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

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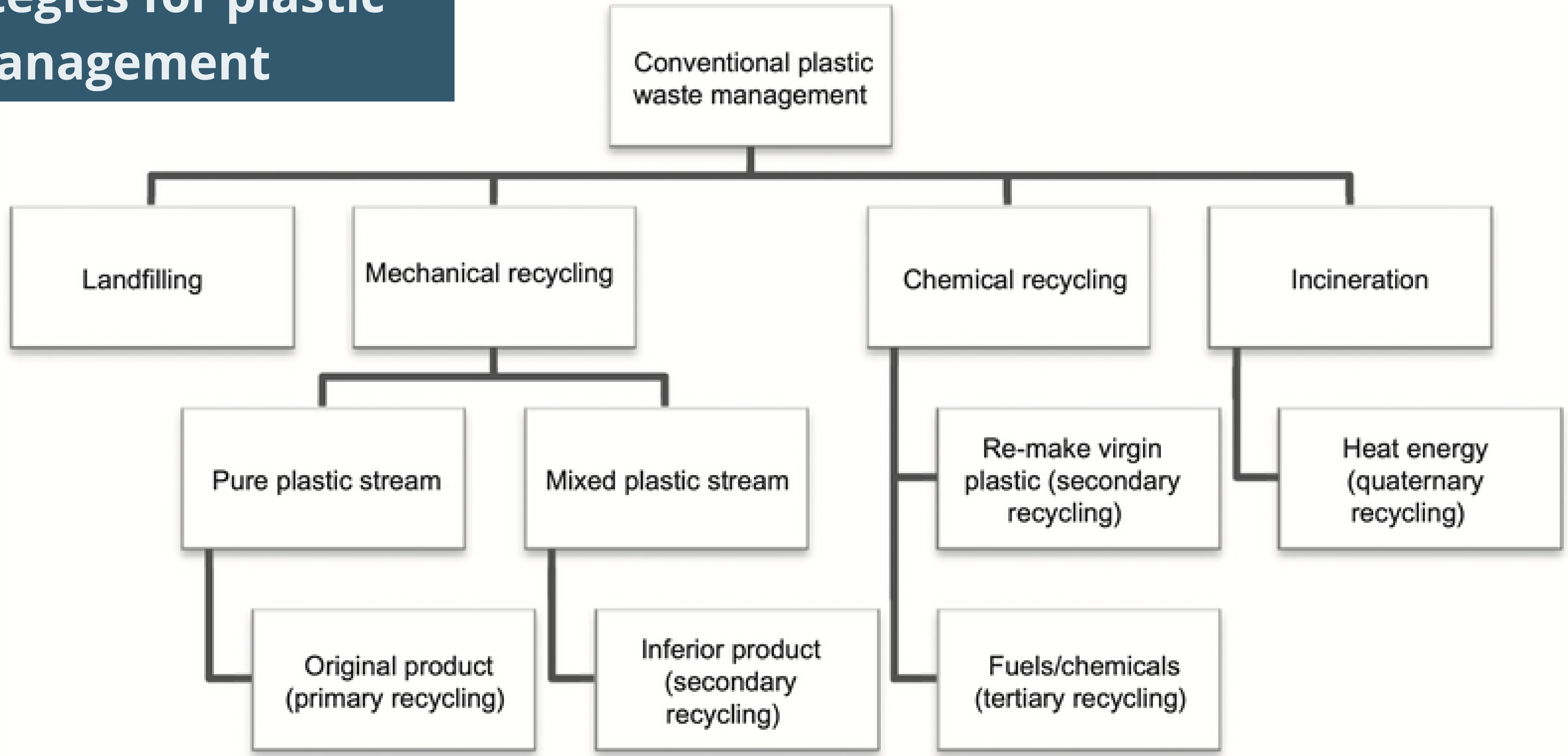
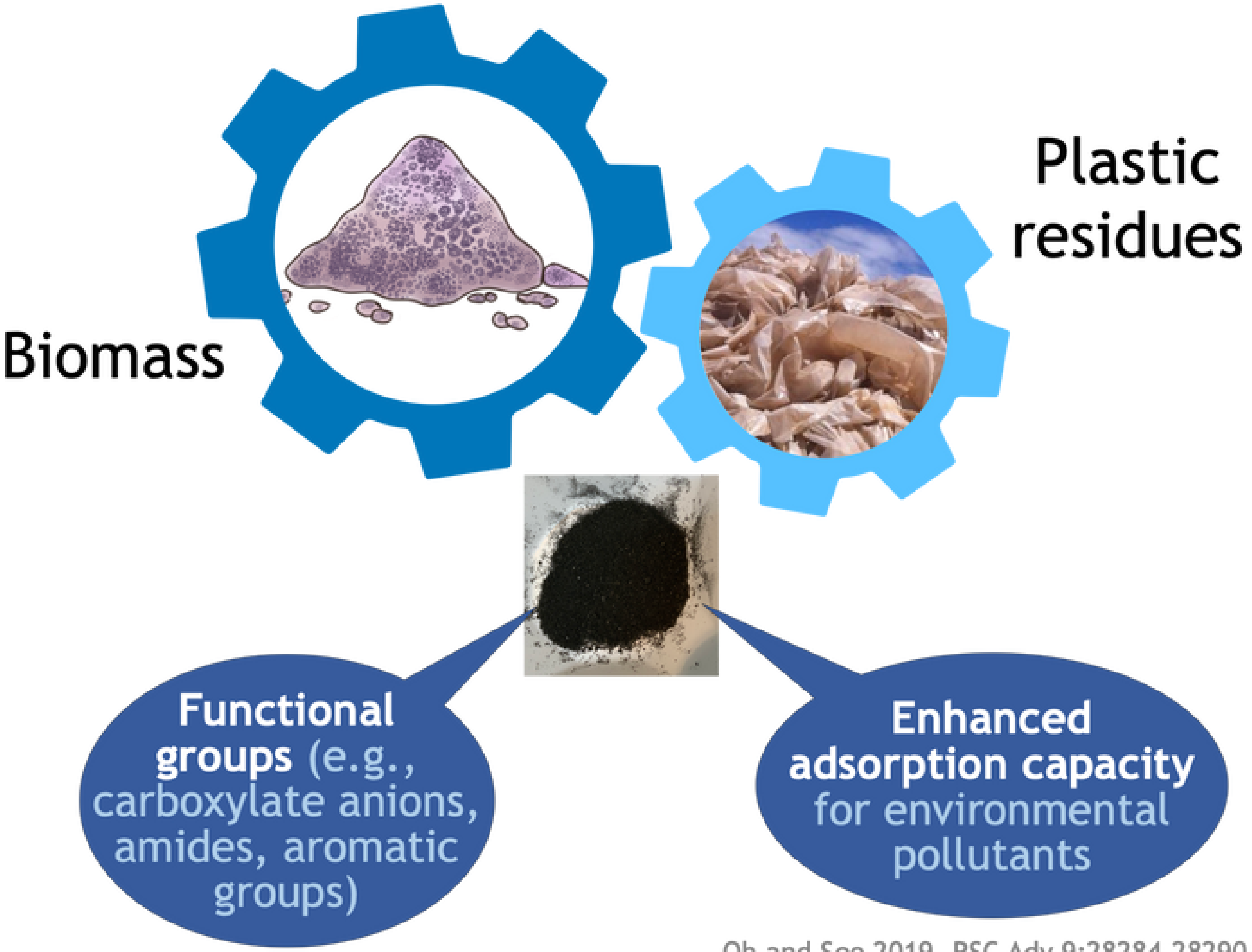
Main strategies for plastic residue management



