Universitat Autònoma de Barcelona

Beneficios ambientales del uso de biocarbón como enmienda en el cultivo de piña de Costa Rica: efectos fisicoquímicos y biológicos en suelo e interacción con agroquímicos

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Tesis Doctoral

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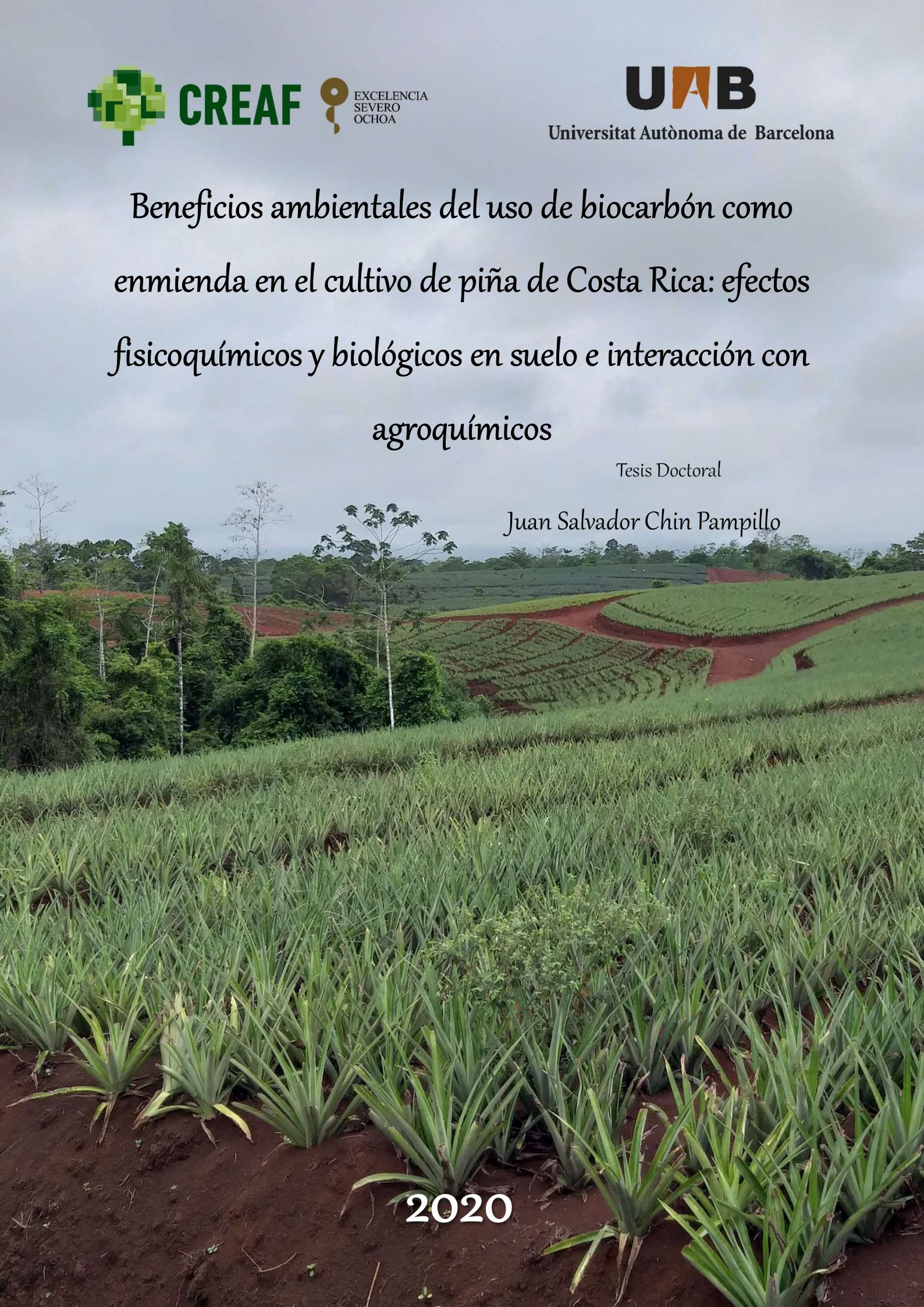

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Environmental benefits of using biochar as an amendment in pineapple cultivation in Costa Rica: soil physicochemical and biological effects and interaction with agrochemicals

Thesis dissertation for fulfillment of the requirement for the degree of Doctor of Philosophy presented by:

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Universidad Autónoma de Barcelona
Departamento de Biología Animal, Biología Vegetal y Ecología
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Disertación de tesis para el cumplimiento del requisito para el grado de Doctor presentada por:

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El presente documento cuenta con el visto bueno de los abajo firmantes y contiene la disertación de la tesis titulada “Environmental benefits of using biochar as an amendment in pineapple cultivation in Costa Rica: soil physicochemical and biological effects and interaction with agrochemicals”, presentada por Juan Salvador Chin Pampillo para optar al título de Doctor en Ecología Terrestre por la Universidad Autónoma de Barcelona.

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"Equipado con sus cinco sentidos, el Hombre explora el Universo que lo rodea y a sus aventuras las llama Ciencia"

Edwin Powell Hubble

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Cuando inicié esta tesis presentía que iba a requerir mucho trabajo y esfuerzo concluirla. Ahora que la he concluido, con certeza puedo decir que estaba en lo correcto, pero muy lejos de imaginar que sin el esfuerzo y apoyo de muchas personas no lo hubiera logrado.

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Abstract

Pineapple production is an important economic activity in Costa Rica as shown by the increase in its cropped area in the last two decades. It involves a high generation of agro-wastes as well as an intensive application of pesticides, including the herbicides bromacil, and diuron. Their use is associated with problems of environmental, health and economic concern, because it has been detected in both surface water and groundwater. However, there is still scarce information about the environmental fate of these herbicides in tropical soils. The transformation of this agro-waste surplus by pyrolysis into biochar, a carbonaceous material, followed by its addition to soil as an amendment, has been suggested as a useful waste management practice. This is because it may improve the soil quality and carbon sequestration and potentially mitigate the mobility of pesticides.

The aim of this thesis was to evaluate the benefits and unexpected effects of biochar addition to a Costa Rican agricultural soil cropped to pineapple, concurrently considering the effects on bromacil and diuron fate and efficiency as well as non-target soil ecotoxicological effects. For this purpose, pineapple stubble (PS), oil palm fiber (PF) and coffee hulls (CH) were pyrolyzed at 300 or 600 °C for one hour and then physically and chemically characterized. Mixtures of the charred materials (CM) with soil were prepared at application rates equivalent to 10 and 20 t ha-1. Sorption, degradation, and biodegradation of both pesticides were evaluated in the laboratory, and the results were used to predict their environmental risk with the Pesticide Impact Rating Index. In addition, lettuce emergence and growth (Lactuca sativa), invertebrates performance (collembolan Folsomia candida; enchytraeid Enchytraeus crypticus), and microorganism functional diversity (Microresp™) were used to test the effects of CM on the herbicides' efficiency and on the non-target soil biological groups.

CM pyrolyzed at 300 °C were classified as torrefied materials (TM), while those pyrolized at 600 °C were classified as biochars (B). Biochars showed higher specific surface area, fixed carbon content and pH values than TM, while PS-B and PF-B presented a higher abundance of surface oxygenated chemical groups than CH-B and all the TM. A weak sorption of both herbicides to soil was observed suggesting a high mobility, while the degradation and biodegradation of bromacil was more limited compared to that of diuron. The addition of biochars increased the persistence of bromacil, while PS-TM and PF-TM increased the sorption of diuron. Despite that, the predicted mobility and aquatic toxicity of the herbicides were unaffected. The addition of CM did not reduce herbicide efficiency but increased seedling emergence without improving growth. The adding of herbicides had no toxic effects on collembolans and enchytraeids as it promoted their reproduction without affecting survival. A generalized avoidance of CM-mixtures by enchytraeids was observed as opposed to the general preference shown in collembolans, irrespective of the supplementation or not of herbicides. Finally, no changes in the microbial functional diversity by the sole addition of herbicides or CM were demonstrated, and a significant increase in the consumption rate of some substrates was observed only in some diuron-treated CM-mixtures.

In summary, the addition of CM did not change the efficiency of the herbicides nor their fate in a tropical clay Ultisol. Under pineapple cropping conditions of the northern region of Costa Rica, bromacil and diuron presented a high estimated environmental risk to surface water and groundwater, and the addition of CM did not change this risk. Simultaneously, no negative effects to the soil ecosystem were observed, but there was an improvement in soil as a habitat for some soil invertebrates.

Resumen

La producción de piña es una actividad económica importante en Costa Rica que ha aumentado significativamente su área cultivada en las dos últimas décadas, con una alta generación de residuos agroindustriales y un uso intensivo de plaguicidas, incluidos los herbicidas bromacil y diurón. Ambos se han detectado en aguas superficiales y subterráneas, lo que ha causado problemas ambientales, de salud y económicos, aunque aún existe poca información sobre su destino ambiental en los suelos tropicales. La transformación de residuos agroindustriales en biocarbón (biochar) mediante pirólisis y su adición al suelo como enmienda, se ha propuesto como una práctica útil de gestión de residuos capaz de mejorar la calidad del suelo, el secuestro de carbono y que podría mitigar la movilidad de los plaguicidas.

El objetivo de la tesis fue evaluar los beneficios y eventuales efectos no deseados de la adición de biocarbón a un suelo costarricense cultivado con piña en combinación con bromacil o diuron, considerando tanto sus efectos ecotoxicológicos como en su destino y eficiencia. Los materiales carbonizados (MC) se obtuvieron pirolizando a 300 o 600 °C durante una hora rastrojo de piña (PS), pinzote de palma aceitera (PF) y cascarilla de café (CH), se caracterizaron física y químicamente y se mezclaron con suelo en dosis de aplicación equivalentes a 10 y 20 t ha-1. Se evaluaron la sorción, la degradación y la biodegradación de ambos plaguicidas en condiciones de laboratorio con lo que se predijo su riesgo ambiental con el Índice de Clasificación de Impacto de Plaguicidas. Además, se midió la emergencia y el crecimiento de la lechuga (Lactuca sativa), el desarrollo de invertebrados (colémbolo Folsomia candida; enquitréido Enchytraeus crypticus) y la diversidad funcional de los microorganismos (Microresp™) para probar los efectos de los MC en la eficiencia de los herbicidas y sobre organismos terrestres no diana.

Los MC a 300 °C se clasificaron como materiales torrefactos (MT) y a 600 °C como biocarbones (B). Los biocarbones mostraron mayor superficie específica, contenido de carbono fijo y pH que los MT, mientras que los PS-B y PF-B presentaron mayor abundancia de grupos funcionales oxigenados superficiales que el CH-B y que todos los MT. Se observó una sorción débil de ambos plaguicidas en el suelo, lo que sugiere una alta movilidad, mientras que la degradación y biodegradación de bromacil fue limitada comparada con el diurón. La adición de biocarbón aumentó la persistencia del bromacil, mientras que los PS-MT y PF-MT aumentaron la sorción del diurón. No obstante, la movilidad y la toxicidad acuática predichas de los herbicidas no se afectaron. La adición de MC no redujo la eficiencia de los herbicidas, pero aumentó la emergencia sin mejorar el crecimiento. La aplicación de herbicidas no tuvo efectos tóxicos sobre colémbolos o enquitréidos pues promovió su reproducción sin afectar la supervivencia. Hubo una evitación generalizada de los enquitréidos de las mezclas suelo-MC opuesta a la preferencia general de los colémbolos, independientemente de la presencia de herbicidas. Finalmente, no se demostraron cambios en la diversidad funcional microbiana por la adición de herbicidas o MC, y solo se observó un aumento en la tasa de consumo de algunos sustratos en algunas mezclas suelo-MC tratados con diurón.

En conclusión, la adición de MC no cambió la eficiencia de los herbicidas ni su destino en el Ultisol tropical arcilloso estudiado. Bajo la gestión habitual del cultivo de la piña en la Región Norte de Costa Rica, ambos herbicidas presentaron un elevado riesgo ambiental para aguas superficiales y subterráneas, y pese a que la adición de MC no le mitigar, no causaron efectos negativos sino una mejora del suelo como hábitat para invertebrados edáficos.

Resum

La producció de pinya tropical és una activitat econòmica important a Costa Rica, l'àrea de cultiu de la qual ha augmentat significativament en les dues darreres dècades, i amb una significativa generació de residus agroindustrials i ús intensiu de plaguicides com els herbicides bromacil i diuron. Tots dos han estat detectats en aigües superficials i subterrànies, causant problemes ambientals, de salut i econòmics, malgrat que hi ha poca informació sobre el seu destí ambiental en sòls tropicals. La transformació de residus agroindustrials en biocarbó (biochar) mitjançant piròlisi i la seva adició al sòl com a esmena han estat proposats com una pràctica útil per a la gestió de residus que permet la millora de la qualitat del sòl, el segrestament de carboni i una possible mitigació de la mobilitat de plaguicides.

L'objectiu de la tesi fou avaluar els beneficis i eventuals efectes no desitjats de l'adició de biocarbó a un sòl costa-riqueny cultivat amb pinya en combinació amb bromacil o diuron, considerant tant els seus efectes en el destí i eficiència com els ecotoxicològics. Els materials carbonitzats (MC) s'obtingueren per piròlisi a 300 o 600 ºC durant una hora, utilitzant com a materials de partida rostoll (rastrojo) de pinya tropical (PS), raquis de raïm de palmera d'oli (pinzote) (PF) i l'endocarp del fruit de café (cascarilla) (CH), que es van caracteritzar fisicoquímicament i es van aplicar al sòl a dosis d'aplicació de 10 i 20 t ha-1. S'avaluà la sorció, la degradació i la biodegradació dels plaguicides en condicions de laboratori, permetent la predicció del seu risc ambiental amb l'índex de classificació d'impacte de plaguicides. Addicionalment, es van avaluar efectes en l'emergència i creixement de d'enciam (Lactuca sativa), el desenvolupament d'invertebrats (el col·lèmbol Folsomia candid i l'enquitreid Enchytraeus crypticus) i la diversitat funcional de la comunitat dels microorganisms (Microresp™) per a provar possibles efectes dels MC en l'eficiència dels herbicides i en organismes terrestres no diana.

Els MC a 300 ºC es classificaren com a materials torrefactes (MT) i a 600 ºC com a biocarbons (B). Els biocarbons presentaren major superfície específica, contingut de carboni fixe i pH que els MT, alhora que els PS-B i PF-B tenien major abundància de grups oxigenats superficials que CH-B i que tots els MT. Es va demostrar una sorció dèbil al sòl en tots dos plaguicides, fet que suggereix una elevada mobilitat, i la degradació i biodegradació del bromacil fou limitada en comparació al diruon. L'adició de biocarbó incrementà la persistència del bromacil, i els PS-MT i PF-MT la sorció del diuron. No obstant, això no va canviar la mobilitat i toxicitat aquàtica predites pels pesticides. L'adició de MC no reduí l'eficiència dels herbicides, però augmentà l'emergència sense incrementar el creixement. L'aplicació d'herbicides no causà efectes tòxics en col·lèmbols o enquitreids, sinó que promogué la reproducció sense afectar la supervivència. S'observà una evitació generalitzada de les barreges sòl-MC en enquitreids contrària a la preferència dominant en col·lèmbols, independentment de la presència d'herbicides. Finalment, no aparegueren canvis en la diversitat funcional microbiana amb l'adició d'herbicides o MC, llevat de l'increment en la taxa de consum d'alguns substrats en algunes barreges sòl-MC tractades amb diuron.

En conclusió, l'adició de MC no canvià l'eficiència dels herbicides ni el seu destí en l'Ultisol tropical argilós estudiat. Sota la gestió habitual del cultiu de la pinya a la regió nord de Costa Rica, tots dos herbicides presentaren un elevat risc ambiental per a aigües superficials i subterrànies, i malgrat que l'adició de MC no ho va mitigar, no van causar efectes negatius sinó una millora del sòl com a hàbitat per a invertebrats edàfics.

List of contents

List of figures

List of tables

List of abbreviations

AF	Attenuation Factor
AIC	Akaike Information Criterion
ANOSIM	Analysis of similarities
BC	Biochar
BMC	Bromacil
14C-BMC	14C radiolabeled Bromacil
C96	Pesticide concentration remaining on day 96
CA	Contact angle
CCE	Calcium carbonate equivalent
CEC	Cation exchange capacity
Cfixed	Fixec Carbon content
CH	Coffee hulls
CIA	Centro de Investigaciones Agronómicas
Corganic	Organic Carbon content
DRN	Diuron
14C-DRN	14C radiolabeled Diuron
DT50	Half-life
EBC	European Biochar Certificate
EC	Electrical conductivity
EDS	Energy dispersive X-ray spectroscopy
FTIR	Fourier transform infrared
GHG	Greenhouse gases
GLM	Generalized Linear Model
GUS	Groundwater ubiquity score
IBI	International Biochar Initiative
ICP	Inductively coupled plasma atomic emission
Kd	Sorption coefficient
KOC	Organic carbon normalized sorption coefficient
KOW	Octanol-Water partition coefficient
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LD	Below the level of detection
LOI	Loss on ignition
MT50	Mineralization half-life time
n.d.	Not determined
n.q.	Not quantified
NOEC	Non-effect observed concentration
OAW	Organic agro-wastes
OECD	Organization for Economic Co-operation and Development
PEC	Predicted Environmental Concentration
PF	Oil palm fiber
PIRI	Pesticide impact rating index
PNEC	Predicted no-effect concentration

PS	Pineapple stubble
S	Clay Ultisol / Soil
SEM	Scanning electron microscopy
SSA	Specific surface area
SSD	Species Sensitivity Distribution
TOC	Total Organic Carbon
UHPLC	Ultra High-Performance Liquid Chromatography
VC	Volatile compounds
VM	Volatile matter
w.a.	Water absorption
ξ	Zeta potential

Preface

This PhD dissertation compiles the information and contains the integral analysis of the results obtained from several laboratory and greenhouse experiments to obtain the PhD Degree in Terrestrial Ecology. It describes the environmental problems associated with the use of the herbicides bromacil (BMC) and diuron (DRN) in pineapple cropping in the Northern Huetar Region (NHR) in northern Costa Rica as well as other impacts associated with the inadequate management of crop residues and the usual land use practices. It proposes the application of biochar as an organic amendment able to improve soil fertility of tropical soils but also to reduce the unintended environmental risk of these pesticides, limiting their mobility in the soil and mitigating their impacts on non-target soil and aquatic organisms. Biochar is a carbonaceous material obtained from pyrolysis of biomass and, due to its porous structure and surface chemical properties, the retention of xenobiotics could be improved by its addition. Furthermore, the production of biochar for this purpose could be completed by using tropical agro-wastes as feedstocks, such as palm oil fiber and coffee hulls produced in large amounts, or by other non-environmentally friendly management pathways such as pineapple stubble that is burnt or abandoned in the field. Moreover, other environmental benefits such as a reduction of greenhouse gases emission and increased carbon sequestration could be reached by this practice. All these benefits point to biochar as being a suitable biotechnology to achieve the shift towards the global adoption of environmentally smart agriculture practices. Following the precaution principle, a burden of proof of non-harmful effects of this biotechnology is needed before its implementation as a regular practice for pineapple production or other crops.

Chapter 1 describes the main problems caused by the intensive use of BMC and DRN in pineapple production in the NHR of Costa Rica and indicate how the use of charred materials could change those pesticides' fate and mitigate their environmental risk, as related to the properties of these materials. Finally, the research question, hypothesis, and the objectives of this research are described.

Chapter 2 presents the characterization of several charred materials, including torrefied ones and biochars, each obtained at two different pyrolysis temperatures, and using three different feedstocks, namely pineapple stubble (PS), palm oil fiber (PF), and coffee hulls (CH). Some of these charred materials have now been characterized for the first time in

the available literature, and their properties assessed to suggest the most suitable environmental use in soil. Those results are already published in the paper entitled "Widespread tropical agro-wastes as novel feedstocks for biochar production: characterization and priority environmental uses"[1].

In Chapter 3, the fate of BMC and DRN in an Ultisol from NHR, in terms of mobility and persistence, are studied in detail under laboratory conditions. Additionally, the effects of the addition of the charred materials, fully characterized in Chapter 2, on these characteristics are also analyzed. These results have been included and submitted as a paper entitled "Amendments with pyrolyzed agro-wastes change the sorption and persistence of bromacil and diuron without mitigating their predicted environmental risks in a tropical soil".

Chapter 4 describes, under greenhouse conditions, how the addition of charred materials to the same tropical soil influences herbicide efficiency and its potential ecotoxicological effect on non-target species. These results are part of the paper entitled "Biochar addition to a tropical agroecosystem does not alter herbicide efficiency and improves soil habitat function".

Finally, Chapter 5 provides the specific and general conclusions and recommendations derived from this research..

[1] Chin-Pampillo, J.S., Alfaro-Vargas, A., Rojas, R., Giacomelli, C.E., Perez-Villanueva, M., Chinchilla-Soto, C., Alcañiz, J.M., Domene, X., 2020. Widespread tropical agrowastes as novel feedstocks for biochar production: characterization and priority environmental uses. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-020-00714-0

Chapter 1. Introduction

1.1. Environmental problems associated with the use of BMC and DRN for the pineapple production in Costa Rica

Pineapple cultivation is an important activity in Costa Rica, both in terms of cultivated area, production, and organic wastes generation. This crop has experienced a rapid growth since 2000, increasing the cropped area from 11 000 to close to 40 000 registered hectares in 2019 (Mora Ramírez et al., 2020). The crop is distributed between three main regions in the country (Figure 1-1), the Northern Huetar Region with 49% of the total cropped surface, the Atlantic Huetar Region (29%), and the Pacific Region (22%)(CANAPEP, 2019).

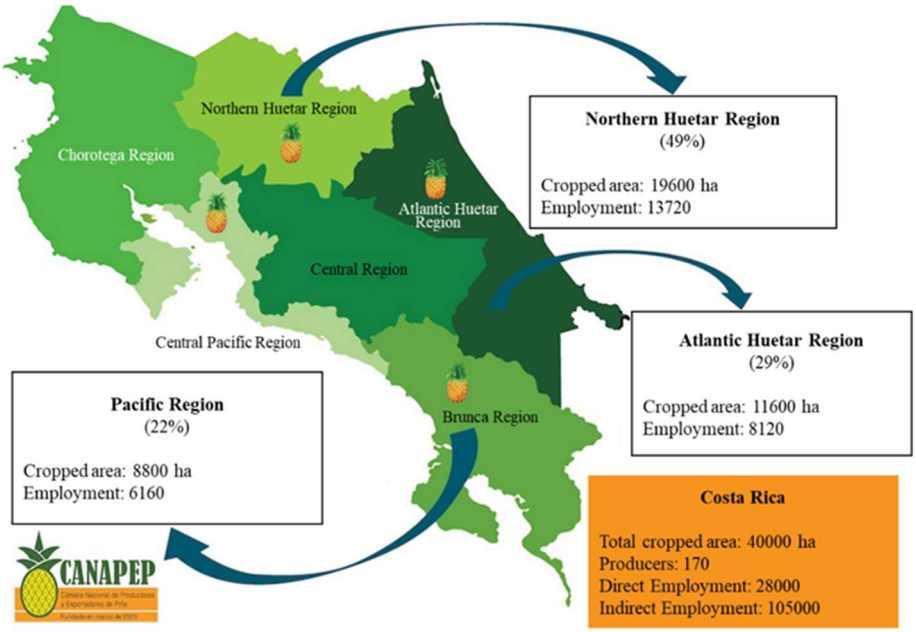

Figure 1-1. Distribution of pineapple cropped areas in Costa Rica (CANAPEP, 2019).

In 2019 the production reached 3.2 million of metric tons, ranking this crop as the second in Costa Rica after sugarcane, and surpassing that of banana, oil palm, and coffee (Table 1-1). In addition, the exportation of fresh pineapple was equivalent to 962 million US$ in 2019, representing 35 % of the total exports of Costa Rican agricultural products, and occupying second place behind banana, with 36 % (Mora Ramírez et al., 2020). As a result, Costa Rica occupies first place as a worldwide exporter of fresh pineapple since 2007 (Manjavacas, 2012; Workman, 2020).

Table 1-1. Cropped area and production of the main crops in Costa Rica in 2019 (Mora Ramírez et al., 2020).

Crop	Cropped area (ha)	Production (t)
Sugarcane	60 000	5 915 822
Pineapple	45 000	3 190 278
Banana	43 050	2 486 236
Oil palm	76 910	1 087 800
Coffee	93 697	449 105

However, several problems of environmental concern have been associated to pineapple production, including deforestation of protected wild areas and wetlands, soil erosion, and soil and surface and ground water pollution by pesticides, particularly in the Northern Huetar Region (CICA, 2019; González Gamboa, 2019; Kellon et al., 2011; Programa Estado de la Nación, 2014). The agrochemical control of weeds involves the intensive use of several herbicides, among them BMC and DRN, which are of widespread use during cropland preparation and after planting, to avoid the competition for nutrients and water caused by weeds (BANACOL, 2011).

BMC is an herbicide belonging to the uracil chemical group. It was registered for the first time in the United States in 1961, for weed control in pineapple and citrus fruits, and non-agricultural uses such as roads margins and sidewalks. In agriculture, BMC is used in the pre- and post-emergence phase and controls a broad spectrum of annual and perennial weeds, as well as brush or bush, woody plants, and vines. (U.S. EPA, 1996). Due to its high solubility in water and its low soil sorption capacity (Table 1-2), BMC has a high leaching potential, plus it is not volatile and its persistence in the soil is moderate to high. The main degradation pathway for BMC in the soil under anaerobic conditions is caused by microorganism action and it is also susceptible to photolysis in water under alkaline conditions, but is not susceptible to decomposition by hydrolysis or photolysis at pH 5 and 7 in water, or by photodegradation and aerobic metabolism in soil (U.S. EPA, 1996). However, other transformation products and metabolites with non-significant toxicity have been identified as results of plant metabolism, abiotic photodegradation and metabolic transformation in animals (Figure 1-2). The U.S. EPA (1996) established a reference dose for BMC of 0.1 mg kg^{-1} d^{-1} based on chronic rat toxicity studies. Furthermore, its acute toxicity is classified as type IV, the lowest toxicity category, and

it is classified as a possible human carcinogen but is not considered mutagenic. (U.S. EPA, 1996).

Table 1-2. General information about the pesticides bromacil and diuron (Lewis et al., 2016)

Parameter	Bromacil	Diuron
IUPAC name	(*RS*)-5-bromo-3-sec-butyl-6-methyluracil	3-(3,4-dichlorophenyl)-1,1-dimethylurea
CAS Registry Number	314-40-9	330-54-1
Chemical formula	$C_9H_{13}BrN_2O_2$	$C_9H_{10}Cl_2N_2O$
Molecular mass (g mol^{-1})	261.12	233.09
Solubility in water at 20 °C (mg L^{-1})	815	35.6
Octanol-Water partition coefficient at pH 7, 20 °C (Log Kow)	1.88	2.87
Vapor pressure at 20 °C (mPa)	4.10×10^{-2}	1.15×10^{-3}
Henry's law constant at 25 °C (Pa m^3 mol^{-1})	1.50×10^{-5}	2.00×10^{-6}
GUS leaching potential index	3.44	2.65
DT50 in soil (days)	60	146.6
Freundlich sorption coefficient normalized (K_{foc})	117	757
Freundlich sorption coefficient (K_f)	2.9	7.0
Constant 1/n	0.917	0.75

In contrast, DRN belong to the chemical family of substituted ureas and it is applied as a pre-emergence control of a wide variety of annual and perennial broad leaved and grassy weeds. DRN is registered for occupational uses on agricultural food as well as non-food crops, fish ponds, roads margins and industrial sites, but also for residential uses in ponds, aquariums and paints (U.S. EPA, 2003). The herbicide presents a low volatility (Table 1-2), low solubility in water, is mobile in soil and has the potential to leach into groundwater (Kogan and Pérez, 2003; U.S. EPA, 2003). It is persistent in soil and the major degradation routes are photodegradation in soil and water and microbial degradation in water, however it is stable to hydrolysis at pH 5, 7 and 9. Two

transformation products Figure 1-3) are of toxicological health concern, 3,4-dichloroaniline (DCA) and tetrachloroazobenzene (TCAB) (U.S. EPA, 2003). DRN is not acutely toxic, and the chronic reference dose is 0.003 mg kg^{-1} d^{-1} based on chronic rat toxicity studies, with DRN having been classified as known/likely to be carcinogenic to humans (Lewis et al., 2016; U.S. EPA, 2003).

