Assessing Microplastic Impact on Soil Microbial Communities: Integrating Metabarcoding Data into Regulatory-Relevant Endpoints

Fabian Essfeld[1], Udo Hommen[1], Sebastian Eilebrecht[2], Cecilia Diaz[3], Karsten Schlich[3]

[1] Department Modelling & Bioinformatics, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany

[2] Department Ecotoxicogenomics, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany

[3] Department Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany

Introduction

Microplastics (MPs) are emerging contaminants in terrestrial ecosystems, yet their effects on soil microbial communities are still insufficiently understood. Soil microorganisms play a key role in nutrient cycling and ecosystem functioning and are therefore relevant targets for ecological risk assessment. Current regulatory tests with soil microorganisms focus on functional endpoints and may overlook subtle community-level changes. High-throughput metabarcoding offers new opportunities to detect structural shifts in microbial communities. Integrating such endpoints with established functional tests may support the development of regulatory-relevant, ecologically meaningful thresholds.

Materials & Methods

Test items: Two fragmented low-density polyethylene (LDPE) microplastics differing in their size were tested. One material was also aged via UV illumination:

A. Fragmented LDPE, particle size range: 0.6 -30.1 µm (average = 4.6 ± 2.9 µm),

B. Manufactured LDPE, particle size up to ~500 µm,

C. As B. but UV-aged using an Atlas SUNTEST XLS+ (wavelength range: 300–400 nm; temperature: ~65°C; UV intensity: ~75 W/m2) for 14 days, which corresponds to around 42 days of sun irradiation.

Test design:

- Concentration: 0, 0.1, 1, 10, 100, 1000 mg/kg dry weight (dw)
- Exposure duration: 56 days

Endpoints:

- Nitrogen transformation according to OECD Test Guideline 216.
- Microbial community structure: High-throughput 16S rRNA gene amplicon sequencing.

Data analysis:

- Assessment of microbial composition and diversity.
- Concentration–response modelling to identify affected taxa.
- Derivation of benchmark concentrations (BMC) on different taxonomic levels.

Results

- Aging of LDPE particles induced measurable effects on nitrogen transformation, whereas non-aged particles showed no functional impact within the tested concentration range (Table 1).
- Family-level composition remained visually similar across test items and concentrations and did not show a clear shift of dominant taxa (exemplary Figure 1).

Table 1: Results of the nitrogen transformation test and number of BMC values identified for each LDPE treatment and taxonomic level FDR = 0.1, Benchmark response = 1 SD

	Nitrogen transformation test	Number of BMC values					
		Phylum	Class	Order	Family	Genus	Species
Aged	EC_{50} = 156 mg/kg dw	5	2	1	1	0	0
Fragmented	NOEC ≥ 1000 mg/kg dw	7	11	22	46	32	7
Manufactured	NOEC ≥ 1000 mg/kg dw	9	7	6	10	1	1

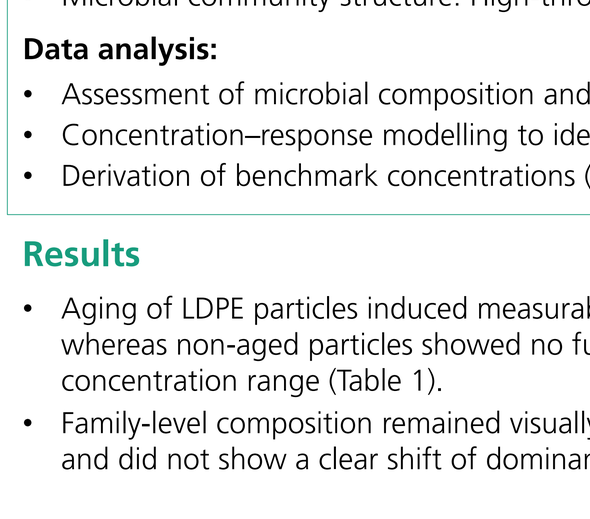

Figure 1: Family-level community composition in manufactured LDPE. Displaying the 25 most prevalent families, with each biological replicate shown separately.

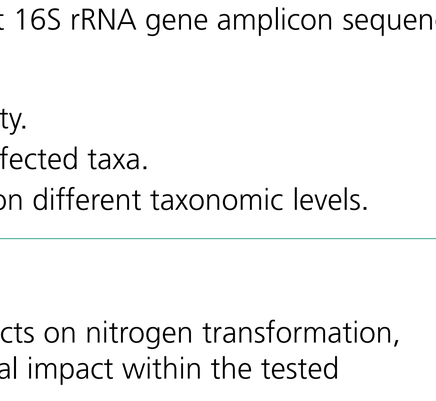

Figure 2: Alpha diversity index on family level. Mean values of biological replicates with standard deviation as error bar. P-values indicate within-treatment Kruskal-Wallis tests across concentrations.

