pyreg GmbH, Dörth, Germany), and digestate (PlanET Biogastechnik GmbH, Vreden, Germany) for the incubation experiment. Both chars were obtained from slow pyrolysis processes using woodchips as feedstock and the digestate was obtained from anaerobic digestion process used chicken manure, beef waste, and maize silage. Additionally, the two types of biochars were also mixed each with digestate. The main physico-chemical properties of the raw substances used for incubation are depicted in TableII.1.

II.2.2 Preparation of soils with organic amendments

Field-moist soil samples were sieved (≤2 mm), and kept at 5 ± 2°C in the dark until further analysis. Raw biochar was also sieved and the fraction between 1.5 to 2.0 mm was selected. The soil amendments were mixed as large portions with 3 kg dry mass equivalent soil in 12 L plastic pots and stored at 20 ± 5 °C in the dark. Soil moisture content was determined separately, and the soil was adjusted to 20% of maximum water holding capacity (WHC_{max}). After rewetting, the soil was stored again in the dark at 20°C for 3 to 4 days to re-establish soil humidity equilibrium and to reactivate the soil microflora. The final moisture content was adjusted to 50% of WHC_{max} by adding de-ionized water. Finally, subsamples of 50 g (dry matter equivalent) each were taken from the pots and transferred to the microcosms (250 mL Schott Duran glass bottles).

The experiment consisted of 14 different treatments in triplicate for each soil type: one control (bare soil without any amendment) and 13 different application ratios of organic residues or amendments. An overview for all samples with the labelling used throughout the study is listed in TablesII.2 and II.3. All mixtures (in triplicate) are based on dry matter basis (W/W) in contrast to most reported studies.

II.2.3 Measurement of microbial respiration

For the respiration measurements an automated 12 channel respirometer was used (Manufacturer: Messtechnik für Gasumsätze bei biologischen Prozessen, 42799 Leichlingen; Model: 12 channel Respiration Monitor equipped with a Zirconium oxydsensor Typ FCX- MCxx-CH and two IR sensors, 5000ppm and 5ppm max. range; madur electronics; madirD01v3). In total 28 different compositions in triplicate were investigated (in total 84 mixtures). The CO_2 efflux of the microcosms was recorded over one day (24 h) before disconnecting the bottles and connecting the

next sample series. Each sample was measured semi continuously by switching the gas flows between the sensors and sample bottles with a multiplex valve. This gave 8 to 10 measuring points for each sample within the given 24 hours. With respect to the turnover of samples within the respirometer device, soil respiration rates of the respective identical aliquots could be measured every 10 days. At each measurement cycle water content was adjusted to 50% WHC_{max} to provide optimal water content and aeration conditions for microbial activity (Skopp et al., 1990). Finally, the arithmetic mean and the standard deviation (STD) of the evolved CO_2 were calculated from the triplicates for each consecutive measurement date. The incubation time was 90 days for all samples and the incubation was performed at 20 ± 5°C.

II.2.4 Characterization of mixtures (DOC, $SUVA_{254}$ and pH measurement)

II.2.4.1 Determination of DOC and $SUVA_{254}$

Dissolved organic C (DOC) from mixtures was characterized according to Cox et al. (2004). Therefore, 10 g of dry mass equivalents soil (mixture) and 20 ml10 mM $CaCl_2$ were mixed in a jar and placed on a horizontal shaker at 225 rpm (SM25, Edmund Bühler) for 10 min at room temperature (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 decanted and filtered through a 0.45-µm sterile cellulose acetatemembrane filter. DOC was measured with a TOC analyser 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 was measured with Uvikon 860 UV/Vis spectrophotometer (Tegimenta AG, Rotkreuz, Switzerland). Specific UV-absorbances at 254 nm ($SUVA_{254}$) (Leenheer and Croue´, 2003; Cox et al., 2004) of the extracts were calculated by dividing the absorptions by the respective DOC concentrations. The pH of the mixtures was determined with 10 mM $CaCl_2$ at a 1:2 soil/solution ratio (w/v) with a portable pH-meter (Orion 3-star, Thermo Electron Co., USA) using a glass electrode.

Table II.1: Main physico-chemical properties of the native soils, biochars and digestate used for incubation. HTB = high temperature biochar, LTB = low temperature biochar.

Material Source /place and texture	Soil 1 Kaldenkirchen (loamy sand)	Soil 2 Merzenhausen (silt loam)	LTB Woodchips	HTB Woodchips	Digestate Maize-silage, chicken manure and beef waste
pH	6.12	6.19	7.8	7.5	8.7
Clay content (%)	4.90	15.40	-	-	-
C_{org}(%)	0.825 ± 0.006	1.15 ± 0.03	75.90	74.40	40
Total N content (%)	0.082 ± 0.006	0.126 ± 0.010	0.536 ± 0.046	0.520 ± 0.016	6.51 ± 0.02
Surface area N_2 (m^2/g)	2.05	2.12	231	225	3.09
Surface area CO_2 (m^2/g)	-	-	634	625	37.90
DOC (mgL^{-1})	3.42 ± 1.10	2.76 ± 0.33	3.97 ± 0.40	3.56 ± 0.75	1301.87
$SUVA_{254}$ (L mg^{-1} m^{-1})	6.52	1.98	1.26	1.06	5.92

-= Not determined

Table II.2: Observed cumulative CO_2-C and degraded C (both after 90 days of incubation), pool sizes (C_1 and C_2), degradation rates (k_1 and k_2) fitted by the double first order in parallel model (DFOP) [Equation II.4], as well as calculated mean residence time (MRT) [Equation II.5] for the Merzenhausen (MRZ) soil (silt loam). HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures.

SubstrateComposition	cum. CO_2-C [mg C g mixture^{-1}]	C degraded [% of added C]	Active carbon pools			Slow carbon pools		
			C_1 [%C]	k_1 [d^{-1}]	MRT [days]	C_2 [%C]	k_2 [d^{-1}]	MRT [days]
MRZ	0.47 ± 0.04	4.75 ± 0.45	2.10	1.70	0.59	97.90	0.0003	3334
MRZ-1.0 % HTB	0.54 ± 0.14	3.16 ± 0.85	1.70	1.70	0.59	98.30	0.0002	5000
MRZ-2.5 % HTB	0.77 ± 0.10	2.75 ± 0.39	0.70	1.70	0.59	99.30	0.0002	5000
MRZ-5.0 % HTB	0.85 ± 0.05	1.89 ± 0.11	2.10	1.00	1.00	97.90	0.0001	10000
MRZ-1.0 % LTB	0.52 ± 0.04	2.94 ± 0.27	1.70	1.43	0.70	98.30	0.0002	5000
MRZ-2.5 % LTB	0.52 ± 0.03	1.80 ± 0.10	0.70	1.43	0.70	99.70	0.0001	10000
MRZ-5.0 % LTB	0.56 ± 0.02	1.20 ± 0.02	2.10	1.43	0.70	97.90	0.0001	10000
MRZ-5.0 % DG	10.45 ± 0.41	36.60 ± 1.44	1.70	0.91	1.09	98.30	0.0034	294
MRZ-15 % DG	11.25 ± 0.66	18.49 ± 1.09	0.70	0.92	1.09	99.30	0.0016	625
MRZ-30 % DG	16.88 ± 5.93	14.16 ± 5.09	2.10	0.83	1.20	97.90	0.0015	667
MRZ-5 % DG: 1 % HTB	7.18 ± 0.55	20.36 ± 1.56	1.70	0.91	1.09	98.30	0.0015	667
MRZ-5 % DG: 5 % HTB	5.63 ± 0.09	9.24 ± 0.16	0.70	0.91	1.09	99.30	0.0006	1667
MRZ-5 % DG: 1 % LTB	1.36 ± 0.40	3.80 ± 1.11	2.10	0.91	1.09	97.90	0.0002	5000
MRZ-5 % DG: 5 % LTB	1.82 ± 0.45	2.89 ± 0.72	1.70	0.91	1.09	98.30	0.0002	5000

II.2.4.2 DOC adsorption study

Equilibrium adsorption experiments were conducted at room temperature (20 ± 2°C) with four different DOC concentrations (10, 20, 30, and 40 mg L^{-1}) gathered from digestate. Three different doses of low temperature biochar (100, 250, and 500 mg) were mixed to the DOC solutions (3.33, 6.66, 10.00, 13.33 mL for four different concentrations of DOC) in 50 mL centrifuge tubes (Oak ridge Nalgene centrifugation tubes, Rochester, NY, USA). Final volume of solution was made with 20 mL 10 mM $CaCl_2$. All tubes were covered by aluminum. Samples were shaken continuously for 72 h on a horizontal shaker at 225 rpm (SM25, Edmund Bühler). After, the samples were 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 decanted and filtered through a 0.45-µm sterile cellulose acetate membrane filter. Concentration of DOC in the extracts was measured with a TOC analyzer and $SUVA_{254}$ was determined with UV/Vis spectrophotometer (Please see II.2.4.1 section for details) and percentage of DOC adsorbed on the three different dosage of LTB was calculated as:

$$Ads\ [\%] = \left[\frac{(C_i - C_e)}{C_i}\right] \times 100 \qquad [II.1]$$

Where, C_i is the initial and C_e (mg L^{-1}) is the equilibrium DOC concentration water phase, respectively. C_s as the amount of sorbed DOC on the LTB (mg kg^{-1}) was calculated by:

$$C_s = (C_i - C_e) \times \frac{V}{M} \qquad [II.2]$$

Where V is the volume of DOC solution (mL) and M is the mass of LTB added (mg).

II.2.5 CO_2 flux calculation

The cumulative amount of CO_2 evolved from the mixtures during the incubation study was calculated as CO_2-C using stepwise integration of the instantaneous fluxes over the entire incubation time period:

$$C = \int_0^T C(t)\, dt \qquad [II.3]$$

With *C* (t) [µg min^{-1}] as the instantaneous CO_2-C flux at time *t* [min], *dt* as the time interval [min], and *T* as the time of sampling.

In a next step, the fluxes were related to soil dry matter of the input mixture for direct comparison and /or related to input C content to calculate the percentage of C degraded.

II.2.6 Kinetics of the carbon turnover

For the description of the dynamics of carbon turnover a double carbon pool or double first order in parallel model (DFOP) was used, whereby the corresponding CO_2-C efflux over time t [d] can be described by:

$$C_t = (C_1 \times e^{-k_1 t}) + (C_2 \times e^{-k_2 t}) \qquad [II.4]$$

where *C* (t) is the mineralized total C stock [%], C_1 is the total percentage of the labile (active) C-pool from total C, C_2 is the percentage of the refractory (slow) C-pool which is basically 1-C_1, k_1 is the first order mineralization rate of the labile C-pool [d^{-1}], and k_2 is the first order mineralization rate of the refractory C-pool [d^{-1}] (Liang et al., 2008; Qayyum et al., 2012).

Mean Residence Time (MRT) (days) for the labile and refractory carbon pools can be calculated from their corresponding mineralization rates, k_1 *and* k_2 respectively by:

$$MRT = \left(\frac{1}{k_1 \ or \ k_2} \right) \qquad [II.5]$$

II.2.7 Statistical Analysis

The parameters providing the best prediction of the measured data were determined by minimizing the sum of squared residuals:

$$SSR = \sum_{i=1}^{n} (x_{obs,i} - x_{sim,i})^2 \qquad [II.6]$$

Where, x_{obs} and x_{sim} are the observed and simulated cumulative CO_2-C fluxes [g CO_2-C g^{-1} mixture] at time step *i* and *n* is the total number of observations. For the minimization of the objective function [EquationII.6] the global optimization routine shuffled complex evolution developed at the University of Arizona (SCE-UA) as

described by Duan et al. (1992 and 1994) was used. This optimization routine has been already successfully applied in a wide range of applications in hydrology (Mertens et al., 2005; Mboh et al., 2011) but also for the estimation of parameters in non-linear C models (Weihermüller et al., 2009 and 2013; Bauer et al., 2012).

To quantify the quality the agreement between measured and fitted data of the inversion the coefficient of determination R^2 was calculated:

$$R^2 = \left[\frac{\sum_{i=1}^{n} (x_{obs} - \bar{x}_{obs})_i (x_{sim} - \bar{x}_{sim})_i}{\sqrt{\sum_{i=1}^{n} (x_{obs} - \bar{x}_{obs})^2_i \sum_{i=1}^{n} (x_{sim} - \bar{x}_{sim})^2_i}} \right]^2 \qquad [II.7]$$

Where, $\bar{x}_{obs}$ and $\bar{x}_{sim}$ are the arithmetic mean of the fitted and measured cumulative CO_2-Cfluxes,respectively.

Table II.3: Observed cumulative CO_2-C and degraded C (both after 90 days of incubation), pool sizes ($C_1$and C_2), degradation rates ($k_1$and k_2) fitted by the double first order in parallel model (DFOP) [Equation II.4], as well as calculated mean residence time (MRT) [Equation II.5] for the Kaldenkirchen (KK) soil (loamy sand). HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures.

SubstrateComposition	cum. CO_2-C [mg C g mixture^{-1}]	C degraded [% of added C]	Active carbon pools			Slow carbon pools		
			C_1 [%C]	k_1 [d^{-1}]	MRT [days]	C_2 [%C]	k_2 [d^{-1}]	MRT [days]
KK	0.31± 0.03	3.60 ± 0.20	1.40	3.77	0.26	98.60	0.0002	5000
KK-1.0 % HTB	0.38 ± 0.04	2.38 ± 0.14	0.70	3.77	0.26	99.30	0.0002	5000
KK-2.5 % HTB	0.60 ± 0.05	2.25 ± 0.10	0.80	2.10	0.48	99.20	0.0001	10000
KK-5.0 % HTB	0.72 ± 0.31	1.65 ± 0.40	0.80	1.30	0.77	99.20	0.0001	10000
KK-1.0 % LTB	0.47 ± 0.05	2.90 ± 0.18	1.10	0.80	1.25	98.90	0.0002	5000
KK-2.5 % LTB	0.75 ± 0.26	2.75 ± 0.53	0.30	1.20	0.83	99.70	0.0002	5000
KK-5.0 % LTB	0.69 ± 0.15	1.52 ± 0.20	0.60	0.80	1.25	99.40	0.0001	10000
KK-5.0 % DG	11.32 ± 0.90	41.55 ± 22.50	18.60	1.20	0.83	81.40	0.0045	222
KK-15 % DG	14.00 ± 2.60	21.42 ± 4.00	9.90	1.20	0.83	90.10	0.0020	500
KK-30 % DG	14.90 ± 2.31	15.06 ± 2.34	7.60	1.20	0.83	92.40	0.0011	910
KK-5 % DG: 1 % HTB	7.48 ± 4.44	22.01 ± 13.08	18.80	0.07	14.28	81.20	0.0004	2500
KK-5 % DG: 5 % HTB	4.83 ± 2.37	8.09 ± 3.90	5.30	0.17	5.88	94.70	0.0003	3334
KK-5 % DG: 1 % LTB	1.87 ± 0.70	1.87 ± 0.70	3.10	0.17	5.88	96.90	0.0003	3334
KK-5 % DG: 5 % LTB	1.09 ± 0.10	1.09 ± 0.10	0.90	0.16	6.25	99.10	0.0001	10000

II.3 Results and Discussion

II.3.1 Cumulative CO_2-C releases

The cumulative CO_2 evolution measured in the microcosms over the course of the incubation experiment for the Merzenhausen (silt loam) and Kaldenkirchen (loamy sand) soil and the corresponding mixtures are plotted in Figure II.1 and II.2. In the following the cumulative CO_2 evolution after 90 days as listed in Table 2 and 3 will be discussed. The values for the two contrasting native soils without amendments were 0.47 ± 0.04 mg CO_2-C g^{-1} soil for Merzenhausen and 0.31 ± 0.03 mg CO_2-C g^{-1} soil for Kaldenkirchen soil, whereby the CO_2 evolved for the Kaldenkirchen was only 66 % of the Merzenhausen soil. The lower CO_2 flux for the Kaldenkirchen soil was in line with the relative difference in the total C content of about 71% of the Kaldenkirchen soil (0.825 % ± 0.006) compared to the Merzenhausen soil (1.15 % ± 0.03).

