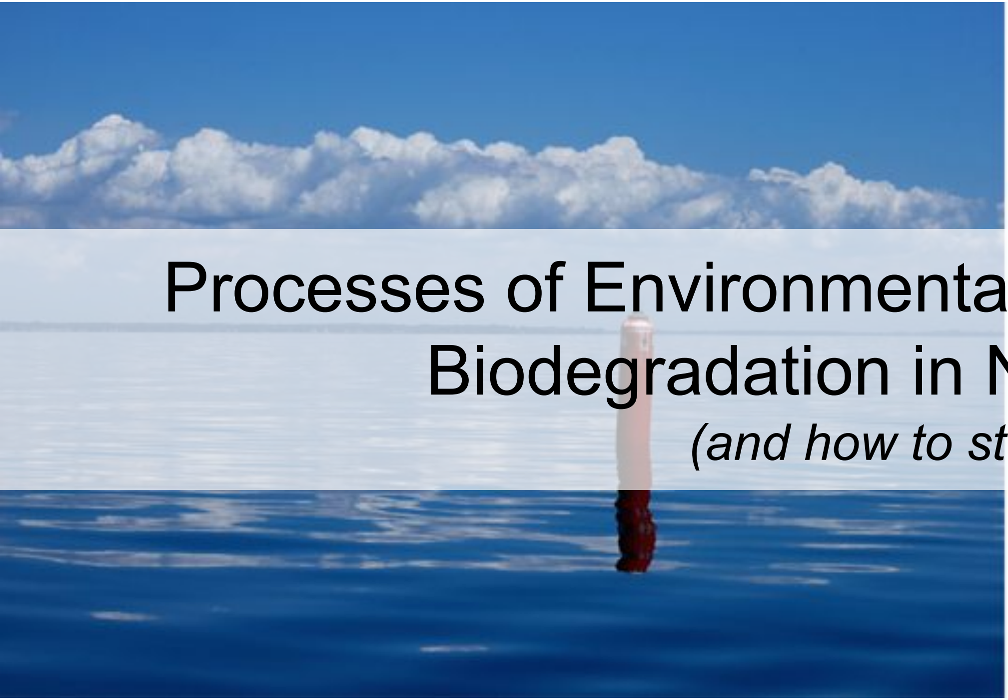
Microplastics Advance Research and Innovation Initiative (MARII) // Session: *Degradation Processes of Microplastics*
Seattle, Washington // June 12-14, 2023



Processes of Environmental Plastic Weathering and Biodegradation in Natural Systems

(and how to study them)

Dr. Melissa Duhaime Assistant Professor, Department of Ecology and Evolutionary Biology

Rachel Cable, Jessica Choi, Elizabeth Michaelson (UM EEB)
Zhan Chen, Shuqing Zhang, Ting Lin, Chengcheng Zhang (UM Chem)
Yuming Lai, Piyush Thakre, Cristina Serrat, Yujing Tan, Jing Hu, David M. Meunier (Dow)

Project Team

University of Michigan

Duhaime Lab

PI: Prof. Melissa Duhaime
Dr. Jessica Choi
Max Murray
Charles Luther Smith
Isabelle Montilla
Rachel Cable
Lizy Michaelson
Adi Mizrahi
Agniva Bhaumik
Cory Plotske

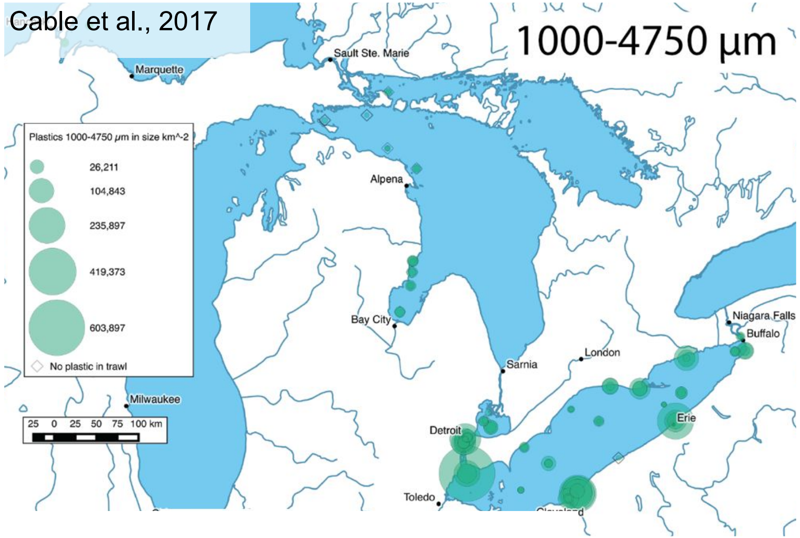
Chen Lab

Co-PI: Prof. Zhan Chen
Shuqing Zhang
Ting Lin
Dr. Chengcheng Zhang (Alumnus)

Dow Chemical Company

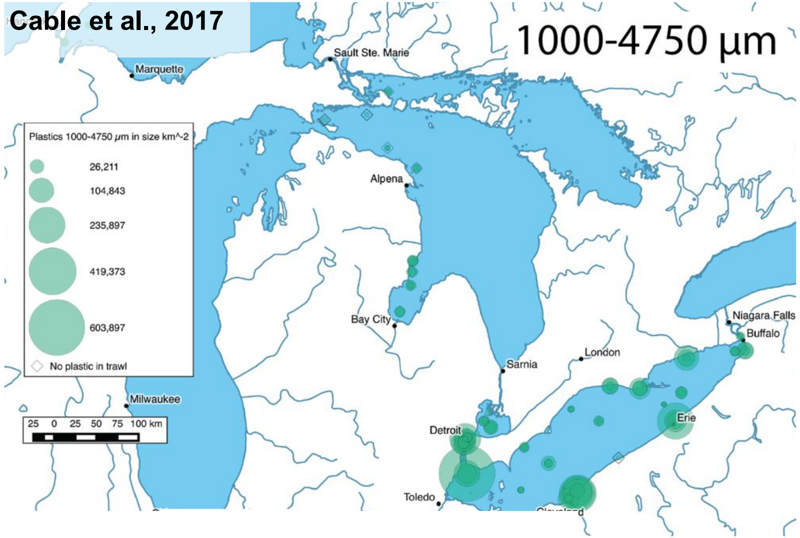
Dr. Yuming Lai
Dr. Piyush Thakre
Dr. Cristina Serrat
Dr. Yujing Tan
Dr. Jing Hu
Dr. David M. Meunier

Cable et al., 2017



Motivation

Urban coastal areas most at risk for microplastics pollution



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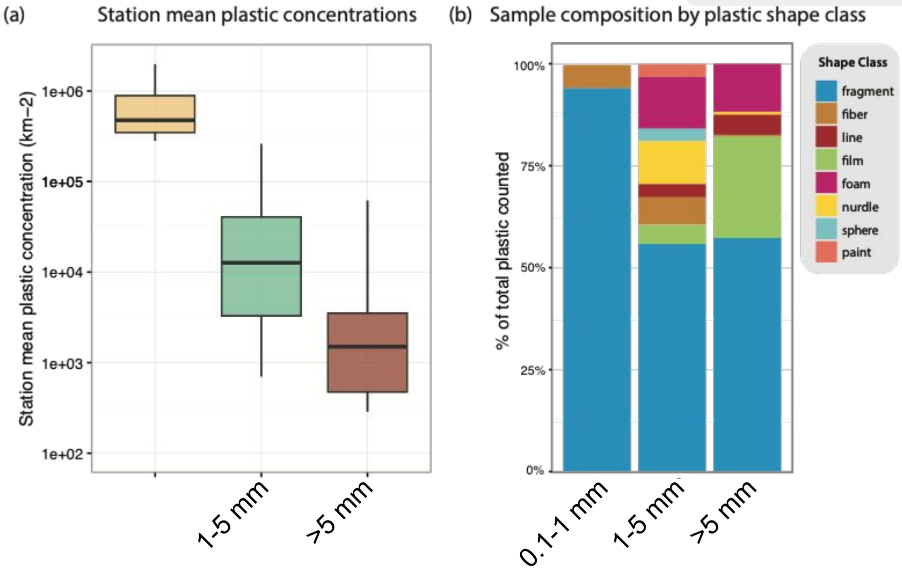

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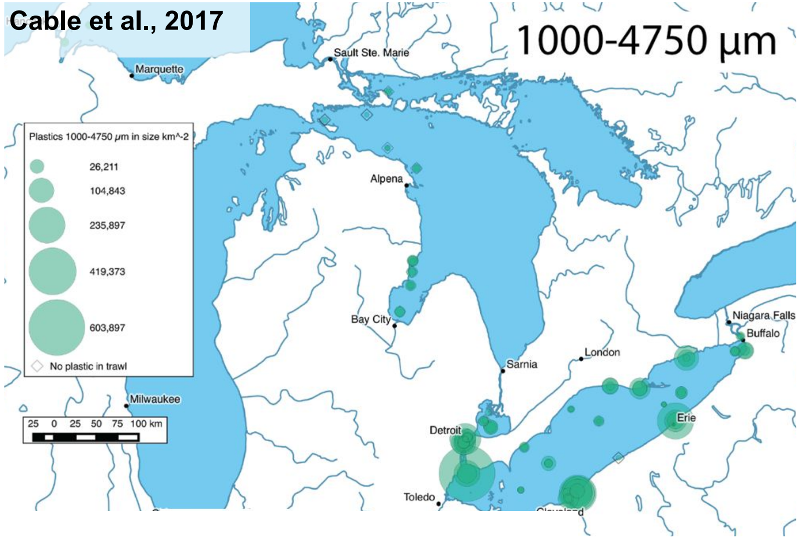

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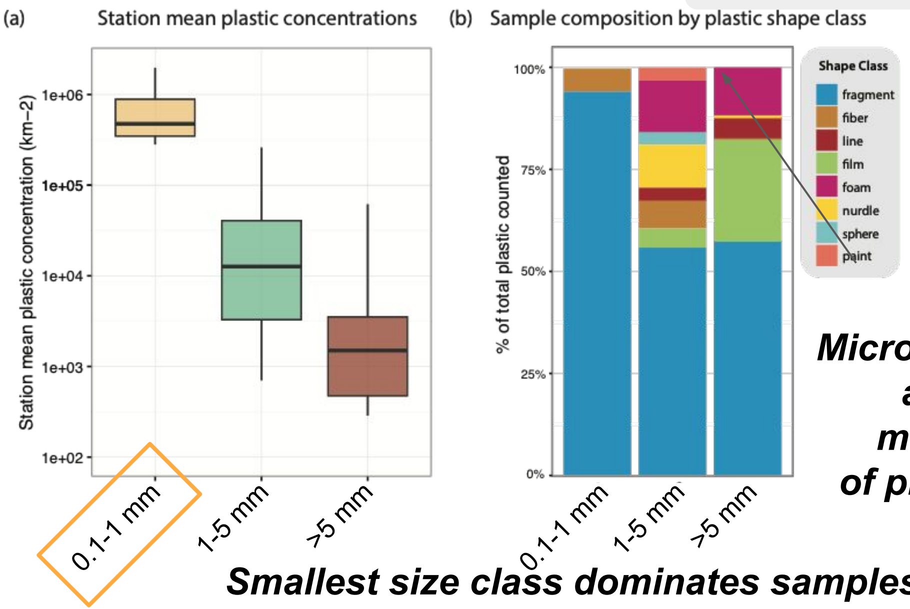
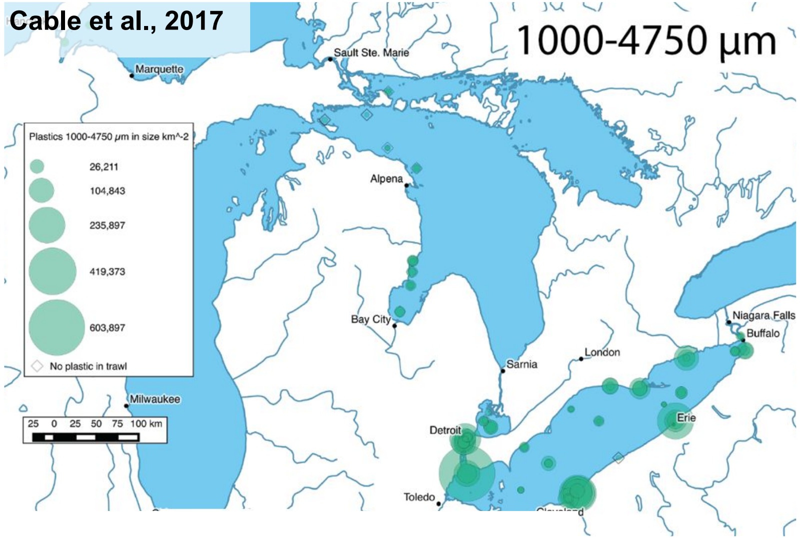


Microbeads are the minority of plastics

Smallest size class dominates samples

Motivation



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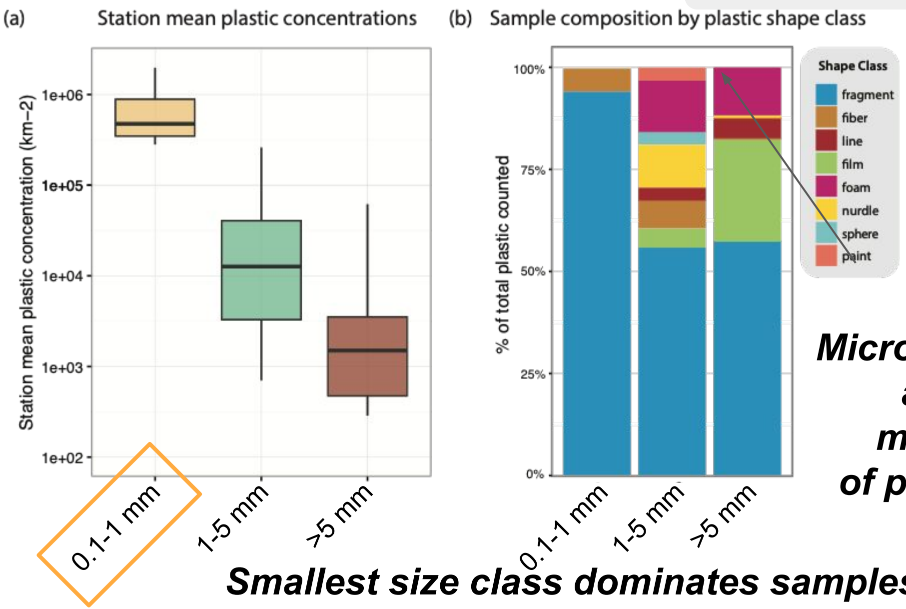

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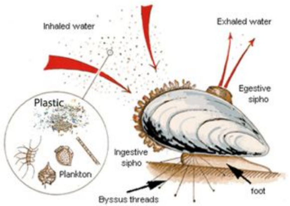
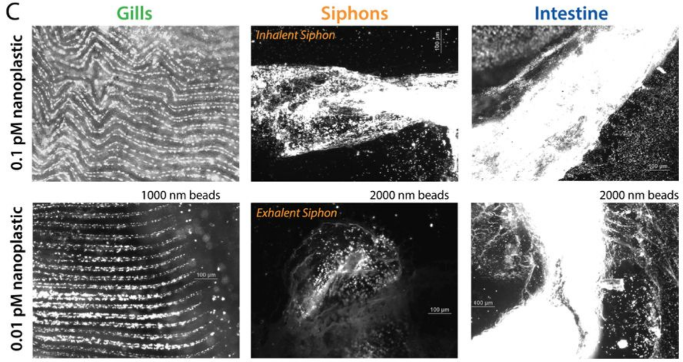
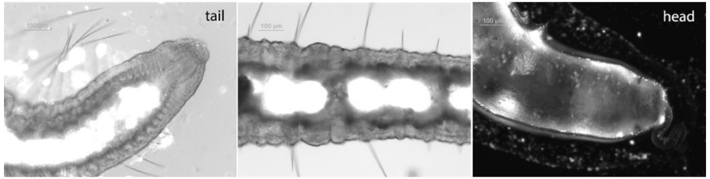




Merzel et al., 2020

Lake Michigan mussels

Great Lakes food web base ingests microplastics



Lake Michigan chironomid

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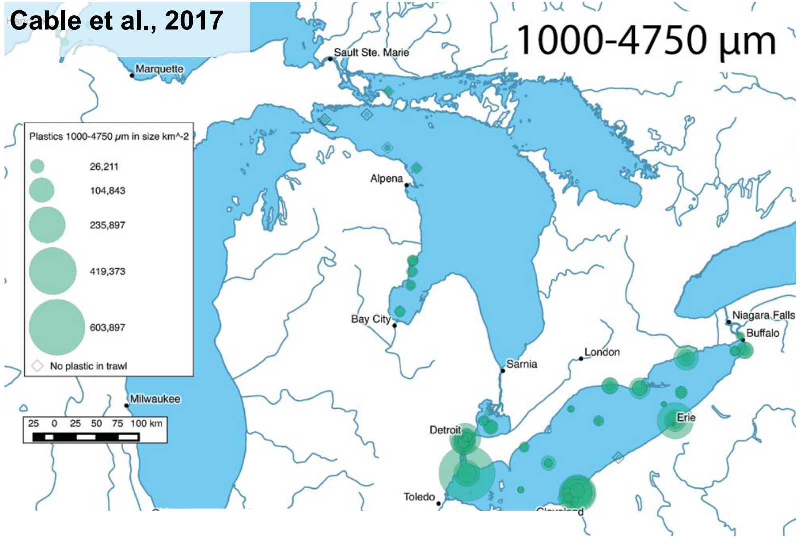

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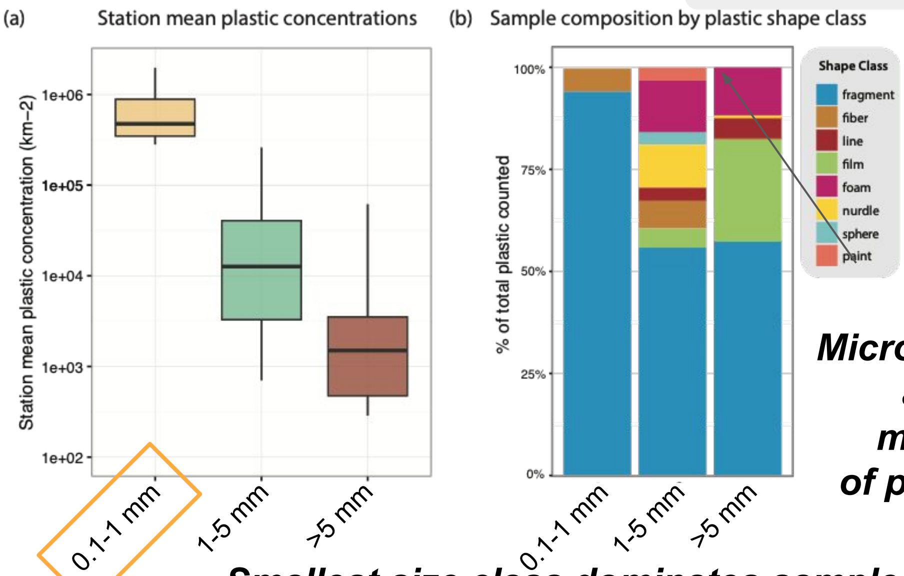

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*Smallest size class extremely important— need research to determine processes and rates of break down***

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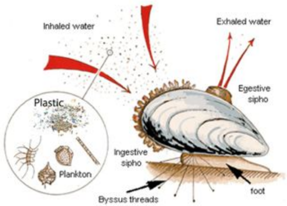

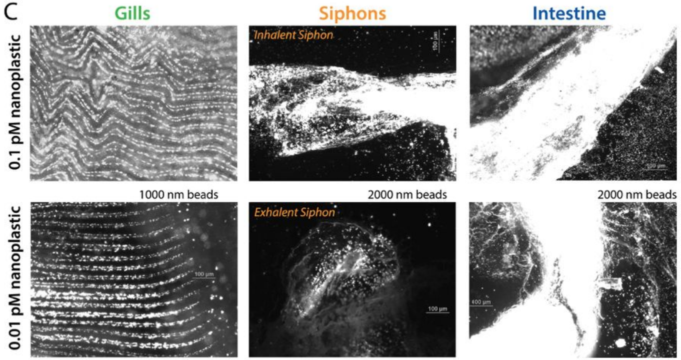

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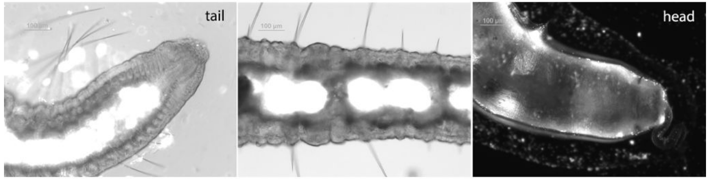
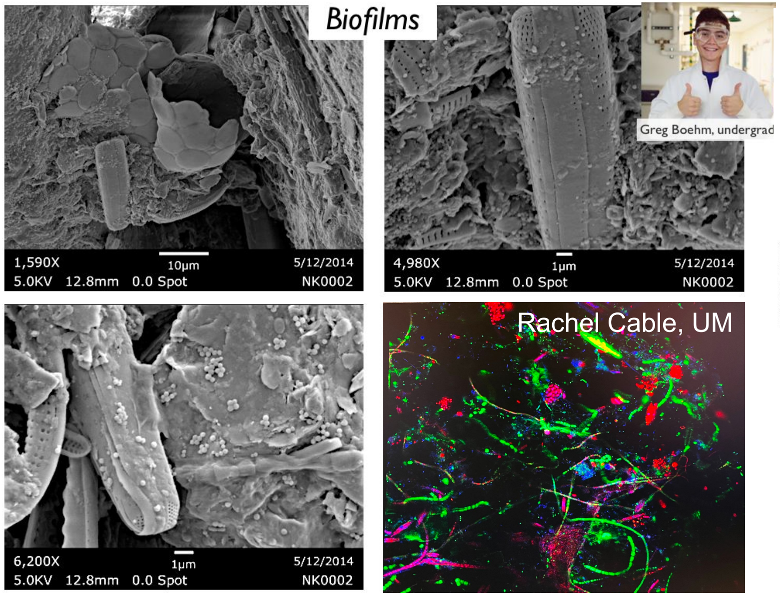
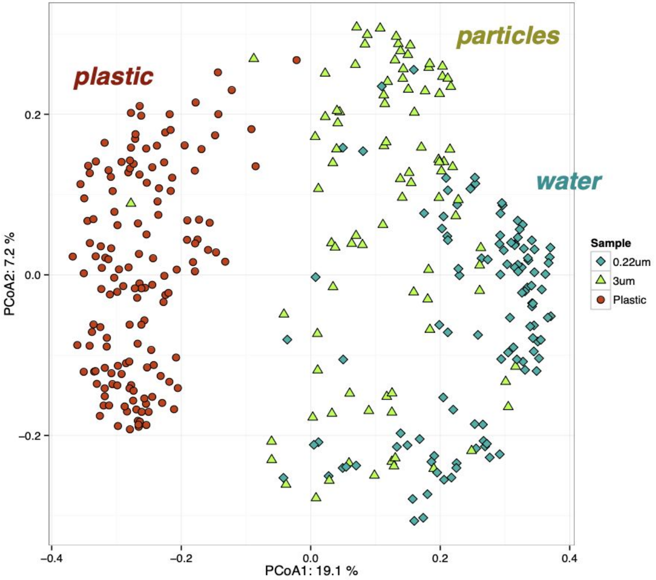


