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Fungi in marine plastisphere: ecological role and biotechnological potential

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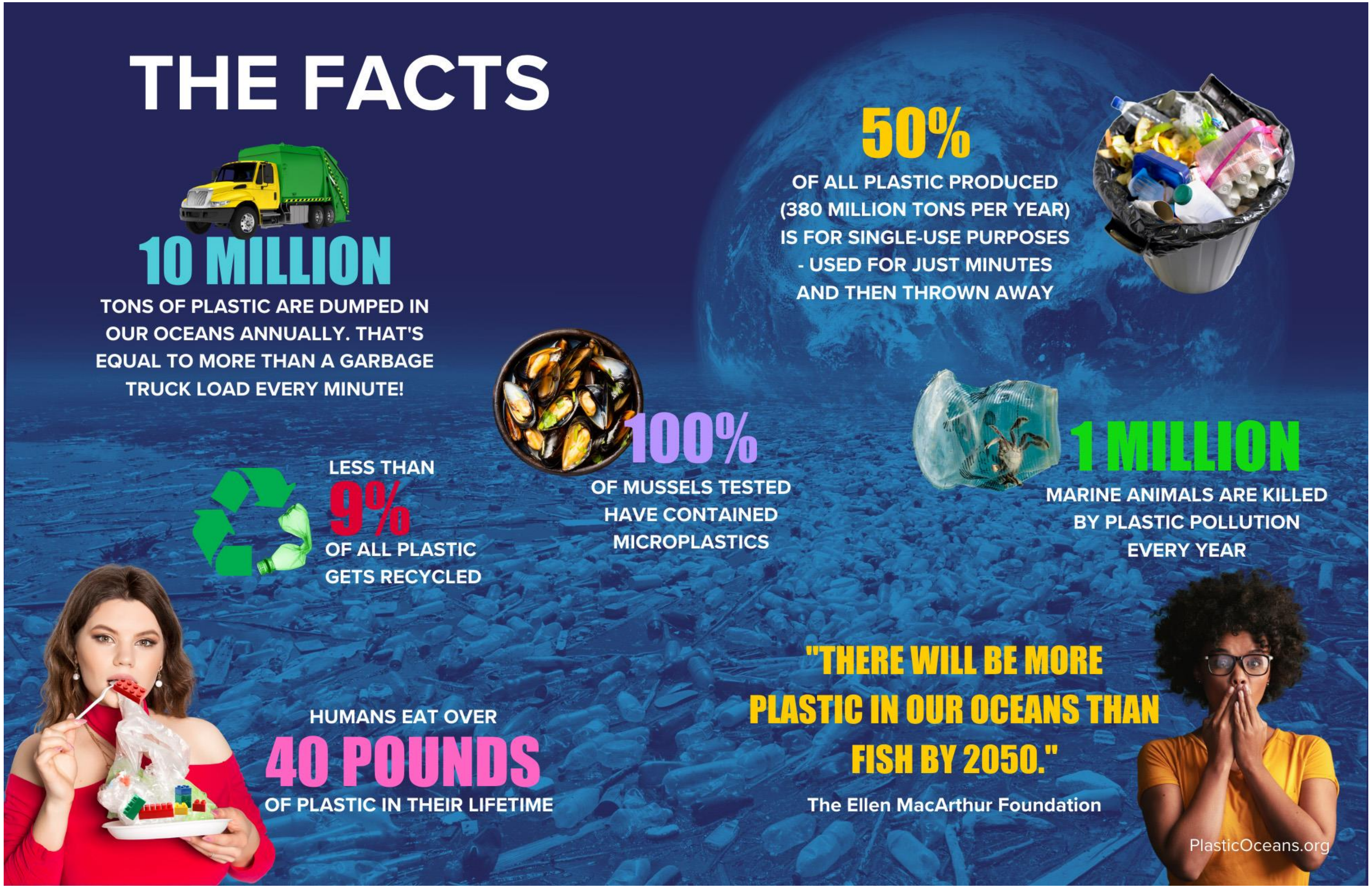
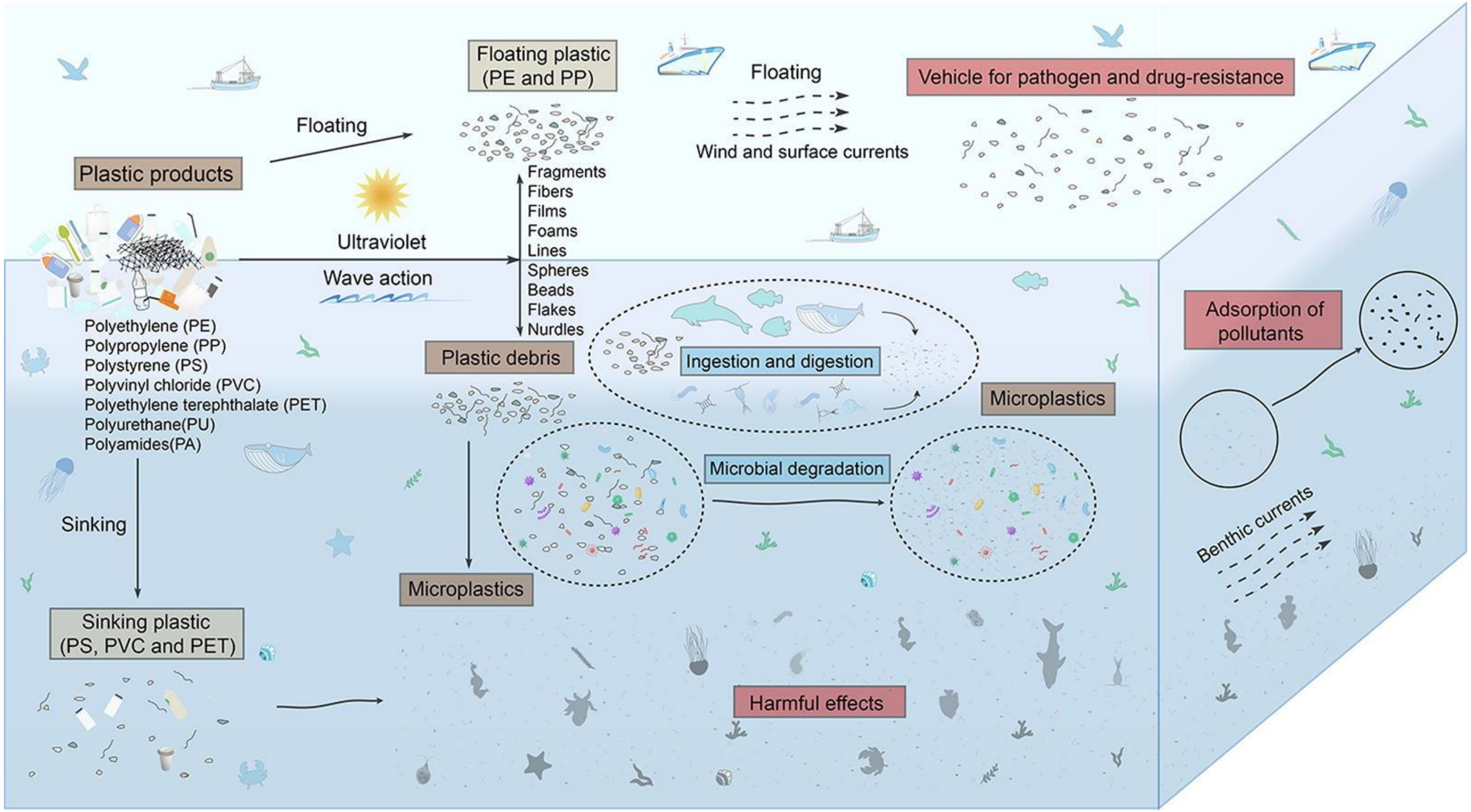
THE PLASTISPHERE

Annual world production: ~ 400 Mt; < 1% bioplastics as eco-friendly alternative



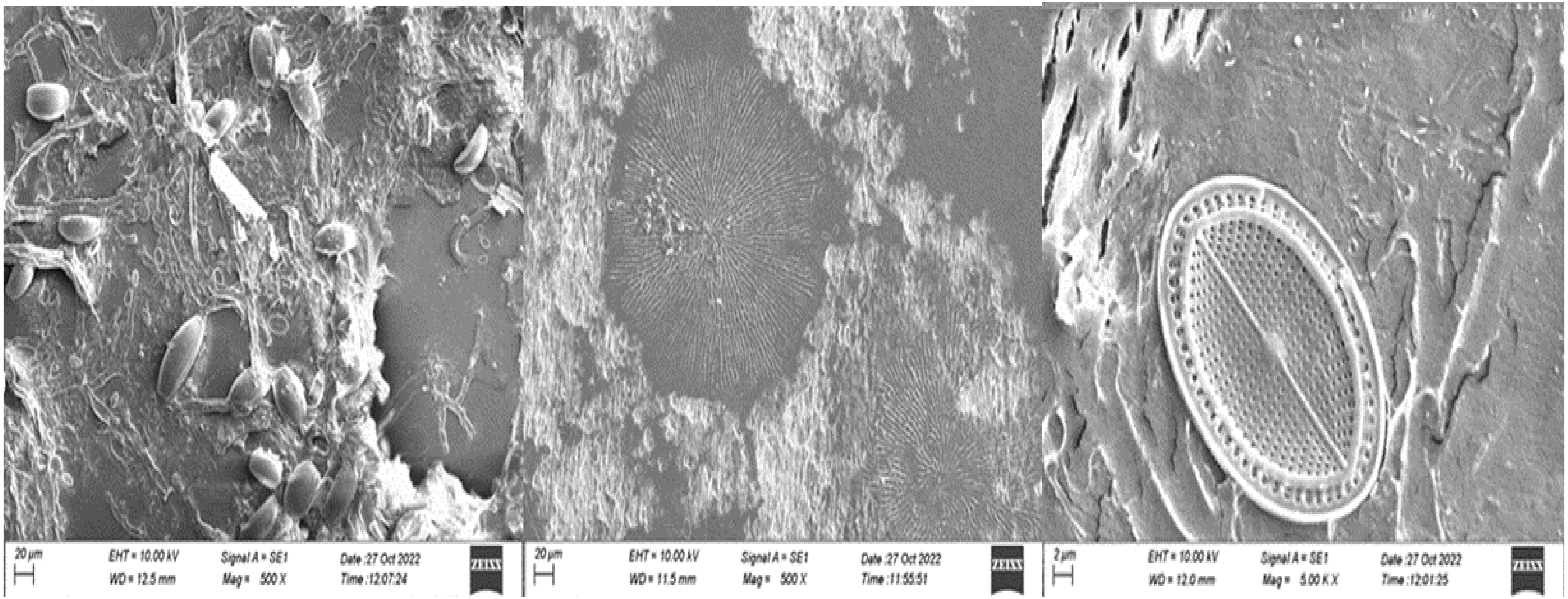
12 million tons of plastic and 51 trillions of plastic particles float in the oceans impacting the functionality and the food chain because of the intrinsic toxicity of plastic and because they can transport different pathogens and pollutants.