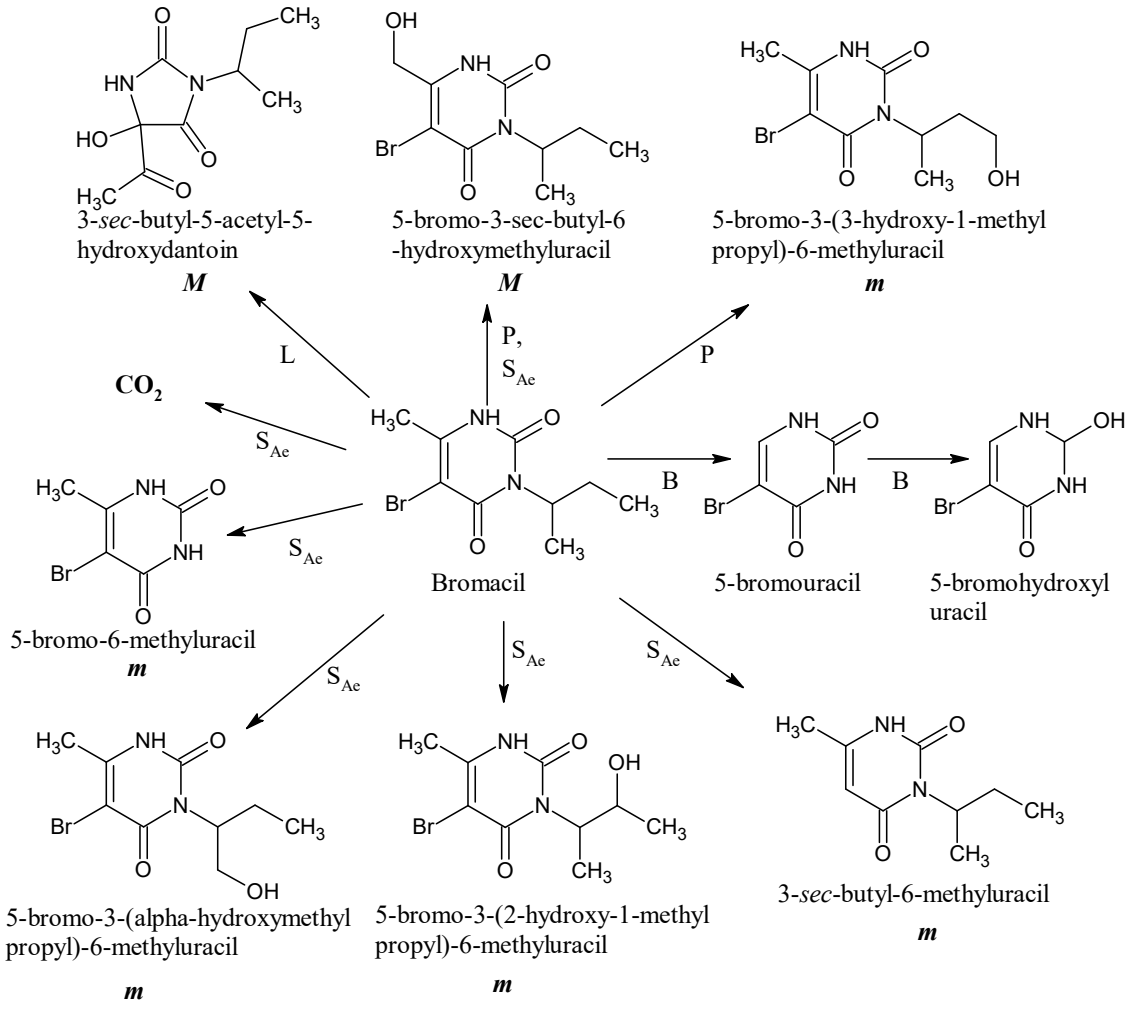

Figure 1-2. Transformation products and metabolites of BMC. Routes of transformation are codified as L (photodegradation by direct outdoor solar irradiation), P (plant metabolism in orange or pineapple), B (biodegradation by bacteria *Pseudomonas* sp. strain 50235 isolated from soil), S_{Ae} (aerobic soil metabolism). ***M*** indicates major and ***m*** indicates minor metabolite (Aisawa, 2001, 1982; U.S. EPA, 1996).

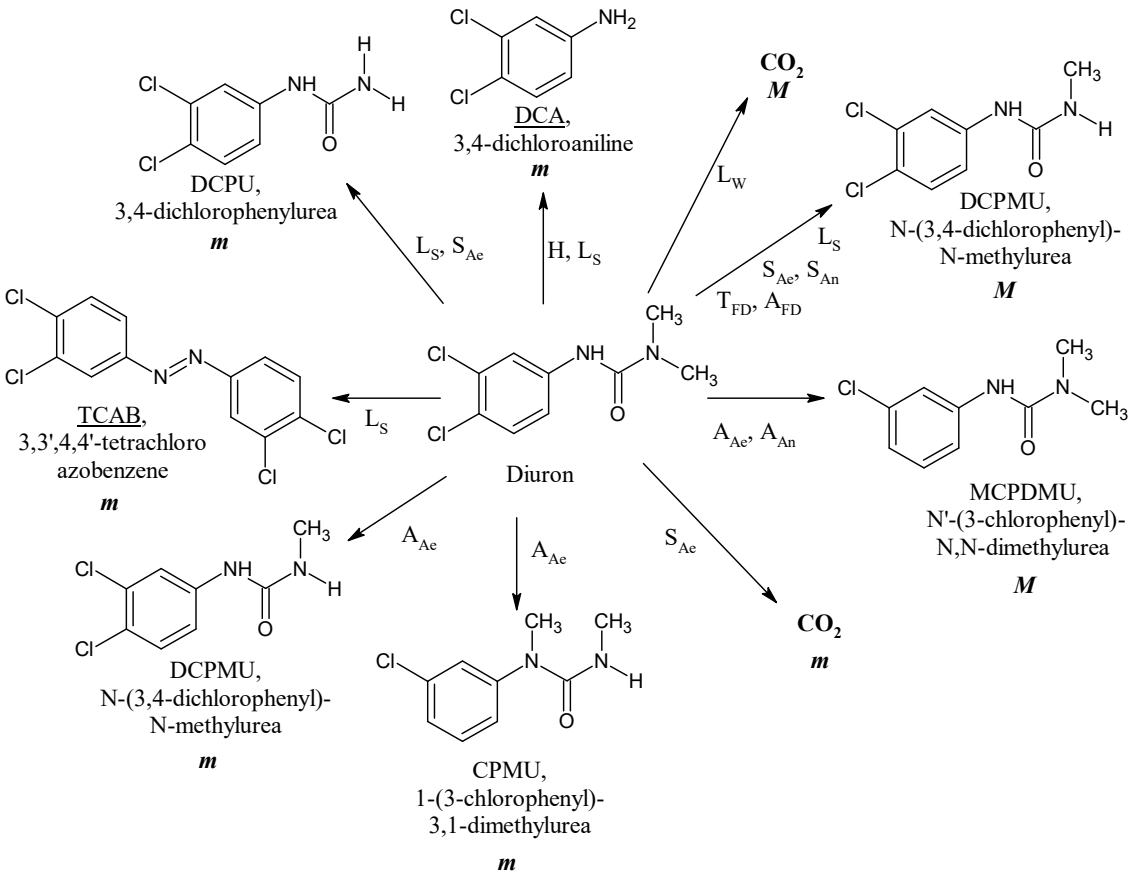

Figure 1-3. Transformation products and metabolites of DRN. Routes of transformation are codified as H (hydrolysis), L_W (photodegradation in water), L_s (photodegradation in soil), S_{Ae} (aerobic soil metabolism), S_{An} (anaerobic soil metabolism), A_{Ae} (aerobic aquatic metabolism), A_{An} (anaerobic aquatic metabolism), T_{FD} (terrestrial field dissipation), A_{FD} (aquatic field dissipation). ***M*** indicates major and ***m*** indicates minor metabolite. Underlined name indicates products of human health concern (Tixier et al., 2001; U.S. EPA, 2003).

BMC is practically non-toxic to avian and reptilian species, mammals, aquatic invertebrates, and estuarine species on an acute basis, and is relatively non-toxic for bees, and slightly toxic to fish and amphibians (U.S. EPA, 1996). In addition, DRN is slightly toxic to birds and rats, practically non-toxic to bees and moderately toxic to aquatic animals, including *Daphnia magna*, but highly toxic to *Oncerynchus clarkii* (freshwater fish) and *Gammarus fasciatus* (freshwater invertebrate) (U.S. EPA, 2003). Because the mode of action of both pesticides is the inhibition of photosynthesis, their high risk of being transported by leaching or surface runoff and their high persistence in soil and sediments (ENSR International, 2005; Field et al., 2003) means that their presence in aquatic ecosystems presents moderate to high risk for primary producers (Rämö et al., 2018). Besides DRN's individual toxicity to aquatic organisms such as green algae

(Escher et al., 2005; Knauer et al., 2007; U.S. EPA, 1996) or phytoplankton (Knauert et al., 2008) it has been observed that it can be increased by the mixture with other herbicides such as BMC (Knauert et al., 2008), or even to have synergistic effects with insecticides like carbofuran which affects heterotrophic organisms (Mansano et al., 2018; Rocha et al., 2018). Toxicity to soil bacteria has also been reported as well as synergistic effects between BMC and DRN which augment phytotoxicity (El-Nahhal and Hamdona, 2017).

Since 2015 in the Northern Huetar Region of Costa Rica, BMC and DRN have been detected in surface water, near pineapple cropped lands, at concentrations between 0.06 to 8.6 μg L^{-1} and 0.24 to 6.9 μg L^{-1} respectively and in groundwater at concentration up to 3.8 μg $L^{-1}$for BMC (CICA, 2019). Presence of pesticides in groundwater in that region constitutes a problem in terms of environmental, economic, and public health concerns. As the main source for water consumption in Costa Rica is the groundwater, the findings of contaminated water sources in that region have resulted in the closure of aqueducts, causing an important social and economic impact. Due to those problems and considering the high risk to leaching of BMC, its use was banned in 2017 (Ministerio de Agricultura y Ganadería et al., 2017; Valverde and Chaves, 2020). However, despite the widespread use of both pesticides, knowledge of its fate in tropical regions is not well understood, and aspects such as persistence and mobility in the soils of the Northern Huetar Region have not been fully studied.

Finally, pineapple cultivation generates an important amount of organic wastes, divided into two main biomass by-products, the stubble, which is the plant biomass remaining after the edible part has been cropped, and the crown, corresponding to the leaves surrounding the fruit. Pineapple stubble is the first agro-waste in terms of quantity among the main crops in Costa Rica (Table 1-3). Currently, the most widespread management is to apply an herbicide, and leave the plants to dehydrate naturally and then till to incorporate this material to the land, with the aim of increasing the amount of organic matter for soil. However, the accumulation of decomposed plant stubble in the field can be used for the stable fly (*Stomoxys calcitrans*) to oviposit and to develop their larvae, which is of concern as this species feed on the blood of cattle in the adult stage, with health and economic impacts (Solórzano et al., 2015; Taylor et al., 2012)

Table 1-3. Estimated amount of agriculture organic waste generated in Costa Rica in 2018 (Coto, 2013).

Crop	Estimated wet biomass wastes (t)	Estimated dry biomass wastes (t)	Waste	Water content (%)	Mass balance relative to production (t waste / t production)
Pineapple	8748000	875700	Stubble	90	3.290
			Crown	79	0.003
Sugarcane	4254000	1671000	Molasses	50	0.350
			Cachaça	74	0.300
			Bagasse	50	0.250
			Field wastes	70	0.232
Oil palm	538370	299180	Fiber	55	0.220
			Mesocarp fiber	37	0.130
			Coquito shell	17	0.050
Banana	421890	63284	Pinch	85	0.94
			Banana rejected	85	0.114
Coffee	402897	96508	Pulp	81	0.416
			Mucilage	81	0.156
			Husk	11	0.043

1.2. The use of biochar as a soil amendment as a potential solution to mitigate the environmental risk of BMC and DRN

The fate of pesticides is influenced by several factors, including properties of the soil, organic matter content, pH, temperature and rainfall, among others (Tiryaki and Temur, 2010). Pesticides and other xenobiotics could (i) exist as free constituents in the soil, (ii) could be degraded or transformed as a result of chemical and/or biological processes or (iii) be fixed by sorption to soil particles, and in fact, the most significant process that governs the bioavailability of those compounds is sorption (Katayama et al., 2010). Addition of organic amendments to the soil could affect the fate of pesticides, as it can limits their mobility, reduces their bioavailability or increases or reduces their persistence (Briceño et al., 2007). By increasing the organic matter content, the sorption of pesticides to the soil could be increased and their mobility limited, and it could also stimulate microbial activity and promote enhanced biodegradation of the pesticide. On the other hand, higher sorption could also extend the time of persistence in soil, with unexpected consequences for their short- to medium- term bioavailability and therefore on their herbicide activity and any impact on non-target groups (Katayama et al., 2010).

The use of biochar as an organic amendment has been demonstrated to improve soil quality and fertility (Agegnehu et al., 2017). In addition, its production by pyrolysis of

organic wastes could be an interesting option for waste management including how to revalorize them. Thus, organic wastes derived from agricultural or livestock sources, without other environmental destinations, could be good candidates for pyrolysis management (IBI, 2015; Quesada Kimzey, 2012). Besides those benefits, it has been observed that pesticides and other xenobiotics interact with the biochar in soil, remaining adsorbed to it and, as a result, limiting their mobility in the soil (Ahmad et al., 2014; Bansal, 2018; Dechene et al., 2014; Gámiz et al., 2019; Haskis et al., 2019). Thus, pyrolysis of agro-wastes for biochar production and subsequent incorporation into the soil as an organic amendment could provide solutions to the environmental problems exposed.

Biochar is a carbonaceous material obtained from biomass through an exothermic process called pyrolysis, which is started by initial heating between 300 and 1000 ºC and in the absence of oxygen, with variable residence times and rates of heating (Lehmann and Joseph, 2015; Verheijen et al., 2010). Besides biochar, during the pyrolysis other by-products such as flammable gases, heat and bio-oil can be used to feed the process and to generate energy. Biomass can be obtained from various sources: agricultural waste, grass, plant material from trees, bamboo, or even animal waste, such as manure, among others. The type of biomass used influences properties such as ash content, C/N ratio and specific surface area (SSA), as well as the yield of the biochar produced (Ahmad et al., 2014; Mukome et al., 2013).

The pyrolyzed biomass is constituted mainly of carbon (C) but also, though at lower contents, of oxygen (O) and hydrogen (H), and generally has a porous structure when derived from plant materials and inherited from plant cell tissues (Kookana et al., 2011). The physical and chemical properties of the pyrolyzed biomass, including the ratios of C, O and H and SSA are strongly influenced by the pyrolysis conditions and the feedstock materials used (Tomczyk et al., 2020). Biochars produced with grass or manure present high ash content while wood derivatives present higher values of SSA, however, wood or grass derived biochar present higher C/N ratios than manure derived biochars (Mukome et al., 2013). Temperature influences surface properties of biochar such as porosity and SSA, increasing both properties as it goes up (Rafiq et al., 2016; Yu et al., 2006; Zhao et al., 2017). High temperature also increases the degree of aromaticity, defined by H/C and O/C ratios, which is a proxy for the expected carbon recalcitrance, and this is why low

degrees of aromaticity, which implies a greater aliphatic character, are linked to a higher fraction of easily biodegradable carbon (Mukome et al., 2013).

Incorporating biochar into the soil improves the soil's characteristics and promotes an increase in its fertility by several mechanisms: it can increase soil pH in acid soils (Berek and Hue, 2016; Wu et al., 2020), enhance soil water-holding capacity and available water (Masís-Meléndez et al., 2020; Nelissen et al., 2015; Peake et al., 2014) and cation exchange capacity, resulting in higher availability of nutrients in the rhizosphere (Glaser et al., 2002; Liang et al., 2006; Steiner et al., 2008), but also can improve the hydraulic conductivity in the soil (Herath et al., 2013), aeration (Laird, 2008), porosity and bulk density (Nelissen et al., 2015) and soil aggregation (Lu et al., 2014).

Furthermore, the production and use of biochar as a soil amendment could have positive impacts on the mitigation of climate change. The process of photosynthesis fixes carbon from the atmosphere as biomass, but in the short- to medium term, this carbon is released again as CO_2 to the atmosphere again by plant and animal respiration and decomposition (Stavi and Lal, 2013). When the biomass is transformed into biochar, it is possible to obtain bioenergy (as flammable gases or liquids) which only add carbon already present in the atmosphere, and therefore it is considered a carbon-neutral process. The energy balance of the process is generally positive, since more energy is produced than is consumed (Roberts et al., 2010). In turn, a solid residue is obtained with pyrolysis, biochar, which contains a significant fraction of the carbon originally present in the feedstock and therefore of a recalcitrant nature. This means that its release as CO_2 is avoided, which explains why pyrolysis is globally considered a carbon-negative technology (Glaser et al., 2002; Laird et al., 2009). The addition of biochar to the soil, therefore, allows long-term carbon sequestration due to the recalcitrant nature of its carbon content. Additionally, biochar supplementation has also been shown to reduce the emissions of the most powerful greenhouse gases such as N_2O, mostly released from agricultural soils (Laird et al., 2009).

Regarding the biochar effect of main interest to this investigation, it has been observed that biochars can adsorb and retain pesticides, as well as other types of organic or inorganic compounds present, and therefore strongly influence their fate and bioavailability (Askeland et al., 2020; Hu et al., 2020). However, sorption does not increase in all cases, because this depends on the chemical properties of both the

pesticides and biochar. Zheng et al. (2010) observed the sorption affinity of green-waste biochar for the triazine herbicides atrazine and simazine and concluded that it is produced by a combination of adsorption and partition mechanisms. On the other hand, Cabrera et al. (2014) reported that the herbicides aminocyclopiraclor and bentazon, of which both present high mobility in soil, increased their retention in soil amended with a biochar produced from wood, characterized by high SSA and low dissolved carbon content, while the sorption and low mobility of the fungicide pyraclostrobin was unaffected. In contrast, Dechene et al. (2014) observed that the addition of biochar did not increase sorption of the herbicide imazamox while sorption was increased by 2.1 to 2.5-fold with respect to the unamended soil for methyl-desfemil-chloridazon. This was attributed to the anionic and neutral character of the pesticides, respectively and their interaction with the negatively charged surface of the biochar. There is a knowledge gap around the validity of the conclusions from studies in temperate soils with respect to agricultural tropical soils, which clearly have different pedoclimatic conditions, such as a high acidity and low cation exchange capacity.

Similarly, there is little research on the biodegradation of pesticides in soils amended with biochar and more specifically in tropical soils. Yang et al. (2006) reported decreased biodegradation of diuron attributed to its strong adsorption to biochar, in a behavior that has been also observed with other pesticides, such as acetamiprid (Yu et al., 2011) and benzonitrile (Zhang et al., 2005). Jones et al. (2011) studying the effect of biochar on the herbicide simazine also found the same conclusion, in this case associated with a reduction in the mobility, biodegradation, and bioavailability of the pesticide.

Despite the potential benefits mentioned for the use of biochar as a soil amendment, it is important to note that this practice could have unexpected effects on the effectivity of the herbicides (Cheng et al., 2016; Nag et al., 2011) or the functions of soil ecosystems, and also have direct negative effects on soil organisms and their ecosystem functions (Kuppusamy et al., 2016). Moreover, information on these topics is also scarce for tropical regions.

Considering all of the previous information, this investigation is of value as a necessary and useful input to assess the implementation of biochar as a regular agricultural practice in tropical conditions. The motivation of this investigation could be summarized in the following questions:

- Due to the important amount and availability of agro-wastes generated from pineapple, oil palm and coffee production, is it possible to obtain biochar from these materials by a pyrolysis process, and importantly, under which temperature conditions? Which are the main properties linked to its potential environmental use for each material (soil amendment versus sequestration)?
- Could the application of biochar as an amendment to a tropical clay soil, typically used in pineapple cultivation, have any effect on the BMC and DRN fate? Might this have consequences on their predicted environmental risks?
- Is it possible that the presence of biochar in the soil coud attenuate the effectiveness of BMC and DRN as herbicides?
- Does the addition of biochar have direct ecotoxicological effects or could it mitigate the ecotoxicological effects of BMC or DRN on non-target soil organisms (microorganisms and fauna)?

Those research questions were then linked to the following initial hypothesis:

- The pyrolysis of dry pineapple stubble, palm oil fiber and coffee hull at two different temperatures will produce biochars with different properties that might allow the characterization of specific optimum environmental solutions for soil fertility improvement or carbon sequestration.
- The addition of the produced biochars to a clay Ultisol will increase the sorption and persistence of the herbicides BMC and DRN and reduce their estimated environmental risks.
- The supplementation of biochars to this soil when treated with BMC and DRN will reduce the herbicides' effectivity.
- Biochar will improve the habitat conditions and will attenuate the negative effects of BMC and DRN on non-target organisms in an edaphic ecosystem.

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Objectives

Main objective

- To evaluate the benefits and unexpected effects of applying biochar to an agricultural soil from Costa Rica dedicated to pineapple cultivation, considering ecotoxicological effects on the edaphic community as well as the effect on the fate and efficiency of the herbicides bromacil and diuron.

Specific objectives

- To chemically and physically characterize the charred materials produced from three widespread tropical agroindustry wastes: pineapple stubble, palm oil fiber, and coffee husk, under two different pyrolysis temperatures.
- To determine, under laboratory conditions, the fate (sorption, degradation of parent compounds and mineralization) of two widespread used herbicides, BMC and DRN, in a tropical clay soil amended with six different charred materials (torrefied or biochars).
- To estimate the environmental risk (mobility and toxicity) of BMC and DRN for surface and groundwater as influenced by the addition of six different charred materials according to pineapple cropping conditions of the northern region of Costa Rica as a case study.
- To determine for both BMC and DRN, under greenhouse and laboratory conditions and in a soil corresponding to a tropical Ultisol, whether the addition of charred materials could i) change their efficiency as herbicides; and ii) mitigate any unintended ecotoxicological effects on non-target soil fauna bioindicator species and microbial functional diversity, in both cases also considering the charred materials effect by itself.

Chapter 2. Widespread tropical agro-wastes as novel feedstocks for biochar production: characterization and priority environmental uses

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2.1. Abstract

Biochar, a carbon-rich pyrolytic product, has demonstrated positive results as a soil improver and carbon sequestration agent. Its production could be an appropriate and innovative practice for agricultural waste management in the context of environmentally smart agriculture. However, considering the relevant effect of the production conditions on the final biochar properties, its characterization is a necessary step, moreover if an unknown feedstock is being used. Coffee hulls (CH), pineapple stubble (PS) and palm oil fiber (PF) are typical tropical agro-industrial wastes and biochar from first two are not reported before. In this work, biochars from them were obtained after one hour of pyrolysis at 600 °C. Surface area and pH of biochars were close to 60 m^2 g^{-1} and 9 respectively (except for PF which was 29 m^2 g^{-1}), while torrefied biomass (charred material prepared at 300 °C) presented a surface area close to 1 m^2 g^{-1} and neutral pH. Fixed C was approximately 80% (PF and CH) and 59% (PS) for biochars, and close to 40% in torrefied biomass. It was concluded that key properties of biochars were mostly determined by the feedstock's origin. Due to its high ash content and surface area, PS biochar was identified as a suitable soil amendment, while PF and CH biochars showed a higher potential for carbon sequestration in soil due to their high fixed carbon content, demonstrating that the production of biochars from widespread tropical wastes tailored for specific environmental uses is possible.

2.2. Introduction

Intensive and extensive tropical agriculture impacts the environment though deforestation, habitat fragmentation, biodiversity loss, freshwater withdrawal, eutrophication, pollution of water and soil ecosystems (due to misuse of agrochemicals), enhancement of greenhouse gases (GHG) emissions, and the generation of large amounts of organic agricultural wastes (OAW) (Carlson and Garrett, 2018). Regarding the latter, several initiatives to revalorize those materials in novel ways have been proposed, ranging from biofuel production to biochar production (Elkhalifa et al., 2019; Saini et al., 2015).

Biochar is a carbon-rich product obtained by a thermochemical conversion of biomass such as OAW, in an oxygen-limited environment (pyrolysis), and constituted mainly of carbon (C) and variable proportion of oxygen (O), hydrogen (H) (Lehmann and Joseph, 2015). Biochar also has good liming capacity via its capacity to provide or exchange basic cations (Ca^{2+}, Mg^{2+}, K^{+}) from biochar particles, and through the carbonates and ashes they can contain (Mosley et al., 2015). Moreover, its porous structure enhances water and nutrient retention due to its high specific surface area (SSA) and the variety of surface functional groups that generate ion exchange capacity (Kookana et al., 2011). Therefore, the use of biochar as a soil amendment in agriculture has demonstrated benefits in terms of soil fertility, including the improvement of nutrient availability in acid soils by increasing pH and cation exchange capacity (CEC), and also because its significant content of recalcitrant carbon offers an opportunity for long-term carbon sequestration (Liang et al., 2006; Steiner et al., 2008). A variety of materials have been readily demonstrated to be suitable as pyrolysis feedstock for biochar production, including animal, forestry and industrial residues, wood, poultry manure, urban wastes, among others.

The International Biochar Initiative (IBI) published guidelines defining a biochar as a carbonaceous material with a molar $H:C_{organic}$ ratio below 0.7 (IBI, 2015). This guideline suggests the use of organic carbon content ($C_{organic}$) in this ratio instead of total carbon to prevent an overestimation of aromatic groups produced by inorganic carbon from carbonates present in high ash biochars. Molar ratios of these elements also provide basic information about the chemical properties of biochars: low $H:C_{organic}$ molar ratios indicate high carbon aromaticity and high chemical stability in soil (resistance to decomposition or recalcitrance); while high $O:C_{organic}$ molar ratios are associated with high polarity due

to a large surface density of oxygenated groups such as carboxyl, phenol, and lactone, among others (Mukome et al., 2013; Sumaraj and Padhye, 2017; S. X. Zhao et al., 2017). Surface oxygenated functional groups have an important role in the surface chemistry of the biochar, which affects functions such as ion exchange capacity, pH, and the capacity for sorption of organic compounds (Liang et al., 2006; Sumaraj and Padhye, 2017). Both the feedstock identity and the pyrolysis temperature used for the biochar production have been identified as the main drivers controlling its final composition and properties (Mukome et al., 2013). These factors are therefore relevant for biochar tailored production approaches, aiming for specific environmental benefits (Boateng et al., 2015) such as carbon sequestration, liming, remediation, or modulation of soil nutrient dynamics. Pyrolysis temperature also influences the extent of release of volatile compounds (VC) and the reorganization by condensation of carbon structures from chemical molecules into aromatic rings (Imam and Capareda, 2012; C. Zhao et al., 2017). Increasing temperatures are known to decrease pyrolysis yield (biochar:feedstock ratio) and VC content, but also increase the fixed carbon content, i.e. a higher proportion of recalcitrant carbon and therefore the carbon sequestration capacity. The higher volatilization of organic compounds is in turn associated with an increased pore size that partly explains the higher SSA generated by bubbles of gases released from the material during pyrolysis, but also with a reduction in the abundance of surface oxygenated groups because of the emission of volatile oxygenated compounds (S. X. Zhao et al., 2017).

In this study, we assessed the chemical and physical properties of biochars produced from pineapple stubble (PS), palm oil fiber (PF), and coffee hulls (CH), under different pyrolysis temperatures. These wastes are highly relevant due to the importance of the respective commercial crops, grown on a large scale in tropical regions, and for the environmental impacts they cause. This is particularly true for the case of pineapple stubble, produced in vast quantities in Costa Rica, with nearly 8.2×10^6 metric tons per year (Coto, 2013), and especially because it requires the use of herbicides at the end of the cropping cycle to speed plant dehydration before tilling for the next cropping season. This is done to avoid the cattle health problems caused by the stable fly (*Stomoxys calcitrans*) that oviposit in decomposing pineapple residues (Solórzano et al., 2015). Regarding PF, the current most common management is composting, while CH are usually used as fuel for coffee bean drying. Thus, pyrolysis is an interesting alternative management option for those wastes able to generate materials with interesting

environmental benefits based on their main properties, which could range from the enhancement of soil fertility, agrochemical pollution attenuation by chemical sorption, to carbon sequestration. While studies that have specifically studied feedstocks of tropical origin are growing, this remains an area requiring further study to fully characterize these biochars and their potential benefits. There is only a handful of papers on the production of biochar using tropical agro-industrial wastes using coffee husk (Asfaw et al., 2019; Domingues et al., 2017; Kiggundu and Sittamukyoto, 2019; Limwikran et al., 2018; Veiga et al., 2017), exhausted coffee residue (Tsai et al., 2012), sugar cane bagasse (Batista et al., 2018; Domingues et al., 2017), rice husk, palm kernel shells (Limwikran et al., 2018), coconut shells (Batista et al., 2018; Limwikran et al., 2018), orange and pineapple peel (Fu et al., 2016; Limwikran et al., 2018), oil palm brunch (Batista et al., 2018), oil palm empty fruit brunch (Samsuri et al., 2014; Shariff et al., 2014; Sukiran et al., 2011) and other palm tree residues (Abnisa et al., 2013), though not for the most widespread waste products.