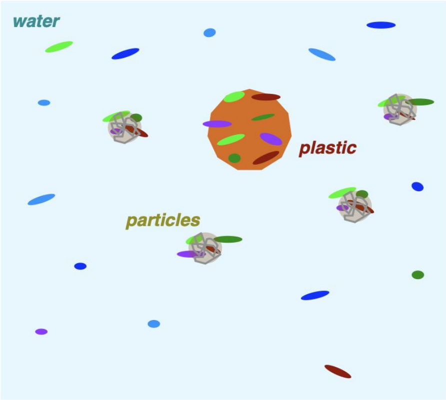
Lake Michigan chironomid

Environmental plastics harbor complex communities of plastic-specific microbes



Plastics teaming with microbial life



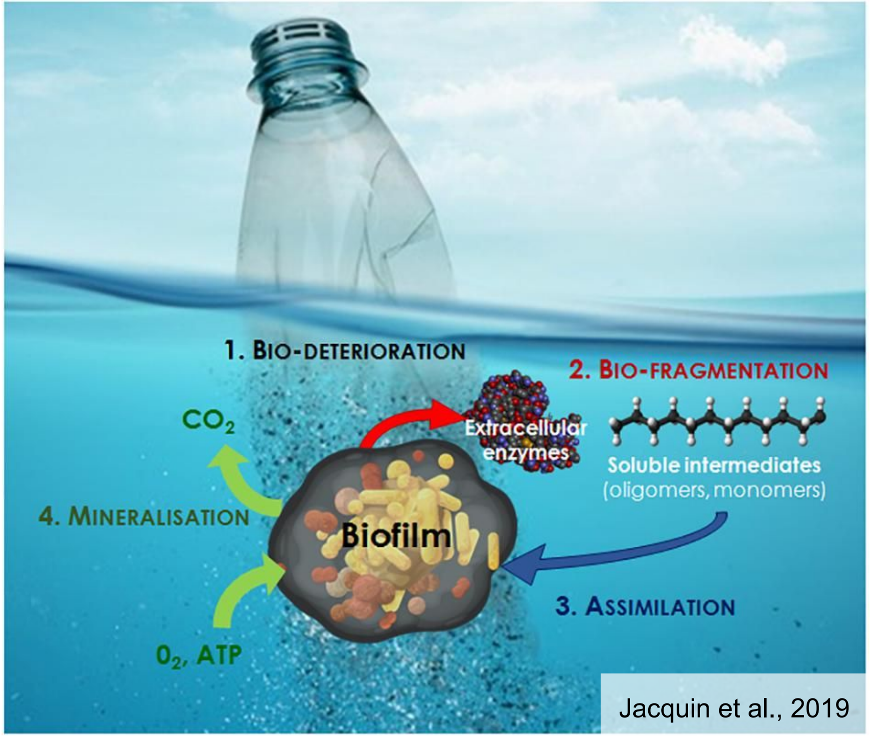


Great Lakes plastics harbor distinct microbes, potential pathogens, toxic bloom-forming algae…

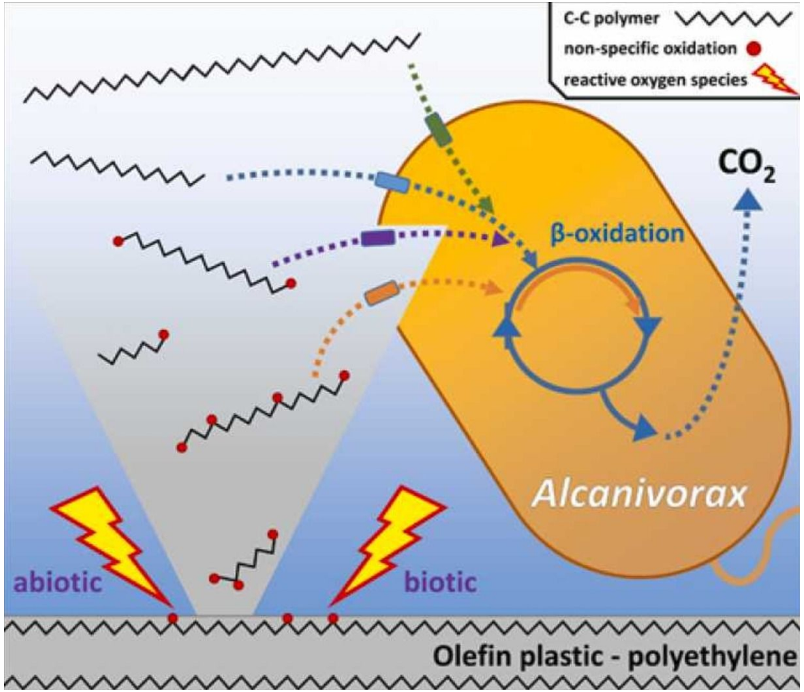
…and plastic-degrading microbes.

Microbial degradation of environmental plastics

degradation - progressive decrease in the bulk molar mass due to macromolecule cleavage; for polymers, this involves chain scission/cleavage due to chemical reactions (UV oxidation, enzymes)



Jacquin et al., 2019



Zadjelovic et al., 2022

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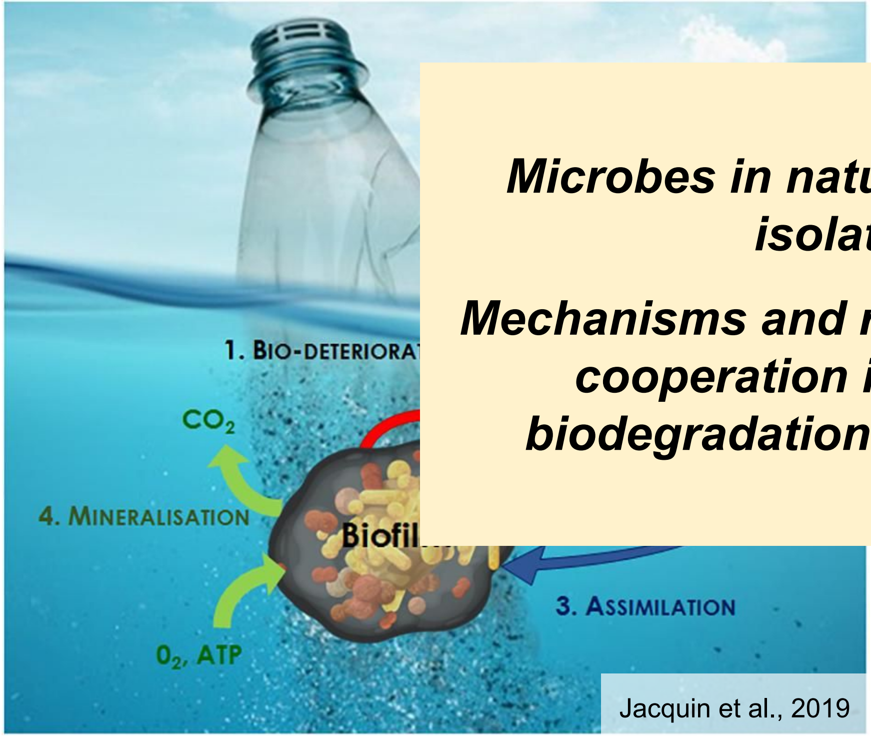
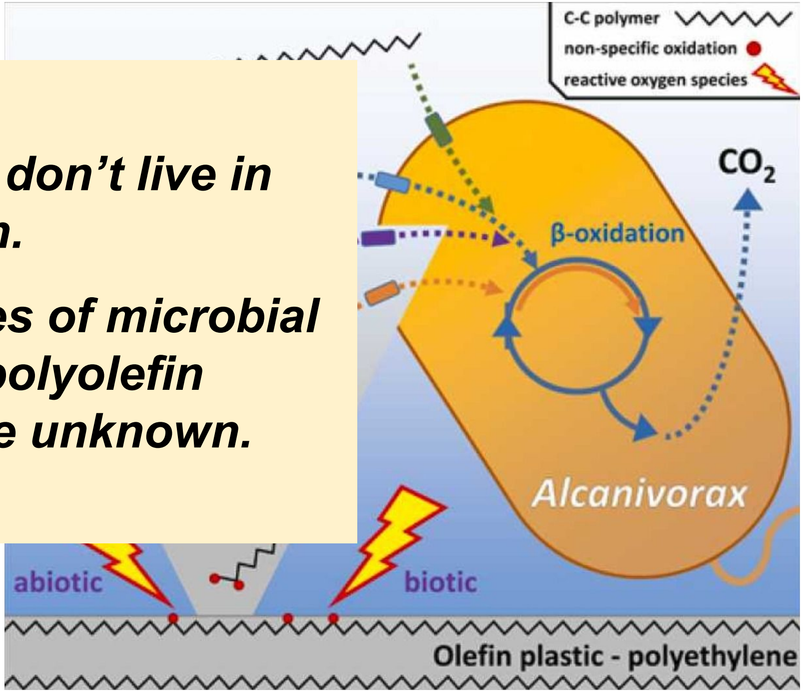


Jacquin et al., 2019

Microbes in nature don't live in isolation.

Mechanisms and rates of microbial cooperation in polyolefin biodegradation are unknown.



Zadjelovic et al., 2022

Project Goals

Better understand and predict environmental fate of plastics.

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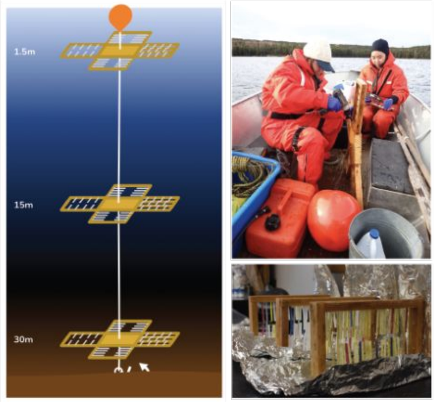
A fundamental tenet of environmental microbiology is that microbes work cooperatively in a collective community metabolism.

To fully understand bio-weathering potential and processes in the environment, study environmental microbial communities *in situ*: (1) *Which microbes persist in biofilms?* (2) *What is their functional potential?* (3) *What is their functional activity?*

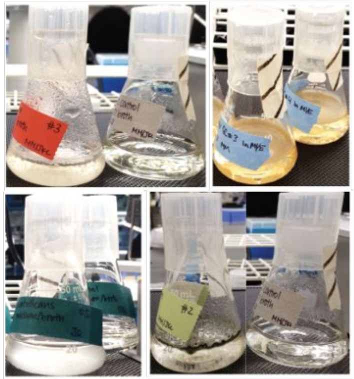
Approach

APPROACH 1
UV Accelerated Aging
(simulated weathering)

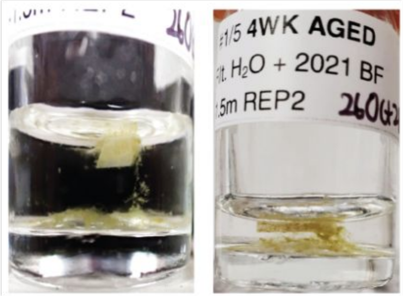
APPROACH 2
Field Deployments
(*in situ* weathering)

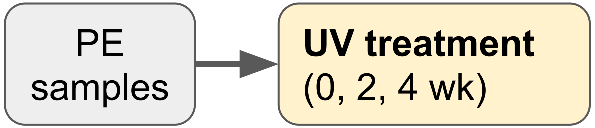


APPROACH 3
Microbial Isolates
(*in vitro* weathering)



APPROACH 4
Microbial Consortia
(*in vitro* weathering)





~15 polymer formulations

PE (UV stabilizers, TPS)

PLA, PHA

Rigid and film types

APPROACH 1
UV Accelerated Aging
(simulated weathering)

Analytical lab analysis
- FTIR
- Raman
- Nanoindentation

PE samples → **UV treatment** (0, 2, 4 wk)

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PE (UV stabilizers, TPS)
PLA, PHA
Rigid and film types

Analytical lab analysis
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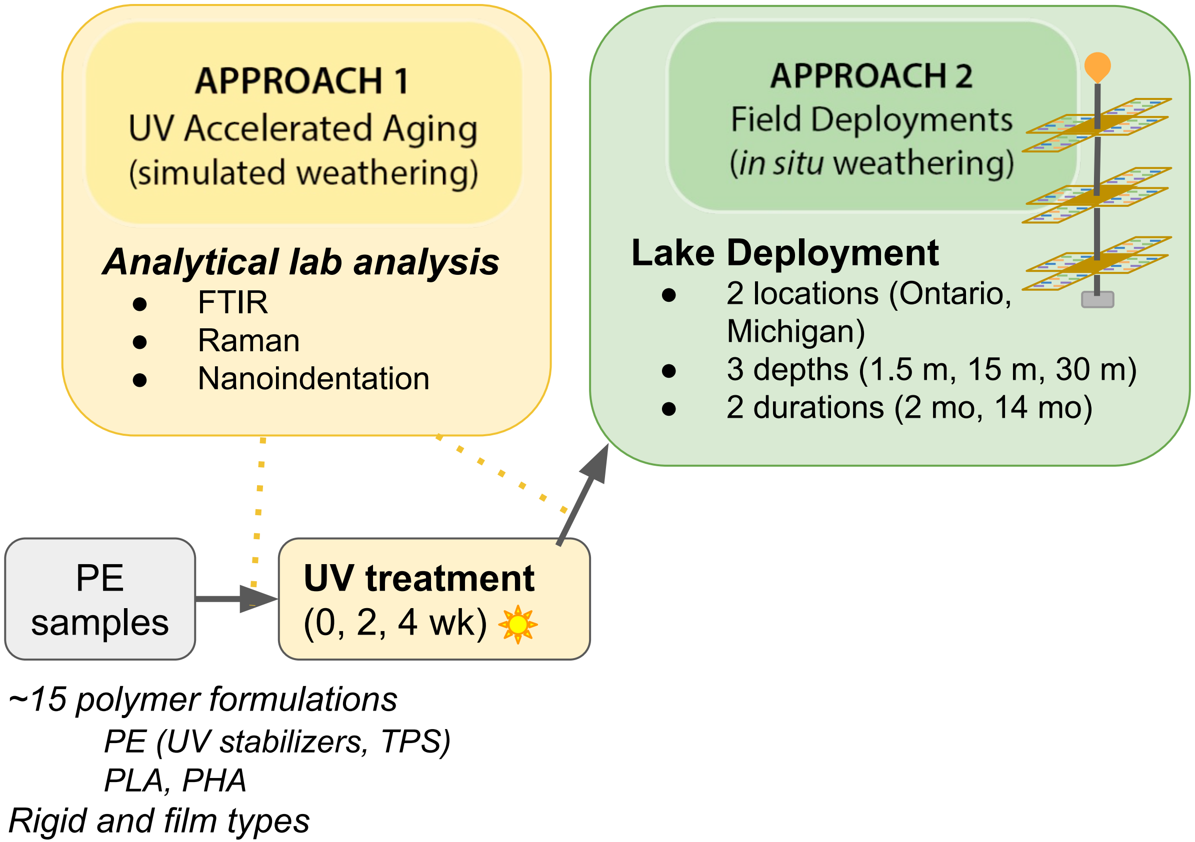
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Lake Deployment
2 locations (Ontario, Michigan)
3 depths (1.5 m, 15 m, 30 m)
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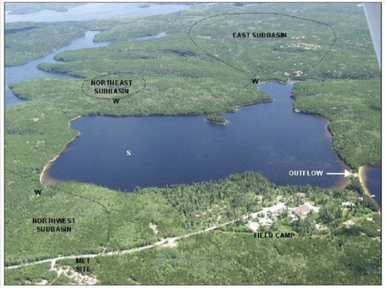
Depth profiles to study natural variability in two independent lake systems

APPROACH 2
Field Deployments
(*in situ* weathering)

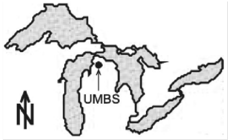
Field Site 1
ELA Lake 239
Ontario, Canada

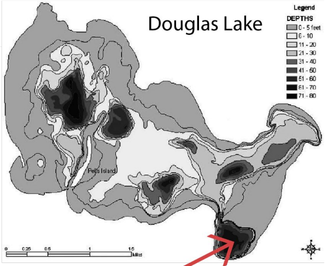
IISD Experimental Lakes Area



Field Site 2
Douglas Lake, UM Biological Station
Pellston, Michigan

UMBS





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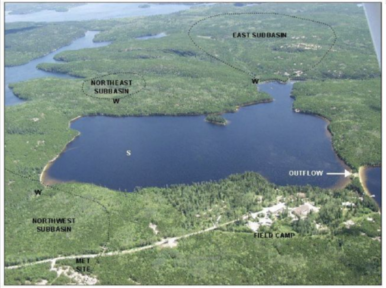

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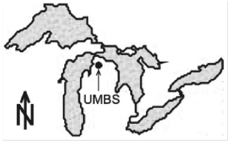
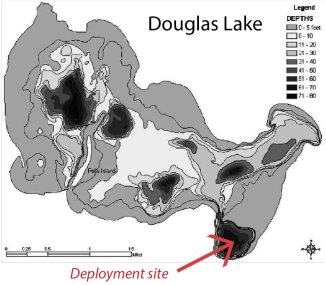
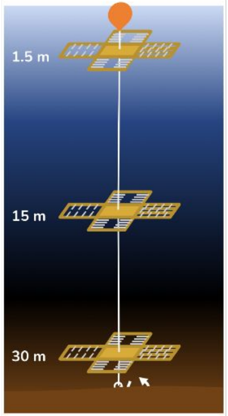
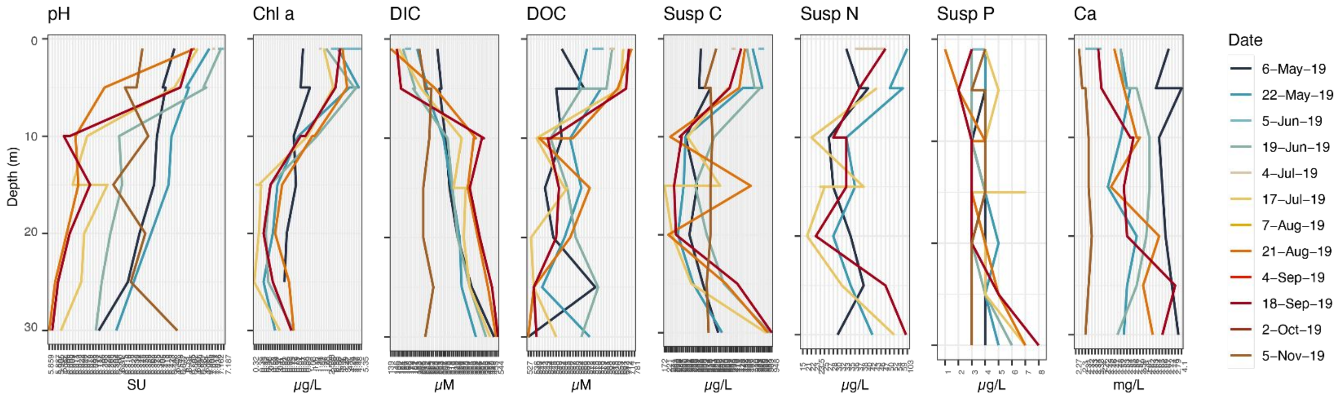






ELA Lake 239 Depth Profiles 2019



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APPROACH 2
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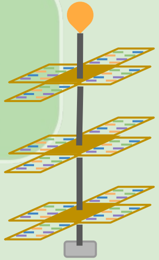

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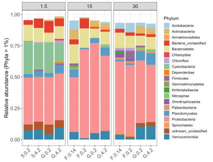
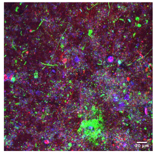


Microbial Community
Sequencing
Functional activity



Microscopy
Biofilm quantity and quality

Biological lab analysis

- Biofilm quantification
- Microbial identity (fungi, bacteria)
- Microbial functions (enzymes)