@https://doi.org/10.1016/j.csbj.2022.02.008

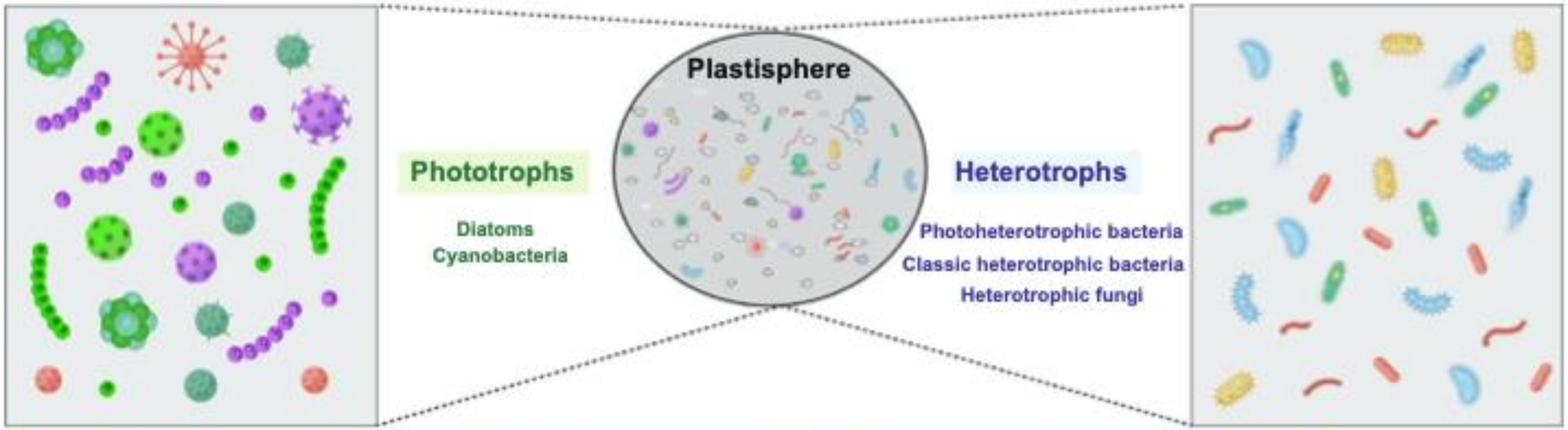
THE PLASTISPHERE

Microorganisms colonize the surface of MPs, forming biofilms called plastisphere, a novel microbial community attached to plastic and distinct from the surroundings.



SEM images showing presence and signs of microorganisms (diatoms, fungi and bacteria) adhered to the surface of the plastic after the washing procedure.

Microscopic and molecular sequence data indicate that plastisphere is composed of primary producers, heterotrophs, symbionts, and predators



Diatoms
Mastogloia
Navicula
Nitzschia
Cyclotella
Pleurosigma
Sellaphora
Amphora
Nitzschia
. . . .

Cyanobacteria
Prochlorotrichaceae:
Phormidium
Rivulariaceae:
Rivularia
. . . .

Photoheterotrophic bacteria
Erythrobacteraceae:
Erythrobacter
Rhodobacteraceae:
Roseobacter
. . . .

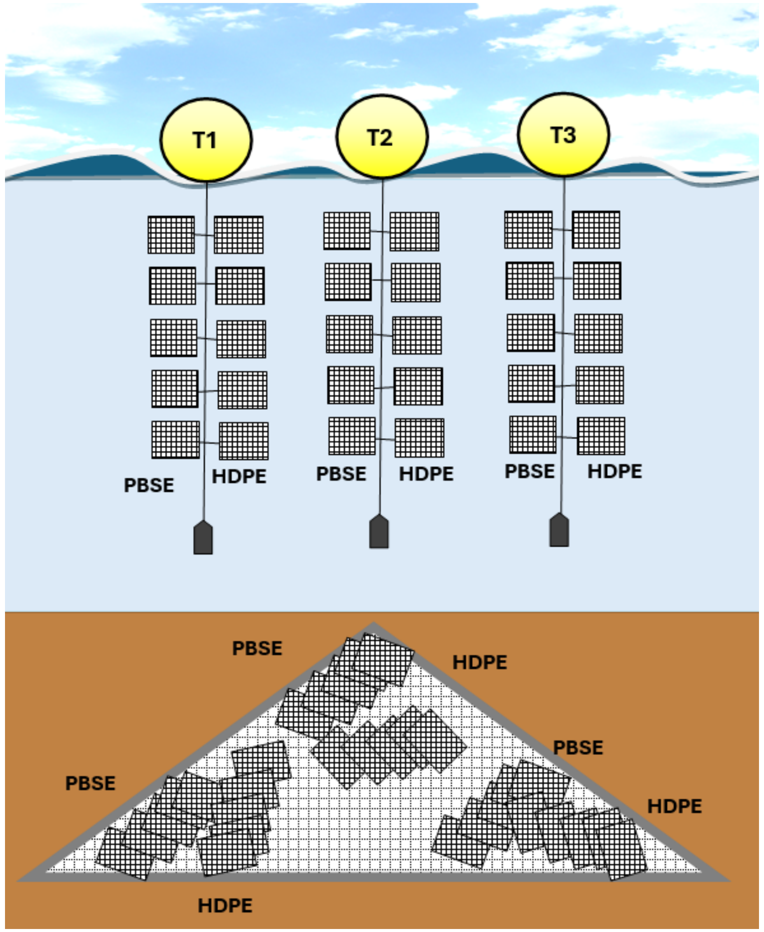
Classic heterotrophic bacteria
Pseudomonadaceae:
Pseudomonas
Azotobacter
Bacillaceae:
Bacillus
Nocardiaceae:
Rhodococcus
Pirellulaceae
Phycisphaerales
. . . .
Cyclobacteriaceae
Acetobacteraceae:
Roseococcus
Rhodobacteraceae
Flavobacteriaceae
Burkholderiales
Vibrionaceae
Mycobacteriacea
Hyphomonadaceae
. . . .

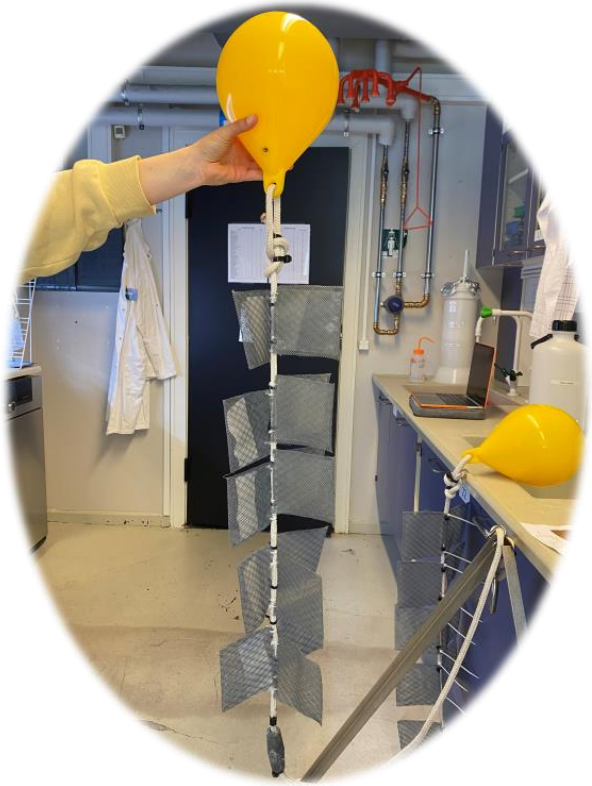
Heterotrophic fungi
Chytridiomycota:
Chytridium
Cryptomycota
Ascomycota:
Saccharomyces
Candida
Kazachstania
. . . .

Follow the fungal colonization and possible involvement in plastic degradation in marine environment

Experimental set up

- bioplastic/HDPE → 10x10 cm flags
- Flags enclosed into nets
- 2 locations in the North Sea
  (different salinity: Ise 11g/l-Faxe 23 g/l)
- Water column + Sediment (80 cm)
- Exposure period: 6 months
- Collection: every 2 months
  (3 timepoints)

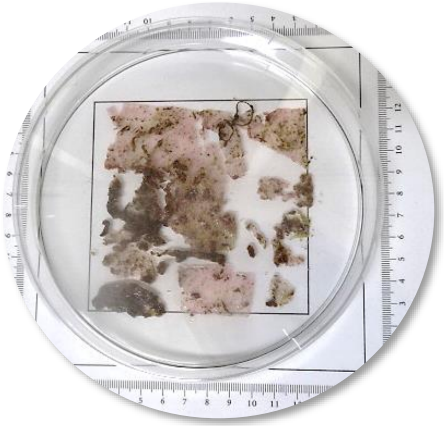




180 flags (90 in each site)

Once in the lab...

