Detecting Low Abundance Plastic Degrading Soil Microbes through Response Ratio Analysis and DNA Stable Isotope Probing

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Introduction

- Some soil microbes utilize plastic as a carbon source despite the inefficiency of this strategy[1, 2].
- Detecting key soil microbes that increase significantly in abundance under soil microplastic pollution (potential degraders) may be challenging with DNA stable isotope probing (SIP) because these microbes are likely to be relatively slow growing.

QUESTIONS

1) Is Response Ratio Analysis effective for determining taxa-level changes in abundance in agricultural soil with low density polyethylene (LDPE) microplastic addition?
2) How do known plastic-degrading soil bacteria respond to higher concentrations of microplastic contamination?

2024 URC Poster: "Year 1" recap

Utilized ^{18}O DNA SIP to observe the active soil microbial community composition under plastic pollution conditions.

Follow the QR code for details on last year's poster →

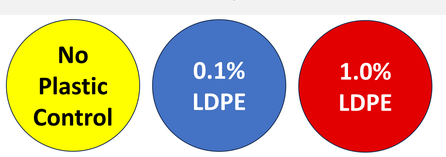


Figure 1: Plastic treatments used in 8-week soil incubation.

Indications of insufficient ^{18}O incorporation led to inconclusive DNA SIP results. Pursued additional analyses:

2025 "Year 2": Methods

Completed a response ratio analysis to determine which specific taxa changed in abundance between plastic and control treatments.

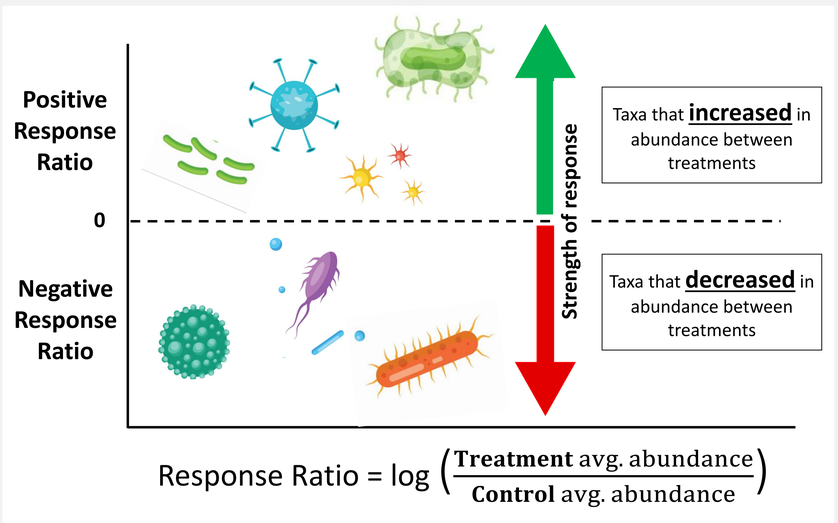


Figure 2: Response ratio analysis diagram. Response ratios above 1 indicate that taxa increased significantly in abundance in the given treatment comparison.

Results/Discussion

Taxa that increased across all plastic additions were primarily known plastic degraders or taxa known to increase in abundance with plastic addition.

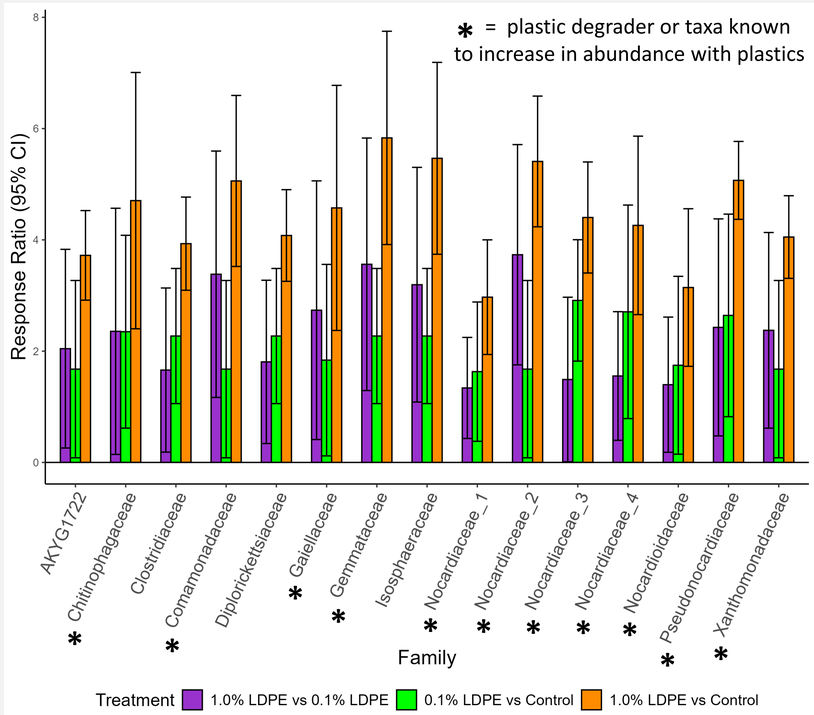
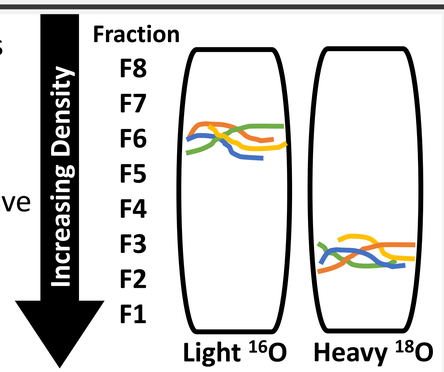