Dilkes-Hoffman L.S. et al. (2019). Plastics to Energy (pp. 469-505). Elsevier.

Co-pyrolisis of blended biomass and plastic waste

BIOCHAR



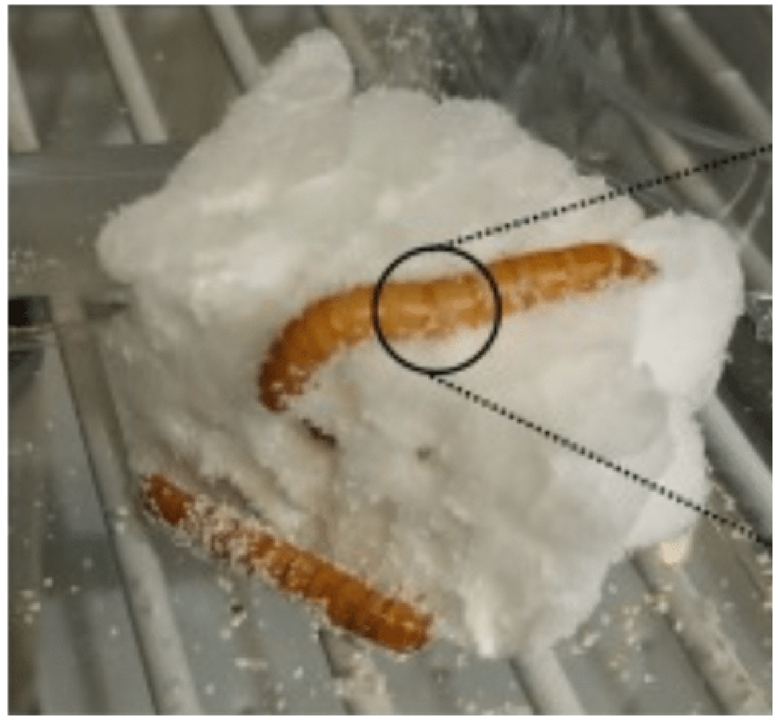
Rathnayake et al. 2021. J Anal Appl Pyrol 155:105029

