Soil and sludge exposures induce measurable morphology and stable isotopic changes in plastics

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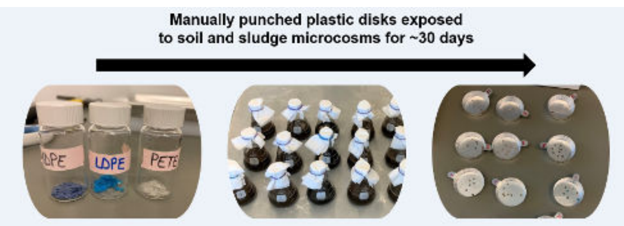
Introduction

Nearly 75% of produced plastic in the world is considered mismanaged waste and is expected to impact the environment. Terrestrial systems are estimated to receive as much or more plastic waste as aquatic systems, however, soils are understudied in comparison. The fate of microplastics in soil is currently debated but they are presumed to impact human and animal health if ingested, damage plants, and alter soil microbial communities. More recent studies have indicated that the presence of plastics in soil may affect the ecosystem cycling of carbon.

We evaluated:

- The degradation of low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polyethylene terephthalate (PETE) using controlled soil and sludge microcosm experiments.
- Plastics were analyzed using a combination of scanning electron microscopy (SEM) and Isotope Ratio Mass Spectrometry (IRMS) techniques.

Materials and Methods

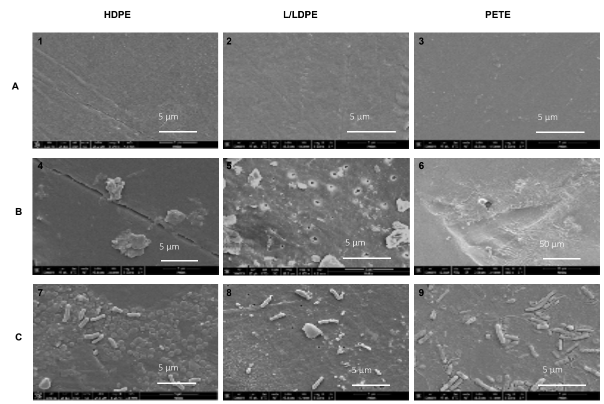


- Disks were punched from each plastic stock material; ~3.4 mm Dia. to match microplastic size criteria.
- Microcosms contained the plastic disks and a digested sludge, a "fresh" soil, or an "aged" soil and mechanically agitated at room temperature for a 32 day period.

Sample Preparations

- **Morphology imaging or ^{13}C IRMS analysis:** samples were removed of soil organic matter with 30% H2O2, a mild oxidizer (12 hrs.).
- **Biofilm and microbial attachment imaging:** samples were chemically fixed using 2.5% glutaraldehyde/phosphate buffer solution (pH 7, 2 hrs.); serially dehydrated using 70%, 90%, 100% ethanol (20 min. each); and treated with 100% hexamethyldisilazane (20 min.) as a final drying agent.
- SEM samples were mounted on stubs and coated with 15 um Au.
- ^{13}C IRMS samples were weighed (± 1 ug) into tin capsules prior to their stable isotopic analysis.

Scanning Electron Microscopy

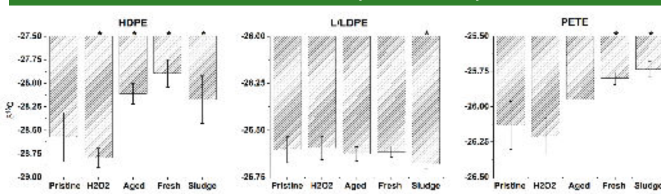


SEM images arranged by plastic polymer and feature; Row A – pristine plastic prior to exposure, Row B – post exposure surface morphology or physical transformation, Row C – post exposure biofilm and microbial cell attachment.

SEM images show:

- Physical and morphological changes occurred to all plastics.
- Attachment of biofilm and presence of microorganisms were observed, but mostly favored PETE and HDPE surfaces.
- Plastics may be degraded through physical, chemical, or biological processes occurring in soil.

$\delta^{13}C$ Stable Isotopic Composition



$\delta^{13}C$ values are a ratio of $^{13}C/^{12}C$ and measured using an Isotope Ratio Mass Spectrometer. Mean values for pristine plastics (n = 10), pristine plastics treated with 30% H2O2 (n = 6), and plastics exposed to fresh soil, aged soil, or digested sludge (n = 3, each) are shown. Significantly different sample measurements compared to the pristine plastic are denoted with asterisks.

Stable isotopic measurements show:

- Discernable positive isotopic enrichments for HDPE and PETE, and variability in values across soils and sludge. This suggests different underlying geochemical variables (pH, soil organic matter, etc.) or microbial communities may influence HDPE and PETE degradation.
- L/LDPE samples had negligible isotopic changes; suggesting its overall recalcitrance to environmental degradation.
- They may be an ideal tracer of plastic dynamics in soil.

Unknowns and Future Considerations

This research is preliminary and ongoing:

- We did not characterize geochemical parameters of the soils and sludge, such as pH or organic matter content. These parameters could be important factors driving the degradation of plastics in soil.
- Observed isotopic changes were not attributed to specific degradation pathways; these will be explored in upcoming work.
- Our continued efforts will incorporate liquid or gas chromatography isotope mass ratio spectrometry to help identify plastic degradation processes and 16S rRNA sequencing to characterize the soil microbiome.

Conclusions

The main objective of this study was to determine if plastics degrade differently given similar biotic and abiotic conditions.

Overall our results show:

- Plastics are physically altered and colonized by microbial communities in soil environments within a short 32-day period.
- Significant changes in stable isotopic values were observed for HDPE and PETE, but these cannot be assigned to any one degradation pathway.
- Degradation may be related to plastics' chemical/physical properties.
- Future isotopic characterizations should identify if plastics are degraded physically, chemically, or biologically, and may assist in broader investigations of plastics' fate in soil or how they affect soil ecosystem processes.

Funding and Acknowledgements

The authors are grateful to the City University of New York Summer Undergraduate Research Program, funded by the Alfred P. Sloan Foundation(G-2018-11286), and by a Professional Staff Congress CUNY research award (Cycle 52, Award# 64443-00 52). S. Cime is a grateful recipient of the Spring 2022 Pfizer/ACS Travel and Research Grant which allowed her to attend this meeting and present this poster.

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