Respiration was substantially higher where 30% digestate was added due to the large amount of fresh C added for both Kaldenkirchen and Merzenhausen soils. Nevertheless, total cumulated CO_2-C was slightly larger for the Merzenhausen soil mixture (with 16.88 ± 5.93 mg CO_2-C g^{-1} soil compared to the Kaldenkirchen soil mixture with 14.90 ± 2.31 mg CO_2-C g^{-1} soil, whereby the relative difference was still 12%. Soil mixtures with less digestate (15 and 5%) had lower respiration rates, which can be expected due to the lower amount of available C in the mixtures. Surprisingly, the height of the CO_2 flux did not correspond linearly to the total amount of C in these mixtures. The Kaldenkirchen soil mixture with 15% digestate evolved 14.00 ± 2.60 mg CO_2-C g^{-1} soil which is only 6% less compared to 30% digestate. In Kaldenkirchensoil mixture with 5% digestate the flux added up to 11.32 ± 0.90 mg CO_2-C g^{-1} soils, which is only 24% less compared to the 30% addition. The same trend can be found for the Merzenhausen soil, whereby the 15% digestate already showed a much lower absolute (11.25 ± 0.66 mg CO_2-C g^{-1}) CO_2 flux. Addition of only 5% digestate reduced CO_2 release even more by 38.1%. The mechanisms for these differences between digestate loading and increase in CO_2 evolution are still unclear but show a kind of saturation effect in the turnover as already observed by Cayuela et al. (2009) and Liu (1998).

For the lowest loading with high temperature biochar (1% w/w), CO_2 evolution is 114 and 122 % compared to the native Merzenhausen and Kaldenkirchen soil and for the highest biochar loadings (5% w/w) 180 and 232 %, respectively. For the low

temperature biochar CO_2 evolution is in the same range and again total CO_2 evolution is slightly higher for the Kaldenkirchen as for the Merzenhausen soil. Again the CO_2 evolution and biochar loadings are not a 1:1 relationship and likewise show a kind of saturation effect as for the digestate. Nevertheless, an increase of CO_2 evolution with higher biochar loadings is detectable, which indicates that part of the biochar can be degraded even during the relatively short incubation period as already shown by Pietikainen et al. (2000) or Zimmerman et al. (2011). On the other hand, the reported higher flux of CO_2 at the beginning of the incubation of biochar amended soils as reported by Kuzyakov et al. (2009) or Steinbeiss et al. (2009) could not be observed.

Mixtures of digestate and biochar indicate a more complex behavior as can be seen from Figure II.1 and II.2 and Table II.2 and II.3. Hereby even relatively low additions of biochar to the soil digestate mixture reduced CO_2 evolution, which could be potentially produced from the digestate in the mixture. For example, 1% of biochar added to the 5 % soil/digestate mixture reduced CO_2 evolution by more than 45% for all soils and biochar types. Increasing the biochar ratio to 5% shows an even smaller flux with less than 83 % of the digestate/soil mixture alone. This reduction in C turnover in addition of biochar has been already reported by Keith et al. (2011) and Zimmermann et al. (2011).

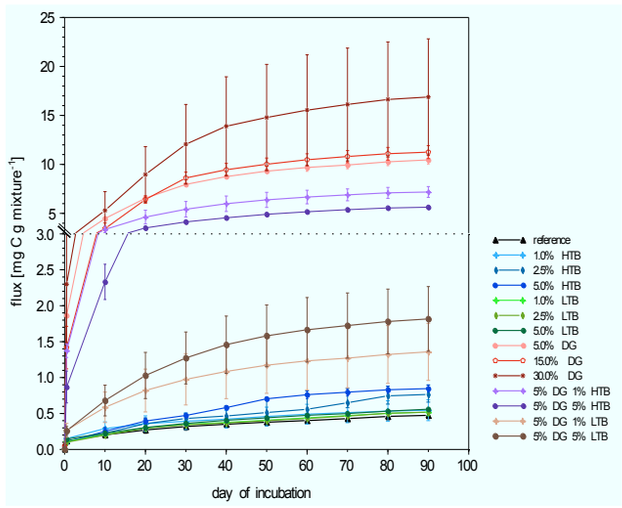


Figure II.1: Cumulative amount of CO_2-C evolution [mg g^{-1} dry mass mixture] for the Merzenhausen soil (silt loam). Control = Merzenhausen soil (silt loam), HTB = high

temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Error bars indicate standard deviation.

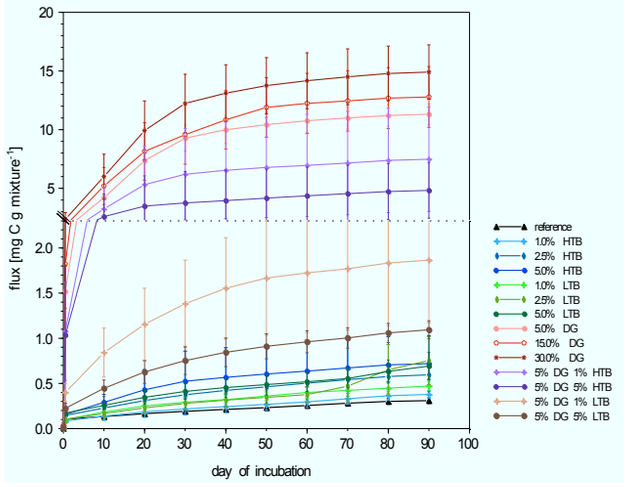


Figure II.2: Cumulative amount of CO_2-C evolution [mg g^{-1} dry mass mixture] for the Kaldenkirchen soil (loamy sand). Control = Kaldenkirchen soil (loamy sand), HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Error bars indicate standard deviation.

II.3.2 CO_2 release with respect to C added

In the following step, the ratio of degraded C was calculated and the results are also listed in Table II.2 and II.3. For both soils, the percentage of degraded C was highest following the addition of 5% digestate (Figure II.3 and II.4), where roughly 40 % of the total added carbon was turned over within 90 days. For higher digestate loadings the turnover was much slower with less than 22% and 17% for the 15 and 30% digestate loadings. A kind of saturation effects occurred leading to fewer turnover for higher digestate based C contents, which may relate to higher N content of the pure digestate (Table II.1). This is supported by the observations of Cayuela et al. (2009) and Tenuta and Lazarovitis (2004), who illustrated that the higher percentage of amendment lead to NH_3 toxicity to different microbial species in soil. They also found an inverse relationship between the percentage of mineralized C and application rate of organic amendments. To account for this effect Liu (1998) proposed a growth yield

model where “energy uncoupling” is the driving mechanism for suppression of microbial growth under “substrate-sufficient” conditions. He also observed the mismatch between fundamental biochemical processes such as anabolism and catabolism.

Relative degradation also dropped for the mixtures where biochar was added to the soil, whereby the differences in C degraded are less pronounced in comparison to the native soil. For both the high temperature and low temperature biochar maximum relative degradation was detectable for the lowest amount of char added to the system compared to highest loadings probably due to sorption of DOC to the biochar surface.

Biochar additions to the digestate/soil mixture reduced not only total CO_2 evolved as discussed before but also the relative proportion of degraded C, whereby for both soils the addition of 1 % high temperature biochar to the 5 % digestate/soil mixture reduced the degradation by > 45 % and 1% low temperature biochar mixed to the 5 % digestate/soil mixture reduced the relative degradation to <13%. For higher biochar additions the relative degradation dropped even more. Again differences between the biochars are detectable, which have to be associated to the pyrolysis temperature and the physico-chemical characteristics of the chars.

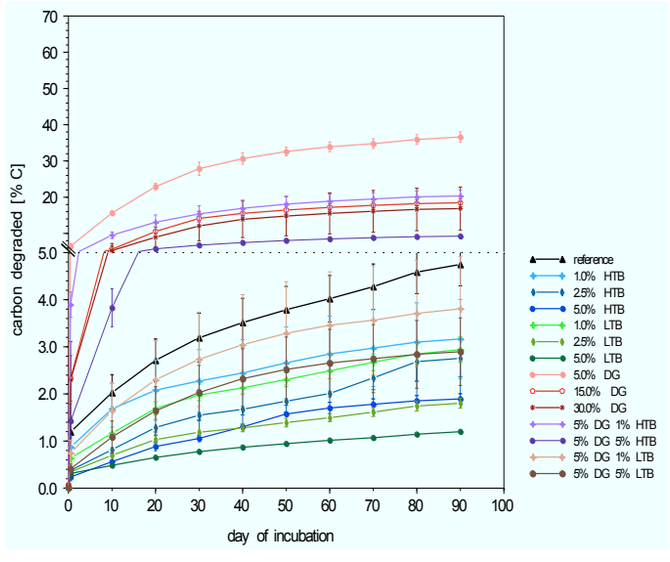


Figure II.3: Percentage of C degraded with respect to total C added to the system for the Merzenhausen soil (silt loam). Control = Merzenhausen soil (silt loam), HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Error bars indicate standard deviation.

II.3.3 Carbon turnover kinetics

Finally, the C turnover kinetics was estimated using the double pool model [EquationII.4].The total percentage of the labile (C_1) and refractory pool (C_2) and their corresponding mineralization rates , k_1 and k_2 [d^{-1}] as well as the mean residence time (MRT) [days] are listed in Table II.2 and Table II.3 for the Merzenhausen and Kaldenkirchen soil based mixtures, respectively. The goodness of the fit expressed by the R^2 (Equation II.7) exceeds 0.98 for all samples, indicating that the DFOP is the adequate model to describe the data sufficiently.

For both reference soils (MRZ and KK) the largest proportion of total C was allocated to the slow C-pool (C_2) with more than 97.9 % of the TOC. Additionally, both soils showed large MRTs for the slow C-pools with 3334 years for the Merzenhausen and 5000 years for the Kaldenkirchen soil. The fast C-pool (C_1) which turned over with MRTs of 0.59 and 0.26 years for both soils indicate that only a small but still active C-pool was detectable. Surprisingly, the slow C-pool seems to turnover faster for the clayic Merzenhausen soil compared to the sandy Kaldenkirchen soil, which is in contradiction to findings that clay stabilized C in the soil (Six et al., 1998). On the other hand, these long-term turnover cannot be precisely described using a short-term incubation experiment of only 90 days.

For the Merzenhausen soil the total percentage of the fast C-pool (C_1), as well as the corresponding rate constants (k_1) and MRTs did not differ much between the reference soil and the mixtures, whereby smallest MRTs were found for the reference soil and low dosage of HTB char (1 and 2.5%). On the other hand, digestate alone based mixtures did not increase the labile C-pool and corresponding MRTs increased slightly. Adding biochar to the digestate did not change the proportion or the MRTs either. For the mineralization of the slow C-pool (C_2) an order in the rate constant k_2 of: digestate > digestate + biochar based mixture ≥ control soil ~ biochar, could be found.

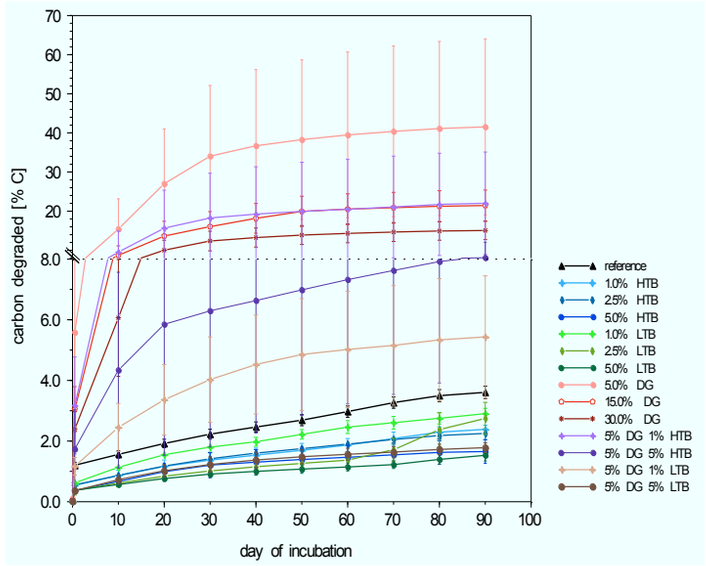


Figure II.4: Percentage of C degraded with respect to total C added to the system for the Kaldenkirchen soil (loamy sand). Control = Kaldenkirchen soil (loamy sand), HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures.

The influence of the amendments is more pronounced in the results of the slow C-pool, where the total fraction stays nearly constant between reference soil and all mixtures but MRTs increased for the biochar based mixtures slightly, whereby no clear trend between the two types of char is detectable. The MRTs decreased substantially for the digestate only based mixtures down to less than 667 day, which is a 5 times reduction compared to the reference soil. This decrease is caused by the carbon added to the soil which is neither fully easily degradable nor recalcitrant. Mixing biochar to the digestate increased again the MRT, whereby the low temperature biochar (LTB) indicted a stronger effect compared to the high temperature biochar. For the slow C-pool turnover MRTs increased in the order: digestate based mixtures, digestate + biochar to the biochar only soils.

For the Kaldenkirchen based soil mixtures the percentage of the labile C-pool varies much stronger and a fraction of more than 18% was fitted for the mixture with 5 % DG as well as 1% DG and 1% HTB. Additionally, MRTs are slightly lower for the fast C-pool except for the digestate + biochar based mixtures, where MRTs are roughly 5 times larger as for the Merzenhausen based mixtures. An extreme high

MRT could be found for the Kaldenkirchen soil mixed with 1 % DG and 1% HTB with 14.28 years. The reasons for these differences are unclear. Again a clearer order is detectable for the slow C-pool, where again MRTs are slowest for the digestate based mixtures followed by digestate + biochar and biochar only mixtures. Also the recalcitrant nature of the biochar is detectable with largest MRTs for these samples.

II.3.4Characterization of Soil, Biochar, and Digestate Mixture

All of the mixtures used for the respiration study showed slightly acidic pH-values ranging from 6.04 to 6.74 (see Table II.4), whereby the Kaldenkirchen soil has slightly lower pH-values due its sandy character. Additionally, digestate based mixtures had highest pH-values, which are caused by the alkaline character of the digestate. The two contrasting soil types contained different amounts of clay, whereby the Merzenhausen soil had >3 times more clay as the Kaldenkirchen soil. Generally, the sorption capacity of a the soil for organic matter is related to the surface area of the soil which in turn is related to its clay content (Nelson et al., 1997), because most clays have a net negative charge, small size and large surface area (Oades, 1988). Additionally, clay rich soils tend to form stable aggregates which physically protect the organic substance (Six et al., 1998). Therefore, our hypothesis was that water extractable DOC content will decrease with increased clay content due to greater sorption of DOC onto the clays. However, this was not the case except for the 15% and 30 % digestate based mixtures. Because this phenomenon cannot be described by the clay content alone other soil properties must also play a role. Clay content also does not affect $SUVA_{254}$, and therefore, does not change DOC quality (see TableII.4).

Table II.4: Main physico-chemical properties of the mixtures for the Kaldenkirchen (KK) soil (loamy sand) and Merzenhausen (MRZ) soil (silt loam) used for incubation. HTB = high temperature biochar, LTB = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures.

Substrate Composition	pH		DOC [mgL^{-1}]		$SUVA_{254}$ [$L\ mg^{-1}\ m^{-1}$]	
	KK	MRZ	KK	MRZ	KK	MRZ
1.0 % HTB	6.04	6.15	1.91 ± 0.20	2.23± 0.43	5.36	1.43
2.5 % HTB	6.05	6.17	2.63 ± 0.06	2.34 ± 0.21	3.26	1.48
5.0 % HTB	6.07	6.22	3.27± 0.70	3.58± 0.19	8.14	2.19
1.0 % LTB	6.09	6.12	1.73 ± 0.13	1.57± 0.32	4.43	1.36
2.5 % LTB	6.14	6.14	2.15 ± 0.58	2.10 ± 0.51	7.50	1.81
5.0 % LTB	6.06	6.06	3.53 ± 0.46	2.14 ± 0.15	13.09	2.33
5.0 % DG	6.16	6.34	9.69 ± 0.24	10.10 ± 0.89	17.31	4.35
15 % DG	6.20	6.29	26.73 ± 5.06	17.53± 1.49	18.62	4.43
30 % DG	6.26	6.74	41.69 ± 3.09	23.63± 1.07	25.02	4.73
5 % DG: 1 % HTB	6.17	6.25	5.25 ± 1.45	6.32± 0.32	8.41	3.27
5 % DG: 5 % HTB	6.15	6.26	4.84± 1.63	5.25 ± 0.47	5.42	2.32
5 % DG: 1 % LTB	6.13	6.20	4.36 ± 1.10	4.63± 0.70	6.81	1.97
5 % DG: 5 % LTB	6.06	6.16	2.64 ± 0.24	2.26 ± 0.34	11.05	2.41

Compared to pH-values extractable DOC differs greatly between the soil mixtures (Table II.4), whereby digestate based mixtures showed highest extractable DOC. For these mixtures extractable DOC increased also with increasing digestate content and mixtures with high and low temperature biochar had much lower extractable DOC with no clear trend between the two biochars. Interestingly, extractable DOC dropped in the biochar/digestate soil mixtures compared to the digestate soil mixtures by a factor of >1.8 and >1.6 for the Kaldenkirchen and Merzenhausensoil, respectively. Based on these data, biochar seems to act as a sink of DOC.

