

## Investigating Roadside Soil Microbiomes: Metagenomic Analyses to Identify Taxa Driving Rubber Degradation

**Alessia Dicesare**<sup>1,2</sup>, Daniela Bucchieri<sup>1,2</sup>, Marcella De Divitiis<sup>1,2</sup>, Immacolata Serra<sup>1</sup>, Luca Giannini<sup>3</sup>, Silvia Guerra<sup>3</sup>, Federica Magaletti<sup>3</sup>, Paola Branduardi<sup>1</sup>

*E-mail:* a.dicesare3@campus.unimib.it

<sup>1</sup> Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy

<sup>2</sup> Department of Material Science and Nanotechnology, University of Milano-Bicocca, Milano, Italy

<sup>3</sup> Pirelli Tyre S.p.a., Viale Piero e Alberto Pirelli, 25, Milano, Italy

**Keywords:** TRWPs, Metagenomics, Rubber Degradation, Microbial Consortia, Roadside Soil

### Abstract:

The significant environmental accumulation of waste tyres and the release of significant amounts of particulate debris, predominantly tyre and road wear particles (TRWP) generated by the friction between tyres and road surfaces, pose a critical challenge. While biodegradation has been extensively studied for other recalcitrant polymers such as plastics, rubber has received little attention. Moreover, current research often focuses on individual rubber-degrading microbial strains, whereas the complexity of rubber suggests that efficient degradation requires synergistic interactions within microbial consortia. A broader overview of rubber degradation processes is therefore essential. In this work, we proposed a combined approach employing metagenomic sequencing alongside tests (plate isolation) on microbial consortia to explore the potential of both culturable and non-culturable, low-abundance microorganisms in rubber degradation.

We investigated this potential by enriching microbial communities derived from roadside soil collected from heavily trafficked road using rubber compounds as the sole carbon source. This selective pressure promoted the growth of rubber-degrading taxa. Subsequently, we utilized two metagenomic approaches - 16S rRNA gene sequencing and shotgun metagenomics - to characterize the taxonomic composition of the whole microbial communities from both the original soil and the enriched samples.

Comparative metagenomic analyses (16S rRNA gene sequencing and shotgun sequencing), demonstrating concordance between taxonomic and functional profiles, revealed significant shifts in microbial community structure and facilitated the identification of key taxa. This allowed us to assess the overall metagenomic divergence driven by the selective pressure of the enrichment conditions. By comparing microbial communities from heavily trafficked road soils with those enriched under controlled, rubber-only incubations, this work provides novel insights into environment's responses to rubber pollutants and the microbial potential for their breakdown.