Oh and Seo 2019. RSC Adv 9:28284-28290

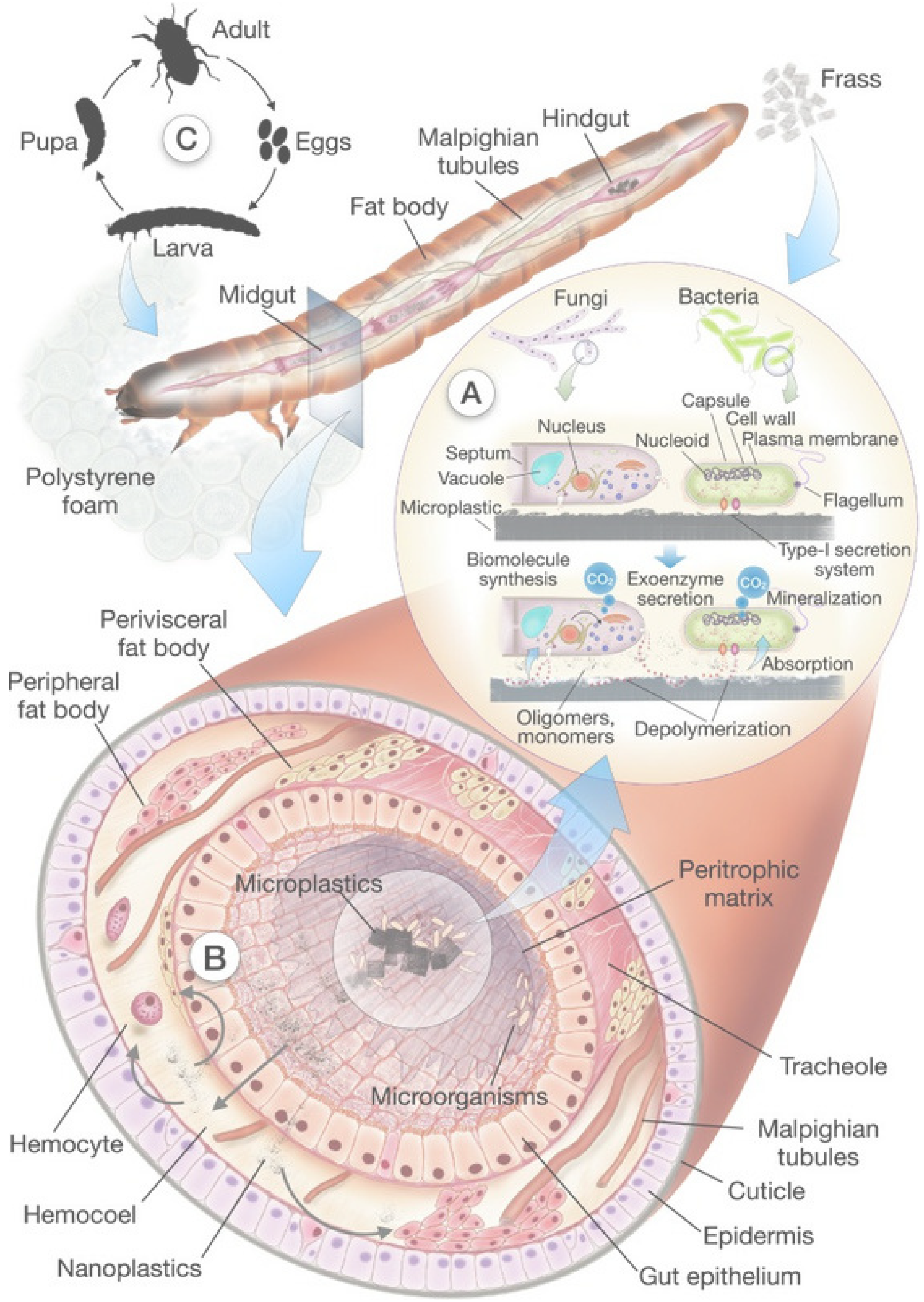
The difference in density between plastic and biomass commits the percentage and homogenization of the mixture, which limits the production of biochar

Bioconversion of plastic residues using insect larvae

Tenebrio molitor



Chew and ingest plastics
polystyrene, polyethylene, polyvinyl chloride, and polypropylene



Biological treatment as a cost-effective option for plastic fragmentation and homogenizing with biomass

Plastic-biodegradation of the mealworm gut biota is limited.

Microplastic still found in the frass.

Juan C. Sanchez-Hernandez, A toxicological perspective of plastic biodegradation by insect larvae, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Volume 248, 2021, 109117, ISSN 1532-0456, https://doi.org/10.1016/j.cbpc.2021.109117.

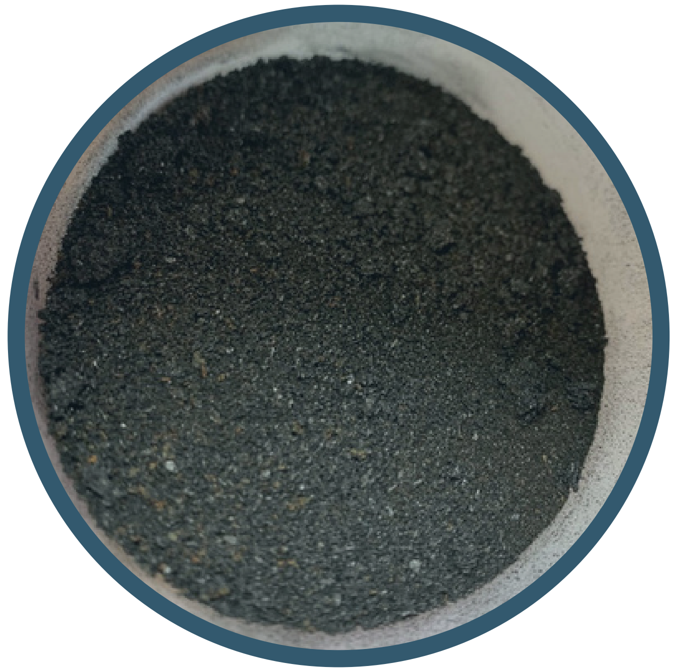
T. Molitor

Plastic diet

Frass rich
in plastic

Biomass

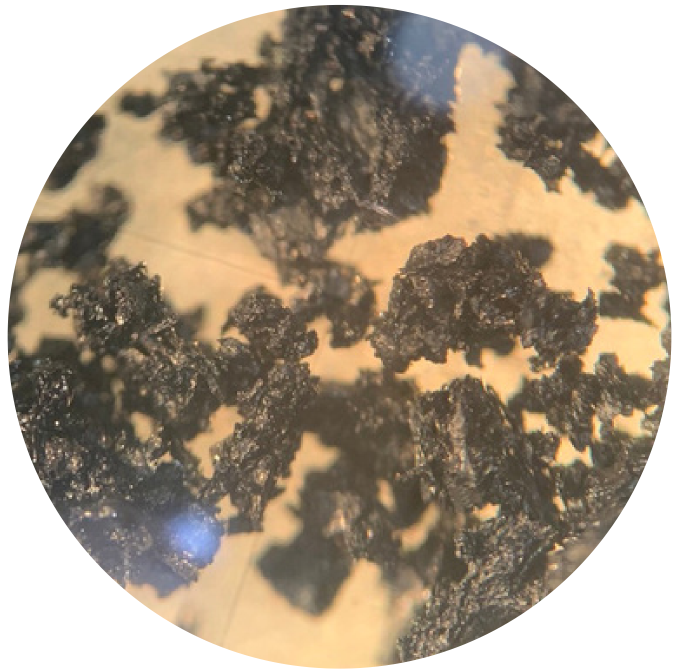
Pyrolysis



300 °C and
600 °C

BIOCHAR +++

Pyrolysis of plastic- containing frass produces non-toxic biochar with upgraded properties to promote soil health

