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Biochar and earthworms working in tandem: Research opportunities for soil bioremediation

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ABSTRACT

Intensive use of agrochemicals is considered one of the major threats for soil quality. In an attempt to mitigate their side-effects on non-target organisms and soil functioning, many engineering and biological remediation methodologies are currently available. Among them, the use of biochar, a carbonaceous material produced from pyrolysing biomass, represents an attractive option enhancing both remediation and soil carbon storage potentials. Currently, activation of biochar with chemical or physical agents seeks for improving its remediation potential, but most of them have some undesirable drawbacks such as high costs and generation of chemical wastes. Alternatively, the use of biological procedures to activate biochar with extracellular enzymes is gaining acceptance mainly due to its eco-friendly nature and cost-effectiveness. In these strategies, microorganisms play a key role as a source of extracellular enzymes, which are retained on the biochar surface. Recently, several studies point out that soil macrofauna (earthworms) may act as a biological vector facilitating the adsorption of enzymes on biochar. This paper briefly introduces current biochar bioactivation methodologies and the mechanisms underlying the coating of biochar with enzymes. We then propose a new conceptual model using earthworms to activate biochar with extracellular enzymes. This new earthworm-biochar model can be used as a theoretical framework to produce a new product "vermichar", vermicompost produced from blended feedstock, earthworms, and biochar that can be used to improve soil quality and remove soil contaminants. This model can also be used to develop innovative in-situ "vermiremediation" technologies utilizing the beneficial effects of both earthworms and biochar. Since biochar may contain toxic chemicals generated during its production stages or later concentrated when applied to polluted soils, this paper also highlights the need for an ecotoxicological knowledge around earthworm-biochar interaction, promoting further discussion on suitable procedures for assessing the environmental risk of this conceptual model application in soil bioremediation.

1. Introduction

Soil health, defined by the Natural Resources Conservation Service (NRCS, USA) as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans" (Bünemann et al., 2018), is under severe threats (Ronchi et al., 2019). Soil degradation processes such as erosion, organic matter decline, contamination, sealing, compaction, biodiversity loss, salinization, landslides and floods, decrease soil quality and ultimately affect soil-based ecosystem services (Bünemann et al., 2018). Soil not functioning to its full capacity, endangers the productivity, sustainability and resilience to climate change of agroecosystems, reduce net farmer profit and negatively affect the environmental quality over the mid and long term (Moebius-Clune et al., 2016). Although poor soil properties may be consequence of soil formation processes, these usually result from inappropriate farming practices or anthropogenic pollution (Yu et al., 2019). For instance, the continuous application of synthetic pesticides and fertilizers in conventional agriculture to maintain high yields of short-rotated crops impacts on soil biodiversity (Tsiafouli et al., 2015), decreases agricultural systems productivity, and progressively degrades the quality of soils (Shennan et al., 2017). Soil becomes a sink for agrochemicals, where a broad variety of physicochemical and biological processes

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determine their persistence, environmental fate and toxicity (Varjani et al., 2019). A recent survey showed the occurrence of multiple pesticide residues in an extremely high percentage (83%) of topsoil samples from European agricultural soils (Silva et al., 2019). These data call for the attention on the environmental risks already identified in the past such as toxicity on non-target organisms (Devine and Furlong, 2007; Gianfreda and Rao, 2008), and contamination of groundwater and surface waters (Arias-Estévez et al., 2008). Recognition of these environmental threats has resulted in a significant advance in engineering and biological methodologies to alleviate the adverse effects derived from pesticide-contaminated soils (Morillo and Villaverde, 2017; Sun et al., 2018; Varjani et al., 2019).

Among the variety of remediation technologies for removing soil pesticide residues, those performed in-situ are preferred because their relatively low cost and the minimum impact on soil properties respect to ex-situ procedures (Morillo and Valverde, 2017). In-situ remediation of pesticide-contaminated soils may involve physical (immobilizing agents such as clays, activated carbon, zeolites or polymeric materials), chemical (ionization, reduction and hydrolysis reactions), and biological (compost and organic green waste addition, phytoremediation, bioaugmentation) methods (Cycoń et al., 2017; Marican and Durán-Lara, 2018; Morillo and Valverde, 2017). The latter are currently the most viable and promising options at large scale, but they presents some drawbacks related to the time needed to achieve significant decrease of pesticide residues (usually years), toxicity of pesticides (or their metabolites) to microorganisms, and pesticide bioavailability, among others (Megharaj et al., 2011). Accordingly, an integrated bioremediation approach that combines several complementary methods is suggested as the best option to accelerate pesticide dissipation while increasing soil quality (Masciandaro et al., 2013). In this scheme, addition of biochar provides multiple synergic options in soil remediation based on its physicochemical properties and its capacity of promoting soil microbial proliferation (Novak et al., 2016; Zhu et al., 2017).

