Istituto Zooprofilattico Sperimentale delle Venezie

Nano e microplastiche: identificazione e caratterizzazione del pericolo microbiologico

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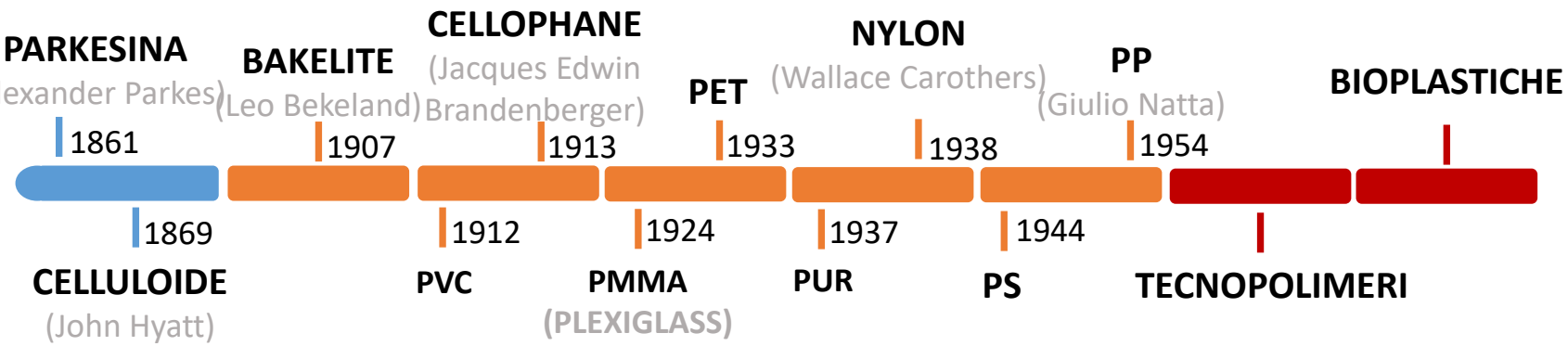
Istituto Zooprofilattico Sperimentale delle Venezie

Di cosa parlerò

- Contesto: origine delle plastiche, produzione e rifiuto
- La plastisfera
- Caratterizzazione del pericolo microbiologico

Contesto: origine delle plastiche, produzione e rifiuto

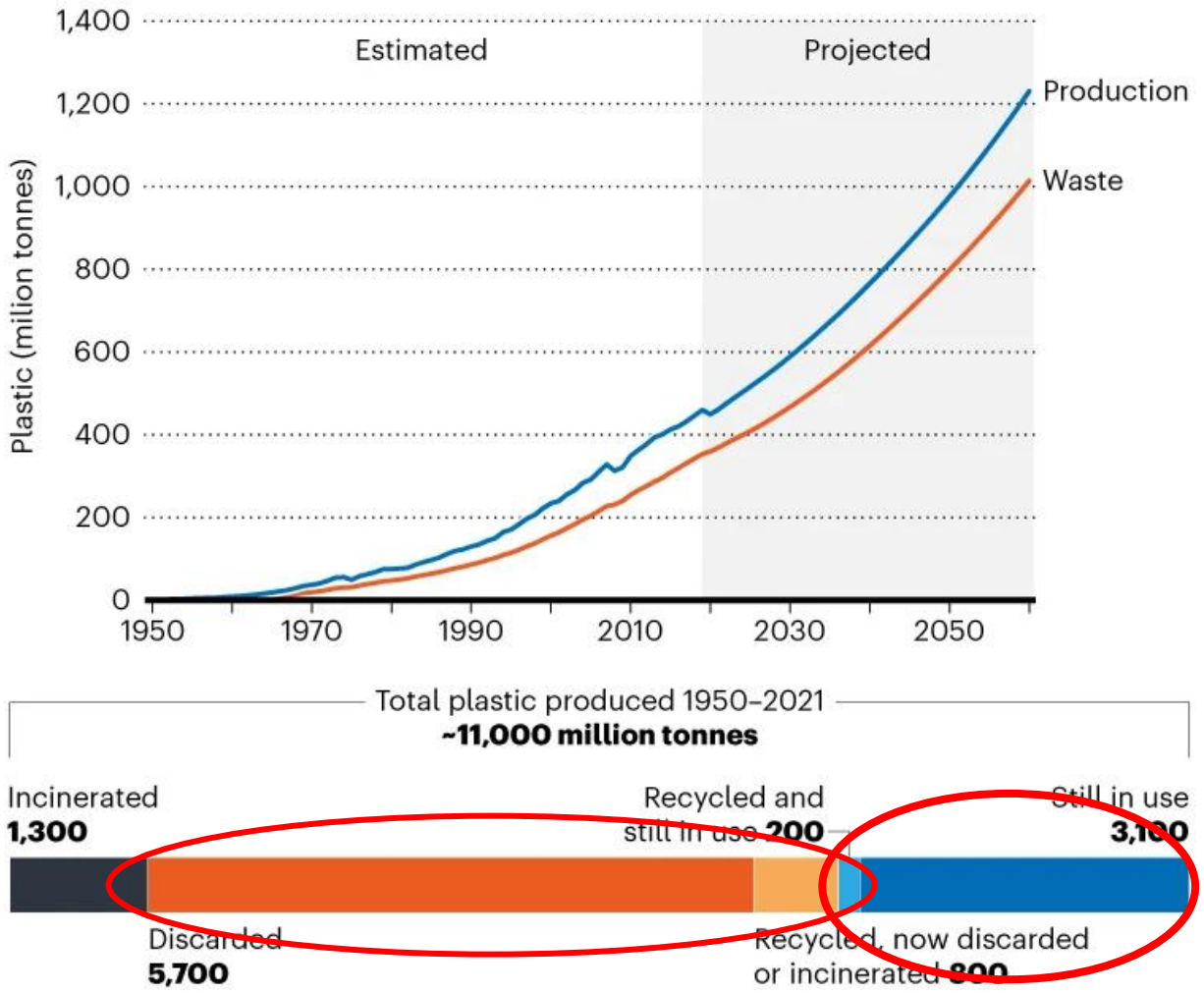
L’origine della plastica



- rapporto resistenza/peso elevato
- elevata modellabilità
- impermeabilità ai liquidi
- resistenza alla degradazione
- basso costo

Possono persistere come rifiuto nell’ambiente per lungo tempo

Produzione e rifiuto

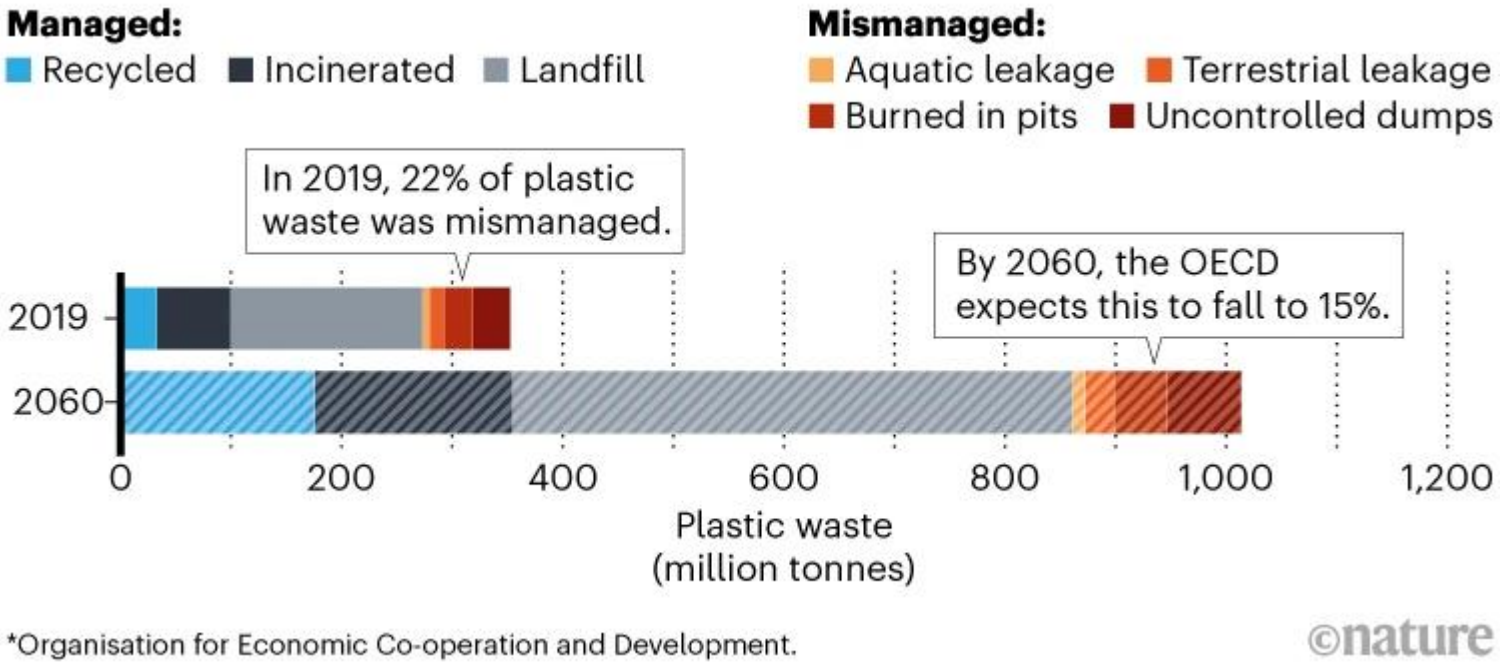


Si stima che, negli anni '50, la produzione e il consumo di plastica a livello mondiale ammontassero a **2 Mt annue**.
Nel 2021, questa cifra è salita a oltre **390 Mt** (Plastics Europe, 2022).

Se questa tendenza continuerà, la produzione globale di plastica supererà **1.000 Mt** nel 2050 (Zheng e Suh, 2019).

Nature 616, 234-237 (2023)
doi: https://doi.org/10.1038/d41586-023-00975-5

Dove finiscono i rifiuti plastici?



Fino al 2019 solamente il **9% dei rifiuti plastici è stato riciclato**, il 19% bruciato tramite inceneritori e il 50% è finito in discariche controllate. Il rimanente **22%** è stato smaltito in discariche non controllate, bruciato in fosse a cielo aperto o **disperso nell'ambiente**.

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La trappola di plastica del Mediterraneo

Il Mar Mediterraneo è oggi uno dei mari con i più alti livelli di plastica nel mondo. Secondo il rapporto "*Out of the Plastic Trap: Saving the Mediterranean from plastic pollution*", pubblicato dal WWF nel giugno 2018, le plastiche rappresentano il 95% dei rifiuti presenti in mare aperto, sul fondo marino e sulle spiagge del Mediterraneo.

MPs, prima descrizione (2004)

Millions of metric tons of plastic are produced annually. Countless large items of plastic debris are accumulating in marine habitats worldwide and may persist for centuries (*1–4*). Here we show that microscopic plastic fragments and fibers (Fig. 1A) are also widespread in the oceans and have accumulated in the pelagic zone and sedimentary habitats. The fragments appear to have resulted from degradation of larger items. Plastics of this size are ingested by marine organisms, but the environmental consequences of this contamination are still unknown.

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Over the past 40 years, large items of plastic debris have frequently been recorded in habitats from the poles to the equator (*1–4*). Smaller fragments, probably also plastic, have been reported (*5*) but have received far less attention. Most plastics are resistant to biodegradation, but will break down gradually through mechanical action (*6*). Many "biodegradable" plastics are composites with materials such as starch that biodegrade, leaving behind numerous, nondegradable, plastic fragments (*6*). Some cleaning agents also contain abrasive plastic fragments (*2*). Hence, there is considerable potential for large-scale accumulation of microscopic plastic debris.

To quantify the abundance of microplastics, we collected sediment from beaches and from estuarine and subtidal sediments around Plymouth, UK (Fig. 1B). Less dense particles were separated by flotation. Those that differed in appearance to natural particulate material (Fig. 1A) were removed and identified with Fourier Transform infrared (FT-IR) spectroscopy (*7*). Some were of natural origin and others could not be identified, but about one third were synthetic polymers (Fig. 1C). These polymers were present in most samples (23 out of 30), but were significantly more abundant in subtidal sediment (Fig. 1D). Nine polymers were conclusively identified: acrylic, alkyd, poly (ethylene:propylene), polyamide (nylon), polyester, polyethylene, polymethylacrylate, polypropylene, and polyvinyl-alcohol. These have a wide range of uses, including clothing, packaging, and rope, suggesting that the fragments resulted from the breakdown of larger items.

To assess the extent of contamination, a further 17 beaches were examined (Fig. 1B). Similar fibers were found, demonstrating that microscopic plastics are common in sedimentary habitats. To assess long-term trends in abundance, we examined plankton samples collected regularly since the 1960s along routes between Aberdeen and the Shetlands (315 km) and from Sule Skerry to Iceland (850 km) (*7*) (Fig. 1B). We found plastic archived among the plankton in samples back to the 1960s, but with a significant increase in abundance over time (Fig. 1E). We found similar types of polymer in the water column as in sediments, suggesting that polymer density was not a major factor influencing distribution.

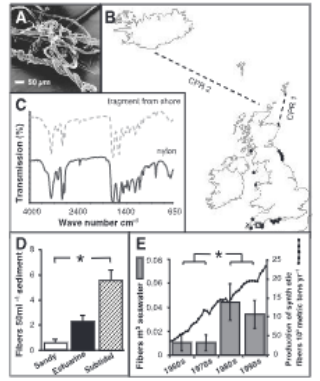

Fig. 1. (**A**) One of numerous fragments found among marine sediments and identified as plastic by FT-IR spectroscopy. (**B**) Sampling locations in the northeast Atlantic. Six sites near Plymouth (▽) were used to compare the abundance of microplastic among habitats. Similar fragments (●) were found on other shores. Routes sampled by Continuous Plankton Recorder (CPR 1 and 2) were used to assess changes in microplastic abundance since 1960. (**C**) FT-IR spectra of a microscopic fragment matched that of nylon. (**D**) Microplastics were more abundant in subtidal habitats than on sandy beaches (*, $F_{2,3} = 13.26$, $P < 0.05$), but abundance was consistent among sites within habitat types. (**E**) Microscopic plastic in CPR samples revealed a significant increase in abundance when samples from the 1960s and 1970s were compared to those from the 1980s and 1990s (*, $F_{3,3} = 14.42$, $P < 0.05$). Approximate global production of synthetic fibers is overlain for comparison. Microplastics were also less abundant along oceanic route CPR 1 than along CPR 2 ($F_{1,24} = 5.18$, $P < 0.05$).