Analytical lab analysis

- FTIR
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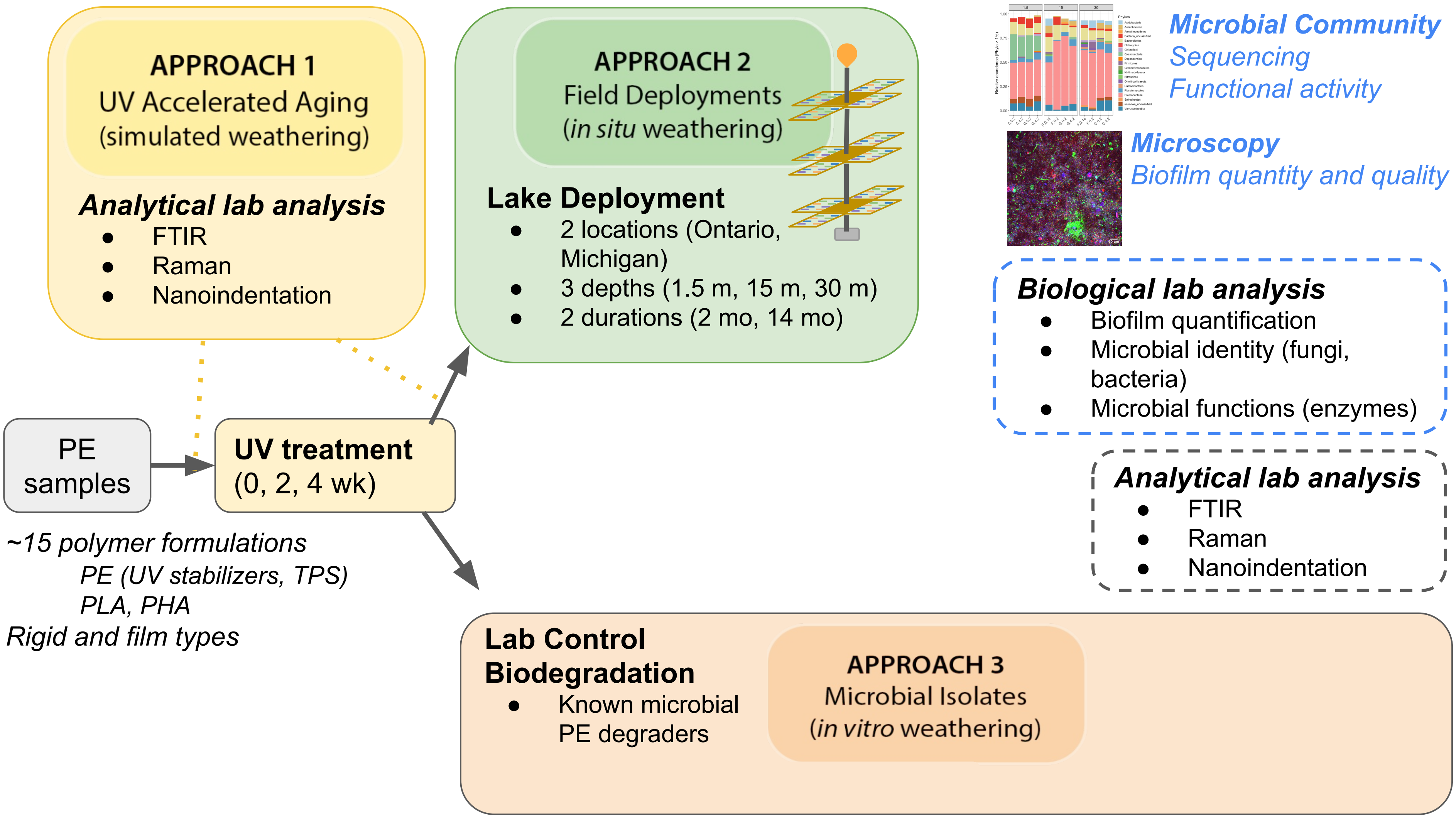
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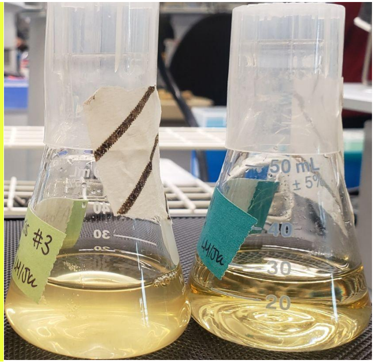
Lab Control Biodegradation
• Known microbial PE degraders
APPROACH 3
Microbial Isolates
(in vitro weathering)

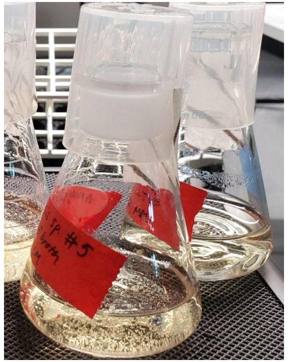
Over 100 described PE degraders in literature, 15 now in culture in our lab

APPROACH 3
Microbial Isolates
(*in vitro* weathering)

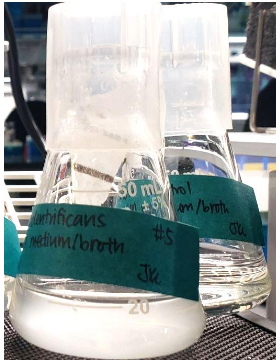
BACTERIA



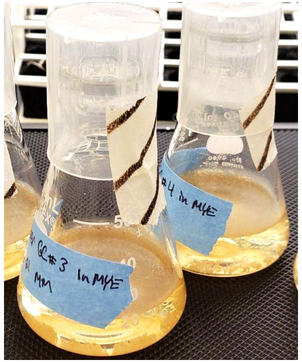
Alcanivorax borkumensis



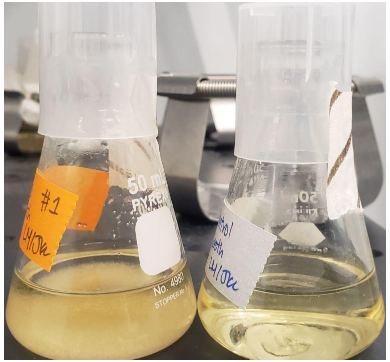
Amycolatopsis sp.



Virgibacillus halodenitrificans

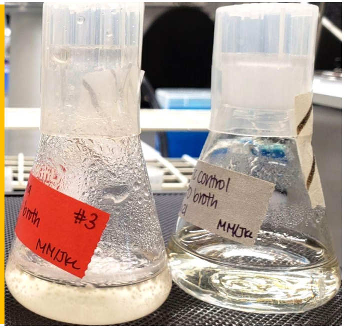


Rhodococcus ruber

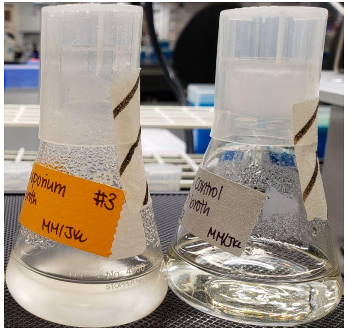


Streptomyces viridosporus

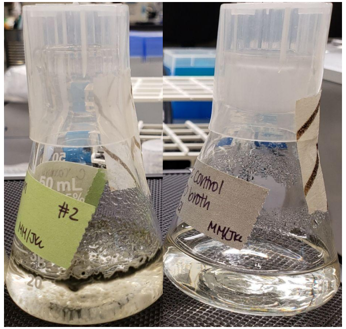
FUNGI



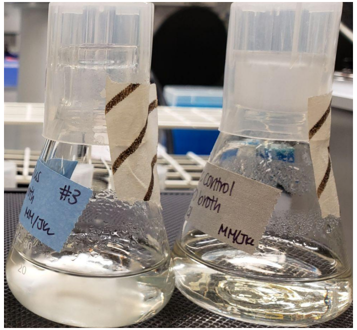
Mortierella alpina



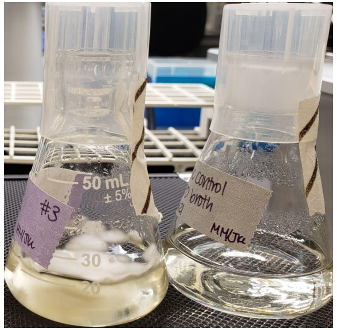
Phanerochaete chrysosporium



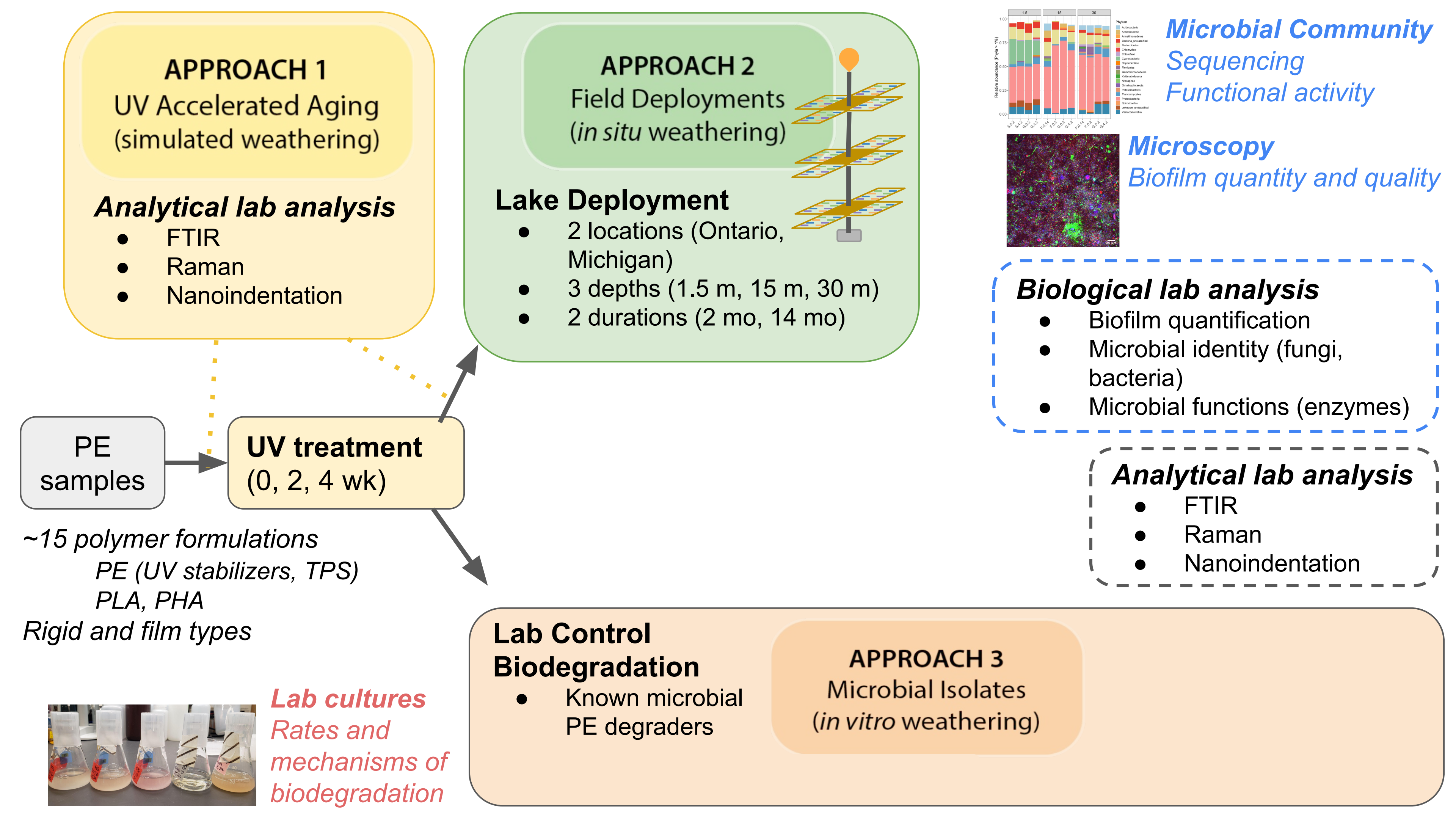
Cladosporium ramotenellum



Talaromyces pinophilus



Aspergillus brasiliensis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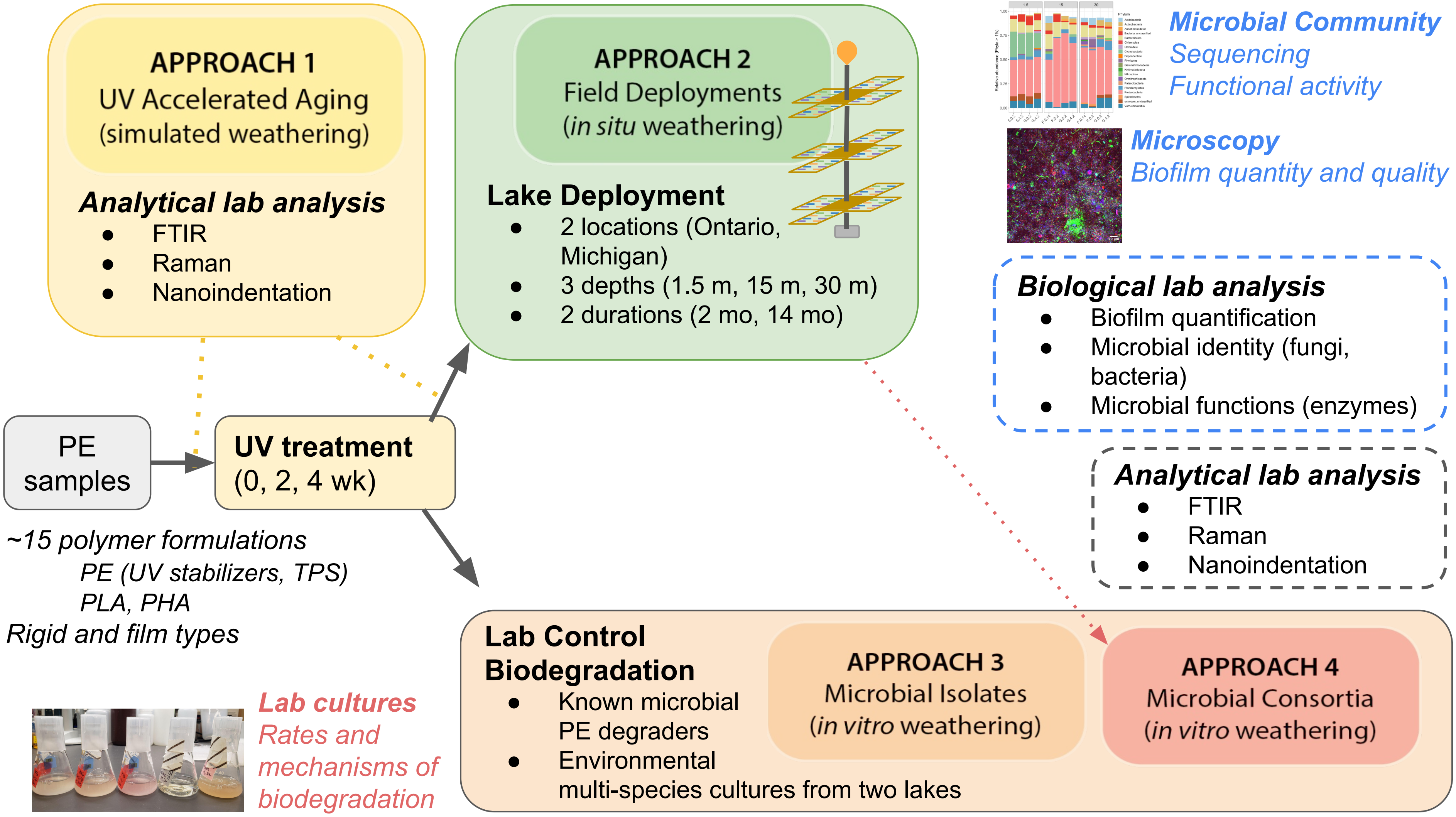
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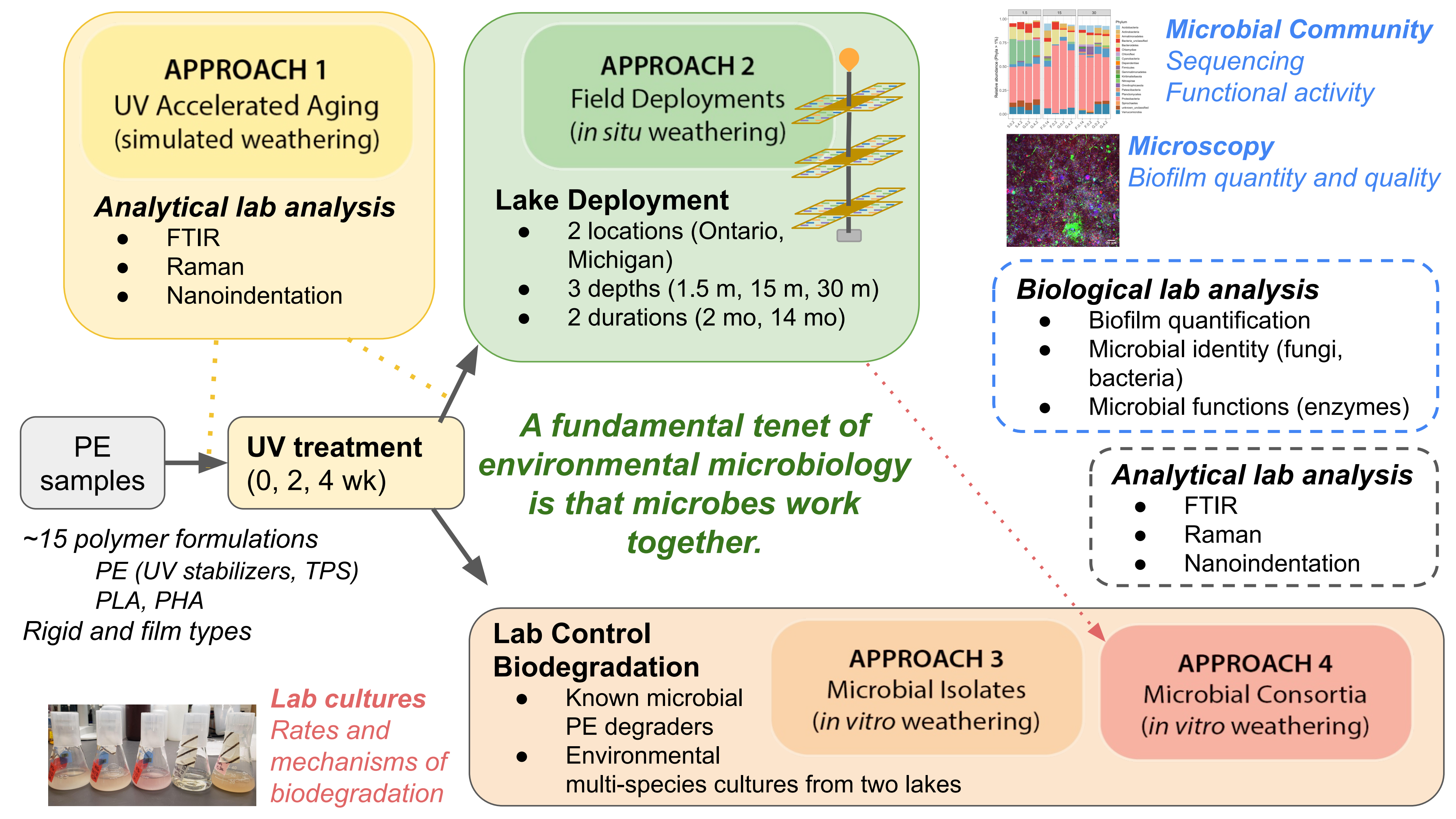
• Nanoindentation
APPROACH 2
Field Deployments
(in situ weathering)
Lake Deployment
• 2 locations (Ontario, Michigan)
• 3 depths (1.5 m, 15 m, 30 m)
• 2 durations (2 mo, 14 mo)
Microbial Community
Sequencing
Functional activity
Microscopy
Biofilm quantity and quality
Biological lab analysis
• Biofilm quantification
• Microbial identity (fungi, bacteria)
• Microbial functions (enzymes)
Analytical lab analysis
• FTIR
• Raman
• Nanoindentation
PE samples
UV treatment
(0, 2, 4 wk)
~15 polymer formulations
PE (UV stabilizers, TPS)
PLA, PHA
Rigid and film types
Lab Control Biodegradation
• Known microbial PE degraders
• Environmental multi-species cultures from two lakes
APPROACH 3
Microbial Isolates
(in vitro weathering)
APPROACH 4
Microbial Consortia
(in vitro weathering)
Lab cultures
Rates and mechanisms of biodegradation



APPROACH 1
UV Accelerated Aging
(simulated weathering)
Analytical lab analysis
• FTIR
• Raman
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APPROACH 2
Field Deployments
(in situ weathering)
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Analytical lab analysis
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APPROACH 1
UV Accelerated Aging
(simulated weathering)

APPROACH 2
Field Deployments
(*in situ* weathering)

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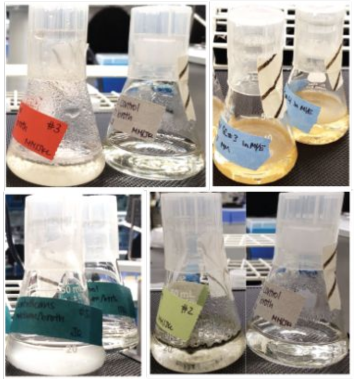
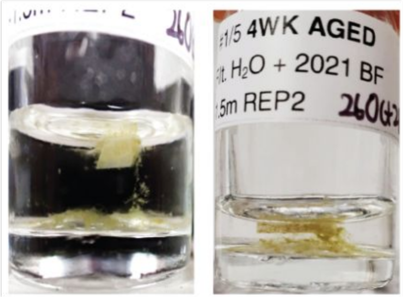
Microbial Isolates
(*in vitro* weathering)



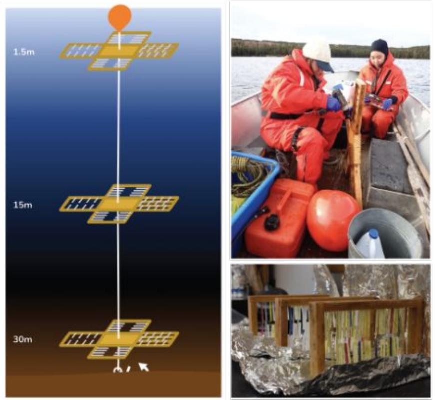
APPROACH 4
Microbial Consortia
(*in vitro* weathering)



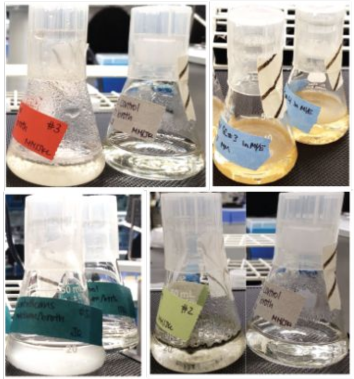
Results

APPROACH 1
UV Accelerated Aging
(simulated weathering)

APPROACH 2
Field Deployments
(*in situ* weathering)



APPROACH 3
Microbial Isolates
(*in vitro* weathering)



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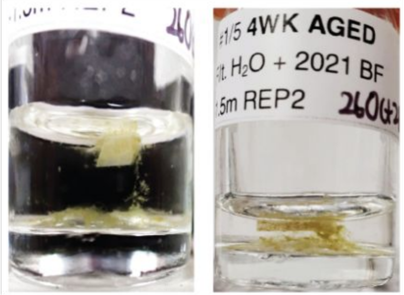

Investigation of polyethylene degradation with a combination of analytical techniques

Shuqing Zhang, Ting Lin, Chengcheng Zhang, Rachel Cable, Jessica Choi, Elizabeth Michaelson, Piyush Thakre, Cristina Serrat, Yujing Tan, Jing Hu, David M. Meunier, Yuming Lai, Melissa Duhaime, Zhan Chen

University of Michigan | Dow Chemical Company

Manuscript *in prep*

Results

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Field Deployments
(*in situ* weathering)

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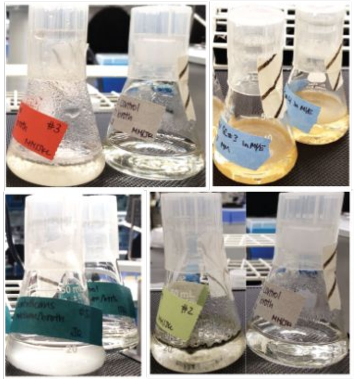