The aim of this study was to report, for the first time, the chemical and physical properties of biochars produced from three widespread tropical agro-industry wastes for which scarce studies exist in the biochar literature. Furthermore, we propose preferential environmental uses of these biochars as related to their chemical and physical properties, that could guide tailored biochar production from tropical wastes.

2.3. Materials and methods

2.3.1. Feedstock materials

PS corresponded to the whole plant, after removing the edible part, that were collected in the field during the seven days after the harvest and cut into small pieces (2–5 cm). PF consisted of the pulp fibers remaining after oil extraction. CH corresponded to dry endocarp separated from coffee bean, obtained from coffee fruits after mechanical separation of the skin and pulp and elimination of mucilage by fermentation and washing. PS and PF were air dried until reaching a moisture content around 5 %. The three dry feedstocks were transformed into pellets of around 8 mm diameter x 20 mm long with a pelleting machine (model “baby” by Picadoras Sanabria, Costa Rica) before pyrolysis.

2.3.2. Feedstock characterization

Water content of raw materials and moisture of dry feedstock were determined gravimetrically at 105 °C after oven-drying for one day (model 6555, Thermo Electron Corporation, USA). Chemical analyses were performed according to standardized protocols at the Centro de Investigaciones Agronómicas (CIA), Universidad de Costa Rica, based on those of the Soil Survey Staff (Soil Survey Staff, 2014). Briefly, total C and nitrogen (N) contents were determined by dry combustion. P, Ca, Mg, K, S were determined by wet digestion with HNO_3 and inductively coupled plasma atomic emission spectroscopy (ICP). Electrical conductivity and pH were determined in a filtered extract of a dispersion of 10 g of the feedstock in water (40-60 mL), until reaching a saturated paste. Lignin, cellulose and hemicellulose content were determined according to Van Soest et al. (Van Soest et al., 1991).

2.3.3. Biochar production

Feedstock materials described in section 2.3.1. were pyrolyzed using a laboratory muffle. Briefly, about 700 g of pelletized feedstock were placed inside the pyrolysis chamber, that was a stainless-steel container (176 mm long x 162.5 mm wide x 150 mm high) quasi-hermetically sealed by allowing pressure release. Then, the pyrolysis chamber was introduced in a pre-heated laboratory muffle at the selected working temperature (300 or 600 °C) for 1 h, and therefore it was considered a slow pyrolysis. After this, the pyrolysis chamber was placed in a cooling air-purged chamber until reaching ambient temperature. The resulting biochars were ground to pass through a 2 mm sieve and stored in a sealed container. The pyrolysis yield was calculated as follows: %Yield = dry mass biochar/dry mass feedstock x 100.

2.3.4. Biochar characterization

2.3.4.1. Proximate analysis and humidity content

The moisture content of the biochar samples was determined at 105 ºC using a moisture analyzer model PMR-50 (RADWAG, USA). Proximate analysis, including volatile matter (VM), fixed carbon (C_{fixed}), and ash content, was determined according to ASTM D3172-13 (ASTM, 2013) with some minor modifications. Briefly, VM was measured as the weight loss of biochar (dry mass) placed into a covered porcelain crucible heated for 7 min at 950 °C. After this, the remaining biochar was combusted at 750 °C for 6 hours to determine ash content, which was calculated from the mass of combusted residue. C_{fixed}

was calculated by subtraction of VM and ash content from the initial mass (dry mass) of biochar (C_{fixed} = biochar - VM - ash content). The thermal stability of biochars was determined by loss on ignition (LOI) analysis in accordance with Raya-Moreno et al. (Raya-Moreno et al., 2017). Briefly, 1.00 g of biochar was heated at 375 °C for 18 h; then, at 550 °C during 5 h, and finally, at 950 °C during 5 h. After each heating cycle, the remaining mass and the mass loss were measured.

2.3.4.2. Elemental and nutrient analysis

C, H and N contents were determined in a CHN 2400 Series II Elemental Analyzer (Perkin Elmer, USA), using cysteine as reference. $C_{organic}$ was estimated by subtraction of inorganic carbon, calculated from $CaCO_3$ determined as calcium carbonate equivalent or CCE (see below), from the total carbon. The remaining elements were determined by energy dispersive X-ray spectroscopy (EDS) using a FE-SEM Sigma instrument (Zeiss, Germany) on samples prepared as follow. A drop of a 0.1 g L^{-1} water dispersion of the corresponding biochar was placed on a silica wafer and dried at 60 ºC and finally covered with a Cr layer. CCE was determined by a modified version of Erich and Ohno (Erich and Ohno, 1992). Briefly, 1.00 g of dried biochar was mixed with 50 mL of HCl 0.50 mol L^{-1} in a glass tube. The dispersion was boiled for 5 min, then filtered using a filter Whatman No.1. A portion of the filtrate was titrated with NaOH 1 mol L^{-1} using phenolphthalein as indicator. Nutrient contents (NO_3^- and PO_4^{3-}) were determined by a colorimetric method using Hach Reagent Powder Pillows (NitraVer5 and PhosVer3) and measured in a Hach DR/700 Colorimeter. Namely, 2.0 g of the sample was dispersed in 5 mL of a KCl (1 mol L^{-1}), and $NaHCO_3$ (0. 1 mol L^{-1}) solutions, for NO_3^- and PO_4^{3-} respectively, and equilibrated for 24 hours. Afterwards, the solids present in the extracts were washed with 3 cycles of successive centrifugation and decantation of the supernatant (5 mL). The supernatants were mixed and brought to a volume of 25 mL and then diluted with water at a 1:100 rate before mixing with the corresponding reagent.

2.3.4.3. Surface properties, pH and salinity of biochars

The pH and electrical conductivity (EC) of biochars were measured at a 1:5 solid:water ratio after shaking for 1 h, according to Singh et al. (Singh et al., 2017).

Zeta potential (ζ) values of biochars were determined on 1 g L^{-1} dispersions in 0.005 mol L^{-1} NaCl solutions using a Delsa Nano C instrument (Beckman Coulter). Contact angle

(CA) measurements, were performed by the sessile drop method using a homemade goniometer, placing deionized water drops over biochar pellets prepared with a hydraulic press at 2 t with a 11 mm die. The specific surface area (SSA) was determined by the 1-point BET method in a Pulse ChemiSorb 2700 (Micromeritics), using samples previously degassed at 150 ºC in flowing N_2/He mixture for 60 minutes. The surface morphology of the biochar was examined using scanning electron images (SEM), obtained using a microscope (Hitachi model S-3700N). Functional groups of biochars were identified using Fourier transform infrared (FTIR) spectrometry analysis, performed in a Perkin Elmer Spectrum 1000 spectrometer using KBr pellets (1:100 sample: KBr ratio).

2.4. Results and discussion

2.4.1. Feedstock properties

The feedstock materials showed important differences in terms of water, ashes and P, Ca, Mg, and K content, and also in terms of polymeric composition (lignin, cellulose, hemicellulose), but presented similar C, H, O, and N content (Table 2-1). Because of the low ash, and the high lignin of CH, this feedstock showed a similar composition to hardwood derived materials. Conversely, PS and PF resembled the composition of herbaceous biomass such as bagasse or grass, due to their high cellulose and low lignin content (Dhyani and Bhaskar, 2018).

The ash content is expected to mostly consist of inorganic elements such as calcium, magnesium, potassium and silicon, and their differential values in the different feedstocks certainly influence properties such as the pH and EC of the derived biochars (Zornoza et al., 2016). While PS showed the highest ash content, PF presented a clearly lower content, and CH, in agreement with previous reports (Bekalo and Reinhardt, 2010), could be considered almost an ash-free material (Table 2-1) when it is compared with other similar agricultural wastes as rice hulls (23.5%) or more common residues as sugar cane bagasse, rice straw or sorghum bagasse, 1.4, 19.8 and 9.5%, respectively (Dhyani and Bhaskar, 2018). This trend explains the higher and lower EC in PS and CH, respectively, while pH did not follow this trend because it was similar in the different materials. The low ash content in CH is explained by the coffee bean extraction process itself, already described in section 2.3.1. that involves the removal of the pulp and any soluble compounds.

H:C molar ratio was similar for all the materials, while PS showed the highest O:C molar ratio. Differences in O:C molar ratio could be either caused by differences in the content of oxygenated functional groups and/or inorganic compounds. These differences are related to the dissimilar fiber composition of the biomass, including lignin composition and content, but also to the composition of PF co-extractives such as lipids, waxes, proteins, sugars, or phenols, that can partly remain in the feedstocks after drying (Rajasekhar Reddy and Vinu, 2018).

Table 2-1. Properties of feedstock materials. Results expressed on a dry weight basis.

Property	Pineapple Stubble (PS)	Oil palm fiber (PF)	Coffee hulls (CH)
Moisture before drying (%)	70.78	n.d.[a]	5.43
Moisture after drying (%)	2.54	5.35	5.43
Lignin (%)	6.34	8.20	21.79
Cellulose (%)	32.68	43.01	41.80
Hemicellulose (%)	20.46	27.4	20.35
Ash (%)	11.24	3.86	0.40
pH (H_2O)	6.5	7.0	5.1
EC (mS cm^{-1})	9.2	4.5	1.4
C (%)	42.2	43.9	46.1
H (%)	5.7	5.8	6.1
O (%)	51.5	49.6	47.4
N (%)	0.7	0.7	0.4
H:C (molar ratio)	1.62	1.58	1.59
O:C (molar ratio)	0.92	0.85	0.77
P (%)	0.10	0.08	0.01
Ca (%)	0.40	0.20	0.10
Mg (%)	0.25	0.11	0.03
K (%)	1.74	1.30	0.26

[a] n.d.= not determined.

2.4.2. Biochar properties

2.4.2.1. Yield, proximate analysis, and thermal stability

The yield, carbon composition and thermal stability values of the different biochars are shown in Table 2-2. The charred materials yield decreased with increasing temperatures, in agreement with previous studies (Weber and Quicker, 2018; Zornoza et al., 2016), and associated with higher VM losses, given that most VM is lost between 300 °C and 500°C (Cantrell et al., 2012; S. X. Zhao et al., 2017). Results showed the increase of fixed carbon and ash when pyrolysis temperature rises, suggesting a concentration of both variables. A portion of carbon is lost in the form of gases as CO and CH_4 during pyrolysis, explaining the decrease of mass and the relative increase of ashes, mainly consisting of

non-volatile alkaline elements. On the other hand, the increase of fixed carbon is explained by the conversion of aliphatic organic structures to aromatic ones. (Sizmur et al., 2017; Wang et al., 2014; Zhao et al., 2018).

Table 2-2 Yield, proximate analysis, and loss on ignition (LOI) analysis of the studied biochars. Results are expressed as dry basis ± standard deviation.

Biochar feedstock	**PS**	**PF**	**CH**	**PS**	**PF**	**CH**
Pyrolysis temp. (ºC)	300			600		
Code	PS300	PF300	CH300	PS600	PF600	CH600
Yield (%)	56±10	43.7±6.4	55.9±12.5	34.0±1.1	29.6±1.8	25.0±1.2
Volatile matter (%)	47.29±1.86	47.39±0.81	59.36±0.33	11.77±0.95	8.69±0.07	8.62±0.17
C_{fixed} (%)	38.47±1.28	41.11±1.30	39.29±0.36	58.60±0.79	75.98±0.19	88.33±0.09
Ash (%)	14.23±0.68	11.49±0.69	1.35±0.02	29.63±0.19	15.34±0.26	3.05±0.08
LOI 350 °C (%)	83.75±0.24	85.37±0.55	85.82±7.36	66.66±0.12	72.43±2.41	85.73±8.67
LOI 350-550 °C (%)	0.72±0.22	1.68±0.55	12.50±7.36	1.26±0.15	11.21±2.48	10.72±8.54
LOI 550-950 °C (%)	1.66±0.11	0.71±0.19	0.36±0.01	1.90±0.04	1.99±0.07	0.85±0.04

Regardless of the pyrolysis temperature, the highest and the lowest ash content values were also found in charcoals elaborated with PS and CH respectively (with an almost 10-fold difference), the same trend observed in the corresponding feedstocks. Moreover, higher fixed carbon was shown in 600 ºC charred materials compared to the corresponding ones produced at 300 ºC, confirming the known effect of temperature on this property (Enders et al., 2012). While no clear effect of feedstock on fixed carbon content was observed for charcoals obtained at 300 ºC, in PF600 and CH600 values were around 1.4-fold higher than PS biochar. These results might support other studies suggesting an inhibitory effect of high ash contents in feedstock on reactions of ring condensation during pyrolysis, producing low fixed carbon content in biochar (Windeatt et al., 2014).

Fixed carbon value has been proposed as indicator of aromaticity of biochars (Brewer et al., 2011). In this study, PF and CH biochars presented a high aromaticity and, consequently, they were suitable for carbon sequestration in soil. CH and PS chars presented the highest and the lowest values of loss on ignition at 550 °C (LOI 550 °C) respectively, especially those produced at 600 ºC, while the loss on ignition between 550

°C and 950 °C (LOI 550-950 °C) presented the opposite trend. This again indicates a higher mineral content in the PS compared to the CH feedstock, in agreement with the higher ash content of pineapple biochar compared to the other feedstocks, without excluding the potential contribution of traces of soil adhered to roots and low parts of the stalk.

2.4.2.2. Elemental composition

Charred materials produced at 300 °C presented higher C_{org}, H, O, N contents and higher molar ratios H:C_{org} and O:C_{org} values than biochars produced at 600 °C (

Table 2-3). As expected, because of the concentration of inorganic elements, the 600 ºC-produced biochars presented higher values of K, Ca, Mg, Si, CCE, PO_3^{-4} and NO_3^- than the low-temperature chars. Similarly, as a result of pyrolysis itself, both organic and inorganic C showed a clear trend to increase as temperature rose. The observed decrease of H and O could be at least partly explained by their preferential loss as part of volatile matter in comparison to C losses (C. Zhao et al., 2017). H:C_{org} molar ratio, indicative of the degree of aromaticity, decreased with the temperature, indicating an increased recalcitrant C content (Leng et al., 2019), coupled to decreases in O:C_{org} molar ratio that suggest the diminution of oxygenated chemical groups present in chars.

Table 2-3. Elemental analysis and chemical properties of chars

	PS300	**PF300**	**CH300**	**PS600**	**PF600**	**CH600**
C_{org} (%)	49.0	61.2	59.3	56.8	63.3	66.2
H (%)	3.7	4.2	5.0	1.6	1.5	1.9
O (%)	36.5	27.6	33.2	25.1	22.5	23.3
N (%)	1.4	1.2	0.6	1.2	0.8	0.6
H:C_{org} (molar ratio)	0.91	0.82	1.01	0.34	0.28	0.34
O:C_{org} (molar ratio)	0.56	0.34	0.42	0.33	0.27	0.26
K (%)	2.7	2.5	0.9	4.3	4.9	4.2
Ca (%)	0.5	0.4	0.5	0.8	0.9	2.1
Mg (%)	0.4	0.2	0.2	0.6	0.3	0.6
Si (%)	1.8	0.9	n.q.[a]	2.5	1.6	n.q.
CCE (%)	4.53	3.87	2.10	7.75	7.87	3.83
P-PO_3^{-4} (mg kg^{-1})	3.2	3.8	1.4	5.8	4.5	0.7
N-NO_3^- (mg kg^{-1})	< LD[b]	< LD	< LD	24.7	9.9	7.4
pH (H_2O)	6.88	7.07	6.90	9.44	9.54	8.71
EC (mS cm^{-1})	59.06	37.85	5.98	64.3	62.77	10.05
Zeta potential	-17.8	-36.6	-16.95	-36.57	-40.6	-27.09
Contact angle (º)	83	91	110	w.a.[c]	w.a.	w.a.
SSA (m^2 g^{-1})	1.22	<LD	<LD	66	29	59

[a] n.q.: not quantified / [b] LD: below the level of detection / [c] w.a.: water absorption

The van Krevelen diagram (Figure 2-1) for all the feedstocks and biochars showed three clear groups of samples conditioned by pyrolysis temperature, corresponding to the feedstocks and the biochars obtained at each temperature, without a strong effect of feedstock identity on their position in the diagram. Considering the International Biochar Initiative (IBI) and the European Biochar Certificate (EBC) criteria for biochar certification, only the materials pyrolyzed at 600 °C fulfilled the requirement to be considered biochars (EBC, 2012; IBI, 2015). The biochars pyrolyzed at 300 °C were subjected to a torrefaction process rather than a true pyrolysis.

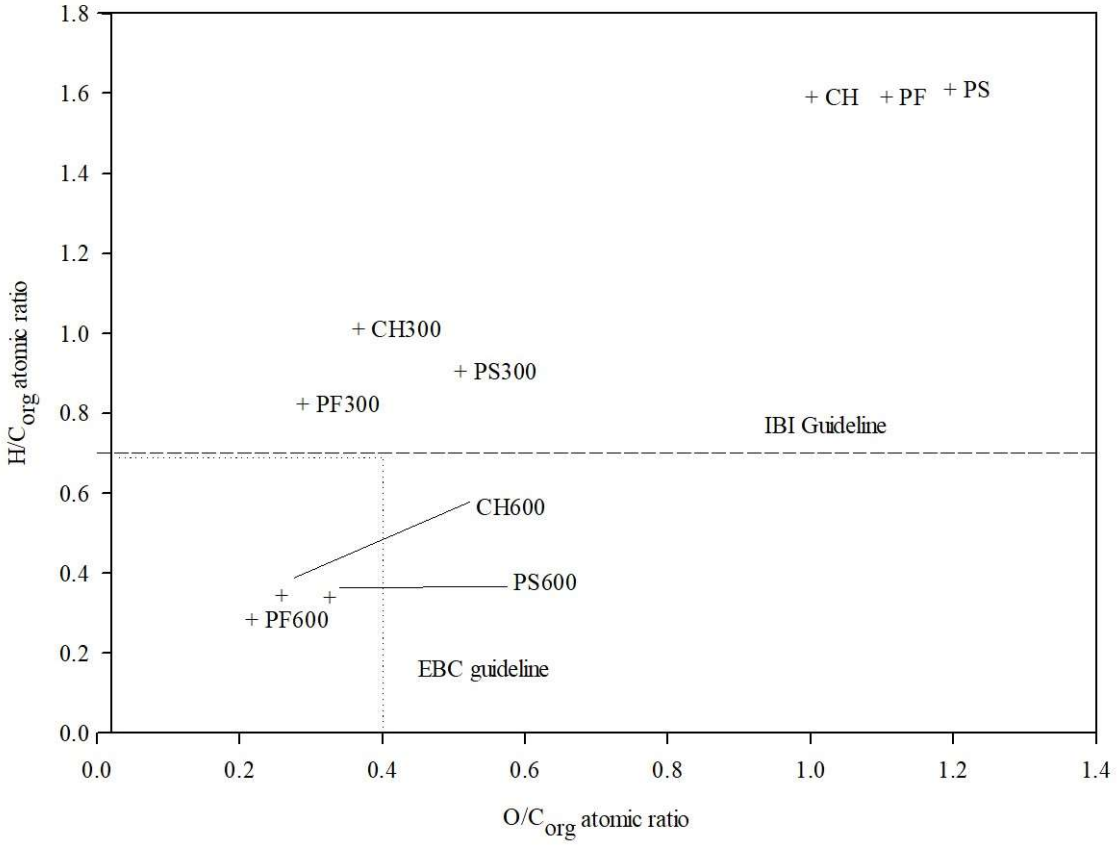

Figure 2-1. Van Krevelen diagram including the biochars and the corresponding feedstocks. Dotted line represents EBC criteria (EBC, 2012) and dashed line represents IBI criteria (IBI, 2015) to classify a charred material as biochar.

Regarding the impact of the original feedstock, with similar elemental contents and elemental ratios, pyrolysis clearly led to higher and similar C_{org} contents in PF and CH biochars compared to PS at both temperatures. The H contents were similar for all the biochars, but PS presented slightly higher N and O contents. This led to higher H:C_{org} molar ratio in CH while PS presented higher O:C_{org} molar ratios at both production temperatures. Those differences are plausibly linked to higher lignin content in PF and

CH, which explains the higher carbon content. Lignin presents fewer oxygenated functional groups and more C-rich structures, mostly aromatic benzene rings, than polysaccharides such as cellulose and hemicellulose (Chen, 2014). On the other hand, PS biochars presented higher mineral content, being consistent with the high ash content of the corresponding feedstock.

2.4.2.3. Interfacial and chemical properties

Figure 2-2 shows the FT-IR spectra from charred materials. The decrease in the abundance of peaks assigned to oxygenated bonds like hydroxyl, carboxy, carbonyl and ether (3415 cm^{-1}, in the higher temperature biochars, which corresponds to O-H stretching; 1692 cm^{-1}, stretching of C=O carbonyl/carboxyl bond; 1034 cm^{-1}, stretching of C-O (ester, alcohol) bond), is indicative of significant oxygen losses (Figure 2-2). This trend is attributable to volatilization, and is also consistent with the measured decrease in the O content and $O:C_{org}$ molar ratio already discussed. The absence of peaks associated with C-H aliphatic bonds (2921 cm^{-1} and 2852 cm^{-1}, stretching) in the 600 ºC biochars could be attributed to cracking and/or condensation reactions during pyrolysis, promoting the reconfiguration of aliphatic chains to condensed carbon rings that increase the aromaticity degree of the biochar. This explanation is supported by the enhancement in peaks assigned to aromatic C-H bonds (peaks between 900 cm^{-1} and 700 cm^{-1}) in the 600 °C biochars spectra and the diminution of the $H:C_{org}$ molar ratios. Interestingly, peaks assigned to oxygenated bonds in BCH300 and BCH600 spectra were slightly lower, suggesting that they remained unchanged and that temperature has a limited effect of on those chemical functional groups.

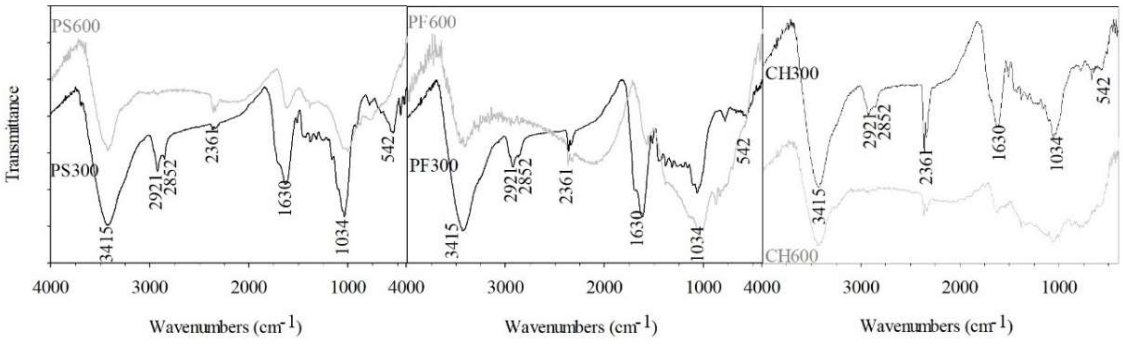

Figure 2-2. FTIR spectra of the biochars studied. PS: pineapple stubble; PF: oil palm fiber; CH: coffee hulls. Number after symbol indicates temperature of pyrolysis in ºC.

Table 2-3 summarizes the different interfacial properties and reactivity parameters of the biochars in this study. The pH of biochar dispersions indicates the acid-base character of the biochars, which in turn determines their net surface charge and liming capacity and, consequently, plays a major role in their performance as soil amendment and pollutant sorbent (Anawar et al., 2015; Chintala et al., 2014). The materials obtained at 300 ºC presented a neutral pH, while those obtained at 600 ºC showed pH values above 8.5. Biochars contain either basic (nitrogen-containing groups such as pyridines and inorganic materials such as carbonates, hydroxides), or acidic (mainly organic, such as carboxylic acids, phenols, phosphonate) components. Due to the preferential loss of acidic sites (Li et al., 2014) with pyrolysis, the increase of fused aromatic rings (Li et al., 2013), and the concentration of salts of alkaline elements (Singh et al., 2017) the basicity increases with temperature. The carboxyl groups can be undissociated and act as cation-exchange sites or undergo OH-consuming saponification reactions and form esters depending on the environmental conditions. Those esters groups are eliminated in high temperature biochars and, although the surface density of the carboxyl groups decreases, the net content increases due to the surface area increment with pyrolysis temperature (Chen et al., 2015).

A higher electric conductivity (EC) was observed with increasing temperature in all cases probably due to the increasing relative content of ash which also explained their higher pH. On the other hand, the lower content of ash and ions (e.g. phosphates, K, Ca, etc.) in CH-based biochars explained the lower conductivity of this biochar, both at 300 and at 600 ºC, compared to those derived from PS and PF.

Zeta potential (ζ) values are used to determine the predominance of surface groups of positive or negative charge, but they are also affected by other factors such as the counter-ions placed at the particle surface. All the charred materials obtained in this study presented negative ζ values, as previously found for biochars (Yuan et al., 2011), which have been related to the predominance of carboxylate groups. The biochars obtained at 600 ºC presented larger ζ absolute values than those obtained at 300 ºC, which was consistent with the transformation of carboxyl groups into carboxylate groups at high temperatures as well as the basicity of the medium, which led to a larger deprotonation of the acidic sites, and the development of negative charges in inorganic materials such as hydroxides (Das and Sarmah, 2015). Finally, the hydrophilicity of the biochars

increased with increasing pyrolysis temperature, coupled to the aromaticity increase as well as the plausible transformation of the ester groups to carboxylate. This hydrophilicity increase in the 600 ºC biochars, together with the development of a larger specific surface and, allegedly porosity (Gray et al., 2014), led to a larger water sorption capacity, which hindered contact angle determinations.

In agreement, SSA increased with the pyrolysis temperature when the 300 to 600 ºC biochars were compared. SSA of torrefied biomass was very low or not detected, as has been similarly reported by other authors (Uchimiya et al., 2011). Biochars showed values comparable with the ones reported for analogous materials (Pagnanelli et al., 2008; Uchimiya et al., 2011) but lower than others produced at a similar temperature (Windeatt et al., 2014). Low lignin content of PS and PF could explain why biochars derived from these materials did not presented a high SSA value, in agreement with Uchimiya et al. (Uchimiya et al., 2011) who indicated that this condition provide low SSA because of the low structural support of pores. In addition, as was observed by other studies with comparable feedstock (Pagnanelli et al., 2008; Windeatt et al., 2014), the presence of residual oil and the decomposition and deposition of tars could explain the SSA found in PF600, being lower than found in PS600. On the other hand, due to the high lignin content it could be expected to find a higher SSA in CH600 than in the other biochars studied, however results showed a similar value. This could be explained by the irregular shape and size of the pores observed in SEM images for this material (Figure 2-3f), that contrasts with the regular shape and size of other biochars (Figure 2-3b, 2-3d). Porosity increased at the higher production temperature, being congruent with the mass loss by volatilization during pyrolysis. Surface morphology of the torrefied biomass showed few open or exposed pores (Figure 2-3), with curved continuous plaques, slit-shaped pores or small and not well-defined pores being more common. In contrast, biochars produced at high temperature showed larger and well-defined pores and more soot, that are consistent with their higher porosity (Mukome et al., 2013).