Our findings demonstrate the broad spatial extent and accumulation of this type of contamination. Given the rapid increase in plastic production (Fig. 1E), the longevity of plastic, and the disposable nature of plastic items (*2*, *3*), this contamination is likely to increase. There is the potential for plastics to adsorb, release, and transport chemicals (*3*, *4*). However, it remains to be shown whether toxic substances can pass from plastics to the food chain. More work is needed to establish whether there are any environmental consequences of this debris.

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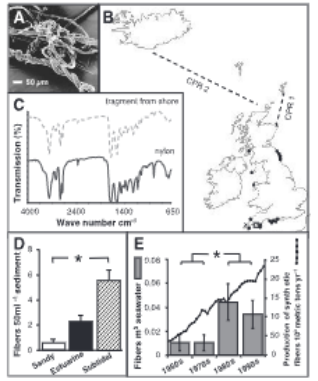

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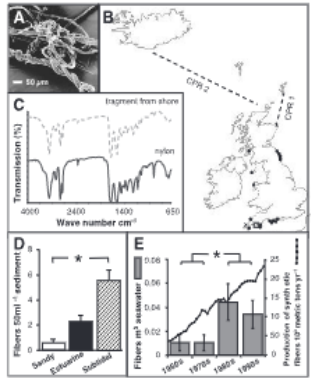

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Microplastiche: definizione

- MPs: dimensioni comprese tra 5 mm e 1 µm

MACROPLASTICHE

> 5000 µm

MICROPLASTICHE

0.1 > 5000µm

NANOPLASTICHE

0.001>0.1µm

Microplastiche primarie

Microplastiche secondarie

Pérez-Méndez MA, Fraga-Cruz GS, Domínguez-García S, Pérez-Méndez ML, Bocanegra-Díaz CI, Nápoles-Rivera F. Microplastic Pollution in Soil and Water and the Potential Effects on Human Health: A Review. Processes. 2025; 13(2):502. https://doi.org/10.3390/pr13020502

Le microplastiche nell'ambiente marino

Microplastiche primarie

Rilasciate direttamente in ambiente, rappresentano il 15-31% delle microplastiche presenti in mare

- **Lavaggio** di capi sintetici (35% delle microplastiche primarie)
- **Abrasione** dei pneumatici durante la guida (28%)
- Microplastiche **aggiunte intenzionalmente** nei prodotti per la cura del corpo (per esempio, le micro-particelle dello scrub facciale) 2%

Microplastiche secondarie

Rappresentano circa il 68-81% delle microplastiche presenti in mare

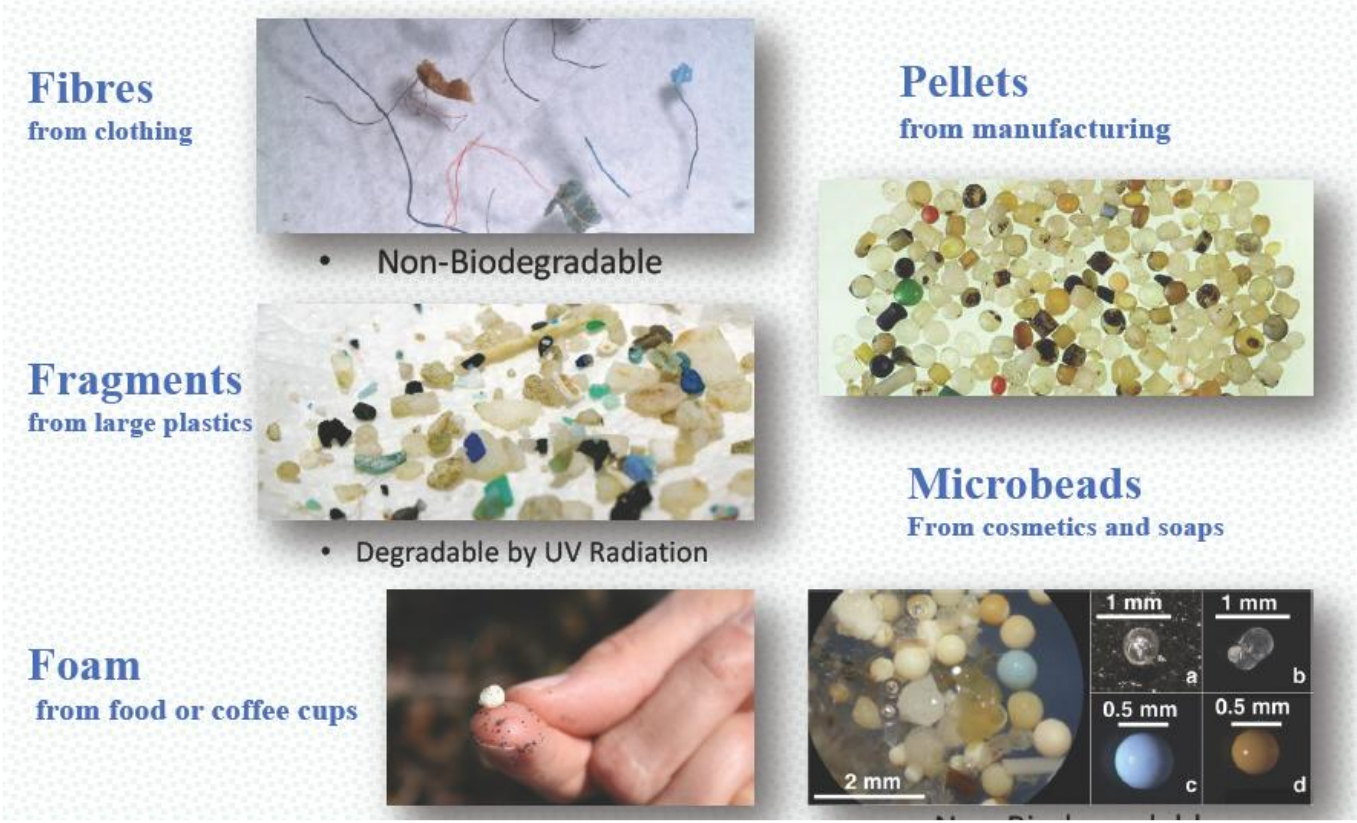
- Prodotte dalla degradazione degli oggetti di plastica più grandi, come buste di plastica, bottiglie o reti da pesca

Le microplastiche nell'ambiente marino: quali?

La frammentazione della plastica è causata da una combinazione di forze meccaniche, ad esempio onde e/o processi fotochimici innescati dalla luce solare.

La maggior parte delle microplastiche contengono polimeri organici :

- ✓ **Polietilene (PE)**
- ✓ **Polipropilene (PP)**
- ✓ Tereftalato di polietilene
- ✓ Polimetilmetacrilato
- ✓ Cloruro di polivinile (PVC)
- ✓ Policarbonato (PC)
- ✓ Poliammidi (PA)
- ✓ Poliestere (PES)



PE e PP sono i materiali più diffusi, essi hanno una densità inferiore a quella dell'acqua di mare e quindi sono galleggianti

La plastisfera

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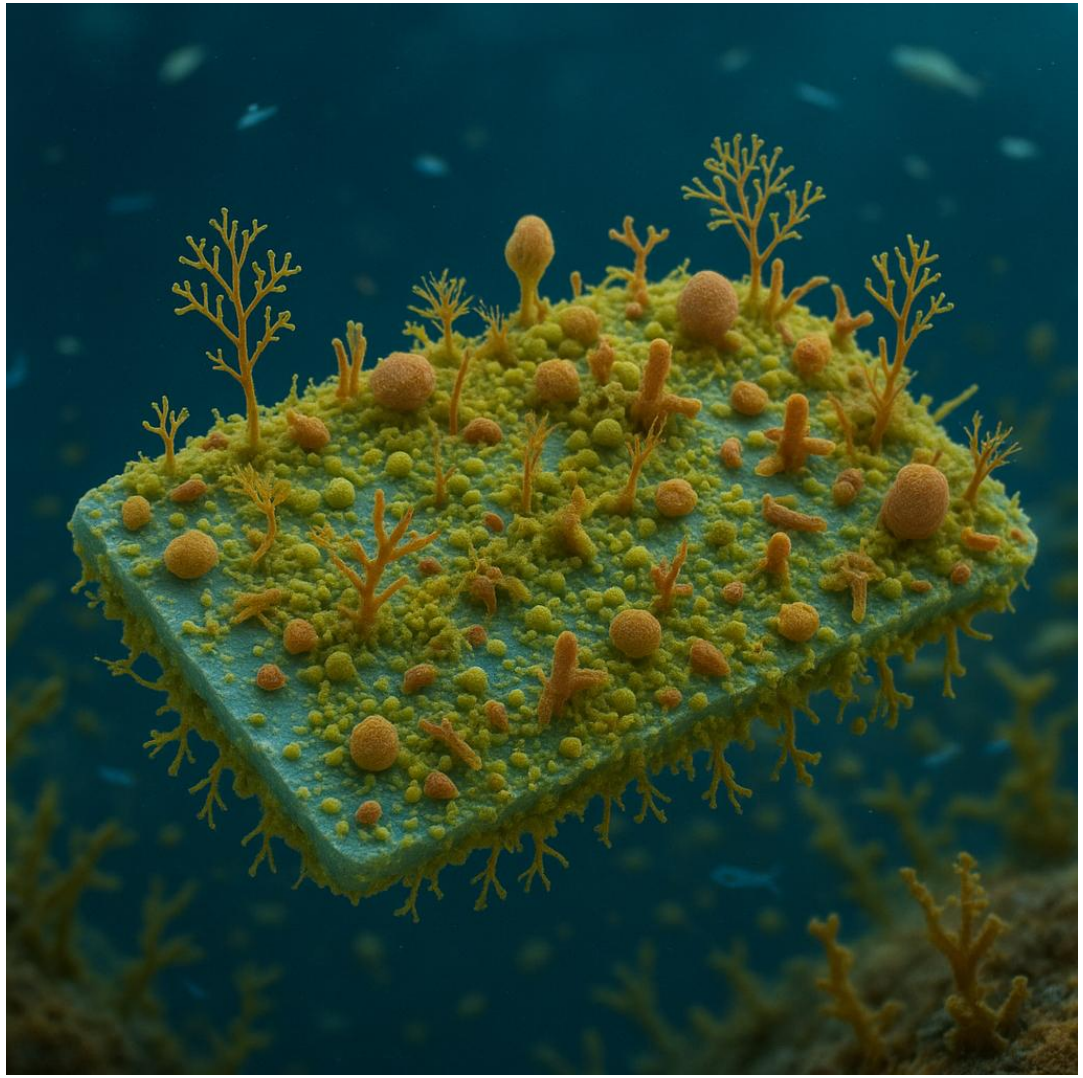
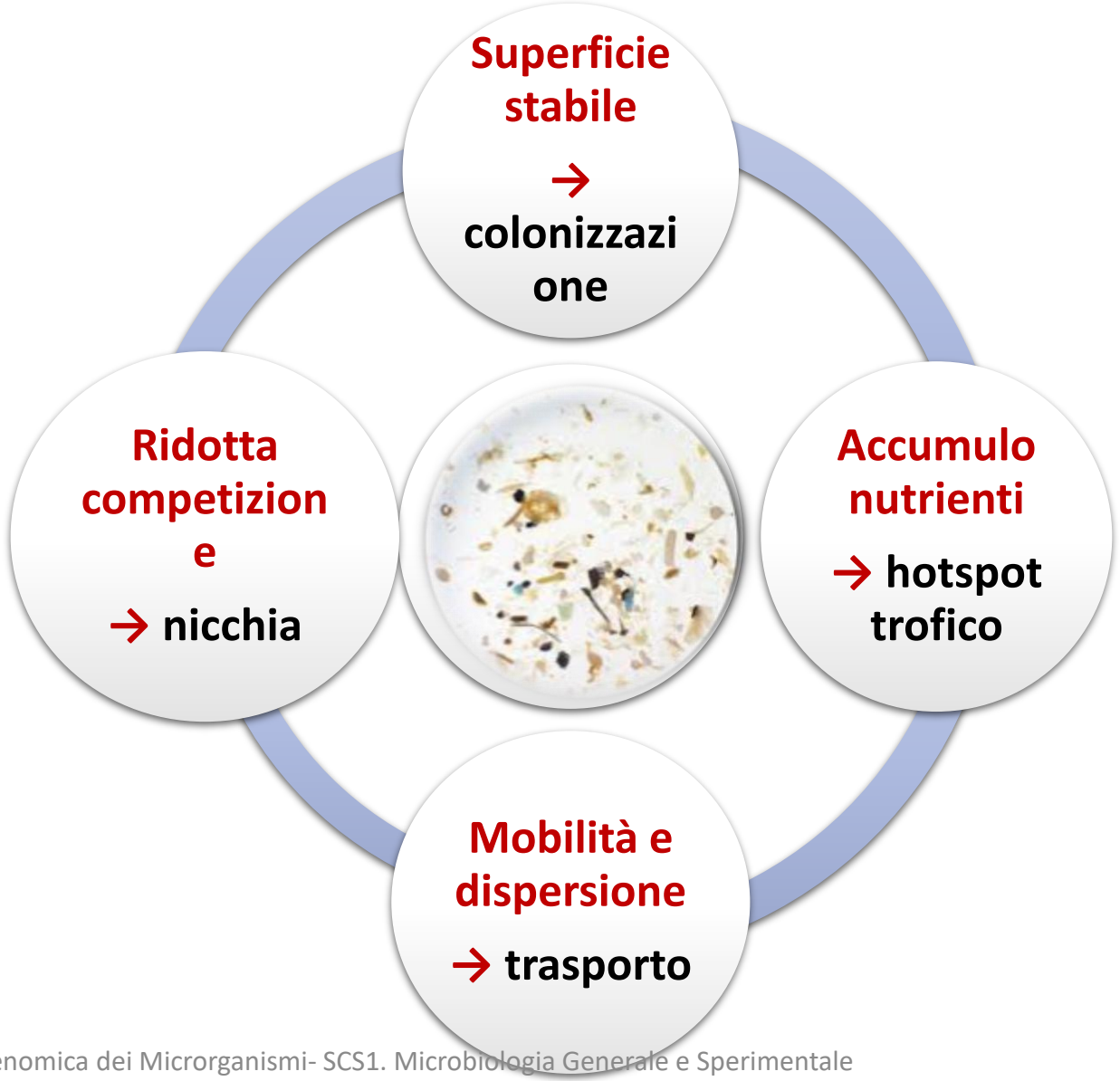


Il termine è stato coniato per la prima volta da *Zettler et al.,* indicando una **nuova nicchia ecologica microbica** che si sviluppa sulle superfici delle microplastiche disperse nell'ambiente.
E' composta da una comunità di microrganismi che aderiscono alla plastica formando un **biofilm stabile.**

Wu, X., et al. (2019). Water research
Zettler, E. R., Mincer, T. J., and Amaral-Zettler, L. A. (2013). Environmental science & technology
Zheng Z., et al. (2023). J. Hazard. Mater

Perché le microplastiche sono hotspot microbiologico?