Figure 3: Taxa that displayed significant (95% CI) response ratios between plastic treatments and controls across all treatment comparisons. Each x-axis Family category represents one taxa (ASV).

Plastic degrading bacteria had the greatest abundance increase in soils incubated with the highest microplastic concentration.

In DNA SIP, active microbes will incorporate the ^{18}O isotope into their DNA, become heavier, and shift into heavier fractions relative to the ^{16}O treatment.

Figure 4: Isotope incorporation in active microbes during DNA SIP.



Active, slow-growing plastic degraders had minimal ^{18}O isotope incorporation due to the limited incubation time (final 7-days) with the isotope.

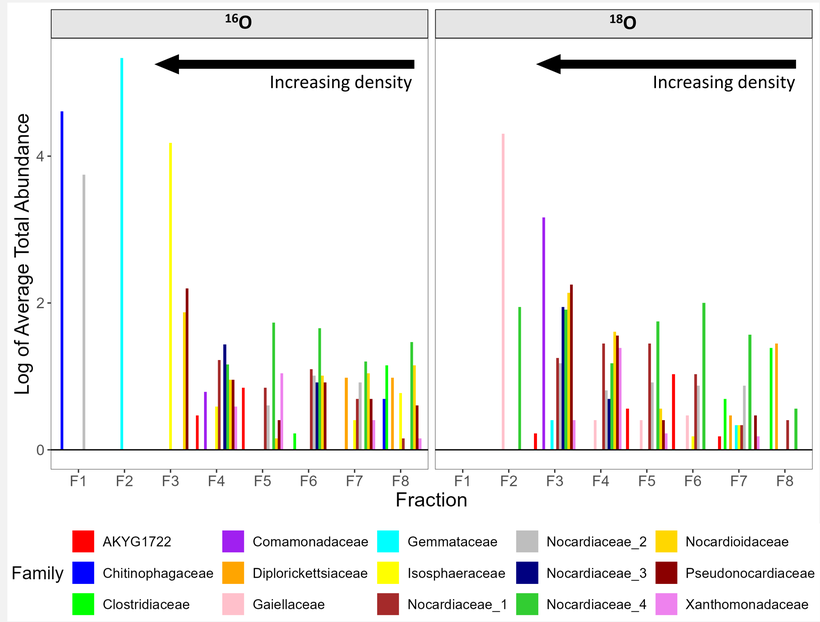


Figure 5: Log of average total abundance of 15 taxa that increased significantly in abundance with increasing microplastics (Fig. 4). Bars show taxa abundance in each DNA fraction from DNA SIP.

Conclusions

- Identified plastic-degrading taxa were slow-growing because plastic is not an energetically favorable source of carbon.
- **Longer isotope incubation is needed during DNA SIP to allow sufficient isotope incorporation in active, slow-growing taxa, such as plastic degraders.**
- Under pollution exposure, abundance changes in particular taxa may be important but can be hard to detect amongst the entire community. Response ratios should be utilized to detect taxa that proliferate, which could have pollutant degradation potential.

Next Steps

- Assess the genomes of taxa that increased with plastics for shared genes that may be key to plastic degradation.
- Run incubations with variable ^{18}O isotope incorporation times to assess how long pollutant-stressed, active microbes must be exposed to isotopes for proper DNA incorporation.

Acknowledgements & References

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1. Cao, J. et al. (2023). Agricultural soil plastic as a hidden carbon source stimulates microbial activity and increases carbon dioxide emissions. *Resources, Conservation and Recycling*, *198*, 107151.
2. Bai, X. et al. (2024). Direct evidence for selective microbial enrichment with plastic degradation potential in the plastisphere. *Science of The Total Environment*, *954*, 176576.