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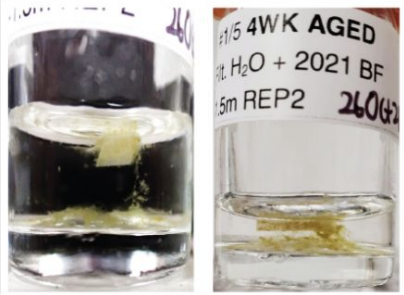
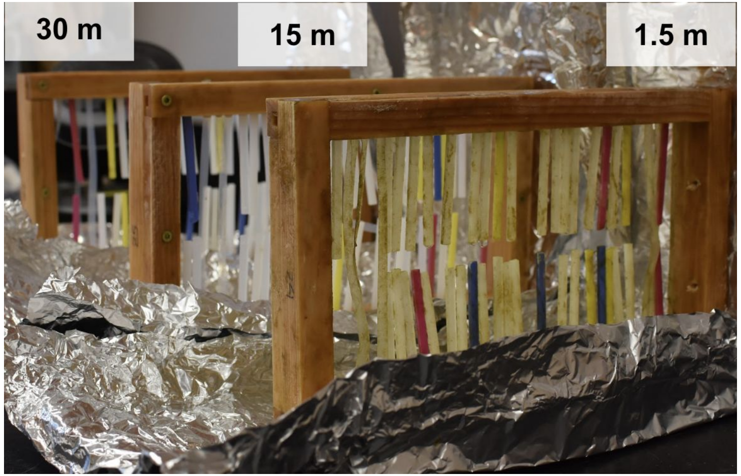
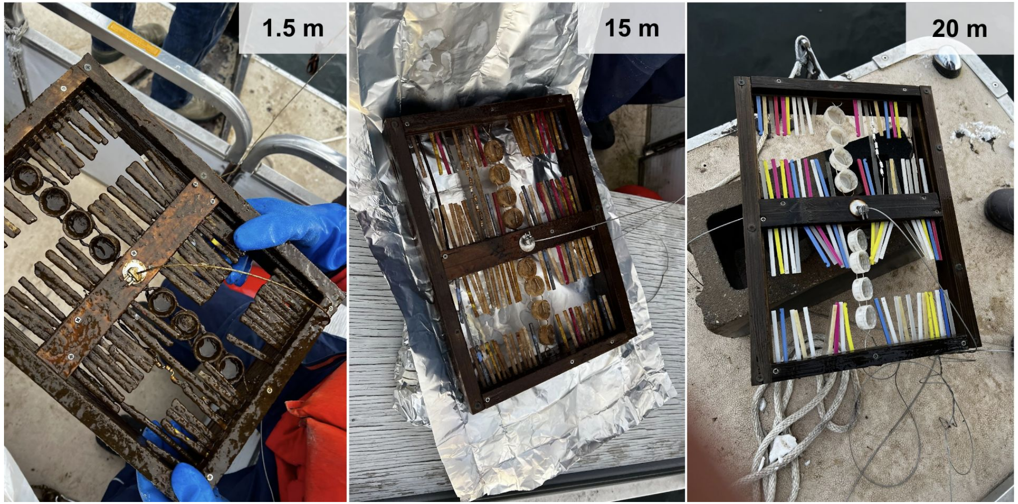


Biological lab analysis

- Biofilm quantification
- Microbial identity (fungi, bacteria)
- Microbial functions (enzymes)

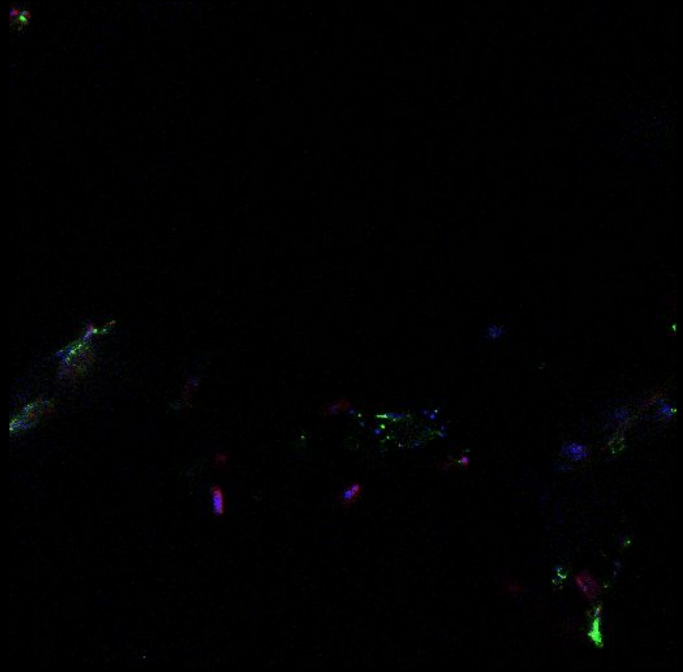
Biomass growth depends on depth





Confocal laser scanning microscopy (CLSM) provides spatial information on plastic biofilms

DNA (bacteria/ archaea)	Chitin (fungi)	Natural pigments (prim. prod.)



Rachel Cable, UM *2 month lake incubation, UMBS*

- We can image the biofilm along its entire cross-section, from the organisms closest to the plastic surface, to the organisms in contact with the environment

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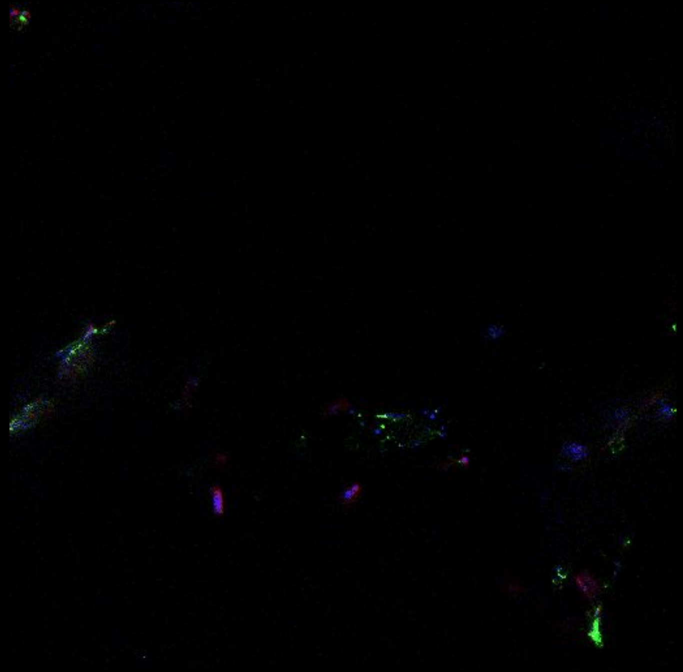

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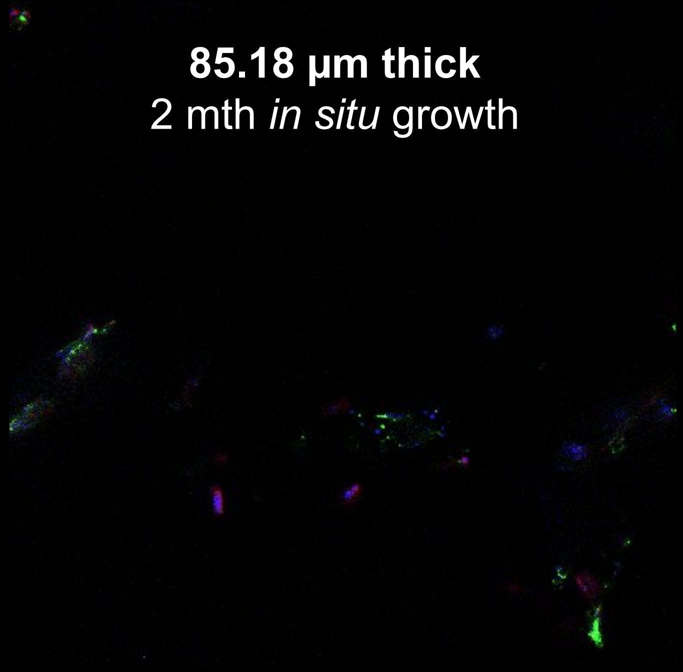

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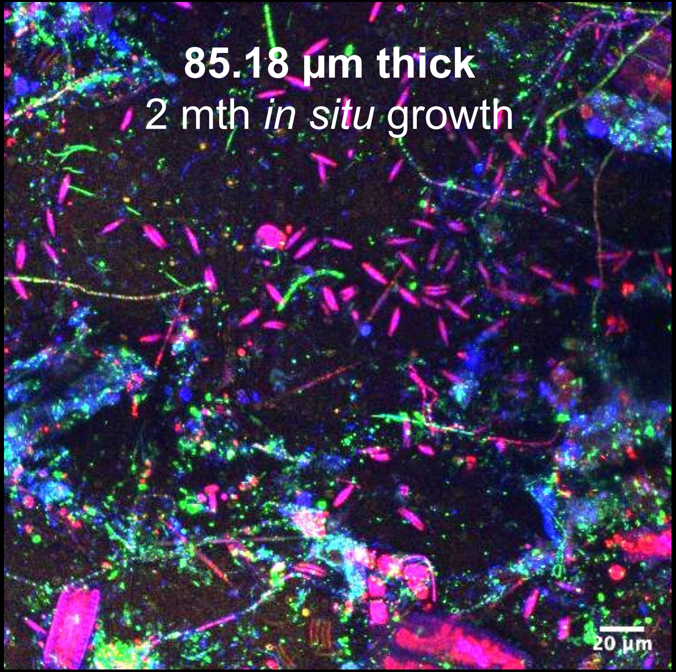

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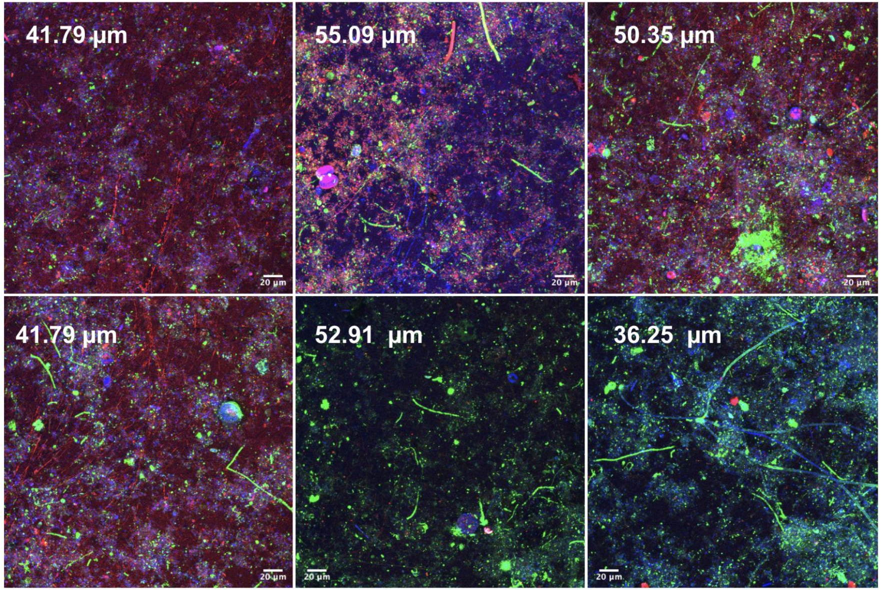
Water depth negatively correlates with biomass growth

APPROACH 2
Field Deployments
(*in situ* weathering)

DNA (bacteria/ archaea)	Chitin (fungi)	Natural pigments (prim. prod.)



Rachel Cable, UM

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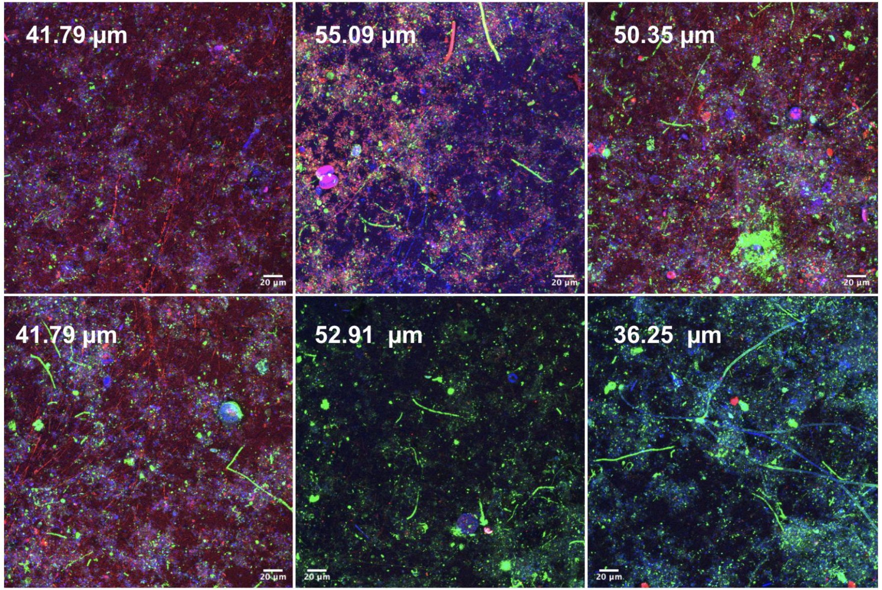

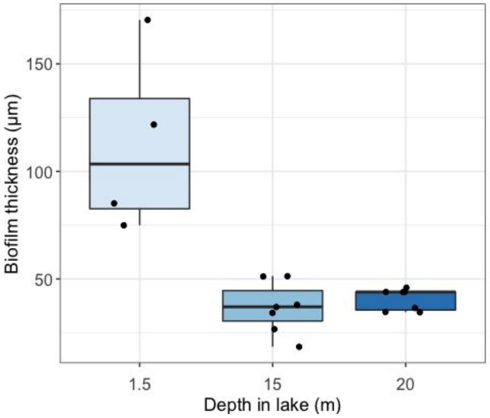

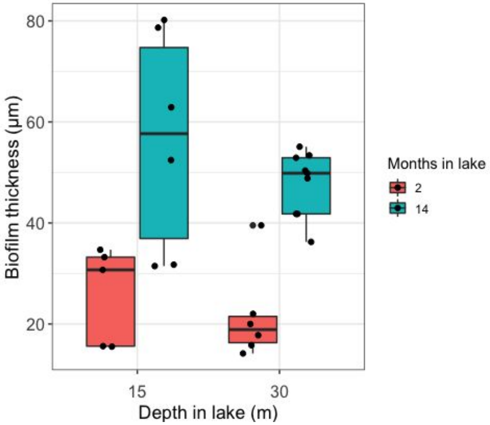

Rachel Cable, UM

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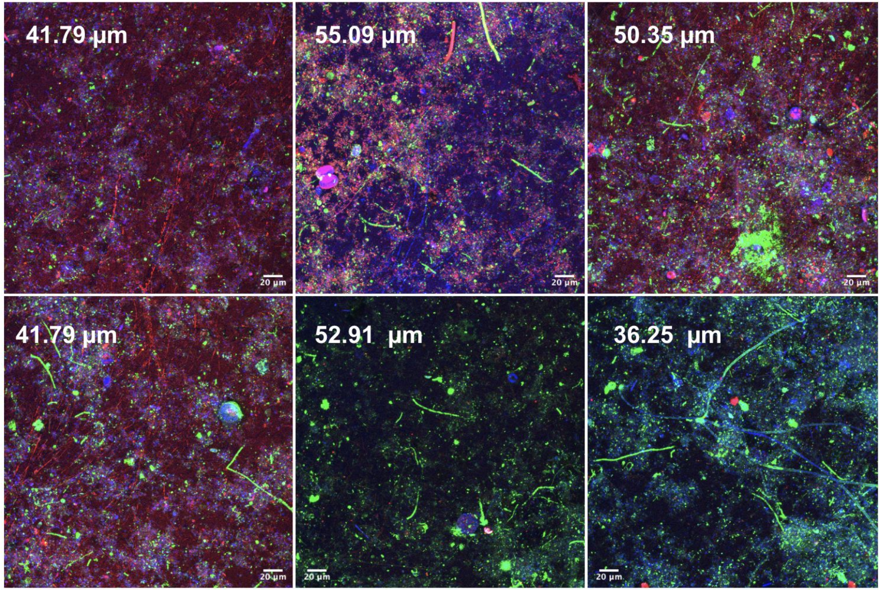

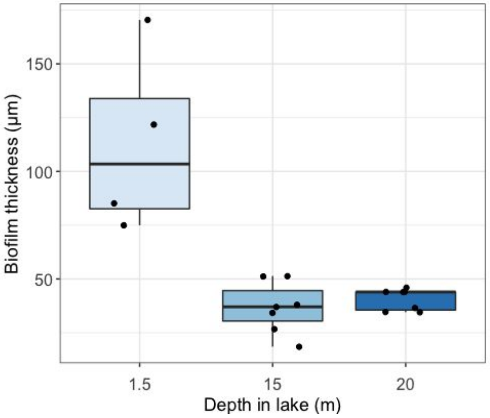

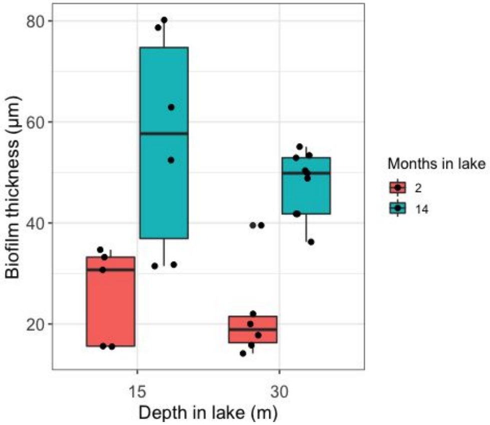

Hydrodynamic models of plastic fate can more accurately account for habitat-specific biofilm thickness and growth rate.

Rachel Cable, UM

Water depth negatively correlates with biomass growth

APPROACH 2
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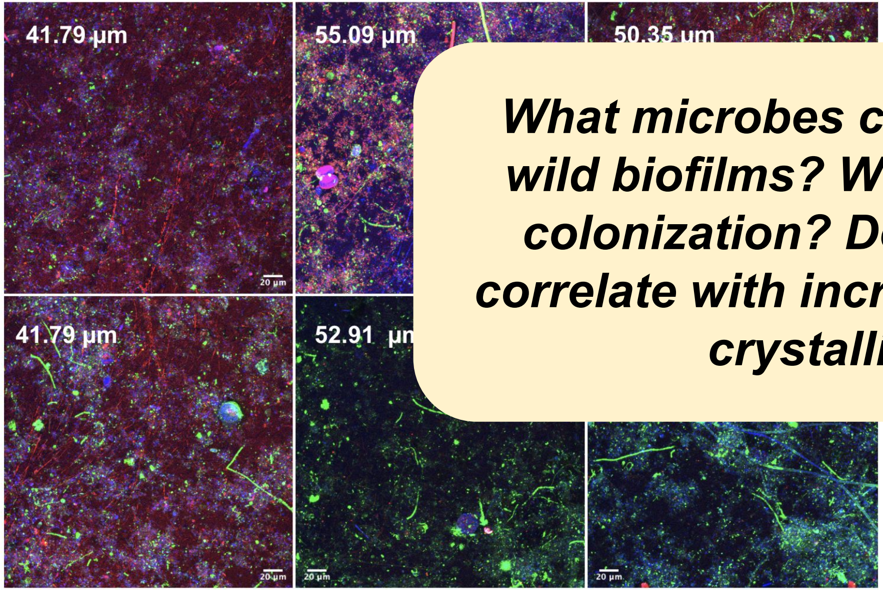
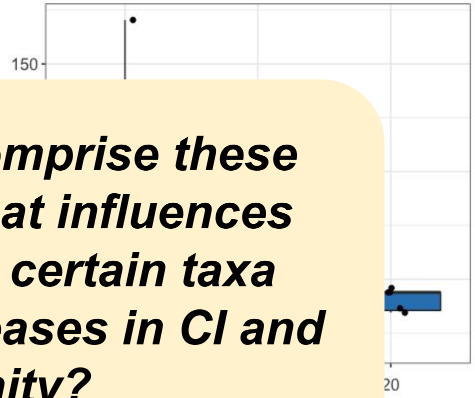
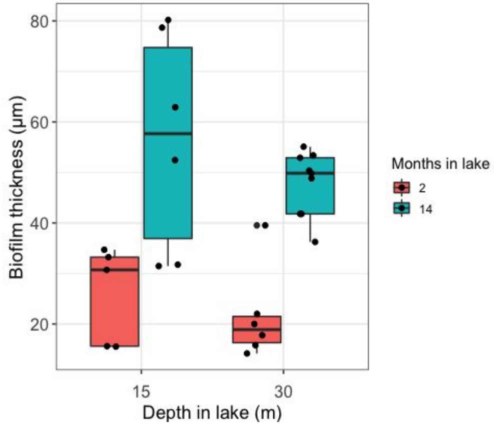




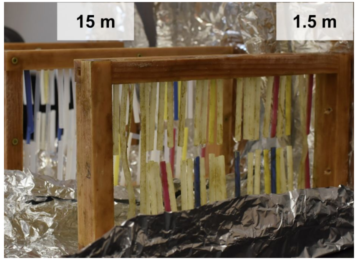


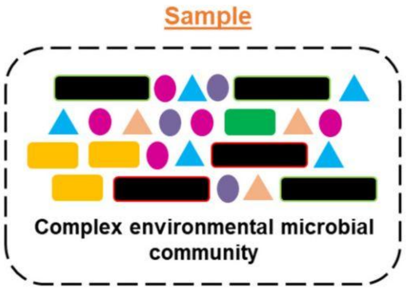
What microbes comprise these wild biofilms? What influences colonization? Do certain taxa correlate with increases in CI and crystallinity?

Hydrodynamic models of plastic fate can more accurately account for habitat-specific biofilm thickness and growth rate.