Digestate based mixtures showed significantly higher $SUVA_{254}$ values than the biochar/soil mixtures and, additionally, Merzenhausen soil based mixtures showed much lower $SUVA_{254}$ values compared to the Kaldenkirchen based soil mixture. This means that DOC extracted from digestate based mixtures is more aromatic compared to the DOC extracted from biochar and that DOC extracted from Merzenhausen soil based mixtures is also less aromatic compared to the DOC extracted from the Kaldenkirchen soil. This can be explained by the fact that the hydrophobic nature of biochars tends to preferentially bind aromatic fractions of the DOC and that the silt-clay rich Merzenhausen soil also adsorbs major fractions of the aromatic DOC.

II.3.4.1 Influence of DOC, SUVA and clay content on CO_2 evolution

As Marschner and Kalbitz (2003) stated in their review paper dissolved organic C might be probably the most bioavailable fraction of soil organic C, since all microbial

uptake mechanisms require an aqueous environment (Metting, 1993). A conceptual model for the forming of DOC from SOC and the microbial turnover of DOC is provided in Figure 6a without the intention to be complete. The microbial turnover of DOC depends by microbes depends not only on the total available DOC but also on its aromaticity and hydrophobicity, which increases its recalcitrance and might inhibit enzyme activities. Additionally, if DOC is hidden in pores, which are present when biochar is added to the soils, DOC will not be accessible for microorganisms (Zsolnay, 1997).

To analyze if the amount of extractable DOC and aromaticity (measured by $SUVA_{254}$) can describe the CO_2 efflux differences as seen in our incubation study (especially between digestate and char/digestate based mixtures) these soil parameters were correlated against total evolved CO_2. As can be seen in Figure II.5 there is a strong logarithmic correlation (R^2 = 0.90) between extractable DOC and total CO_2 evolved over the 90 days of incubation. It has to be noted that not the regression function itself is of high importance because it may change with the extraction procedure applied, but the overall shape of the function plays an important role. As discussed earlier, higher DOC values could be extracted in digestate based mixtures followed by char/digestate and char based ones. This is in good agreement with the CO_2 evolution measured in the incubation study. A comparable correlation for Australian pasture topsoil over an incubation time of 21-days was found by Marschner and Noble (2000), whereby their relationship was more linear-like. Based on the information which can be deduced from the regression (low DOC leading to low CO_2 and high DOC to high CO_2) the question arise which mechanisms and parameters influence extractable DOC amounts. The simplest explanation for height of extractable DOC would be the total amount of available C in the soil. The Lowest SOC contents were in the native soil and increased with biochar, digestate, and digestate/biochar based mixtures. Unfortunately, total mass of carbon cannot explain the full behavior because mixing a small proportion of biochar to the same amount of digestate shows that the biochar addition will reduce CO_2 evolution but also extractable DOC. Therefore, it seems that either DOC production is limited in systems where biochar was added or that the biochar sorbed some of the DOC which will then not be available for the microbes. To illustrate the mechanism which might be responsible for the lower CO_2 production in biochar amended soils the conceptual model in Figure II.6b can be used.

To prove that significant amounts of DOC can be sorbed to the biochar a DOC sorption experiment was performed using DOC produced from raw digestate. This DOC was diluted to four different concentrations and three different amounts of low temperature biochar were added to the system.

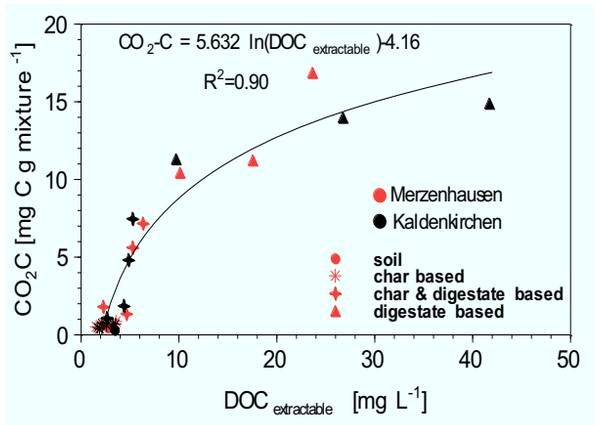


Figure II.5: Logarithmic regression function between extractable dissolved organic C (DOC) and cumulative amount of CO_2-C after 90 days of incubation [mg g^{-1} dry mass mixture] for the Kaldenkirchen soil (loamy sand) and Merzenhausen soil (silt loam).

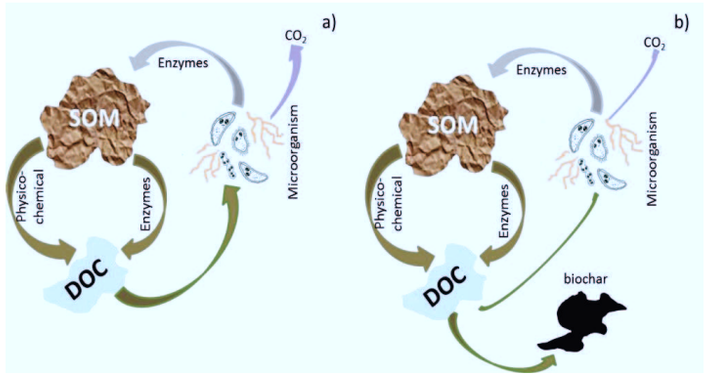


Figure II.6: Conceptual model for SOC turnover a) without biochar and b) with biochar added to the system.

As can be seen from Table II.5 and FigureII.7 DOC sorption to the biochar increased more or less linear by increasing DOC concentration. This means that DOC can be withdrawn from the microbial accessible liquid phase at high quantities in the presence of biochar. Several other studies already reported that DOC can be

significantly sorbed to biochar, (Smith et al., 1992; Jin, 2010; Liang et al., 2010), leading to stabilization of organic matter in biochar amended soils. Looking at the percentage of DOC which can be potentially sorbed to the biochar it turns out that up to 70 % of the DOC can be sorbed at high biochar load. Normalizing the absorption of DOC on the amount of biochar added to the system (DOC absorbed in mg DOC per kg biochar added) shows a slightly different picture with lower relative amounts of DOC which can be sorbed to high biochar additions (see also Figure II.7). Finally, aromaticity is lower for those batches where higher biochar additions were used indicating, that aromatic DOC will be preferentially sorbed to the char leading to an enrichment of less aromatic DOC in the microbially accessible liquid phase. Therefore, two opposing mechanisms occur simultaneously in the liquid phase in presence of biochar: i) reduction of DOC leads to lower CO_2 production and ii) enrichment of less aromatic DOC which might favor DOC degradation and CO_2 formation.

As already mentioned, CO_2 evolved for the Kaldenkirchen was only 66 % of the Merzenhausen soil despite the difference in clay content, which indicates that clay is not playing a major role in C mineralization at short time scales. Also for the other biomixtures higher CO_2 fluxes were found for Merzenhausen soil. These findings contradicted with the observation by Liang et al. (2008) who observed that old black carbon mineralized at similar rates in soils of different texture. On the other hand, Kuzyakov et al. (2009) observed enhanced mineralization of biochar in silt loam soil (mostly during the first 3 months) over a total incubation period of >3 yrs. The hypothesized that mechanical disturbance which occurred during mixing of the soil with the biochar lead to release of labile organic matter from protected sites, which facilitated faster mineralization rates of the biochar at the beginning of the experiment.

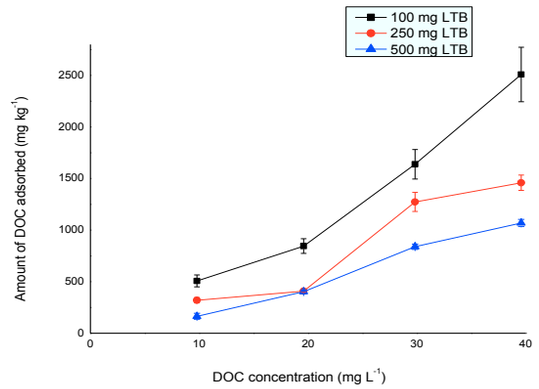


Figure II.7: Adsorption isotherm for DOC on LTB (*n* = 3).

II.4Conclusions

In the study presented incubation experiments with different soil amendments, namely biochar and digestate, were performed over the course of 90 days. Hereby not only the amendments were used in different application ratios but the amendments were also mixed together with the soil to analyze their interactions with soil texture. Additionally, two contrasting soil types (loamy sand and silt loam soil) were used. The dynamics of C mineralization followed a biphasic pattern which leads to rapid decomposition at the early incubation periods and then decomposition gradually slowed down in a comparatively steady stage. This mineralization pattern could be well described by a bi-exponential or two pool model.

Expected the highest addition of a fresh C source (digestate) lead to the largest CO_2 fluxes, whereby the increase in CO_2 flux was not proportional to the amount of digestatate.

Table II.5: DOC and $SUVA_{254}$ values for the three different amounts of low temperature biochar (LTB) added to different DOC concentrations

Mixture composition		DOC in aqueous phase	$SUVA_{254}$	DOC adsorbed	
Digestate DOC [mg L^{-1}]	LTB [g L^{-1}]	[mg L^{-1}]	[L mg^{-1} m^{-1}]	% adsorption	[mg kg^{-1}] sorbed
10					
(9.79 ± 0.17)	5	7.22 ± 0.19	1.38 ± 0.11	25.92	507.5 ± 57.10
12.5		5.79 ± 0.68	1.25 ± 0.24	40.86	320 ± 26.1
25		5.68 ± 0.30	0.87 ± 0.08	42.01	164.5 ± 28.9
20					
(19.60 ± 0.33)	5	16.39 ± 0.12	1.98 ± 0.15	21.57	845.4 ± 71.5
12.5		14.60 ± 0.42	0.92 ± 0.04	26.00	407.4 ± 10.5
25		9.53 ± 0.22	0.83 ± 0.01	51.33	402.3 ± 18.0
30					
(29.88 ± 0.49)	5	21.65 ± 0.67	2.36 ± 0.10	27.46	1638 ± 143
12.5		13.92 ± 1.08	2.01 ± 0.27	53.35	1273 ± 92.1
25		8.86 ± 0.62	1.57 ± 0.07	70.28	838 ± 26.5
40					
(39.59 ± 1.20)	5	27.04 ± 1.24	2.54 ± 0.13	31.69	2508 ± 264
12.5		21.34 ± 0.87	2.19 ± 0.16	46.09	1460 ± 74.3
25		12.84 ± 0.82	1.97 ± 0.03	67.55	1069 ± 34.7

Surprisingly, mixtures of digestate and biochar indicated a profound suppression of CO_2 evolution even at relatively low biochar additions (1 % W/W).In this context, both soil types reacted in the same way. To analyze the mechanism of this reduction in soil C turnover additional measurements were performed to characterize the soil/digestate/biochar mixtures. It was found that extractable DOC content highly correlates with the total CO_2 evolved over 90 days and that the addition of biochar to the system significantly reduced microbial accessible DOC in the liquid phase by DOC sorption. Additionally, more aromatic DOC seems to be favorably sorbed to the biochar, and therefore, the microbially accessible liquid phase is enriched with more labile DOC which on the other hand can be turned over more easily. In consequence, two contrasting mechanisms compete in the C turnover if biochar is added to the soil. i) DOC sorption to the biochar and therefore, reduction of the degradable DOC pool, and ii) enrichment of labile (or less aromatic) DOC in the microbial accessible liquid phase which favors C (DOC) turnover. It seems that the DOC reduction overcompensates the enrichment of less aromatic DOC and consequently totals C-turnover is reduced in presence of biochar. To quantify these effect and for generalization more and specific research is needed, where the DOC production (quality and quantity) should be studied not only at the end of the experiment, but also over the course of incubation. This increase of understanding of C turnover in biochar amended soils will help to improve the assessment of the environmental and economic benefits of biochar addition to agricultural soils.

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

Modified on the basis of

Mukherjee, S., Tappe, W., Weihermüller, L., Hofmann, D., Koeppchen,S., Laabs, V., Schroeder, T., Vereecken, H., Burauel, P., 2016b. Dissipation of bentazone, pyrimethanil and boscalid in biochar and digestate based soil mixtures for biopurification systems. Sci.Total Environ. 544,192-202.

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

III.1Introduction

Inappropriate use of pesticides can cause high concentrations in soils, ground and surface-waters with significant environmental consequences (Kolpin et al., 1995 and 1998; Acevedo et al., 2011). In general, pesticide pollution of water stemms either from diffuse source pollution caused e.g. by pesticide leaching to goundwater or by surface runoff from fields to water bodies (Carter, 2000). Pollution may also origin 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 CO_2) and dissipation (sum of mineralisation, metabolization, 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 ($MinT_{50}$) or to dissipate (DT_{50}). Hereby the DT_{50}, 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 (DT_{50}) and mineralization ($MinT_{50}$) half-life times have to be determined for each pesticide and soil combination individually.

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

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 Chenivesse, 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 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 matterto 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 controversally 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., 2016a). 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 and2013) 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 ^{14}C from pesticide labelling 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 ^{14}C is out of scope of this paper. As different pesticides react diversely in the soil systems 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 ^{14}C labelled 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.

III.2 Material and Methods

III.2.1 Substrates

For the experiment, loamy sand topsoil (0 to 10 cm depth) from Kaldenkirchen, Germany (51°19'13 N 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. (2016a). The main physico-chemical properties of the raw substances and soil mixtures used for the experiments are listed in Table II.1 and Table III.1, 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.

III.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 labelled (^{14}C 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 III.2.

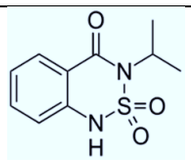
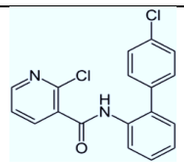
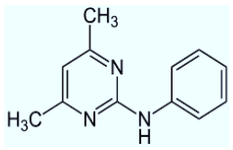
III.2.3 Characterization of used soil-mixtures

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 $CaCl_2$ 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 analyser 5050A equipped with an autosampler ASI-5000A from Shimadzu (Kyoto, Japan) after acidification and sparging the samples for 1 min.

Table III.1: 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)	C_{org} (%)	Surface area N_2 ($m^2 g^{-1}$)	Extractable DOC ($mg\ L^{-1}$)	$SUVA_{254}$ ($L\ mg^{-1}\ m^{-1}$)
Reference soil (KK)	6.1	4.3	0.82	2.0	3.4 ± 1	6.5
5.0 % BC	6	8.2	4.8	8.6	3.5 ± 0.5	13
5.0 % DG	6.1	8.2	2.8	3.5	9.7 ± 0.2	17
30 % DG	6.2	17	12	3.3	42 ± 3	25
5 % DG & 5 % BC	6.0	10	6.7	6.9	2.6 ± 0.2	11
30 % DG & 5 % BC	6.5	18	16	4.2	4.9 ± 0.3	9

Table III.2: Physico-chemical and degradation properties ofused pesticides (PPDB, 2016)

	Bentazone	Boscalid	Pyrimethanil
Structure			
Type	Herbicide	Fungicide	Fungicide
Molecular formula	$C_{10}H_{12}N_2O_3S$	$C_{18}H_{12}Cl_2N_2O$	$C_{12}H_{13}N_3$
Molecular weight (g mol^{-1})	240	343	199
Melting point (°C)	140	143	96
Vapour pressure (25 °C, mPa)	0.17	72 X 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

UV absorbance at 254 nm (UVA_{254}) 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 ($SUVA_{254}$) (Leenheer and Croué, 2003; Cox et al., 2004) of the extracts were obtained by dividing the UVA_{254} values by therespective DOC concentrations. The pH of the soil/soil-mixtures was determined by equilibrating soil/soil-mixture with 10 mM $CaCl_2$(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 Lüer and Böhmer (2000): In a first step 2.5 g soil was equilibrated with 10 mL 1 M NH_4Cl for 24 h. Subsequently, a folded paper filter (640d, Macherey-Nagel, Düren, Germany) was wetted with 1 M NH_4Cl and placed in a filter funnel. The wet soil was completely transferred to the filter and percolated with 1 M NH_4Cl until a volume of 100 mL percolate was collected. Exchangeable cations (Al^{+3}, Ca^{+2}, K, Mg^{+2}, Na) were determined in the filtrate using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Ciros CCD, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

III.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 III.1. 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.