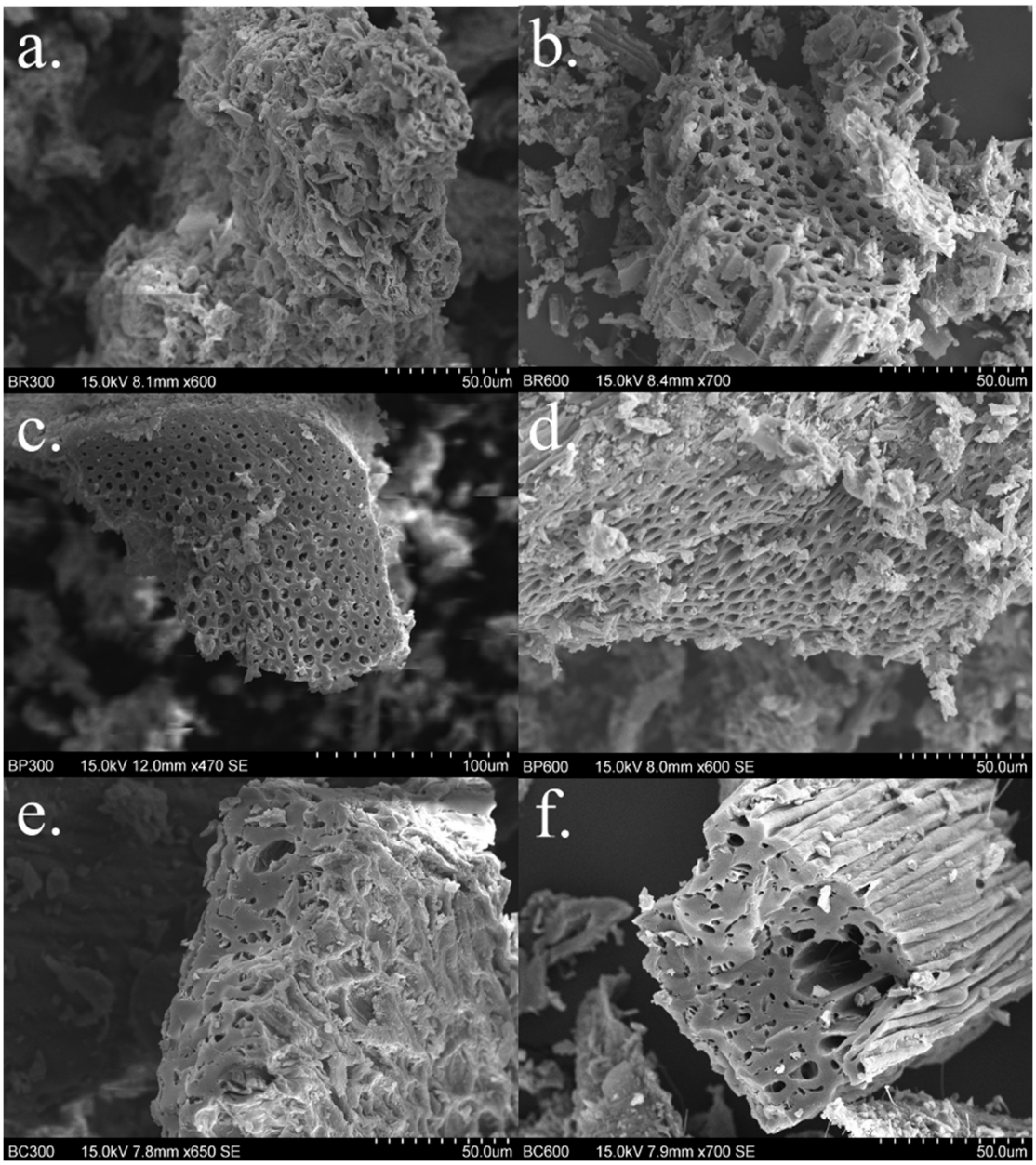

Figure 2-3. SEM images of biochars studied. a: PS300; b: PS600; c: PF300; d: PF600; e: CH300; f: CH600.

In terms of the effect of feedstock material, 300 ºC biochars showed similar pH values, and the same was found for those produced at 600 ºC except for CH600, with a pH that was clearly lower, in agreement with it having the lowest ash content of the corresponding feedstock. On the other hand, PS and PF biochars presented more net negative surface charges, except for PS300, being congruent with their higher content of oxygenated functional groups shown by the FTIR spectra, possibly derived from the corresponding biomass, with higher oxygen content from cellulose and hemicellulose. Biochar derived from PS and CH presented high and similar values of porosity, which is noteworthy

because the corresponding feedstocks had the opposite values of lignin, cellulose and ash contents.

In summary, CH and PF have a higher capacity to contribute to carbon sequestration in soil, due to their high fixed carbon content yield, while this is not true for the PS, which had a lower fixed carbon yield. (Weber and Quicker, 2018). Nonetheless, due to the high ash content and porosity of PS its use as a potential soil amendment could be considered via the short-term provision of nutrients and the long-term increase water retention. The results obtained in this work provide, to our knowledge, novel information of pyrolysis products obtained from widespread tropical feedstocks, giving details on their properties, as a first step to provide tailored biochar production schemes to achieve specific environmental benefits. Additionally, this study demonstrates the feasibility of coffee hulls, pineapple stubble and palm oil fiber lignocellulosic wastes as biochar feedstocks, and the promising role of pyrolysis as a new tool for the management of tropical agro-industrial wastes that are currently causing environmental threats.

2.5. Conclusion

Pyrolysis temperature was the most important parameter for biochar obtention, since only the charred materials obtained at 600 °C, and not those produced at 300 °C, fit with the biochar defined by international standards. Meanwhile, many key properties of the biochars were mostly determined by the feedstock's origin: all the biochars showed a similar pH near 9.5, except for coffee hulls that showed the lowest pH (8.7) probably related to the lower ash content of the feedstock. On the other hand, pineapple and coffee biochars showed similar surface area, near 60 m^2g^{-1}, despite the initial differences in the original feedstock, while oil palm and coffee hulls biochars (not pineapple) presented a fixed carbon content near 76 and 88%, respectively, that was higher than that of pineapple chars (approximately 38 and 59 %). Considering their properties, CH and PF biochars could be more useful for soil carbon sequestration purposes considering their elevated fixed carbon content. Alternatively, PS biochar might be more suitable as a soil amendment, considering its high ash content and surface area, that could provide a short-term provision of nutrients, liming, and a long-term increase in nutrient and water retention, with special interest in tropical arable soils, with low carbon and nutrient contents, and low pH.

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Chapter 3. Amendments with pyrolyzed agro-wastes change the sorption and persistence of bromacil and diuron without mitigating their predicted environmental risks in a tropical soil

3.1. Abstract

Knowledge on the fate of pesticides in tropical soils and how it could be affected by pyrolyzed biomass as amendments is limited. Combining conventional and radiotracer methods, as well as risk assessment tools, the effects of several charred agro-wastes on the sorption, persistence, and ecological risk of the herbicides bromacil (BMC) and diuron (DRN) were evaluated in a tropical agricultural soil under laboratory conditions. Pineapple stubble (PS), palm oil fiber (PF), or coffee hulls (CH) were charred at 300 °C (torrefied) and 600 °C (biochar) and applied at two application rates to the soil. It was found that K_{oc} in unamended soil for BMC and DRN were 18.4 and 212.1 L kg^{-1}, respectively, and the addition of torrefied PS and PF caused a 3 to 4-fold and a 3 to 6-fold increase in BMC and DRN sorption, respectively, while PS biochar increased DRN sorption around 3.5 times. No significant effects were observed with CH materials. On the other hand, BMC degradation in unamended soil was limited compared to DRN, with a half-life of 300 and 73 days, and a mineralization half-life time, as an indicator of biodegradation, of 1278 and 538 days, respectively. While only PF and CH torrefied increased the persistence of BMC, all the torrefied caused the same for DRN. However, despite the effects observed, the predicted ecological risk was not mitigated. Our results highlight the need for scientific evidence on the use of pyrolyzed organic amendments to assess potential benefits and prevent unintended impacts in tropical agroecosystems.

3.2. Introduction

The fate of pesticides in the environment is mainly explained by its persistence and mobility within and between different environmental compartments (Bonmatin et al., 2015). Several transformation and degradation processes are involved in persistence, while mobility in soil is mainly ruled by leaching and runoff (Katagi, 2013). Factors such as pesticide and soil physical and chemical properties, in combination with agricultural practices and climatic conditions, are the main factors determining the fate of the pesticides (Carazo-Rojas et al., 2018; R. Don Wauchope et al., 2002). Several parameters are used to characterize the fate of pesticides, including degradation (half-life, DT50), biodegradation (mineralization half-life time, MT50), binding affinity (soil-water

sorption coefficient, K_d or K_{oc}), potential to leaching (Groundwater Ubiquity Score, GUS) and lipophilicity or hydrophobicity (octanol-water partition coefficient, K_{OW}). Soil organic amendments, such as compost, sewage sludge, pig slurry digestate, paper mill wastes, and more recently biochar, has been demonstrated to affect the pesticides efficiency (either positively or negatively) as a plant protection products, but also their fate when they finally reach the soil (Alvarenga et al., 2015; Liu, Lonappan, Brar, & Yang, 2018). Therefore, ecotoxicological impacts of pesticides for non-target organisms is of interest and how organic amendments like biochar could modulate such undesired effects.

Ecotoxicological risks can be empirically quantified but also estimated by tools that relate the predicted environmental concentrations with the expected effects, such as the Pesticide Impact Rating Index (PIRI) used in this study. This risk assessment tool is focused on the risks for surface and ground waters, and considering the pesticide fate, toxicology, environmental conditions, and agricultural practices (R. S. Kookana, Correll, & Miller, 2005). However, there is still limited empirical information regarding the environmental fate of pesticides in tropical regions for a good prediction, and more specifically about the plausible biochar-pesticide interactions that could strongly influence any ecotoxicological risk.

Biochar (BC) is a carbon-rich and porous material obtained by pyrolysis of organic materials as agro-industrial wastes (Lehmann, Gaunt, & Rondon, 2006). It has been studied intensively in recent decades, as a result of the discovery of its use since prehispanic times in the Amazonian region in the areas known as “Terras pretas” as amendment able to turn poor typical tropical soils into long-term fertile soils for agriculture (DeLuca & Gao, 2019). Due to its physicochemical properties, the addition of BC as soil organic amendment has shown to improve soil quality, fertility, and crop yield in modern times (Drake, Carrucan, Jackson, Cavagnaro, & Patti, 2015; Jeffery, Verheijen, van der Velde, & Bastos, 2011; Sika & Hardie, 2014), but it has also shown to reduce greenhouse gases emissions from soil and/or offset them by carbon sequestration due to the recalcitrant nature of their carbon content (Lehmann et al., 2006; G. Xu, Lv, Sun, Shao, & Wei, 2012). Because its high surface and sorption capacity, BC can also interact with nutrients, emergent pollutants, heavy metals and pesticides, and therefore affects its environmental fate (Bair et al., 2016). Increased BC soil addition rates has been shown to

proportionally increase the sorption of some herbicides while decreasing its photodegradation and leaching (Gámiz, Velarde, Spokas, Celis, & Cox, 2019; Haskis, Mantzos, Hela, Patakioutas, & Konstantinou, 2019; Yu, Mu, Gu, Liu, & Liu, 2011). Similarly, a decreased persistence and bioavailability of some organophosphates pesticides in BC amended soils has been reported (Ali, Khan, Yao, & Wang, 2019; Yavari, Sapari, Malakahmad, & Yavari, 2019).

Pineapple is an important crop in tropical regions and a major source of income and employment, but also requires an intensive use of herbicides before the plants develops its maximum soil cover. As a result, significant amounts of two the most widely used herbicides for this crop, bromacil (BMC) and diuron (DRN), have been reported at trace levels in surface and groundwater of pineapple cropping areas (CICA, 2019; Field, Reed, Sawyer, Griffith, & Wigington, 2003; Hidalgo et al., 2019). BMC is a broad-spectrum systemic herbicide, highly soluble in water and moderately persistent that belongs to the substituted uracil chemical group, and therefore prone to leaching (Lewis, Tzilivakis, Warner, & Green, 2016). DRN is a broad-spectrum substituted phenyl urea herbicide which presents a low solubility in water, being persistent and only slightly mobile in soil (Lewis et al., 2016). Since herbicide leaching to groundwater is of sanitary and environmental concern, the use of biochar as soil amendment could be a potential management solution to reduce the movement of these pesticides. However, there is still little information about their fate and ecotoxicological risks in tropical clay soils, such as Ultisols used in this study, located on heavy rainfall areas that are extensively used for pineapple cropping. Ultisols cover 21% of Costa Rica's surface, have a low pH values, high concentration of Fe and Al, and with kaolinite as the predominant clay, with low nutrient retention capacity. When used for pineapple production, these soils need an intensive liming and mechanization, the last causing important soil erosion (Bertsch, Alvarado, Henriquez, & Mata, 2000). On the other hand, several typical tropical agro-wastes produced at high quantities are promising substrates for biochar production, such as palm oil fiber (usually composted), coffee hulls (used as fuel for coffee bean drying) and pineapple stubble itself (which are usually abandoned in the fields). In the last case, the lack of management poses a risk for cattle, as it is used as breeding substrate by the pathogen stable fly (*Stomoxys calcitrans* L.) (Solórzano et al., 2015), so its use for biochar production could help in reducing their mass and their risk as pathogen host.

The aim of this study was to determine, under laboratory conditions, the fate (sorption, degradation of parent compounds and mineralization) of two widespread used herbicides, BMC and DRN, in a tropical clay soil amended with six different pyrolyzed materials, torrefied or biochars, obtained from three typical tropical crop residues (pineapple stubble, palm oil fiber and coffee hulls) and produced at two different pyrolysis temperatures (300 and 600 ºC). Furthermore, with the information obtained, we estimated the environmental risk (mobility and toxicity) of those pesticides for surface and groundwater as influenced by those charred materials additions according to pineapple cropping conditions of the northern region of Costa Rica as case of study.

3.3. Materials and methods

3.3.1. Soil, biochar, and soil-biochar mixtures

The topsoil (0−20 cm) of a clay Ultisol (herein referred as S; sand 13%, silt 10%, clay 77%) was collected from a pineapple field in Pital (Alajuela, Costa Rica). The soil was air-dried and sieved to 2 mm before being mixed with charred materials later described. Pineapple stubble (PS), palm fiber (PF) and coffee hulls (CH) were used as feedstock to produce each of the materials: PS corresponded to the whole plant after removing the edible part, PF consisted of the fibers of empty fruit brunches remaining after oil extraction and CH was the dry endocarp separated from coffee bean after the fermentation of the mucilage. Feedstock materials were air-dried, chopped, and then pyrolized by placing them in a container that was placed in a pre-heated laboratory muffle at two working temperatures (300 or 600 °C) for 1 h. The resulting charred materials were slightly grounded < 2 mm and then stored in a sealed container. Because their characteristics (Chin-Pampillo et al., 2020) the materials obtained at 600 ºC were classified as biochar according to the International Biochar Initiative criteria (IBI, 2015), while the material obtained from the same process at 300 ºC did not satisfied the standard criteria to be designated as biochar, and hence thereafter referred as torrefied material (Chen, Hsu, Kumar, Budzianowski, & Ong, 2017).

Soil-biochar mixtures were prepared at two different application rates of pyrolyzed materials (0.5 and 1% w/w), which corresponds approximately to an application rate of 1 and 2 kg m^{-2} (10 and 20 t ha^{-1}). Then, each mixture was moistened to 40% of field capacity (30% water content dry basis) and pre-incubated in darkness for 28 days at 25 °C before its use for the herbicide tests.

3.3.2. Pesticides formulates, radiolabeled standards and chemicals

All the reagents used were of analytical grade with the indicated exceptions. Commercial formulates of bromacil (BMC) and diuron (DRN) were purchased from a local supplier. Radiolabeled BMC, [2-^{14}C]-Bromacil (^{14}C-BMC; 1.177 × 10^9 Bq g^{-1}; initial isotopic purity 98.8%) was provided by DUPONT AG PRODUCTS (Wilmington, DE, USA), and radiolabeled DRN, [Ring-U-^{14}C]-Diuron (^{14}C-DRN; 5.937 × 10^9 Bq g^{-1}; radiochemical purity 98.57%; chemical purity 96.12%) was obtained from Izotop (Institute of Isotopes Co., Budapest, Hungary). Physico-chemical properties of the pesticides are shown in Table 3-1. More details regarding the reagents used can be found in the Annex A.

Table 3-1. Physico-chemical properties of the pesticides bromacil and diuron (Lewis et al., 2016). K_{OW}: octanol-water partition coefficient; GUS: groundwater ubiquity score; DT50: half-life of parent compound; PNEC: predicted no-effect concentration.

Parameter	Bromacil (BMC)	Diuron (DRN)
IUPAC name	(RS)-5-bromo-3-sec-butyl-6-methyluracil	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Chemical formula	$C_9H_{13}BrN_2O_2$	$C_9H_{10}Cl_2N_2O$
Solubility - water 20 °C (mg L^{-1})	815	35.6
Log Kow (pH 7, 20 °C)	1.88	2.87
pKa (25°C)	9.27	No dissociation
GUS	6.77 [a]	3.11 [a]
DT50 - lab, 25 °C (d)	300 ± 81 [b]	73 ± 4 [b]
PNEC, growth (mg L^{-1})	6.2×10^{-4} [c]	1.9×10^{-4} [c]

[a] Value obtained as described in section 3.3.3.1.
[b] Value obtained as described in section 3.3.3.2. Mean ± standard deviation.
[c] Value obtained as described in section 3.3.4. .

3.3.3. Experimental setup and sampling procedure

3.3.3.1. Sorption experiments

Adsorption tests were performed using the batch equilibrium method (OECD, 2000). Three replicates per mixture (2 g) were prepared and each treated with ^{14}C-BMC (4000 dpm g^{-1}) and BMC (4 points, 1 mg kg^{-1} to 4 mg kg^{-1}), or ^{14}C-DRN (10000 dpm) and DRN (4 points, 1 mg kg^{-1} to 4 mg kg^{-1}), and completed with $CaCl_2$ 0.01 M until reach 10 mL of the liquid phase. Samples were mixed in a rotator ATR, model RKVS (Laurel, MD) during 24 h in darkness at 25 °C and then centrifugated at 6000 rpm during 10 min at 20 ºC. The amount of BMC and DRN (measured as ^{14}C activity, later described) retained on the solid fraction was evaluated based on the concentration remaining dissolved in the aqueous phase at equilibrium. Sorption was then assessed by adjusting the measured concentrations to the Freundlich model, from which two parameters were obtained by

logarithmic linearization of the resulting Freundlich equation ($\log C_s = 1/n_f \log C_{aq} + \log K_f$), where C_s and C_{aq} are the equilibrium concentration of the pesticide in the soil and aqueous phase; K_f is the constant indicating the sorption capacity of the pesticide in the material, and the term $1/n_f$ describes the degree of deviation from linearity of the relationship between the amount pesticide dissolved and adsorbed on soil that ranges between 0 and 1 (Martins, de Freitas Melo, Bohone, & Abate, 2018). K_f values were used to calculate the partition constant normalized with organic carbon ($K_{oc} = K_f/f_{oc}$), where f_{oc} correspond to the fraction of organic carbon in the soil. Then, K_f values of each charred material were plotted against the organic carbon content at each soil-charred material mixture instead of the nominal charred material addition rates to more properly assess pesticide sorption patterns, due the variable carbon content in each material. The groundwater ubiquity score (GUS = log (DT50) x (4 – log (K_{OC})), a parameter that characterize the potential pesticide loss by leaching was calculated according to Gustafson (1989). Values above 2.8 indicates high potential to leaching (Goss & Wauchope, 1991).

3.3.3.2. Degradation experiments

To determine the half-life (DT50) of BMC and DRN in soil, the test of aerobic transformation of chemicals in soil was used (OECD, 2002) with the modifications consecutively described. Briefly, 5 g (dry weight) of moisture soil (40% of field capacity, 30% water content dry basis) were added to 50 mL polypropylene tubes, by triplicated and per time-point. Then, the soil was spiked with commercial BMC (2 mg kg^{-1}) or DRN (2 mg kg^{-1}), manually homogenized, and incubated in the dark at 25 ± 1 °C for 120 d. The set of triplicated samples was withdrawn at each time-point and pesticide concentration quantified by LC-MS/MS (sections 3.3.3.4.) after 0, 42, 56, 84, 96 and 120 days of incubation. The concentration of pesticides at different incubation time were modelled using a first order kinetic model in order to estimate the DT50 of each pesticide (see Table 3-1).

To determine the pesticide concentration remaining on day 96 (C_{96}) in the soil+charred material mixtures, two sets of triplicate samples for each solid matrix were prepared and incubated as described previously, to compare the pesticide concentration on day 0 with that on day 96, expressed as percentage.

3.3.3.3. Mineralization experiments

The mineralization half-life times (MT50) of ^{14}C-BMC and ^{14}C-DRN were determined by measuring $^{14}CO_2$ production separately for each pesticide at each soil-charred material mixture and in unamended soil (control). The incubation was carried out in biometric systems constituted by a 400 mL glass jars having a CO_2 trap prepared with a 50 mL flask added with KOH (10 mL, 0.1 M) and suspended by a copper wire. A catheter was placed in the lid of each jar and used to replace the KOH solution when needed. 50 g of soil were weighed into biometric flasks and spiked with commercial BMC (2 mg kg^{-1}) + ^{14}C-BMC (5000 dpm g^{-1}) or DRN (2 mg kg^{-1}) + ^{14}C-DRN (5000 dpm g^{-1}). Three replicates were prepared for each pesticide and soil-charred material mixture. The incubation was carried out in the dark at 25 ± 1 °C for 120 d. The KOH in the flasks was withdrawn every 4 days after treatment and replaced with the same amount of fresh KOH. Air exchange was performed pumping air with syringe into the jar. Activity of ^{14}C were measured in aliquots of 2 mL of KOH (section 3.3.4.) and total cumulative $^{14}CO_2$ activity evolved (mineralized) calculated, to estimate the percentage of total ^{14}C-pesticide mineralized. Data were modelized using a first order kinetic model in order to obtain the mineralization half-life time (MT50 = ln 2/k).

3.3.3.4. Analytical procedures

The determination of the ^{14}C activity was carried out by measuring it in 2 mL aliquots from the respective liquid phases of the samples (KOH or $CaCl_2$ solution) placed in Scintillant liquid (8 mL), and measured by liquid scintillation using a Beckman LS6000SC counter (Beckman Instruments Inc., USA).

For the extraction of pesticides from soil and mixtures, 5 g were taken and added with 10 mL of water and 15 mL of a mixture of acetonitrile:acetic acid (1% v/v), then vortexed during 1 min and shaken manually during 1 min. Then, the water was eliminated from the extract and the organic phase was separated by centrifugation. An aliquot of the dry extract was prepared for analysis by LC-MS/MS. Additional samples were spiked with surrogates and internal standard as part of the quality assurance of the results. A detailed description of the procedure can be found in Annex B.

The quantification of BMC and DRN in the extracts was performed using an Agilent 1290 Infinity II LC System (Santa Clara, CA, U.S.) Ultra High-Performance Liquid

Chromatography (UHPLC) coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was done in a Poroshell 120 EC-C18 column (100 mm x 2.1 mm i.d., particle size 2.7 µm; Agilent Technologies, CA, USA), using water containing 0.1% formic acid (A) and methanol containing 0.1% formic acid (B) as mobile phases. The mass spectrometer was operated in dynamic-MRM positive and negative mode. Data acquisition was performed using the MassHunter software (Santa Clara, CA, U.S.). A more detailed description of the procedure can be found in Annex B.

3.3.4. Risk assessment of pesticides mobility and ecotoxicology

The effect of the addition of the torrefied or biochar materials on the mobility and toxicological risks of the pesticides, as compared to the unamended soil, were estimated by the Pesticide Impact Rating Index (PIRI) obtained with a software developed by Kookana et al. (2014), a widely used tool for the estimation of pesticides risk to groundwater and surface water (R. S. Kookana et al., 2005). PIRI assesses the risk based on the estimation of the mobility and the potential impacts for aquatic organisms (toxicity). In both cases, this results from the software estimation of the expected environmental concentrations (thereafter referred as predicted environmental concentrations or PEC), as a function of the pesticides load and its ease of transport, and toxicological information. The PEC is calculated taking in consideration several input values describing the site of concern, namely soil, land use, site, environmental and pesticide characteristics. The PEC in groundwater was estimated by using the Attenuation Factor (AF) index proposed by Rao et al. (1985) with some modifications (Jury, Spencer, & Farmer, 1983), that improve the AF by considering the organic carbon content and microbial abundance with soil depth, while that for surface waters was obtained considering also the load and its transport by erosion, direct-runoff and drift instead of the AF index.

In this study the input values correspond to those of a typical pineapple crop in northern Costa Rica and considering two weather scenarios: dry season (February thru March, 251 mm cumulative rainfall) and rainy season (April thru January, 3239 mm cumulative rainfall) (see the exact input values in the Annex C. Table A- 2, Table A- 3, Table A- 4). The crop was assumed to be developed in a clay soil with a 1º slope, without a buffer zone (worst scenario), and located at 15 m of a water body of 2 m-width and 8 m-depth to the water table. The herbicide was assumed to be applied in a unique event before

planting in a field with no plant cover, sprayed in 100% of the cropping area, at the following rates: BMC (0.6 g m^{-2}, 0.80 active ingredient fraction) or DRN (0.4 mL m^{-2}, 0.80 active ingredient fraction) as the common practice on the field. The pesticide load was calculated considering the management of the pesticides in the agricultural practices (i.e. application rates and frequency, and the treated area size). In addition to the expected annual rainfall at each season, the irrigation associated to pesticide and fertilizer application was considered, consisting of 0.2 L m^{-2} per event, with a total of 20 events per year.

The toxicity risk (ecotoxicity in this study), was then assessed by the quotient PEC/PNEC, being PNEC the predicted no-effect concentration (Table 3-1), which corresponds to the maximum tolerable exposure of a chemical for a whole ecosystem (European Chemicals Bureau, 2003), and estimated from the available toxicological information for a particular chemical in a particular environmental compartment, in this case surface waters. The PNEC in this study was calculated using the MS Excel application "ETX-2000" (Van Vlaardingen, P., Traas, T. P., and Aldenberg, 2003) using non-effect observed concentration (NOEC) on chronic toxicity endpoints for aquatic organisms available collected from the EnviroTox Database (Health and Environmental Sciences Institute (HESI), 2020). Ten NOEC values were available for BMC (3 algae, 1 invertebrate, 1 fish) and 19 NOEC values for DRN (7 algae, 3 invertebrates, 1 fish) (see Annex C. Table A-5, Table A- 7, respectively). Using ETX-2000, a species sensitivity distribution (SSD) was constructed from these datasets, validated by goodness-of-fit tests and then the fifth percentile of the distribution (the hazardous concentration or HC5) was taken as PNEC either for BMC (Annex C. Figure A- 1, Table A- 6) and for DRN (Annex C. Figure A- 2, Table A- 8). The ecotoxicity risk was again labeled as extremely low (EL), very low (VL), low (L), medium (M), high (H), very high (VH), extremely high (EH).

3.3.5. Statistical analysis

All the statistical tests were performed using InfoStat software (Di Rienzo et al., 2018). A non-parametric analysis of variance based on the Kruskal-Wallis test (1952) was performed at 5% level of significance to look for differences between each treatment (each of the charred materials mixtures and controls), while pairwise comparisons were carried out following the procedure proposed by Conover (1999).

3.4. Results

3.4.1. Sorption of the pesticides

By comparing the pesticides K_f values on each charred material and application rate with that of controls (pairwise test, $p<0.05$), it was shown that BMC and DRN sorption increased with torrefied materials addition (except PS300/10, CH300/10 and CH300/20), while only biochars derived from PS were able to do it and only for DRN (see abbreviations and values in Table 3-2). An analogous trend was observed for K_{oc} and torrefied materials. In addition, a closer inspection of the results revealed that mean BMC K_f values were clearly lower, indicating a weaker sorption to the solid matrix compared to DRN. This in agreement with the calculated K_{oc} values (Table 3-2), that qualify BMC as mobile pesticide and DRN as moderately mobile (FAO, 2018).

Similarly, in both pesticides, sorption (K_f) presented a positive relationship with total organic carbon (TOC) in PS and PF torrefied materials, but also in PF biochar, though in this case with a clearly lower slope (Figure 3-1). For all the other materials there was not such relationship or there was slightly negative, indicating no effects of the charred materials or other major mechanisms affecting sorption.

It is worth noticing that addition of PS and PF torrefied materials decreased the heterogeneity of sorption of BMC, reflected with changes in the $1/n_f$. The trend of $1/n_f$ to reach values close to 1 for BMC showed greater availability active sorption sites, resulting that sorption increased linearly with the pesticide concentration. Regarding to DRN, no change in the trend of 1/nf was observed. On the other hand, for both pesticides, the 1/nf value is below 1 in the control soil.

Table 3-2. pH and TOC and sorption parameters of BMC and DRN in soil mixtures of a tropical soil amended with charred agro-wastes. The standard deviation is shown in brackets, n = 3. Each soil-char mixture is codified using the label feedstock+pyrolysis temperature/application rate. Feedstock: Pineapple stubble (PS), palm fiber (PF), coffee hull (CH). Pyrolysis temperature: 300 or 600 ºC. Application rate: 10 (1 kg m-2) or 20 (2 kg m-2). Values with * showed significant differences respect to the control (pairwise test, $p<0.05$).