Rachel Cable, UM

Generate a microbial species list of complex environmental samples using 16S rRNA gene sequencing





Ortiz-Estrada (2018) *Rev. in Aquacult.*

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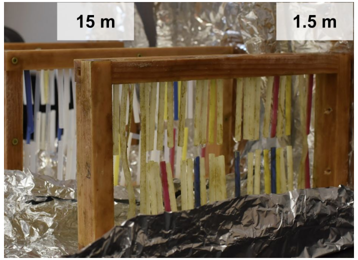

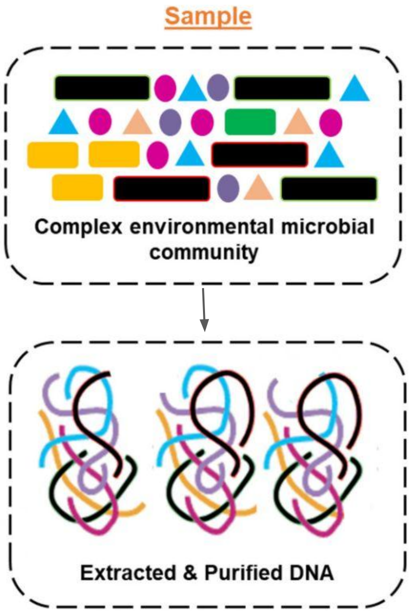

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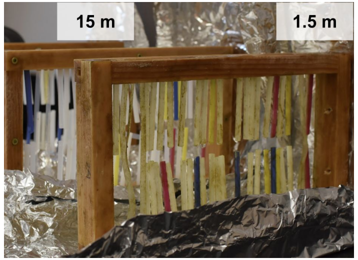

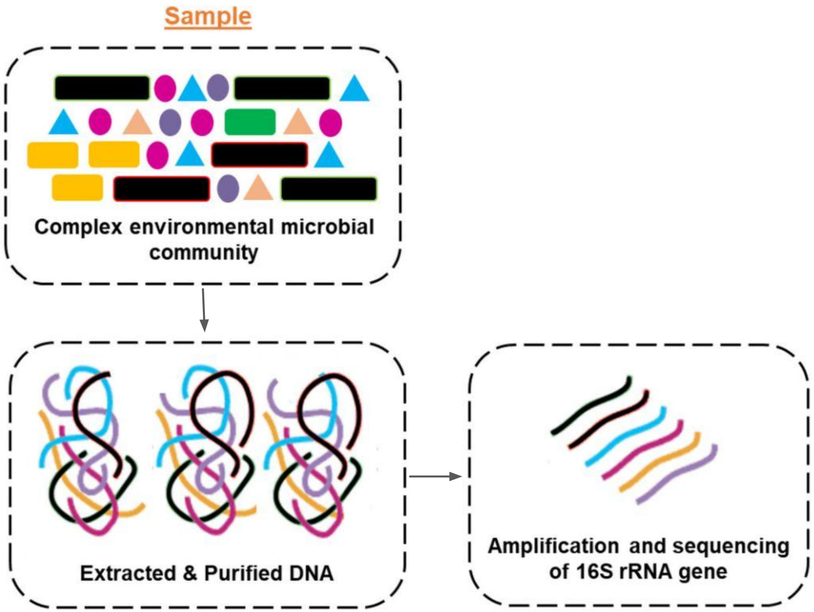

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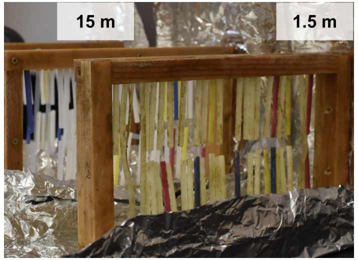

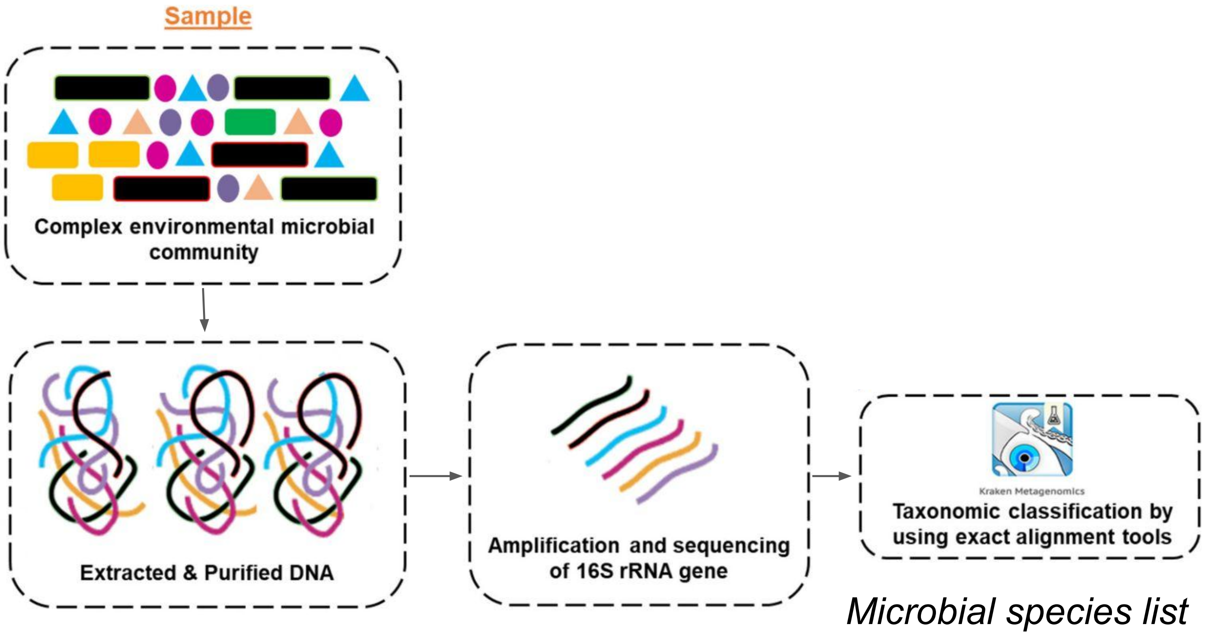

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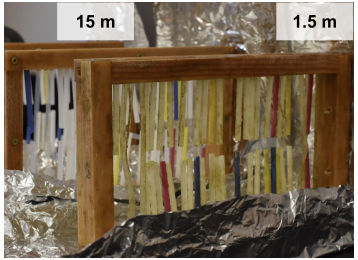
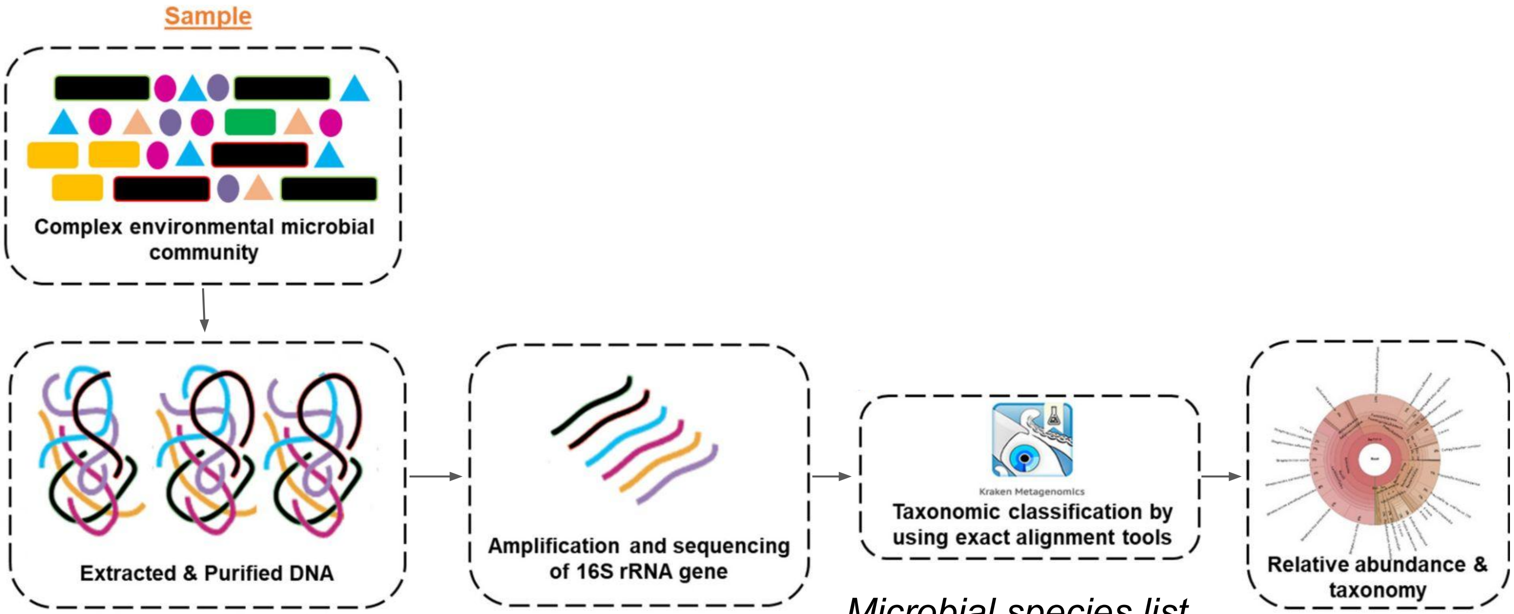


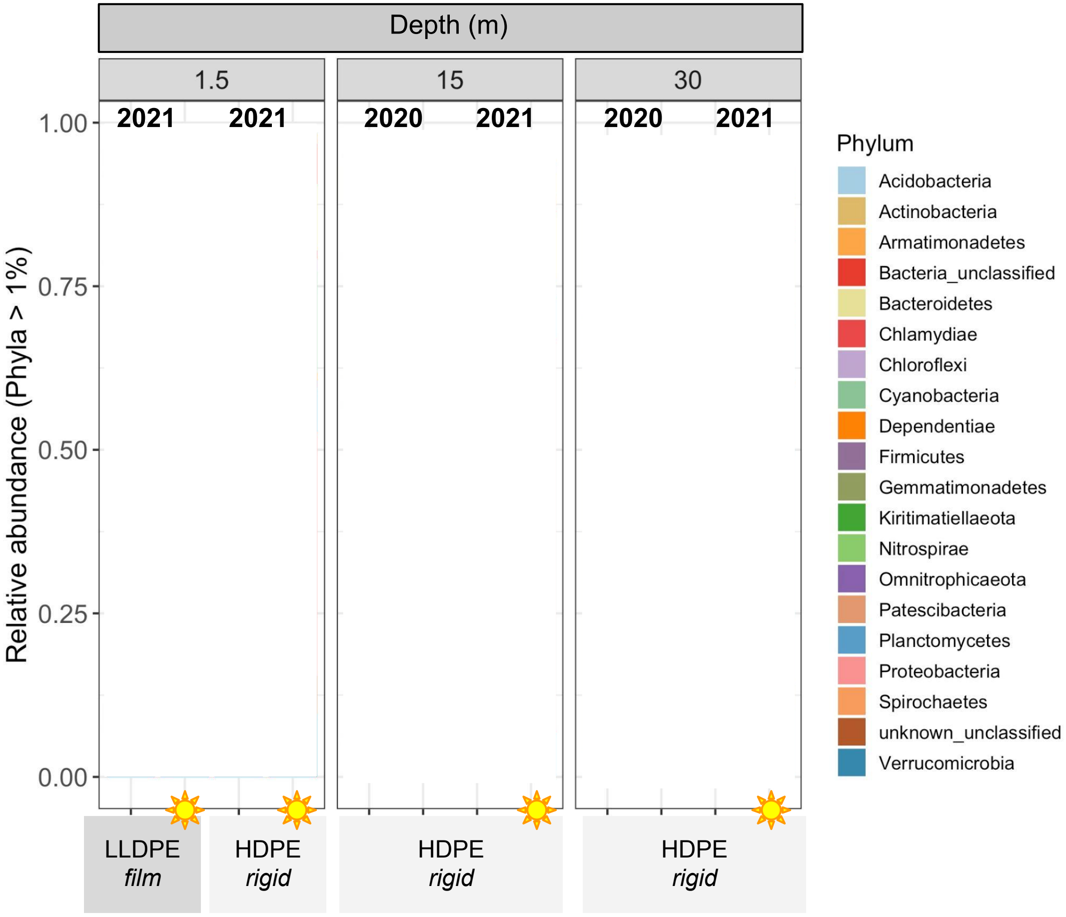


Microbial species list

Ortiz-Estrada (2018) *Rev. in Aquacult.*

Differences in community composition between samples

APPROACH 2
Field Deployments
(*in situ* weathering)



Rachel Cable, UM

Differences in community composition between samples

APPROACH 2
Field Deployments
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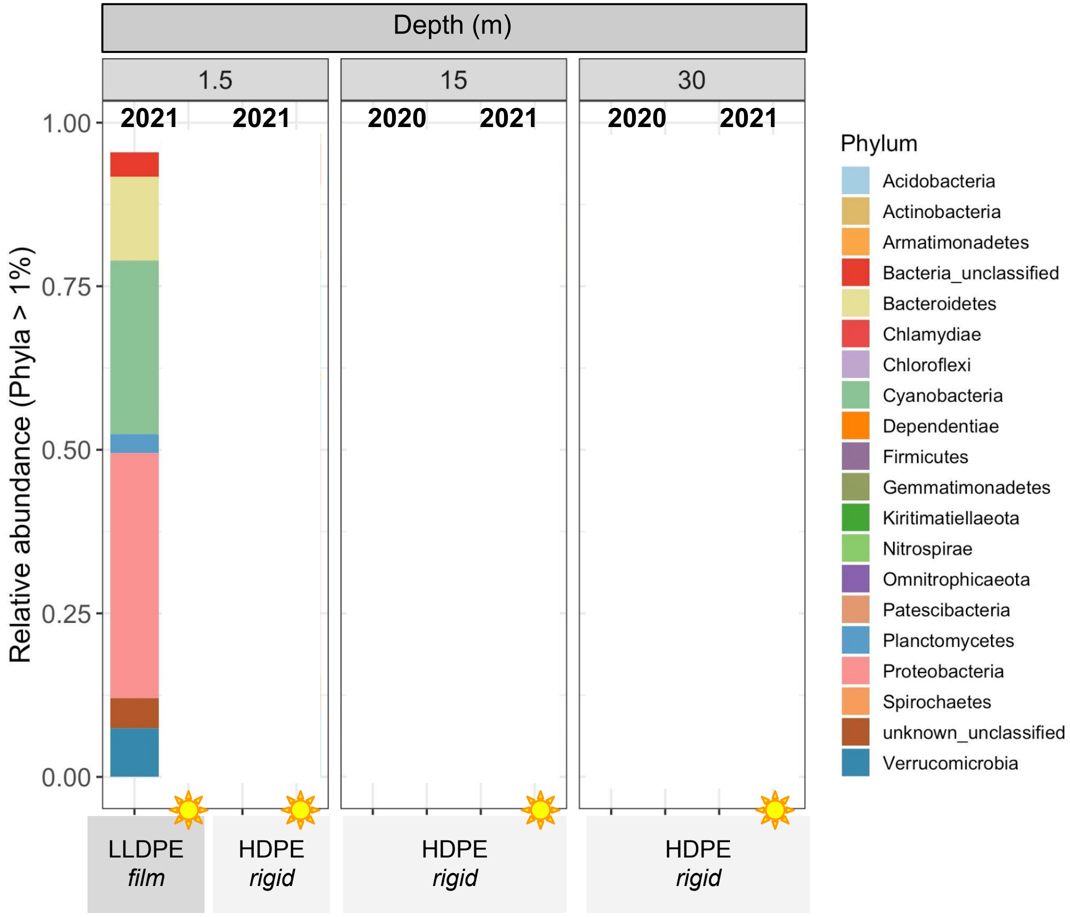
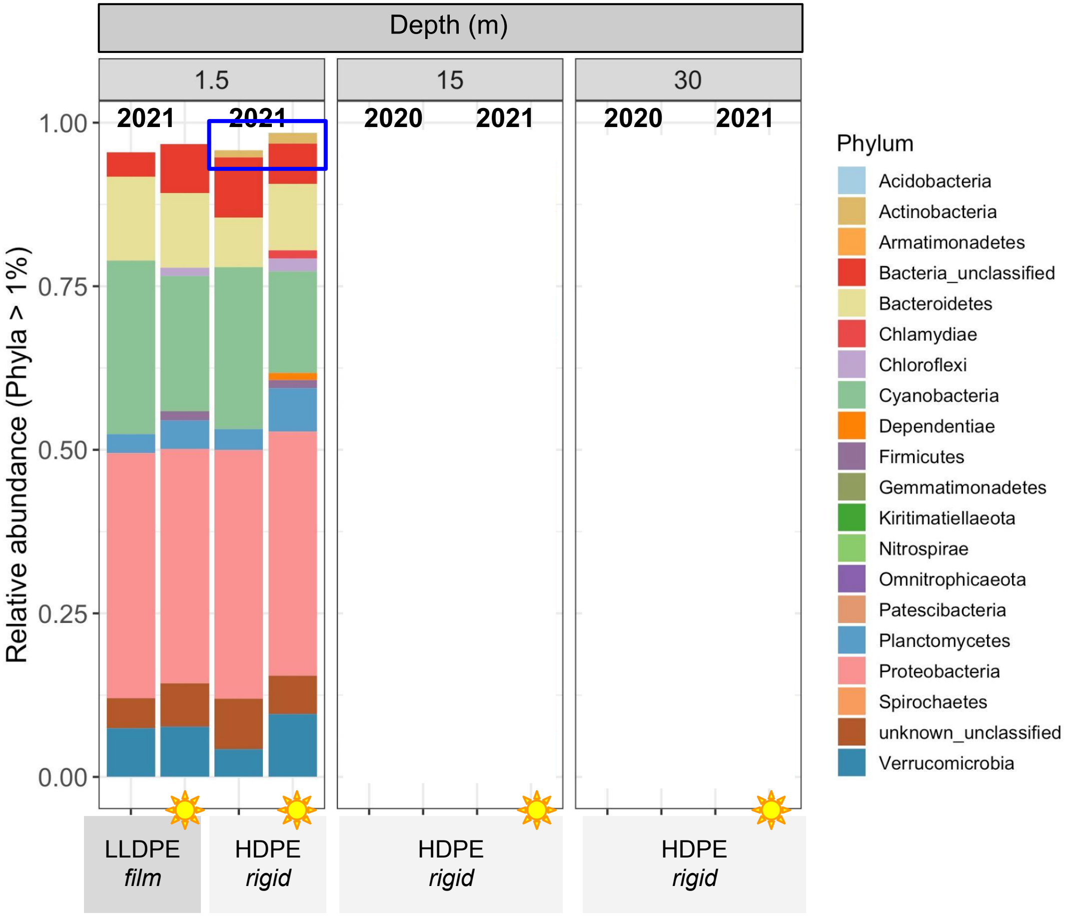


- Plastic biofilms consist of members of many bacterial phyla

Rachel Cable, UM

Differences in community composition between samples

APPROACH 2
Field Deployments
(*in situ* weathering)

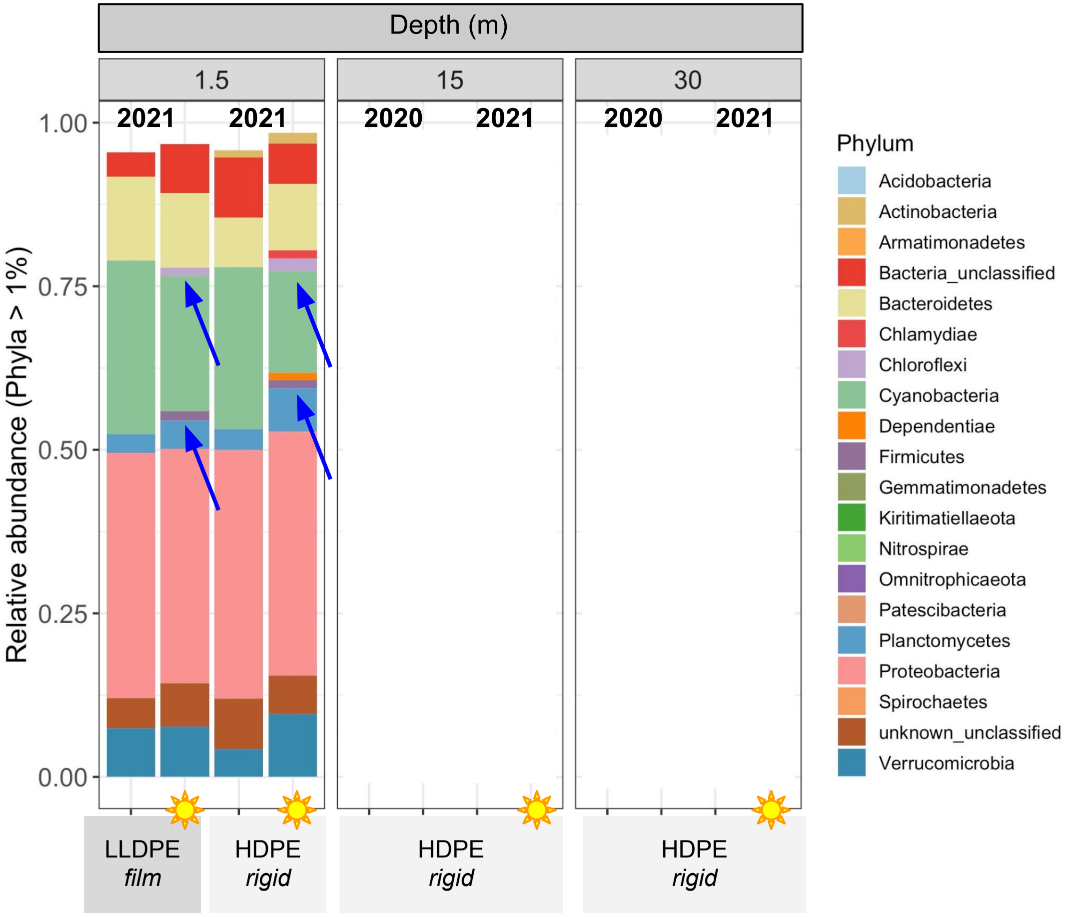


- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
  - Polymer effects

Rachel Cable, UM

Differences in community composition between samples

APPROACH 2
Field Deployments
(*in situ* weathering)



- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
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  - 4 week aging effects