Biochar is a carbonaceous material generated by the thermochemical conversion of biomass in an oxygen-limited environment at relatively low temperature (<700 °C) (Lehmann and Joseph, 2015; Han et al., 2018). It is considered as a mean of sequestrating carbon, but its popularity is mainly associated with environmental applications such as improving soil quality (Shaaban et al., 2018), reducing ammonia and greenhouse gas emissions from soil (Ro et al., 2015; Kammann et al., 2017), removing environmental pollutants via sorption (Han et al., 2016; Sun et al., 2016; Wang et al., 2016), and remediating polluted soil (Liu et al., 2018), among other applications and ecosystem services summarized in Fig. 1. Notably, over the past two decades the number of studies dealing with biochar capability for adsorbing environmental pollutants has increased exponentially (Liu et al., 2018; Yuan et al., 2018; Varjani et al., 2019). Moreover, its large surface area and open porosity make biochar an attractive support for microbial colonization and proliferation (Lehmann et al., 2011), leading researchers to postulate that biochar may act as a microbial carrier for contaminant degradation (Zhu et al., 2017).

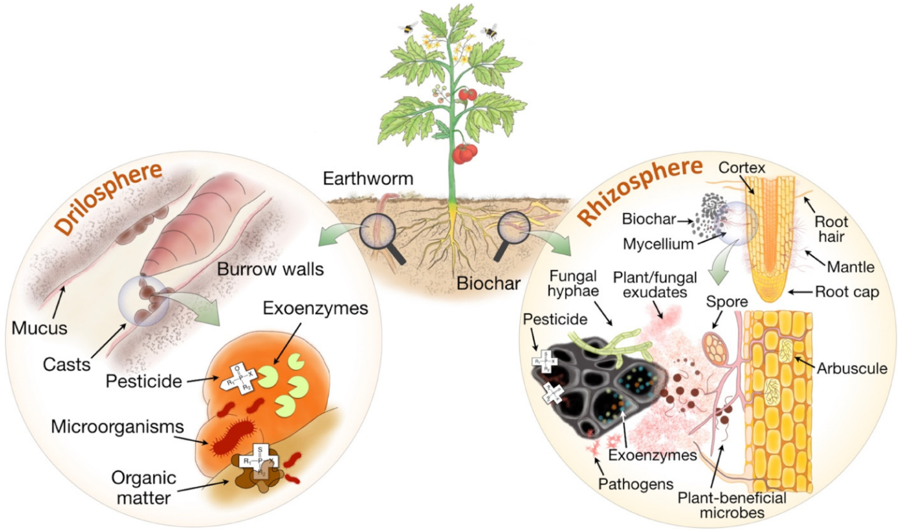
Among biological technologies focused on recovering degraded soils, the use of earthworms and products derived from its activity (vermicompost) has emerged as a promising eco-friendly approach (Rodriguez-Campos et al., 2014). Under favorable conditions, these annelids may reach a biomass up to 200 g m^{-2} (Curry, 2004), and population densities up to 1300 individuals m^{-2} in the agroecosystem (Whalen and Fox, 2006). Their continued feeding, burrowing and casting activities (i.e., geophages earthworms ingest 850–1350 t dry soil ha^{-1} $year^{-1}$; Blouin, 2018) substantially improve soil physicochemical properties (Brown et al., 2000), and create microhabitats that alter both below- and above-ground systems (Wurst, 2010). Accordingly, they improve plant productivity by indirectly stimulating root and shoot development (Scheu, 2003; Gavinelli et al., 2018), as a consequence of changes in soil structure, nutrient mineralization and microbial communities (Brown et al., 2000). A meta-analysis study concluded that earthworm occurrence in agroecosystem leads to a 25% increase in crop yield and a 23% increase in aboveground biomass (van Groenigen et al., 2014). Furthermore, earthworms, particularly endogeic, have been used as promoters for contaminant removal through their continue soil ingestion (Rodriguez-Campos et al., 2014; Morillo and Villaverde, 2017). Fig. 1 displays the main effects of earthworm activity in soil quality and remediation.

Considering the environmental benefits derived from both biochar and earthworms, this discussion paper questions whether these organisms may be used as promoters of activating biochar with extracellular enzymes benefiting soil nutrient cycles and bioremediation of polluted soils. With the intention of motivating further discussion, we present two approaches of earthworm-assisted activation of biochar: i) ex-situ bioactivation, which consists of vermicomposting biomass in the presence of biochar and producing innovative byproduct called "vermichar" (i.e., activated biochar-containing vermicompost), and ii) in-situ bioactivation, which consists in directly applying both earthworms and biochar to soils (in-situ "vermiremediation" process). Both strategies are based on the capacity of earthworms to boost microbial activity. Recent results showed that earthworms were able to facilitate enzyme adsorption on biochar surface (Sanchez-Hernandez, 2018). The first part of the paper uses examples in the literature to describe how biochar can be coated with microorganisms and extracellular enzymes. Next section introduces a new conceptual model about the potential mechanisms underlying the biochar bioactivation with earthworms. We then propose two innovative scenarios that utilize this new conceptual model in producing "vermichar" and developing in-situ "vermiremediation" technologies for soil quality control and buildup. Finally, discussion moves towards ecotoxicological aspects around the "earthworm-biochar" interaction in order to apply this methodology under an environmentally safe scheme.