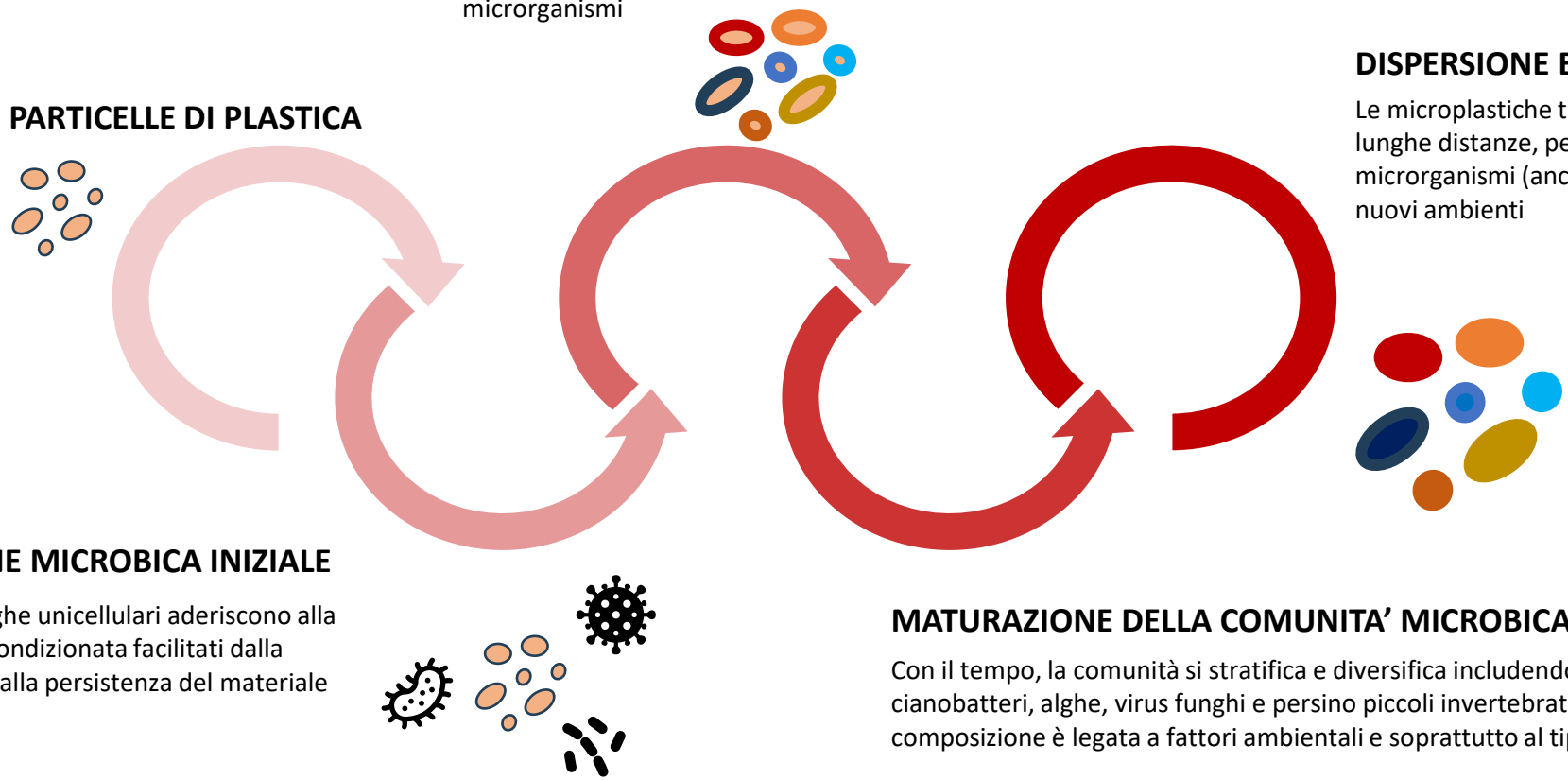
La Plastisfera biosintesi

FORMAZIONE DEL BIOFILM

I microrganismi pionieri iniziano a produrre esopolisaccaridi che aumentano l'adesione alla superficie plastica e facilitano la colonizzazione di altri microrganismi

CONDIZIONAMENTO DELLE PARTICELLE DI PLASTICA

Sostanze organiche e ioni presenti nell'ambiente formano un film molecolare che rende più appetibile la plastica per i microrganismi



DISPERSIONE E TRASPORTO

Le microplastiche trasportano la plastisfera su lunghe distanze, permettendo la diffusione di microrganismi (anche patogeni o invasivi) in nuovi ambienti

ADESIONE MICROBICA INIZIALE

Batteri e alghe unicellulari aderiscono alla superficie condizionata facilitati dalla rugosità e dalla persistenza del materiale plastico

MATURAZIONE DELLA COMUNITA' MICROBICA

Con il tempo, la comunità si stratifica e diversifica includendo batteri, cianobatteri, alghe, virus funghi e persino piccoli invertebrati. La composizione è legata a fattori ambientali e soprattutto al tipo di plastica

SCIENZA: RC IZSVe 02/20 e RF PLASTI@Risk

produrre una **valutazione esaustiva del rischio microbiologico per la salute umana associato all'esposizione a microplastiche (MPs) ingerite attraverso il consumo di molluschi bivalvi filtratori**, che permetterà l'individuazione di strategie di mitigazione del rischio.

> *"Il progetto mira a fornire un quadro ampio e realistico del pericolo microbiologico associato all'esposizione alle microplastiche attraverso il consumo di molluschi bivalvi"* afferma Carmen Losasso, responsabile del progetto, *"Si propone di suggerire strategie di mitigazione del rischio derivante sia dalle microplastiche attualmente circolanti che dalle bio-plastiche che si prevede circoleranno in futuro a seguito dell'applicazione della normativa comunitaria sull'uso delle plastiche".*

Le microplastiche rappresentano un rischio emergente per la sicurezza alimentare, introdotto dall'antropizzazione. Il trasferimento di agenti patogeni e di geni di resistenza agli antibiotici dall'ambiente antropizzato alla catena alimentare attraverso la circolazione di microplastiche, rappresenta un nuovo scenario di rischio per la sicurezza alimentare, consentendo lo stabilirsi di nuove vie di trasmissione dei patogeni e/o promuovendo la circolazione di nuovi patogeni o varianti di resistenza agli antibiotici.

Caratterizzazione del pericolo microbiologico

Caratterizzazione del pericolo microbiologico

- La biosintesi della Plastisfera include anche taxa potenzialmente patogeni?
- La comunità microbica delle MPs è diversa da quella dell'ambiente naturale?
- C'è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?

Comunità microbica e patobiota delle microplastiche prodotte e invecchiate in ambiente

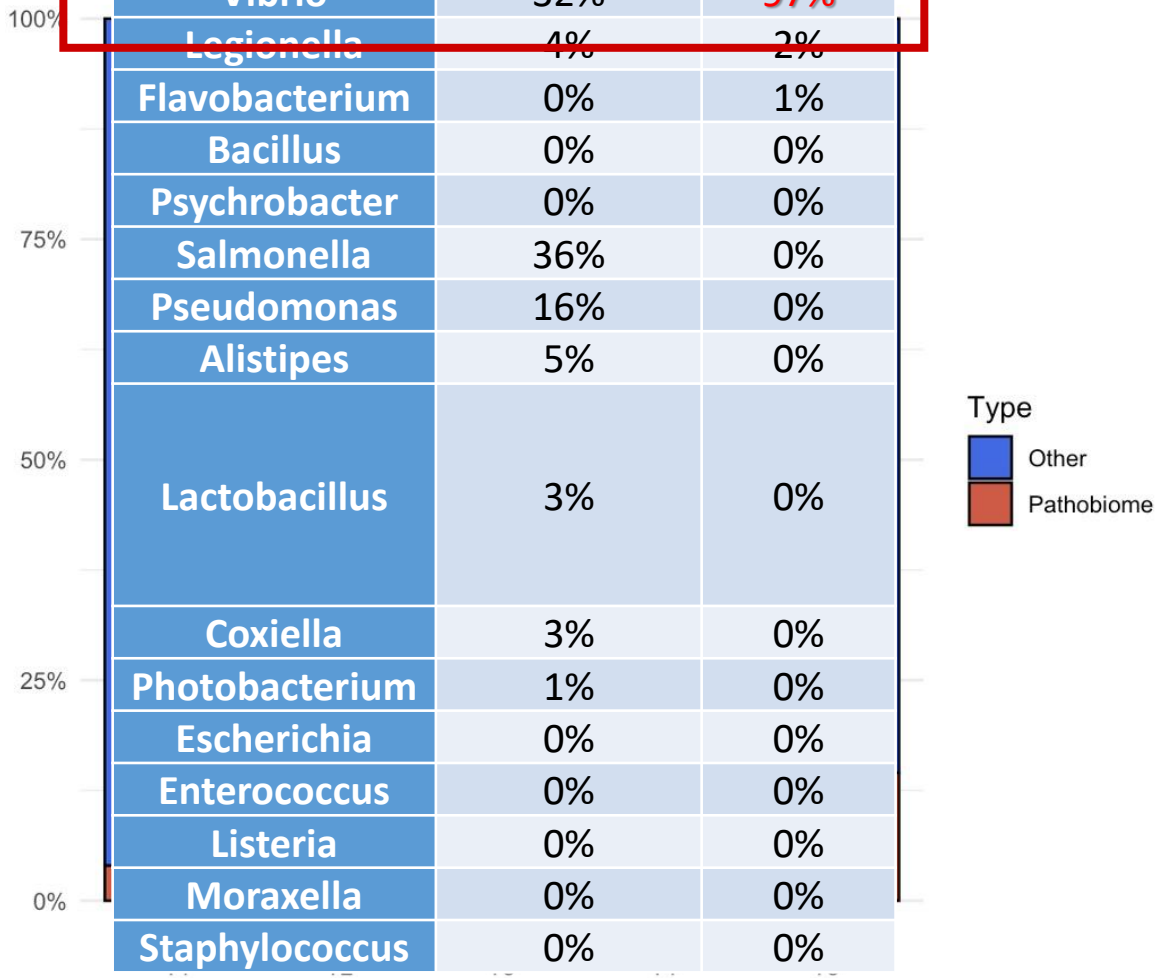
Comunità microbica, patobiota e resistoma delle microplastiche in ambiente

Comunità microbica, patobiota e resistoma delle particelle naturali flottanti in acqua marina

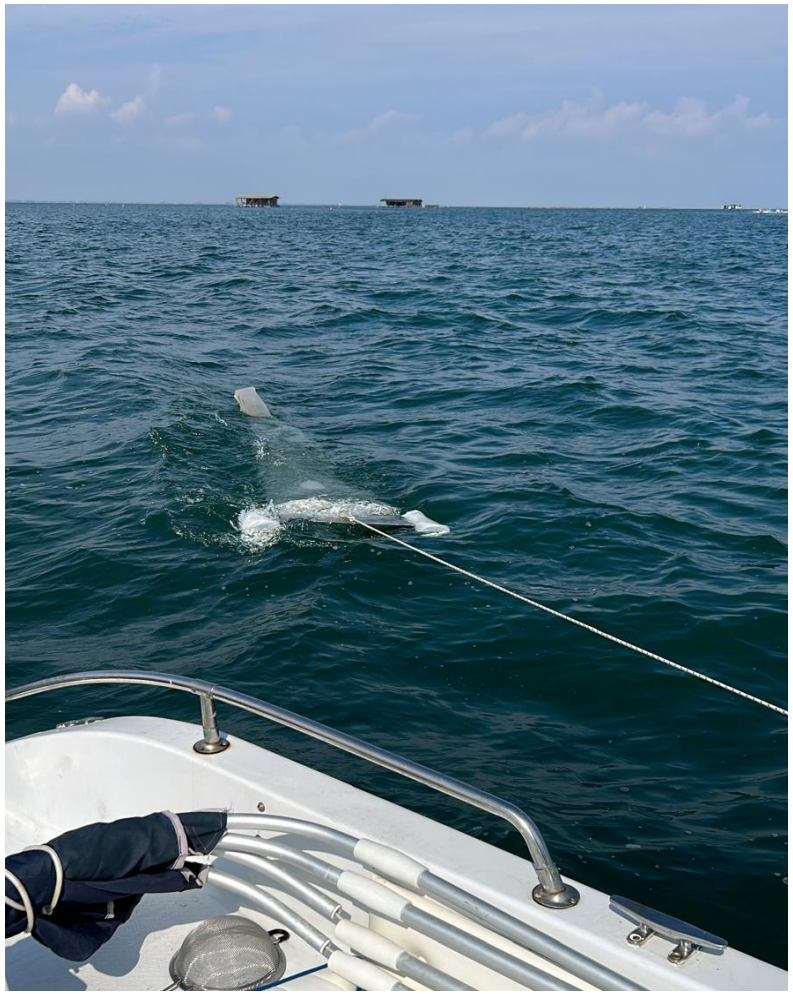
La biosintesi della Plastisfera include anche taxa potenzialmente patogeni?