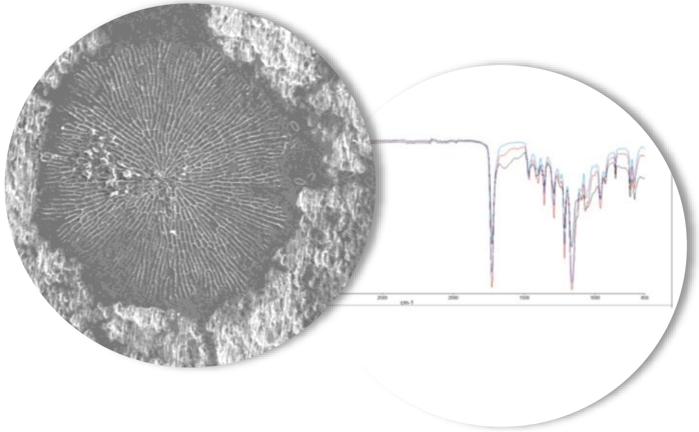
Image analysis



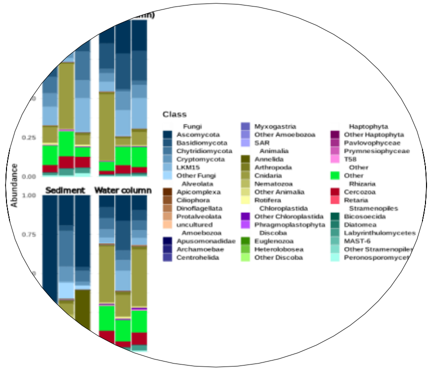
Culturomics

Biodegradation trials

SEM and ATR-FTIR



Metabarcoding



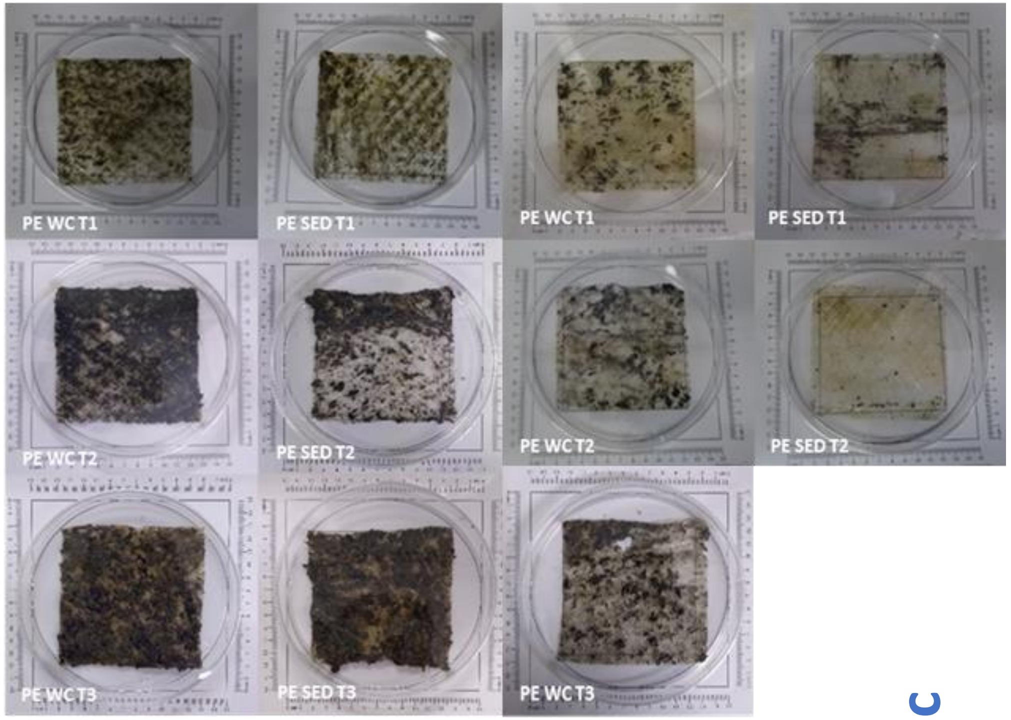
Genome Sequencing

Image analysis

HDPE

ISE FJORD | FAXE LADEPLADS

2 MONTH | 4 MONTHS | 6 MONTHS

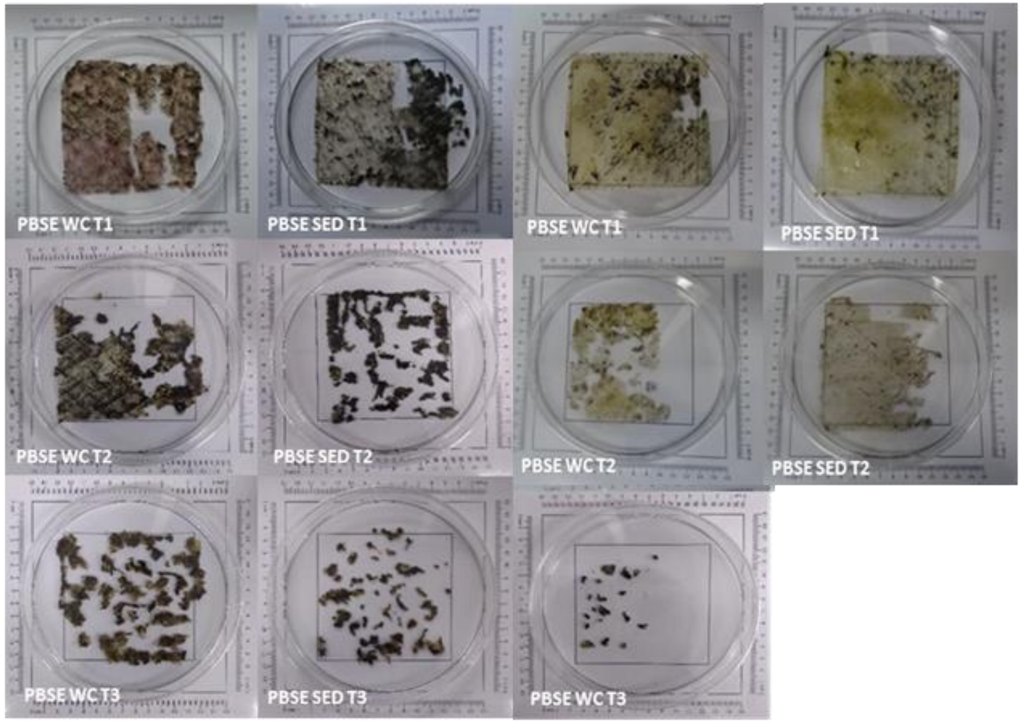


the plastic was almost completely intact

bioplastic

ISE FJORD | FAXE LADEPLADS

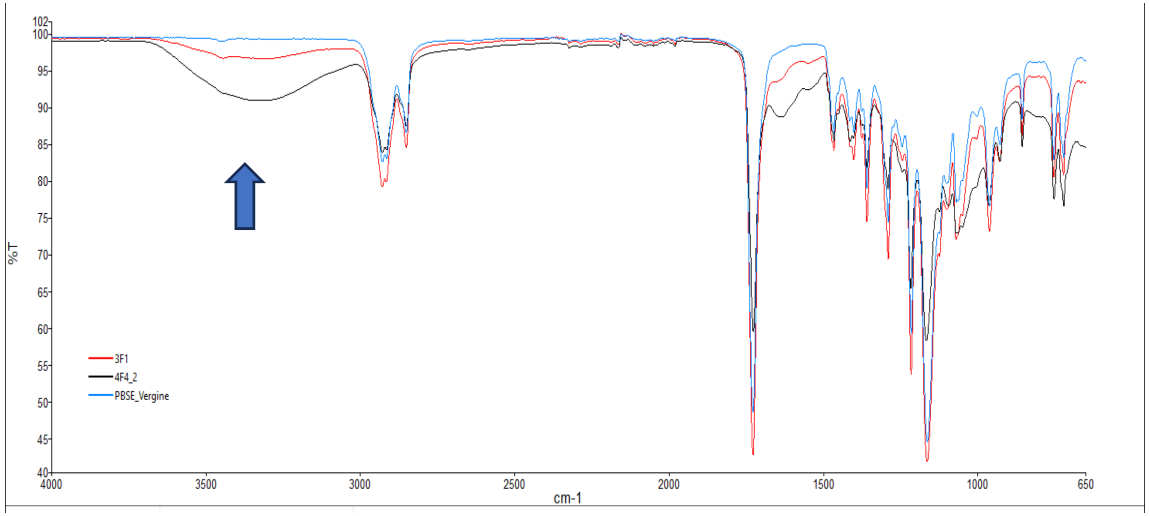
2 MONTH | 4 MONTHS | 6 MONTHS



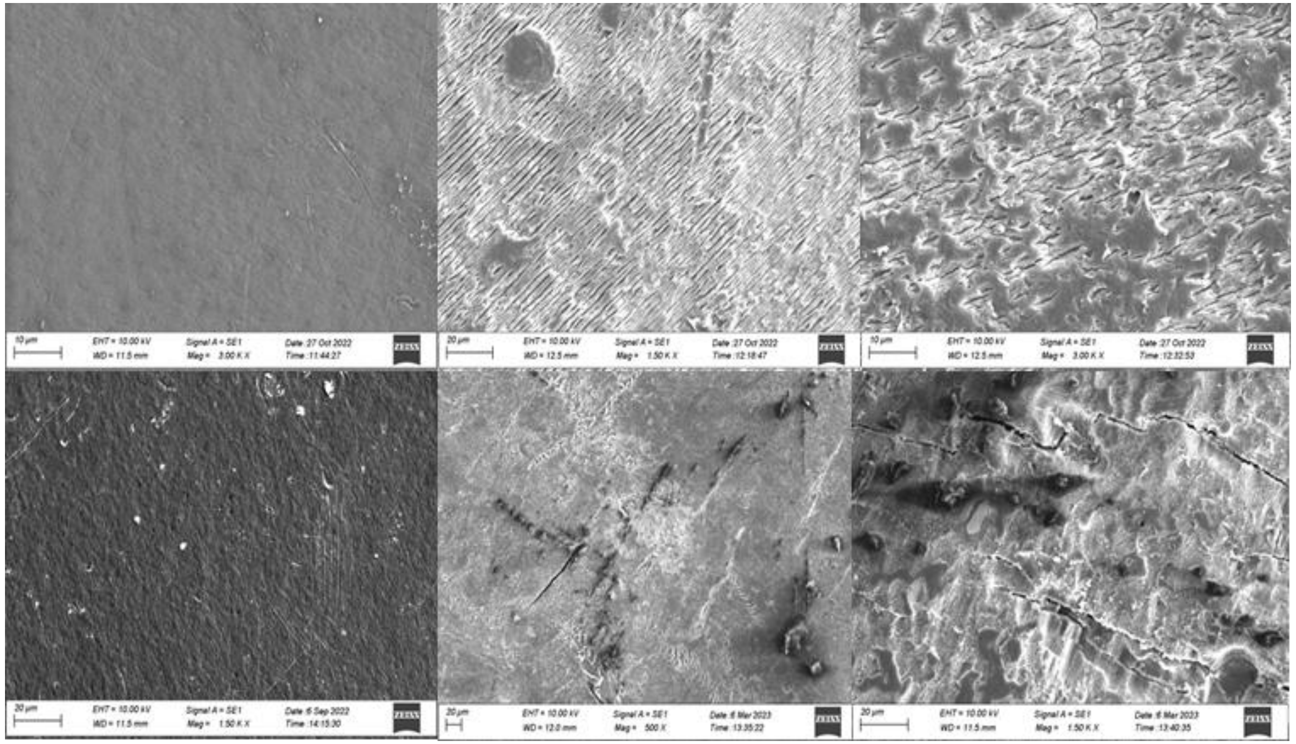
85-50% degradation after 6 months

Chemical and SEM analyses

No degradation of HDPE
Biodegradation of bioplastic



Control **2 months** **4 months**



- The ATR-FTIR spectra showed the appearance of a **very broad signal** (3000-3600 cm-1) typical of hydroxyl groups and two other signals between 1680 and 1500 cm-1 attributable to hydrolysis of the polymer