2. Current activation methodologies

The enhancement of surface area and reactivity of biochar upon thermal and chemical treatment receives the term of "activation" (Ahmad et al., 2014). Currently, alteration of biochar characteristics follows two main strategies based on chemical or physical processes. The former option implies the treatment of biochar or the feedstock with alkaline solutions, acids, or oxidized agents, whereas the latter method submits the biochar to steam, CO_2 or ozone to develop both micropores and mesopores of the biochar with an enhanced surface area (Rajapaksha et al., 2016; Sizmur et al., 2017; Tan et al., 2017). Nevertheless, the associated economical costs to these physico-chemical activation methods, as well as the generation of chemical wastes during production, are main drawbacks for its viability at a large scale. In recent years, simultaneous pyrolysis of multiple feedstocks (co-pyrolysis) is gaining acceptance as an alternative to generate value-added biochar while treating various waste streams (Ro et al., 2014; Hassan et al., 2016). Although the primary aim of co-pyrolysis is obtaining high quality fuels characterized by a low oxygen content (Uzoejinwa et al., 2018), the biochar yield and its abundance in chemical functional groups (e.g., carboxylic acids and esters) are higher than those obtained by pyrolyzing the biomass alone (Tang et al., 2018). These effects are attributed to the high content in hydrogen and carbon of residues as plastics added to co-pyrolysis (Hassan et al., 2016).

Alternative to those engineered methodologies, biochar can also be activated with microorganisms and extracellular enzymes (Sizmur et al., 2017). For example, the filamentous green algae *Klebsormidium flaccidum* and the cyanobacteria *Anabaena cylindrica* efficiently grown on biochar surface, resulted in bioactivated biochar for improving soil fertility (Kholssi et al., 2018). Similarly, biochar was coated with a biofilm composed of the microbial community from aqueous residues generated by mining operations (Frankel et al., 2016). This bioactivated biochar efficiently removed environmental contaminants from water. Likewise, some studies have described the capacity of biochar to bind



Soil quality improvement

Earthworms	Biochar
Increase water infiltration and soil aeration, alter soil aggregate distribution, and reduce water runoff.[1] Stimulate microbial activity, nutrient cycling, and soil C mineralization and stabilization.[2] Burrow system represents hotspots for microbial activity because of deposition of nutrient-rich materials (mucus, cast, and litter).[3] Middens created by anecic species are hotspots for microbial activity and faunal diversity.[4] Stimulate plant growth and development,[5,6] with potential benefits for resistance against herbivore damages,[7] and plant pathogens.[8] Also alter plant community by feeding and dispersion of seeds,[9] and promote agriculture sustainability.[8]	Stimulates soil microbial proliferation and extracellular enzyme (or exoenzymes) production.[10] Reduces soil greenhouse gas emissions.[11] Decreases nutrient losses and triggers priming effect on soil C mineralization.[12] Increases soil water holding capacity, thus favoring plant growth and development.[13] Some types of biochar exert a phytohormone-type effect on plants,[14] and stimulate phytohormone production.[15] Reduces soil-borne disease indirectly by binding extracellular pathogenic enzymes implied in the breakdown of cell walls.[16]

Soil remediation

Earthworms	Biochar
Contribute to breakdown organic pollutants.[17] Lining of burrows (*L. terrestris*) reduces vertical transport of pesticides.[18] Facilitate metal uptake by plants (phytoremediation).[19] Induce pesticide-detoxification enzymes in soil.[20]	Immobilizes metals and organic pollutants on its surface.[21] Stimulates biodegradation of organic pollutants by induction of microbial pollutant degraders.[22] Alleviates adverse effects of soil salinization.[23]

Fig. 1. Description of the main beneficial effects of earthworms and biochar on soil quality and remediation at the drilosphere (soil environment under the influence of earthworms, Andriuzzi et al., 2013) and rhizosphere (soil narrow zone that surrounds and it is influenced by plant root secretions, Berendsen et al., 2012). Pictorial representation using Autodesk© SketchBook software (the rhizosphere illustration was drawn taken as model the figure 6 in McNear D.H., 2013. The Rhizosphere - roots, soil and everything in between, *Nature Education Knowledge* 4[3]:1). References: [1]Shipitalo and Le Bayon, 2004; [2]Brown et al., 2000; Zhang et al., 2013; [3]Hoang et al., 2017; [4]Stroud et al., 2016; [5]Scheu, 2003; [6]Blouin, 2018; [7]Wurst, 2010; [8]Plaas et al., 2019; [9]Forey et al., 2011; [10]Lehmann et al., 2011; [11]Kammann et al., 2017; [12]Whitman et al., 2015; [13]Kavitha et al., 2018; [14]Spokas et al., 2010; [15]French and Iyer-Pascuzzi, 2018; [16]Jaiswal et al., 2018a; [17]Rodriguez-Campos et al., 2014; [18]Edwards et al., 1992; [19]Kaur et al., 2018; [20]Sanchez-Hernandez et al., 2018; [21]Liu et al., 2018; Shaaban et al., 2018; [22]Zhu et al., 2017; [23]Saifullah et al., 2018.

extracellular enzymes. Laccase is an example of oxidoreductase enzyme that, covalently bound to biochar using several molecular cross-linking agents, maintained its catalytic properties and was resistant to thermal denaturation (Naghdi et al., 2018). Holm oak-derived biochar was a suitable support to immobilize laccases from *Myceliophthora thermophila* and *Pleurotus eryngii*, using aminopropyltriethoxysilane and glutaraldehyde as cross-linkers (García-Delgado et al., 2018). The resultant laccase-activated biochar degraded 100% of tetracyclines and 54–100% of sulfonamides. In addition, the laccase-activated biochar designed by Lonappan et al. (2019) removed 88% of the drug diclofenac when packed into a column (20 cm long × 1 cm inner diameter). However, most of those studies have been performed at lab-scale using cross-linking agents (e.g., glutaraldehyde) and purified laccases obtained from microorganisms, which would imply time-consuming and high cost procedures if these bioactivation methods are scaled up for field applications.