Rachel Cable, UM

Differences in community composition between samples

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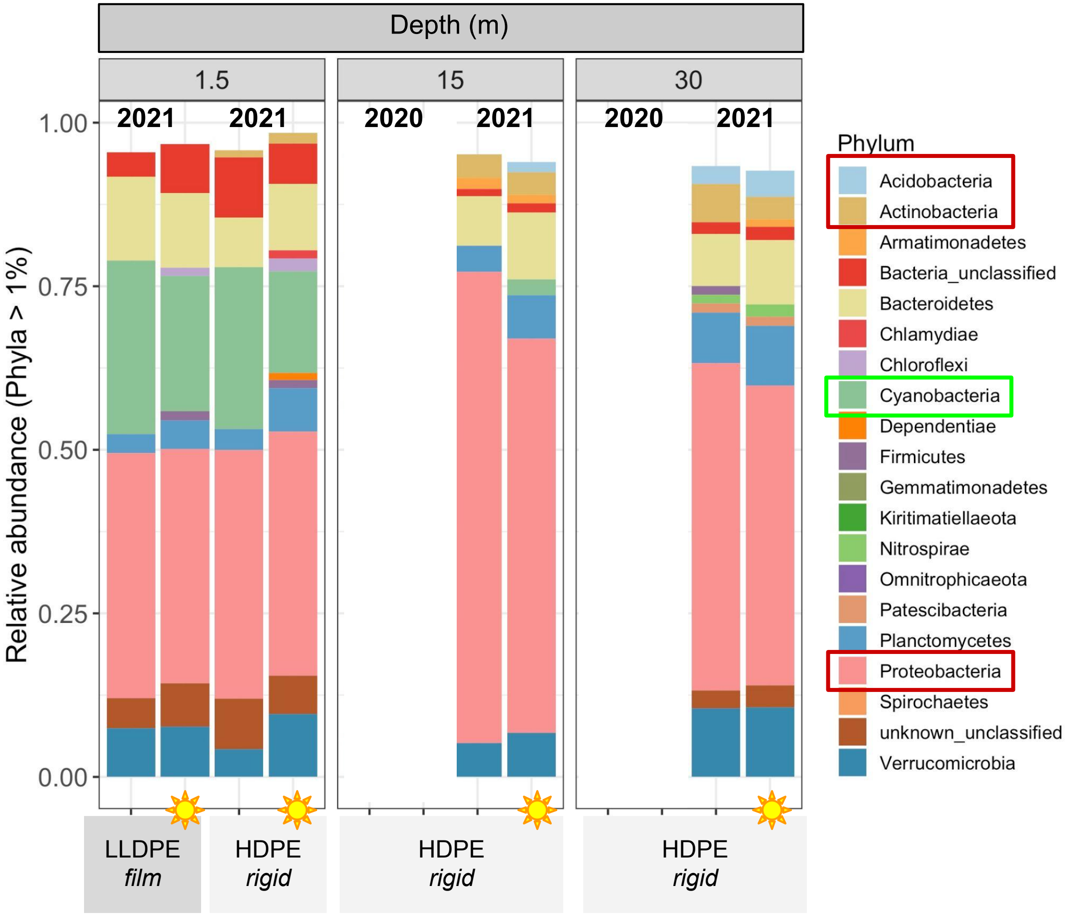
Field Deployments
(*in situ* weathering)

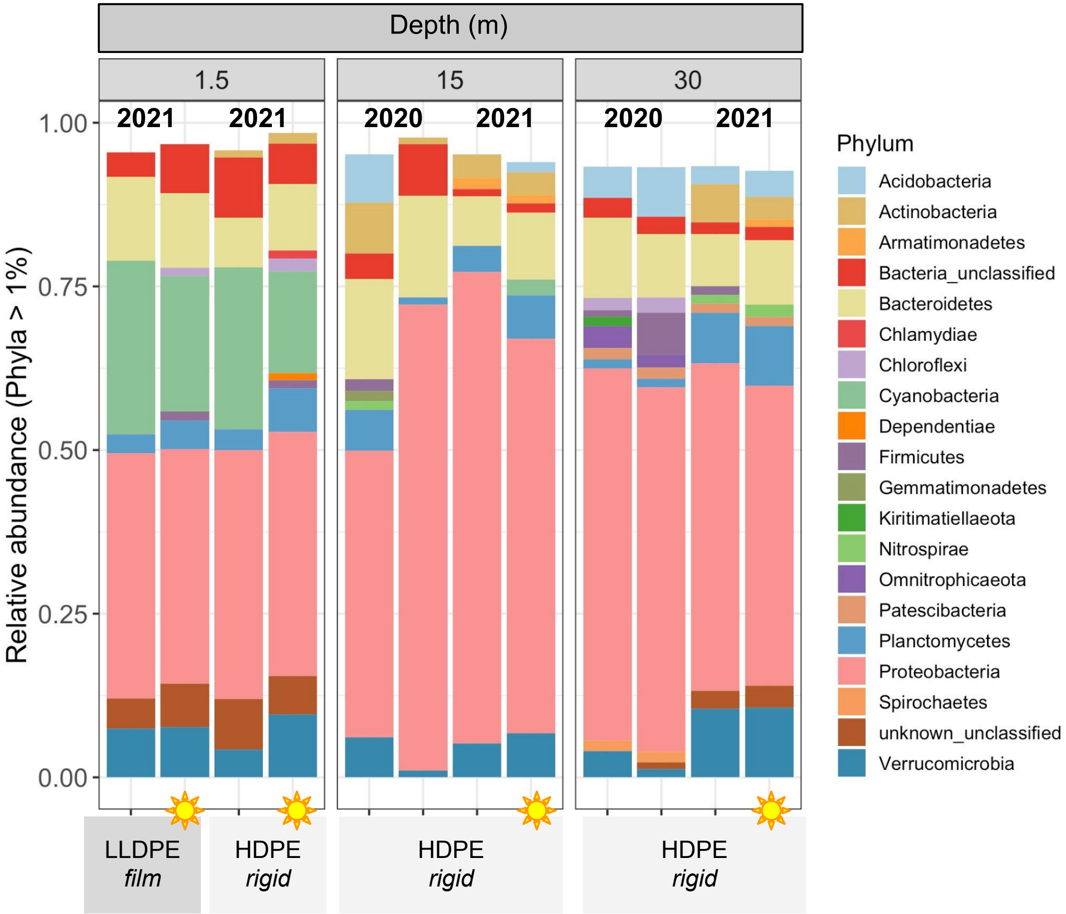


- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
  - Polymer effects
  - 4 week aging effects
- Community composition driven most strongly by depth

Rachel Cable, UM

Differences in community composition between samples

APPROACH 2
Field Deployments
(*in situ* weathering)



- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
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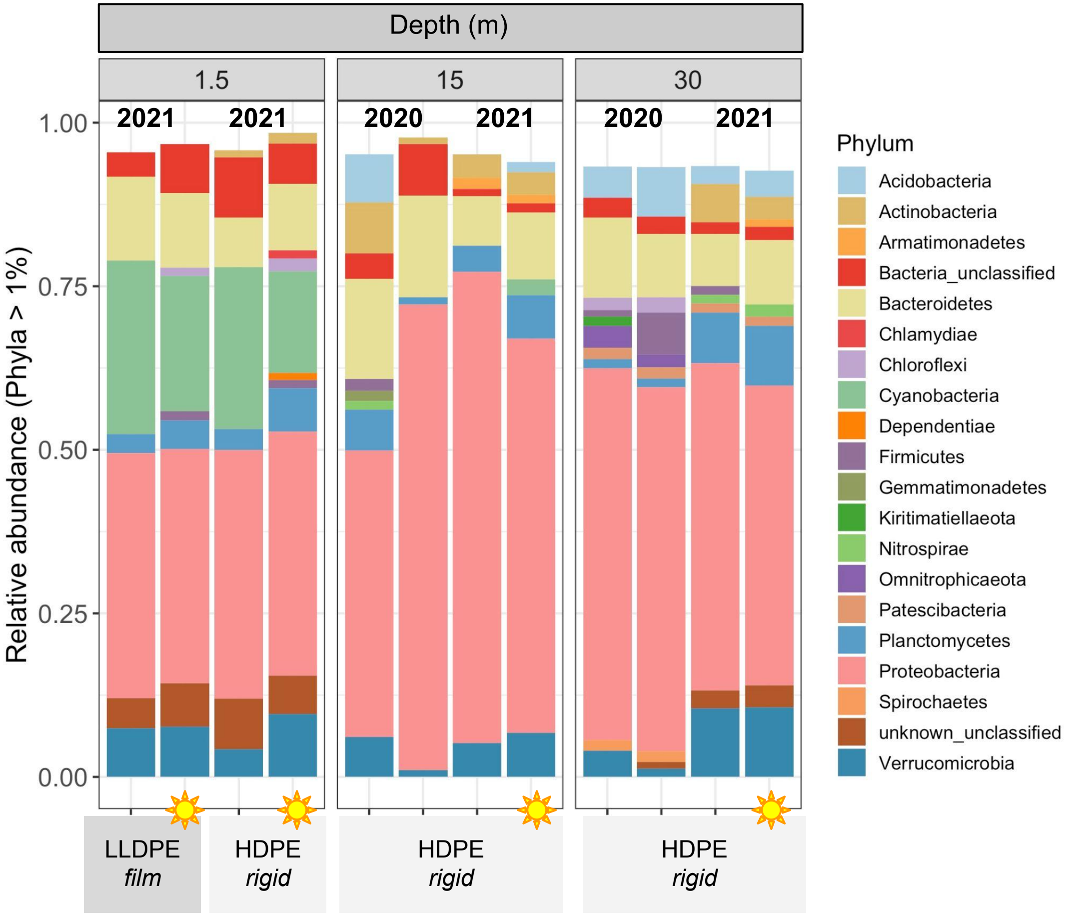
- Community composition driven most strongly by depth
- Evidence of “founder effect”

Rachel Cable, UM

Differences in community composition between samples

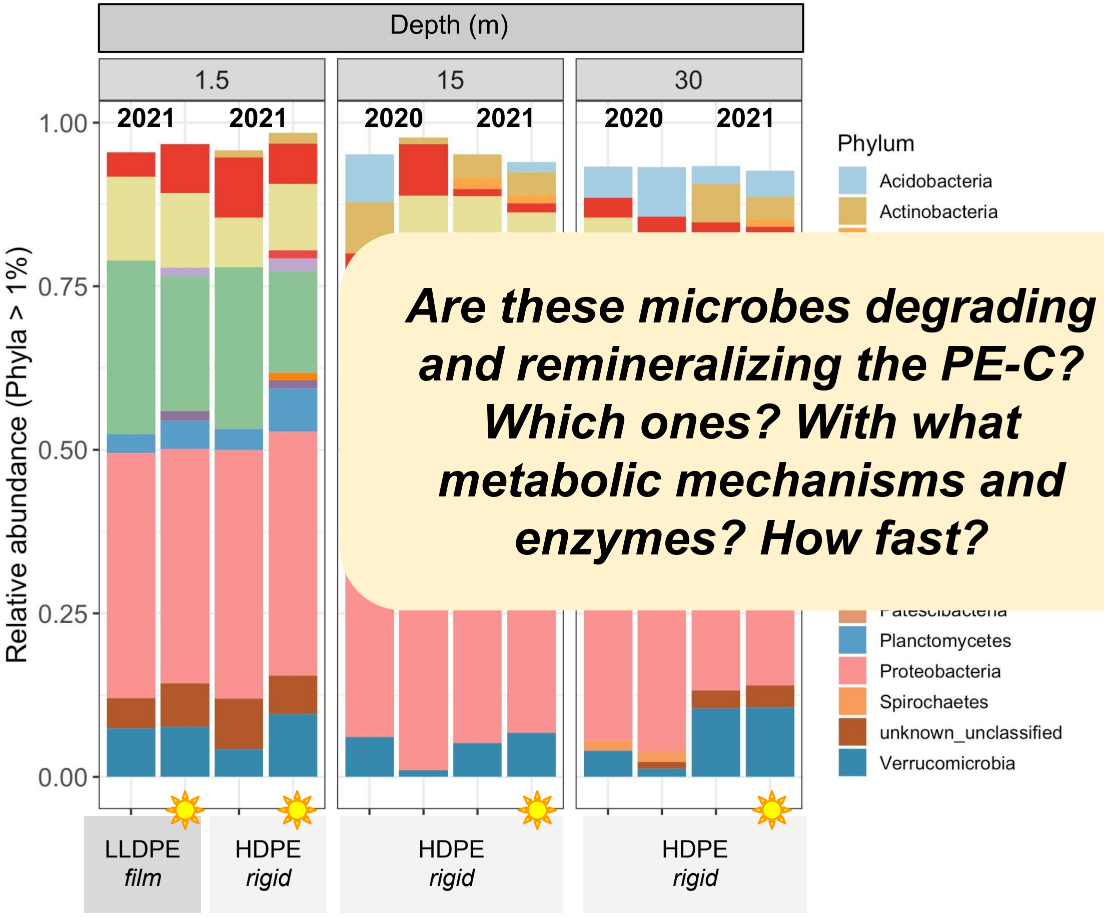
APPROACH 2
Field Deployments
(*in situ* weathering)



- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
  - Polymer effects
  - 4 week aging effects
- Community composition driven most strongly by depth
- Evidence of “founder effect”
- 100s of more communities now being analyzed
- Can connect species identified to lab control culture experiments

Differences in community composition between samples

APPROACH 2
Field Deployments
(*in situ* weathering)



Are these microbes degrading and remineralizing the PE-C? Which ones? With what metabolic mechanisms and enzymes? How fast?

- Plastic biofilms consist of members of many bacterial phyla
- Surface communities similar after 2 months
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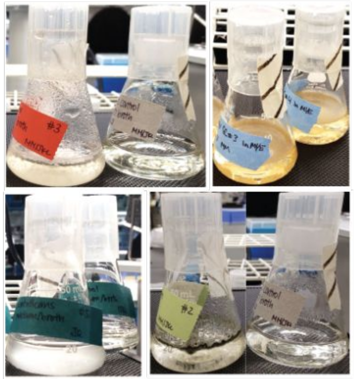
Results

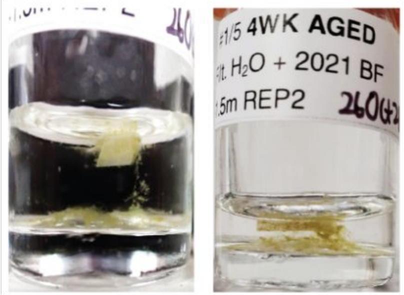
APPROACH 1
UV Accelerated Aging
(simulated weathering)

APPROACH 2
Field Deployments
(*in situ* weathering)

APPROACH 3
Microbial Isolates
(*in vitro* weathering)



APPROACH 4
Microbial Consortia
(*in vitro* weathering)



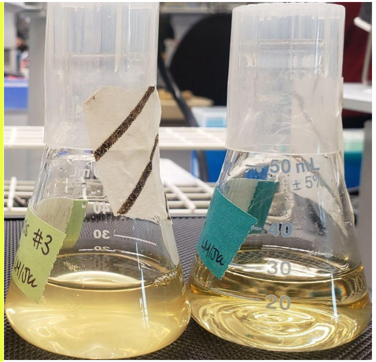
Biological lab analysis

- Biofilm quantification
- Microbial identity (fungi, bacteria)
- Microbial functions (enzymes)

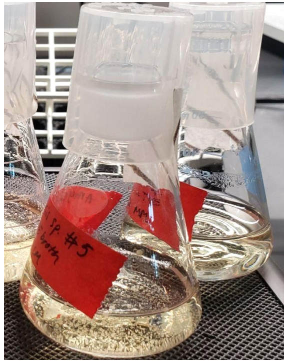
Over 80 described PE degraders in literature, 15 now in culture in our lab

APPROACH 3
Microbial Isolates
(*in vitro* weathering)

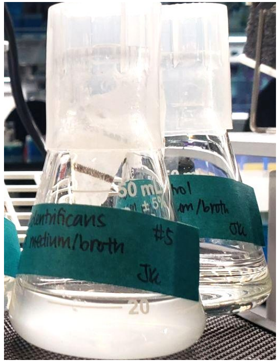
BACTERIA



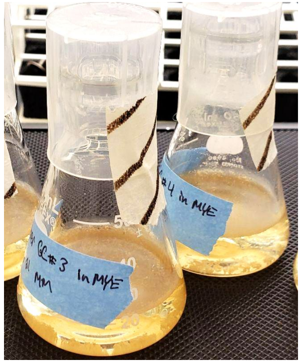
Alcanivorax borkumensis



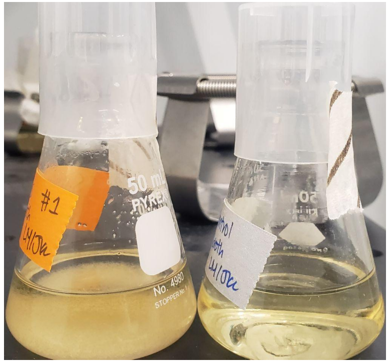
Amycolatopsis sp.



Virgibacillus halodenitrificans

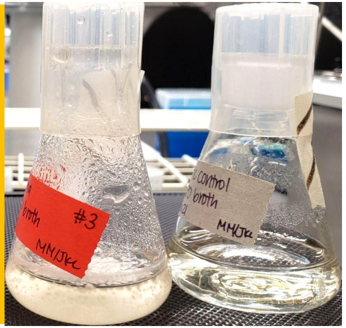


Rhodococcus ruber

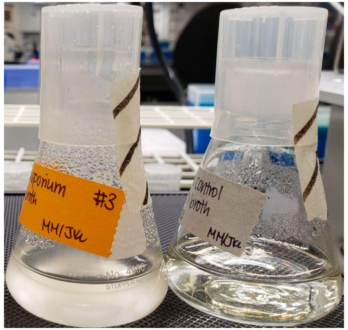


Streptomyces viridosporus

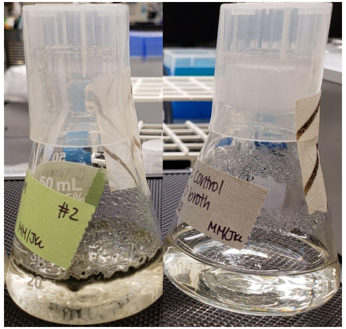
FUNGI



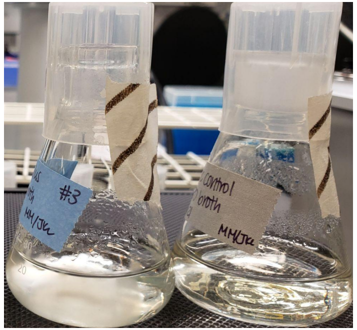
Mortierella alpina



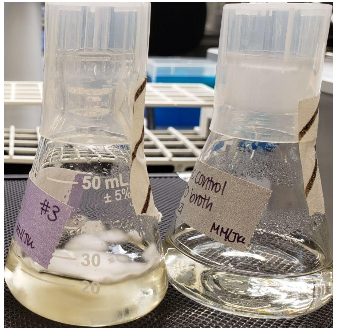
Phanerochaete chrysosporium



Cladosporium ramotenellum



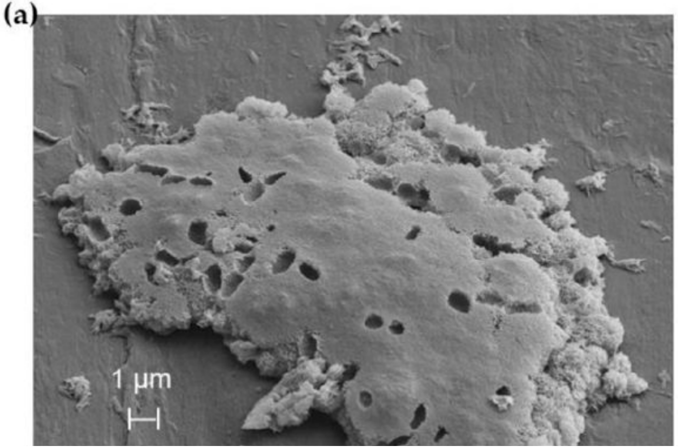
Talaromyces pinophilus



Aspergillus brasiliensis

Evaluate potential for microbial cultures to remineralize PE-C to CO_2 gas as metabolic end-product

Evaluate potential for microbial cultures to remineralize PE-C to CO_2 gas as metabolic end-product



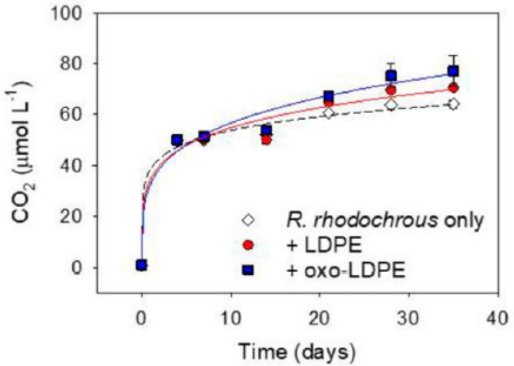
International Journal of *Molecular Sciences*

MDPI

Article

Microbial Degradation of Plastic in Aqueous Solutions Demonstrated by CO_2 Evolution and Quantification

Ruth-Sarah Rose [1,*], Katherine H. Richardson [1], Elmeri Johannes Latvanen [1], China A. Hanson [1,2], Marina Resmini [1] and Ian A. Sanders [1]

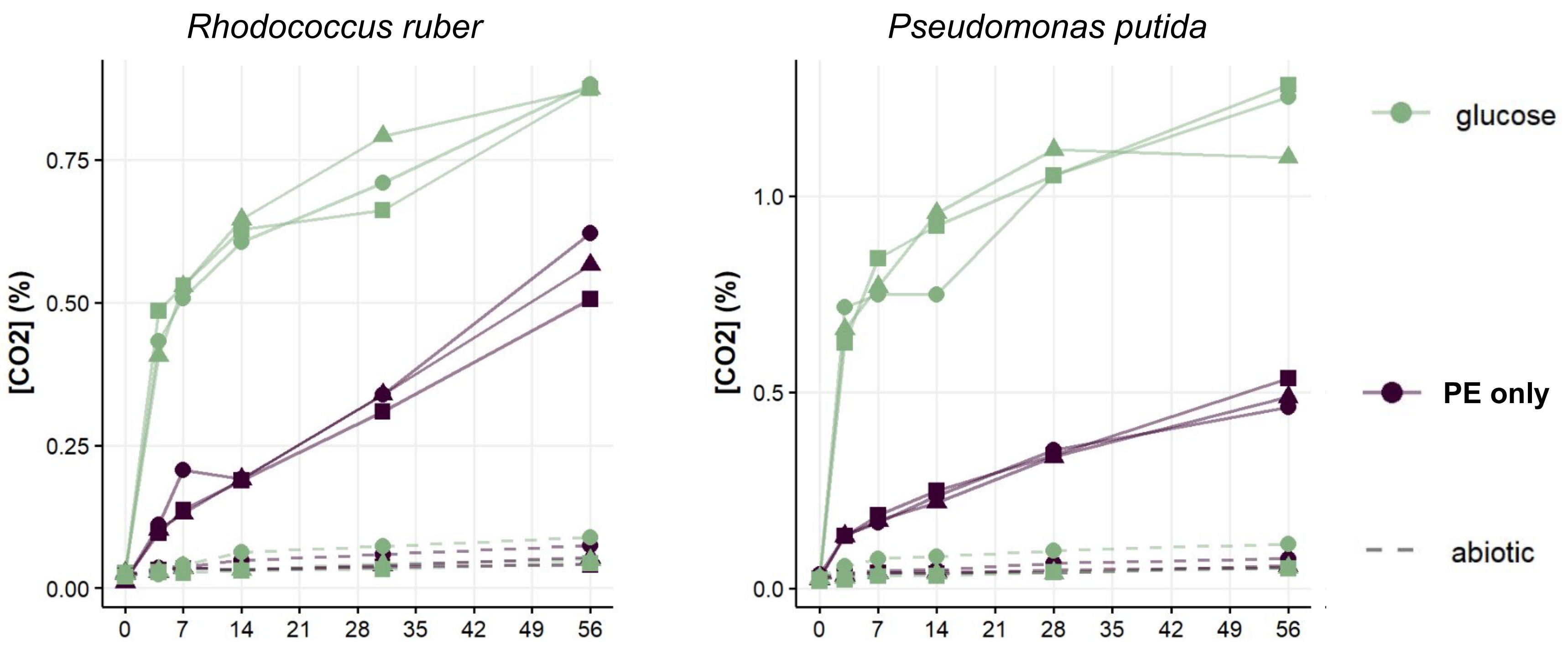


Use CO_2 production measurements as a proxy for microbial growth on plastic as sole carbon source