^{14}C 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 biomixures. 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 hrs 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 CO_2-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.

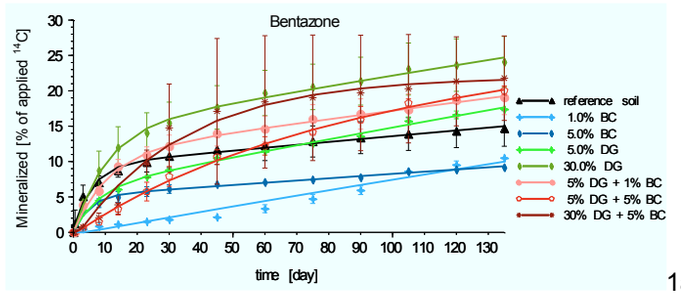
Table III.3: Mathematical expressions for different kinetic models used in the incubation study and estimation of $MinT_{50}$ and DT_{50}.

model	Mathematical equation	$MinT_{50}$ /DT_{50} determination
Simple first order (SFO)	$M_t = M_O e^{-kt}$	$MinT_{50}$ /DT_{50} = ln 2/ k
Bi-Exponential (DFOP)	$M_t = M_1 e^{-k_1^d t} + M_2 e^{-k_2^d t}$ Where, M_2 = 100- M_1	iterative method

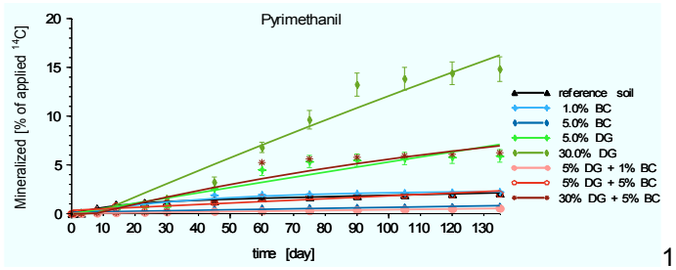
To determine any pesticide losses over the course of preparation of the incubation system, soil subsamples were taken immediately from each incubation flask and combusted via an biological oxidizer (OX 500, R.J.Harvey Instrument Corp., Tappan, NY, USA). Evolving $^{14}CO_2$ was trapped in Oxysolve C-400 oxidizer scintillation cocktail (Zinsser Analytic, Germany), and analyzed using liquid scintilation 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.

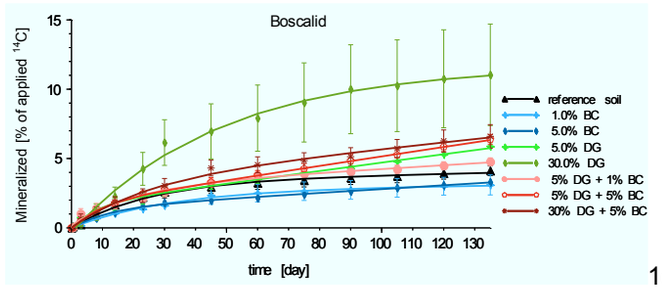
Pesticide mineralization from the incubation flasks was measured by trapping evolved $^{14}CO_2$ in 2 M NaOH solution, whereby the NaOH traps were replaced after 0, 3, 8, 14, 23, and 30 days after application, and thereafter twice a month until day 135. Quantification of trapped $^{14}CO_2$ was done via LSC.Based on a preliminary study (Mukherjee et al., 2016a) and calculations, it was ensured that all evolved CO_2 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.



1a



1b



1c

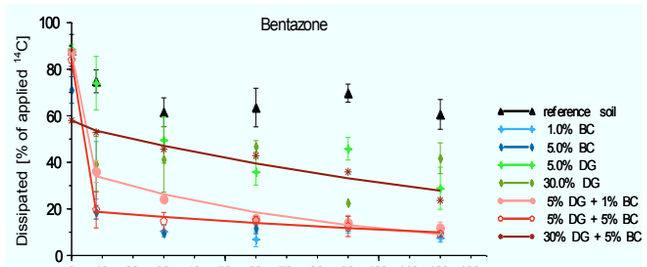
FigureIII.1a-1c: Pesticide residues calculated from complete mineralization of ^{14}C-bentazone, pyrimethanil, and boscalid in % for the different soil/amendment mixtures. Error bar represents standard deviation (n = 3). Reference soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Note that the y-axis does not have the same scale for better visualization. Points indicate measurements and line the best fitting model as listed in Table A1.

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 50ml 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 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 sequestions/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 $^{14}CO_2$ and quantified via LSC.

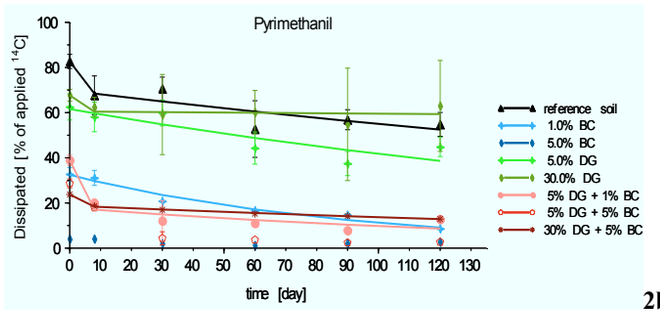
III.2.5 Analytical procedures

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 × 4.6 mm × 5 µm 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, ≥99.9% purity) was used for bentazone and pyrimethanil and acetonitrile (Merck Lichrosolv, ≥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

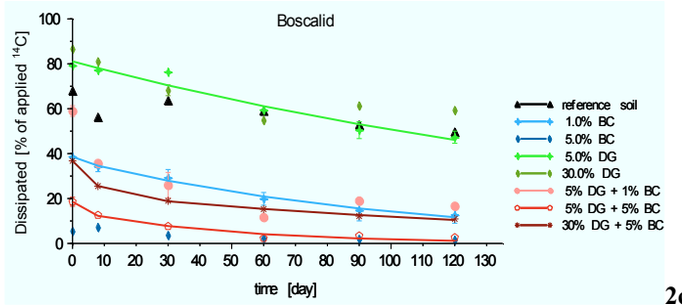
100% solvent B for 11 min. Hold 100% B for 16 min, switch back to 70% A and hold for 25 min. The UV detector was adjusted to 219, 243, and 270 nm for bentazone, boscalid, and pyrimethanil, respectively. Quantification of active ingredients via radio-HPLC was performed by calculating the measured radioactivity 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 based on an injection volume of 0.25 ml. Therefore, LOQs for the labelled 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).



2a



2b



2c

FigureIII.2a-2c: 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 III.4.

III.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 ($MinT_{50}$andDT_{50}). For each data set, the 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 $14CO_2$ evolution curves (Figure III.1a-1c).

III.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 residuals (SSR) was evaluated (FOCUS, 2006) and the endpoints $MinT_{50}$ for the mineralization and (DT_{50}) for

dissipiation were reported. For all fittings a χ^2 error threshold was set to 15%, which corresponds to a probability level of p = 0.05. That means that a calculated χ^2 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 χ^2 error and SSR, no kinetic parameters and end-points are reported. For the χ^2 test Equation III.1was used:

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

where, the error is model error at which the χ^2 test is passed, $\chi^2_{tabulated}$ is tabulated value of χ^2 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 Equations (III.2 and III.3), a single-sided t-test was used:

$$t = \frac{parameter - value}{SD(parameter - value)} \quad [III.2]$$

$$type - I\ error\ rate = t - distribution(t, dof, 1) \quad [III.3]$$

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. χ^2 error level and type-I error rate, were calculated within the KinGUI runs and documented in the respective output files. The fit passed the χ^2 test if the calculated χ^2 is lower than the tabulated χ^2 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 using the least-squares method.

III.3 Results and Discussion

III.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. Figure III.1a-1c shows the $14CO_2$ evolution curves in percentage of total applied ^{14}C 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 (Figure III.1a).

Pyrimethanil (see Figure III.1b) is less mineralized compared to bentazone as it can be expected from its known properties (Table III.2). 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% (Figure III.1c) untl 135 d after application. Mineralization is clearly increased in mixtures with digestate contents ≥ 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 et al. (2003a and 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 (Fernandez 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 II.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 and 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.

Table III.4: 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 letters 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*	*3.70x*		
	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**

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 II.1). This is supported by the observations of Cayuela et al. (2009) and Tenuta and Lazarovitis (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 III.1) 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 behaviour (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, there by 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 (Figure III.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 III.3) were tested to identify which best describes the mineralization (based on cumulative $^{14}CO_2$ 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 A1). 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 descibe 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 appendix, Table A1). 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 (Figure III.1a-1c) 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 d 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.

III.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 (Figure III.2a-2c). 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 et al., 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 Sopeña 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 nano-porous 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 III.4. 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 III.4, 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.

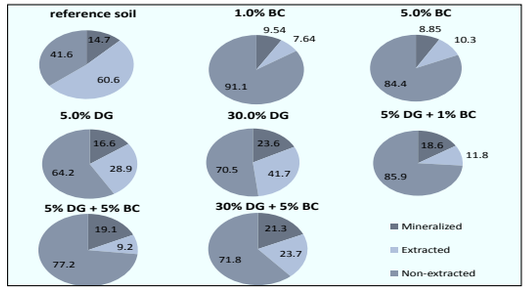
Nevertheless, even from these sparse data it can be seen that the addition of biochar accelerated dissipation of the pesticides, which is mainly driven by the sequestrations of pesticides onto the biochars and corresponding low extractability. The influence of sequestration/strong sorption on the dissipation 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).

III.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 Figure III.3a-3c. 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-transpirated to a sufficient degree), and therefore, a high overall water purification rate.

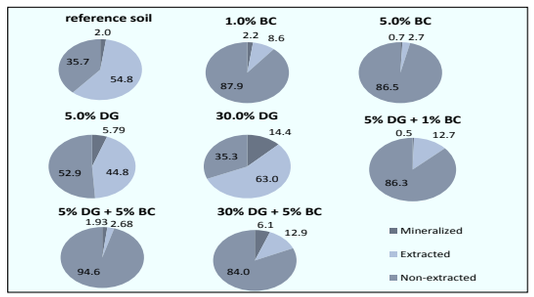
For all studied pesticides the amounts of non-extractable residues increased for bentazone from 0 to 120 d after application from 4.38 to 91.1%, for pyrimethanil from 8.73 to 94.6%, and for boscalid from 10.5 to 93.7% (detailed data not shown) (Figure III.3a-3c), as reported previously for other compounds (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.

Bentazone



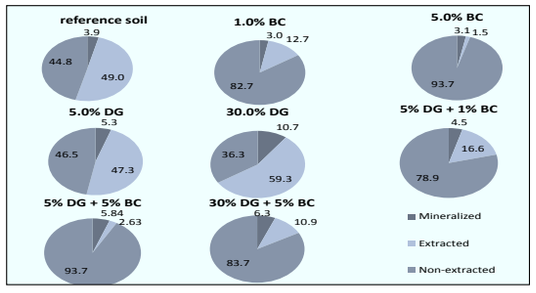
3a

Pyrimethanil



3b

Boscalid



3c

FigureIII.3a-3c: Cumulated $^{14}CO_2$ 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.

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 III.2). 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.

III.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 onagriculturalfields.

IV. Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems

Modified on the basis of

Mukherjee, S., Weihermüller, L., Tappe, W., Hofmann, D., Koeppchen,S., Laabs, V., Vereecken, H., Burauel, P. Sorption-desorption behavior of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems. Sci.Total Environ. 559,63-73.

Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems

IV.1Introduction

Pesticide pollution caused by point or diffuse sources may lead to the contamination of ground and surface water. Point sources typically contribute 40 to 90% of contamination of natural water resources (Castillo et al., 2008, Karanasios et al., 2010). They mainly arise from on-farm activities, such as filling, mixing, and washing of sprayer equipment (De Wilde et al., 2009). The contamination potential is larger when farmers are located close to any open water body or if washing activities are performed on gravelly or sandy soils with low retention capacity for any spilled pesticides (Karanasios et al., 2010). Mitigation or prevention of point sources can on one hand be achieved by implementing best management practices, on the other hand by using advanced depurification systems based on sophisticated physical, chemical, and/or biological methods to treat any remnants of pesticides on farm (De Wilde et al., 2008). Unfortunately, many methods for remnant treatment (e.g., chemical coagulation, sedimentation, oxidation and photocatalysis) are cost and/or labour intensive (Spanoghe et al., 2004). To overcome these limitations the “biobed” concept was developed in Sweden in the early 1990s to establish an environmentally sustainable low cost technology, which can be easily installed and maintained by the farmers (Torstensson and Castillo, 1997). The principal of the biofilter is that pesticide remnants (aqueous solutions of pesticides stemming from sprayer dead volume, washing operations, spillages, etc.) are percolated over a bioactive matrix, in which pesticides are sorbed and degraded. Biofilters may function without any outflow of water, if enough evaporation occurs from the system to eliminate the excess water in the system, or a certain amount of treated water may exit at the bottom of the biofilter (if the water retention capacity of the biofilter is exceeded at certain times).

In general, two processes occur simultaneously within the biobed system: i) sorption of the pesticide to the biomixture material, which reduces the pesticide concentration within the liquid phase and therefore reduces leaching and toxic effects for microbes, and ii) degradation which reduces the load directly (Castillo et al., 2008; Karanasios et al., 2010). Adsorption is considered to be one of the most effective physical processes for pesticide removal (De Wilde et al., 2009; El Bakouri et al., 2007). Hence, there is a growing demand to find relatively efficient, low cost and easily available adsorbents for the adsorption of pesticides for such setups. In natural soils

organic matter and clay are the main soil components contributing to the sorption of pesticides (El Bakouri et al., 2007; Spark and Swift, 2002). Because sorption is one of the main processes reducing the mobility of these chemicals in soils, the addition of exogenous organic matter to soil has been suggested as a possible method to reduce pesticide leaching (Singh, 2003; Si et al., 2011). Although the conventional biomixture used in this system is soil, peat and straw, several recent publications reported the use of low-cost and locally available adsorbents e.g. garden waste compost, cow manure, coconut chips, raw and bio transformed olive cake (Delgado-Moreno et al., 2010; De Wilde et al., 2008), which improved the sorption and degradation behaviour of the studied pesticides even when the pesticides were added in repeated applications and high dosage. Even if some studies already analyzed the sorption and mobility of pesticides in different substrates used for biopurification concepts (e.g., Albarrán et al., 2004; El Bakouri et al., 2007) more investigations are needed for new substrate combinations and different target pesticides.