Another bioactivation alternative consists of incubating biochar in microbially rich environments such as composting piles, or taking advantage of soil microbiome. Several recent reviews have provided detailed accounts about the impact of biochar addition to the composting process or even to the final product to obtain a value-added compost (Sanchez-Monedero et al., 2018; El-Naggar et al., 2019). Its main effects are summarized as follows: (i) boosting the composting process by increasing microbial activity which, in turn, leads to decrease in composting time; (ii) chemically activating biochar by oxidative processes during composting, thus increasing the abundance of functional groups on its surface (e.g., acidic carboxylic groups); (iii) increasing water retention, pH, and nutrient availability of final compost; (iv) increasing feedstock aeration, which favors the aerobic organic matter decomposing; (v) decreasing greenhouse gases (N_2O and CH_4) emissions from the composting piles; and (vi) decreasing metal mobility and bioavailability when added as an ingredient to metal-rich feedstocks (e.g., sewage sludges). These biochar-induced benefits are achieved with doses between 3 and 5% (w/w dry mass) biochar. Furthermore, the biochars produced at 500–600 °C are recommended for composting because of their larger surface area and porosity, and more resistance to decompose during composting than the biochars produced at lower pyrolysis temperatures (Sanchez-Monedero et al., 2018).

With regards to soil microbiome use, recent research suggests that such strategy could be workable in horticultural soils (Jaiswal et al., 2018b). These authors compared the microbial communities developed in commercial plant growth media (peat: tuff, 7: 3 v/v mixture) treated with 0, 0.5, 1 and 3% w/w biochar, and kept permanently wet and NPK-fertilized for 6 weeks at 26 °C before planting. Results showed that this pre-conditioned phase increased the beneficial microorganism community for plant growth (bacteria and filamentous fungi), reduced soil-borne diseases and removed potential phytotoxic compounds initially present in biochar. Although biochar coating with microorganisms and/or extracellular enzymes was not demonstrated in that study, further investigations confirmed that soil-borne disease decreased because of adsorption and deactivation of pathogenic enzymes (cellulase and pectinase) onto the biochar surface (Jaiswal et al., 2018c).

As discussed above, current biochar bioactivating methods are mostly limited to using lab-cultured microorganism strains and the native microorganisms of composting and soil. However, the use of other potential biological vectors of microbial proliferation such as earthworms to bioactivate biochar has not been explored to date. Earthworms exert a strong impact on soil microbiota. Their feeding, burrowing and casting activities alter and disperse soil microbial communities and organic matter decomposition. In fact, the drilosphere (the soil environment under the influence of earthworms) and vermicomposting are two environments that share an overwhelming number of microorganisms whose foraging activity and exoenzyme production are stimulated by earthworms (Edwards et al., 2011; Domínguez, 2011; Hoang et al., 2016; Hoang et al., 2017). In addition, earthworms can enhance the binding of extracellular enzymes onto biochar surface (Sanchez-Hernandez, 2018). Taken together, these studies open new exciting possibilities for utilizing earthworms to bioactivate biochar and to promote soil quality improvement and restoration.

3. “Vermichar” production scenario (ex-situ bioactivation)

Vermicomposting is defined as an aerobic, mesophilic process in the presence of earthworms by which organic matter is transformed into a fine and porous peat-like material with high content in humic substances, nutrients and microorganisms called vermicompost (Edwards et al., 2011). In contrast to composting, vermicomposting takes advantage of epigeic, detritivorous earthworm species such as *Eisenia fetida* and *E. andrei.* According to Domínguez (2011), vermicomposting can be described as a two-phase decomposing process operating simultaneously: the earthworm gut-associated processes (GAPs) and the cast-associated processes (CAPs). During GAPs, the organic matter ingested by earthworms undergoes physical transformations; grinding and mixing of the ingested material, and complex biochemical transformations facilitated by the action of digestive enzymes originated from both the microbial symbionts and the earthworm gut epithelium (Garvín et al., 2000; Drake and Horn, 2007; Nozaki et al., 2009). Moreover, changes in the chemical composition of the ingested organic matter imply absorption of nutrients mainly in the foregut (or anterior intestine), and secretion of compounds such as mucus, urea, ammonia and enzymes. A vast variety of digestive enzymes such as β-glucosidase, urease and phosphatases participates in the GAPs as evidenced by studies on the enzymatic dynamic of vermicomposting