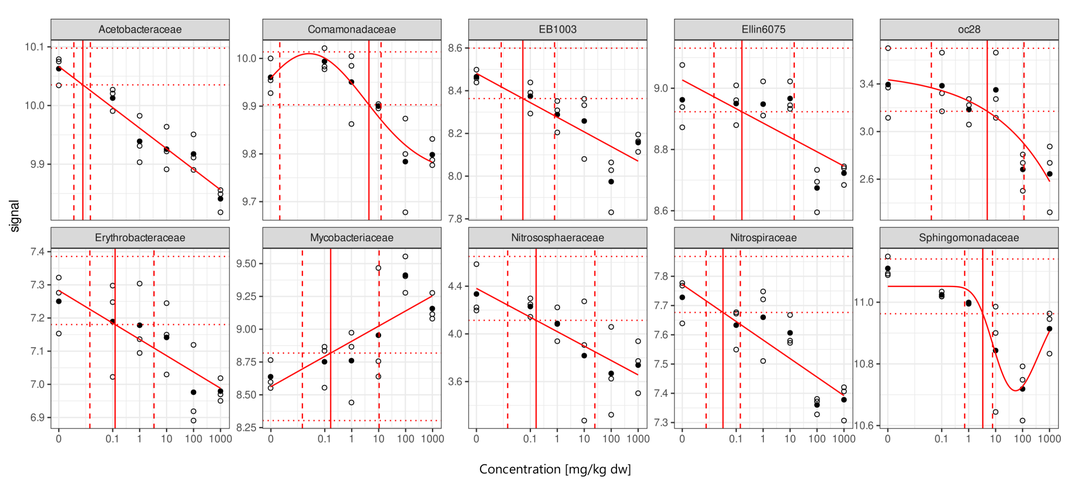

Figure 3: Example concentration-response curves for family-level manufactured LDPE. Open circles denote individual replicate data points, whereas filled circles represent the mean values. The best-fit model was used to estimate the benchmark concentration (shown as a solid vertical line with the 95% confidence interval indicated by dashed lines), defined as 1SD from the control level (marked by the horizontal dotted line). Note: Truncated y-axes are used to visualize small effect sizes; apparent differences may represent only minor deviations from control levels.

- Fragmented LDPE at 1000 mg/kg dw led to a notable reduction in Shannon diversity at the family level (Figure 2).
- Concentration–response modelling was more sensitive in detecting changes at different taxonomic levels (Figure 3).
- The highest count of benchmark concentrations (BMC) was detected at the family level, while aged samples produced only a few valid BMCs (Table 2).
- BMCs density distribution-based scores (10th percentile and 1st peak) for manufactured LDPE were slightly lower than fragmented LDPE; for aged LDPE, no density distribution could be established.
- Overall, BMC-derived scores were lower than the functional endpoint and alpha diversity. This was probably due to a low response threshold.

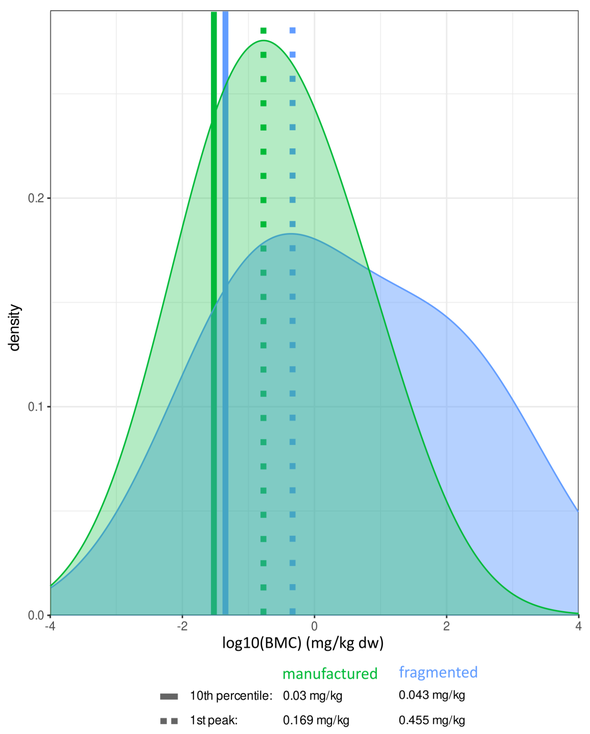

Figure 4: Density distributions of BMC values for fragmented and manufactured LDPE.

Conclusion:

- The contrasting outcomes highlight the value of combining functional soil tests with metabarcoding-based concentration–response modelling.
- Concentration-response modelling provided lower taxon-related changes than functional endpoints or diversity indices. However, BMCs were often associated with very small (e.g., 1%) differences from controls.
- The distribution of taxon-level BMC is closely related to an SSD concept.
- However, this dataset is not sufficient to define a robust regulatory threshold because results rely on rarefied read counts rather than absolute abundances.
- A point of departure-like quantitative metabarcoding endpoint would require absolute scaling (spike-ins, qPCR normalization, or comparable approaches) and stronger cross-study calibration.
- Until then, molecular readouts should be used as complementary evidence alongside established functional endpoints, not as an independent regulatory standard.

Contact:
Dr. Cecilia Díaz
Fraunhofer IME
Phone: +49 2972 302 138
Mail: cecilia.andrea.diaz.navarrete@ime.fraunhofer.de

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