MP-A

Bacterial genus	Lagoon Water	MP-A
Vibrio	32%	97%
Legionella	4%	2%
Flavobacterium	0%	1%
Bacillus	0%	0%
Psychrobacter	0%	0%
Salmonella	36%	0%
Pseudomonas	16%	0%
Alistipes	5%	0%
Lactobacillus	3%	0%
Coxiella	3%	0%
Photobacterium	1%	0%
Escherichia	0%	0%
Enterococcus	0%	0%
Listeria	0%	0%
Moraxella	0%	0%
Staphylococcus	0%	0%

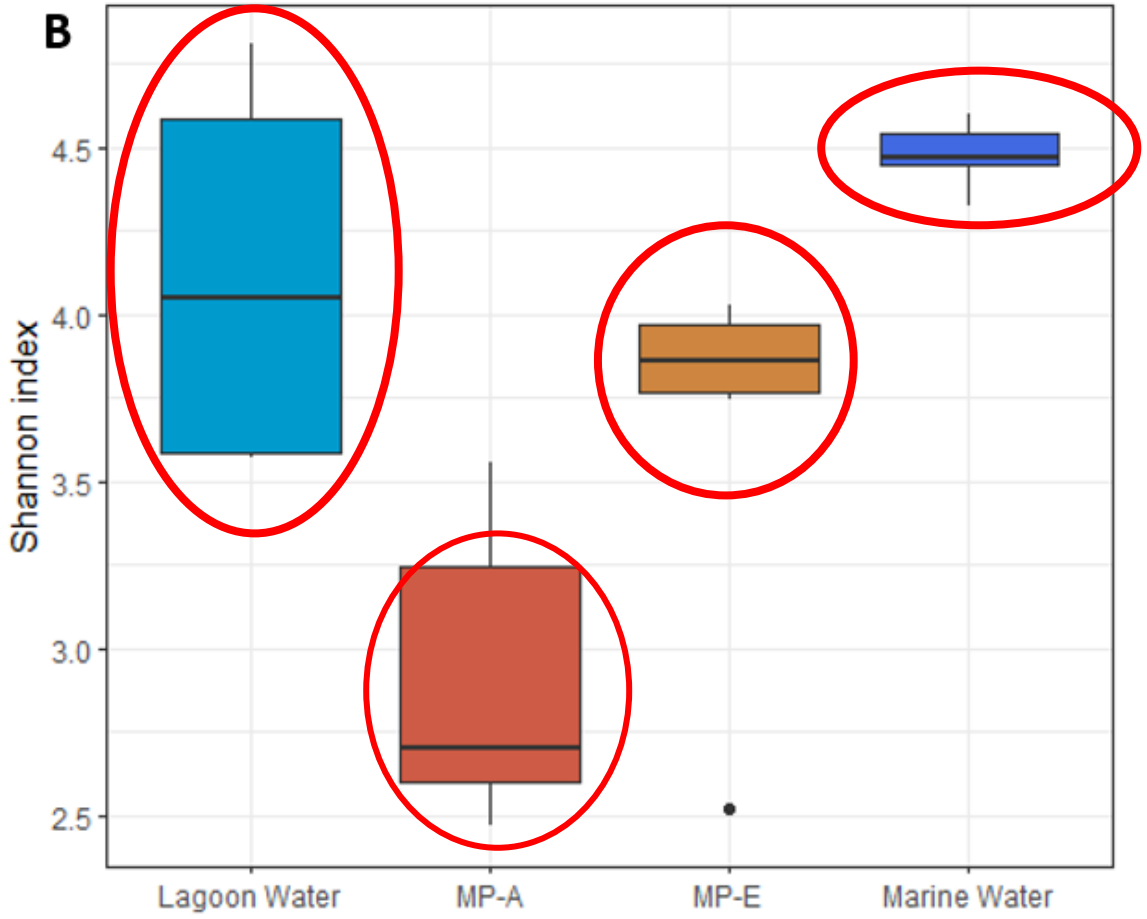


La comunità microbica delle MPs è diversa da quella dell'ambiente naturale?

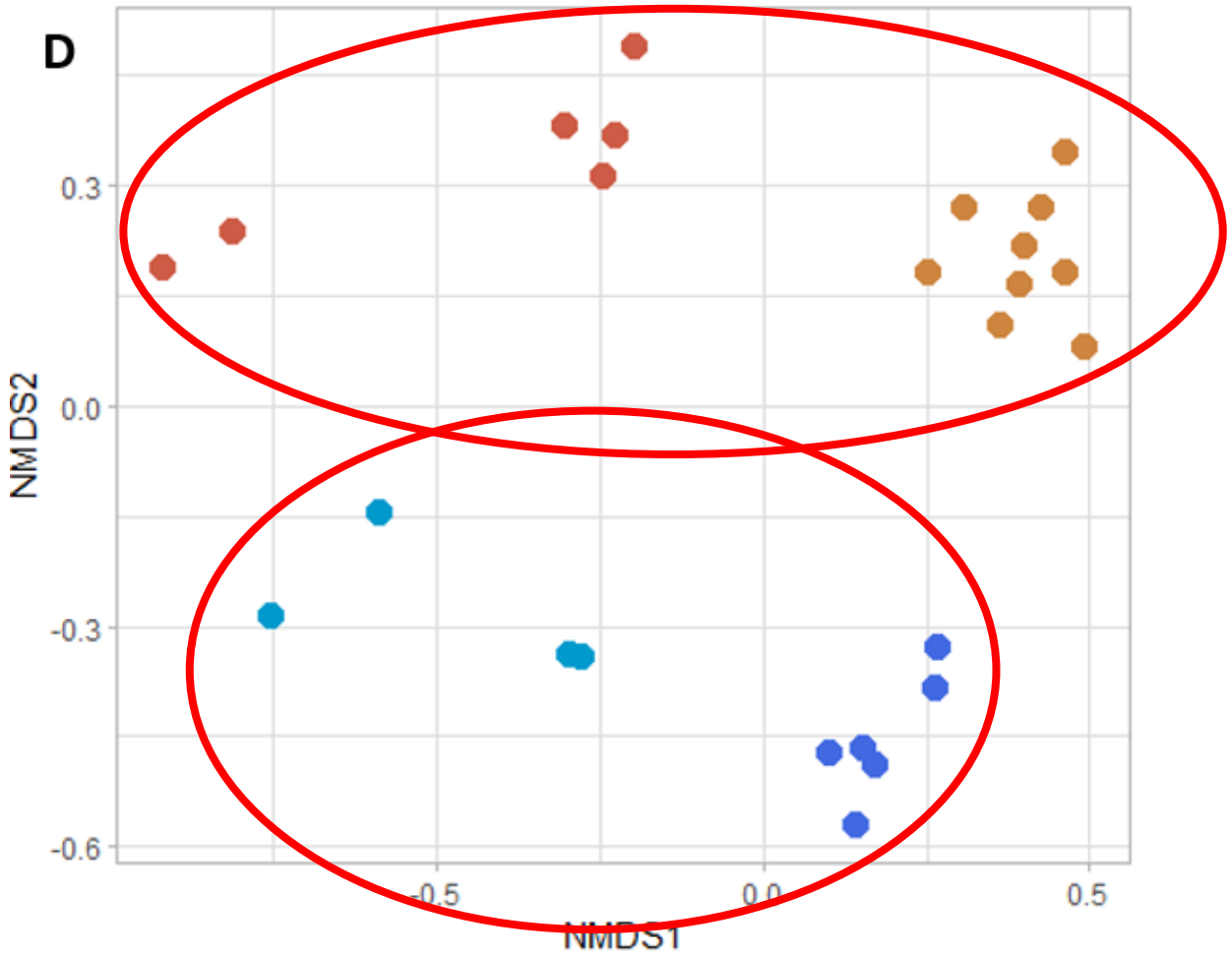


Diversità del microbiota: MPs vs Acqua

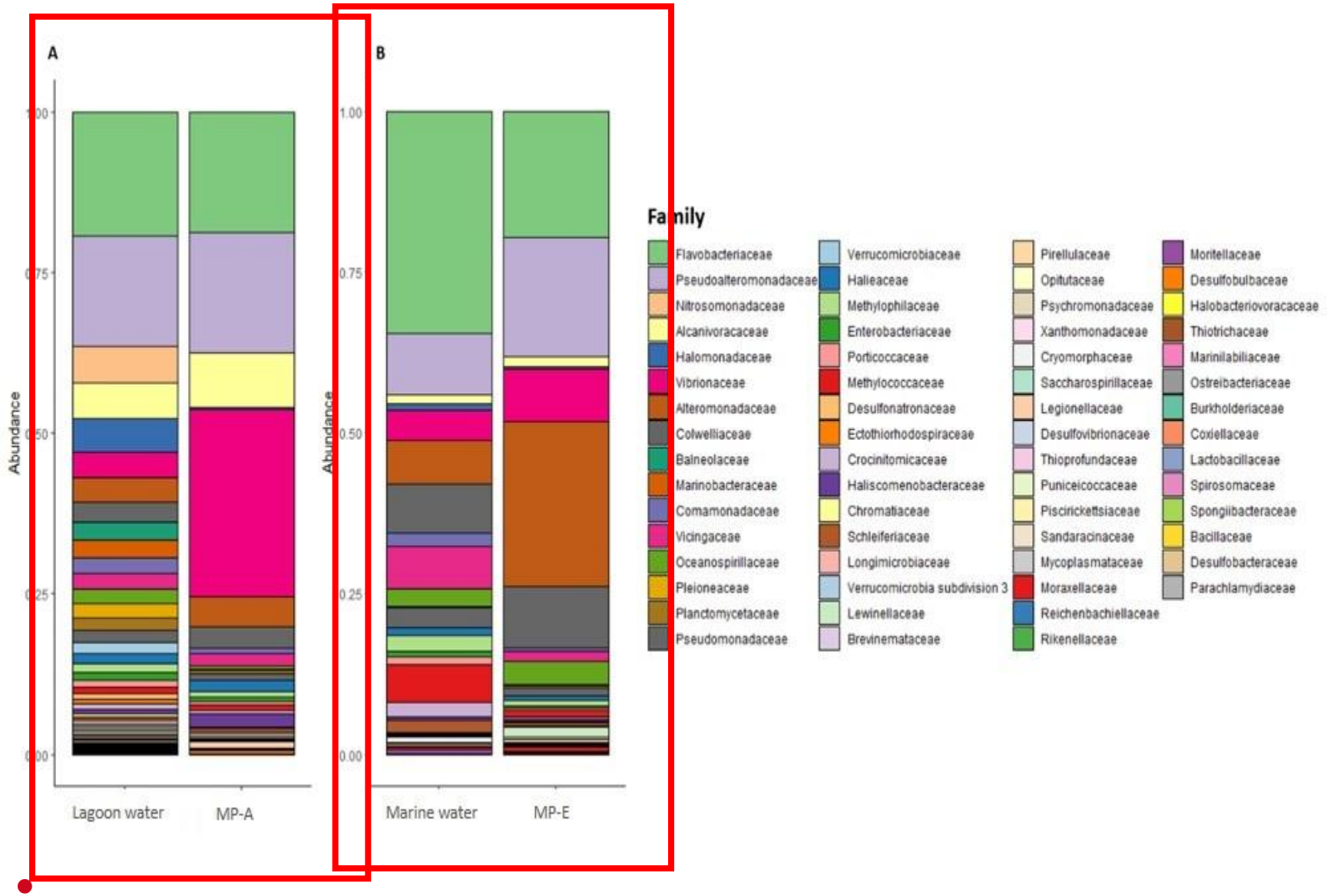
Alpha diversità



Beta diversità



Composizione microbiota: MPs vs Acqua

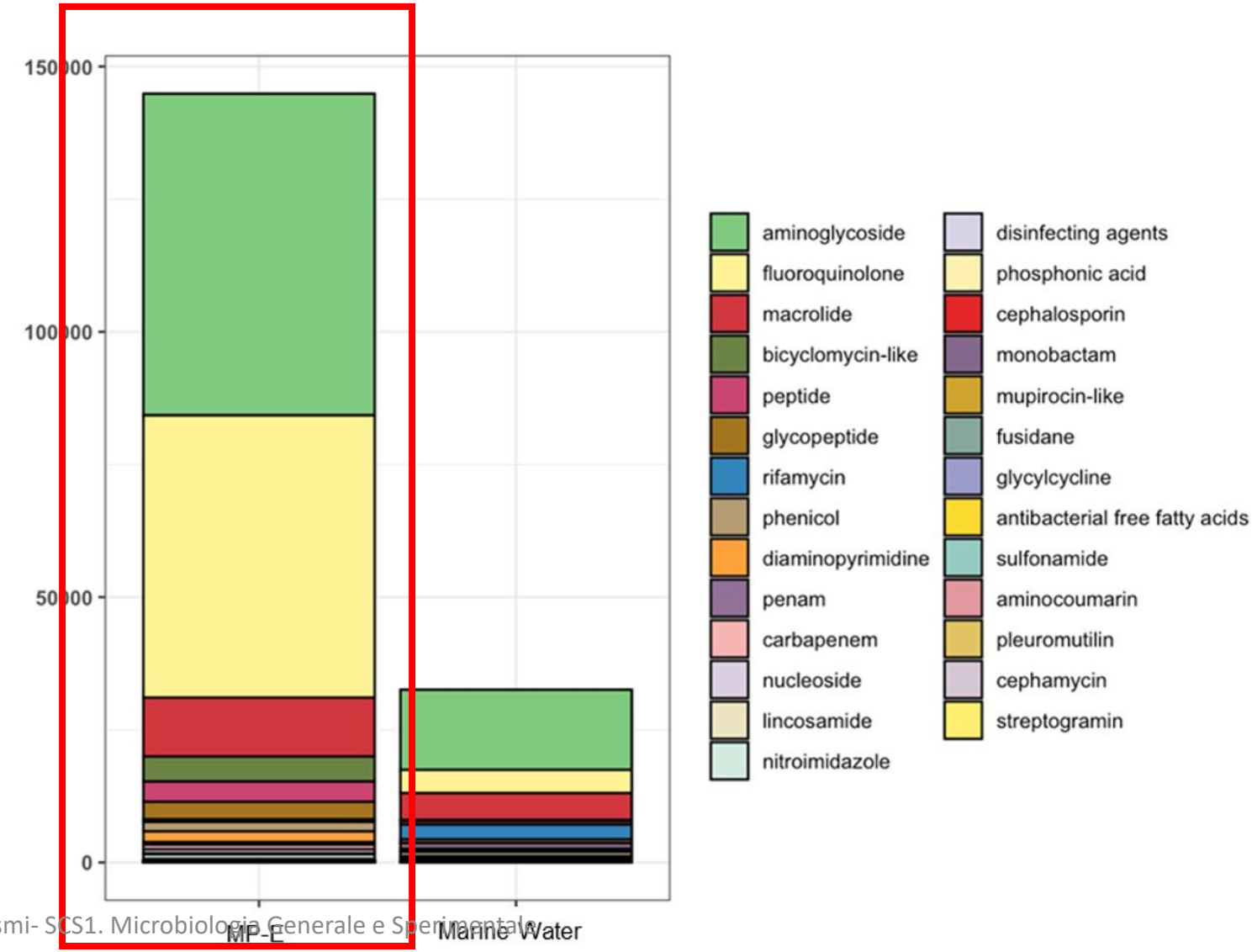


Nelle MPs si osserva un **aumento nell'abbondanza dei taxa** appartenenti alla famiglia **delle Alteromonadaceae.** e **delle Vibronaceae.**