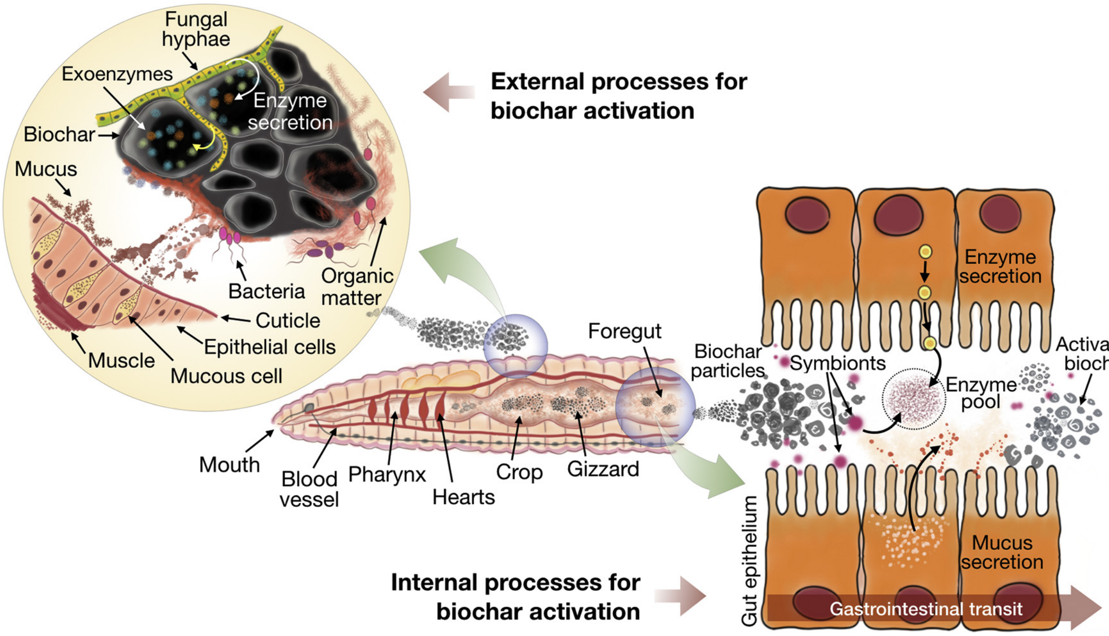
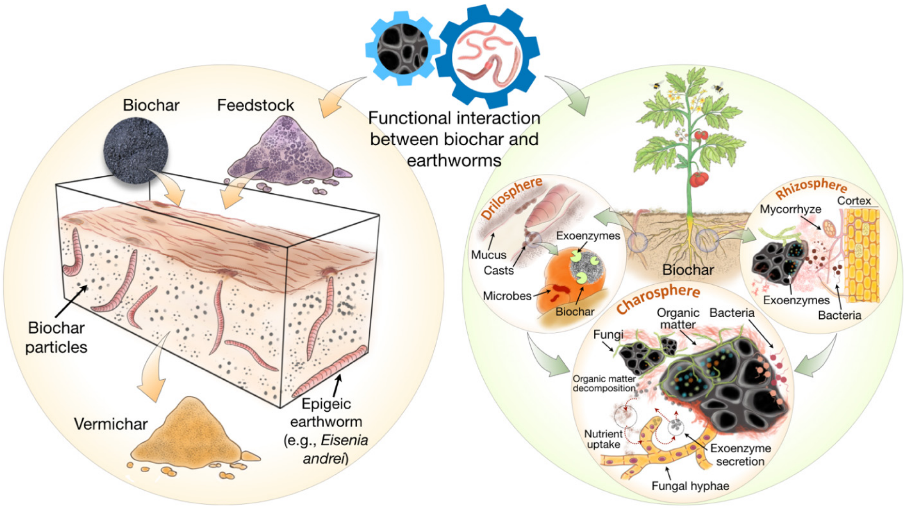


Fig. 2. Conceptual model on biochar bioactivation. Adsorption of extracellular enzymes onto biochar surface may take place in the burrow walls, casts and middens because of the high microbial activity and faunal diversity of these structures created by earthworms (external processes for biochar activation), and also during the gastrointestinal transit of ingested biochar particles (internal processes for biochar activation). In both cases, two components are essential to obtain enzymatically activated biochar: i) microorganisms which are significant sources of extracellular enzymes in the drilosphere structures and in the luminal microenvironment of earthworm digestive channel, and ii) mucus which is secreted by the gastrointestinal epithelium and earthworm skin. Mucus would have the role of cross-linker to facilitate enzyme retention onto biochar surface. Pictorial representation using Autodesk© SketchBook software. The earthworm anatomy was drawn from figure 2.2 in Gaddie and Douglas (1976), with modifications.

(Castillo et al., 2013; Ghosh et al., 2018). The CAPs occur in the earthworm casts (i.e., excreta in the form of pellets), and microorganisms and other decomposer fauna (e.g., springtails, enchytraeids, detritivore mites, isopods and millipides) participate in the further decomposition of organic waste (aging or maturation stage). The high content in organic matter of casts (molecules refractory to rapid digestion plus the earthworm gastrointestinal secretions) provides a nutritive cocktail for microbiota and decomposer fauna. Therefore, CAPs extend the feedstock decomposition although earthworms move away seeking for fresh and non-ingested organic waste or intentionally removed from the vermicomposting system.