			BMC			DRN		
Mixture	**pH**	**TOC (%)**	**K_f (L kg^{-1})**	**$1/n_f$**	**K_{oc} (L kg^{-1})**	**K_f (L kg^{-1})**	**$1/n_f$**	**K_{oc} (L kg^{-1})**
Soil	4.2	2.03	0.37 (0.07)	0.64 (0.26)	18.43	4.30 (0.36)	0.81 (0.04)	212.06
PS300/10	4.5	2.13	0.91 (0.05)	0.94 (0.17)	44.86	12.45 (0.82) *	0.88 (0.03)	613.13 *
PS300/20	4.4	2.5	1.29 (0.05) *	0.90 (0.07)	63.54*	18.62 (3.35) *	0.86 (0.07)	917.12 *
PS600/10	4.3	2.23	0.61 (0.03)	0.66 (0.08)	29.95	9.28 (0.97) *	0.74 (0.03)	457.27
PS600/20	4.4	2.5	0.89 (0.05)	0.81 (0.11)	43.89	15.04 (2.01) *	0.77 (0.04)	740.71 *
PF300/10	4.4	2.34	0.98 (0.04) *	0.86 (0.03)	48.47	17.24 (0.93) *	0.86 (0.02)	849.31 *
PF300/20	4.4	2.7	1.67 (0.16) *	0.90 (0.07)	82.22*	26.57 (4.64) *	0.87 (0.06)	1308.95 *
PF600/10	4.4	2.46	0.34 (0.05)	0.84 (0.16)	16.79	5.60 (0.66)	0.83 (0.04)	276.02
PF600/20	4.5	2.53	0.29 (0.09)	1.17 (0.35)	14.17	7.25 (1.66)	0.79 (0.11)	357.14
CH300/10	4.3	2.38	0.41 (0.04)	1.10 (0.28)	20.26	6.24 (0.80)	0.85 (0.06)	307.59
CH300/20	4.3	2.57	0.56 (0.04)	0.80 (0.19)	27.8	8.26 (1.07)	0.87 (0.06)	406.83
CH600/10	4.3	2.44	0.30 (0.14)	1.10 (0.47)	14.92	5.65 (1.21)	0.91 (0.11)	278.41
CH600/20	4.4	2.91	0.31 (0.01)	1.09 (0.30)	15.03	5.35 (0.23)	0.83 (0.03)	263.76

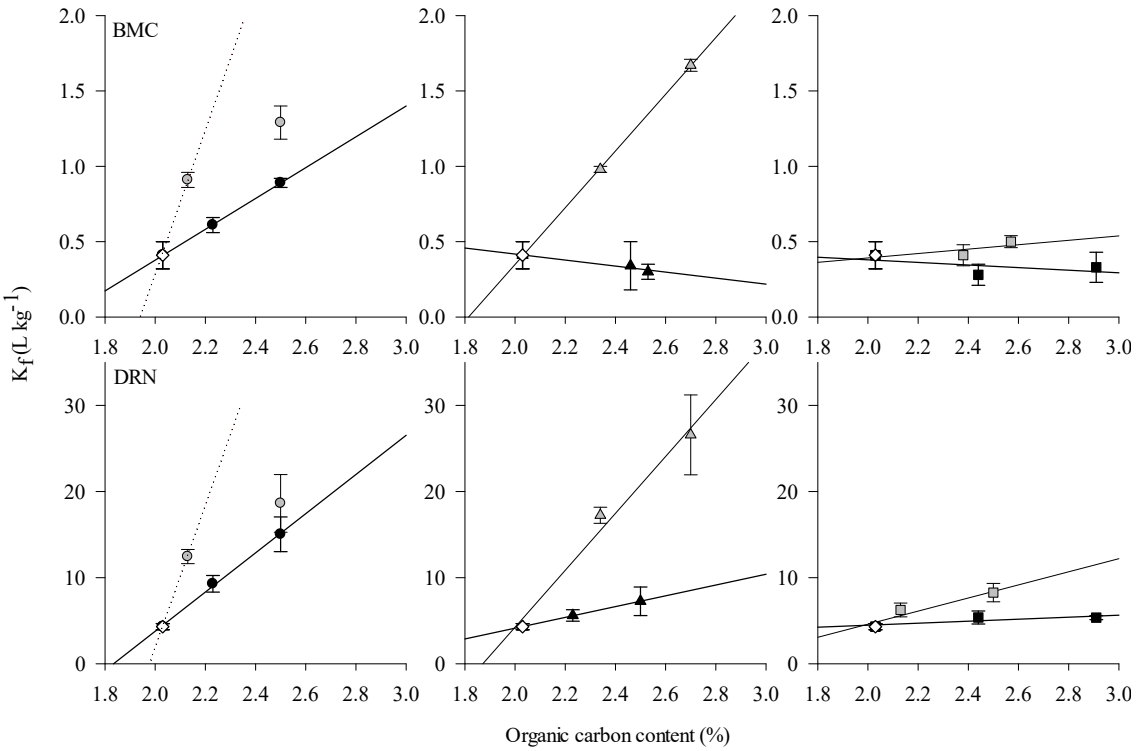

Figure 3-1. Distribution of K_f values of the pesticides bromacil (BMC, top) and diuron (DRN, bottom) as function of the total organic carbon content (TOC) in amended soil. Grey (▒) and black (▉) symbols correspond to materials charred at 300 °C and 600 °C, respectively. Circle represents pineapple stubble (○/●, PS), triangle palm fiber (△/▲, PF), and square coffee hull (□/■, CH). White diamond (◊) corresponds to the values in control soil. Error bars correspond to standard deviation of three replicates. Dotted lines indicate an estimation of the expected linearity.

3.4.2. Persistence of the pesticides

The C_{96} of both pesticides was unaffected by biochar amendments but affected by some torrefied materials: BMC C_{96} values were higher in PF300/10 and CH300/10 (pairwise test, $p<0.05$), while DRN C_{96} values was significantly higher in PS300/10 PS300/20, PF300/10, PF300/20 and CH300/10 (Figure 3-2), indicating that torrefied materials were slowing down pesticide degradation.

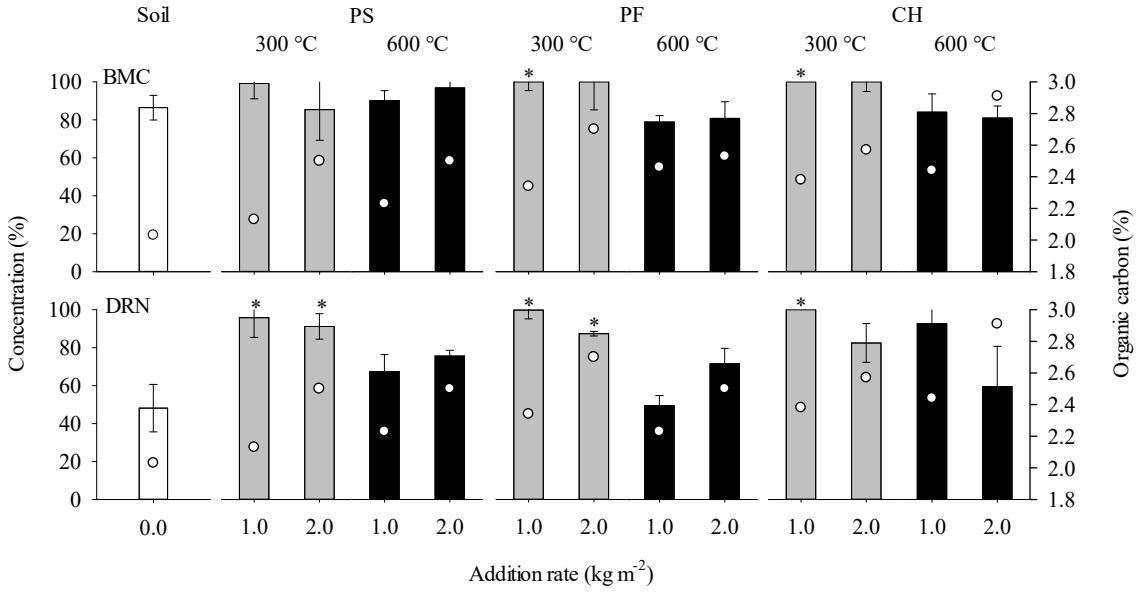

Figure 3-2. Pesticide concentration remaining on day 96 (C_{96}) of the incubation for bromacil (BMC, top) and diuron (DRN, bottom) in the different treatments. White bars (□) correspond to control soil, and grey (▒) and black (■) bars to mixtures with torrefied and biochar materials, respectively. The white circle (○) corresponds to TOC in the mixture (see right Y-axis). Error bars correspond to standard deviation of three replicates. Values with * were significantly different to the control (pairwise test, $p<0.05$).

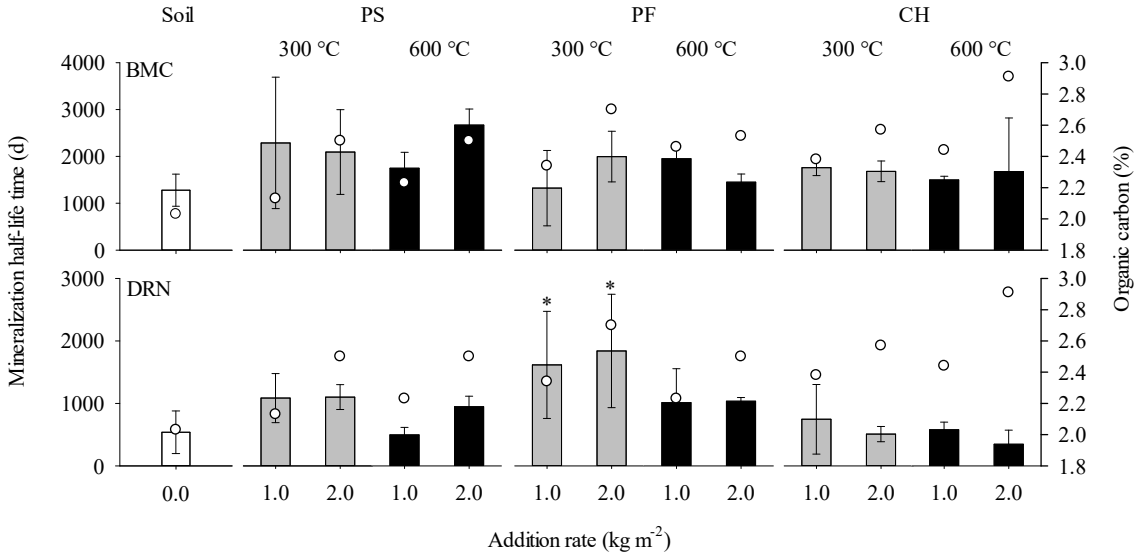

Figure 3-3. Mineralization half-life time (MT50) of pesticides bromacil (BMC, top) and diuron (DRN, bottom) in the different treatments. The white bars (□) correspond to control soil, and the grey (▒) and black (■) bars to mixtures with torrefied and biochar materials, respectively. The white circle (○) corresponds to TOC in the mixture (see right Y-axis). Error bars correspond to standard deviation of three replicates. Values with * were significantly different to the control (pairwise test, $p<0.05$).

Regarding the mineralization half-life time (MT50) for both pesticides, the amendments with charred materials generally did not increase persistence measured as MT50, with the exception of DRN in PF300/10 and PF300/20 with higher MT50 values when compared to those of control soil (Figure 3-3).

An unanticipated finding was that degradation, measured as concentrations after 96 days (C_{96}), of BMC in soil (86.4 ± 6.5%) was almost twice than that of DRN (48 ± 12%), given that DRN was demonstrated to be more strongly adsorbed than BCM. Similarly, (MT50) values for BMC (1278 days) were nearly 2.4 times higher than those observed for DRN (538 days), in agreement with the trend observed in the C_{96} values.

3.4.3. Estimation of the environmental risk to waterbodies

The mobility and toxicity risk of these pesticides in the different amendment scenarios showed no differences with control soil (Table 3-3)

. However, while the mobility to surface water of BMC increases from high (H) in dry season to very high (VH) in rainy season, mobility risk to groundwater was low (L) under all scenarios. Finally, the risk of toxicity of both pesticides was extremely high (EH) in both seasons and water compartments and was unaffected by charred material addition.

Table 3-3. Risk assessment for mobility and toxicity of the pesticides BMC and DRN estimated for groundwater and superficial water for dry and rainy season using the PIRI model. The levels of the risk are presented using the following abbreviations according to the categories of the model: extremely low (EL), very low (VL), low (L), medium (M), high (H), very high (VH), extremely high (EH).

		Surface water				Groundwater			
		Mobility		Toxicity		Mobility		Toxicity	
Pesticide	Season / Mixture	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
BMC	Soil	H	VH	EH	EH	L	L	EH	EH
	PS300/10	H	VH	EH	EH	L	L	EH	EH
	PS300/20	H	VH	EH	EH	L	L	EH	EH
	PS600/10	H	VH	EH	EH	L	L	EH	EH
	PS600/20	H	VH	EH	EH	L	L	EH	EH
	PF300/10	H	VH	EH	EH	L	L	EH	EH
	PF300/20	H	VH	EH	EH	L	L	EH	EH
	PF600/10	H	VH	EH	EH	L	L	EH	EH
	PF600/20	H	VH	EH	EH	L	L	EH	EH
	CH300/10	H	VH	EH	EH	L	L	EH	EH
	CH300/20	H	VH	EH	EH	L	L	EH	EH
	CH600/10	H	VH	EH	EH	L	L	EH	EH
	CH600/20	H	VH	EH	EH	L	L	EH	EH
DRN	Soil	H	H	EH	EH	L	L	EH	EH
	PS300/10	H	H	EH	EH	L	L	EH	EH
	PS300/20	H	H	EH	EH	L	L	EH	EH
	PS600/10	H	H	EH	EH	L	L	EH	EH
	PS600/20	H	H	EH	EH	L	L	EH	EH
	PF300/10	H	H	EH	EH	L	L	EH	EH
	PF300/20	H	H	EH	EH	L	L	EH	EH
	PF600/10	H	H	EH	EH	L	L	EH	EH
	PF600/20	H	H	EH	EH	L	L	EH	EH
	CH300/10	H	H	EH	EH	L	L	EH	EH
	CH300/20	H	H	EH	EH	L	L	EH	EH
	CH600/10	H	H	EH	EH	L	L	EH	EH
	CH600/20	H	H	EH	EH	L	L	EH	EH

3.5. Discussion

3.5.1. Pesticides fate as affected by charred materials addition

3.5.1.1. Effects on sorption are linked to the abundance of surface functional groups

One interesting finding is that Sorption measured as K_f and K_{oc} of BMC and DRN generally increased with addition rates of torrefied materials (except PS300/10, CH300/10 and CH300/20), while only biochars derived from PS were able to do it and only for DRN. Furthermore, this sorption was strongly dependent of total carbon (TOC) content in the soil or mixtures in most cases, as previous reports have indicated for pesticides such as DRN (D. Wang et al., 2015) and others with increasing biochar content (Aziz, Murtaza, Usman, Basra, & Niaz, 2018; Yu et al., 2011). Our findings agree only partially with that, since sorption was stronger with torrefied but generally not with biochars. While TOC increases in the different materials K_f values were different (Figure 3-1), suggesting that sorption is not only affected by TOC but also by the quality of pyrolyzed material, as already highlighted in other studies (Ahmad, Kookana, Alston, & Skjemstad, 2001; Motoki, Iwafune, Seike, Otani, & Asano, 2014), which is reflected with the important differences on its physical or chemical properties.

A previous study (Chin-Pampillo et al., 2020) revealed higher presence of functional chemical groups containing oxygen (such as carbonyls and hydroxyl) in PS300 and PF300 than in PS600, PF600, CH300 and CH600, on the other hand a similar total surface was measured in the PS and CH biochars. All that said, our results suggest that the higher sorption observed in mixtures with torrefied materials for the studied pesticides is mainly explained by the formation of hydrogen-bonds between the BMC and DRN molecules and surface oxygenated functional groups, as occurs with soil humic substances (Senesi, 1992; X. Wang, Guo, Yang, Tao, & Xing, 2011) and reported with other biochars (Sun et al., 2012) than a simple effect related with the magnitude of total surface. Furthermore, the low sorption of pesticides showed by the CH300 amended soil, like biochars, support this explanation. These results suggest that hydrogen-bonding could be a major mechanism of sorption for BMC and DRN in soils amended with torrefied materials, considering its hydrophilic character, as suggested for other herbicides (J. Wang, Zhang, Xiong, Liu, & Pan, 2011). Conversely, in the studied biochars, the hydrogen-bonding could not have a high influence because their low presence of oxygenated functional groups. The higher sorption of DRN respect to BMC in the biochars could be explained

by the hydrophobic moiety of its molecule that interact with the hydrophobic regions of the biochar-soil system (Liu et al., 2018; Petter et al., 2016).

Regarding the pesticides sorption deviation from linearity observed in the PS300+soil mixtures (Figure 3-1), it could be explained by the release of dissolved organic carbon (DOC) from biochar, demonstrated to disrupt the adsorption of pesticides to soil (Cox, Velarde, Cabrera, Hermosín, & Cornejo, 2007). According with previous reports, pyrolyzed materials between 350 °C and 500 °C can release organic substances (measured as DOC) enriched with carboxylic and polyphenolic groups (Uchimiya, Ohno, & He, 2013). Therefore, DOC can decrease the sorption of the pesticides to the soil, because on the one hand adsorb pesticide, and on the other compete for adsorption sites in the soil, increasing the concentration of dissolved pesticide (Cox et al., 2007).

3.5.1.2. Persistence of the pesticides was very high but generally unaffected by charred materials

BMC persistence was comparatively higher than DRN, with around 85% and 50% remaining after 96 days, and with MT50 of 1278 days and 538 days, respectively. When measured as DT50, the value observed for DRN (Table 3-1) is consistent with previous reports, which indicates values between 78 and 128 d in tropical acid clay, loamy sand and sandy loam soils with OC between 1 and 2% under laboratory conditions at 20 °C (Guimarães et al., 2018). It is also worth noticing since the persistence in this tropical soil is much higher than similar studies that have indicated BMC DT50 values between 12 and 44 days under laboratory conditions at 20 °C (Sarmah, Close, & Mason, 2009), 4 to 5 months in field conditions (Gardiner, Rhodes, Adams, & Soboczenski, 1969) and 5 to 6 months (Lewis et al., 2016), all determined in silt loam soil belonging to temperate regions. Similarly, Zhu et al. (2002) observed a high residual concentration after one-year of BMC application to a field clay soil and Sanders et al. (1996) found phytotoxic levels of BCM in soil up to 11 months after the application.

Regarding the effect of the addition of charred materials measured as C_{96}, the torrefied or biochars did not changed it for BMC (except PF300 and CH300), while DRN persistence generally increased in torrefied materials and PS and PF biochars (Yang et al., 2006, Cheng et al., 2016). The limited effects on BMC persistence could not be explained by its higher sorption capacity that could limits their bioavailability and biodegradation, as discussed previously. Sorption is the key process as a previous step limiting the

occurrence of several other phenomena, such as dissipation, biodegradation, or bioavailability (R. Don Wauchope et al., 2002). The results from this study only agree partly with others, pointing out that increments of organic matter in soils can increase persistence by slowing down its degradation (Dube, Lesoli, & Fatunbi, 2009), since this was only validated in DRN. Microbial degradation is the main degradation mechanisms for BMC in the soil rather than any abiotic factor (Dube et al., 2009; Sarmah et al., 2009). As an example, Corbin and Upchurch (1967) failed to find any influence of soil pH in its degradation between 4.3 and 7.5 for isocil, an uracil herbicide chemically similar to BMC. Therefore, the high persistence of BMC observed in the soil could be either explained by its slow degradation, explained by the expected low microbial activity caused by its low organic matter content; but also as a result of the direct reduction of microbial activity with BCM addition demonstrated by Sanders et al. (1996), although the mechanism behind is out of the scope of this study.

When assessed as MT50, no effects of charred materials were found for BMC persistence, and for DRN it increased only in the PF materials, as shown for other pesticides and as related to carbon addition (Si et al., 2011; Tatarková et al., 2013). Again the DRN lower sorption is the explanation which allows some degree of bioavailability and biodegradation (Yang et al., 2006, Cheng et al., 2016).

3.5.2. The addition of charred materials does not mitigate the environmental risks of the pesticides to waterbodies

The mobility and toxicity risks of pesticides to groundwater and surface water under the modelled conditions were non-significantly affected by the addition of charred materials when those addition scenarios were simulated by using as input values the sorption data at each application scenario.

Concerning the predicted mobility risk, it was high (dry season) to very high (rainy season) in all the treatments for surface waters, but low for groundwaters, and for both BMC and DRN, in agreement with their already reported classification as mobile and moderately mobile, respectively. Moreover, the GUS values for BMC and DRN in literature (Table 3-1), used as indicator of leaching ease, suggest high and moderate risk of leaching, respectively. In both cases, such a divergence between seasons: firstly, in the input value where pesticides were supposed to be applied in a unique event before planting, that implies a lower load to several application practice; and secondly, the

transport risk was estimated for the dry (2 months) or rainy season (10 months) separately. This predicted limited mobility might agree with the study by Correia et al. (2007), who evaluated the leaching of the herbicide atrazine, with similar sorption properties (K_{oc} = 80 mg L^{-1}), in lysimeters under laboratory conditions on an Ultisol from Brazil. After two months, around 7% of the initial applied pesticide was leached and 75% was found in the first 5 cm of soil. In addition, when they evaluated leaching under field conditions, atrazine concentrations of the upper layer decreased exponentially, and traces reached 50 cm depth after two months.

Concerning the risk for surface water, and the predicted high to very high risks of both pesticides, it is mainly explained by the specificity of tropical climates (Daam & Van Den Brink, 2010) and the pineapple cropping practices. The typical short but intense rains (Daam & Van Den Brink, 2010) and artificial irrigation in for agrochemical application in bare soils require draining systems aiming to quickly evacuate any water excess that impairs this culture development, therefore maximizing agrochemicals transportation by runoff. In a similar study, Correia et al. (2007) found that in the first rainfall event two days after an atrazine application, 2.1% of the initially pesticide applied was removed by runoff water. Moreover, 75% of pesticide in runoff water was dissolved while the remaining 25% was associated with soil particles. In fact, according to Wauchope (1978) pesticide dissolved fraction could range between 65% and 95%. All that said, this might explain the elevated mobility risk of the pesticides in our study considering their relatively high-water solubility and low sorption, as also shown by the increase in mobility risk of BMC in the rainy season.

Regarding the toxicity risk, it was extremely high in all the considered amendment scenarios and pesticides, indicating the inability of charred materials to mitigate the ecological risks of pesticides. This partly result from the conservative approach taken, not based on mortality data, since PNEC values were used as assessment factor (AF), which represents a pesticide concentration for which 95% of the organisms in an aquatic community have a lower NOEC for chronic endpoints (reproduction and growth), and therefore plausibly protecting most of the species present and, by extension, the ecosystem functioning. The estimated risk agrees with results of monitoring data available for rivers of the north region of Costa Rica (CICA, 2019) showing that the usual concentrations for those pesticides were higher than the calculated PNEC. In our

conceptual approach we considered the groundwater and surface water as an integrated system, where both waterbodies interact as an unique and continuous riverine system (Korbel & Hose, 2011; Sophocleous, 2002; Ward, 1989). Because their toxicity mechanism of photosynthesis inhibition, the presence of BMC and DRN has an important impact on photosynthetic aquatic organisms, as shown by their low NOEC values used for the derivation of PNEC, and quickly translated to ecosystem levels impacts as they are a key trophic group as primary producers for the whole aquatic community functioning (Brock, Lahr, & Van den Brink, 2000; F. L. Xu, Jørgensen, & Tao, 1999).

As a final though, the results of our study highlight the need for an experimental assessment of unintended effects of new agronomic practices expected to be beneficial, of any new agronomical practice purposes before its inclusion as a regular crop practice, to avoid negative to no positive outcomes. In the specific case of pesticides, the use tools such as PIRI is helpful as preliminary information for decision-making of innovative practices, but also as a previous step to scale-up experimentation to field conditions and the implementation as a regular practice. Despite of the high inherent toxicity risk of BMC and DRN for aquatic ecosystems that might overcome any mitigation effect of organic amendments or other practices, the results in this study provide new knowledge on the impact of pyrolyzed materials on two widespread pesticides fate and environmental risk in the relatively understudied tropical regions. More research is still required to better understand the fate of pesticides under tropical conditions as well as the potential benefits and unintended impacts of the addition of charred materials.

3.6. Conclusion

BMC degradation in this tropical soil was very limited compared to DRN, with around 85% and 50% remaining after 96 days, DT50 of 300 days and 73 days, and MT50 of 1278 days and 538 days, respectively.

Torrefied materials delayed the degradation of both pesticides, measured at day 96 of incubation (but not most of the biochars tested) but especially that of diuron, which comparatively was the easiest to degrade. A similar trend was observed for persistence measured as MT50, though only for DRN and PF materials.

The increased sorption and persistence effects exerted by these materials can be explained by their abundance of surface oxygenated functional groups, rather than their total surface or the total organic carbon addition.

The predicted mobility risk was unaffected by charred materials addition and only seasonal differences were found: increased from high (H) in dry season to very high (VH) in rainy season for BMC, and was high (H) in both seasons for DRN; for groundwater, risk was low (L) under all scenarios for both pesticides. For both pesticides the toxicity risk was estimated to be extremely high (EH) in all the seasons and water compartments for aquatic organisms and was unaffected by charred material addition.

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Chapter 4. Biochar addition to a tropical agroecosystem do not alter herbicides efficiency and improves soil habitat function

4.1. Abstract

In the last decade, the use of biochar, a carbonaceous material, has been claimed as a win-win agricultural amendment able to improve soils fertility. However, little is still known about the effect of biochar on the efficiency of the herbicides as well as to non-target organisms of tropical agroecosystems.

In this study, by using an ecotoxicological approach under greenhouse conditions, the effects of different charred materials on the herbicides bromacil and diuron is assessed in an agricultural Ultisol, by direct assessment of their herbicide activity on lettuce and their ecotoxicity for two soil invertebrate species (collembolans and enchytraeids) and for microbial soil functional diversity. Pineapple stubble (PS) and coffee hulls (CH) pyrolyzed at 300 °C (torrefied materials) or 600 °C (biochar) were applied at two different rates to soil (10 and 20 t ha^{-1}) were combined or not with the herbicides.

The efficiency of the herbicides was unaffected by the addition of charred materials as well as plant emergence and growth, except for the increment of emergence in the PS300 mixture. No effects of the herbicides on fauna species survival were observed, and on the contrary their reproduction was promoted. In contrast, a generalized avoidance of amended soil was observed in enchytraeids opposed to the preference for collembolans. Moreover, microbial functional diversity remained generally unaffected, but the degradation rates of some substrates decreased, suggesting some toxic effects but also a strong functional redundancy. Despite the promising benefits observed, further studies are still required to better known the unintended effects of addition of charred materials in tropical agroecosystems.

4.2. Introduction

The fate of pesticides in the environment is defined by their physical and chemical properties together with those of the receiving soil, but also by the current agricultural practices, pedoclimatic conditions and biotic elements (Carazo-Rojas et al., 2018). The particular fate of a pesticide in a given scenario determines their efficiency for the target biological group through changes in their bioaccessibility and bioavailability, it also

determines the degree of interaction with soil ecosystems, thus causing unintended effects on non-target organisms inhabiting the crop soils and the neighboring watercourses.

Soil organic amendments, like compost, sewage sludge, pig slurry digestate, paper mill wastes and more recently biochar, as reactive solid materials and by changing soil properties, can also cause shifts in the pesticides efficiency and fate (Alvarenga et al., 2015; Liu et al., 2018). However, there is still a knowledge gap regarding the environmental fate of widely used pesticides in tropical regions and more specifically on the interaction between charred materials and pesticides.

Biochar (BC) is a carbon-rich and porous material obtained by pyrolysis of organic materials as agro-industrial wastes (Lehmann et al., 2006) that has been intensively studied in the last 20 years for their potential, as soil amendment for the sustainable agricultural use on tropical and low fertility soils, among other benefits, reproducing the fertile Anthrosols known as “Terras pretas”, man made in pre-Hispanic times in the Amazonian region (DeLuca and Gao, 2019). The thermal conversion by pyrolysis of biomass to BC or other charred materials could be a revalorization pathway for tropical agro-wastes produced at high amounts such as coffee hull or the pineapple stubble. In the last case this might mitigate the health concern for cattle caused by the stable fly (*Stomoxys calcitrans*) (Solórzano et al., 2015), which grows in pineapple stubble when it is abandoned in the fields after harvest.