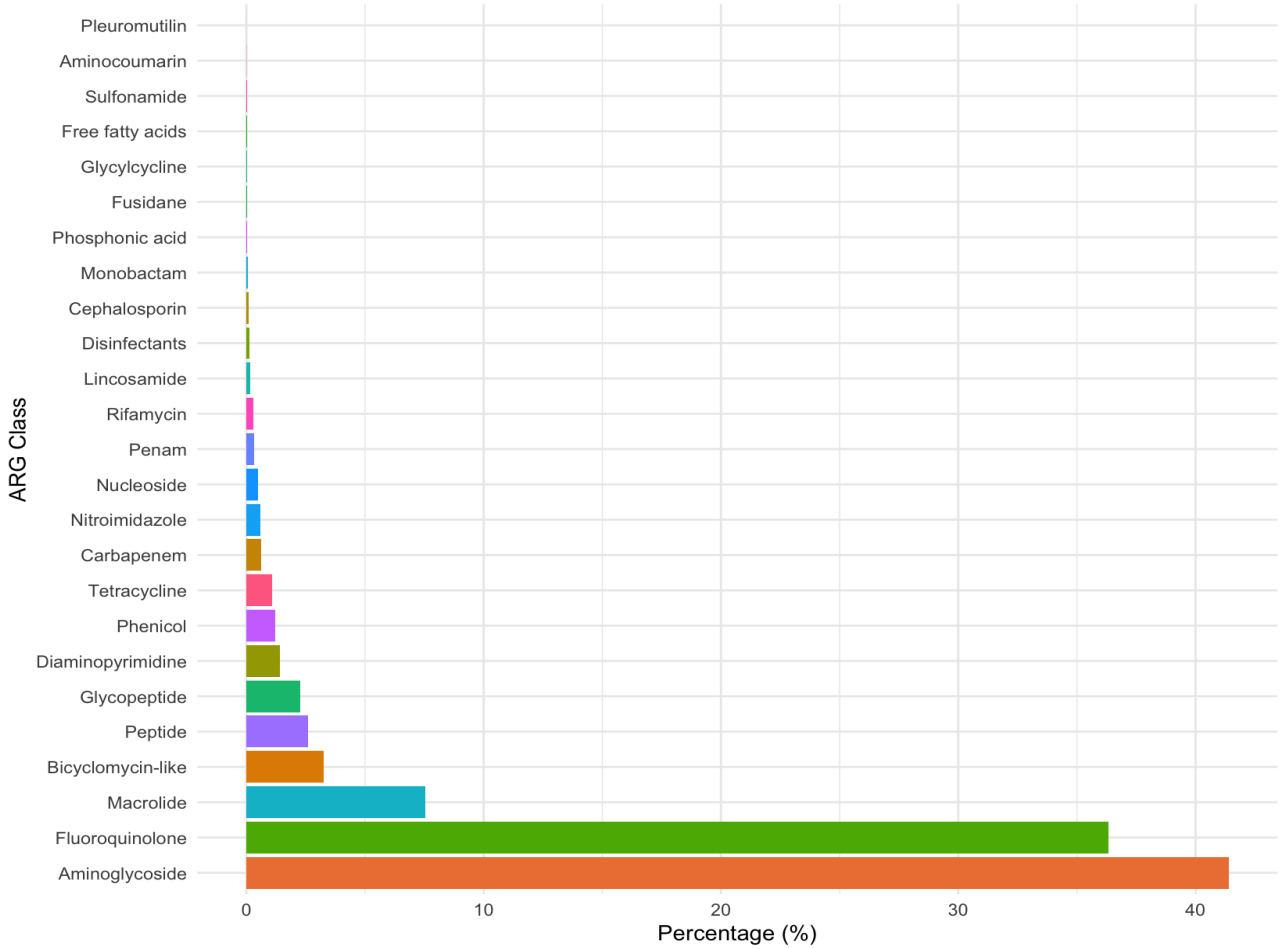
C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?

→ Resistoma diversificato associato alle particelle di MPs

→ Sono stati identificati in totale 245 diversi ARG, appartenenti a 26 diverse classi.



C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



Il resistoma era dominato da geni che conferiscono resistenza a:

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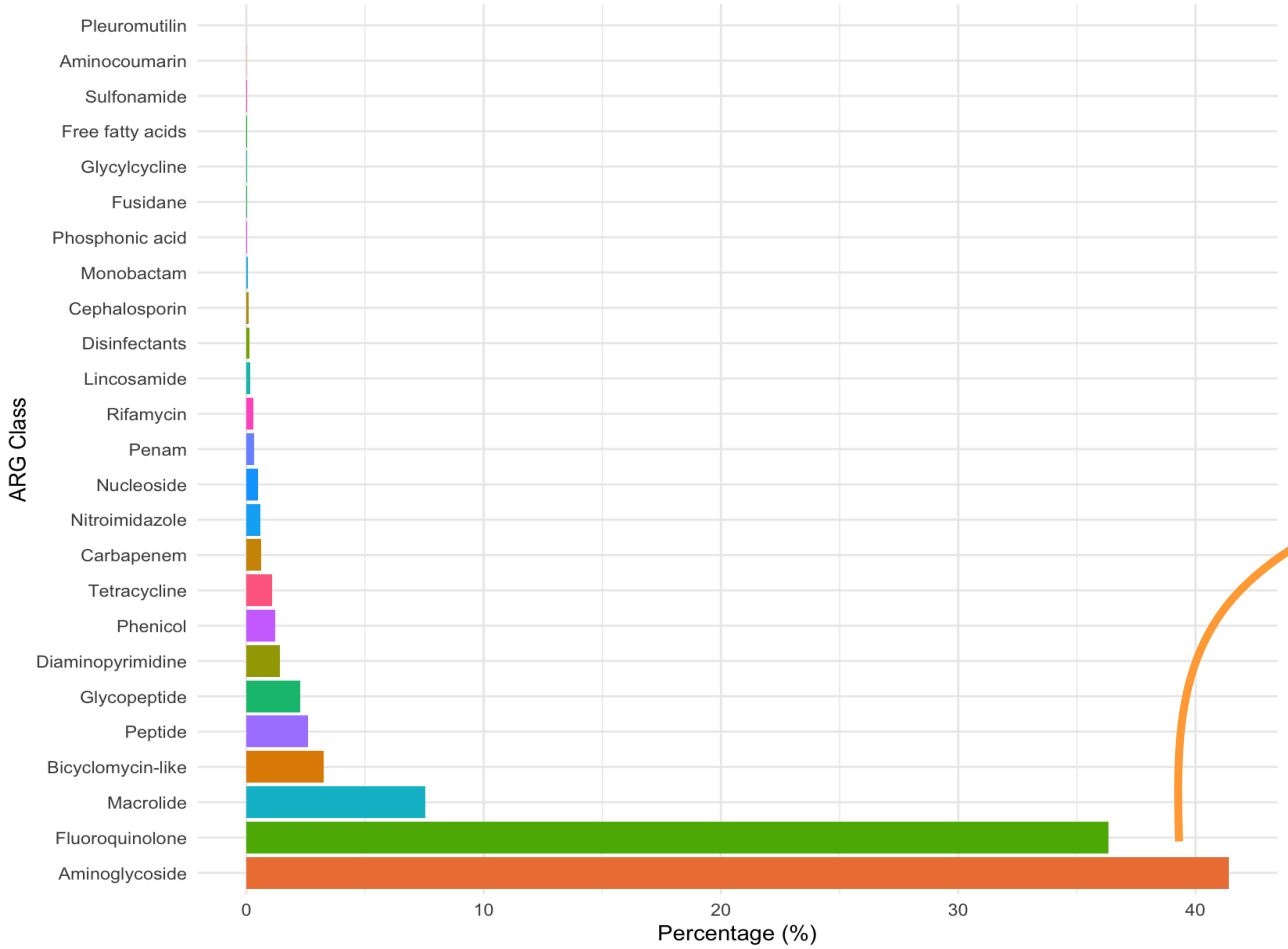
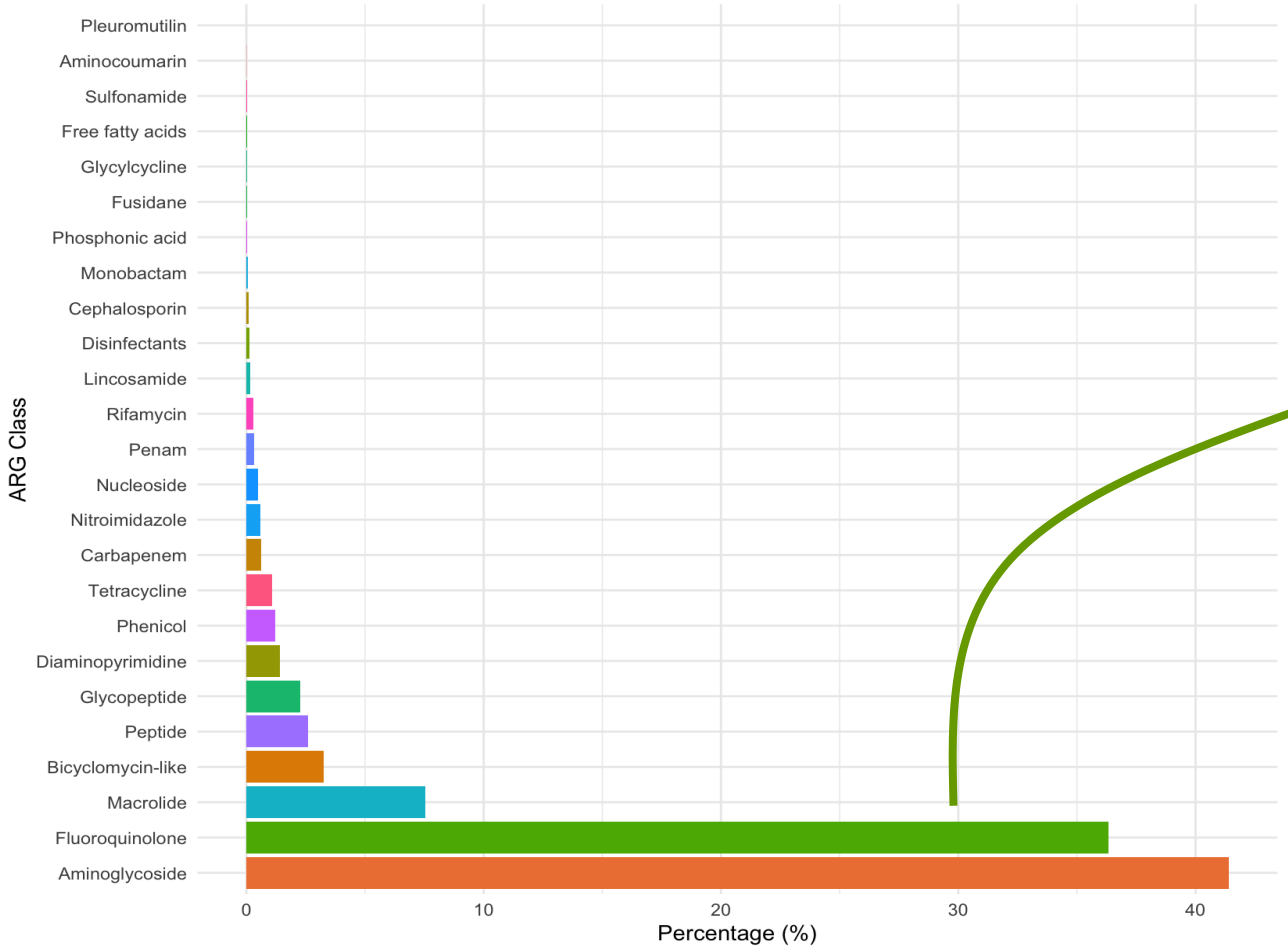
C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



Il resistoma era dominato da geni che conferiscono resistenza a:

Aminoglycosidi (41,8%)