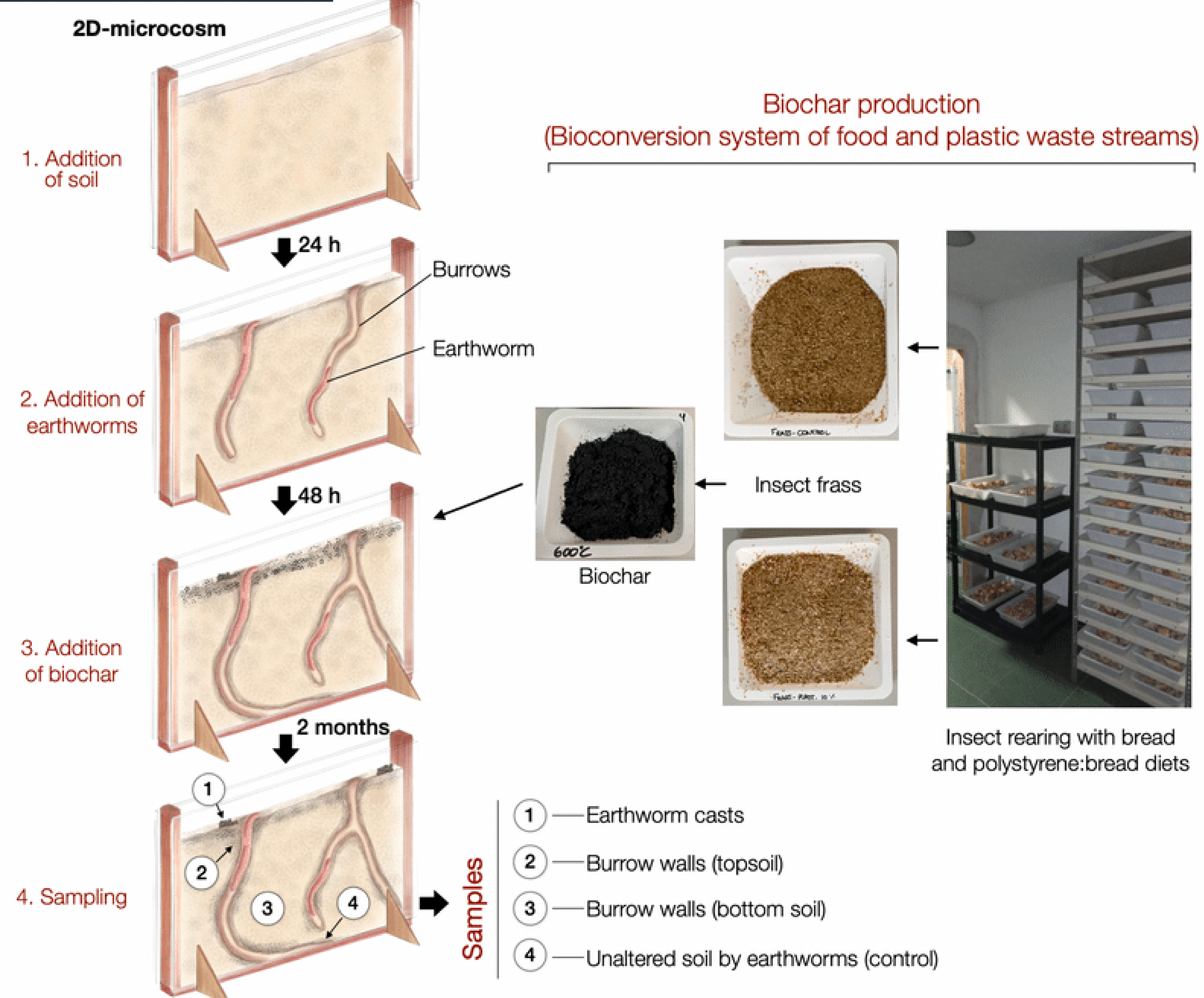


Objectives

Evaluate if the biochar produced from insect frass- containing polystyrene residues (300°C and 600 °C) increased the microbial and enzymatic properties of soil

Evaluate the toxicity of biochar in microcosms with earthworms (*Lumbricus terretris*)

Experimental design



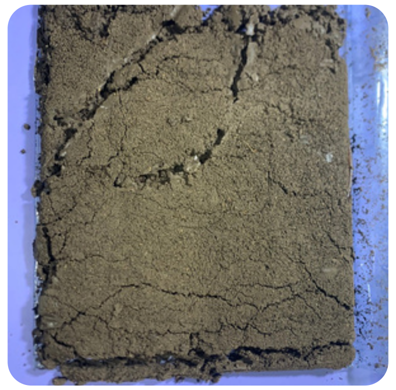
Pyrolysis was performed in a muffle heater at two temperatures (**300°C and 600°C** for 90 min, heating rate of 15°C/min).