It has been reported that using BC as organic amendment improved soil fertility and in several cases reduced the irrigation needs and increased the crop yield (Drake et al., 2015; Jeffery et al., 2011; Sika and Hardie, 2014), it also offset/reduced greenhouse gases through carbon sequestration (Lehmann et al., 2006; G. Xu et al., 2012), and was able to mitigate soil GHG emissions (Fidel et al., 2019). Because its high adsorption power, some studies also observed that BC is able to change the environmental fate of substances such as emergent pollutants, heavy metals and pesticides, including herbicides (Bair et al., 2016; Gámiz et al., 2017; Nag et al., 2011). Additionally, a decrease in persistence and bioavailability of some organophosphates has been reported (Ali et al., 2019; Yavari et al., 2019). However, some BC have also shown to indirectly inhibit plant growth by promoting the competition with microorganisms for nitrogen as a result of the provision of labile organic carbon of freshly applied char (Deenik et al., 2010; Marks et al., 2014a), but also by directly releasing toxic compounds or by changing the soil environment that

detrimentally affect plants, microorganisms or soil fauna (Domene et al., 2015b; Domene, 2016; Marks et al., 2014b; Mccormack et al., 2013). The last effect is of concern given the role of soil fauna in the regulation of soil ecosystems key processes such as decomposition and primary production (Domene, 2016).

In this study, bromacil (BMC) and diuron (DRN), two widespread used herbicides in tropical areas cropped with pineapple, are used as model agrochemicals to test the effects of the addition of charred materials on their efficiency and on eventual unintended effects of non-target organisms. The first one, BMC, is a substituted uracil broad-spectrum herbicide which exhibits an important persistence in soil and is prone to leaching (Lewis et al., 2016). On the other hand, DRN is a substituted phenyl urea herbicide, persistent and slightly mobile in soil with a low solubility in water (Lewis et al., 2016). However, despite its wide use in tropical agriculture there is still little information about the environmental fate and especially their unintended ecotoxicological potential effects of its use in tropical Ultisols, developed in high rainfall areas. Because their chemical properties such as water solubility, low sorption and high potential of leaching, both pesticides present high environmental risk to migrate by surface runoff or leaching to water bodies. Tropical Ultisols typically contain 1:1 clays (mainly kaolinites), low pH values and high concentration of Fe and Al, being cultivated under intensive liming and mechanization for the pineapple production (Bertsch et al., 2000).

The aims of this study were determining for both BMC and DRN, under greenhouse and laboratory conditions using a tropical Ultisol, whether biochar addition could: i) change their efficiency as herbicides; and ii) mitigate any unintended ecotoxicological effects for non-target soil fauna bioindicator species and for microbial functional diversity.

4.3. Materials and methods

4.3.1. Soil, charred materials, and their mixtures

The topsoil (0−20 cm) of an acid clay Ultisol (S) (sand 13%, silt 10%, clay 77%) was collected in Pital (Alajuela, Costa Rica) from a field devoted to pineapple cultivation. It was air-dried and sieved to 2 mm before mixing with charred materials. Charred materials were produced using pineapple stubble (PS) or coffee hulls (CH) as feedstocks. PS corresponds to the whole plant after removing the edible part. CH corresponds to the dry endocarp separated from coffee bean by wet separation of the skin, the pulp and the

mucilage. Briefly, feedstock materials were air-dried, chopped and then pyrolyzed in a pre-heated laboratory muffle at 300 or 600 °C for 1 h in a O_2-depleted atmosphere. The resulting charred materials were grounded at 2 mm and then stored in a sealed container. After its characterization, the materials were considered as biochars (600 ºC) or torrefied materials (300 ºC) according to International Biochar initiative standards (IBI, 2015) as described by Chin-Pampillo et al. (2020). Mixtures of soil and torrefied or biochars were prepared with application rates of 0.5 or 1 % (weight dry basis), equivalent to 10 and 20 t ha^{-1}. $CaCO_3$ was added to soil at an application rate equivalent to 5.8 t ha^{-1} (2.7 g $CaCO_3$ kg^{-1} content dry basis) for pH and acidity corrections, as the usual practice in the area during the land preparation before planting.

pH and electrical conductivity (EC) of soil and mixtures were determined from 1:5 (w/v) aqueous extracts. Briefly, 10 g of solid sample were added with 50 mL of deionized water and mixed during 1 h at 60 rev min^{-1}. Then, the aqueous phase was decanted, centrifuged, and filtered through Whatman 42 filter paper. Soluble ions were determined from a 1:10 (v/v) dilution of the aqueous soil extract. To determine Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+ a CS12A Dionex cation column on a Dionex ICS-1100 ion chromatograph (Dionex, Sunnyvale, USA) was used, while to determine concentration of Cl^-, NO_2^-, NO_3^- and SO_4^{2-} a AS4A-SC Dionex anion column on a Dionex DX-100 ion chromatograph (Dionex, Sunnyvale, USA) was installed.

4.3.2. Pesticide spiking and application rates

All reagents used were of analytical grade. Commercial formulates of diuron (DRN) and bromacil (BMC) were purchased from a local market. Before carrying out the bioassays later described, each soil-organic amendment mixture was moistened to 30% of its field capacity (22.5% moisture w/w) using a pesticide spiked aqueous solution (9.7 mg BMC L^{-1} or 7.9 mg DRN L^{-1}), to reach the recommended equivalent application rates for pinneaple, which was 2.3 mg kg^{-1} for BMC and 1.8 mg kg^{-1} for DRN. For plant emergence and growth tests, and for microbial functional diversity measurements, the emulsion addition was carried out by direct application on the pots used for plant growth, by the slow addition of 70 mL of the spiked solution avoiding any leaching, while for fauna, the addition was carried out in glass tray then manually homogenized. In both cases, the spiked mixtures were left overnight to equilibrate pesticide concentration with the soil solution before adding seeds or fauna in the corresponding bioassays.

4.3.3. Greenhouse plant emergence and growth tests

Plant tests for herbicide efficiency assessment were conducted according to the OECD standardized protocol (OECD, 2006) and using *Lactuca sativa* as test species. Each replicate consisted of a squared plastic pot of 190 cm^3 (7 x 7 x 6.5 cm) filled with 300 g of soil or soil-charred materials mixture. Then, pesticides were added as already described and left overnight to equilibrate, and then ten seeds were planted in each pot. Five replicates of each treatment were prepared and the pots randomly distributed. A dropping system was applied to each pot for automated irrigation, adjusted to daily watering of 20 mL per pot. The average temperature was 17.5 °C, while maximum and minimum temperatures during the experiment were 31.9 °C and 7.5 °C, respectively, and the average of day light during the whole experiment was 13h.

Emergence was monitored daily until 50% of the initial seeds in controls emerged. Then, seedlings were thinned to only two individuals per pot for growth assessment. After 21 days, plants were collected and gently washed in tap water to remove soil particles from the roots. Then, plants were placed in a paper bag and oven-dried at 60 ºC during 48. Finally, root and aerial plant parts were separated and weighted as dry biomass measure.

4.3.4. Laboratory faunal and microbial bioassays

4.3.4.1. Folsomia candida avoidance and reproduction tests

The avoidance tests were conducted according with a standardized protocol (ISO, 2011) using the *Folsomia candida* (Isotomidae, Collembola, Hexapoda) as test species. Briefly, each replicate consisted of a 100 mL polypropylene vessel with sealable tops. Before the soil addition a plastic sheet division in the test vessel and 15 g of control soil was placed in one side and 15 g of test soil in the treatment side. Once the soil was similarly leveled in both sides, the plastic division was retired, and the vessel gently was moved until each soil sides were in contact. Then, fifteen juveniles aged 2 to 10 days old were placed on the soil surface. Vessels were closed and kept at 25 ± 1 ºC in the dark. After 48 h the vessel was opened, and the plastic sheet was introduced to separate the two soil portions. Control and test soil were poured with care and placed in separate vessels. Then each vessel was flooded with water colored with a dark ink to refloat the organisms and allow their counting. Five replicates of each treatment were prepared. Vessels containing control soil in both sides (dual tests) were also prepared and used for the validity of the

test as it allows to discard any avoidance behavior not directly related to the treatments assessed.

The reproduction tests were conducted according to a standardized protocol (ISO, 1999). Briefly, 30 g of moistened soil substrate were added to each test vessel (100 mL polypropylene vessels with sealable tops), and then ten juveniles aged 10-12 days were introduced. Six replicates of each treatment were prepared. Vessels were kept at 25 ± 1 ºC and under a 12:12 h light:dark cycle, and were opened and aerated weekly. Baker's yeast was added as food source on day 0 and 14. On day 28, the vessels were flooded with a water containing a dark ink to refloat the organisms and high-resolution photos were taken for the adults and their offspring counting using the software ImageJ 1.52n (National Institutes of Health, Bethesda, MD, USA).

4.3.4.2. Enchytraeus crypticus avoidance and reproduction tests

Avoidance tests were conducted according to a standardized protocol (ISO, 2008) using the *Enchytraeus crypticus* (Enchytraeidae; Oligochaeta; Annelida) as test species. The same test vessels and setup methods of the collembolan tests were used for enchytraeids but using 15 g of fresh soil in each size instead. Five replicates of each treatment were prepared, as well as dual test replicates containing control soil in both sides for the validity assessment of the test. Vessels were closed and stored at 25±1 ºC in the dark and after 48 h the vessel was opened, and the plastic sheet introduced to separate the soils. Control or test soil was poured with care to separated vessels what then were then filled with 70 % alcohol solution until it reached 1 cm above substrate level to fix the organisms, and then 10 drops of 1% alcoholic solution of Bengal red were added for their dyeing. Vessels were closed with the cap, gently agitated, and the individuals allowed to stain a minimum of 12 h, and then the individuals were counted by eye after passing all the vessel content through a 0.2 mm mesh sieve with the help of tap water to clean fine particles.

Reproduction tests were conducted according to a standardized protocol (ISO, 2014). Briefly, 30 g of moistened soil subtract were added to test vessel and then ten adults (individuals with clitella) were introduced. Six replicates of each treatment were prepared. The vessels were kept at 25 ± 1 ºC and under a 12:12 h light:dark cycle, and were opened and aerated weekly. Finely grounded oat was added as food source at day 0 and weekly. On day 28 all the vessels were filled with alcohol solution to fix the individuals and Bengal red added, as already described, to fix and dye the individuals

4.3.4.3. Microbial functional diversity assay

Using fresh soil samples collected at the same day of the end of plant tests, the functional diversity of microbial community was assessed by the MicroResp™ assay, and following the protocol described by Campbell et al. (2003). Briefly, soils were loaded into 1.2 mL deep-well plate (ca. 0.35 g soil per well). Then, water was added as well other different carbon substrates to each well and the plate was sealed hermetically face to face with a second plate containing a colorimetric gel CO_2 trap and stored for 5 days at 25 ºC. The microbial activity was determined by the intensity of the emission of CO_2 from soil, that was trapped causing a proportional color change of the cresol red indicator that was determined by measuring the absorbance at 570 nm with a microplate spectrophotometer (model Sunrise™, Tecan Trading AG, Switzerland) before and after 6 h of incubation at 25 ºC. The physiological profiles were determined as a response of the addition to 7 different C-substrates: one simple sugar (D-glucose); one polysaccharide (Cellulose); two amino acids (γ-aminobutyric acid, L-arginine) and three carboxylic acids (α-ketoglutarate, citric acid, L-malic acid).

4.3.5. Statistical analysis

Differences between the treatments of the different biological endpoints assessed were evaluated by using the Mann-Whitney pair-wise test (McKean and Ryan, 1977; MINITAB LLC, 2019) performed at 5% level of significance. Avoidance behavior was statistically determined by Fisher's test (MINITAB LLC, 2019) $p < 0.05$. Microbial functional diversity was assessed calculating the Shannon diversity index (Kennedy and Smith, 1995) and differences between treatments again assessed by Mann-Whitney pair-wise tests. The significant shifts in the microbial functional profile with different soil treatment were assessed by a one-way ANOSIM analysis based on Bray-Curtis coefficient (9999 permutation) using the PAST software (Hammer et al., 2001).

The response of the test organisms as different endpoints (faunal avoidance, survival, and reproduction, plant emergence and biomass, and microbial functional diversity) was modeled using generalized linear models (GLMs) and using pH, electrical conductivity, water-soluble ions and microbial activity (as the basal respiration and as glucose-induced respiration obtained in the MicroResp™ assay) as explanatory variables. For modeling, the avoidance response was expressed as percentage and calculated as $A = [(C-T)/N]$, where C corresponds to the number of individuals in the control soil, T is the number of

individuals in the test soil, and N corresponds to the total number of individuals collected at the end of the experiment, i.e., C + T. This leads to positive values when avoidance is present, and therefore negative values indicating attraction behavior.

The normality of each response variable was tested using the *shapiro.test* function of the *stats* package and a therefore Gaussian distribution for the GLM construction was assumed. When not, a Poisson distribution was assumed (*E. crypticus* survival, and lettuce shoot and root biomass). Then, for each response variable, a full GLM was constructed containing all the explanatory variables using the *glm* function of the *stats* package, and then, using the *dredge* function of the MuMIn package, the model with the lowest AIC was selected as the best. Then this best model was tested for their terms significance using the *summary* function, the fraction of the variance in the response variable estimated by calculating the pseudo-R^2 [1-(Residual Deviance/Null Deviance)] and by the inspection of residual diagnostic plots obtained using the *glm.diag.plots* function of the *boot* package (Canty and Ripley, 2020).

4.4. Results

4.4.1. Effect of charred materials on the herbicides' effectivity

The addition of charred materials itself did not globally affect the emergence and growth of *Lactuca sativa* as no differences were observed regarding to the control soil in most of the untreated or herbicide-treated samples with the exception of torrefied PS applied at the highest rate (PS300/20), which improved emergence (Figure 4-1-I).

As expected, either in the bromacil- and diuron-treated unamended soils (with no biochar), a decrease of emergence was observed (Figure 4-1-II and Figure 4-1-III). When the last treatment was compared with their corresponding biochar-amended treatment, no effects of the addition of charred materials was found, and the only differences were within charred materials produced from the same feedstock. Namely, for bromacil, higher emergence at the highest dosage of torrefied PS biochar (PS300/20) was observed but only when that was compared with the lowest addition rate (PS300/10) (Figure 4-1-II). For diuron, the only difference was between the higher emergence with the biochar PS at the higher rate (PS600/20) compared to the torrefied PS at the lowest rate (PS300/10)

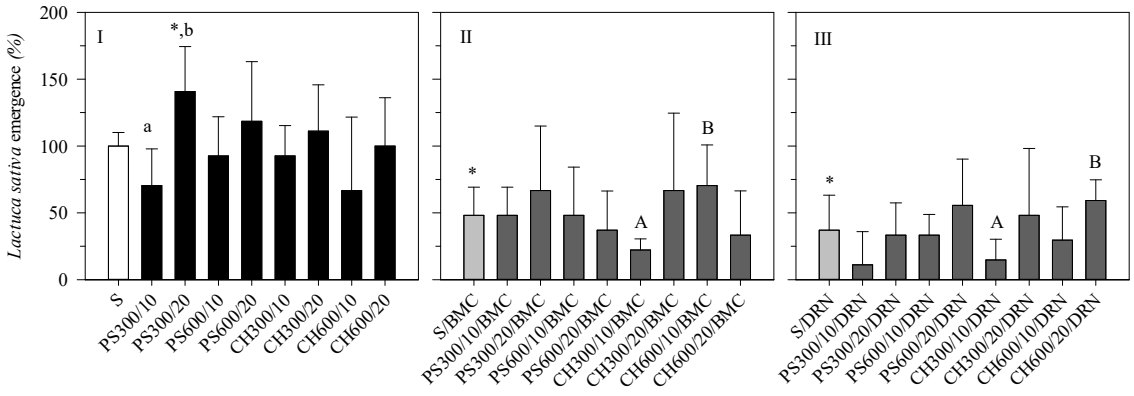

Figure 4-1. Emergence of Lactuca sativa expressed as percent with respect to the untreated and unamended control soil: I. No pesticide treatments. II. Treated with BMC. III. Treated with DRN. Labels in the X-axis reflects the treatment identity: S=unamended soil (without charred materials); charred materials feedstock (PS=pineapple stubble, CH=coffee hulls), pyrolysis temperature (300/600 ºC), and application rate (10/20 t ha^{-1}; and the herbicide treatment supplemented (DRN=diuron, BMC=bromacil). White bars (□) represent the control soil, black bars (■) represent the pesticide untreated amended soil, light grey bars (▒) represent the herbicide treated unamended (with no charred materials) soil and dark grey bars (▓) represent herbicide treated amended soil (mixed with charred materials). Error bars correspond to standard deviation of five replicates. Values with * were significantly different to the control (Mann-Whitney pairwise test, $p<0.05$). Lowercase letters indicated difference between PS amended soils, and capital letters report differences between CH amended soils (Mann-Whitney pairwise test, $p<0.05$).

Regarding plant shoot and root biomass, none of the charred materials affected those endpoints compared to control soil, and within feedstocks the only difference was the two-fold increase in shoot biomass in torrefied CH at the highest application rate (CH300/20) when compared to the lowest addition rate of the corresponding biochar (CH600/10). In herbicide treated soil-charred materials mixtures, none of the emerged seedlings survived, and therefore any mitigation effect of the charred materials could not be observed, with the exception of the CH600/20 treated with BMC, the only treatment with surviving plants, were a slight significant increase in shoot biomass was apparent (Figure 4-2-I). Furthermore, in CH600/20/BMC was observed a higher growth of roots respect to the untreated sample CH600/20 (Figure 4-2-II).

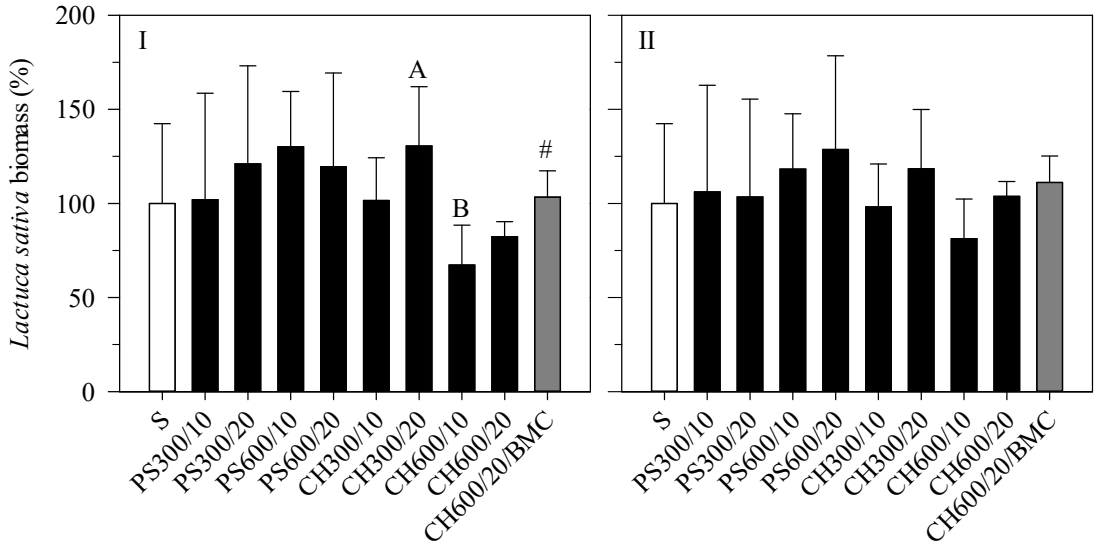

Figure 4-2. Lactuca sativa shoot and root biomass as bioindicator expressed as percent with respect to the untreated and unamended control soil: I. Shoot biomass. II. Root biomass. See Figure 4-1 for treatment label abbreviations. White bars (□) represent the unamended control soil, black bars (■) represent the pesticide untreated amended soil and dark grey bars (■) represent treated amended soil. Error bars correspond to standard deviation of five replicates. Values with capital letters indicate difference between CH amended soils and with # indicate differences with the respective pesticide untreated amended soil (Mann-Whitney pairwise test, $p<0.05$).

4.4.2. Effect of charred materials on the mitigation of unintended effects of herbicides on soil invertebrates

It was observed that in almost all the cases collembolans preferred soils treated with herbicides while for enchytraeids were observed the opposite preference. However, the addition of charred materials produced opposed effects as were observed in the two bioindicator species, with *F. candida* generally preferring biochar-amended mixtures over control and *E. crypticus* avoiding them, and irrespective of the presence of the herbicides (Figure 4-3).

Regarding the soil- charred material mixtures without herbicides, *F. candida* preferred the biochar-amended mixtures in half of the cases, namely PS600/20, CH300/10, CH600/10 and CH600/20 (Fisher test, $p < 0.05$; Figure 4-3-I). Conversely, *Enchytraeus crypticus* showed a clear avoidance of biochar mixtures and their preference for control soil, in a trend that was significant in PS300/20, CH300/10, and all the PS600 and CH600 addition rates (Fisher test, $p < 0.05$; Figure 4-3-IV). In contrast, neither *F. candida* nor *E. crypticus* showed avoidance behavior for herbicide-treated unamended soil when offered

with untreated unamended soil with the exception of the preference for DRN-treated soil in *F. candida* (Figure 4-3-III).

Regarding the BMC-treated amended soils, PS300/10 were the only preferred by collembolans (Figure 4-3-II), while enchytreids avoided both PS600 addition rates (Figure 4-3-V). The CH mixtures treated with BMC were always preferred by *F. candida*, while *E. crypticus* was unaffected by treatments except for the CH300/10 mixture, which was significantly avoided. Concerning the DRN-treated biochar mixtures, a clear preference over the control was observed by *F. candida* (Figure 4-3-III), except for the PS600/10 mixtures. *E. crypticus* generally did not responded and only avoided some diuron-treated biochar mixtures (PS300/10, CH300/10, CH600/10) (Figure 4-3-VI).

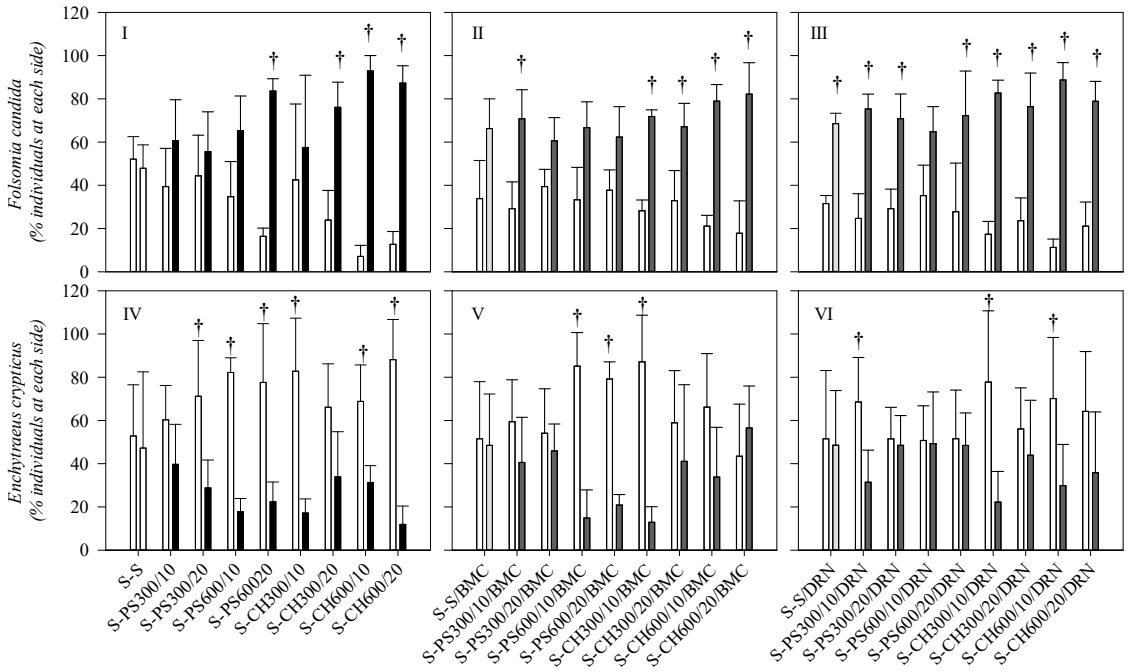

Figure 4-3. *Folsomia candida* (top) and *Enchytraeus crypticus* (bottom) distribution (%) in the two soil sections corresponding to untreated and unamended control soil versus charred material-amended mixtures (I. and IV); in control soil versus bromacil-treated bromacil-treated soil and soil- charred material mixtures (II and V); and control soil versus diuron-treated soil versus diuron-treated soil- charred material mixtures(III and VI).White bars (□) represent the unamended control soil, black bars (■) represent the herbicide untreated amended soil, light grey bars (▒) represent the herbicide treated unamended soil and dark grey bars (▓) represent herbicide treated amended soil. See Figure 4-1 for treatment label abbreviations. Error bars correspond to standard deviation of five replicates. Values with † indicate avoidance of one of the two soil sections, i.e. a non-random distribution (Fisher test, $p<0.05$).

Concerning the effects on survival, the charred materials addition did not affect survival of *Folsomia candida* (Figure 4-4-I) while in contrast significantly increased for

Enchytraeus crypticus (Figure 4-4-IV). Within charred materials, the only difference in *F. candida* survival is that was higher in PS300/20 than in PS300/10 survival (Figure 4-4-I). The addition of BR did not caused differences in survival between the unamended soil and soil- charred material mixtures either for collembolans and enchytraeids (Figure 4-4-II and IV), and surprisingly, and increased enchytraeid survival was observed in the bromacil-treated unamended soil compared to unamended soil without pesticides (Figure 4-4-IV). In contrast, the addition of DRN to some charred material mixtures reduced *F. candida* survival (PS300/20, PS600/10, PS600/20, CH300/10, CH600/20) (Figure 4-4-III), but increased *E. crypticus* ones (PS300/20, CH300/20, CH600/10, CH600/20) (Figure 4-4-VI). Within charred materials, some significant differences in collembolans survival were observed, without a clear pattern linked to feedstock, pyrolysis temperature or addition rate (Figure 4-4-III).

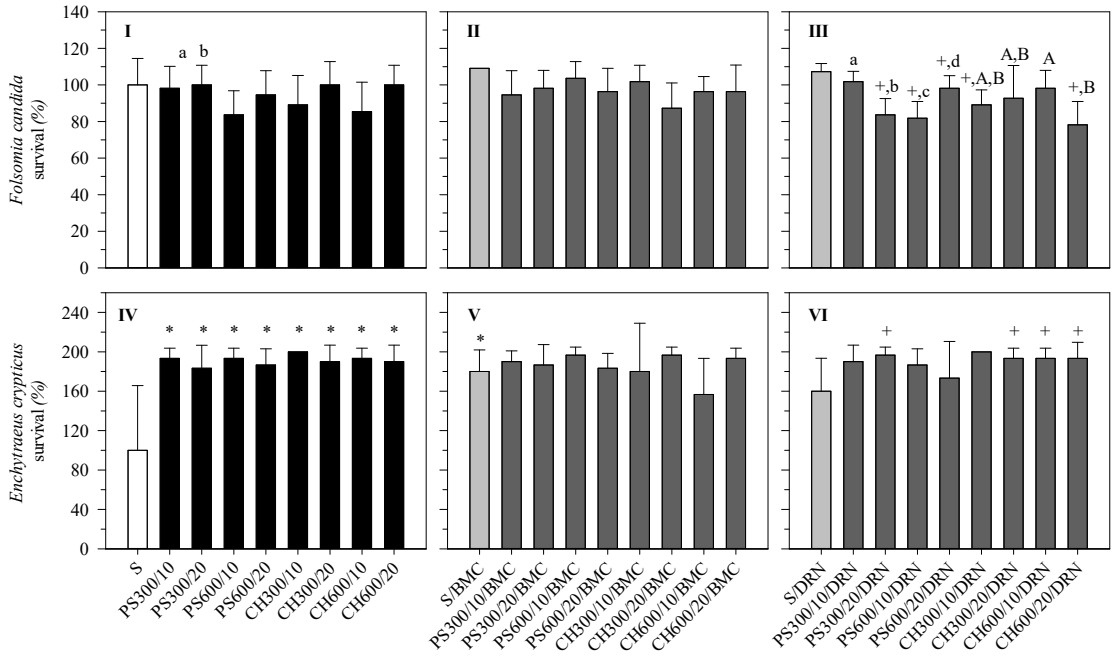

Figure 4-4. Survival of *Folsomia candida* (top) and *Enchytraeus crypticus* (bottom) expressed as percent with respect to the untreated and unamended control soil: I. and IV. Untreated amended soils, II. And V. Amended soils treated with BMC. III. And VI. Amended soils treated with DRN. See Figure 4-1 for treatment label abbreviations. White bars (□) represent the unamended control soil, black bars (■) represent the herbicide untreated amended soil, light grey bars (▒) represent the herbicide treated unamended soil and dark grey bars (▓) represent herbicide treated amended soil. Error bars correspond to standard deviation of six replicates. Values with * indicate difference respect to control soil, + respect to the pesticide treated amended soil, lowercase letters indicate difference between PS amended soils and capital letters between CH amended soils (Mann-Whitney pairwise test, $p<0.05$).

