Impact of biochar produced from plastic-eating insect frass on microbial hotspots created by earthworms

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Biochar is the solid residue generated during the pyrolysis of biomass. Although its primary use is fertilizing agricultural soils (Jeffery *et al* 2015), biochar also has a prominent use in the remediation of polluted water and soil (Bandara *et al* 2019). The capacity of biochar to retain environmental contaminants and biomolecules (enzymes) largely depends on its physicochemical properties, which, in turn, are defined by the type of biomass and pyrolysis conditions (e.g., temperature and time) (Ippolito *et al* 2020). Recent studies suggest that co-pyrolysis of lignocellulosic residues and non-biodegradable plastic wastes improves the adsorptive properties of biochar (Hassan *et al* 2016). To this, the biomass-plastic mixture requires a pre-treatment to increase plastic density and, consequently, facilitate a good homogenization with biomass, especially if high plastic proportions are required. These pre-treatment methods (i.e., mechanical or thermal treatments) need an external energy supply and specific apparatus. However, bioconversion of plastic residues using insect larvae is recently emerging as a cost-effective option for plastic fragmentation and homogenizing with biomass.

Larvae of some insect species such as *Tenebrio molitor* can chew and ingest plastics, particularly polystyrene (Pivato *et al* 2022). However, the biodegradation capacity of this organism is limited and, consequently, a high proportion of the ingested plastic is found in the frass (excreta mixed with non-ingested organic waste, chewed fragments, and small molt fragments). Taking advantage of this biological treatment of plastics, we hypothesized that the pyrolysis of plastic-containing frass produces non-toxic biochar with upgraded properties to promote soil health. The aim of this study was to evaluate whether biochar produced from insect frass-containing polystyrene residues increased the microbial and enzymatic properties of soil.

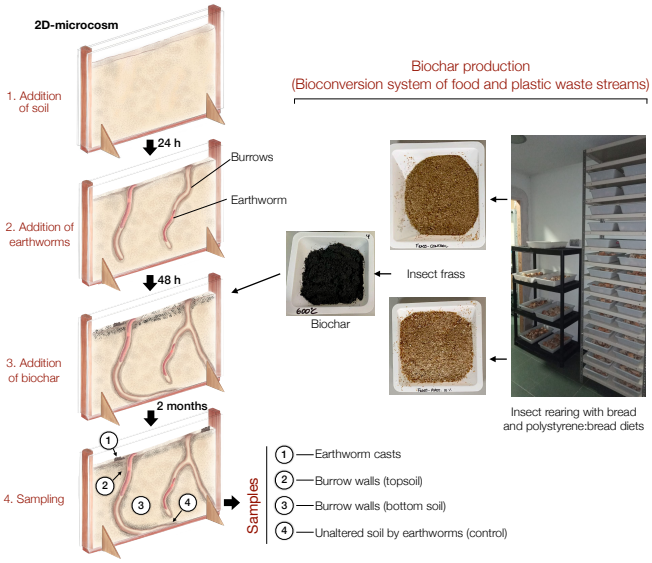

Figure 1. A pictorial representation of biochar production from insect frass, and the microcosm system used for biochar impact on soil microbial and enzymatic activities.

Biochar was obtained from the frass of insect larvae (*T. molitor*) fed with bread alone (control) and bread-polystyrene (10% w/w) co-diet. Pyrolysis was performed in a muffle furnace at two temperatures (300ºC and 600ºC for 90 min, heating rate of 15ºC/min). The impact of biochar on soil microbial and biochemical properties was assessed through a two-dimensional (2D) microcosm (two methacrylate panels, 50×25 cm, leaving an interior space of 15 mm with the help of wooden rods placed at the edges of the panels), which allowed us to evaluate the interaction between biochar and earthworms through the hotspots (burrow walls and casts) created by the earthworm activity (Fig. 1). Wet soil (< 2mm particle size) was poured inside the 2D microcosm, generating a 45-cm height soil layer. The microcosms (n=20) were kept at 15ºC and dark for 24 h, and one earthworm (*Lumbricus terrestris*) was released in each one. The microcosms were again incubated for a 48-h additional time to allow earthworms to burrow in the soil. Afterward, a fine soil layer (45 g) mixed with biochar (2.5% w/w) was poured on the soil surface. Control microcosms received 45 g of biochar-free soil. Therefore, the treatments (4 replicates) were the followings: i) control, ii) soil mixed with 300ºC biochar produced from frass (B300), iii) soil mixed with 300ºC biochar made from frass+polystyrene (BP300), iv) soil mixed with 600ºC biochar made from frass (B600), and v) soil mixed with 600ºC biochar produced from frass+polystyrene (BP600). After a 2-month incubation, microcosms were opened to collect samples of the casts deployed on the soil surface and burrow walls at two depths (topsoil 0–5 cm or topsoil, and 40–45 cm or bottom soil) (Fig. 1). We measured the potential activity of esterase, alkaline phosphatase, β-glucosidase, protease, urease, dehydrogenase, and catalase according to Sanchez-Hernandez *et al* (2017). Dehydrogenase and catalase activities together with soil respiration were used as indicators of microbial activity. The results of the enzyme activities were used for assessing the soil enzyme functional

diversity through two enzymatic indexes: the treated-soil quality index or T-SQI (Mijangos *et al* 2010) and the integrated biological response index or IBR (Sanchez *et al* 2013).