In the present study, the biomixture was prepared using two bioenergy residues, namely biochar and digestate. Biochar as an anthropogenic pyrogenic solid carbon source has been proven to be good replacement of peat in horticultural media (Tian et al., 2012) and might be therefore also suitable for biopurification systems. The main process induced by addition of biochar into the matrix for biopurification systems is strong sorption of the pesticides which lead to the development of non-extractable residues and reduced bioavailability over time (Spokas et al., 2009; Tatarkova et al., 2013). Several studies reported that biochar enhanced the sorption of pesticides by 400-2500 times compared to soils without biochar addition (Yang and Sheng, 2003; Yu et al., 2010), whereby Loganathan et al. (2009) and Kookana (2010) observed that biochar amendment was even effective in low dosages (<1 % w/w) for the sorption of polar and non-polar pesticides if compared to the sorption in the reference soil. The high sorption capacity of biochar for different pesticides is mainly attributed to its aromaticity and high specific surface area (Accardi-Dey and Gschwend, 2003). Additionally, the biochar sorption properties primarily depend on the pyrolysis conditions, mostly by production temperature (Keiluweit et al., 2012). For example, high temperature biochar is characterized by highly condensed aromatic structures, which will lead to surface adsorption of the pesticides whereas partitioning into the amorphous carbon and different site specific interactions with

functional groups can be the principle adsorption mechanisms for low-temperature biochar (Chun et al., 2004). This indicates that biochar can sorb different compounds which may vary in their polarity and planarity (Chun et al., 2004). Even if high pesticide sorption was reported in several studies Martin et al. (2012) stated that the sorption capacity of the biochar might be reduced over longer incubation time periods (>1 year) due to aging. Additionally, most studies focused on the adsorption processes but did not analyze the desorption mechanism, which is a key process affecting pesticide behavior in soils and controls the predisposition of a pesticide to be degraded and/or leached at different times (Boivin et al., 2005). This process is equally essential in the assessemnt of biochar addition in biopurification systems. Especially, the entrapment of organic compounds in biochar micropores can cause pore deformation and changes, which may induce desorption hysteresis.

Digestate as a source of easily available carbon has been investigated with respect to its influence on the microbial activity and microbial growth by e.g. respiration studies (e.g., Mukherjee et al., 2016a). Yet, to our knowledge no study reported on pesticide sorption-desorption properties for digestate amended soils so far.

Therefore, the aim of this study is to analyze the pesticide sorption-desorption behaviour in six different soil/amendment (biochar and digestate) mixtures including reference soil (without amendment) in a laboratory experiment. Additionally, the effects of different biochar and digestate dosages were tested 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 will be provided, which will help to set up efficient biopurification (biobed) systems for a wide range of pesticides.

IV.2. Material and Methods

IV.2.1 Substrates

A loamy sand topsoil (0 to 10 cm depth) from Kaldenkirchen, Germany (51°19’13 N and 6°11’47E) (Gleyic Cambisol) was used as basis for the soil biomixtures. The soil contained 73.3% sand, 23.1% silt, and 4.9% clay. A full description of the test site can be found in Karlsson et al. (2016). The soil was mixed with two different organic amendments namely, low temperature biochar (BC) and digestate, each in different mixing ratios. The BC originates from slow pyrolysis processes (400°C) using Pine woodchips as feedstock and the digestate 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). The main physico-chemical properties of the raw substances and soil mixtures used for the experiment are listed in Table II.1 and III.1. It has to be noted that for the experiments already aged soil-biomixtures were used for being more representative for the long-term use of the biopurification matrix. All soil-biomixtures had been stored at room temperature in the dark for 6 months prior the experiments.

IV.2.2 Pesticides

Three different pesticides were used in the experiments, two fungicides (pyrimethanil and boscalid) and one herbicide (bentazone). These pesticides were selected based on their different environmental propertiese, namely persistence in soil and extent of sorption to soil. All pesticide standards including internal standard (Pyrimethanil-d5) (>99% purity) were purchased from Dr. Ehrenstorfer GmbH (Bayern, Germany). Stock solutions were prepared in methanol (MeOH, Merck Lichrosolv, ≥ 99.9 % purity). Working solutions were prepared by dilutions of stock solutions with an aqueous 10 mM $CaCl_2$ solution. The percentage of solvent in the final pesticide solution was less than 0.1%. The standard stock and working solutions were stored at 4ºC prior to the experiment. An overview of the physico-chemical characteristics of the three compounds is provided in Table III.2.

IV.2.3 Characterization of used soil-biomixtures

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 $CaCl_2$ 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 through a 0.45-µm sterile cellulose acetate membrane filter. DOC was measured with a TOC analyser 5050A equipped with an autosampler ASI-5000A from Shimadzu (Kyoto, Japan) after acidification and purging the samples for 1 min.

UV absorbance at 254 nm in water-based soil extracts provides information on the presence of specific bonding arrangements in the DOC molecules. Spectra obtained for a complex mixture of molecules, such as DOC, are generally considered to represent the average of individual compounds that comprise the mixture. In our experiment it was measured with a Uvikon 860 UV/Vis spectrophotometer (Tegimenta AG, Rotkreuz, Switzerland), measuring specific DOC UV-absorbances at

254 nm ($SUVA_{254}$) (Cox et al., 2004) of the extracts and by dividing the measured absorption by the respective DOC concentrations. The pH of the soil/-mixtures was determined by equilibrating soil/-mixture with 10 mM $CaCl_2$ at a 1:2 soil/solution ratio (w/v) and measuring pH 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 Lüer and Böhmer (2000): In a first step 2.5 g soil was equilibrated with 10 mL 1 M NH_4Cl for 24 h. Subsequently, a folded paper filter (640d, Macherey-Nagel, Düren, Germany) was wetted with 1 M NH_4Cl and placed in a filter funnel. The wet soil was completely transferred to the filter and percolated with 1 M NH_4Cl until a volume of 100 mL percolate was collected. Exchangeable cations (Al^{+3}, Ca^{+2}, K, Mg^{+2}, Na) were determined in the filtrate using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Ciros CCD, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

The specific surface area (SSA) of the soil and biomixtures was determined by The Brunauer, Emmett and Teller (BET) gas adsorption method for dry surface area measurement on a previously degassed 0.2 g sample at 80 °C for 24 h. The principle of measurement based on nitrogen adsorption-desorption isotherms at 77 K within the 0.03-0.3 relative pressure range (AUTOSORB-1, Quanta chrome apparatus).

The measurement of the organic carbon of the soil and biomixtures were performed with a Leco RC 612 multiphase carbon determinator (LECO instrumentation GmbH, Germany) at the central chemical laboratory (ZEA-3) of the Forschungszentrum Jülich GmbH.

IV.2.4 Equilibrium adsorption experiments

All equilibrium sorption-desorption experiments were performed in accordance to the OECD guideline 106 (OECD, 2000). The experiment consisted of five different biomixtures and one reference soil (see Table III.1), whereby all combinations were analyzed in triplicates. The blank soil (-biomixtures) in 10 mM $CaCl_2$ (without any pesticides) was included in the experiments to check for artifacts and matrix effects caused by them in the analytical method. Additionally, control samples without sorbent (pesticides in 10 mM $CaCl_2$) were analyzed on all equipments (shaken for 168 h) to test the stability and their possible adsorption on the batch container surfaces, but no sorption and no metabolization could be detected.

Pesticide loads were calculated 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 a mixing depth of 5 cm into the soil and a soil bulk density of 1.5 g cm^{-3}. To cover a broader spectrum of concentrations for the sorption/desorption study these concentrations were multiplied by a factor of 0.5, 1, 2, 4, and 6. The resulting initial pesticide concentrations (C_i) for the experiment were therefore 7.10, 14.2, 28.4, 57.0, and 85.2 µg L^{-1} for bentazone, 7.0, 13.0, 23.0, 43.0 and 66.0 µg L^{-1} for pyrimethanil and 0.71, 1.43, 2.85, 5.70, and 8.54 µg L^{-1} for boscalid, respectively assuming a 1:100 soil (and biomixtures)/solution ratio. This ratio was selected due to preliminary experiments, which indicated that strong sorption of the pesticides in biochar based biomixtures occurred and that at least 50 % of the added pesticide should not be adsorbed, and therefore, be available for analysis as recommended by the OECD guideline.

Equilibrium adsorption experiments were conducted at room temperature (20 ± 2°C). Therefore in total 270 centrifuge tubes (Falcon Corning centrifugation tubes, Corning, NY, USA) were filled with 1 g biomixture on dry mass basis and the final volume was filled with 100 mL 10 mM $CaCl_2$. Preliminary studies indicated that sorption equilibrium was not reached before a contact time of 168 h for the 1:100 soil/-mixture solution ratio and all pesticide concentrations. According to Aubee and Lieu (2010), Boivin et al. (2005) and Vanni et al. (2006), no measurable degradation occurred for these studied pesticides over the equilibration time of 168 h. Based on a preliminary study (Mukherjee et al., 2016b) and calculations, it was ensured that <5 % degradation could be reached for all pesticides during this time period. Samples were shaken continuously for 168 h on a horizontal shaker at 225 rpm (SM25, Edmund Bühler). After that, the samples were centrifuged for 15 min at 2910×g and the supernatant was decanted. Equilibrium concentrations (C_e) of pesticides in the supernatant were measured with ACQUITY UPLC (Ultra Performance Liquid Chromatography) system coupled to a Xevo TQ-S triple quadrupole mass spectrometer (both Waters, Eschborn, Germany). Finally, a 10 mL aliquot from supernatant was stored as backup for pH measurement. Percentage of pesticides adsorbed on the different soil/-mixtures was calculated by:

$$Ads\ [\%] = \left[\frac{(C_i - C_e)}{C_i}\right] \times 100 \qquad [IV.1]$$

Where C_i is the initial and C_e (µg L^{-1}) is the equilibrium pesticide concentration in water phase, respectively. $C_s$as the amount of sorbed pesticides on the soil/-mixtures (µg kg^{-1}) was calculated by:

$$C_s = (C_i - C_e) \times \frac{V}{M} \quad [IV.2]$$

Where V is the volume of pesticides solution (L) and M (kg) is the mass of soil/-mixture.

IV.2.5 Equilibrium desorption experiments

Equilibrium desorption experiments were conducted immediately after the sorption experiments according to the OECD guideline 106 (OECD, 2000) by the decant and refill method. For all three steps of the desorption study 60 mL 10 mM $CaCl_2$ solution was added to centrifugation bottles, shaken for 24 h, centrifuged and solution was sampled as described before. The shorter time period for desorption was chosen due to practical reason. Centrifugation tubes were weighed at the start and end of each sorption-desorption step to account for residual solution in the centrifugation tubes. For the desorption study the maximum initial pesticide concentrations (85.2 µg L^{-1} for bentazone, 66.0 µg L^{-1} for pyrimethanil and 8.54 µg L^{-1} for Boscalid) were chosen. The lower concentrations of the adsorption study were not used for desorption experiment because expected concentrations were lower than the limit of detection of the method.

IV.2.6 Analytical procedures

The analysis of pesticides in the supernatant from both experiments were carried out by Ultra Performance Liquid Chromatography (UPLC) – electrospray (ESI) - mass spectrometry (MS) using an ACQUITY UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer.

UPLC analyses were run at 40°C using a reversed-phase Kinetex Core Shell PFP (pentafluorophenyl) column with TMS endcapping (100 mm × 2.1 mm × 2.6 µm, Phenomenex, Aschaffenburg, Germany). Solvent A was Millipore water (Millipore GmbH, Schwalbach, Germany) buffered with 0.1 % formic acid (pH 3.0) for all pesticides. As solvent B methanol (Merck Lichrosolv, ≥ 99.9 % purity) was used for pyrimethanil, acetonitrile (Merck Lichrosolv, ≥ 99.9 % purity) for bentazone and boscalid. The separation was performed with following program: 0 to 1.7 min: 34 % solvent B, 1.7 to 2.9 min: linear from 34 to 100 % solvent B, 2.9 to 3.3 min hold 100

% solvent B, 3.3 to 4.5 min switch back to starting conditions and hold for 2 min. The flow rate was 0.60 mL min^{-1}, injection volume 10 µL.

Electrospray ionization parameters were: desolvation temperature 600 ºC, capillary voltage 3.6 kV, cone voltage 45 V, source temperature 150 ºC. Nitrogen was used as desolvation and cone gas at a flow of 1000 and 150 L h^{-1}, argon was used as collison gas at flow of 0.15 mL min^{-1}. Positive ESI mode was applied for boscalid and pyrimethanil, negative ESI mode for bentazone. Three transitions were considered for each compound: Bentazon 239 Da →**132 Da** (26 V), 175 Da (18 V) and 197 Da (24 V); Boscalid 343 Da → 112 Da (18 V), **140 Da** (20 V) and 307 Da (18 V) and Pyrimethanil 200 Da → 82 Da (26 V), **107 Da** (22 V) and 183 Da (22 V), in brackets corresponding collision energies, respectively. As internal standard D5-pyrimethanil was used: 206 Da → 173 Da (26 V), **108 Da** (24 V) and 187 Da (26 V). Calibration curves (R^2 > 0.99) were established from 6 concentrations respectively. Limits of quantification (LOQ) were 1 pg mL^{-1} for bentazone and 5 pg mL^{-1} for boscalid and pyrimethanil.

IV.2.7 Equilibrium adsorption-desorption isotherms

Equilibrium sorption-desorption isotherms were used to describe the sorption / desorption characteristics of the different soil/-mixtures. Three different sorption models (Henry, Freundlich, and Langmuir) were used to fit the experimental data. The simplest sorption model (Henry-model) assumes a linear sorption behavior over the entire concentration range and can be expressed by:

$$C_S = K_d . C_e \qquad [IV.3]$$

where C_s and C_e are the equilibrium pesticide concentration in is the solid (µg kg^{-1}) and liquid phase (µg L^{-1}) and K_d (L kg^{-1}) is the distribution coefficient.

The second model tested was the Freundlich model, which theoretically accounts for heterogeneous binding surfaces and infinite surface coverage (sorption) resulting from extremely strong matrix and/or solute–solute interactions. The Freundlich model can be written as:

$$C_S = K_f . C_e^{1/n} \qquad [IV.4]$$

where K_f (µ$g^{1-1/n}$ $L^{1/n}$ kg^{-1})is the adsorption coefficients and 1/*n* (-) is the Freundliche exponent. Hereby, K_f refers to the multilayer adsorption capacity and the Freundlich exponent referes to the adsorption intensity (Hussein et al., 2004). In consequence, different K_f values cannot directly compared without taking the 1/*n*-value into account.

Therefore, the sorption distribution coefficients K_d were determined as C_s / $C_{e,}$ by taking values from each concentrations studied in the batch sorption.

The Langmuir model assumes monolayer sorption on a set of different localized sorption sites with uniform energies and can be expresssed by (Langmuir, 1918):

$$C_S = \frac{C_{S\max}\ K_L\ C_e}{1 + K_L\ C_e} \qquad [IV.5]$$

where C_{Smax} (µg kg^{-1}) is the maximum sorption capacity of the adsorbent, $K_L$is the Langmuir sorption coefficients (L kg^{-1}) (constant related to the affinity between the adsorbent and the adsorbate).

All models were fitted on the experimental data using the Excel solver routine.

The influence of the organic matter on the sorption behavior has been discussed in many studies (Correia et al., 2007; Delgado-Moreno et al., 2010). Consequently, the sorption partition coefficient K_d is generally related to the fraction of organic carbon associated with the sorbent to yield an organic-carbon-partition coefficient, K_{oc} (Majumdar and Singh, 2007) and was calculated by:

$$K_{OC} = \frac{K_d\ .100}{\%\ OC} \qquad [IV.6]$$

where, % OC is the percentage of organic carbon. The C-normalized partitioning coefficient (K_{OC}) is generally assumed to be constant for a particular chemical when sorption is only occuring on the soil organic matter (De Wilde et al., 2009).

As the isotherms of the Freundliche and Langmuir model are not linear, the K_d values were calculated for all concentration ranges. Therefore, mean K_{OC} were determined from their corresponding mean K_d values. As a consequence the K_{oc} values cannot be generalized and only indicate differences in sorption between substrates normalized to the organic carbon content at these concentrations level. Desorption isotherms were calcualted using the same models as for the adsorption. Hysteresis coefficient were determined according to Cabrera et al. 2014 by:

$$H = \frac{1/n_{des}}{1/n_{ads}} \qquad [IV.7]$$

In general, lower H values indicate increased difficulty of the sorbed pesticide to be desorbed from the matrix (Barriuso et al., 1994; O'Connor et al., 1980).

IV.2.8. Statistical Analysis

For the reliability of individual parameters Equations (IV.8 and IV.9), a single-sided t-test was used:

$$t = \frac{parameter - value}{SD(parameter - value)} \quad [IV.8]$$

$$type - I\ error\ rate = t - distribution(t, dof, 1) \quad [IV.9]$$

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 $p<0.05$.