Regarding the effects on invertebrate reproduction, a generalized promotion with charred materials addition was observed for collembolans and enchytraeids, but in the last species case only not seen in CH600/20 (Figure 4-5-I and Figure 4-5-IV). Remarkably, the sole addition of herbicides promoted the reproduction of both invertebrates as shown with the significantly higher number of juveniles in the herbicide treated unamended soil (Figure 4-5-II and Figure 4-5-VI). In agreement, in the herbicide treated charred material mixtures for F. candida for either BMC and DRN reproduction was clearly potentiated over the corresponding herbicide treated control (Figure 4-5-II and Figure 4-5-III). On the contrary, this was not observed for *E. crypticus*, which only showed such reproduction potentiation in PS600/20 (Figure 4-5-V).

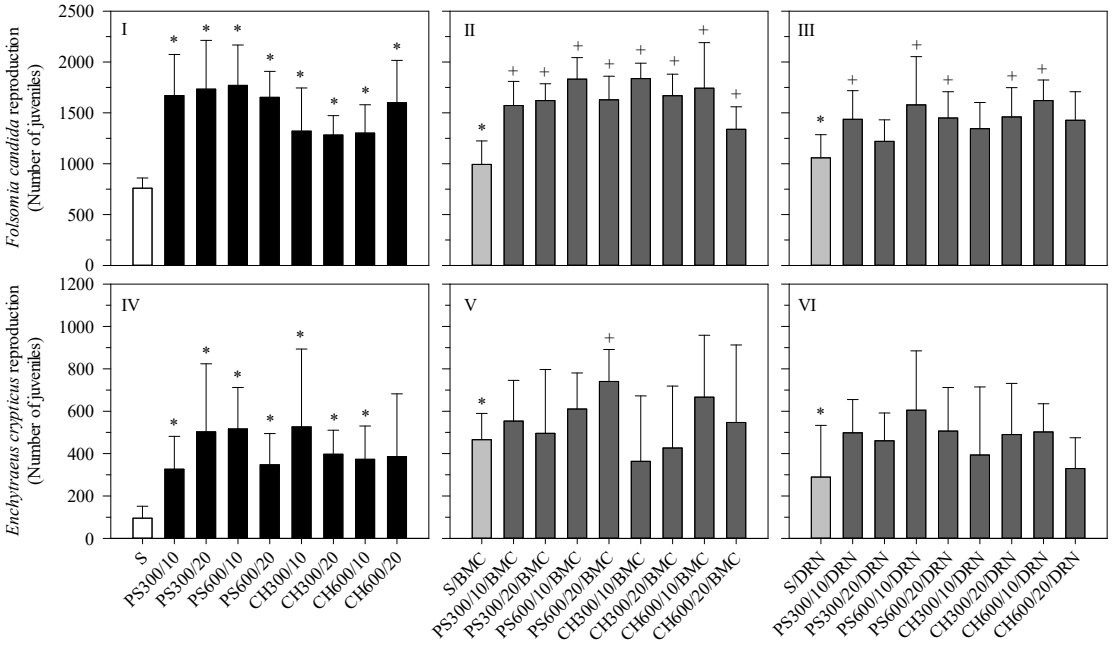

Figure 4-5**.** Reproduction of *Folsomia candida* (top) and *Enchytraeus crypticus* (bottom) expressed as percent with respect to the untreated and unamended control soil: I. and IV. Herbicide untreated amended soils, II. And V. Amended soils treated with BMC. III. And VI. Amended soils treated with DRN. See Figure 4-1 for treatment label abbreviations. White bars (□) represent the unamended control soil, black bars (■) represent the herbicide untreated amended soil, light grey bars (▒) represent the herbicide treated unamended soil and dark grey bars (▓) represent herbicide treated amended soil. Error bars correspond to standard deviation of six replicates. Values with * indicate difference respect to control soil, + respect to the pesticide treated unamended soil (Mann-Whitney pairwise test, $p<0.05$).

4.4.3. Effect of charred materials on the mitigation of unintended effects of herbicides on microbial functional diversity

Either the sole treatment with herbicides (Figure 4-6-II and Figure 4-6-III) and charred materials (Figure 4-6-I) did not caused changes in microbial functional diversity, but some changes were observed when applied in combination. Namely, Shannon index increases were observed in DRN-treated charred material-mixtures (Figure 4-6-III), though not for BMC (Figure 4-6-II). The only significant differences were between charred material-soil mixtures, without clear patterns associated to feedstock, pyrolysis temperature or addition rate.

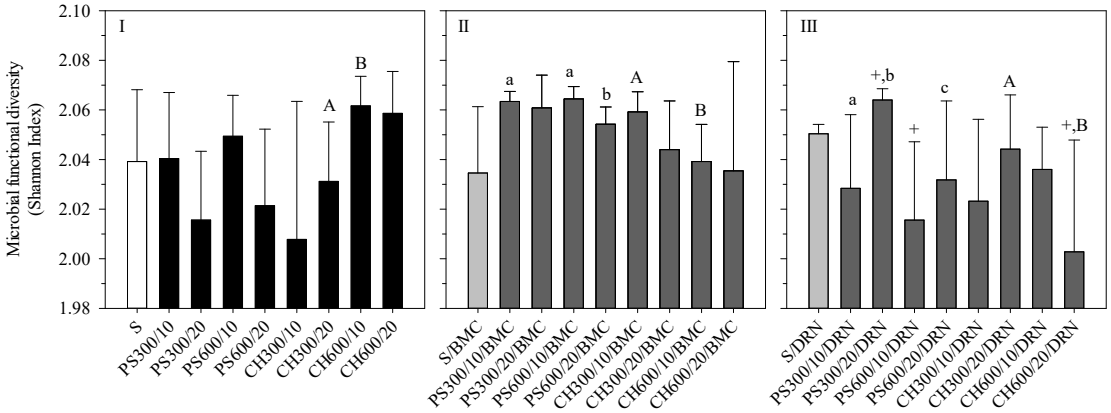

Figure 4-6. Microbial community functional expressed as Shannon index: I. Unamended and amended soils, II. BMC-treated unamended and amended soils. III. DRN-treated unamended and amended soils. See Figure 4-1 for treatment label abbreviations. White bars (□) represent the unamended control soil, black bars (■) represent the herbicide untreated amended soil, light grey bars (▒) represent the herbicide treated unamended soil and dark grey bars (▒) represent herbicide treated amended soil. Error bars correspond to standard deviation of five replicates. Values with + indicate difference respect to the pesticide treated unamended soil, lowercase letters indicate difference between PS amended soils and capital letters between CH amended soils (Mann-Whitney pairwise test, $p<0.05$).

Treatment with sole pesticides did not revealed differences in the substrates use rate with respect to the pesticide-untreated control soil, but substrate use generally decreased, namely with the addition of biochars and CH300 addition (Figure 4-7-I.). Charred materials combined with the BMC treatment decreased substrate use rate when compared to unamended treated soil, with exception of CH600 (Figure 4-7-II.). In contrast, the treatment with DRN did not affect substrate use rate, and moreover a global stimulation was observed with the addition of CH600 (Figure 4-7-III.).

4.4.4. GLM models

In almost all the models performed the degree of prediction was low or not significative with exception of seedling emergence that explain the 72 % of the variance where the significant parameters were the addition rate of charred materials and the treatment with herbicides (Annex E. Table A- 10). The significant parameters for the model for reproduction of *E. crypticus* were treatment with herbicides, pH and NO_2^- content (Annex E. Table A- 18), while the significant parameters for the microbial diversity were Ca^{2+}, NH_4^+, NO_2^- and Mg^{2+} (Annex E. Table A- 19) contents that explain the 46 % of the variance. The model for reproduction of *F. candida* that explain close the 30 % of the variance where the pH and application rate are significative parameters (Annex E. Table A- 15), while the significative parameters were electrical conductivity, application rate and Na^+ contents, and soil basal respiration for the models for avoidance of *F. candida* and of *E. crypticus*, respectively (Annex E. Supplementary material, table SM-5 and table SM-7). The rest of the models explain less than 13 % of the variance (Annex E.).

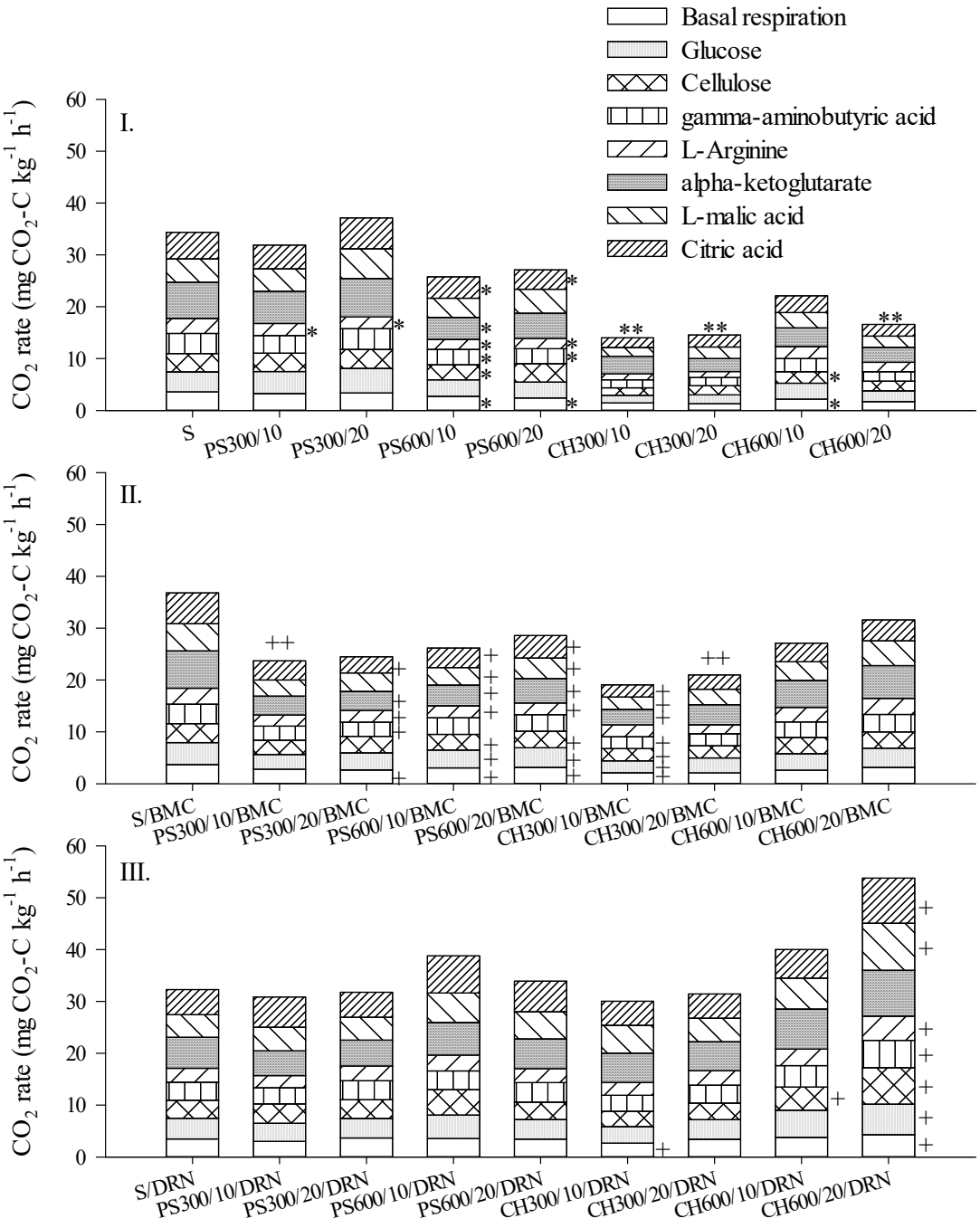

Figure 4-7. Substrate use measured as induced respiration in the different treatment: I. Unamended and amended soils, II. BMC-treated unamended and amended soils. III. DRN-treated unamended and amended soils. See Figure 4-1 for treatment label abbreviations. Values with * indicate differences in the use of the particular substrate compared to that in the corresponding control and values with ** indicate differences in all the substrates use in the treatments with respect to the control soil. Values with + indicate differences in the use of the particular substrate compared to the pesticide-untreated control and values with ++ indicate differences in all the substrates with respect to the pesticide-untreated soil (Mann-Whitney pairwise test, $p<0.05$).

4.5. Discussion

4.5.1. Charred materials addition did not change herbicides efficiency

Our results demonstrate that both BMC and DRN retained their herbicide activity when charred materials were added to this tropical soil, since no survival of plants was observed in any of the herbicide treatments. In previous experiments, it was demonstrated that both BMC and DRN presented a remarkable low sorption to the soil and to charred materials of this study (unpublished results of the main authors). This is in disagreement with other studies (Gámiz et al., 2017; Nag et al., 2011) reporting biochar strong effects on herbicides fate, namely by increasing its sorption, and reducing their bioavailability. Therefore, the low sorption of those pesticide might explain why herbicides activity is unaffected by charred materials addition, and therefore their bio-accessibility, bioavailability and their efficiency. The only exception to this observed trend were the BMC-treated CH biochar mixtures added at high application rate (Figure 4-1-II), where all the plants survived, suggesting an attenuation of the phytotoxicity by this biochar at this high rate, and not observed in the other materials. Alternatively, it cannot be ruled out a heterogeneous application of the herbicide that would not have affected all plants equally.

Despite the fact that herbicides have a post-emergence mechanism (photosynthesis inhibition), a reduced seedling emergence was observed in herbicide treated controls and amended treatments compared to the untreated ones, indicating that charred materials were unable to mitigate such effect (Figure 4-1-II and Figure 4-1-III). These results agree with the reported by Konlan et al. (2016), who observed emergence and survival of seedling for several weeks following a preemergence application of diuron before plants death. A plausible explanation to our emergence inhibition is the fact that emergence was assessed when 50% of the seeds germinated in controls, when some seedlings had already started the production of chlorophylls.

It is also worth noticing that the addition of charred materials did not affected seedling emergence as it could affect seedling imbibition by their impact on water retention or other limiting soil properties. As an example, Anyanwu et al. (2018) observed that addition of rice husk biochar applied at 1 and 10 % enhanced and caused faster seedling emergence of *Oryza sativa* and *Solanum lycopersicum* in an acid soil under plant house conditions. However, the modeling of this response suggests a hidden effect of char

addition together with herbicides addition, with 72% of the emergence variance explained by the negative influence of herbicide addition and a positive influence of char addition rate (Annex E. Table A- 10).

In herbicide-free treatments, neither the emergence or the shoot and root biomass was affected by the presence of charred materials when compared with the controls (Figure 4-2). These results indicate the lack of effects on growth in the short-term, which is surprising as it has been shown that alkaline biochar addition to acid soils can improve lettuce growth (Carter et al., 2013), since at pH below 5.5 there are serious limitations to plant development, including toxicity caused by Al and Mn that become bioavailable and the lower availability of Ca, Mg and P under acidic soil conditions (Yu et al., 2019; Zheng, 2010; Zulfiqar et al., 2019). The liming capacity of some biochars can increase soil pH and reduce its exchangeable acidity therefore improving the soil conditions (Masulili et al., 2010; R. kou Xu et al., 2012; Yuan et al., 2011) and potentially enhancing plant growth in acid soils. Wu et al. (2020) explained that liming capacity of biochar could be attributed to two aspects, the presence of carbonates and oxides of Ca, Mg, and K formed during pyrolysis from minerals presents in the feedstock, and the presence of -COO^- and -O^- surface groups, especially in biochars produced at 300-500 C, that could react with H^+ present in soil. As an example (Van Zwieten et al., 2010) with a paper mill biochar added at 10 Mg ha^{-1} to an acid soil that elevated pH and increased biomass of radish, soy and wheat. Similarly, Zulfigar et al. (2019) observed increased growth of *Syngodium podophyllum* after supplementing wheat straw biochar, an effect attributed by the authors to physiological responses such as enhanced uptake of mineral nutrients, elevated chlorophyll contents and increased net photosynthetic rates, caused by amelioration of soil conditions such as increase of pH, and N, P and K contents. The fact that soil pH only increased between 0.4 and 0.6 pH units with the addition of the charred materials, reaching near 5.2 at most (Annex D. Table A- 9), is therefore the reason why seedling's growth was unaffected as it was not different to control soil (Carter et al., 2013). The GLM models derived for shoot and biomass variation also did not included pH, and only identified the negative effect of microbial activity, measured as soil basal respiration, though only explain 13% of the variance and therefore not further discussed.

4.5.2. Charred materials supplementation does not change the unintended ecotoxicological effects of herbicides on soil faunal groups and microbial functions

Herbicide's sole addition to unamended soil did not had clearly negative effects in collembolans and enchytraeids survival and avoidance behavior that could result in unintended effects of these herbicides addition, again indicating the already mentioned low capacity of the torrefied and biochars of this study to change the herbicides bioavailability in this soil. This is shown in lack of satisfactory GLM models for survival or those derived for avoidance, not including herbicide as significant term. The values of NOEC associated to diuron were 15.78 mg kg^{-1} for *Eisenia foetida* and 22.35 mg kg^{-1} for *Folsomia candida* (Lewis et al., 2016), both higher than the tested in this work, could explain the no affectation of survival, but no data were found for bromacil.

On the contrary, an increased reproduction was observed in both species in controls where herbicide was added, which do not necessarily are interpreted as a positive or neutral effect, and attributable to eventual to hormetic effects at low levels of exposure resulting from the activation of detoxification metabolic pathways that end up activating the whole metabolism and promoting higher reproduction (Forbes, 2000) or less plausibly to indirect trophic effects via microbial growth using the herbicide as substrate, since as an example, diuron application has been linked to decreased microorganisms activity (Prado and Airoldi, 2001). The lack of drastic toxicity is congruent with the fact that both pesticides have the photosynthesis activity-inhibition as action mechanism and therefore not applicable to animals, although unexpected effects of pesticides on non-target groups is not unusual. In fact, Campiche et al. (2006) found toxic effects on *F. candida* when was exposed to DRN at 20 mg kg^{-1} in a soil, a concentration 10-fold higher than used in our experiments, and namely linked to the transformation product of DRN to 1,3-dicloroaniline, that has higher toxicity (Jiang et al., 2000). However, herbicide is only included in the GLM models derived for reproduction.

On the other hand, very strong effects were observed with the sole addition of charred materials on avoidance behavior and reproduction, but not on survival. Namely, a wide preference for charred material mixtures over the controls in collembolans and the generalized avoidance shown in enchytraeids, but also an increased reproduction in both species in nearly all the charred material mixtures.

The effects of charred material addition on fauna did not drastically changed with the application of herbicides, suggesting a major influence of chars over herbicides in the observed trends. While herbicides did not change drastically the avoidance behavior of collembolans to charred material mixtures, they strongly attenuated the enchytraeids avoidance of chars. Similarly, collembolan reproduction was again higher in charred mixtures reproduction with herbicides addition compared to the corresponding unamended control, while such reproduction promotion was strongly attenuated or suppressed in enchytraeids as a result of BMC and DRN supplementation, respectively. The GLM models derived for avoidance and reproduction reinforce that idea, with collembolan reproduction explained (34% of the variance) by the positive effect of char rate and soil pH, and that of enchytraeids (46% of the variance) positively influenced by the presence of pesticide and soil pH, in both cases the pH only possibly linked to char addition. In the GLM models for avoidance, collembolans avoidance was negatively affected by char rate (that meaning more attraction to the higher the char rate in the mixture) and positively affected by EC (27% of variance), and enchytraeids avoidance only by the negative effect of basal respiration, suggesting a microbial-based preference (27% of the variance).

The importance of soil pH for *F. candida* had already been claimed by Crouau et al. (2002), whom demonstrated that reproduction was either affected by the exposure to pollutants an environmental conditions such as pH, moisture and organic matter content of the soil. This is why its plausible linking reproduction promotion to the slight liming caused by charred materials (Annex D. Table A- 9). It has been observed that *Folsomia candida* showed a preference for settle in soils with pH values close to 5.6 where females presented reproduction levels higher than the observed in more acid or alkaline soils (Fountain and Hopkin, 2005). Excessive increases in pH have been also linked to reproduction reductions (Marks et al., 2014b). Such reproduction promotion also seem to discard toxic effects of the chars in this study, together with the low application rates (0.5 and 1%) compared to other studies and char materials, such as the toxic effects for the same species by gasification biochars added at higher application rates of 10 to 50 % (Conti et al., 2018; Domene et al., 2015a; Marks et al., 2014b) or effects on reduction of reproduction by rice husk and wood biochars added at 10 % (Bielská et al., 2018). Concerning the demonstrated pH positive effect on *E, crypticus* offspring production, it is also plausible since despite its wide tolerance to different soil pH, they do not reproduce

below a pH of 4.4 (Kuperman et al., 2006) although a relationship between more reproduction and pH has not been demonstrated elsewhere.

Regarding the avoidance tests, contrasted behavior of faunal species, collembolans preferring and enchytraeids avoiding charred material mixtures, can only be interpreted in terms the quality of habitat of control soil for each species. *F. candida* preference for charred material mixtures only suggest a lower quality as habitat of the control soil in this study for this species, since in a similar studies with a temperate neutral soil and a corn stover biochar avoidance to biochar mixtures was demonstrated, while *E. crypticus* shown no preference or avoidance for biochar (Domene et al., 2015b). The exact reasons for these preferences of the species remain unclear by inspecting the GLM models derived for this response, the attraction to some char-related feature in collembolans, and a preference for soils with higher microbial activity in *E. crypticus*.

Concerning the impacts on microbial functional diversity, no significant changes were observed after the sole addition of herbicides or biochars, or when combined, suggesting a high degree of functional redundancy. Despite the pesticides treatment did not produced differences in the microbial use rates of the tested substrates by itself when compared with the control soil without pesticide, a global reduction in the rates of use were observed with the sole addition of most of the charred materials and those amended and treated BMC, suggesting toxicity effects in some cases, while some DRN treated soils increased those rate. The effect of those pesticides in soils microorganism community is diverse and too scarce to find equivalent studies., Madhun and Fred (1987) was unable to link the addition of BMC and DRN to any microbial activity impact in an acid mucky peat and neutral loamy sand soils, while on the other hand Prado and Airoldi (2001) reported toxic effects to microbial activity in a red Latosol. Similarly, El Fantroussi et al. (1999) reported that diuron application reduced the diversity and functions of microbial populations, while linuron, another urea family herbicide, showed the opposite effect. The higher functional diversity observed in DRN-treated PS300/20 mixture compared to DRN-treated unamended soil, and PS600/10/DRN and BC600/20/DRN, suggest that the pesticide should have boosted functional diversity by an unknown mechanism. However, this effect seems to be more related to the particular biochars concerned than the pesticide itself, shown not to reduce functional by itself. Such increase might be explained similarly to the study by Yuan et al., Yuan et al. (2019) whom using the MicroResp™ method did not

observe differences in Shannon index after the addition of wheat straw biochar to a paddy soil, but did reported an increased use of carboxylic acids, also observed by Tian et al. (2016). in a similar soil. This is also in agreement with our results, when charred materials increased the global substrate use rates, the higher increases correspondent to citric acid.

4.6. Conclusions

The addition of charred materials did not affect the efficiency of the herbicides BMC and DRN in the tropical Ultisol used in this study, in terms of emergence and also of biomass in the only treatment were seedling growth was possible (CH600/20). It is also worth noticing that when no herbicide was applied, no effects on emergence or biomass were observed, with the exception of significant increased emergence in the PS300/20 mixture, contradicting the expected positive effects on tropical acid soils fertility reported in the literature. The tested herbicides showed no unintended toxic effects on soil invertebrates, but on the contrary, promoted their reproduction at the applied rates. Even more clearly, the sole addition of charred materials, promoted the reproduction of both collembolans and enchytraeids, without affecting survival. However, such reproduction promotion was slightly attenuated by the addition of herbicides in collembolans, and strongly in enchytraeids. It is also worth of notice the generalized avoidance of charred material mixtures by enchytraeids opposed to the wide preference for collembolans, irrespective of the supplementation of herbicides or not. Regarding microbial functional diversity, remained unaffected by the sole addition of herbicides or charred materials, and some significant increases were only observed in DRN-treated charred material-mixtures. However, charred materials, but not pesticides, generally decreased the rates of use of the organic substrates used to assess such functionality impacts, suggesting toxic effects not affecting global functional diversity, with the only exception of the stimulation in some treatments, and mainly attributed to an increased use of carboxylic acids. More studies on the pesticide's effects of charred materials addition on tropical agroecosystems are of interest to reconcile their expected fertility improvements with pesticides efficiency preservation and the mitigation of any unintended ecotoxicological effects.

4.7. References

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Chapter 5. Conclusions and recommendations

5.1. General conclusions

- The addition of biochar produced from pineapple stubble (PS), oil palm fiber (PF) or coffee hulls (CH) did not mitigate the efficiency of bromacil (BMC) and diuron (DRN) and also did not modify their fate in a tropical clay Ultisol.

- Under the pineapple cropping conditions of the northern region of Costa Rica, these pesticides were estimated to cause a high environmental risk to surface water and groundwater because of their chemical properties, and the addition of biochar was unable to mitigate this. Nevertheless, biochar addition did not cause negative effects to the soil ecosystem, as measured using bioindicators based on faunal species and microbial soil functions, and on the contrary, it had positive effects on the habitat condition for some soil invertebrates while it also contributed to carbon sequestration.

- The mixed approach implemented in this study, combining chemical indicators and biological and ecotoxicity tests, gave a global view of the benefits and risks of the addition of an organic amendment to tropical soil, and the effects on two pesticides of widespread use. This approach generated useful and novel information needed prior to the implementation of biochar application as a regular agricultural practice. Furthermore, the results obtained in this study highlight the need for more studies on this topic, and specifically in tropical areas. Further research is therefore needed with the final aim of reconciling the expected fertility improvement of biochar addition, together with the other benefits claimed in the literature, with the preservation of pesticide efficiency and the mitigation of any unintended ecotoxicological effects..

5.2. Specific conclusions

- Pyrolysis temperature, rather than feedstock, is the most important parameter for obtaining biochar, since only the charred materials pyrolyzed at 600 °C, and not those produced at 300 °C, fulfilled the requirements of international biochar standards. On the other hand, many key properties of the biochars were associated with the feedstock's composition. Thereby, all the biochars showed a similar pH near 9.5, except for CH that showed the lowest pH (8.7) probably related to the lower ash content of the original feedstock. Despite the initial differences in the original feedstock, PS and CH biochars showed a similar surface area, near 60 m^2g^{-1}, which was higher than the other charred materials, and PF and CH biochars (not PS) presented a fixed carbon content near 76 and 88%, respectively, that was higher than PS biochar (approximately 59%).

- Considering the six different charred materials obtained from pyrolysis of PS, PF and CH, a variety of properties were observed, that suggest different priority environmental uses in each case. For example, CH and PF biochars could be more useful for soil carbon sequestration purposes due to their elevated fixed carbon content. On the other hand, PS biochar might be more suitable as a soil amendment, considering its high ash content and surface area, that could provide short-term provision of nutrients, liming, and a long-term increase in nutrient and water retention, with special interest in tropical arable soils, which have low carbon, nutrient contents, and pH. Considering that torrefied materials presented similar properties among them and particular surface chemical characteristics, their use as an amendment to improve the soil fertility could be considered.

- We demonstrated an unexpected increased persistence of the two pesticides studied compared to that reported in the literature, which could be of environmental concern and must be considered to evaluate their fate under tropical conditions. Consequently, BMC degradation in the tropical soil studied was limited compared to DRN, with estimated half-lives of 300 days and 73 days, respectively.

- On the other hand, the addition of charred materials as organic amendments was demonstrated to affect the environmental fate of the pesticides in different ways. Torrefied materials delayed the degradation of both pesticides, but this was not true of most of the biochars tested, especially for diuron, which comparatively was the easiest to degrade. A similar trend was observed for persistence, though only for DRN applied on the soil treated with PF charred material. The increased sorption and persistence effects exerted by these charred materials can be explained by their abundance of surface oxygenated functional groups, rather than their total surface or the total organic carbon input.

- Despite the fact that addition of charred materials to soil could affect the environmental fate of tested pesticides, the predicted mobility risk was unaffected and only seasonal differences were found. Thus, it increased from high in the dry season to very high in the rainy season for BMC, and was high in both seasons for DRN. For groundwater, mobility risk was low under all scenarios for both pesticides. Equally for both pesticides, the toxicity risk was estimated to be extremely high in all the seasons and water compartments for aquatic organisms and was unaffected by charred material addition.

- The addition of charred materials did not affect the efficiency of the herbicides BMC and DRN in the tropical soil used in this study, both in terms of lettuce emergence and also for biomass in the only treatment where seedling growth was possible (CH600/20). It is also worth noting that when no herbicide was applied, no effects on emergence or biomass were observed, with the exception of the significant increased emergence in the PS300/20 mixture.