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



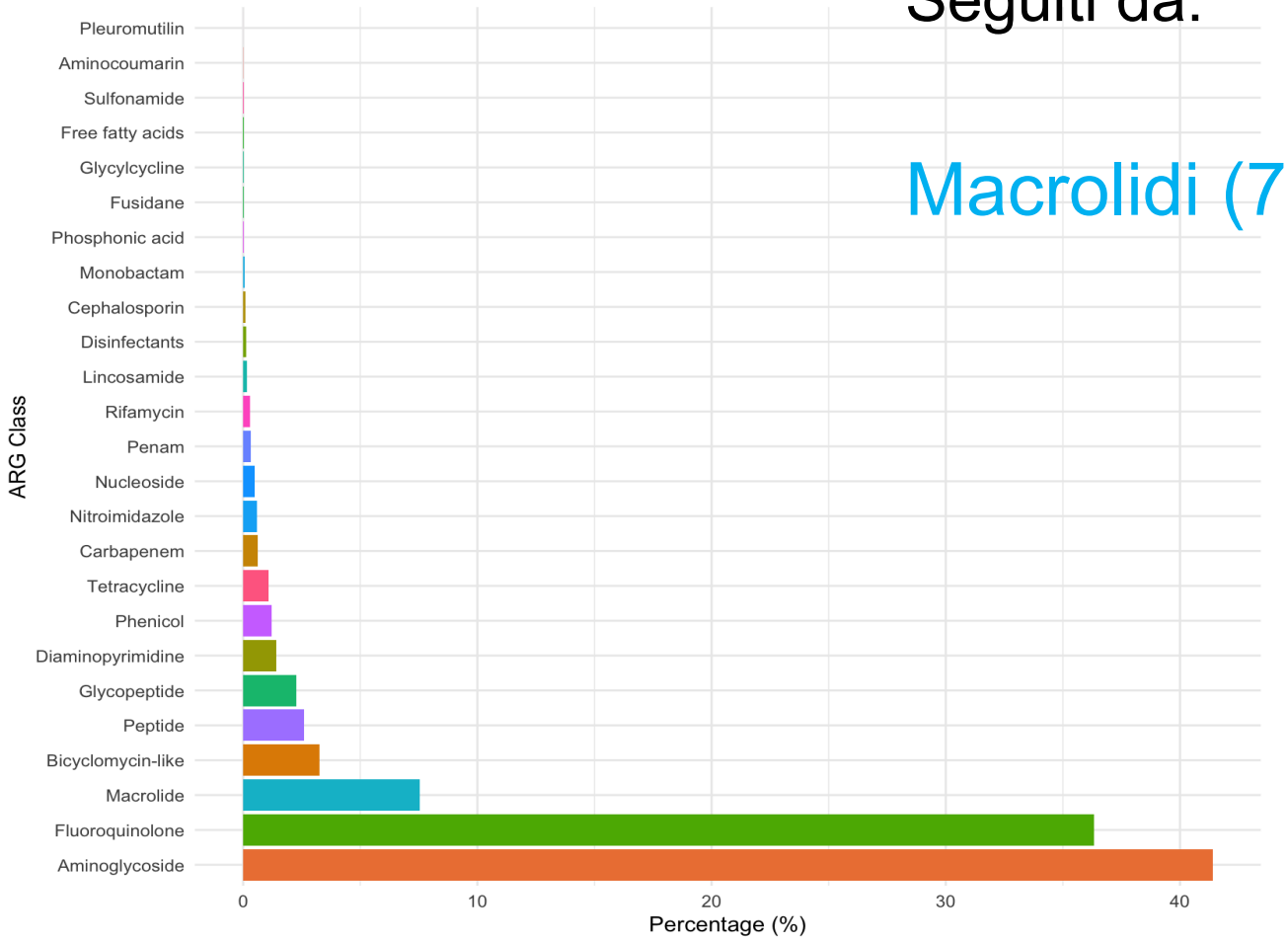
Il resistoma era dominato da geni che conferiscono resistenza a:

Fluorochinoloni (36,7%)

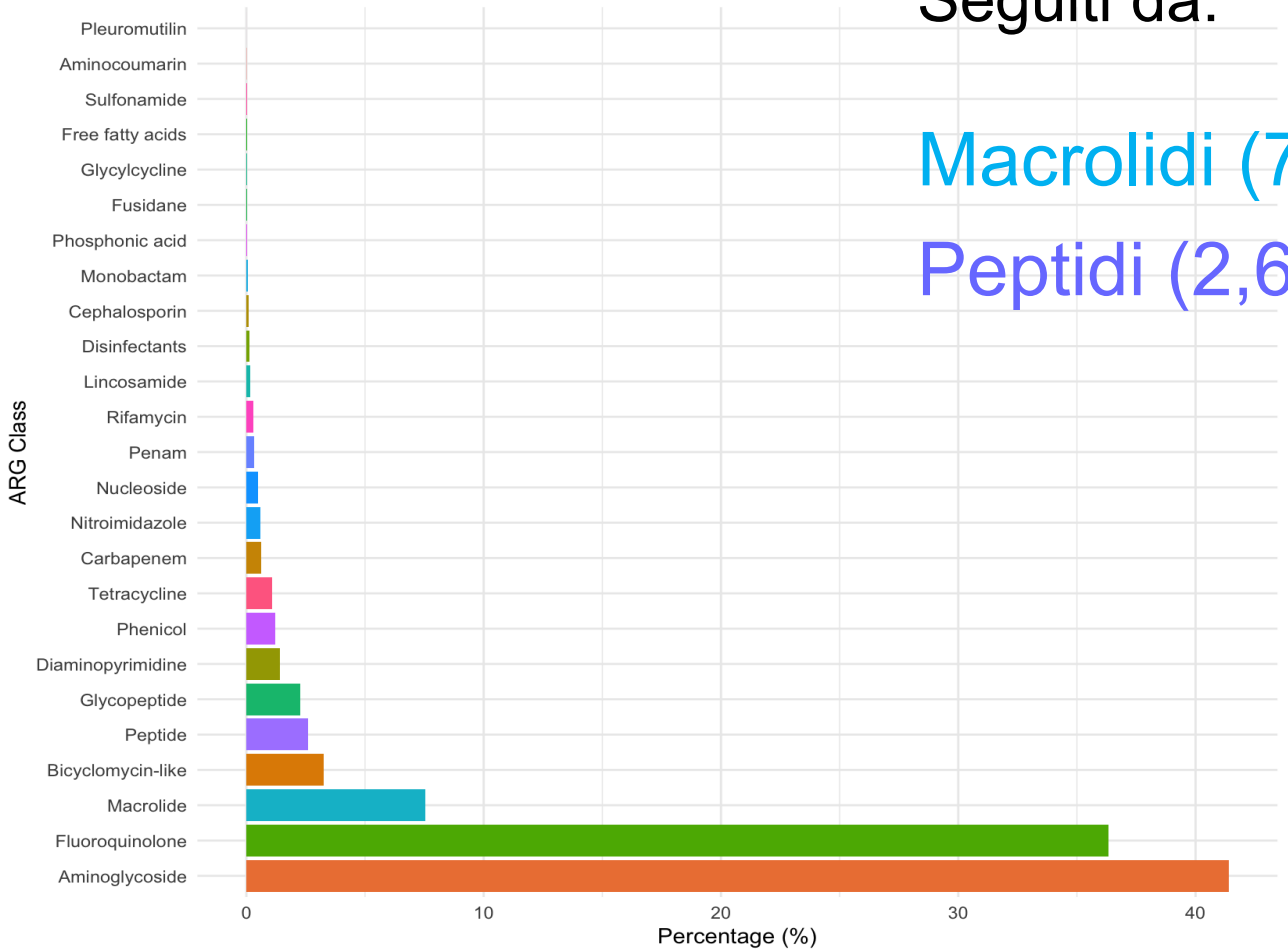
C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?

Seguiti da:

Macrolidi (7,6%)



C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?

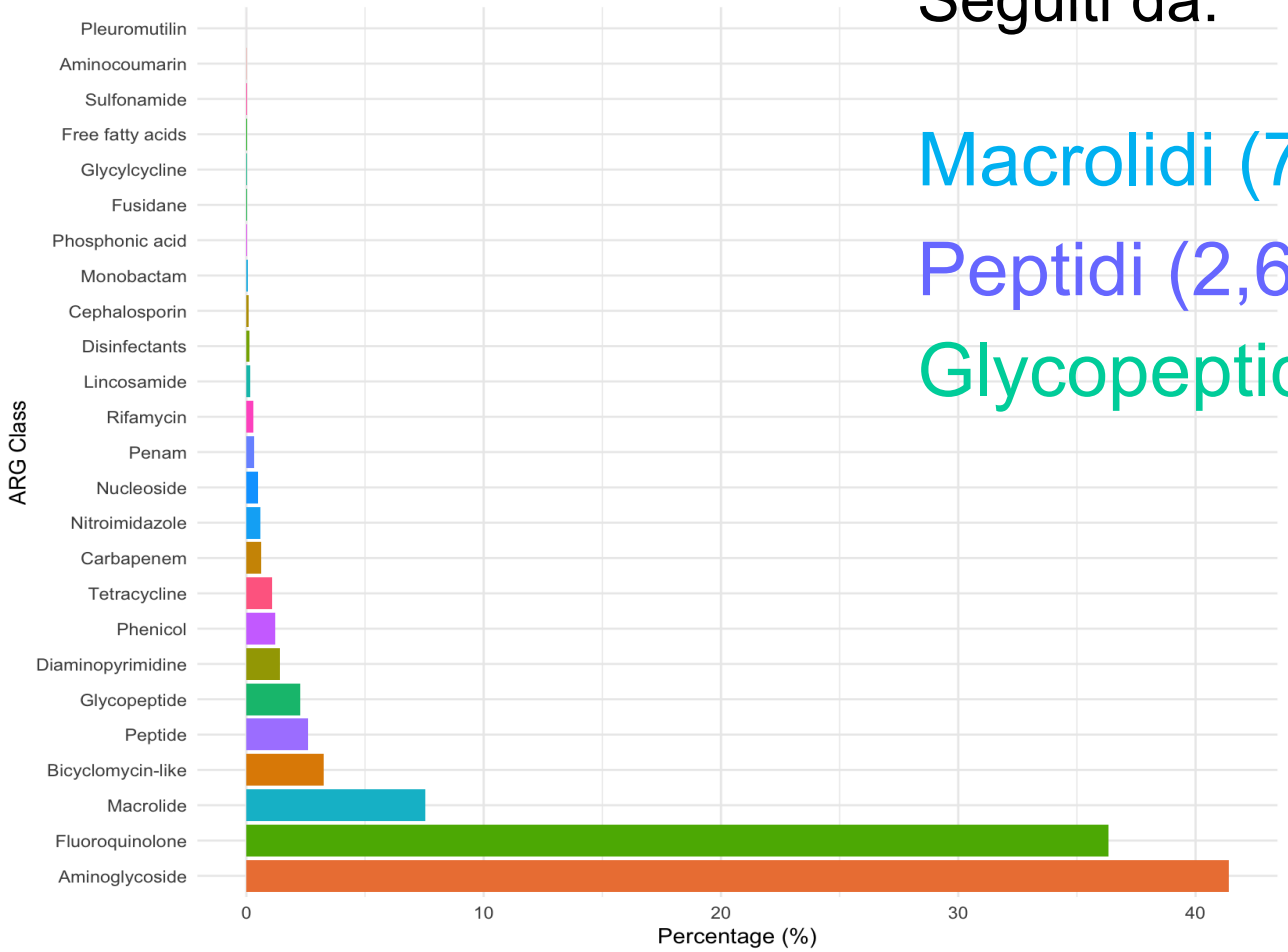


Seguiti da:

Macrolidi (7,6%)

Peptidi (2,6%)

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



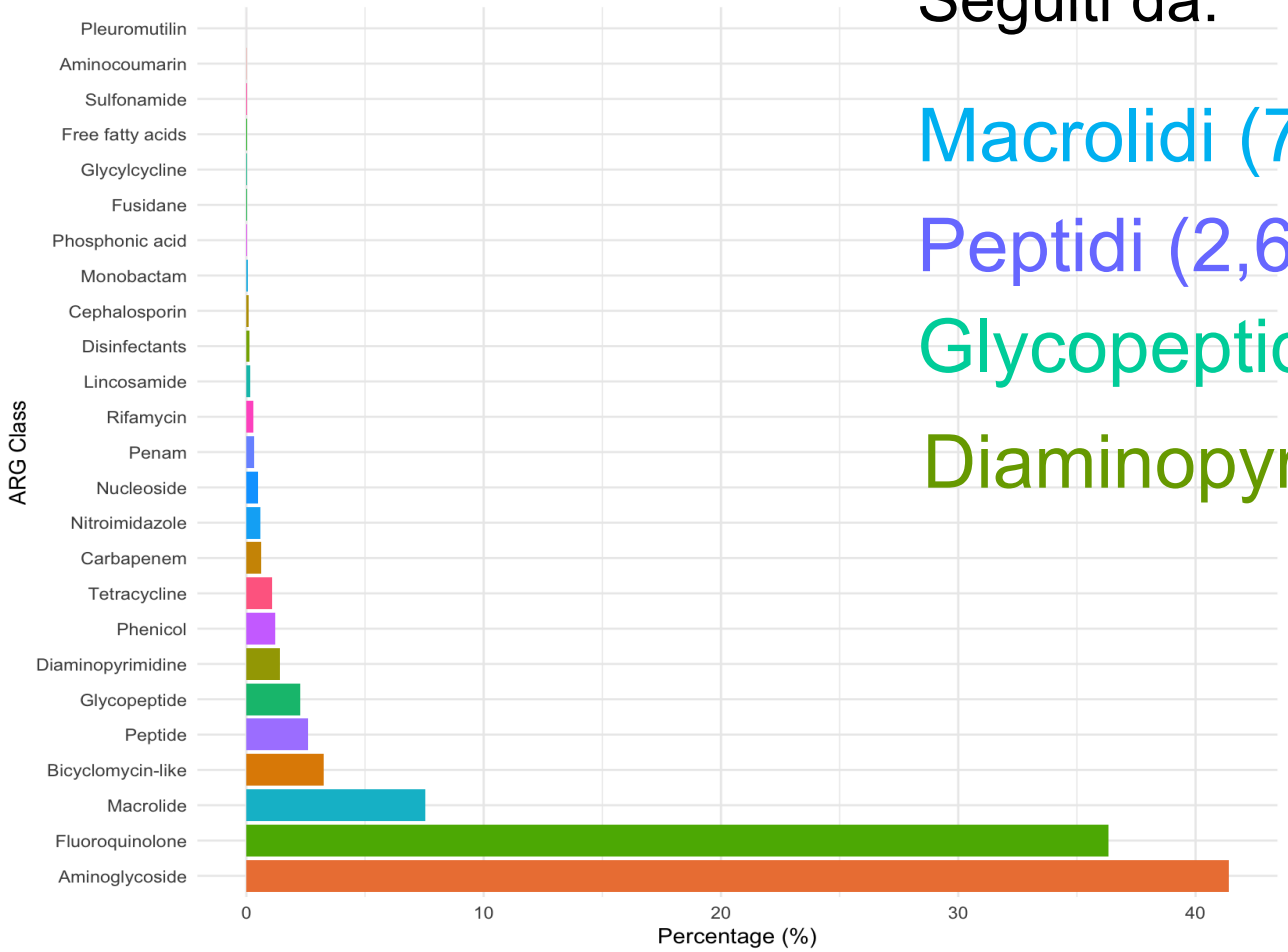
Seguiti da:

Macrolidi (7,6%)

Peptidi (2,6%)

Glycopeptidi (2,3%)

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



Seguiti da:

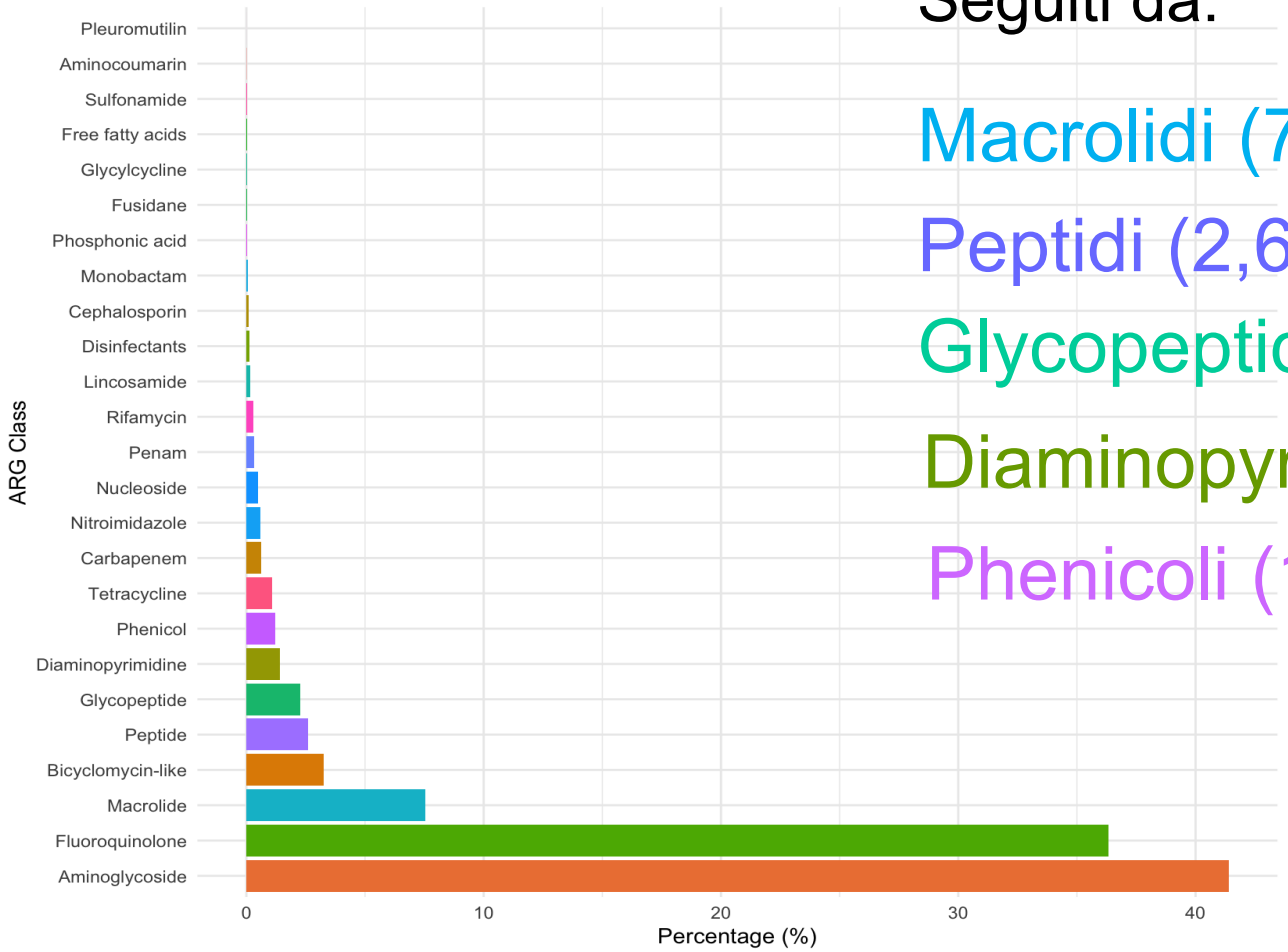
Macrolidi (7,6%)

Peptidi (2,6%)

Glycopeptidi (2,3%)

Diaminopyrimidine (1,4%)

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



Seguiti da:

Macrolidi (7,6%)

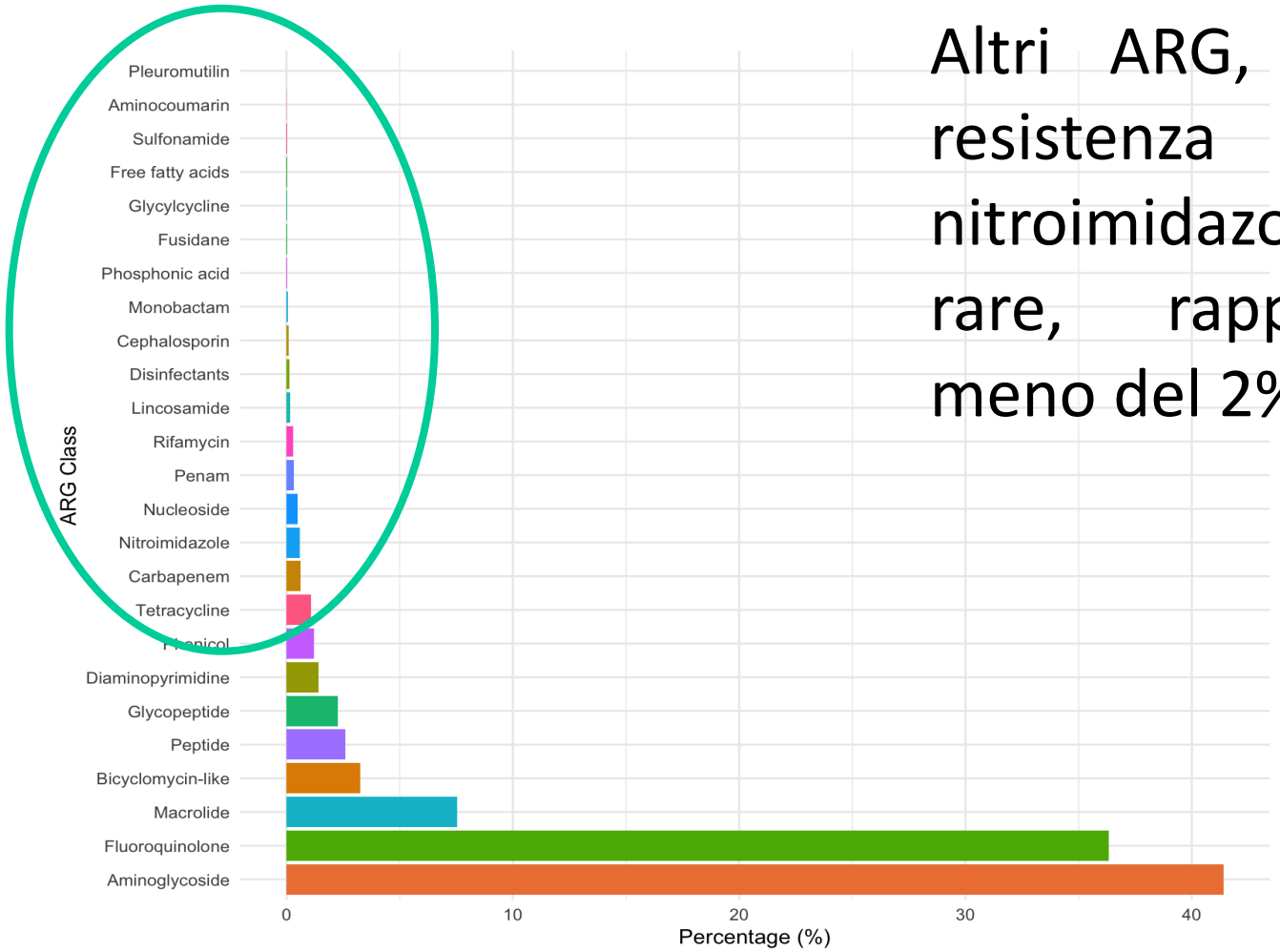
Peptidi (2,6%)

Glycopeptidi (2,3%)

Diaminopyrimidine (1,4%)

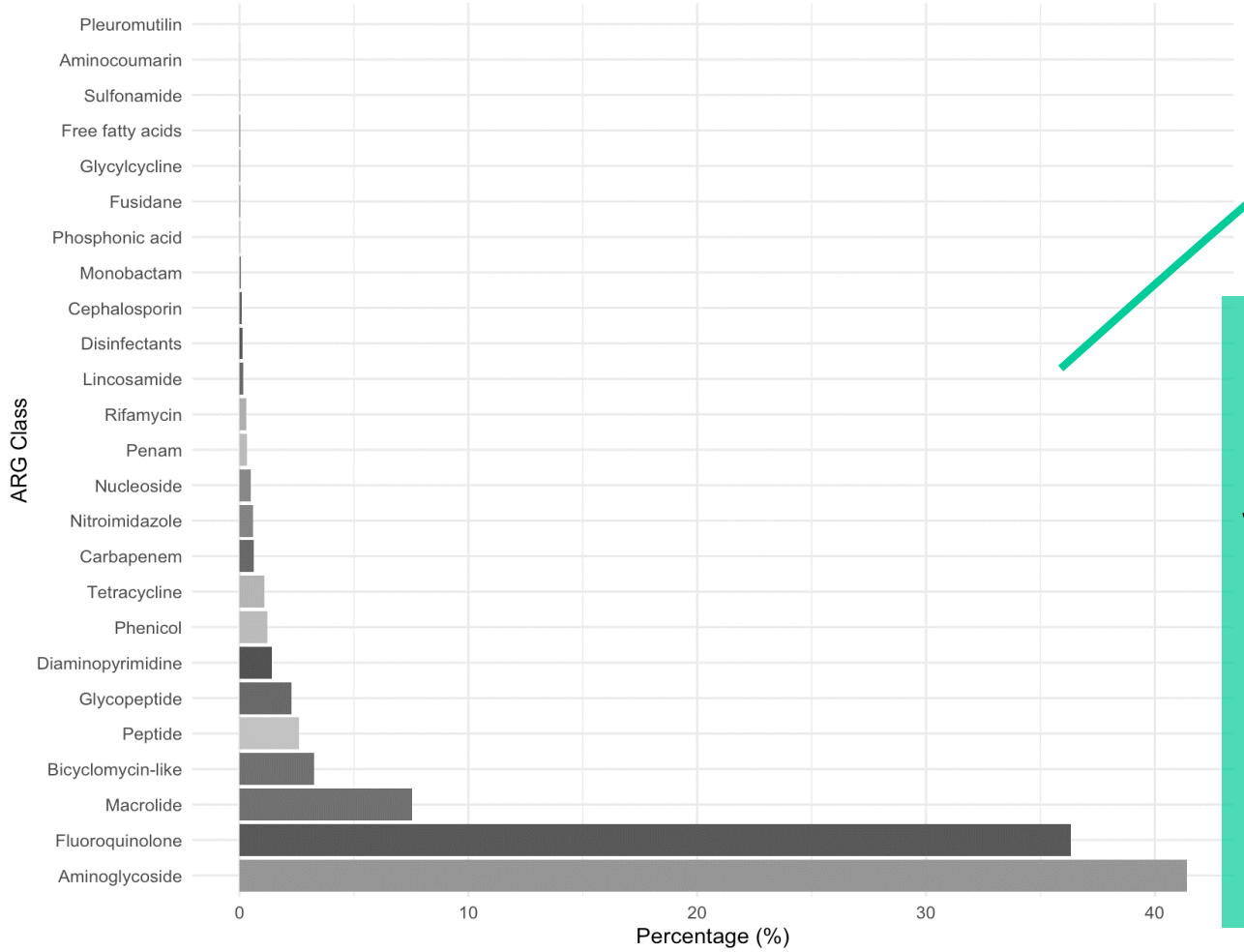
Phenicoli (1,2%)

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



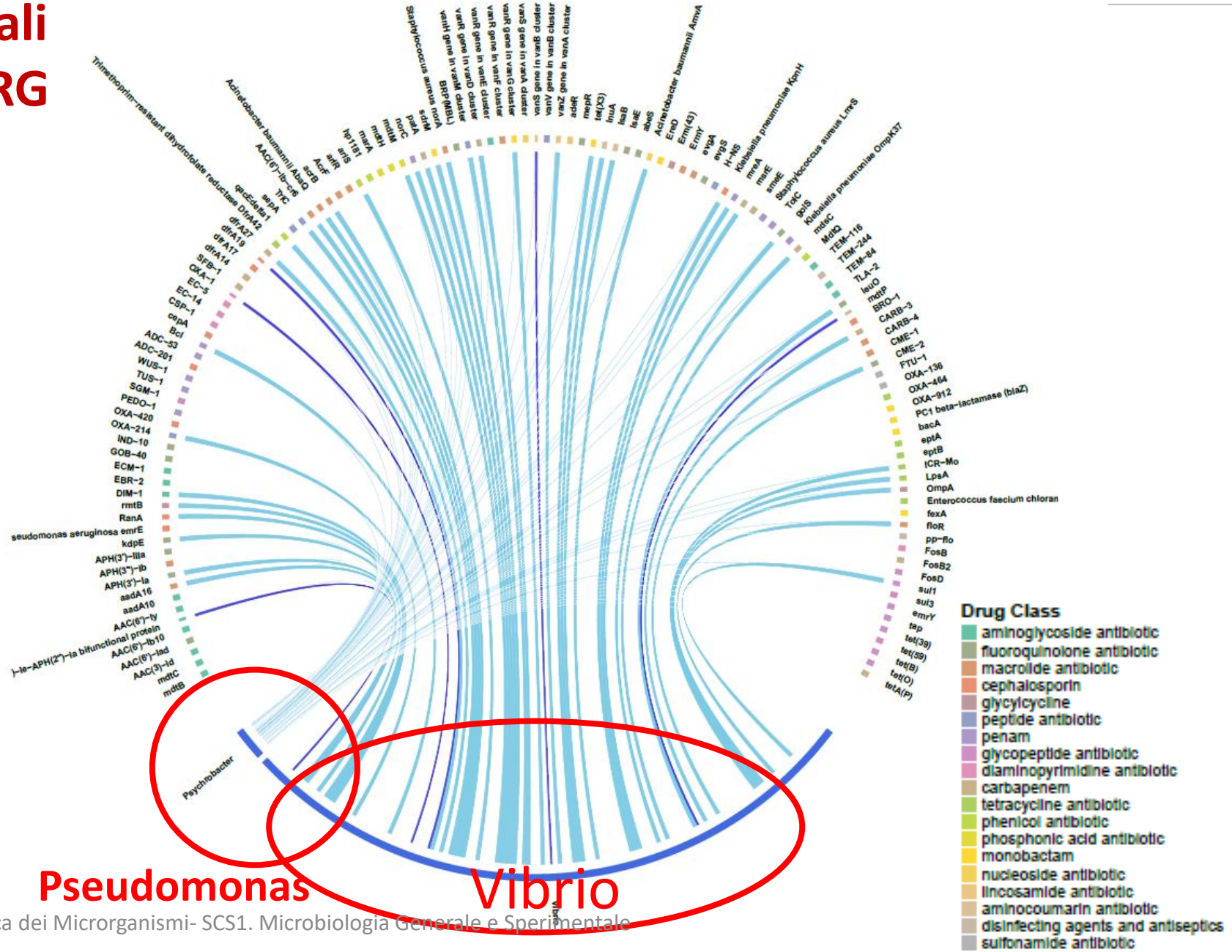
Altri ARG, inclusi quelli che conferiscono resistenza a tetracicline, carbapenemi, nitroimidazoli, penami, lincosamidi e classi rare, rappresentavano complessivamente meno del 2% del totale

C’è un arricchimento di geni di resistenza agli antimicrobici nella Plastisfera?