Because composting and vermicomposting share many functional characteristics (Lim et al., 2016), it is reasonable to assume that the addition of biochar to vermicomposting will have comparable effects as those described for co-composting feedstock with biochar (Kammann et al., 2015; Sanchez-Monedero et al., 2018; El-Naggar et al., 2019). Indeed, biochar appears to be an excellent additive for increasing vermicomposting efficiency (Malińska et al., 2016), and reducing metal toxicity of hazardous feedstocks such as sewage sludges (Malińska et al., 2017). Colonization of biochar surface by microorganisms may explain the synergistic effects of biochar and these decomposing processes. Additionally, the presence of earthworms in the aerobic decomposing phase may introduce further benefits. In particular, earthworm mucus could act as a cross-linking substance, favoring extracellular enzyme binding to biochar surface. Mucus is a viscous secretion with a high content of glycoproteins, mucopolysacharides, and aminoacids (Pan et al., 2010; Zhang et al., 2016), secreted by subcutaneous and gastrointestinal epithelium cells to facilitate movement



"Vermichar" production scenario	"Vermiremediation" scenario
Advantages	**Advantages**
Vermicomposting conditions (moisture and temperature of raw material, earthworm density, and blending of raw material and biochar) can be easily controlled.	Improving soil biological (microbiome, faunal diversity), physical (content and distribution of soil aggregates) and chemical (pH, organic matter content, cation exchange capacity) properties by earthworm-biochar synergic effects.
Biochar activation may be continuously monitored during vermicomposting and maturation (earthworm free) phases by measuring enzyme activities in biochar particles.	Earthworm-induced distribution of activated biochar in a larger soil volume respect to biochar-treated, earthworm-free soils.
Extracellular enzymes would remain long-term active because of stabilization potential by biochar.	Increased bioremediation potential because of interactive effects of earthworms and biochar on pollutant bioavailability, biodegradation and/or immobilization.
Increase of metal bioavailability during vermicomposting of hazardous feedstocks may be compensated by metal immobilization onto biochar surface.	
Limitations	**Limitations**
Vermicomposting of hazardous feedstocks (e.g., sewage sludges) may be compromised by high concentrations of toxic chemicals accumulated in biochar.	Permanent soil moisture and food supply are required to maintain earthworm population.
High biochar dosage in the initial blended feedstock may disrupt vermicomposting because of adverse impact on earthworm population dynamic.	Risk of invasion of exotic earthworms into ecosystems inhabited by native earthworms, and potential negative impacts on soil processes.

Fig. 3. Potential advantages and limitations in the methodologies for activating biochar using epigeic ("vermichar" production scenario) and endogeic/anecic earthworms ("vermiremediation" scenario). Pictorial representation using Autodesk© SketchBook software.

Table 1
Future lines of research on ecotoxicology of earthworm-biochar interaction.

Research area	Background
A) Ex-situ bioactivation (“vermichar” production and use)	
Dynamic of metal speciation in vermicomposting supplemented with biochar.	Feedstocks used for vermicomposting such as sewage sludge (Swati and Hait, 2017), aquaculture sludge (Kouba et al., 2018), silk effluent sludge (Paul et al., 2018) and tannery sludge (Goswami et al., 2018), generally contain metal concentrations that may be toxic to earthworms, or become higher in the final vermicompost (Swati and Hait, 2017). Addition of biochar at the appropriate dose in vermicomposting could reduce metal bioavailability and toxicity via immobilization on biochar. The measurement of variables related to earthworm life cycle traits (e.g., earthworm density, cocoon production, hatching success, juvenile growth rate) and microbial activity could be used as toxicity endpoints to assess the benefits of biochar addition.
Impact of vermichar on the environmental fate of soil pollutants.	Vermicompost is a substrate riches in molecular binding sites (organic matter) and microbial activity (e.g., Domínguez, 2011; García-Sánchez et al., 2017) that facilitate contaminant removal. Vermichar could further improve these vermicompost qualities because of the additional binding sites provided by biochar. Knowledge on degradation rate, mobility and bioavailability of contaminants in soils treated with vermichar is needed to assess whether this upgraded vermicompost is suitable for remediation.
Impact of biochar on earthworm digestive function.	Biochar seems to modulate the activity of digestive enzymes of anecic earthworms (Sanchez-Hernandez et al., 2019b), so similar interaction could also occur in the digestive function of composting earthworms such as *E. fetida*. Moreover, knowledge on biochar toxicity on earthworm gut symbionts is also required to understand bioactivation during vermicomposting.
Impact of soil pollutants on enzymatically activated biochar.	Biochar has demonstrated to be an excellent remediating substrate (e.g., Zhu et al., 2017; Yuan et al., 2018). In order to use vermichar for remediating polluted soils, some uncertainties arise: i) potential alterations of catalytic properties of exoenzymes boudn to biochar by soil pollutants, and ii) improved remediation due to additional molecular ligands (exoenzymes).
B) In-situ bioactivation (“Vermiremediation”)	
Earthworm species.	Interaction between biochar and earthworm is highly dependent on species (Domene, 2016). Factors such as earthworm tolerance to biochar, behavior, and its impact on soil ecology in the case of using exotic species, should be criteria for selecting the most suitable earthworm species to achieve vermiremediation.
Biochar bioactivation with contaminant-detoxifying enzymes.	Soil enzymes such as laccases and carboxylesterases are able to degrade organic pollutants (Gianfreda and Rao, 2008) and bind to biochar (García-Delgado et al., 2018; Kholssi et al., 2018; Naghdi et al., 2018; Sanchez-Hernandez, 2018). Impact assessment of variables such as contaminant type and concentration on biochar bioactivation is highly recommended before initiating an in-situ vermiremediation action.
Biochar toxicity on drilosphere.	The burrow walls, casts and middens created by earthworms are essential components of drilosphere, contributing to improve soil quality (Brown et al., 2000; Hoang et al., 2016). Knowledge of biochar effects on the microbial activity of those structures is required to predict recovery of soil quality via earthworm and biochar jointly.
Biochar-earthworm interaction side-effects.	Biochar adsorbs pesticides (e.g., Liu et al., 2018; Varjani et al., 2019). Therefore, it is needed to know whether progressive accumulation of contaminants (and metabolites) in biochar becomes toxic to earthworms, and consequently could led to failure in the long term. Likewise, earthworms are able to disperse biochar in soil (Elmer et al., 2015). This activity may widen contaminated areas in soil due to transport of contaminant-sorbed biochar particles. This potential side-effect needs to be explored to assess whether earthworms expand polluted area or, by contrary, reduces pollutant toxicity by dilution effect.