- The tested herbicides did not cause unintended toxic effects on soil invertebrates (collembolans and enchytraeids), but on the contrary, promoted their reproduction at the applied rates. Even more clearly, the sole addition of charred materials, promoted the reproduction of both collembolans and enchytraeids, without affecting survival. However, such reproduction promotion was slightly attenuated by the addition of herbicides in collembolans, and strongly in enchytraeids. It is also worth noting the generalized avoidance of charred material mixtures by

enchytraeids, as opposed to the large preference by collembolans, irrespective of the supplementation of herbicides or not.

- Regarding microbial functional diversity, measured as the rate of use of several organic substrates with the MicroResp™ method, it was unaffected by the sole addition of herbicides or charred materials, and only some significant increases were observed in DRN-treated charred material-mixtures. However, charred materials, generally decreased the substrates rates of use, suggesting toxic effects not affecting global functional diversity, with the only exception being the increased use of carboxylic acids in some charred material treatments.

5.3. Recommendations

This research demonstrated positive effects, as well as an absence of negative effects, of the addition of charred materials derived from PS, PF and CH to a tropical clay Ultisol on the edaphic ecosystem and the fate of the herbicides BMC and DRN. Nevertheless, this research had a limited scope and did not attempt to be an exhaustive study of the effects of the use of biochar or torrefied materials as organic amendments in tropical soil conditions. However, results obtained serve as basis to propose some recommendations:

- Pyrolysis of organic agro-wastes with no defined uses must be considered as an option of waste management practice with the potential to revalorize them. In this way, the application of the charred materials as organic amendments to the soils could be part of a smart climate strategy, addressed to develop practices for mitigation of the emissions of greenhouse gases, and contributing to the circular economy.

- Addition of charred materials to the soil improved the habitat conditions for the bioindicators of the edaphic ecosystem, however, it would be useful to scale up the studies to more complex systems such as microcosms or field assays, in order to evaluate the effects as a whole.

- Although key environmental effects were evaluated in this study, it may be advisable and convenient to evaluate the charred materials at the field scale, to explore the possible agronomical benefits of their application as well as other important aspects such as the mid- and long-term carbon stability in field conditions.

Annex A. Chemical reagents description

Analytical standards of bromacil (BMC; (RS)-5-bromo-3-sec-butyl-6-methyluracil, >99% purity) and diuron (DRN; 3-(3,4-dichlorophenyl)-1,1-dimethylurea, >99% purity) were obtained from Dr Ehrenstorfer (Augsburg, Germany) and from Chemservice (West Chester, Pennsylvania, USA), respectively. Acetonitrile and methanol of HPLC grade, formic acid (purity 98–100 %) and glacial acetic acid (purity ≥ 99.7%) were obtained from Merck (Darmstadt, Germany). Water was purified with a Direct-Q UV3 (resistivity 18.2 MΩ cm) water purification system (Millipore, Bedford, MA). Magnesium sulfate anhydrous, sodium chloride, and sodium acetate anhydrous analytical grade were purchased from J.T. Baker (Phillipsburg, NJ); Bondesil-PSA (primary secondary amine, 40 μm) was bought from Varian (Palo Alto, CA), Sepra-C18 was acquired from Phenomenex (Torrance, CA, U.S.). Potassium hydroxide analytical grade was purchased from Merck (Darmstadt, Germany). Ultima Gold cocktail Liquid Scintillation Counting was purchased from Perkin Elmer (Waltham, Massachusetts, USA). Diuron-d6 (surrogate standard, 99.0%) was purchased from Sigma Aldrich (Darmstadt, Germany), bromacil-d3 (surrogate standard, 96.5%), carbofuran-d3 (surrogate standard, 98.0%) and linuron-d6 (internal standard, 99.5%) were purchased from Dr Ehrenstorfer (Augsburg, Germany).

Annex B. Analytical procedures

B.1. Extraction of BMC and DRN

Each sample of soil or mixtures (5 g) were spiked with BMC-d3 and DRN-d6 as surrogate standard, mixed with 10 mL water and 15 mL acetonitrile containing 1% acetic acid (v/v), and vortexed during 1 min and shaken manually during 1 min. Then, 1.5 g of sodium acetate anhydrous, 6 g of anhydrous magnesium sulfate, and 1 g of sodium chloride were added and shaken at 2500 rpm by mechanic equipment for 30 min. Samples were then centrifuged at 4500 rpm for 7 min at 10 ºC. A 3 mL aliquot of supernatant was pipetted into a 15 mL polypropylene centrifuge tube containing 900 mg of anhydrous magnesium sulfate, 150 mg of Bondesil-PSA and 75 mg silica-C18, shaken during 1 min, and subsequently centrifuged at 4500 rpm for 7 min at 10 ºC. A 1.5 mL aliquot of supernatant was evaporated to dryness under a stream of nitrogen at 25-30 ºC. Linuron-d6 was added as internal standard and then dry residue was reconstituted in 1.5 mL of water containing 0.1 % formic acid (v/v), filtered through a 0.45 µm syringe Teflon filter (Agilent Technologies, Santa Clara, CA, U.S.), and collected in a 2 mL HPLC glass vial before analysis by LC-MS/MS.

B.2. Quantification of BMC and DRN

Analyses of BMC and DRN from the extracts were performed using an Agilent 1290 Infinity II LC System (Santa Clara, CA, U.S.) Ultra High-Performance Liquid Chromatography (UHPLC) coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was done by injecting 6 µL sample (2 µL loop) in an Poroshell 120 EC-C18 column (100 mm x 2.1 mm i.d., particle size 2.7 µm; Agilent Technologies, CA, USA), and water containing 0.1 % formic acid (A) and methanol containing 0.1 % formic acid (B) as mobile phases. The mobile phase flow was 0.3 mL min^{-1} at the following conditions: 50% B for 2 min, followed by a 7 min linear gradient to 100% B, 5 min at 100% B and 0.1 min gradient back to 50% B, followed by 5 min at initial conditions. The mass spectrometer was operated in dynamic-MRM positive and negative mode. The mass spectrometer used a jet stream (electrospray) ionization source operating with gas temperature 300 °C; gas flow 7 L min^{-1}; nebulizer 45 psi; sheath gas temperature 250 °C and sheath gas flow 11 L min^{-1}; the capillary voltage was 3500 V (for

positive and negative); nozzle voltage 500 V (for positive and negative); heater MS1 and MS2 100 °C Table A- 1. Data acquisition was performed using the MassHunter software (Santa Clara, CA, U.S.).

Table A- 1. Chromatography conditions for the molecules of study

Molecule	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision cell energy (V)	LOD (µg/kg)	LOQ (µg/kg)
Bromacil	259	203	116	13	7.89	14.79
	259	79		33		
Diuron	233	160	90	25	60	116
	233	72		20		
Bromacil-d_3	262	206	130	15	--	--
	262	79		30		
Diuron-d_6	241	78	110	25	--	--
	241	52		15		
Carbofuran-d_3	225	165	86	9	--	--
	225	123		21		
Linuron-d_6	255	185	92	13	--	--
	255	160		17		

Annex C. Pesticides mobility and toxicity risk for waters

C.1. Input parameters for Pesticides Impact Risk Index (PIRI)

Table A- 2**.** Input parameters about weather conditions used in PIRI model to estimate the risk to water bodies of the use of the pesticides.

Parameter	Dry season	Rainy season
Start month for the period of interest	February	April
End month for the period of interest	March	January
Loss of soil	Sediment very evident	Sediment very evident
Usual moisture condition of the soil during the period of interest	Dry	Moisture
Total rainfall during the period of interest (mm)	251	3239
Average minimum air temperature during the period of interest (°C)	20.4	20.4
Average maximum air temperature during the period of interest (°C)	32	32

Table A- 3**.** Input parameters about land and water bodies used in PIRI model to estimate the risk to water bodies of the use of the pesticides.

Parameter	Dry and rainy season
Organic matter expressed as	% organic carbon
Soil type	Clay
Field cover	Bare
Content of organic matter (% organic carbon)	values from Table 3-2
Total irrigation during the period of interest (mm)	0.6
Soil pH	values from Table 3-2
Diameter of the nearest water body (m)	2
Distance from the edge of crop to water body (m)	15
Slope of land to the water body (°)	1
Width of buffer zone (m)	0
Minimum number of days from application (d)	1
Deep of water table (m)	8

Table A- 4**.** Input parameters about application practices of BMC and DRN used in PIRI model to estimate the risk to water bodies of the use of the pesticides.

Parameter	Dry and rainy season	Dry and rainy season
Pesticide	Bromacil	Diuron
Persistence in environment, half-life (d)	300[a]	73[a]
Koc (L kg^{-1})	values in Table 3-2	values in Table 3-2
Dissociation	9.27	No dissociation
Toxicity (PNEC, mg L^{-1})	6.2 x 10^{-4}	1.9 x 10^{-4}
Classification	Herbicide	Herbicide
Spray type (mg L^{-1})	80	80
Product application rate (kg ha^{-1} or L ha^{-1})	6	4
Fraction active ingredient	0.80	0.80
Frecuency of use (times/period of interest)	1	1
Percent area treated	100	100

[a] Values taken in control soil

C.2. Derivation of Predicted No Effect Concentration (PNEC)

PNEC derivation for BMC

Table A- 5. No Observed Effect Concentration (NOEC) for BMC used to derivation of PNEC. •

Latin name	Trophic Level	Effect	NOEC (mg L^{-1})	Duration
Chlorella vulgaris	Algae	Population	0.1	96 hours
Chlorella vulgaris	Algae	Population	0.1	96 hours
Desmodesmus subspicatus	Algae	Population	0.024	24 hours
Desmodesmus subspicatus	Algae	Population	0.045	72 hours
Desmodesmus subspicatus	Algae	Population Growth	0.045	3 days
Pseudokirchneriella subcapitata	Algae	Population	0.01	96 hours
Pseudokirchneriella subcapitata	Algae	Mortality/Growth	0.0011	4 days
Oncorhynchus mykiss	Fish	Growth	3	90 days
Daphnia magna	Invertebrate	Growth	8.2	21 days
Daphnia magna	Invertebrate	Reproduction	8.2	21 days

• Data were obtained from the EnviroTox Database (Health and Environmental Sciences Institute (HESI), 2020)

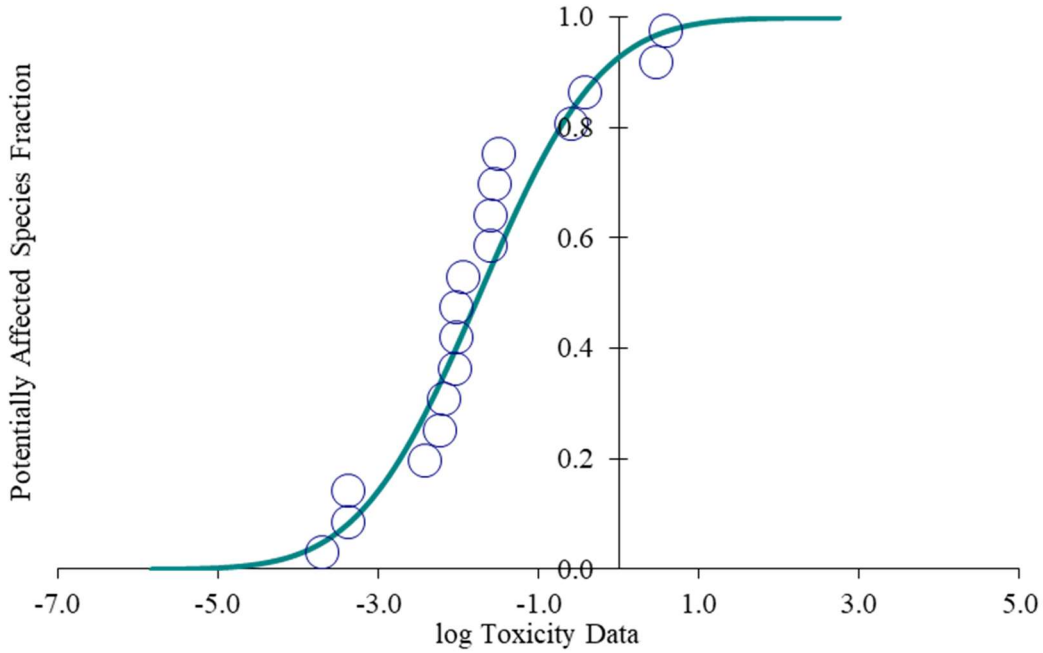

Figure A- 1 Species Sensitivity Distribution (SSD) of species sensitivity to toxicity for BMC.

Table A- 6**.** Predicted No Effect Concentration (PNEC) equivalent to HC5 (hazardous concentration for 5% of species) for BMC derived for an aquatic ecosystem

Name	Value (mg L^{-1})	log(value)	Description
LL HC5	1.32×10^{-5}	-4.89	Lower estimate of the HC5
HC5 (PNEC)	6.19×10^{-4}	-3.21	Median estimate of the HC5
UL HC5	5.02×10^{-3}	-2.30	Higher estimate of the HC5

PNEC derivation for DRN

Table A- 7. No Observed Effect Concentration (NOEC) for DRN used to derivation of PNEC [•]

Latin name	Trophic Level	Effect	NOEC (mg L^{-1})	Duration
Chroococcus minor	Algae	Population	0.00044	7 days
Desmodesmus subspicatus	Algae	Population	0.007	24 hours
Desmodesmus subspicatus	Algae	Population	0.01	72 hours
Desmodesmus subspicatus	Algae	Population Growth	0.01	3 days
Eolimna minima	Algae	Population	3.01	96 hours
Gomphonema clavatum	Algae	Population	0.403	96 hours
Pseudokirchneriella subcapitata	Algae	Mortality/Growth	0.00044	4 days
Pseudokirchneriella subcapitata	Algae	Population	0.0094	72 hours
Scenedesmus acutus var. acutus	Algae	Population	0.004	24 hours
Synechococcus sp	Algae	Population	0.00021	72 hours
Synechococcus sp	Algae	Population	0.012	96 hours
Pimephales promelas	Fish	Growth	0.029	60 d post-hatch
Pimephales promelas	Fish	Growth	0.0334	63 days
Pimephales promelas	Fish	Mortality/Growth	0.0264	35 days
Pimephales promelas	Fish	Growth	0.0264	60 days
Pimephales promelas	Fish	Reproduction	0.0264	60 days
Americamysis bahia	Invertebrate	Mortality/Growth	0.27	28 days
Daphnia magna	Invertebrate	Mortality/Growth	0.006	21 days
Daphnia pulex	Invertebrate	Reproduction	4	7 days

[•] Data were obtained from the EnviroTox Database (Health and Environmental Sciences Institute (HESI), 2020)

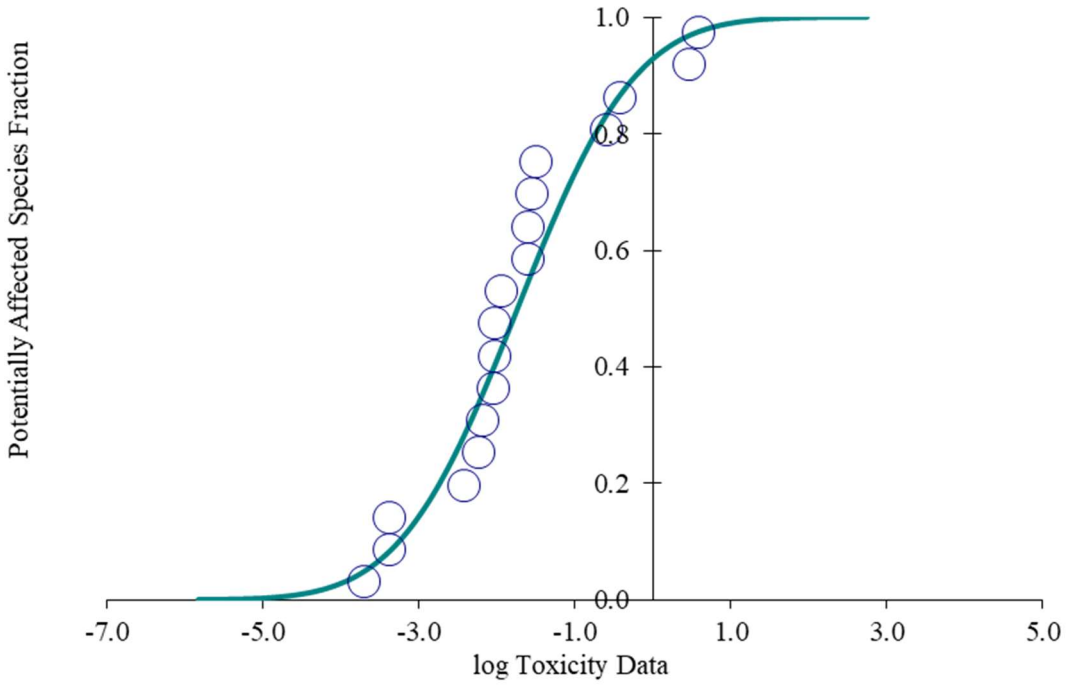

Figure A- 2 Species Sensitivity Distribution (SSD) of species sensitivity to toxicity for DRN.

Table A- 8. Predicted No Effect Concentration (PNEC) equivalent to HC5 (hazardous concentration for 5% of species) for DRN derived for an aquatic ecosystem

Name	Value (mg L^{-1})	log(value)	Description
LL HC5	2.32×10^{-5}	-4.64	Lower estimate of the HC5
HC5 (PNEC)	1.94×10^{-4}	-3.71	Median estimate of the HC5
UL HC5	8.01×10^{-4}	-3.10	Higher estimate of the HC5

Annex D. Characterization of the soil

Table A- 9. Chemical properties of the soil and mixtures soil:charred materials.

Code	pH	CE	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
		µS cm^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
S	4.6±0.5	413.0±49.1	17.5±2.1	3.9±2.4	2.8±1.6	14.4±2.6	98.6±33.4	31.8±3.6	0.4±0.0	43.1±6.9	69.6±53.8	4.7±3.0
BR300/10	5.1±0.4	412.4±40.1	17.6±3.0	2.4±1.0	3.1±3.7	10.5±1.3	113.8±25.4	31.7±8.0	0.3±0.0	34.3±3.1	299.4±132.1	n.d.
BR300/20	5.2±0.3	482.6±29.6	14.6±6.5	2.9±2.0	4.2±3.5	11.3±5.2	118.3±39.2	39.8±8.1	0.4±0.2	42.7±11.6	170.9±214.8	16.3±3.7
BR600/10	4.9±0.6	421.2±46.5	18.2±2.0	2.7±2.0	4.0±2.3	12.6±2.2	104.7±33.0	33.2±4.0	0.4±0.4	37.2±9.9	55.2±61.8	12.1±6.4
BR600/20	5.1±0.4	423.6±84.9	18.1±3.5	2.2±1.4	1.3±0.4	11.3±2.8	125.8±39.0	29.1±4.1	0.3±0.1	40.2±10.2	224.6±162.8	n.d.
BC300/10	5.2±0.2	468.0±101.2	17.4±1.5	2.1±1.2	2.8±1.4	13.2±3.9	137.4±47.9	36.2±6.0	0.4±0.1	41.9±16.1	298.0±202.3	14.6±7.1
BC300/20	5.0±0.6	435.4±61.1	16.6±1.7	2.6±1.6	2.3±2.4	13.1±2.2	116.8±35.8	29.8±5.0	n.d.	42.3±11.1	203.5±246.2	5.4±2.8
BC600/10	5.1±0.4	436.6±72.2	17.9±1.6	1.3±0.9	3.4±1.7	12.8±2.6	125.3±34.4	33.6±6.4	0.3±0.1	40.0±9.0	298.2±175.5	10.1±0.6
BC600/20	5.0±0.5	455.8±67.7	18.0±1.6	3.1±2.0	3.4±2.4	13.1±3.8	120.8±34.7	35.6±6.1	0.3±0.1	42.1±12.0	180.3±78.8	n.d.
S/BMC	4.8±0.5	438.4±91.1	16.7±1.7	2.3±1.9	3.4±2.5	14.9±2.2	121.0±44.1	34.3±8.9	0.2±0.1	44.0±10.6	35.6±45.3	8.8±4.0
BR300/10/BMC	5.0±0.5	436.6±33.8	16.7±0.8	2.4±1.3	3.5±3.3	12.9±2.2	117.8±34.7	33.5±4.9	n.d.	39.3±6.8	291.0±134.3	n.d.
BR300/20/BMC	5.2±0.5	466.2±56.2	17.2±1.7	1.7±1.1	3.3±1.9	14.2±1.8	129.5±39.6	35.2±4.6	0.2±0.0	40.6±7.4	452.0±93.4	10.2±6.1
BR600/10/BMC	4.9±0.5	419.8±71.1	17.6±2.0	2.4±2.6	2.5±1.8	14.2±3.8	104.6±32.5	31.3±7.2	0.3±0.2	40.2±8.9	61.5±40.9	12.5±1.3
BR600/20/BMC	5.0±0.5	446.6±47.3	18.4±2.0	2.1±1.1	3.4±3.1	12.7±2.2	117.4±35.8	34.0±4.7	0.3±0.2	40.3±5.6	293.1±136.9	n.d.
BC300/10/BMC	4.9±0.6	472.8±64.8	17.5±1.0	3.8±2.5	4.3±3.1	13.9±1.3	120.5±53.3	39.0±3.0	n.d.	41.6±11.3	238.5±154.5	9.6±9.1
BC300/20/BMC	4.8±0.4	418.0±54.7	16.4±3.7	1.6±1.7	4.1±2.9	13.4±3.4	111.1±35.1	32.0±8.1	0.3±0.2	40.9±5.9	37.5±63.6	7.5±3.7
BC600/10/BMC	5.1±0.4	450.2±69.1	19.9±4.1	2.0±2.0	19.4±37.2	12.8±2.4	118.7±33.7	55.5±53.6	0.3±0.2	41.0±13.6	188.7±249.2	7.6±1.1
BC600/20/BMC	4.7±0.4	405.4±29.8	15.9±3.4	4.0±3.3	5.5±3.7	12.8±1.9	91.5±36.2	34.4±8.7	0.3±0.2	37.9±8.4	67.9±68.7	6.7±2.9
S/DRN	4.8±0.3	400.2±88.7	16.4±2.3	2.8±2.1	4.2±2.8	12.4±3.0	108.0±49.7	34.0±4.8	0.2±0.1	36.2±15.9	99.4±114.3	8.6±3.2
BR300/10/DRN	5.1±0.4	459.8±86.3	17.3±1.7	2.7±1.2	3.0±3.1	12.5±1.4	133.6±41.2	36.6±11.9	0.3±0.0	41.3±8.7	292.5±144.4	n.d.
BR300/20/DRN	4.9±0.6	436.8±85.8	17.1±1.5	2.9±2.7	4.1±2.7	13.9±2.7	113.1±46.9	34.9±6.9	0.3±0.1	38.4±9.5	114.0±157.0	15.0±1.5
BR600/10/DRN	5.3±0.1	480.6±76.7	17.6±1.7	2.3±0.6	1.4±0.3	11.2±3.5	153.5±25.3	33.2±5.8	0.2±0.2	44.4±10.4	337.2±98.2	n.d.
BR600/20/DRN	4.7±0.6	424.6±89.2	18.0±2.0	3.1±2.2	2.3±0.9	14.9±3.9	102.1±47.4	31.4±5.4	n.d.	43.1±11.9	140.0±155.7	7.7±6.3
BC300/10/DRN	5.0±0.5	472.0±40.7	18.7±3.2	3.3±2.1	3.7±3.7	13.5±0.8	123.3±16.2	35.9±10.7	0.3±0.2	45.8±3.7	187.0±154.9	n.d.
BC300/20/DRN	5.0±0.5	461.2±98.9	16.9±1.2	2.1±1.9	4.1±2.7	14.3±2.1	125.2±45.7	35.1±5.7	0.3±0.1	41.8±16.1	177.7±231.8	11.1±3.5
BC600/10/DRN	4.9±0.3	452.8±46.4	16.9±2.2	2.1±1.8	3.5±3.3	12.8±1.8	128.9±34.1	35.0±7.3	0.3±0.1	43.1±10.9	171.9±145.6	9.0±1.1
BC600/20/DRN	4.8±0.6	395.6±73.5	16.6±2.7	4.0±1.6	4.0±3.5	12.6±2.7	94.4±28.6	33.0±8.6	0.4±0.1	34.3±9.9	217.4±156.2	n.d

Annex E. General linear models

Table A- 10. GLM of the seedling emergence of *Lactuca sativa* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	84.3621	9.2387	9.131	2.81×10^{-9} *
Application rate	1.1111	0.5238	2.121	0.0444 *
Herbicide	-56.7901	7.4074	-7.667	6.65×10^{-8} *

Null deviance: 28733.4 on 26 degrees of freedom.
Residual deviance: 7901.2 on 24 degrees of freedom.
AIC: 237.95
R^2: 0.72

Table A- 11. GLM of the shoot biomass of *Lactuca sativa* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	5.51738	0.10234	53.91	$<2\times10^{-16}$ *
Basal respiration	-0.67584	0.03883	-17.41	$<2\times10^{-16}$ *

Null deviance: 2139 on 26 degrees of freedom.
Residual deviance: 1842.7 on 24 degrees of freedom.
AIC: Inf
R^2: 0.13

Table A- 12. GLM of the root biomass of *Lactuca sativa* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	5.45515	0.10337	52.77	$<2\times10^{-16}$ *
Basal respiration	-0.65548	0.03904	-16.79	$<2\times10^{-16}$ *

Null deviance: 2138 on 26 degrees of freedom.
Residual deviance: 1862.7 on 24 degrees of freedom.
AIC: Inf
R^2: 0.13

* Indicate significant parameters.

Table A- 13. GLM of the avoidance percentage in the *Folsomia candida* as explained by the soil physico-chemical properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-13.0977	78.0144	-0.168	0.8681
EC	0.3429	0.1593	2.152	0.0422 *
Na^+	-7.5613	3.9925	-1.894	0.0709 *
Application rate of charred materials	-1.3582	0.6160	-2.205	0.0377 *

Null deviance: 12112 on 26 degrees of freedom.
Residual deviance: 8858.1 on 23 degrees of freedom.
AIC: 243.04
R^2: 0.268

Table A- 14. GLM of the survival of *Folsomia candida* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	100.1347	3.2513	30.80	$<2\times10^{-16}$ *
Application rate of charred materials	-0.3838	0.2181	-1.76	0.0907 *

Null deviance: 1603.9 on 26 degrees of freedom.
Residual deviance: 1427.1 on 25 degrees of freedom.
AIC: 189.75
R^2: 0.04

Table A- 15. GLM of the reproduction of *Folsomia candida* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-545.7681	326.0629	-1.674	0.1072
pH	143.9240	65.8780	2.185	0.0389 *
Rate	1.6376	0.8967	1.826	0.0803 *

Null deviance: 31393 on 26 degrees of freedom.
Residual deviance: 21525 on 24 degrees of freedom.
AIC: 265.01
R^2: 0.314

Table A- 16. GLM of the avoidance percentage in the *Enchytraeus crypticus* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	83.488	17.366	4.807	6.14×10^{-5} *
Basal respiration	-17.952	5.773	-3.110	0.00463 *

Null deviance: 16968 on 26 degrees of freedom.
Residual deviance: 12236 on 25 degrees of freedom.
AIC: 247.76
R^2: 0.278

Table A- 17. GLM of the survival of *Enchytraeus crypticus* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	5.30971	0.05717	92.870	$<2\times10^{-16}$ *
Basal respiration	-0.03176	0.01911	-1.663	0.0964 *

Null deviance: 63.795 on 26 degrees of freedom.
Residual deviance: 61.041 on 25 degrees of freedom.
AIC: Inf
R^2: 0.04

Table A- 18. GLM of the reproduction of *Enchytraeus crypticus* as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-3805.09	1226.66	-3.102	0.005024 *
Herbicide	179.57	47.03	3.818	0.000882 *
pH	813.53	240.62	3.381	0.002574 *
NO_2^-	329.47	181.84	1.812	0.083094 *

Null deviance: 482149 on 26 degrees of freedom.
Residual deviance: 258711 on 24 degrees of freedom.
AIC: 334.15
R^2: 0.46

Table A- 19. GLM of the microbial functional diversity as Shannon index as explained by the unstandardized soil physico-chemical and biological properties.

Parameter	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	2.1063332	0.0557039	37.813	$< 2\times10^{-16}$ *
Ca^{2+}	-0.0006459	0.0002561	-2.522	0.01942 *
Mg^{2+}	0.0047384	0.0024752	1.914	0.06867 *
NH_4^+	-0.0109037	0.0045520	-2.395	0.02555 *
NO_2^-	-0.0766170	0.0236026	-3.246	0.00371 *

Null deviance: 0.0076656 on 26 degrees of freedom.
Residual deviance: 0.0041110 on 24 degrees of freedom.
AIC: -148.71
R^2: 0.46