- PBSE appeared **more opaque and brittle** than virgin material
- SEM images showed numerous cracks in the films
- An **'accelerated' degradation study** (5 weeks with sterilised seawater) ruled out the possibility of this being attributable to the abiotic component of the marine environment alone

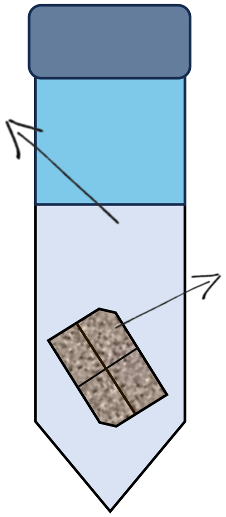
Culturomic

More than 2500 fungi were isolated during the entire experiment

Sonication

Surnatant

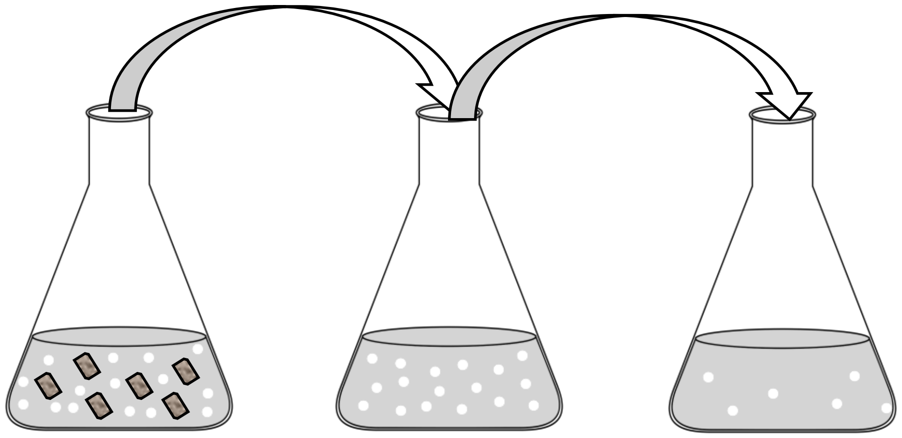
Fungi **not strictly** associated to HDPE/PBSE surface



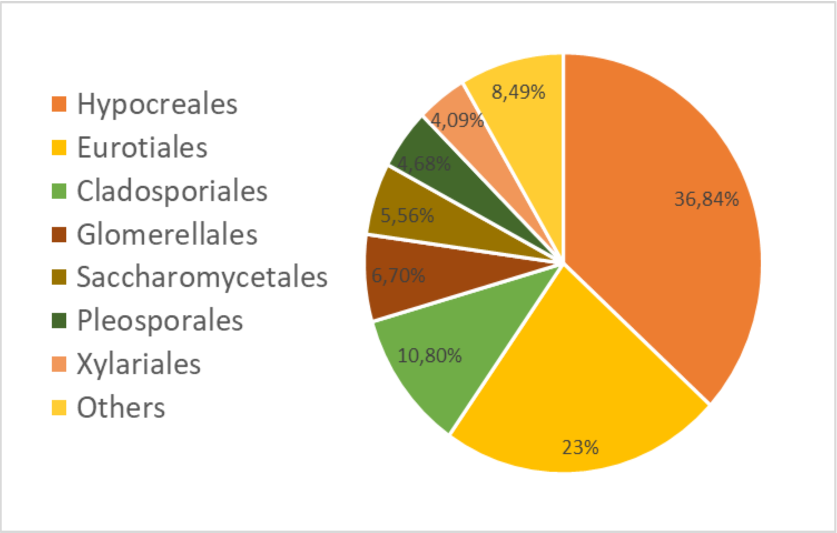
Direct

Fungi **tightly adhered**

Enrichment

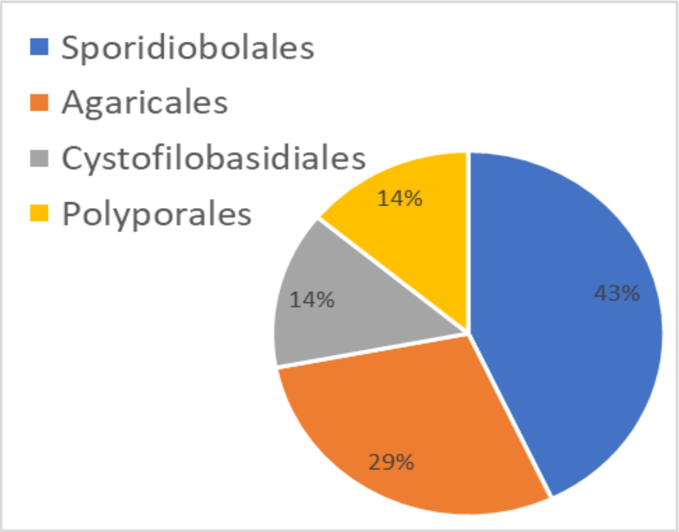


Ascomycota orders



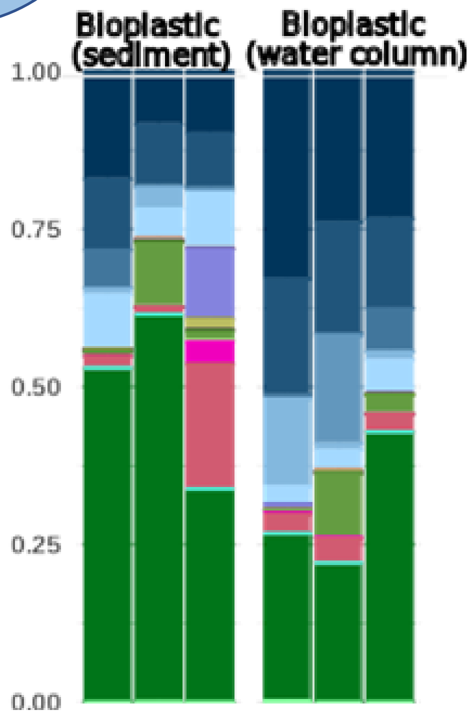
Culturable community was affected by the isolation methods, the type of plastic, the site and the matrix