through soil and transit of ingested material through the alimentary canal, respectively (Brown et al., 2004), among other physiological functions (i.e., innate immunity). For instance, the luminal content of earthworm gut contains up to 80% of mucus which is an important source of nutrients to anaerobic symbionts in that body zone (Wüst et al., 2009). Likewise, epidermic mucus of *E. fetida* is able to accelerate decomposition and humification of organic matter because of its capacity of altering microbial communities (Huang and Xia, 2018). Therefore, it would be reasonable to assume that both internally and externally secreted mucus may contribute to biochar activation with extracellular enzymes (Fig. 2). In the case of the gastrointestinal tract, the foregut generally displays the highest enzymatic activity in its luminal content corresponding to hydrolases such as protease, esterase, lipase, chitinase, invertase, cellulase and amylase (Ordoñez-Arévalo et al., 2018).

Considering the known benefits of biochar mixed in compost (Sanchez-Monedero et al., 2018), we hypothesize that vermicomposting of blended organic feedstock with biochar may lead to an improved vermicompost or *vermichar* characterized by a higher content of enzyme-coated biochar. Many studies have reported that enzymes reach a maximum activity in the first 2–3 weeks of vermicomposting and decrease progressively by the end of decomposing maturation phases (García-Sánchez et al., 2017; Sudkolai and Nourbakhsh, 2017; Usmani et al., 2018). The reduction of enzyme activity is explained by the decline in microbial biomass and its activity linked to the decrease in the earthworm activity as the final vermicompost is stabilized (García-Sánchez et al., 2017; Cui et al., 2018). Thus, addition of biochar in vermicomposting could serve as a physical storage for retaining extracellular enzymes actively produced in the first phase of this process, avoiding their further degradation in the later composting stages. Main advantages and limitations of this potential model of biochar bioactivation are summarized in Fig. 3.

4. “Vermiremediation” scenario (in-situ bioactivation)

The term “vermiremediation” denotes the use of earthworms in the removal of soil pollutants (Sinha et al., 2008; Rodriguez-Campos et al., 2014; Morillo and Villaverde, 2017). In this bioremediation option, both endogeic and anecic earthworms are preferred because of their strong impact on soil properties (Gavinelli et al., 2018). The former are geophagous, soil-dwellers that burrow intensively in the uppermost 10–15 cm of soil, creating temporary horizontal burrows while ingest large amounts of soil to obtain nutrients (Römbke et al., 2005). Anecic earthworms construct long (up to 3 m length), permanent, vertical burrows. Species belonging to this ecological group such as *Lumbricus terrestris* are detritivores, feeding on decaying organic residues that drag into their burrows, although they may also ingest mineral soil. These earthworms create a deposit called "midden" at the entrance of their burrows, which is mainly composed of organic residues mixed with casts (Stroud et al., 2016; Gavinelli et al., 2018). It is well known that the burrow system and middens are hotspots for microbial proliferation and extracellular enzyme production (Stromberger et al., 2012; Hoang et al., 2016). Therefore, it is reasonable to hypothesize that both endogeic and anecic earthworms would be able to activate biochar by creating a microbial biofilm on its surface and binding extracellular enzymes, as long as biochar toxicity is minimal and earthworms do not avoid biochar.

In the last few years, earthworms have been mainly used in biochar research in two main ways: i) as indicators for the assessment of pollutant bioavailability in biochar-amended soils (Gomez-Eyles et al., 2011; Sizmur et al., 2011; Wang et al., 2012; Zhang et al., 2019), and ii) as biological targets for biochar toxicity assessment, which may imply standardized avoidance behavior response tests (Li et al., 2011; Elliston and Oliver, 2019), or toxicity endpoints such as mortality, reproduction rate, and weight loss (reviewed in Weyers and Spokas, 2011). Yet, only a few studies have investigated the beneficial and synergistic effects of earthworms and biochar together on improving soil quality (Beesley and Dickinson, 2011; Paz-Ferreiro et al., 2015). Despite high doses of biochar (e.g., 10%–20% w/w), which could potentially be toxic to earthworms (Elliston and Oliver, 2019; Zhang et al., 2019), some studies have demonstrated that these organisms accidentally ingest biochar particles and disperse them during casting in the bulk soil (Ameloot et al., 2013). Nevertheless, emerging evidence has shown that some earthworm species such as *L. terrestris* are actually attracted to biochar instead of avoiding it. For example, Elmer et al. (2015) found that certain types of biochars with a high content in ash, Ca, Mn and Si were preferentially displaced from the soil surface by *L. terrestris* to deeper soil layers.