IV.3. Results and Discussion

IV.3.1 Characterization of Soil, Biochar, and Digestate Mixture

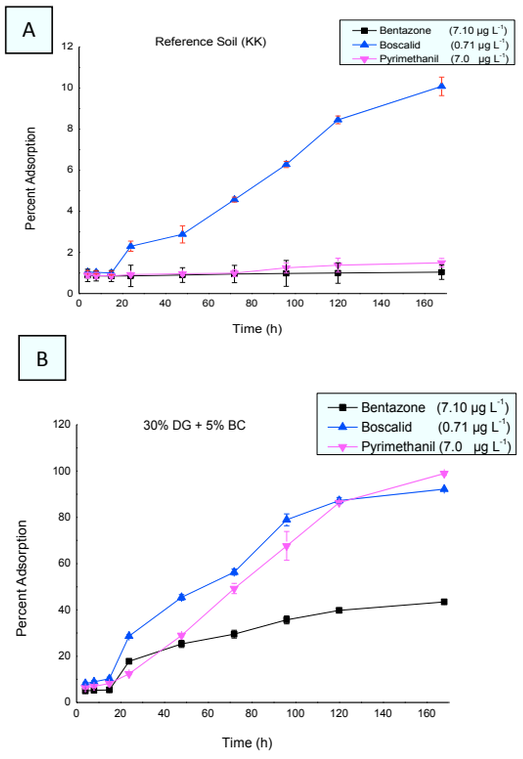
All of the mixtures as well as the native soil showed slightly acidic pH-values ranging from 6.0 to 6.5 (see Table III.1), which is expected due to the sandy character of the Kaldenkirchen soil. Additionally, digestate based mixtures had highest pH-values, which are caused by the alkaline character of the digestate. The biochar mixture had the highest surface area of 8.56 $m^2 g^{-1}$, whereas the pure biochar has a surface area of 231 $m^2 g^{-1}$.

Compared to pH-values extractable DOC differs greatly between the soil/-mixtures (Table III.1), whereby digestate based mixtures showed highest extractable DOC. For these mixtures extractable DOC increased also with increasing digestate content, whereas biochar based mixtures had much lower extractable DOC. Interestingly, extractable DOC dropped in the biochar/digestate soil mixtures compared to the digestate alone soil mixtures by a factor of >1.8. Based on these data, biochar seems to act as a sink of DOC as already suggested by Mukherjee et al. (2016a). Digestate based mixtures showed significantly lower and higher $SUVA_{254}$ values with and without biochar than the biochar/soil mixtures ($p<0.05$; t-test). This means that DOC extracted from digestate based mixtures is more aromatic compared to the DOC extracted from biochar. This can be explained by the fact that the hydrophobic nature of biochar tends to preferentially bind aromatic fractions of the DOC.

IV.3.2 Determination of suitable soil: solution ratio

Four different soil/-mixture/solution ratios (1:10, 1:25, 1:50, 1:100) and nine equilibration time lengths (4, 8, 15, 24, 48, 72, 96, 120 and 168 h) were tested in preliminary study for selecting the suitable ratio and time for the batch equilibrium adsorption experiment. Sorption capacity (%) of reference soil and 30 % DG and 5 % BC biomixture was plotted as a function of the equilibrium time intervals (h) with a

lowest initial pesticide concentrations(Figure IV.1) and it was observed that pesticides removal capacity increased with time up to adsorption equilibrium. The shorter equilibration times did not explain sorption equilibrium particularly for boscalid and pyrimethanil in the 30 % DG and 5 % BC biomixture, as can be seen in the plots in Figure IV.1.



FigureIV.1. Sorption kinetics of bentazone, boscalid and pyrimethanil (for 168 h, 1:100 soil/solution mixtures) on reference soil (A) and soil amended with 30% DG and 5 % BC (B). Data points represent means and error bars indicate standard errors of triplicate samples (symbols in part cover smaller error bars). Reference soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Note that the x and y-axis do not have the same scale for better visualization.

It can be hypothesized that, the adsorptions of these pesticides on the studied organic amendment were multi-step processes, involving adsorption on the external surface, intra-particle diffusion and chemical interaction (adsorption of the pesticide at the active sites via hydrophobic and/or hydrophilic interaction) which are in line with

the observations of El Bakouri et al., 2007 and 2009. After 168 h of shaking the amount of bentazone sorbed on the reference soil was 69 % of the initial concentration (matrix to solution ratio = 1:10), and decreased to 5 % when the ratio was set to 1:100. Sorption of bentazone in the 30 % DG and 5 % BC biomixture decreased from 72 % to 45 % when biomixtures/solution ratio changed from 1:10 to 1:100.

Boscalid and pyrimethanil showed strong sorption affinity towards biomixture. For the reference soil, adsorption of boscalid was 49 % of the initial concentration (8.54 µg L^{-1}) when the soil/solution ratio was set to 1:10 and dropped to 18 % when ratio changed to 1:100. But for the biomixture studied (30 % DG and 5 % BC) adsorption of boscalid decreased from 98 % (1:10) to 96 % (1:100). Sorption of the pyrimethanil changed from 72 % (1:10) to 3 % (1:100) on the reference soil. For 30 % DG and 5 % BC same trend was followed (99 % to 95 %). According to these results, the ratio of 1:100 was selected for all studied pesticides.

IV.3.3 Equilibrium adsorption isotherms

The sorption (and desorption) behavior as well as the fitted isotherms of all pesticides on each soil-/mixtures are depicted in Figure IV.2a-2c and the fitted sorption parameters are listed in Table IV.1. As an indicator of the goodness of the fits the R^2 as well as the sum of squared residuals (SSR) are also listed. Irrespectively, of the carefully performed prelimenary experiments, recording sorption data of all pesticides to the 5 % BC mixture was not possible due to analytical problems, and therefore, no sorption-desorption coefficients could be determined for this combinations. The values of the coefficient of determination (R^2) for almost all other combinations were moderate to high, and quite similar between Freundlich and Langmuir models.

For pyrimethanil and boscalid sorption could be described using the linear Henry model with R^2 exceeding 0.88 (see Table IV.1) as well as the Freundlich and Langmuir model. Even if the R^2 is already high for the linear model fit, fitting error decreased for the more complex models as indicated by a decrease of the sum of squared residuals (SSR). Additionally, the fits are much closer to the measured/observed values and represent the adsorption over the concentration range much better as can be seen in the plots in Figure IV.2. The reason for the better fitting results of the non-linear models can be explained by the specific interactions between polar groups of the pesticide and the organic matter of the substrate as described by De Wilde et al. (2009). Spectroscopic observations

emphasized the prominent role of hydrogen bonding and electron donor-acceptor reactions (via charge-transfer processes through free radical intermediates), in phenylurea-soil organic matter interactions (Senesi and Testini, 1983; Spurlock and Biggar, 1994). It was shown that specific interactions dominate at low concentrations, whereas the relative contribution of hydrophobic and van der waals forces increases with increasing concentrations of sorbates in the solid-solution phase. Basically, natural chemical and photochemical transformations of pesticides in soil appears to be dependent upon the amount and the adsorption capacity of soil organic matter, and in particular of the humic fractions. Khan and Mazurkevich (1974), described that adsorption of polar pesticides on humic acid is mostly goverened through physical forces (ionic bonding and charge transfer complexes), rather than weak chemical bonds such as dipole-ion (cation bridges) or dipole-dipole (hydrogen bonds) due to coordination to cations on the humic acids. Hydrophobic interactions found to be the most vital interaction mechanisms for non-polar pesticides (Torrents et al.,1997). Boscalid and pyrimethanil are more hydrophobic pesticides with low water solubility and consequently their affinity for organic matter is higher, which makes these compounds less mobile than more soluble pesticide like bentazone which is supported by their Log K_{ow} and K_{oc} values from Table III.2. A comparison of the adsorption capacity of each pesticide revealed that the sorption ($K_{f\ ads}$ value) of the pesticides was higher for the more hydrophobic compounds (pyrimethanil and boscalid) and lower for the more polar one (bentazone,Table IV.1). Similar results were found by Rojas et al. (2013), who studied the pesticide sorption capacity of unmodified organic residues and a soil and found an increase in sorption of six pesticides, which depended on the hydrophobic characteristics of the compounds. The results obtained in this study were different than results reported by Rouchaud et al. (1996) and Tejada et al. (2011) who showed the higher effectiveness of the organic soil amendments (cow manure, pig slurry, compost , green manure and municipal solid wastes) for the removal of the pesticides.

For Boscalid the isotherm pattern looks differently. Again, all combinations could be fairly well described (in statistical sense) by the linear model with R^2 exceeding 0.92 and only the biomixtures based on digestate and biochar yielded better results (seen from SSR values) for the Freundlich and Langmuir model.Looking at the plotted data and the fitted model results it becomes clear that the linear model describes the system well compared to the pyrimethanil data, where better fits were obtained by the

Freundlich and Langmuir models. This good fit is also indicated by the fairly low sum of SSR. Compared with pyrimethanil and boscalid, bentazone indicated a different sorption pattern, which could not described by the linear model except for the combined digestate and biochar mixture. All other combinations could be described using the Freundlich and Langmuir concept, whereby the R^2 is much lower and rangesbetween0.61and0.75.

Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems

Table IV.1: Estimated model parameters for the adsorption isotherms of the different pesticides (bentazone, pyrimethanil, boscalid) for the KK = loamy sand soil, 5% DG, 30 % DG, KK+ 5% DG + 5% BC, KK+ 30% DG + 5% BC (BC = low temperature biochar, and DG = digestate) obtained from fitting to a simple Henry (linear), Freundlich and Langmuir model.

		Henry			Freundlich					Langmuir					
Pesticide	Substrate	$K_{d\ ads}$ (L kg^{-1})	R^2	SSR	K_{OC} (L kg^{-1})	$K_{f\ ads}$ (μg$^{1-1/n}$ L$^{1/n}$ kg^{-1})	$1/n_{ads}$	R^2	SSR	K_d (L kg^{-1})	K_{OC} (L kg^{-1})	C_{Smax} (μg kg^{-1})	K_L (L kg^{-1})	R^2	SSR
Bentazone	KK	NA	NA	NA	NA	49.8	0.53	0.67	45011	1.1-17.5	133-2132	699	0.03	0.67	31218
	5% DG	NA	NA	NA	NA	74.6	0.50	0.65	69176	6-24.2	207-870	856	0.04	0.65	47543
	30% DG	NA	NA	NA	NA	75.2	0.54	0.61	54877	8.1-27	65-212	980	0.03	0.75	38059
	5% DG + 5% BC	65.1	0.98	515966	966	NA	NA	NA	NA	16.1-74.2	240-1101	NA	NA	NA	NA
	30% DG + 5% BC	77.8	1.00	23217	470	NA	NA	NA	NA	59-79	354.3-477	NA	NA	NA	NA
Pyrimethanil	KK	4.54	0.90	5847	550	13.2	0.72	0.83	3241	1.5-9	182-1045	544	0.01	0.88	2436
	5% DG	4.93	0.92	6534	177	14.3	0.72	0.85	3263	2.1-9	76-322	574	0.01	0.90	2275
	30% DG	9.53	0.94	35383	76	47.8	0.58	0.88	7025	7.8-18.2	63-146	791	0.03	0.95	3026
	5% DG + 5% BC	2153	0.99	1695262	13000	2950	0.65	0.99	124568	2022-9125	12206-55101	12443	0.31	0.97	547730
Boscalid	KK	19.3	0.98	635	2337	NA	NA	NA	NA	11.4-21.4	1385-2591	NA	NA	NA	NA
	5% DG	41.6	0.93	6728	1497	NA	NA	NA	NA	16-51	569-1824	NA	NA	NA	NA
	30% DG	67.1	0.92	9577	535	NA	NA	NA	NA	38-81	303-645	NA	NA	NA	NA
	5% DG + 5% BC	1061	0.96	27473	15732	928	0.72	0.96	12835	1028-2457	15255-36447	1530	1.24	0.94	15790
	30% DG + 5% BC	2142	0.94	41777	12934	4718	1.60	0.97	9002	1073-2424	6481-14836	NA	NA	NA	NA
	5% DG + 5% BC	1584	1.00	1422997	23500	2329	0.67	0.99	126670	1487-6880	22069-102077	13405	0.20	0.98	459309

NA = Not applicable; $K_{d\ ads}$: lineal sorptionconstant K_{OC}: normalized organic carbon coefficient, calculated for each and every sorption concentration as (K_d/%OC)*100, where $K_{d\ ads}$= C_s/C_e. $1/n_{ads}$: Freundlich exponent correlated to adsorption intensity. $K_{f\ ads}$ (μg$^{1-1/n}$ L$^{1/n}$ kg^{-1}): Freundlich constant correlated to the maximum multilayer adsorption capacity. C_{Smax} (μg kg^{-1}): Langmuir constant representing the maximum sorption capacity relative to the total surface coverage. K_L: Langmuir constant representing the enthalpy of sorption.

Looking again at the plotted data shown in Figure IV.2a-2c, it becomes obvious that a systematic problem is detectable, where sorption greatly increased for the third concentration used (28.4 µg L^{-1}) and stayed nearly constant for all higher concentrations. This already indicates a kind of sorption saturation pleateau, which should be best described by the Langmuir model, which assumes a saturation of the sorption sites. An indicator of the better fitting using the Langmuir concept can be found in the slightly smaller SSR values for this fit.

Analyzing the fitted sorption parameters is becomes evident, that the different mixtures behave differently in their sorption capacity. For pyrimethanil the K_d value calculated from the linear model did not increase for the 5 % digestate addition compared to the native soil and only double in case of 30 % DG addition. Addition of biochar on the other hand significantly increased K_d values to 1584 for the 5 % DG + 5 % BC and even to 2153 for the adding of 30 % DG + 5 % BC ($p<0.05$; t-test). To account for the different amounts of organic carbon available for sorption the K_{OC} was also calculated and indicated that the addition of digestate (5 and 30 %) did not increase normalized sorption capacity compared to reference soil. Moreover, K_{OC} values dropped by more than three times (~3.11) for the low DG addition and even maximum to >7 times for the higher DG loads. On the other hand, mixing of biochar to the digestate increased K_{OC} values substantially with an increase of 4173 % for the 5 % DG + 5 % BC and 2264 % for the 30 % DG + 5 % BC. The reduction for the latter mixture can be explained by the large fraction of digestate added and the low sorption capacity of digestate already shown before.

The boscalid data show the same general trend for the K_d and K_{OC} values, whereby K_d values are generally higher than for the pyrimethanil. For example K_d for the native soil is 4.54 for pyrimethanil and 19.3 for boscalid. The stronger sorption of boscalid has been already reported in several studies (Chen and Zhang et al., 2010; Karlsson et al., 2016), and can be explained by the lower water solubility and higher hydrophobicity of this substance (see also Table III.2). The changes in normalized K_{OC} values are siginificantly lower ($p<0.05$; t–test) in relative terms for the boscalid compared to pyrimethanil. For the addition of 5 % DG the K_{OC} values drops only to 36% and decreases with higher loads (30 %) to 77 % compared to the native soil. Adding biochar and digestate at the same time leads to an increase of the K_{OC} to 573 and 453 % for the 5 % DG + 5 % BC and 30 % DG + 5 % BC mixtures respectively. This means that the normalized sorption capacity is by more than a factor 1.4 smaller

for the boscalid in these mixtures compared to pyrimethanil. Therefore, the high sorption on these substrates cannot be attributed mainly to their high organic carbon content. Other factors, such as the nature of the organic matter or physicochemical characteristics of the surface could play vital role. Moreover, it is now widely recognized that chemical sorption is also affected by the quality or nature of the OC (De Wilde et al., 2009; Delgado-Moreno et al., 2010). This is mainly due to aromatic C content, which increased K_{oc} values, and O-alkyl C and alkyl C content which make K_{oc} values usually decreased. These negative correlations may reflect a lower affinity of these carbon types for the studied pesticide, but they may also be due to blocking of higher affinity sites by organic matter constituents rich in these functional groups. But not only organic carbon content or carbon quality can lead the sorption of contaminants; other factors have been reported previously also played a vital role.

Bentazone sorption could not be described by one model for all mixtures, which makes the interpretation much more difficult but the general sorption can be described as less strong (compared to boscalid and pyrimethanil) with K_d (K_{OC}) values. For the most sorbing biochar + digestate mixtures, 65 (966) and 78 (470) values of K_d (K_{OC}) can be estimated for the lower and higher digestate loads.