Basidiomycota orders



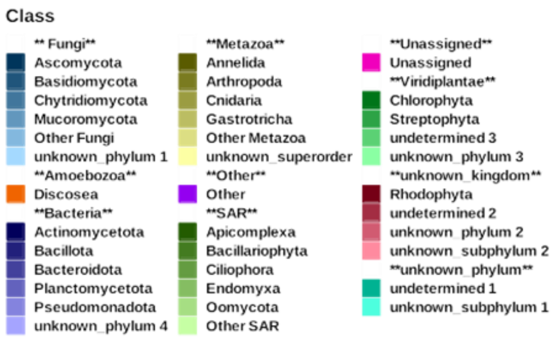
Metabarcoding

Metabarcoding approach for fungal marine plastisphere: ITS vs 18S



ITS
987 Taxa/ASV

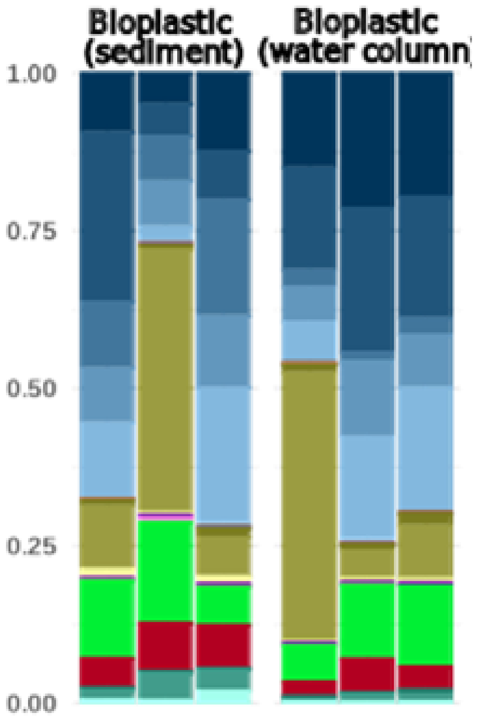
Algae



18S
3789 Taxa/ASV

Fungi

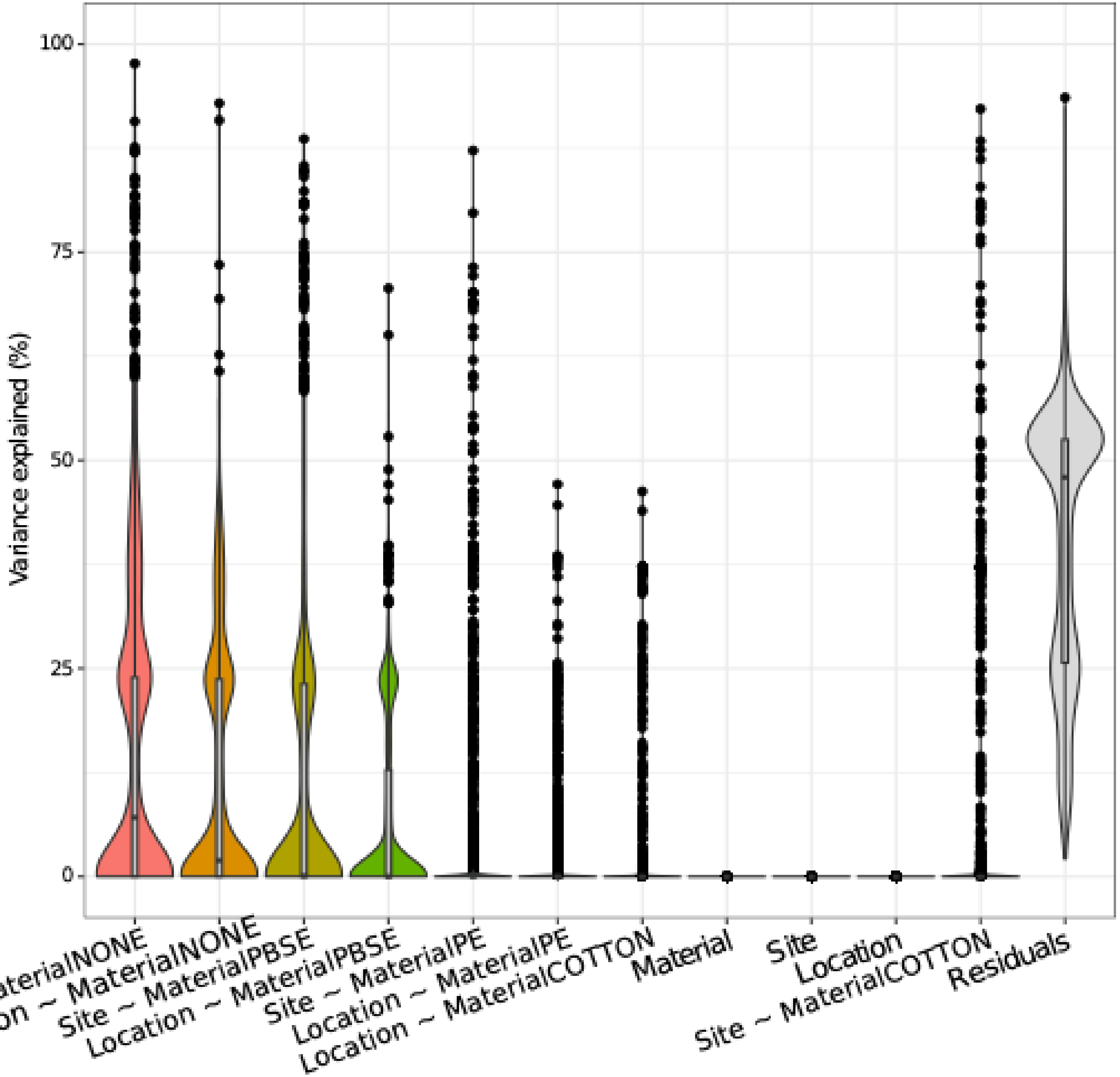
Chytridiomycota, Cryptomycota



The 18s blocking primers highlight a greater fungal biodiversity suggesting that fungi are prominent members of the plastisphere and gave a clearer overview of Chytridiomycota, Criptomycota, and Zoosporomycota populations and of the supergroup LKM15.

Metabarcoding

The fungal community organization mostly changed based on a combination of material type and site, while the location of plastics (e.g. water column/sediment) had a lower impact.



Residual variation suggests other parameters might influence the abundance of the fungal taxa, e.g. sporadic presence of invertebrate or algal host, etc.

The fungal plastisphere is complex with a huge network of interaction among the different components.

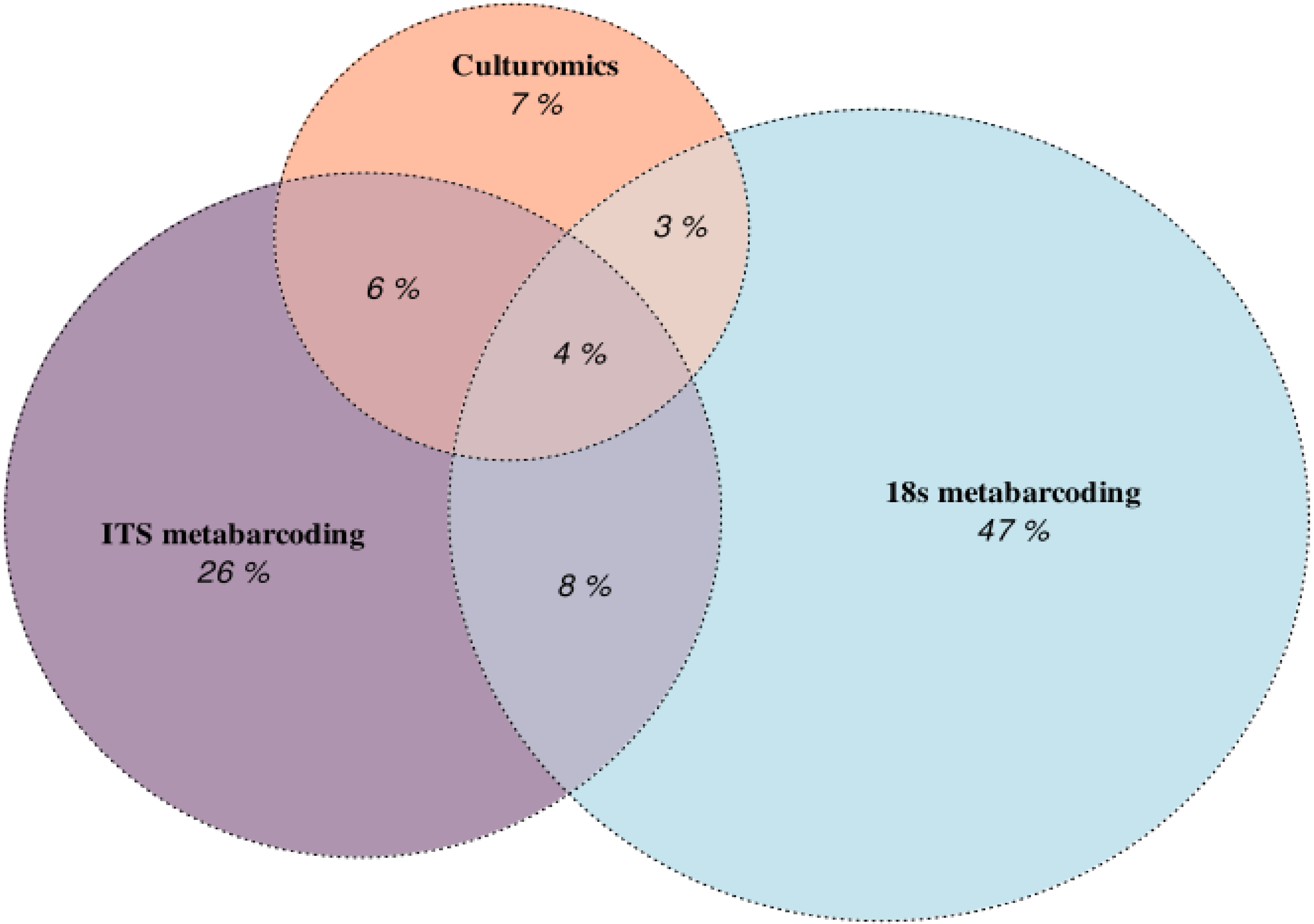
Network of putative interactions among fungi/pseudofungi in the plastisphere

Keystone taxa

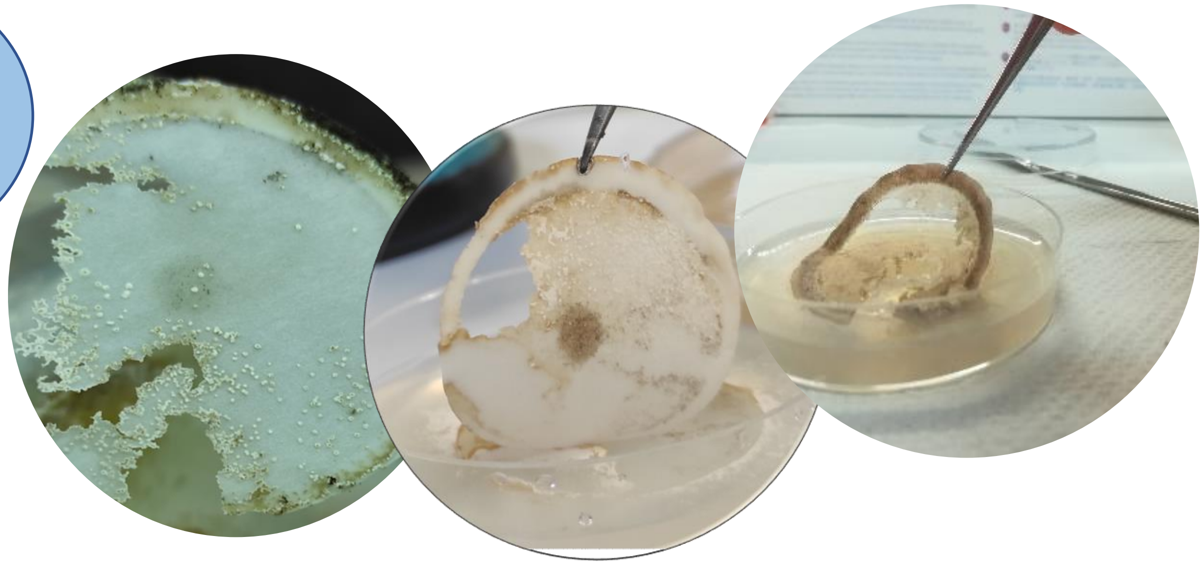
Metabarcoding (ITS vs 18S) vs Culturomic in fungal marine plastisphere

Metabarcoding vs Culturomic

Only a combination of techniques can provide a comprehensive vision of the fungal communities inhabiting plastisphere.