Evaluating PE-C remineralization via CO_2 evolution

APPROACH 3
Microbial Isolates
(*in vitro* weathering)

Two bacterial strains

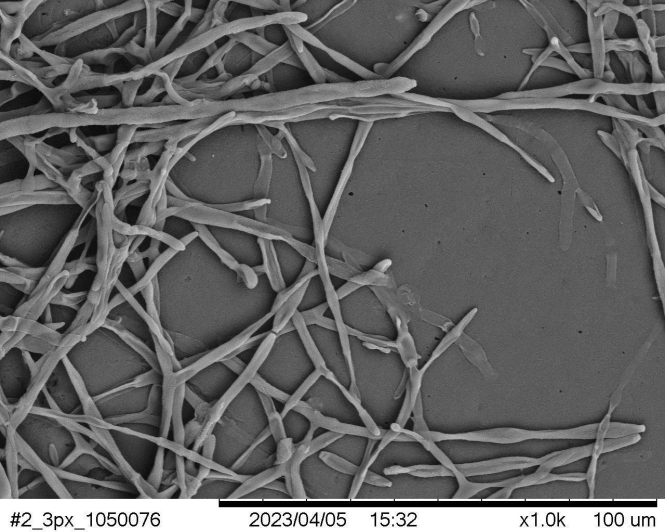


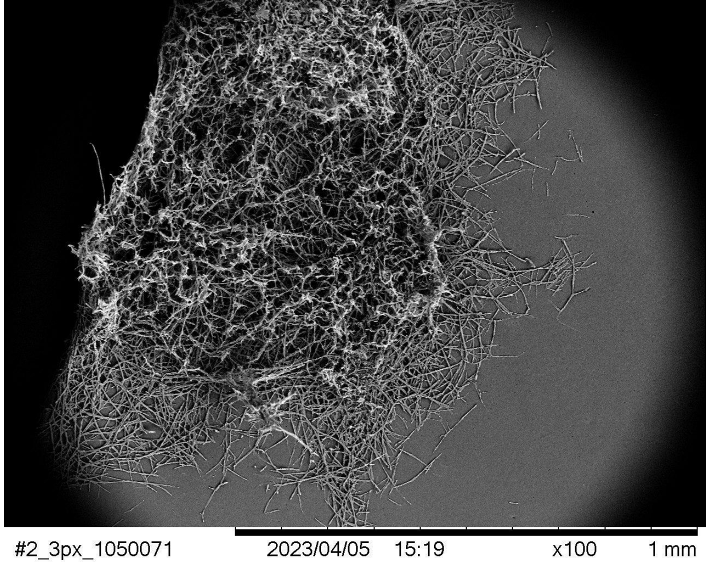
Fungi

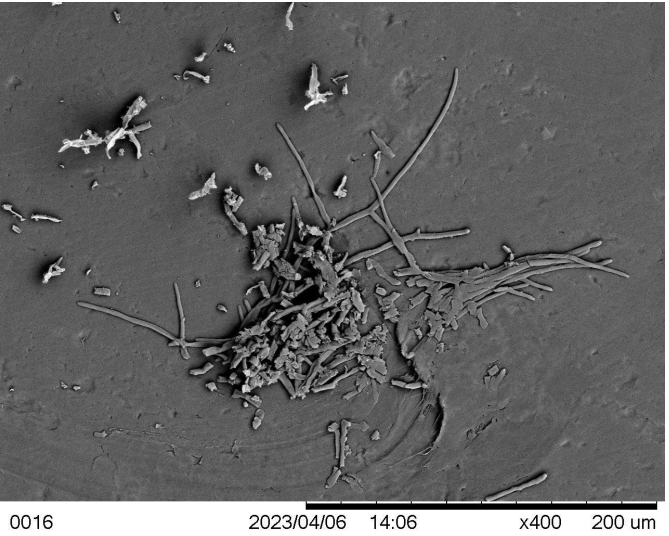
Cladosporium ramotenellum (C.r.)

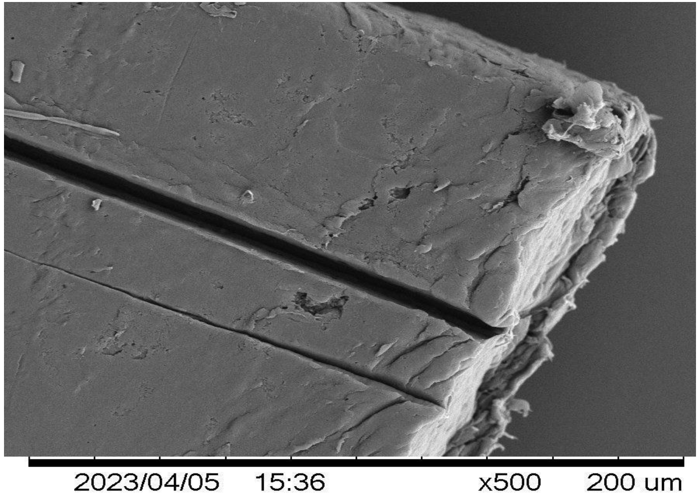
3 days growth on PE film

SEM (EMSL)





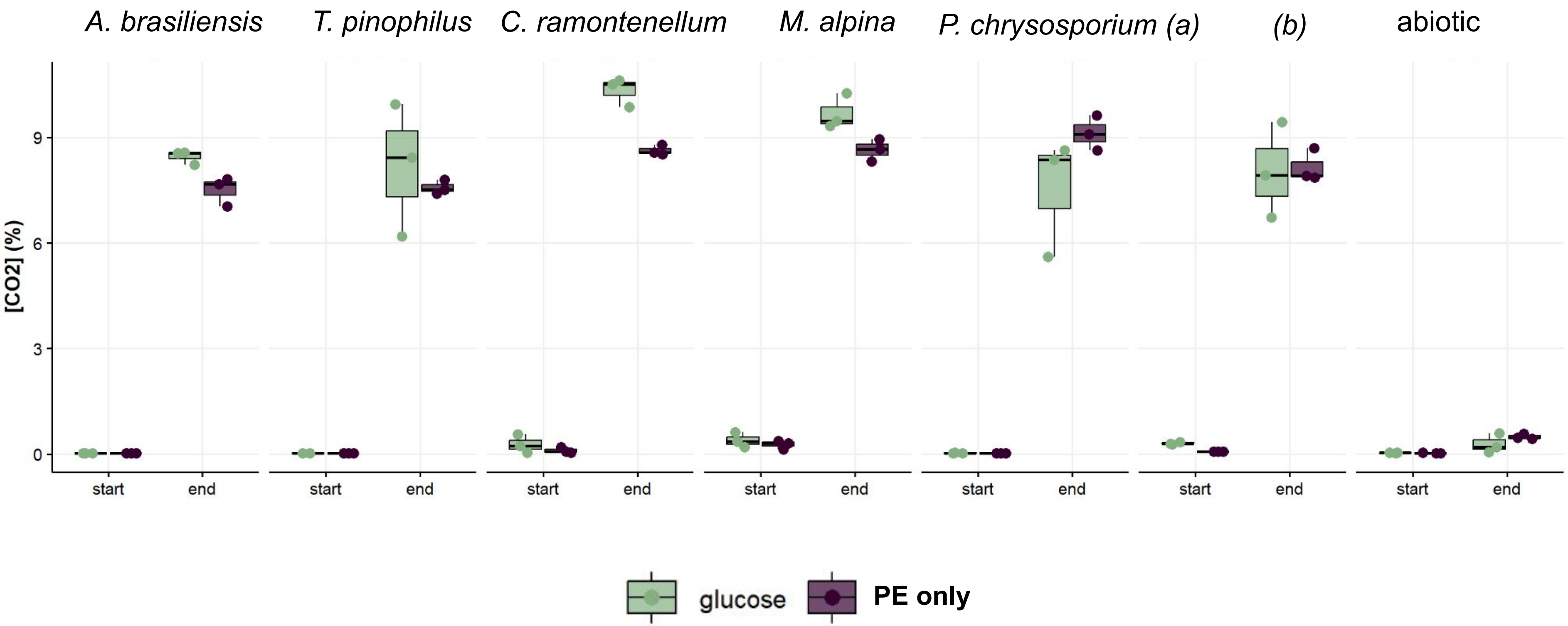




Evaluating PE-C remineralization via CO_2 evolution

Five Fungal strains

APPROACH 3
Microbial Isolates
(*in vitro* weathering)



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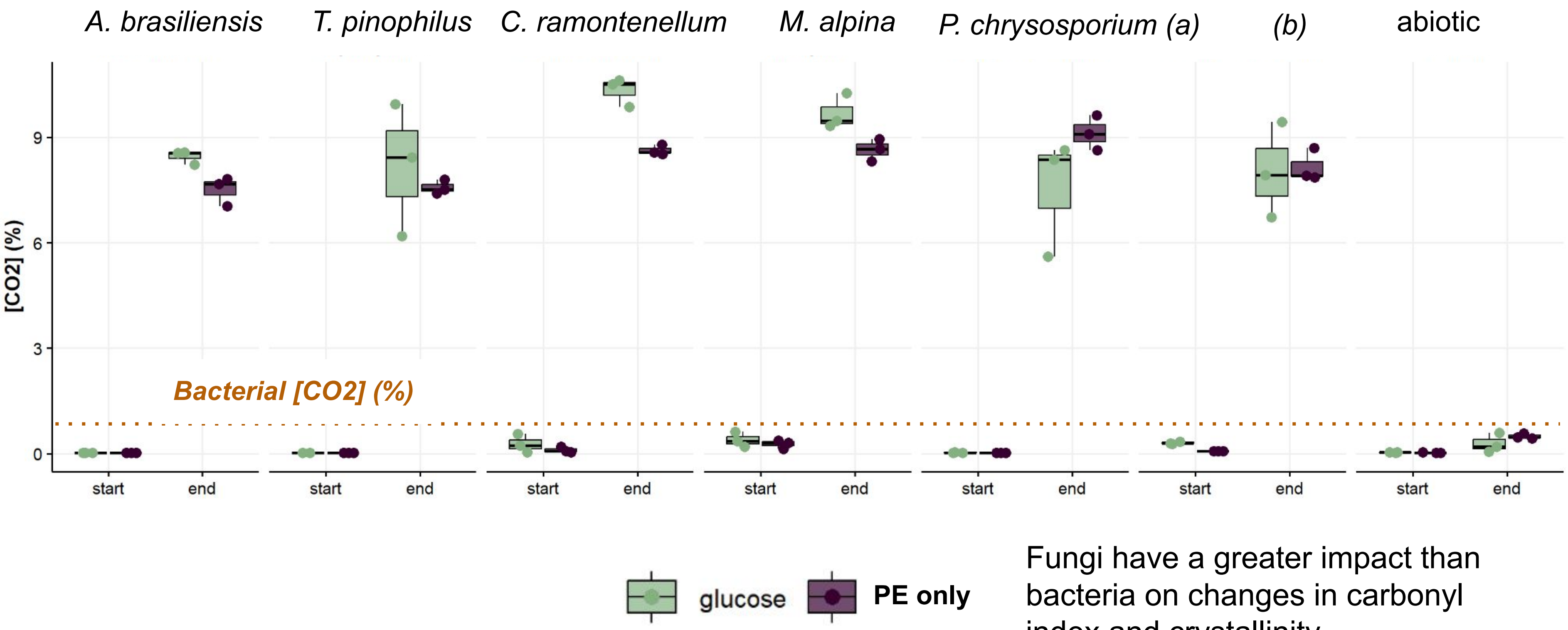
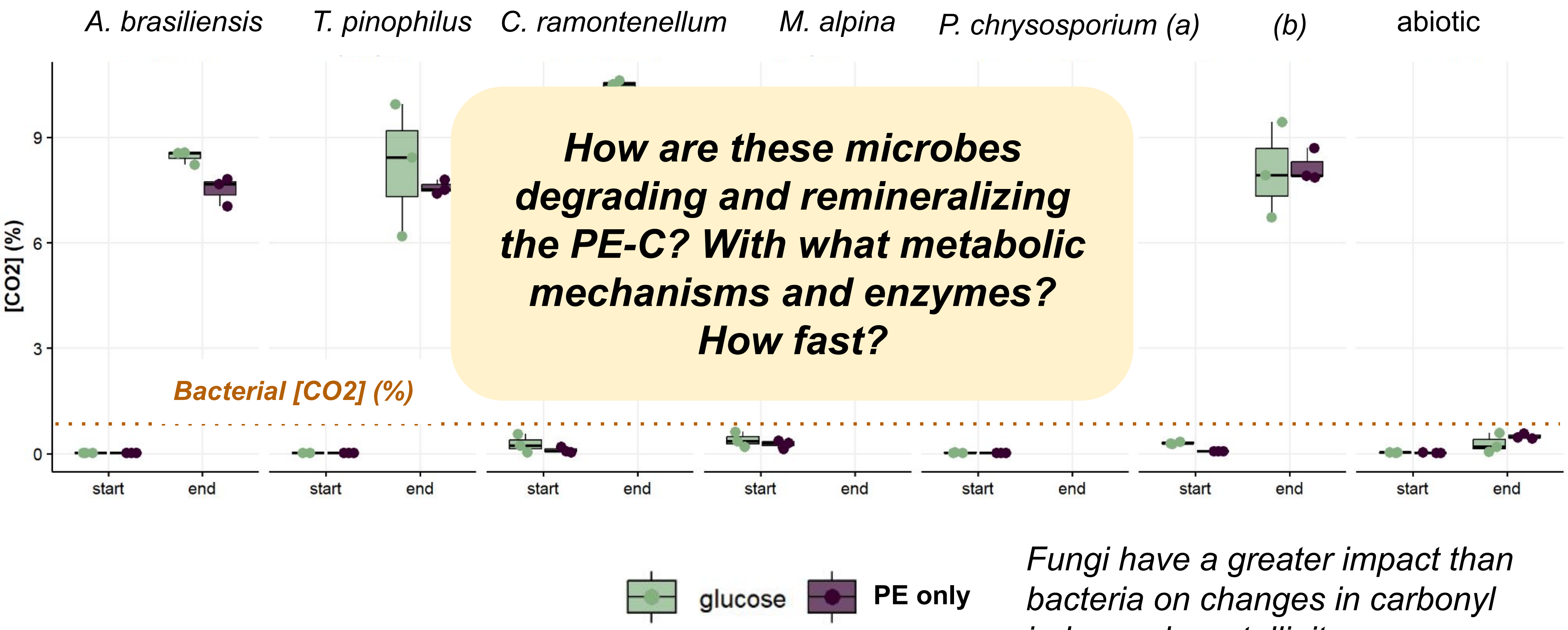


Fungi have a greater impact than bacteria on changes in carbonyl index and crystallinity

Evaluating PE-C remineralization via CO_2 evolution

Five Fungal strains

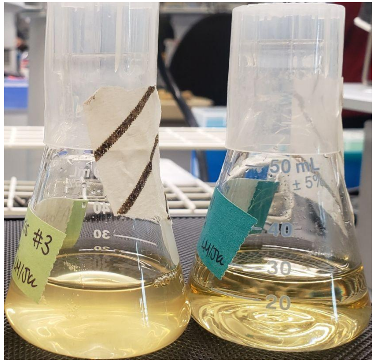


How are these microbes degrading and remineralizing the PE-C? With what metabolic mechanisms and enzymes? How fast?

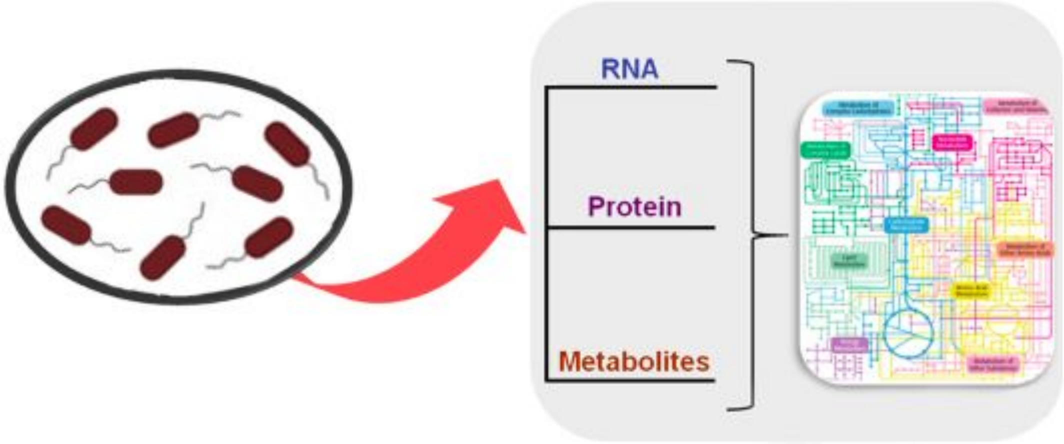
Fungi have a greater impact than bacteria on changes in carbonyl index and crystallinity

‘Omics’ approaches are informing the enzymes involved in PE biodegradation in pure culture

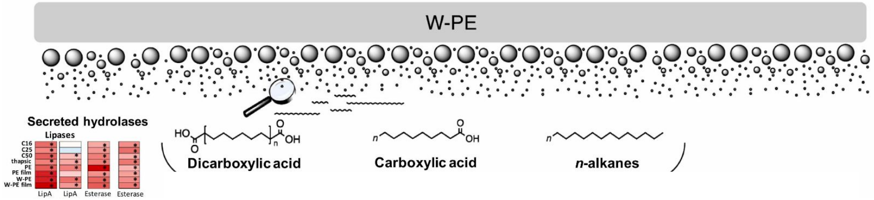
APPROACH 3
Microbial Isolates
(*in vitro* weathering)



Alcanivorax borkumensis



Identify metabolic pathways, mechanisms, and enzymes involved in biodegradation

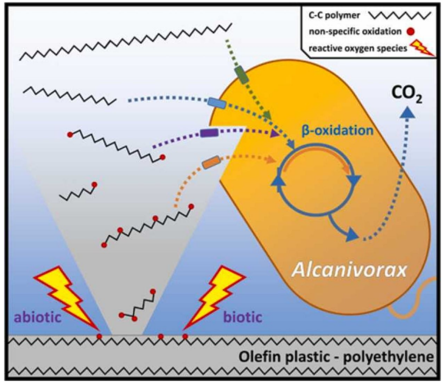


Journal of Hazardous Materials
Volume 436, 15 August 2022, 129278

Research Paper

A mechanistic understanding of polyethylene biodegradation by the marine bacterium *Alcanivorax*

Vinko Zadjelovic [a] [1], Gabriel Erni-Cassola [a] [b] [2], Theo Obrador-Viel [c] [3], Daniel Lester [d], Yvette Eley [e] [4], Matthew I. Gibson [f] [5], Cristina Dorador [g] [h] [i] [6], Peter N. Golyshin [j] [7], Stuart Black [k] [8], Elizabeth M.H. Wellington [a] [9], Joseph A. Christie-Oleza [a] [c] [10]



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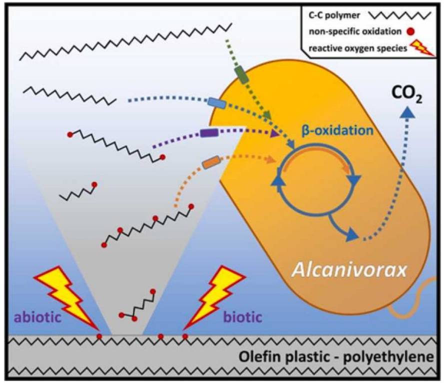

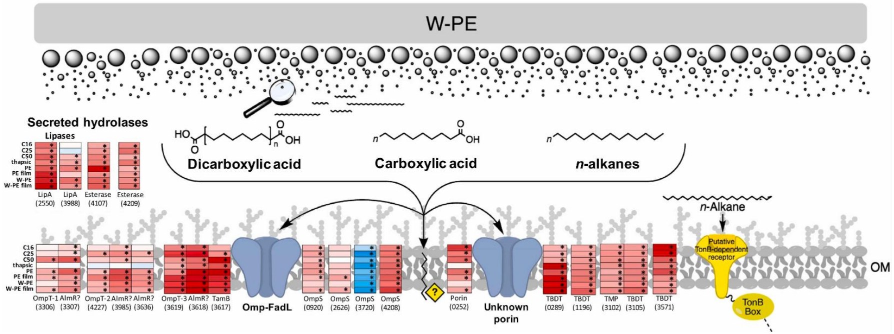

ELSEVIER

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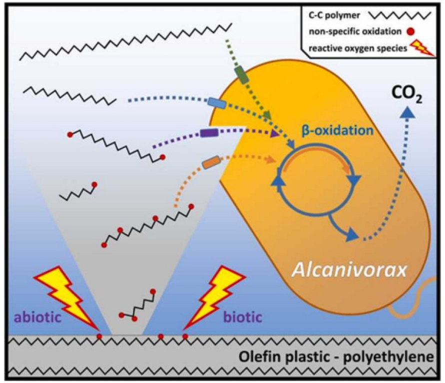

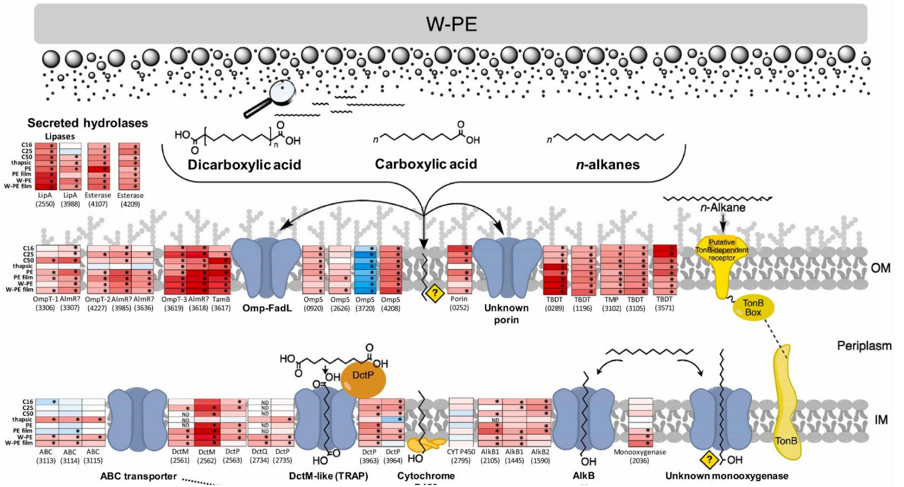

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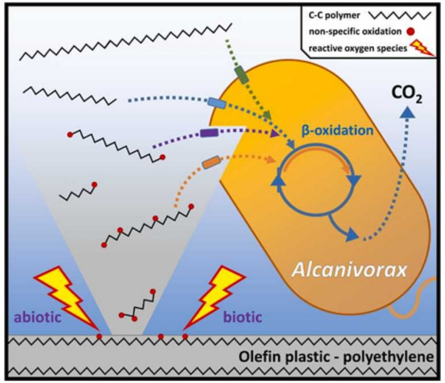