For bentazone, the Langmuir model was not applicable for describing sorption on blended mixture of digestate and biochar, as negative values for Langmuir constants C_{smax} and K_L were obtained, which is improbable (De Wilde et al., 2009). Additionally, soil and digestate based combinations for boscalid could not be described either using this model. This may indicate that monolayer adsorption, assumed in this model, was not valid for these specific experiments (De Wilde et al., 2009; El Bakouri et al., 2009). On the other hand, Freundlich model was applicable to describe three biomixture combinations for bentazone and 2 combinations for boscalid.

Based on the Freundlich exponent, or more precisely on the inverse of the exponent (1/*n*), isotherms can be classified as an *L* (non-linear or Langmuir), *S* (side-by-side association) , or *C* (constant partitioning) type according to Giles et al. (1960). These are an indication that different mechanisms of sorption may exist between pesticides and soil components and/or biomixture moieties (Chiou et al., 2000). L, S or C types of isotherm have frequently been found to describe the sorption of other pesticides on soils, such as triazines, organophosphates, or phenylureas (Wauchope et al. 2002). For the studied pesticides/biomixtures combinations, it was observed that

isotherms were of the L-type (*1/n* < 1), which indicates that the pesticides molecules are adsorbed in a horizontal orientation on sorbents/biomixtures with strong intermolecular attraction, without being affected from a strong competition by the solvent molecules, which explains the high affinity of sorbent for solute at low concentrations (Giles et al., 1960).

Basically, sorption of pesticides on the biomixtures is related also to the DOC and SSA content of the mixtures. Although, the effects that DOC exerted on the sorption of pesticides and hydrophobic compounds by soils were discussed contradictory by previous researchers (Barriuso et al., 1994; Müller et al., 2007). Andrades et al. (2004) reported an increase in the sorption of pesticides if organic soluble compounds from DOC are sorbed by soils and give rise to the formation of new hydrophobic surfaces. A decrease in sorption might occur if pesticides interact with the soluble moieties of organic matter in the soil-solution interface (Luo et al., 2009) or when the pesticides compete with the soluble organic molecules for the same sorption sites (Cox et al., 2000). These effects could explain our results, which indicated decreased pesticides sorption by the amended soil mixtures with the highest DOC load (30 % DG mixture). Additionally, many authors reported smaller pores for organic amendments than soil, and found that the larger proportion in small non conducting pores in organic wastes than in soil increase the residence time of the herbicides in the immobile water phase (Cañero et al., 2012; Cox et al., 1997). High micropores proportion in rice husk residue was reported by Yuzer et al. (2013). In our study, micropores proportion was not studied, but BET equation revealed a SSA of 8.56, 6.87 and 3.31 for 5 % BC, 5% digestate and 5 % biochar and 30 % digestate respectively (Table III.1), which were in agreement with reported values for the other organic matrices (Méndez et al., 2013; Thinakaran et al., 2008). Basically, biochar contains active carbon which is one of its characteristics which give its high adsorbent capability. Uchimiya et al. (2012) and Yu et al. (2010) have also doccumented the increase of sorption of pesticides with the increase of the SSA of the biochars added to soils. However, for polar pesticides and metabolites it was shown that the influence of black carbon addition to soil with regard to sorption on soil was rather limited (Dechene et al., 2014).

IV.3.4Equilibrium desorption isotherms

The adsorption behavior as well as the corresponding equilibrium desorption isotherms are plotted in Figure IV.2a-2c. The desorption isotherms were fitted using

the Henry (linear) and Freundlich equation [Equations IV.3 and4]. The Henry desorption ($K_{d\ des}$ and K_{ocdes}) and Freundlich coefficients ($K_{f\ des}$ and $1/n_{\ des}$), the coefficient of determination (R^2), as well as the hysteresis coefficients (H) are listed in Table IV.2.

For pyrimethanil, desorption could be described using the linear model for the soil and 5 % DG mixture, whereas for the 30 % digestate and digestate/biochar based mixtures the Freundlich model was used. For the Freundlich based desorption, the isotherm is always higher as for the adsorption, which indicates that pyrimethanil cannot be desorbed well from the 30 % digestate and digestate/biochar soil matrix. On the other hand, bentazone desorption seems to be influenced strongly by the sorbent properties. For the reference soil and digestate mixtures (without biochar) desorption is easier than adsorption, as indicated again by the desorption isotherms lying below the adsorption ones, which is in line with the observations of Loganathan et al. (2009). From the physicochemical characteristics (e.g., high water solubility), bentazone would be expected to sorb only weakly and also to be desorbed better as compared with the other two pesticides studied. Additionally, our findings corroborated with the observations of Gebremariam (2011) and Zhang and He (2013), who hypothesized a higher desorption (no hysteresis) for polar compounds due to presence/interference of dissolved organic matter. This is particularly important for the sorption of acidic (anionic) pesticides like bentazone, where this effect can be also attributed due to repulsion between negatively charged bentazone molecules and COO^- groups of the DOC derived from biomixtures. On the other hand, mixing biochar into the soil resulted in stronger sorption and in comparison even lower desorption. The reason for the observed strong sorption to digestate/biochar based mixtures cannot be explained easily.

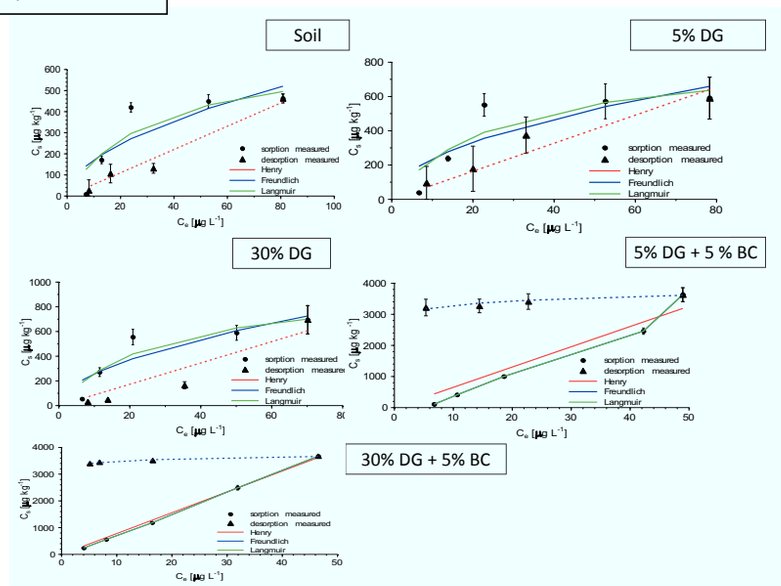
Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems

Table IV.2: Estimated model parameters for the desorption isotherms of the different pesticides (bentazone, pyrimethanil, boscalid) for the KK = loamy sand soil, 5% DG, 30 % DG, KK+ 5% DG + 5% BC, KK+ 30% DG + 5% BC (BC = low temperature biochar, and DG = digestate) obtained from fitting to a simple Henry (linear) and Freundlich model.

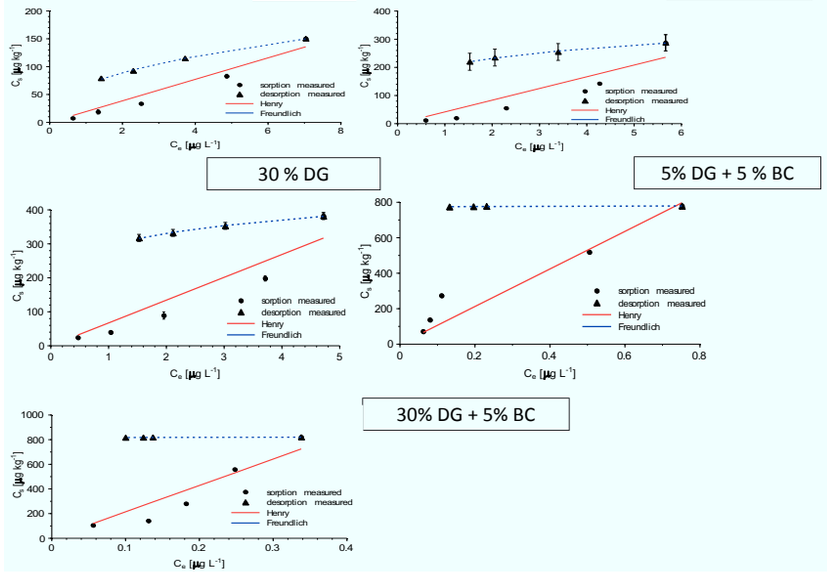
		Henry				Freundlich						
Pesticide	Substrate	$K_{d\,des}$ (L kg^{-1})	R^2	SSR	$K_{OC\,des}$ (L kg^{-1})	$K_{f\,des}$ ($\mu g^{1-1/n} L^{1/n} kg^{-1}$)	$1/n_{des}$	R^2	SSR	K_d (L kg^{-1})	K_{OC} (L kg^{-1})	H
Bentazone	KK	5.52	0.98	3115	669	NA	NA	NA	NA	3.3-6.5	404-795	NA
	5% DG	8.18	0.94	14107	295	NA	NA	NA	NA	7.5-11.3	271-408	NA
	30% DG	8.62	0.94	34665	69	NA	NA	NA	NA	4-10	28-79	NA
	5% DG + 5% BC	NA	NA	NA	NA	2878	0.06	0.78	11487	74.2-602	2226-8927	NA
	30% DG + 5% BC	NA	NA	NA	NA	3215	0.04	0.96	819	79-664	1285-4006	NA
Pyrimethanil	KK	3.78	0.96	1272	459	NA	NA	NA	NA	3-4	328-474	NA
	5% DG	4.21	0.78	6109	152	NA	NA	NA	NA	3.4-10	124-362	NA
	30% DG	NA	NA	NA	NA	432	0.03	0.54	411	200-302	1594-2409	0.05
	5% DG + 5% BC	NA	NA	NA	NA	6070	0.01	0.83	231	2127-3831	31561-56838	0.01
	30% DG + 5% BC	NA	NA	NA	NA	5017	0.20	0.93	882	2021-3246	14217-19601	0.31
Boscalid	KK	NA	NA	NA	NA	67	0.41	1.00	6.7	31-56	3748-6776	NA
	5% DG	NA	NA	NA	NA	202	0.20	0.98	14.8	51-144	2698-5179	NA
	30% DG	NA	NA	NA	NA	295	0.16	1.00	5.5	81-208	930-1655	NA
	5% DG + 5% BC	NA	NA	NA	NA	780	0.004	0.85	1.93	3367-5865	49954-87014	0.005
	30% DG + 5% BC	NA	NA	NA	NA	823	0.003	0.70	1.02	5962-8166	36000-49309	0.005

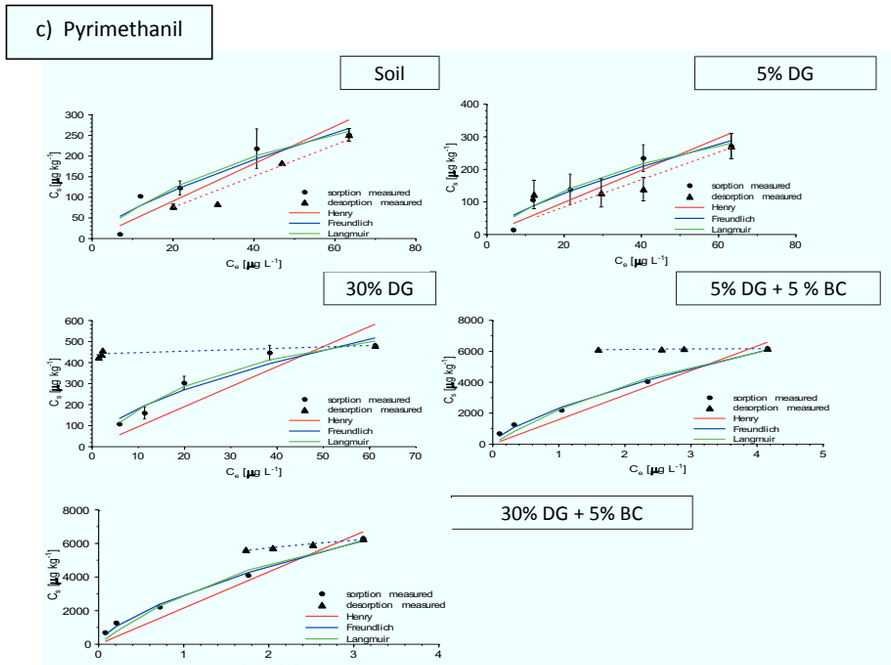
Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems

a) Bentazone



b) Boscalid





FigureIV.2a-2c. Adsorption (solid lines fitted with Henry, Freundlich and Langmuir model) and sequential desorption (dashed lines fitted with Henry and Freundlich model) isotherms of bentazone, boscalid, and pyrimethanil for the different soil/amendment mixtures. Data points represent means and error bars indicate standard errors of triplicate samples (symbols in part cover smaller error bars). C_s denotes sorbed amount and C_e indicates equilibrium water phase concentration. Soil = loamy sand, BC = low temperature biochar, and DG = digestate. The percentage indicates the mass ratios in the mixtures. Note that the x and y-axis do not have the same scale for better visualization.

Yet, it can be speculated that adsorption of biomixture derived DOC by biochar could provide additional sorption sites for bentazone, whereas the high surface area of biochar could contribute to a multiplication of sorption sites for bentazone.

IV.4. Conclusions

The selection of appropriate substrates in biobed systems, used for elimination of pesticides from aqueous remnants, is crucial for their effectiveness. Biochar and digestate, from bioenergy production seem to be a promising novel organic amendment for effective biofilter systems because they are widely available and might replace traditional compounds such as peat.

In our batch sorption experiments the best sorption capacities were obtained by pyrimethanil and boscalid when sorbed on digestate and biochar based mixtures. In contrast, for both pesticides, blank soil was the worst adsorbate. Bentazone showed highest adsorption by blended mixture of digestate and biochar followed by digestate based mixture. 5 and 30 % digestate combinations showed almost similar sorption capacity for bentazone and pyrimethanil respectively. We conclude that a blended mixture of biochar and digestate significantly increases the adsorption and decreases the desorption potential of pesticides compared to bare soil ($p<0.05$; t-test).

However, more work is required to analyze the quality of organic carbon as well as other physico-chemical characteristics (hydraulic responses) and their interactions which are fundamental for the setup of an optimal biobed system. It is also imperative to study desorption potential of the metabolites in aged biomixtures for longer time periods (>1 year). This information will be crucial to assess the availability of aged pesticide residues in biofilter matrix for plant uptake and leaching, after their potential return to topsoil in agricultural fields.

V. Synopsis

V.1 Extended summary

The overall aim of the present study was to identify and quantify the processes and factors that influence the fate of three different pesticides in biochar and digestate based biomixtures used for biopurification systems and to give recommendations of a potentially suitable biomixture for biopurification systems. Several recent publications reported the use of low-cost and locally available adsorbents for pesticide removal: e.g., peat mix, garden waste compost, straw, cow manure, coconut chips, raw and bio transformed olive cake (De Wilde et al., 2008; Delgado-Moreno et al., 2010) but information covering the purification capacity of each individual new adsorbent (or mixture) has to be studied individually for a wide range of pesticides.