It is interesting to assess the impact of earthworms on the fate of biochar in soil. Domene (2016) postulated that interaction of biochar with earthworms may reduce its persistence in the environment because of grinding of ingested particles in the gizzard or, by the contrary, biochar may be incorporated within soil aggregates or buried in depth, thus increasing its persistence. In any case, the contact with earthworms may have significant consequences on biochar in terms of its activation with microorganisms and extracellular enzymes. Recently, Sanchez-Hernandez (2018) reported that incubation of earthworms (*L. terrestris* and *Aporrectodea caliginosa*) in biochar-amended soils increased carboxylesterase, β-glucosidase, alkaline phosphatase and arylsulfatase activities of the biochar particles recovered from the earthworm-treated soils. Furthermore, incubation of *L. terrestris* in soil columns spiked with biochar on top (2.5–5% w/w dry biochar) caused an accumulation of enzyme-rich biochar particles in the burrow walls, with maximum enzyme activity in the biochar recovered from the burrow bottom (Sanchez-Hernandez et al., 2019a). These studies also showed that carboxylesterase-coated biochar was an effective physical carrier to inactivate organophosphorus pesticides, suggesting this enzymatically bioactivated biochar as a promising strategy for bioremediating organophosphorus-contaminated soils. The oxygen-analog metabolites of this class of pesticides display a high affinity to the active site of carboxylesterases (Wheelock et al., 2008), so the enzyme-coated biochar would reduce bioavailability and toxicity of these highly toxic metabolites. Additionally, it was found that skin mucus of earthworms largely increased the retention of carboxylesterase enzyme in biochar, thus suggesting mucus as a cross-linking agent in the enzymatic activation of biochar (Sanchez-Hernandez et al., 2019a). Taken together, these studies encourage to continue investigating the underlaying mechanisms that explain this dynamic, functional interaction between biochar and earthworms, and the ways to improve soil quality and remediation efficiency. Nevertheless, the vermiremediation model is constrained by favorable environmental conditions for maintaining earthworm population, and ecological restrictions that may discourage soil inoculation with exotic species (Fig. 3).

5. Research opportunities

Both biochar and earthworms are potential promoters for remediating degraded soils. Biochar removes environmental contaminants via sorption and by promoting microbial degradation of soil organic pollutants (Rajapaksha et al., 2016; Sun et al., 2016; Wang et al., 2016; Shaaban et al., 2018). Likewise, earthworms are used in bioremediation mainly to increase the availability of contaminants to microorganisms, therefore stimulating the proliferation of contaminant degraders (Rodriguez-Campos et al., 2014; Morillo and Villaverde, 2017). In this paper we have proposed that both entities can be used simultaneously to develop a functional dynamic biotic platform for bioremediation purposes. However, suitability of this strategy requires an ecotoxicological assessment to ensure compatibility between biochar and earthworms, and to avoid adverse side-effects on soil biological processes. Although pyrolysis itself generally removes organic pollutants originally presented in the feedstock (Paz-Ferreiro et al., 2018), biochar may still contain traces of pollutants such as metals and polycyclic aromatic hydrocarbons (PAHs) depending on pyrolysis conditions and the chemical nature of the feedstock (Schimmelpfennig and Glaser, 2012), that could be potentially toxic to soil biota (Lehmann and Joseph, 2015). Notwithstanding, data in the literature reveal that earthworms ingest biochar particles (Topoliantz and Ponge, 2005), and may display preference for biochar-amended soils (Domene, 2016), although reasons for such behavior are still unknown. Microbial growth on biochar surface (reviewed in Lehmann et al., 2011), changes in the pH (Domene, 2016) and the moisture levels (Li et al., 2011) following biochar addition, biochar type and dosage (Liesch et al., 2010), soil type (Weyers and Spokas, 2011), chemical composition of biochar (Elmer et al., 2015), biochar size (Prodana et al., 2019), and earthworm species (Elliston and Oliver, 2019), could have a significant influence on biochar toxicity. Therefore, generalizations about adverse effects of biochar upon earthworms are difficult to establish. Accordingly, we suggest the topics listed in Table 1 as priority lines of ecotoxicological research for obtaining an environmentally-safe activated biochar.

6. Conclusions

The synergistic effects of using earthworms and biochar together can be a viable option to boost soil biodiversity and improving soil quality. Although still scarce, the available studies strongly suggest that earthworms may activate biochar with extracellular enzymes. Therefore, in this paper we propose to develop a new product called "vermichar", a vermicompost containing biochar as an ingredient, which is bioactivated during the vermicomposting process and can be used to treat contaminated soils and improve soil quality. In addition, we also propose a new class of vermiremediation technology utilizing both earthworms and biochar to create an in-situ *enzymatically biodynamic platform* to remediate contaminated soils and build up soil quality. The peculiarity of this procedure is the bioactivation of biochar by biological processes naturally occurring in soil (in-situ) but promoted by earthworms. Biochar will act as a carrier (*the platform*) retaining and stabilizing soil extracellular enzymes that are mainly produced by microorganisms. Earthworms have the role of microbial-stimulating vectors to increase extracellular enzyme production and facilitate their adsorption onto biochar surface (*enzymatic bioactivation*), as well as to disperse this activated biochar in the bulk soil (*dynamic*). We have identified future research lines that will strengthen the proposed strategies using biochar and earthworms together for bioremediation and soil quality improvement.

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