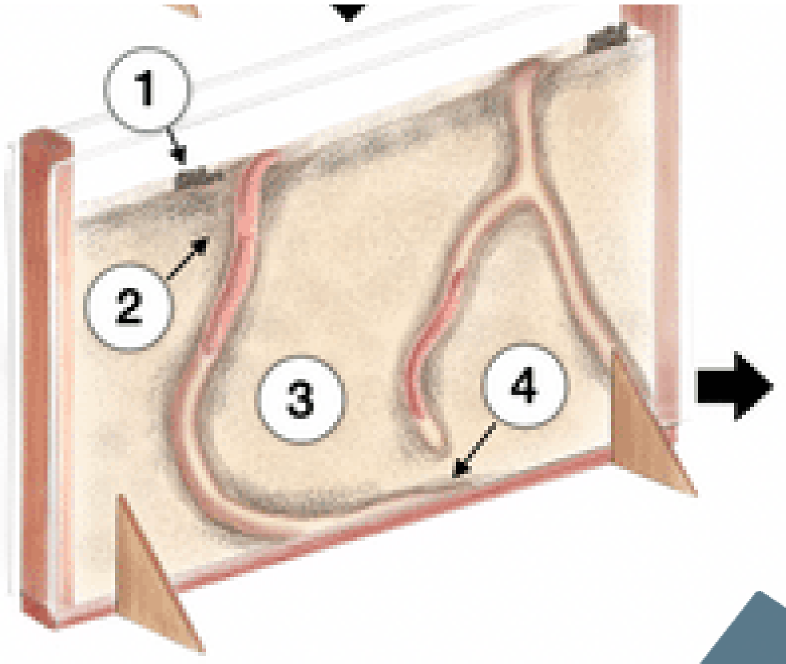
The biochar was produced from frass of insect larvae (T. molitor) fed with **bread alone (control) and bread-polystyrene (10% w/w) co-diet**.

Treatments

- Control (Only soil)
- Biochar 300°C
- Biochar 300°C+ polystyrene 10%
- Biochar 600°C
- Biochar 600°C+ polystyrene 10%

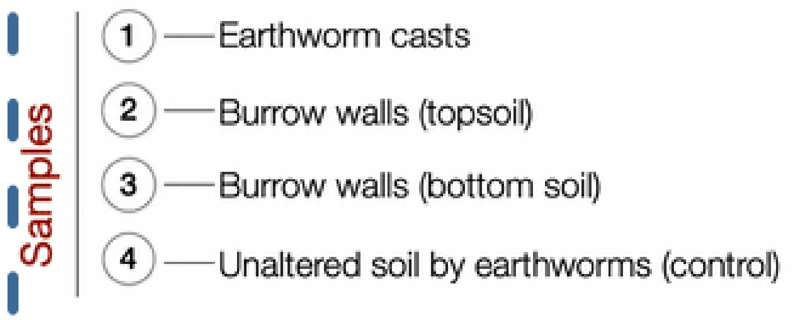
Biochar



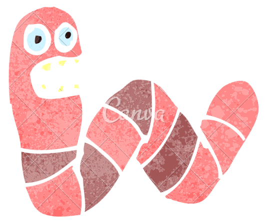


- (2D) microcosm (two methacrylate panels, 50×25 cm)
- Wet soil (< 2mm particle size)
- 45cm height soil column
- Microcosms (n=20)
- 15°C and dark
- 1 Earthworm (*Lumbricus terrestris*) per microcosm
- Fine soil slide mixed with **biochar (2.5% w/w)** was poured on the soil surface.
- 2 month incubation