The main results were: 1) biochars were not toxic to earthworms as no deaths were recorded after 2 months of incubation, although the earthworm weight decreased in the group incubated with biochars produced at 300ºC (67–68% with respect to *t*=0 d). 2) Most enzyme activities were higher in the casts produced by earthworms incubated in soils amended with biochar from frass-polystyrene produced at 300ºC (Fig. 2). 3) The enzymatic indexes revealed that higher activities were found in the casts from the 300B and 300BP experimental groups, despite the enzyme activity levels being lower in the burrow walls with respect to controls (biochar-free microcosms) (Fig. 3). 4) Pearson's correlations (r^2>0.68, p<0.05) showed that organic carbon, probably derived from biochar, was a key driver for stimulating microbial activity (catalase activity and soil respiration) and the enzyme urease.

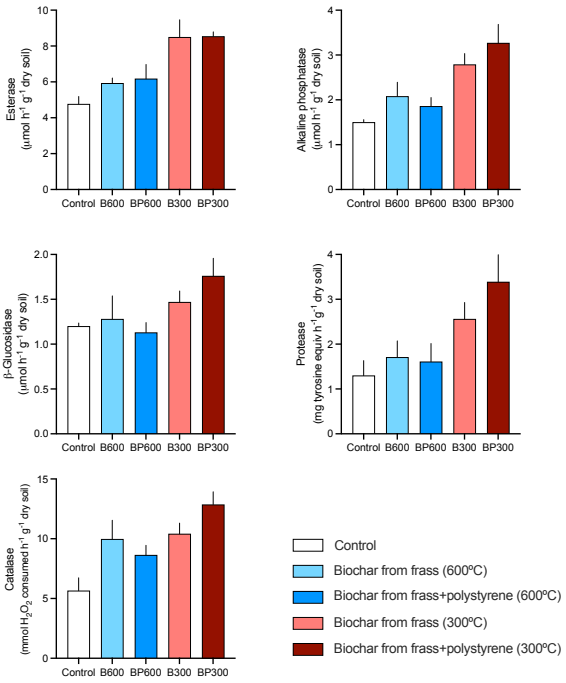

Figure 2. Variation of mean (±SEM) activity of soil enzyme activities in hotspots created by the earthworm *Lumbricus terrestris*.

Our results suggest that the bioconversion of plastic and food waste streams using insect larvae is a workable system for obtaining a plastic-rich, well-homogenized feedstock suitable for obtaining upgraded biochar. Furthermore, biochar produced at 300ºC from plastic-rich feedstock is suggested as an ideal promoter of soil biological processes, probably because of the organic carbon input through this recalcitrant material. Finally, the use of anecic earthworms (*L. terrestris*) incubated in a 2D-microcosm is a recommended lab-scale assay for assessing toxicity and biochar benefit in hotspots of soil microbial activity.

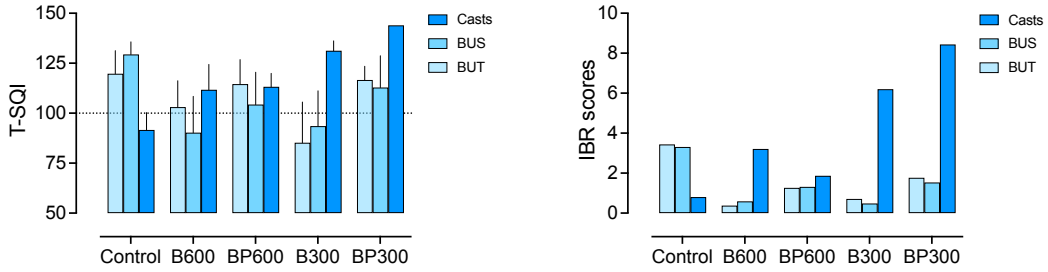

Figure 3. Response of the enzymatic indexes of soil functional diversity T-SQI (treated-soil quality index) and IBR (integrated biological response) in the earthworm casts, and burrow wall samples collected at the topsoil (BUS) and bottom soil layer (BUT).

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