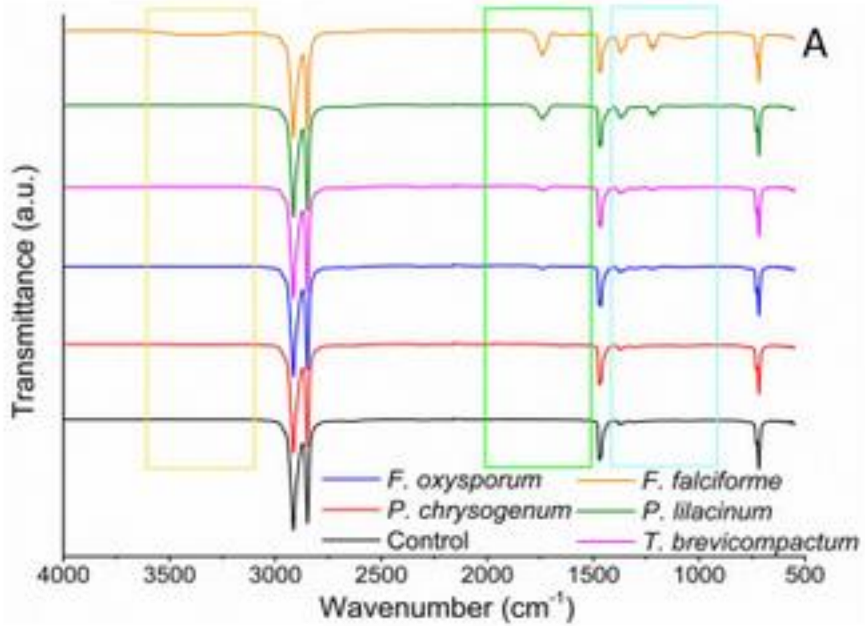


Degradation test

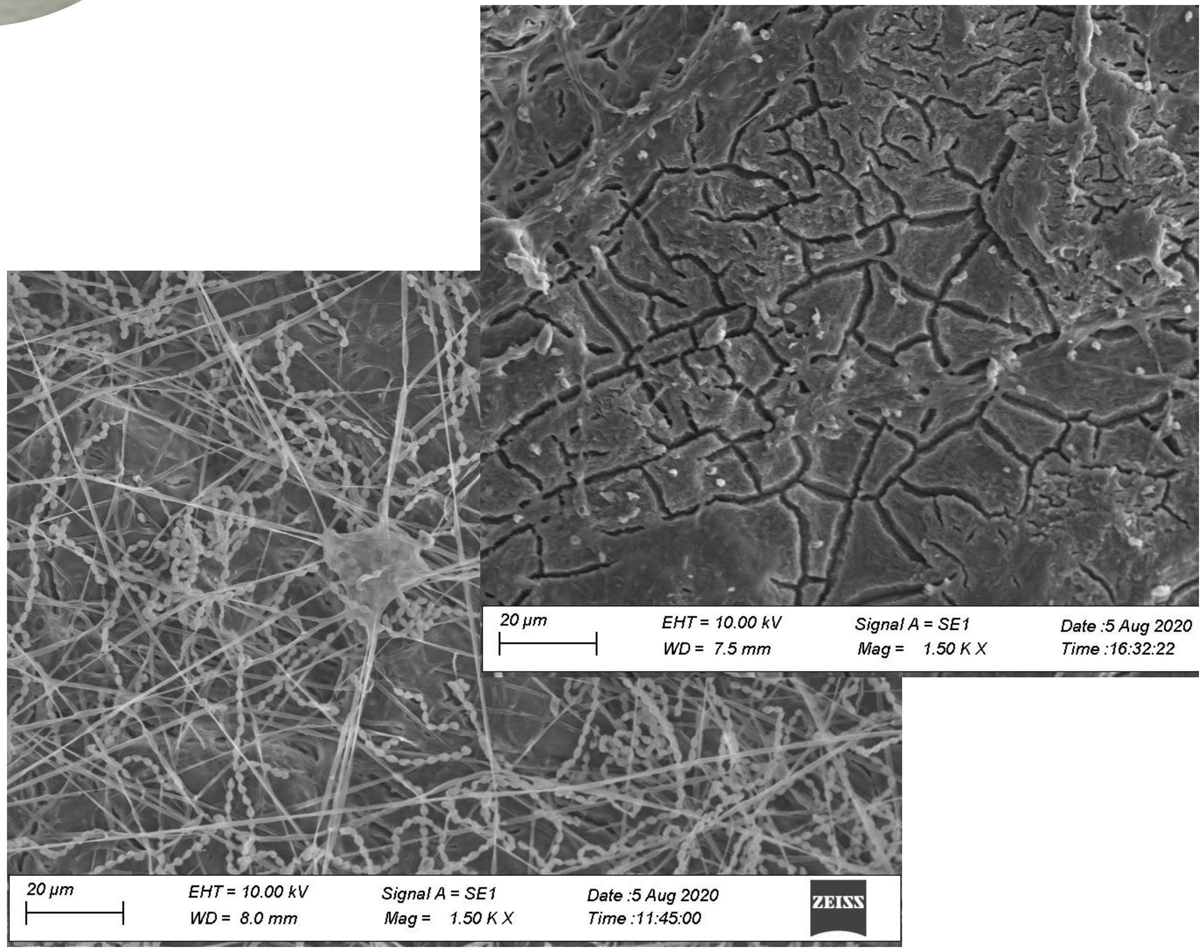


In several cases, degradation of (**bio)plastic** was massive.

