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Nutrient-Enriched Biochar and Ectomycorrhizal Fungi Inoculation Effects on Tree Establishment in Fire-Affected Soils

Olena Dorosh

LAQV REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal | anelo@isep.ipp.pt

Wildfires are a natural component of ecosystem dynamics, yet their frequency, extent, and severity have increased considerably in southern Europe, placing Mediterranean ecosystems at extreme risk of biodiversity loss and desertification. High-severity fires can induce persistent changes affecting soils physical, chemical and biological properties. Soil biota is also affected, especially ectomycorrhizal fungi (EMF) that form symbiotic relations with the tree’s roots. While some early successional EMF taxa may recover rapidly, late-successional and fire-sensitive species often recover slowly or incompletely. Biochar, produced through pyrolysis of organic biomass, is a stable, carbon-rich material that can improve soil structure, nutrient retention, and microbial habitat. However, due to their low nutrient availability, enrichment strategies have been developed, resulting in slow-release fertilizers with the physical benefits offered by these materials. Producing biochar from agricultural residues also supports circular economy practices. EMF inoculation can further enhance seedling growth by improving water and nutrient acquisition, particularly for pioneer species.

In this study, biochar derived from vineyard prunings, both pristine and nutrient-enriched, was applied to unburnt and burnt soils planted with birch (*Betula spp.*) seedlings, half of which were inoculated with *Pisolithus tinctorius*. Nutrient enrichment was performed by optimizing the adsorption conditions of N and P from $NaNO_3$ and KH_2PO_4 solutions, and soil column experiments confirmed that the nutrients were released from the biochars matrix over time. In unburnt soils, seedling growth was not considerably affected. In contrast, in burnt soils, nutrient-enriched biochar significantly increased seedling height (by 24–36%) and aboveground biomass (by 26–37%), with the strongest responses observed when combined with EMF inoculation. EMF inoculation had limited effects in unburnt soils but markedly enhanced root colonization in burnt soils (from 32–46% to 52–61%). DNA metabarcoding, however, revealed that *Pisolithus tinctorius* did not dominate roots, suggesting that colonization was performed by native microbial communities. Fire shifted root-associated EMF communities from late-successional *Thelephora* toward early-colonizing taxa such as *Inocybe* and *Sphaerosporella*, consistent with post-fire successional patterns.

Overall, nutrient-enriched biochar enhanced soil functioning, mycorrhizal re-establishment, and early tree growth in fire-affected soils. The results also suggest that the addition of medium growth may further improve root colonization, without the need of using an inoculum. This integrated approach represents a promising, circular strategy for accelerating forest recovery following wildfire.

Short Bio

Olena Dorosh holds a master's degree in Biotechnology for Sustainability (2019) from ITQB (NOVA University of Lisbon), and a bachelor's in Biochemistry (2017) from the NOVA School of Science and Technology. She worked as a Master Student Fellow on two research projects at REQUIMTE/LAQV-ISEP, Polytechnic University of Porto (Portugal), focusing on the valorisation of agricultural by-products. In 2022 she began a PhD in Sustainable Chemistry. Her thesis project, *"Burnt soils: sustainable remediation strategies"*, focuses on the production of biochar from agricultural by-products to accelerate the rehabilitation of burnt soils in combination with plant symbiotic fungi. She (co)authored 12 Web of Science-indexed papers and 20 communications presented at national and international conferences.

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