Assessment



-Enzimatic activity



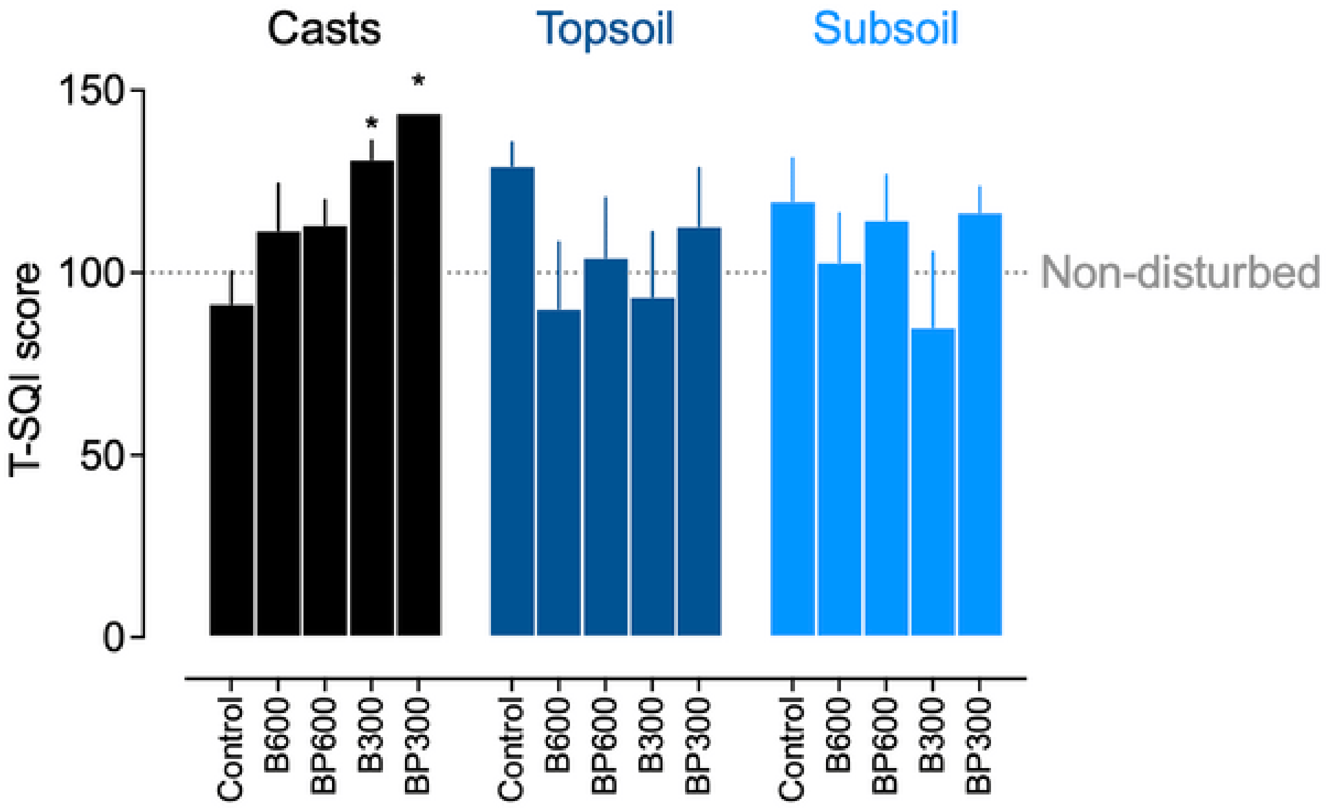
-Survival rate

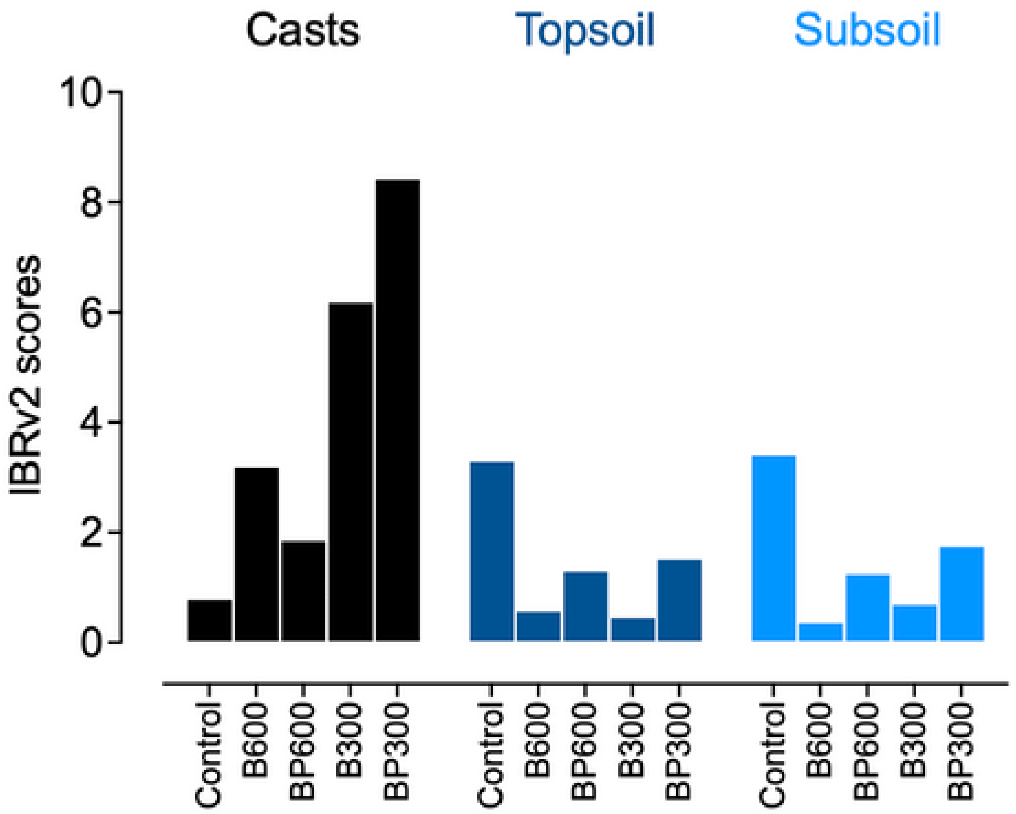
-Biomarkers

Results

INDEXES

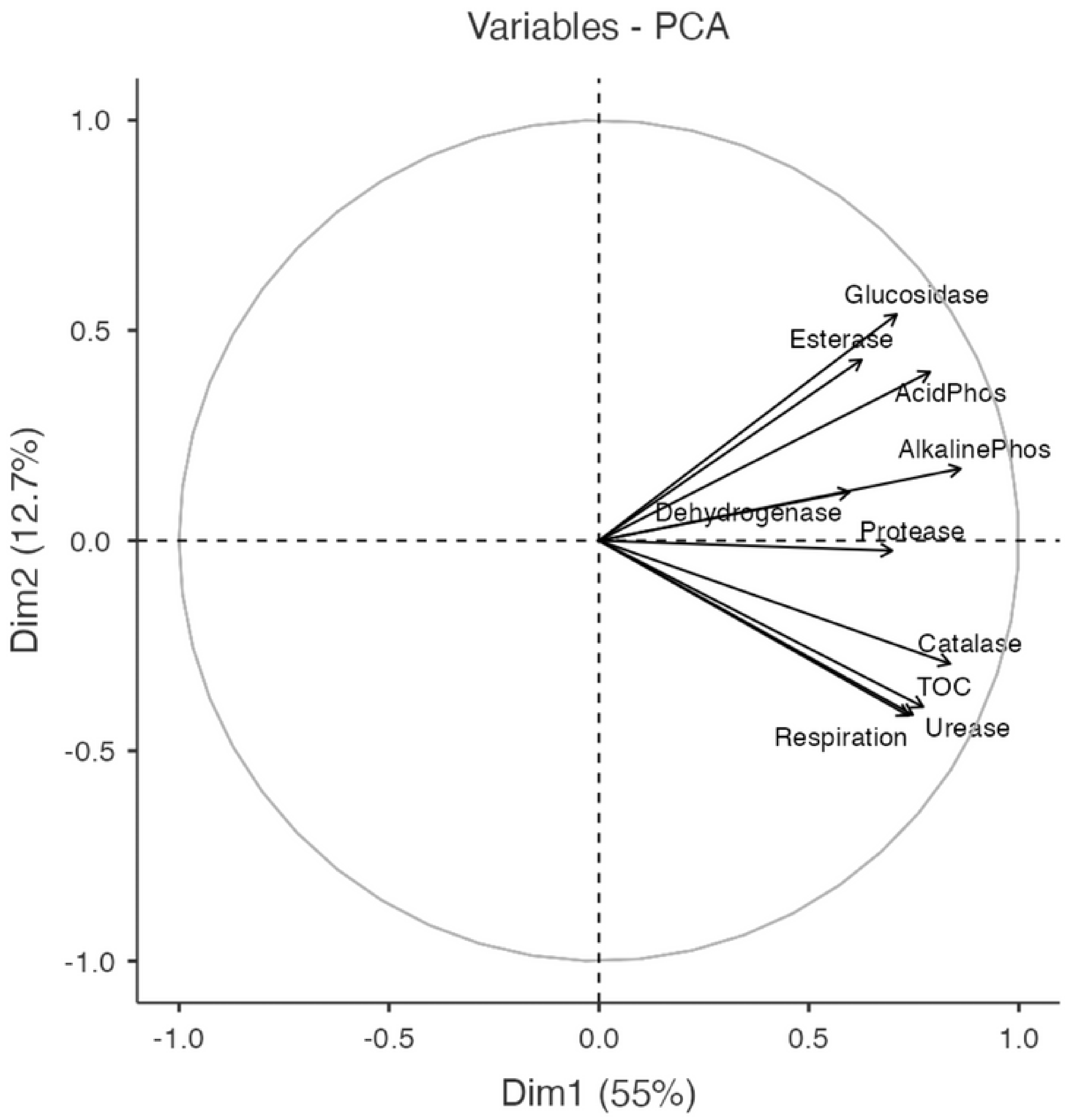
We measured the potential activity of esterase, alkaline phosphatase, β-glucosidase, protease, urease, dehydrogenase, and catalase according to Sanchez-Hernandez et al (2017).