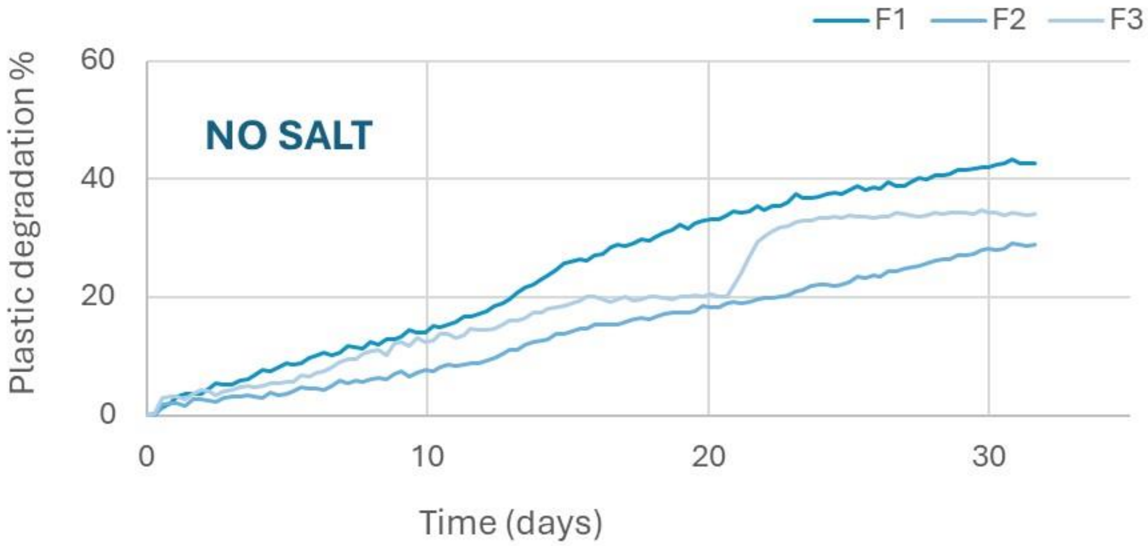
Confirmed by SEM and FT-IR analyses

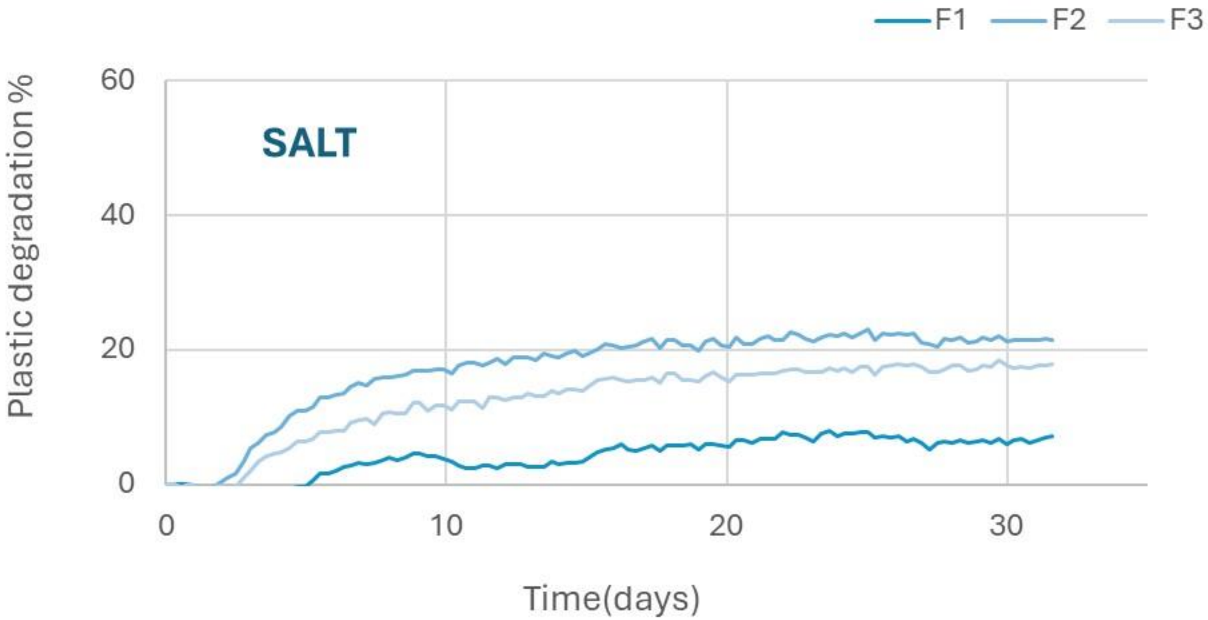


PE film biotransformation



Respirometric test

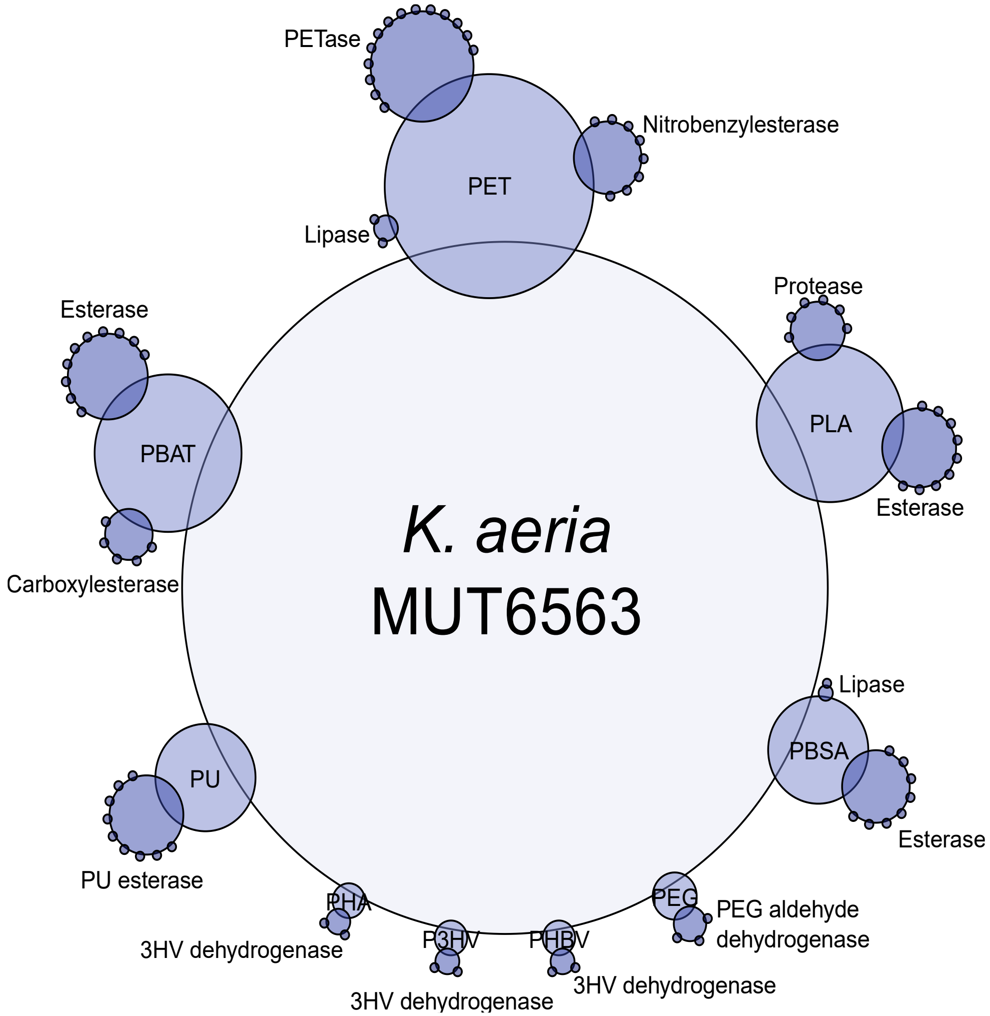


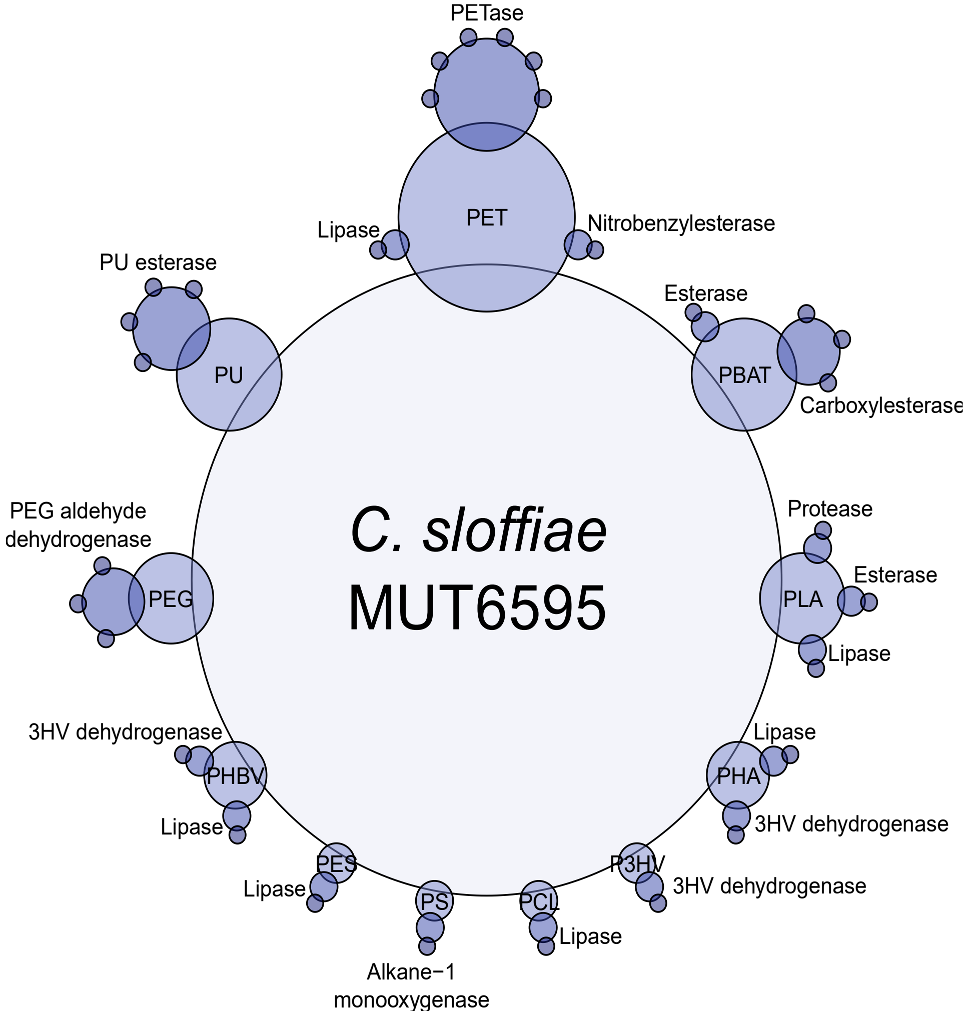


Respirometric trials demonstrated the actual bioplastic degradation (40%), but also the strong impact of salt in the process.

Genome sequencing

The genome analysis showed they have a great variety of putative Plastic Degrading enzymes (PAZy) able to target many plastic polymers





Venice and Florio Furno et al., 2024, in preparation

Conclusions and Perspectives

- This study represents **one of the first studies of plastic degradation in the natural environment**.

- **Different degradation behaviour** between traditional polymer (PE) and biodegradable bioplastic (PBSE): PE showed no molecular change over 6 months while PBSE showed high **biotransformation** (biodegradation < 80%).

- The **results** obtained with culturomic approach **confirmed those of previous studies:** Ascomycota are the most abundant *phylum* followed by Basidiomycota almost all yeasts.

- **The fungal plastisphere displayed a great biodiversity** with a complex networking.

- Degradation and respirometric tests showed **interesting biodegradation capabilities** of several strains that mirror the genetic peculiarities of these strains (Pazy enzymes).