Therefore, this study was aligned along with three major points providing essential information about the suitability of digestate and biochar for the purification of pesticide contaminated wastewaters from on farm activities.

i) **How resistant are biochar- and digestate- based mixtures in soil to degradation and how do they affect biological and chemical soil properties?**

As a proxy for the pesticide degrading potential and to gain information about the temporal evolution of the degradation of the materials themselves, soil respiration was measured over 3 months using different biochar and digestate based mixtures added to a sandy and silt loam. To our knowledge the influence of different biochars (high and low temperature), contrasting soils (light to heavy), and amounts of biochar and digestate addition (low to high), and their response if added are not studied yet within one experiment. The results indicated that an easily available C-source like digestate leads to high CO_2 evolution from the mixture in comparison to other mixtures, whereby the rate of CO_2 evolution was not proportional to the amount of digestate applied. The addition of biochar to the native soil resulted in CO_2 fluxes comparable to the fluxes of the native soil, irrespectively of the higher carbon content in these mixtures. Additionally, adding biochar and digestate simultaneously decreased CO_2 fluxes compared to the addition of the same amount of digestate only, which could be explained by the sorption of DOC onto the reactive biochar surface. Finally, the results revealed the recalcitrant

nature of the biochar and proved the suitability of biochar for long term C-storage in soils.

ii) **How does the biomixtures affect the fate (dissipation and degradation) of three different pesticides (bentazone, boscalid and pyrimethanil) use for biopurification systems?**

For the purification processes pesticide sorption and degradation are essential and both largely depend on the type of filling material and the pesticide in use.In a135 day dissipation and degradation study, seven different biomixtures comprised of two bioenergy residues (low temperature biochar and digestate) in combination with a loamy sand soil were used. The results indicated that the addition of digestate increased pesticide mineralization, whereby the mineralization was not proportional to the digestate loads in the mixture. Biochar addition, on the other hand, decreased the mineralization and led to larger sorption/sequestration, resulting in faster decrease of extractable residues. Largest differences between the mineralization was found for pyrimethanil, where the half-life time was more than 27 times smaller for the digestate based mixture compared to the biochar addition. Among the mixtures tested, a mixture of digestate (5%) and biochar (5%) gave optimal results with respect to degradation and simultaneous sorption for all three pesticides.

iii) **How do these novel mixtures affect the adsorption-desorption of studied pesticides used for biopurification systems?**

The composition and types of organic material present in the biobed system are crucial for the retention of agro-chemicals. Matrix substrates that can be used in a biopurification system can have different organic carbon contents in terms of quality and quantity and more importantly, differing pesticide sorption capacities. In general, higher adsorption coefficients were obtained for all pesticides for the digestate and biochar based mixtures, which are characterized by high organic carbon content. However, lower sorption of the pesticides was observed in blank soil compared to the other biomixtures, which was attributed to the lower organic carbon content of the blank soil. Our results showed that boscalid and pyrimethanil are highly sorbed to the mixture of digestate and biochar.

Based on the three studies presented, the most suitable mixture of biochar and digestate could be identified for the setup of a novel biobed system, namely 5% biochar along with 5 and 30% digestate due to its long-term stability, and balance between mineralization and sorption.

V.2 Synthesis

If biochar and digestate based mixtures are increasingly recommended for use in biopurification systems, it must hold the promise of both: maintaining stability of organic C content of the biomixtures and improved dissipation and sorption/desorption potential for the pesticides to be purified.

V.2.1Responses of the soil biota to biochar and digestate

Despite the recalcitrant nature of biochar, several studies have reported increased soil respiration rates when biochar was added to soils (Kuzyakov et al., 2009;Pietikainen et al., 2000). Zimmerman (2011) reported higher oak biochar mineralization rates (approximately 20 mg C g^{-1} char) in non-sterilized incubation compared to sterilized incubation (mineralization rates of approximately 10 mg C g^{-1} char), emphasizing the importance of soil microorganisms for biochar degradation. In many cases, C mineralization after biochar addition shows an initial flush, after which CO_2 evolution continues at much lower rates, similar to the biphasic mineralization rates observed after addition of non-pyrolyzed organic materials to soils. After mineralization of the labile biochar-C pool in the short-term, mineralization rates in biochar-amended soils drop dramatically and are nearly equal to rates in treatments without biochar. The time lag is highly dependent on the biochar type, biochar application rate, and soil characteristics. On the other hand, digestate as a byproduct of biogas industry is getting popular now-a-days in the emerging economy of bioenergy sector. Although, digestate is used as a fertilizer to agricultural field it is depleted in total C and enriched in nitrogen compared to the initial feedstock (Möller et al., 2008), and therefore, less organic C is available for growth and activity of the soil microbial community, which might lead to a gradual depletion of the soil organic matter stocks with time (Arthurson, 2009). Marchetti and Castelli (2013) reported that heterotrophic respiration will increase directly after digestate amendment due to the easily available carbon. In some cases both biochar and digestate might be applied to the soil simultaneously or at different years. Both amendments seem to influence each other by co-metabolism or suppression and their overall turnover is not well

studied. There are only few studies reported in literature describing the soil respiration response with respect to simultaneous biochar and digestate amendment. To assess the persistence of the digestate and biochar based novel biomixtures used for biopurification systems, a double C pool or double first-order in parallel (DFOP) model was used (Chapter II). The results of the present study nicely showed that the mineralization rate of biochar /soil mixtures is slower compared to the turnover of digestate based mixtures (even if the same amount of biochar and digestate was used), which reflects the recalcitrant nature of the biochar and probability of sorption of DOC to biochar surface. Our findings are corroborated by findings of Das et al. (2008) who reported very low soil respiration rates after the addition of biochar which further decreased over time, while for the addition of wheat straw respiration rates increased. Besides that, it was also shown that the input of complex structured organic matter in soil stabilized the soil organic carbon.

V.2.2 Influence of biochar and digestate on fate (dissipation and sorption/desorption) of pesticides used for biopurification setups

Biochar and digestate materials could successfully replace peat and straw in the traditional biomixture used in northern Europe. This is based on the significantly higher degrading capacity of blended mixture of biochar and digestate compared to only bare soil. Guo et al. (1991) suggested that atrazine and alachlor degradation could be inhibited by the presence of activated carbon, and stimulated by other uncharred amendments, such as municipal sewage sludge and manure. An increase on atrazine degradation by the addition of organic amendments to a sandy loam soil was also reported by Mukherjee (2009).To our knowledge, there was no study concerning digestate or combined effect of digestate and biochar on pesticide dissipation behavior. To address this issue, in the present study (chapter III) kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. Kinetic analysis and calculation of $DegT_{50}$ and $MinT_{50}$ values was performed following the recommendations of the FOCUS Kinetics workgroup.For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document (FOCUS, 2006) were tested in order to identify the best-fit model and the appropriate model to derive modeling endpoints, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC) and bi-exponential (DFOP) kinetics. The present study (chapter III) showed that after 135 days, the lowest mineralization of all studied pesticides were

found in the biochar amended soils (1 and 5% biochar) with <11% (bentazone), and <7% for boscalid and pyrimethanil. Addition of 30% digestate enhances the mineralization of bentazone (24%), whereas 11% and 15% of boscalid and pyrimethanil was mineralized. In general, biochar-only mixtures showed the lowest mineralization (and lower extractability) while the digestate-biochar mixtures again showed an increased mineralization (and higher extractability compared to biochar) of these two pesticides (Mukherjee et al., 2016b).

In the present study, pesticide sorption increases in all cases, when soils are amended with the blended mixtures of biochar and digestate (chapter IV, Table 4). K_d and K_{oc} values were much higher for the most hydrophobic pesticides (pyrimethanil and boscalid) for digestate and biochar based mixtures than the more hydrophilic one (bentazone) (chapter IV, Tables 3 and 4). When comparing K_{oc} values between blank soil and soil/digestate based mixtures for pyrimethanil and boscalid, it was found that digestate based mixtures possess much lower K_{oc} values in spite of having higher K_d values and organic carbon content. Therefore, the high sorption on these substrates cannot be attributed mainly to their high organic carbon content. Other factors, such as the nature of the organic matter or physicochemical characteristics of the surface could play vital role. Our observations are corroborated by the findings of Wang and

Xing (2007), who hypothesized that the sorption of organic compounds to un-charred biomass is dominated by absorption mechanisms, whereas adsorption becomes the dominant process with charred materials, largely due to the newly created atomic surfaces and micropores. Basically, we found that (chapter IV) sorption of pesticides on the biomixtures is related also to the specific surface area (SSA) and dissolved organic carbon (DOC) content of the mixtures. Nevertheless, 5% digestate and 5% biochar based mixture among other combinations showed highest K_{oc} values for all pesticides. So, this mixture probably contains organic matter with a better sorption capacity than the other studied organic mixtures for the sorption of all studied pesticides.

V.3 Outlook

Bioenergy residues, namely biochar and digestate, were investigated at different mixing ratios with respect to their effects on the fate of pesticides in soils.

Experiments were performed at the laboratory scale through measuring microbial respiration in the mixtures and investigating the dissipation/degradation as well as

sorption-desorption behaviour of the three pesticides. The results contribute to a deeper knowledge about the fundamental processes and factors that might impact the fate of pesticides in soil/biomixtures and they will be relevant for the proper operation of biopurification systems with such alternative biomixtures.

Further studies should investigate the influence of different hydraulic regimes and chemical inputs on the fate of contrasting pesticides in biopurification systems. Desorption potential of metabolites should also be assessed in aged biomixtures (>3 years) before they are disposed on fields. This information will give further insights in the potential bioavailability, plant uptake, and leaching behavior of aged mixtures, which might be essential for studying their suitability as a substrate for composting.

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VI.Appendix

Appendix A

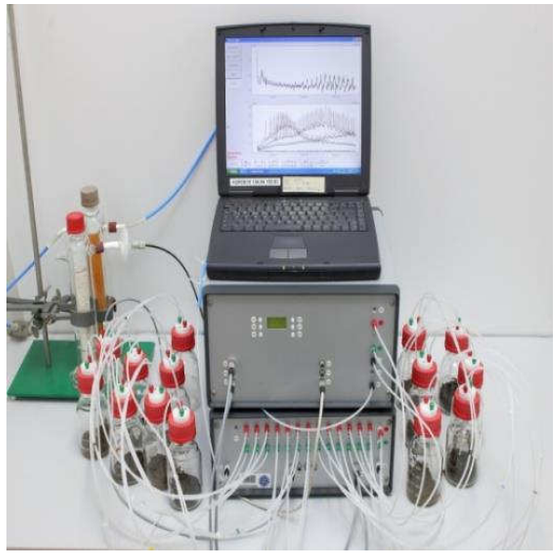

Figure A1: The respirometer device used for the incubation experiment of the biomixtures.

Appendix B

Figure A2: Thermostat Incubator for Degradation Experiment (~ 25 °C).

Appendix C Table A1: Kinetic parameters for the mineralization (derived from cumulative $^{14}CO_2$ fluxes) 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 letters indicate fairly good fit and italics indicate no good fit to the described models).

		Kinetic model																	
		SFO							DFOP										
Pesticide	Substrate	M_o (% of initial)	k (day^{-1})	$DegT_{50}$ (days)	R^2	χ^2	SSR	χ^2 passed	M_o (% of initial)	g	k_1 (day^{-1})	k_2 (day^{-1})	$DegT_{50}$ (days)	R^2	χ^2	SSR	χ^2 passed		
Bentazone	KK	*94.76*	*0.001*	*714.40*	*0.90*	*2.20*	*80.71*	*x*	**99.5**	**0.91**	**0.00047**	**0.171**	**1273**	**0.99**	**0.40**	**2.00**	**x**		
	1% BC	**100.28**	**0.001**	**870.80**	**0.90**	**0.50**	**5.81**	**x**	**100.2**	**1.00**	**0.00080**	**0.162**	**866**	**0.96**	**0.60**	**5.81**	**x**		
	5% BC	*97.15*	*0.001*	*1172.50*	*0.93*	*1.20*	*28.09*	*x*	***100.1***	***0.95***	***0.00034***	***0.212***	***1881***	***0.99***	***0.20***	***0.71***	***x***		
	5% DG	*96.97*	*0.001*	*521.20*	*0.90*	*1.40*	*34.82*	*x*	***99.93***	***0.94***	***0.00097***	***0.132***	***650***	***0.99***	***0.30***	***1.73***	***x***		
	30% DG	*94.01*	*0.002*	*349.90*	*0.94*	*3.30*	*169.66*	*x*	**100.1**	**0.86**	**0.00096**	**0.098**	**560**	**0.99**	**0.40**	**2.46**	**x**		
	5% DG + 1% BC	*95.33*	*0.001*	*471*	*0.92*	*2.50*	*99.54*	*x*	**100.01**	**0.90**	**0.00072**	**0.095**	**8001**	**0.97**	**0.40**	**1.93**	**x**		
	5% DG + 5% BC	**98.98**	**0.002**	**391**	**0.92**	**1.10**	**19.44**	**x**	**100.3**	**0.84**	**0.00059**	**0.016**	**887**	**0.99**	**0.40**	**1.88**	**x**		
	30% DG + 5% BC	*97.07*	*0.002*	*340.40*	*0.92*	*3.20*	*162.20*	*x*	**101.1**	**0.23**	**0.02928**	**0.00**	**NA0.97**	**1.20**	**20.98**		**x**		
Pyrimethanil	KK	*99.59*	*0.00*	*4576.60*	*0.90*	*0.20*	*1.15*	*x*	**100.07**	**0.99**	**0.00007**	**0.07309**	**9177**	**0.99**	**0.10**	**0.06**	**x**		
	1% BC	*99.69*	*0.00*	*3815.10*	*0.92*	*0.30*	*1.64*	*x*	**100.1**	**0.98**	**0.00001**	**0.02584**	**128691**	**0.96**	**0.10**	**0.38**	**x**		
	5% BC	*99.85*	*0.00*	*13869.90*	*0.90*	*0.10*	*0.13*	*x*	**99.86**	**1.00**	**0.00005**	**0.019**	**13753**	**0.84**	**0.10**	**0.13**	**x**		
	5% DG	*100.02*	*0.001*	*1268.20*	*0.95*	*0.60*	*6.90*	*x*	**100.02**	**1.00**	**0.0005**	**0.022**	**1269**	**0.92**	**0.60**	**6.90**	**x**		
	30% DG	*101.08*	*0.001*	*497.50*	*0.94*	*1.00*	*20.36*	*x*	**101.07**	**0.61**	**0.00132**	**0.00150**	**498**	**0.96**	**1.10**	**20.36**	**x**		
	5% DG + 1% BC	*99.98*	*0.00*	*17997.80*	*0.91*	*0.00*	*0.00*	*x*	**99.98**	**0.59**	**0.00004**	**0.00004**	**18131**	**0.99**	**0.00**	**0.00**	**x**		
	5% DG + 5% BC	*99.66*	*0.00*	*4623.00*	*0.92*	*0.20*	*0.99*	*x*	**99.66**	**0.56**	**0.00027**	**0.00**	**7719**	**0.87**	**0.40**	**1.88**	**x**		
	30% DG + 5% BC	*100.31*	*0.001*	*1162.10*	*0.91*	*0.80*	*12.52*	*x*	**100.6**	**0.87**	**0.00**	**0.0066**	**557397**	**0.90**	**0.80**	**11.37**	**x**		
Boscalid	KK	**99.14**	**0.00030**	**2337.50**	**0.83**	**0.50**	**4.93**	**x**	**99.99**	**0.97**	**0.000046**	**0.03893**	**14322**	**0.91**	**0.10**	**0.14**	**x**		
	1% BC	**99.43**	**0.00023**	**2978.50**	**0.86**	**0.30**	**2.41**	**x**	**99.99**	**0.97**	**0.000025**	**0.03110**	**26613**	**0.92**	**0.10**	**0.08**	**x**		
	5% BC	**99.43**	**0.00023**	**3012.30**	**0.95**	**0.30**	**1.66**	**x**	**100.03**	**0.98**	**0.000142**	**0.08449**	**4780**	**0.91**	**0.00**	**0.04**	**x**		
	5% DG	**99.20**	**0.00041**	**1697.00**	**0.95**	**0.40**	**2.55**	**x**	**100.00**	**0.98**	**0.000316**	**0.12365**	**2141**	**0.95**	**0.10**	**0.10**	**x**		
	30% DG	**98.81**	**0.00094**	**737. 0**	**0.90**	**1.10**	**22.52**	**x**	**100.33**	**0.88**	**0.000003**	**0.02074**	**188521**	**0.92**	**0.30**	**1.84**	**x**		
	5% DG + 1% BC	**98.98**	**0.00033**	**2077.60**	**0.86**	**0.50**	**4.65**	**x**	**99.83**	**0.97**	**0.000139**	**0.05087**	**4774**	**0.91**	**0.20**	**0.42**	**x**		
	5% DG + 5% BC	**99.26**	**0.00046**	**1497.90**	**0.95**	**0.40**	**2.94**	**x**	**100.11**	**0.98**	**0.000360**	**0.11391**	**1876**	**0.97**	**0.10**	**0.09**	**x**		
	30% DG + 5% BC	**99.12**	**0.00050**	**1399.40**	**0.93**	**0.50**	**5.28**	**x**	**99.98**	**0.96**	**0.000248**	**0.04060**	**2658**	**0.98**	**0.20**	**0.41**	**x**		

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