Dehydrogenase and catalase activities together with soil respiration were used as indicators of microbial activity.

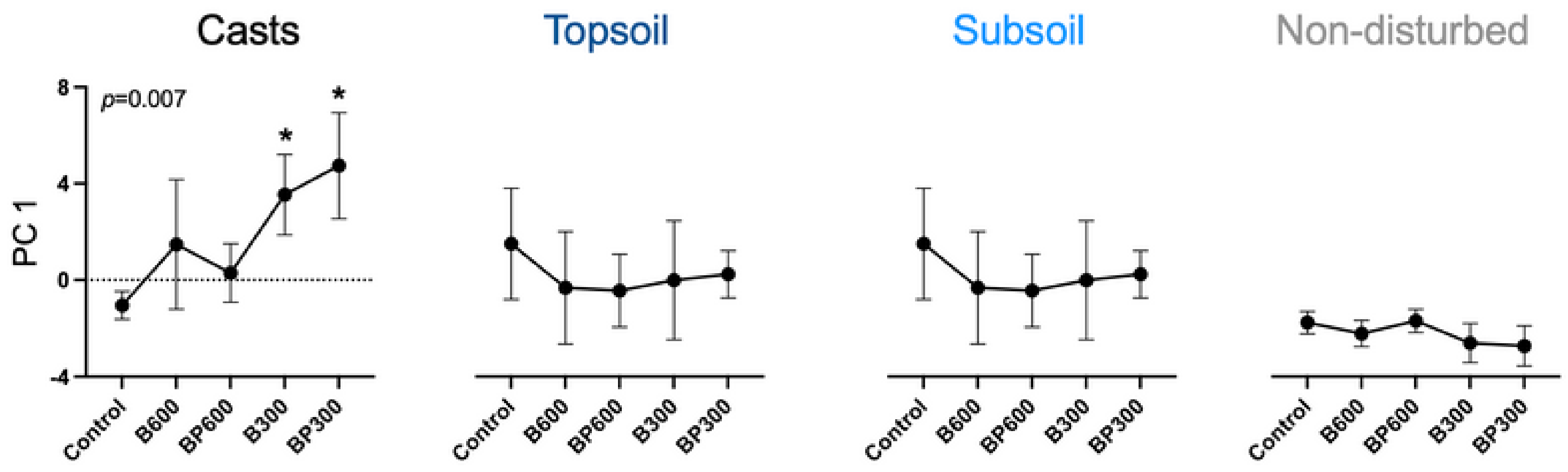




We used two **enzymatic indexes** to assess the **impact of biochar on soil enzyme activities**: the **treated-soil quality index (T-SQI)** and the I**ntegrated Biological Response Index,** version 2 (IBRv2).