- Some of these results could could have some **ecological** and **biotechnological applications** such as the **development of more biodegradable plastics, the use of fungi or their enzymes for bioremediation or the reuse of plastics under controlled conditions (circular economy).**

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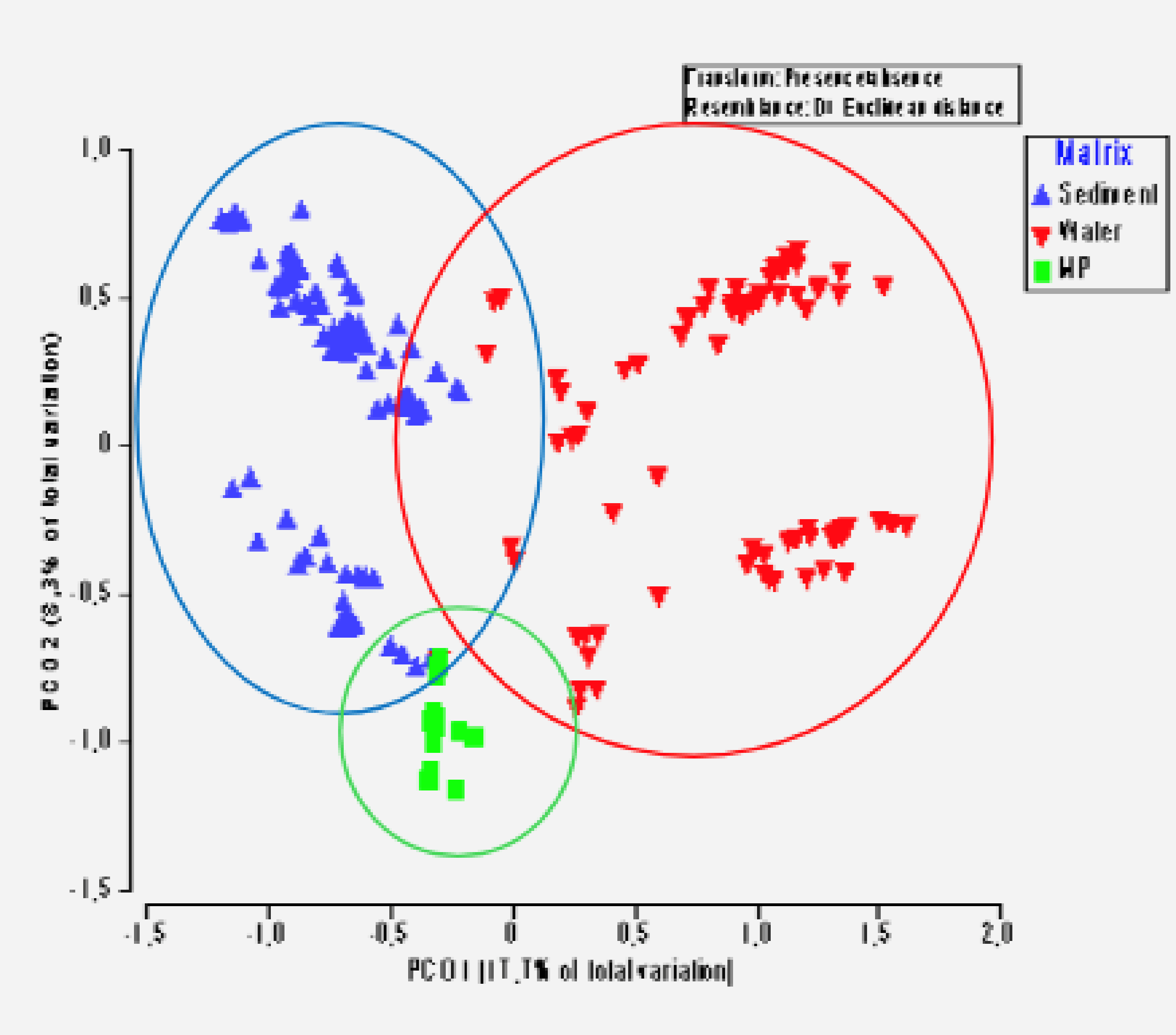
Thank you

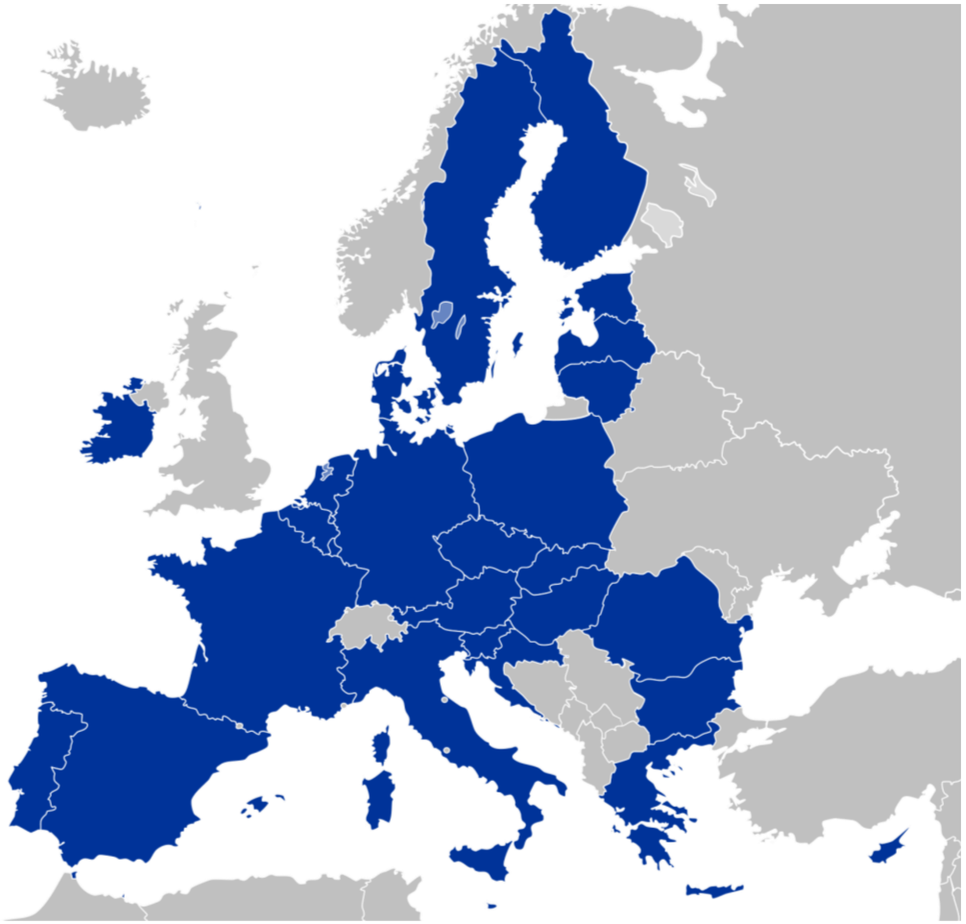
MUT team
Copenhagen University
Novamont Spa

Fungal communities associated to microplastic, sediment and water collected in the Tirrenian Sea

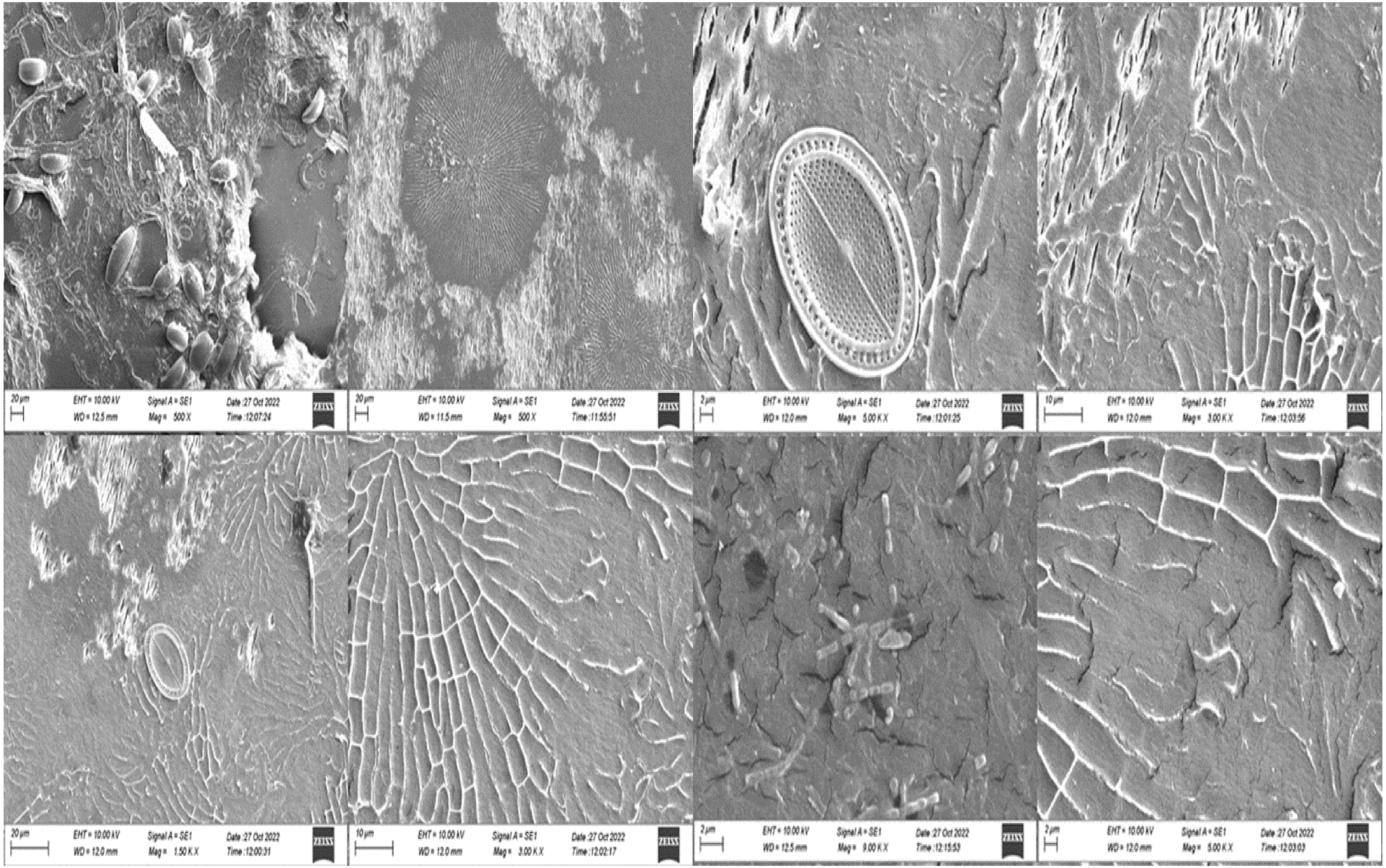


@Florio Furno et al., 2022 https://doi.org/10.3390/jof8090927

The organic compounds of the plastic coupled with the various substances adsorbed make MPs a unique substrate for microbial attachment in the ocean.
Assuming one plastic particle with a diameter of 1 mm per cubic meter of seawater, these MPs could provide a surface area of 4.2 million square kilometers, which would correspond to the EU area!



SEM images showing presence and signs of microorganisms (diatoms and fungi) adhered to the surface of the PBSE after the washing procedure



5. Culturomic approach

Fungal community composition