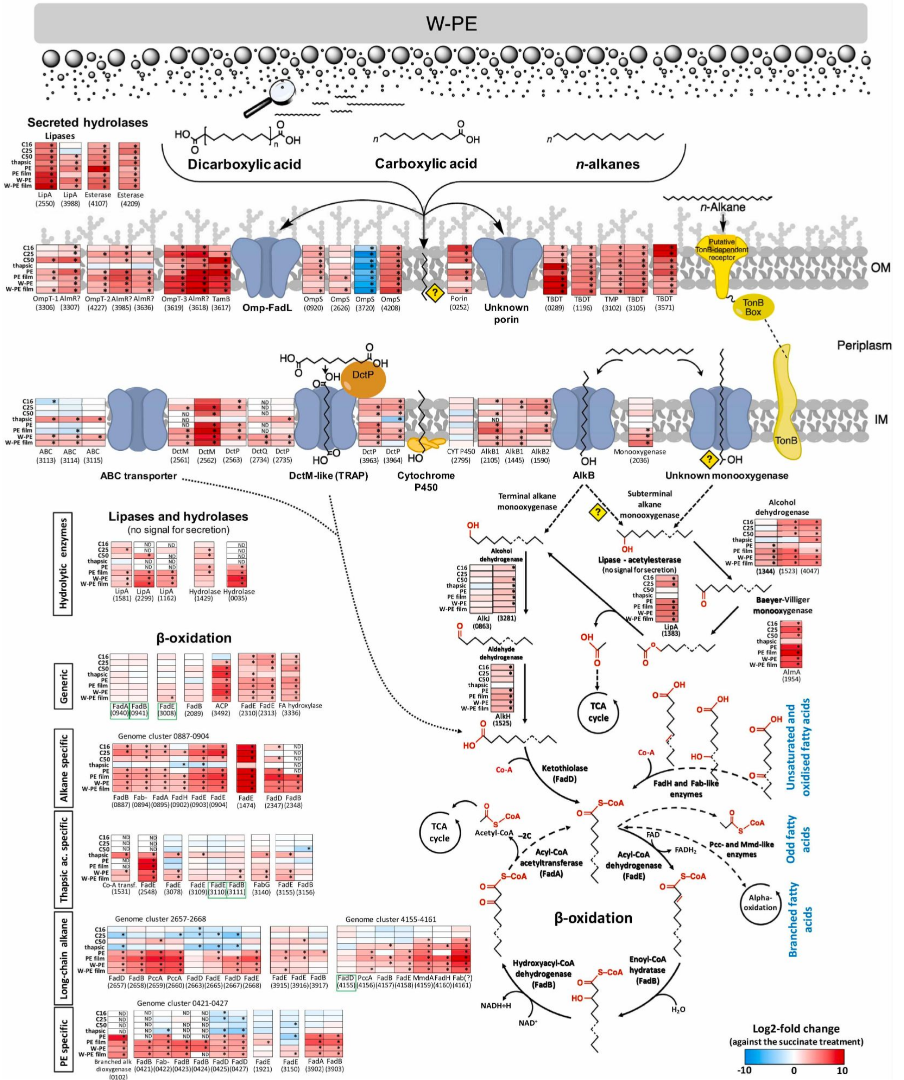

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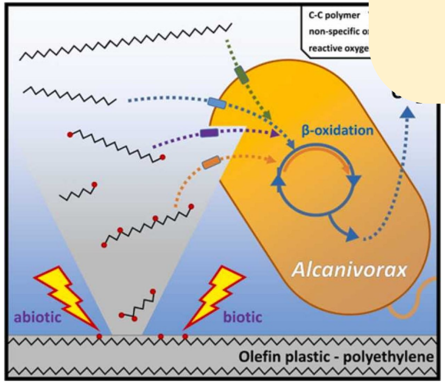
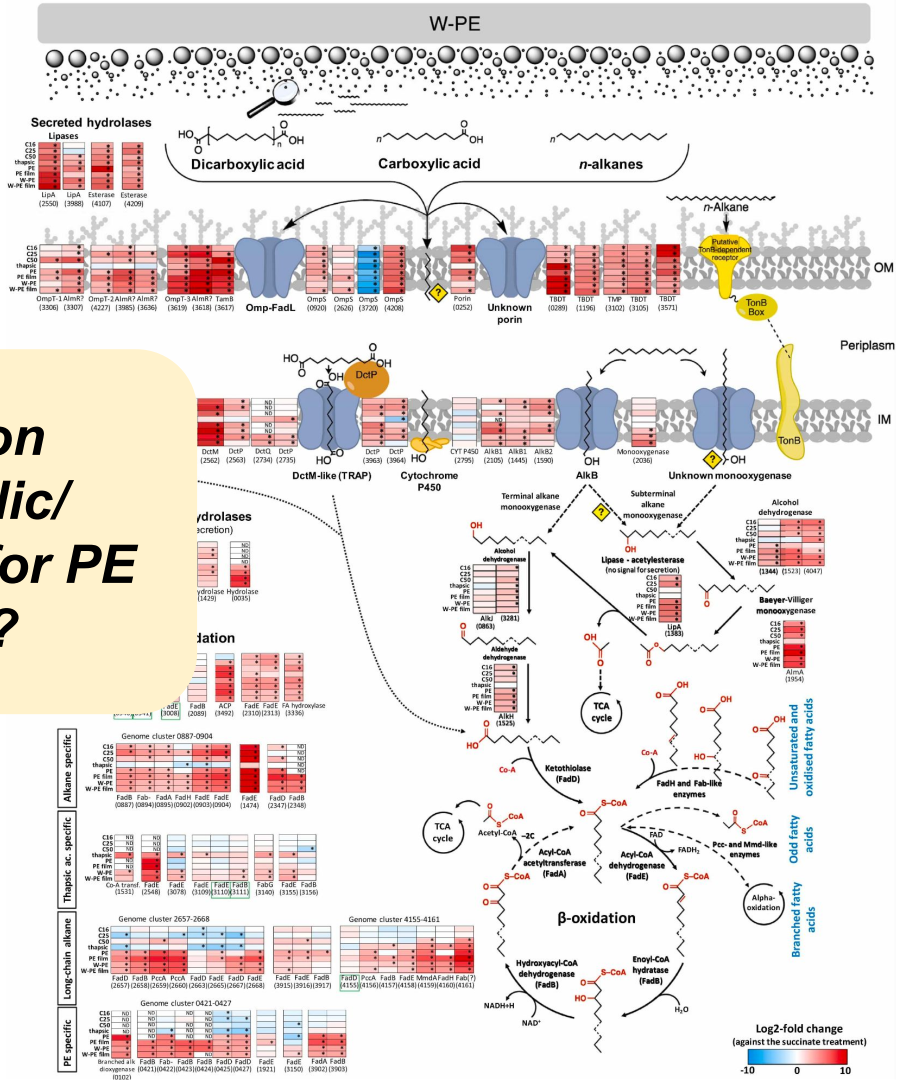


Is there a common microbial metabolic/ enzymatic strategy for PE biodegradation?



Comparative genomics of PE degraders to identify metabolic mechanisms of PE-degradation

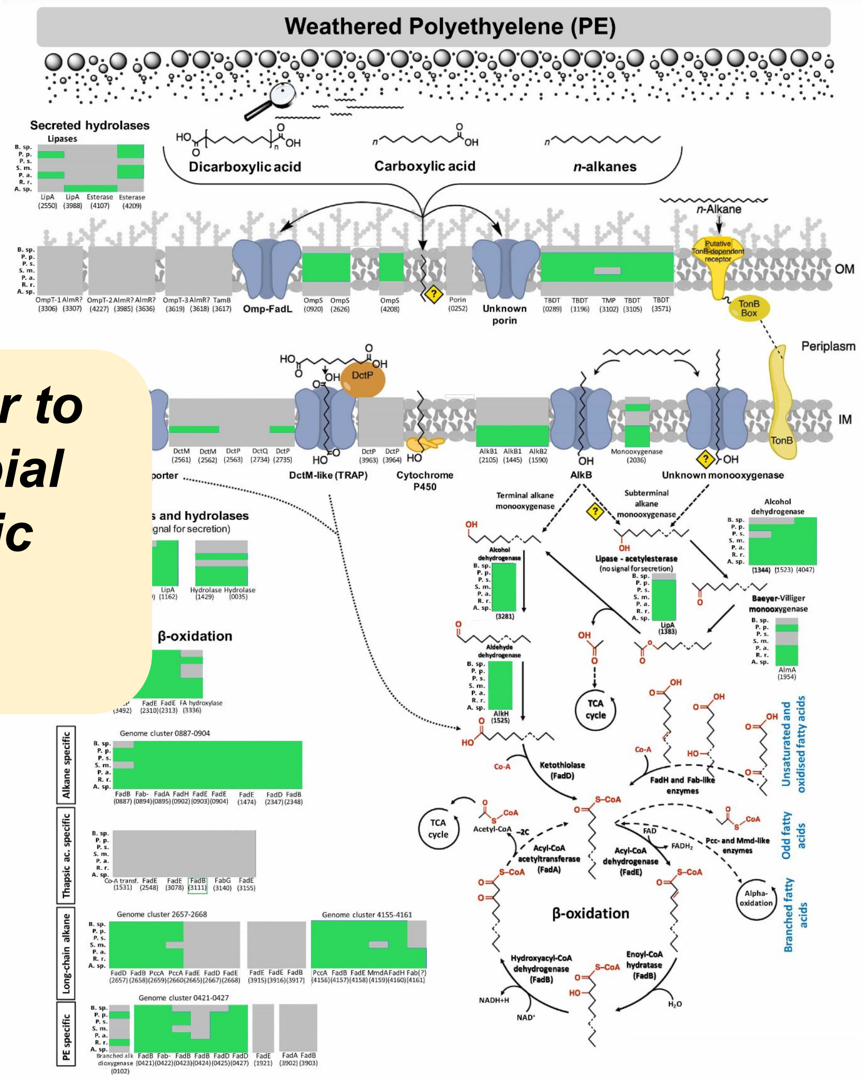
Organism Name Key

B. sp.	*Bacillus* sp. YP1
P. p.	*Pseudomonas putida*
P. s.	*Pseudomonas syringae*
S. m.	*Serratia marcescens*
P. a.	*Pseudomonas aeruginosa*
R. r.	*Rhodococcus ruber**
A. sp.	*Amyolatopsis* sp. 75Vi2*

No Ortholog Encoded
Ortholog Encoded

Max Murray
Honors Thesis
Duhaime Lab

Comparative genomics of PE degraders to identify metabolic mechanisms of PE-degradation



Organism Name Key

B. sp.	*Bacillus* sp. YP1
P. p.	*Pseudomonas pu*
P. s.	*Pseudomonas s*
S. m.	*Serratia marcesc*
P. a.	*Pseudomonas ae*
R. r.	*Rhodococcus rub*
A. sp.	*Amyolatopsis* sp. 75vi2

There does not appear to be a common microbial metabolic/ enzymatic strategy for PE biodegradation…

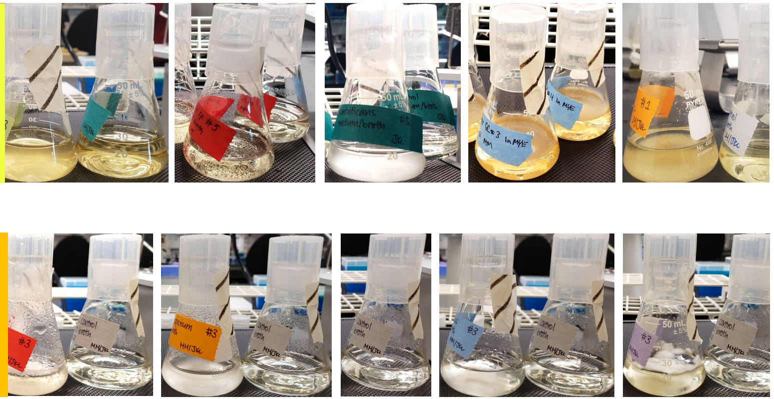
Max Murray
Honors Thesis
Duhaime Lab

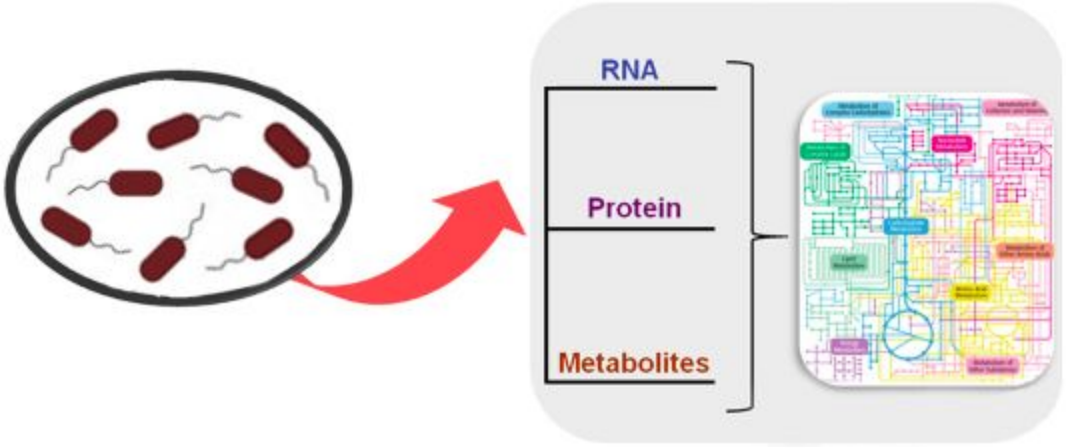
‘Omics’ approaches are informing the enzymes involved in PE biodegradation in pure culture

APPROACH 3
Microbial Isolates
(*in vitro* weathering)

BACTERIA

FUNGI





Identify metabolic pathways, mechanisms, and enzymes involved in biodegradation

Look for activity of these organisms and enzymes in our environmental samples.

(APPROACH 2)

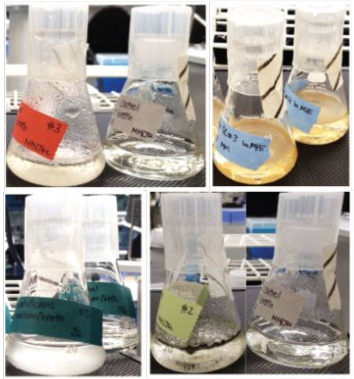
Jessica Choi, Lizy Michaelson, Adi Mizrahi, UM

Results

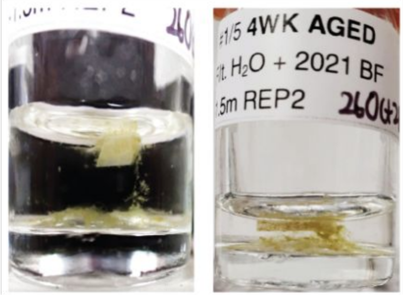
APPROACH 1
UV Accelerated Aging
(simulated weathering)

APPROACH 2
Field Deployments
(*in situ* weathering)

APPROACH 3
Microbial Isolates
(*in vitro* weathering)



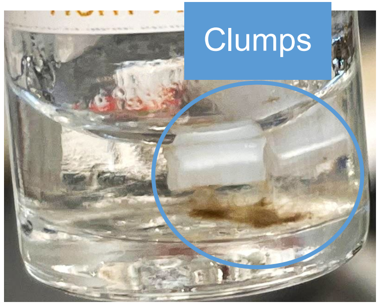
APPROACH 4
Microbial Consortia
(*in vitro* weathering)



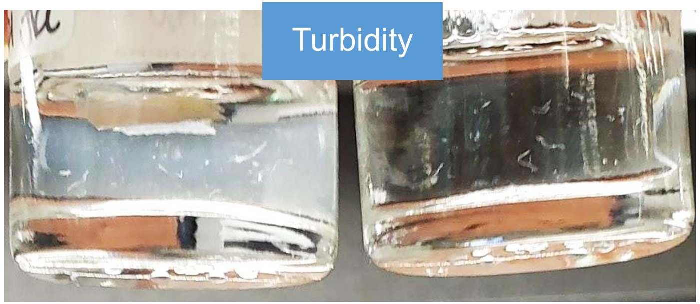
Biological lab analysis
- Biofilm quantification
- Microbial identity (fungi, bacteria)
- Microbial functions (enzymes)

Enrichment cultures grow >14 months after inoculation from the environment

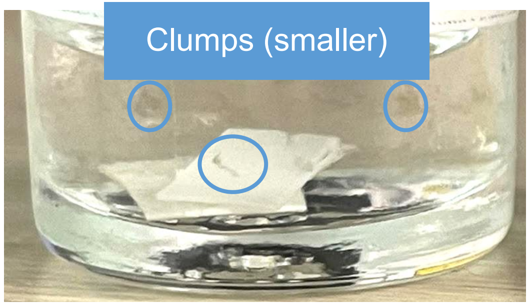
APPROACH 4
Microbial Consortia
(*in vitro* weathering)



HDPE with ELA 1.5m 2-mo biofilm (2021)

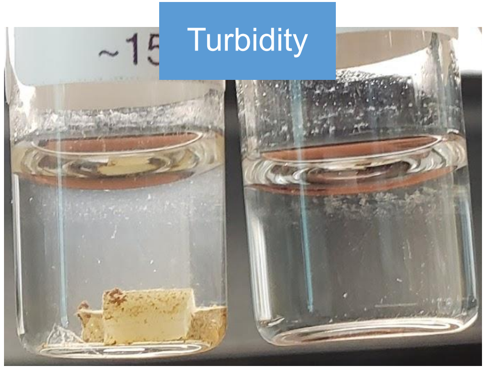


LLDPE 4 weeks aged with ELA 15m 14-mo biofilm (2020) & No plastic control (right)

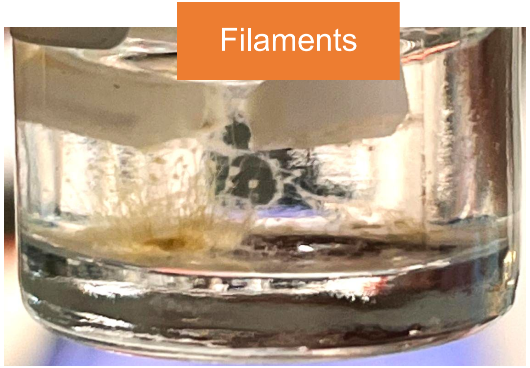


LDPE with ELA 30m 14-mo biofilm (2020)

LDPE with UMBS 1.5m 2-mo biofilm (2021)



LDPE with UMBS 15m 2-mo biofilm (2021) & No plastic control (right)

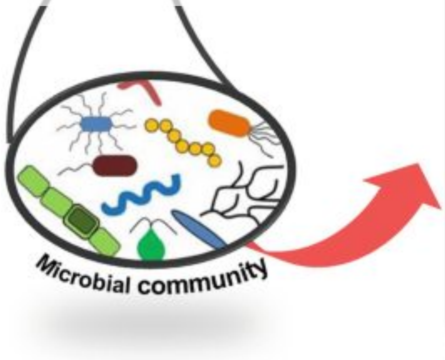


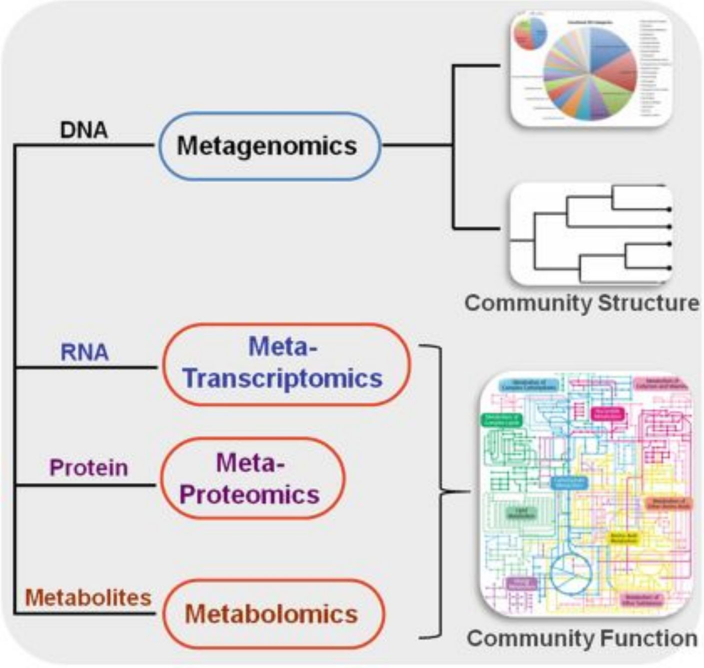
HDPE with ELA 1.5m 2-mo biofilm (2021)

‘Omics’ to identify mechanisms of *multi-species metabolic cooperation* in PE biodegradation

APPROACH 4
Microbial Consortia
(*in vitro* weathering)

LDPE with UMBS 1.5m
2-mo biofilm (2021)





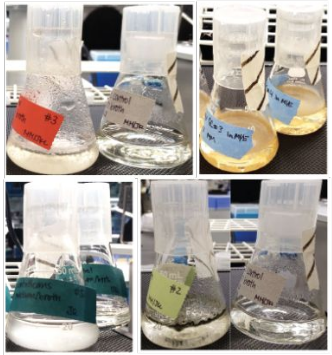
- Combine with stable isotope probing of 13C-PE to track polymer carbon fate in complex communities
- Mass-spec imaging (NanoSIMS) to localize PE degradation in the biofilm structure and determine cell-specific PE-C uptake rates

Future

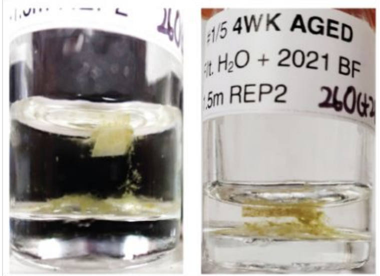
APPROACH 1
UV Accelerated Aging
(simulated weathering)

APPROACH 2
Field Deployments
(*in situ* weathering)

APPROACH 3
Microbial Isolates
(*in vitro* weathering)



APPROACH 4
Microbial Consortia
(*in vitro* weathering)



Looking to naturally evolved systems for novel engineered strategies for depolymerization of plastics

Acknowledgments

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University of Michigan

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Dow Chemical Company

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