Both indexes showed that **the overall response of enzymes was significantly higher in the casts collected from treatments B300 and BP300.**





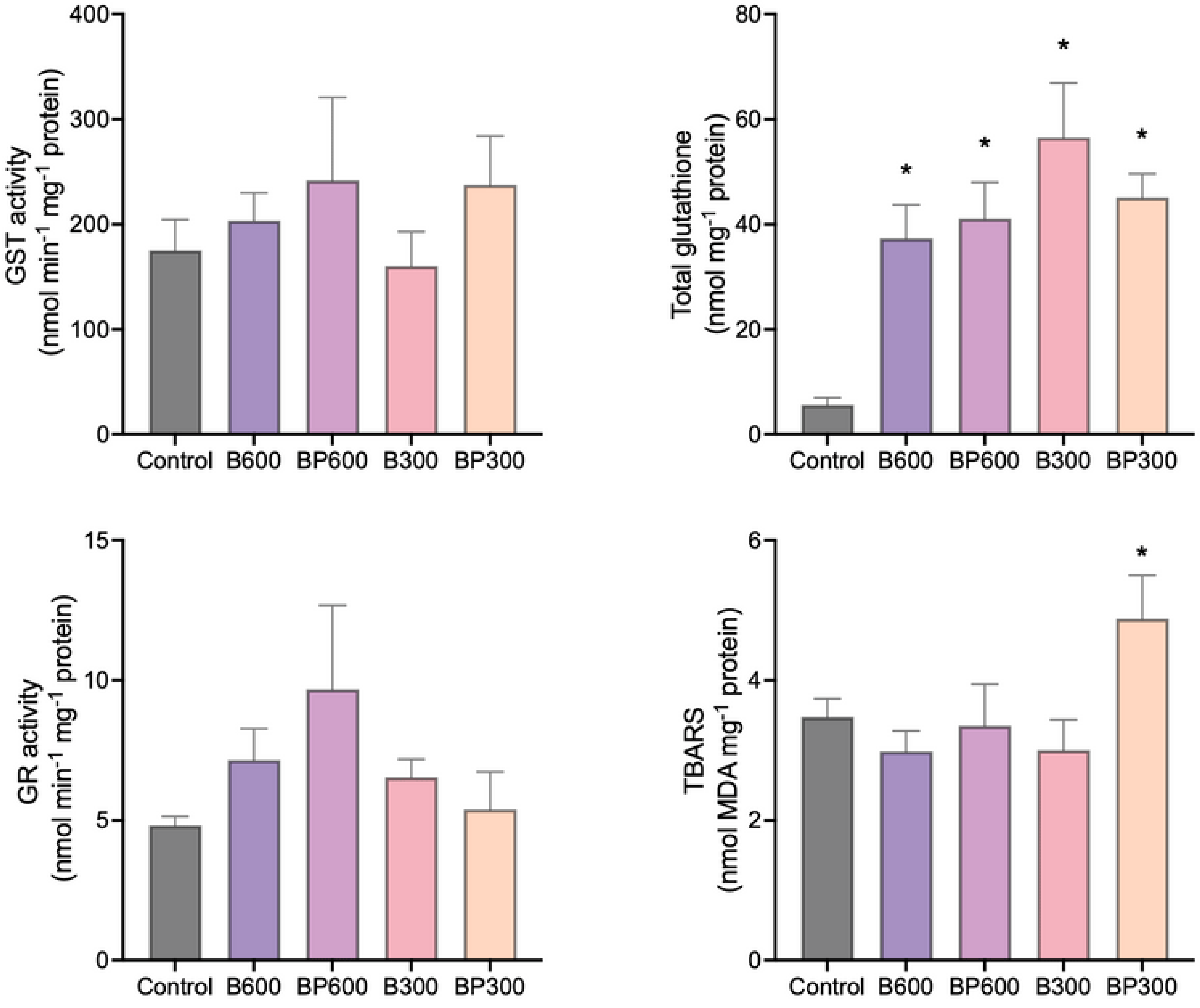
PC 1: microbial respiration, total organic carbon, catalase, urease and protease.
PC 2: Esterase, glucosidase, acid and alkaline phosphatase activities.

Component 1, which grouped microbial activity and N-cycling enzymes was **significantly higher in casts from treatments B300 and BP300.**

We also used the Principal Component Analysis (PCA) to reduce the number of variables (enzyme activities) to a small number of uncorrelated variables or factors. Univariate ANOVAs were then run over the factor scores generated from the PCA.

Biomarkers

We also measured some biomarkers of oxidative stress in earthworms (muscle tissue) to assess potential sublethal toxicity derived from biochar exposure.



We found a strong **increase of glutathione** concentration in the muscle of earthworms exposed to all biochar treatments respect to controls.

Lipid peroxidation (a biomarker of oxidative damage) was significantly **higher in the BP300** group than the rest of treatments

No deaths were recorded after 2 months of incubation. We don't found significant differences in the earthworm weight between treatments.

1

Biochar derived from frass of insect fed with spent bread and polystyrene and produced at 300°C **stimulated the enzyme activities of casts deployed on soil surface**. The enzymes responsible for this increase were urease and protease, which suggest nitrogen-containing organic compounds as the primary energy source for microorganisms as revealed by the PCA analysis.

2

The use of enzymatic indexes, particularly the **IBRv2**, are suggested as suitable tools for **assessing the impact of biochar on soil enzyme activities**, which may complement other multivariate analysis such as PCA.

3

Biochar treatments caused oxidative stress in the earthworms but the dose of 2.5% of biochar in the soil was not lethal for earthworms.

In the light of these preliminary results, **we recommend the addition of polystyrene in biochar production**

Conclusions

Thanks for you attention

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Supplementary results