I risultati evidenziano il potenziale ruolo delle microplastiche (MP) come vettori per la diffusione della resistenza antimicrobica negli ambienti marini, con possibili implicazioni per la salute umana attraverso il consumo di prodotti ittici

Chi sono i principali detentori degli ARG identificati?



Pseudomonas **Vibrio**

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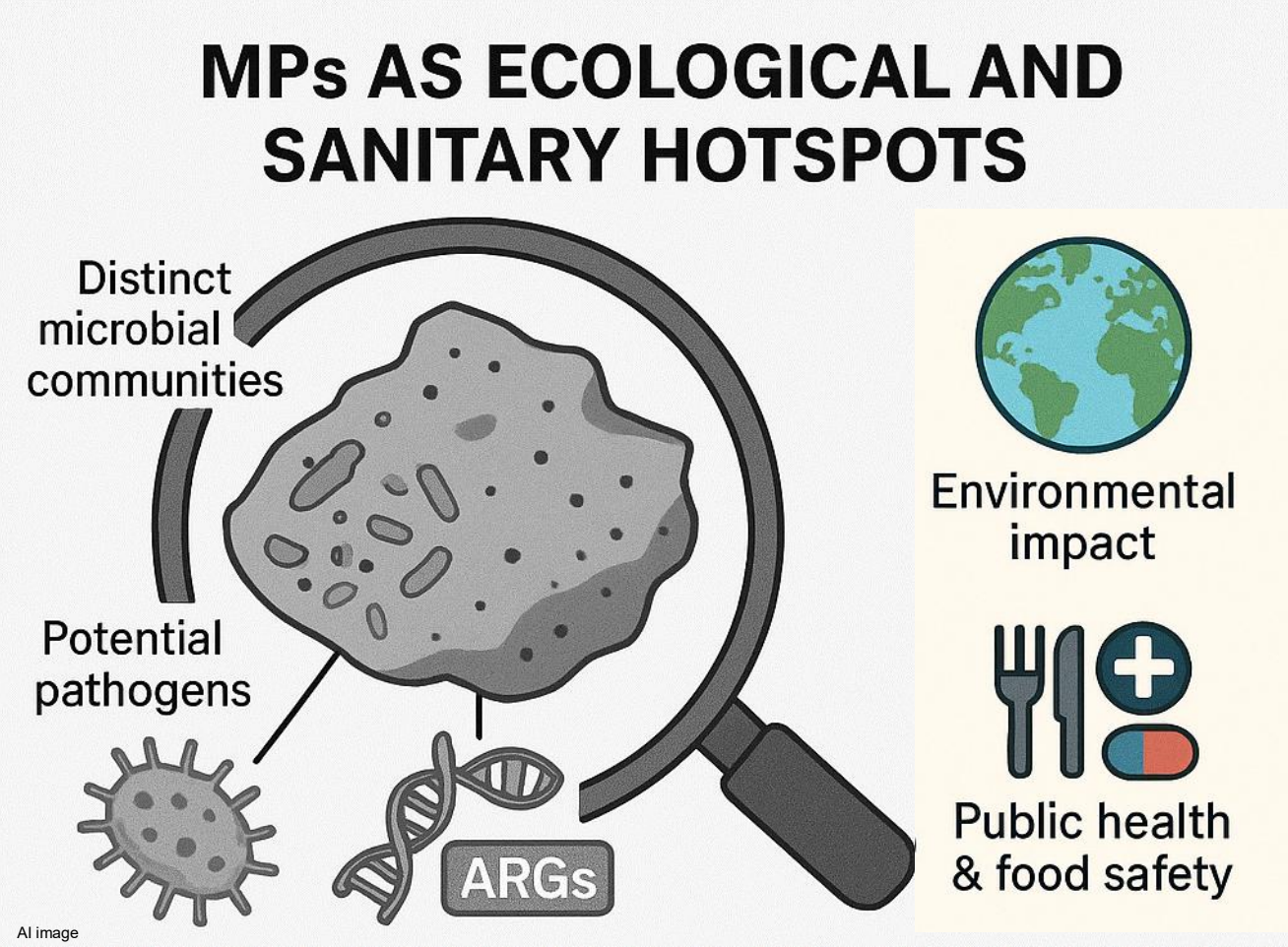
Conclusioni

- Le comunità microbiche sulla plastisfera, sia su microplastiche ambientali (MP-E) sia su microplastiche invecchiate sperimentalmente (MP-A), risultano **diverse dalla comunità planctonica dell'acqua** in termini di biodiversità e composizione tassonomica, indicando la formazione di **nicchie ecologiche uniche**.
- **Gammaproteobatteri**, inclusi patogeni alimentari (Salmonella, Listeria, Vibrio), sono distribuiti in maniera non uniforme, con maggiore abbondanza sulle MPs, evidenziando potenziali rischi per la salute pubblica attraverso la catena alimentare.
- La plastisfera mostra una maggiore presenza di **patogeni del genere Vibrio**, evidenziando il ruolo delle MPs come serbatoio di batteri potenzialmente pericolosi.
- La plastisfera, inoltre, è un **serbatoio di geni associati a resistenze antimicrobiche (resistoma)**



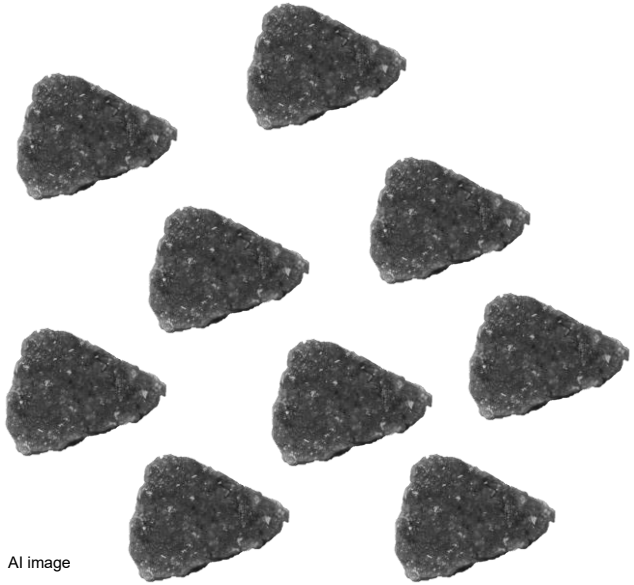
→ Necessità di piani di monitoraggio e di strategie di mitigazione che affrontino **non solo** l'impatto ambientale delle plastiche, **ma anche le implicazioni dirette per la salute pubblica e la sicurezza alimentare**

Istituto Zooprofilattico Sperimentale delle Venezie

closasso@izsvenezie.it
Laboratorio di Ecologia Microbiologia Microbica e Genomica dei Microrganismi

MP-E

Materiali:

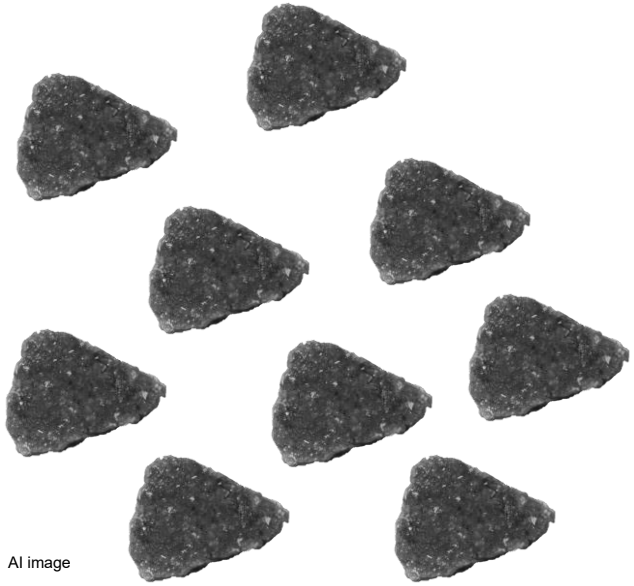


AI image

- HDPE / LDPE (polyethylene)
- PP (polypropylene)
- PS (polystyrene)
- Polyolefin
- Materiali compositi

MP-E

Materiali:



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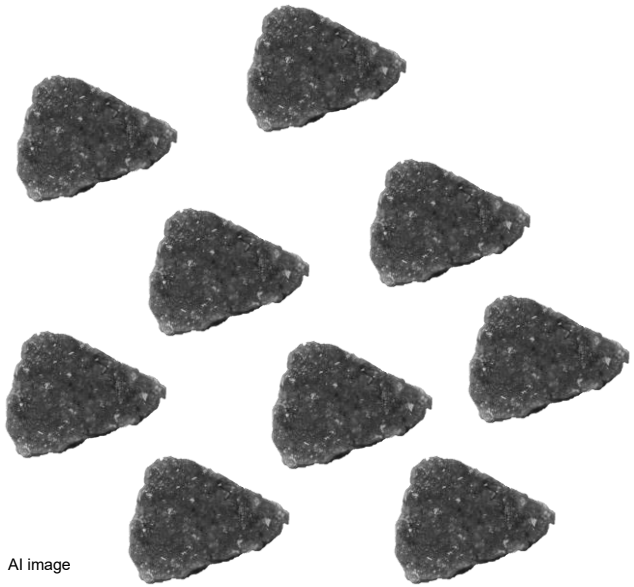

AI image

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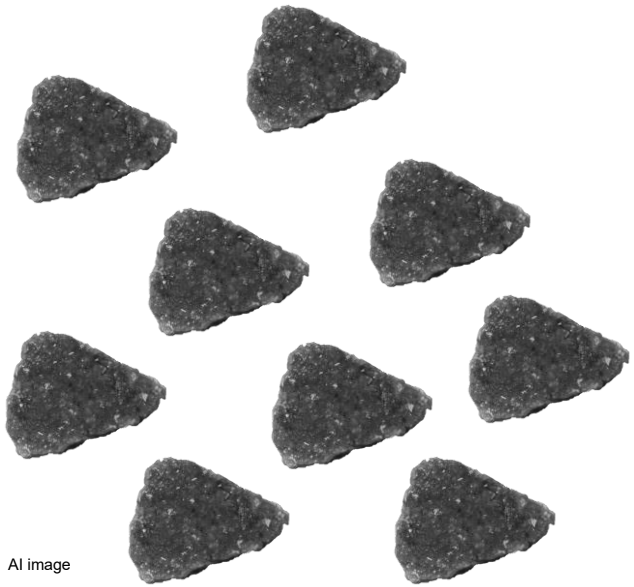
- Polyolefin
- Composite materials

MP-E

Materiali:



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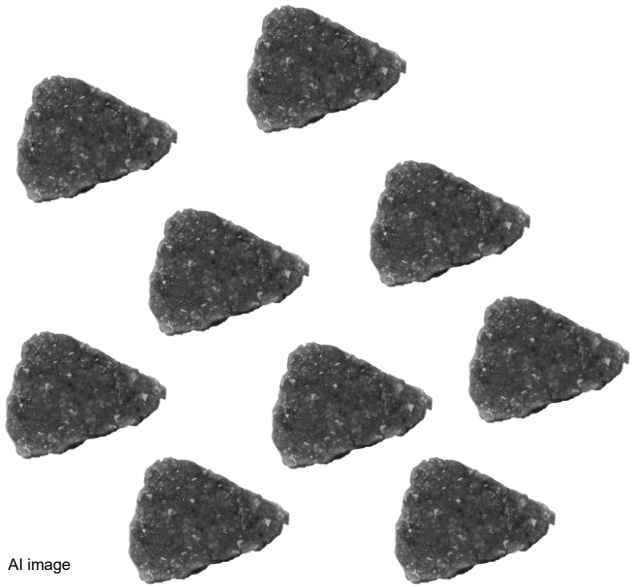
MP-E

Materiali::



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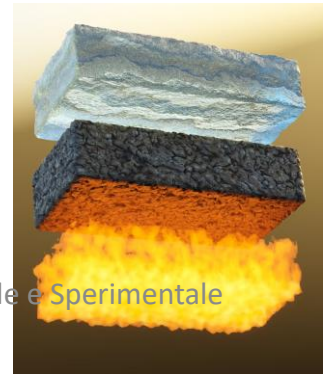

MP-E

Materiali:

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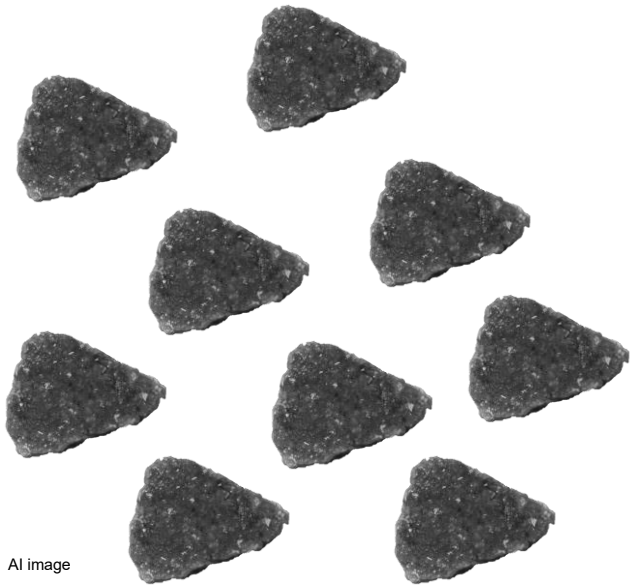
Grandezza:

- 1 µm → >5 mm



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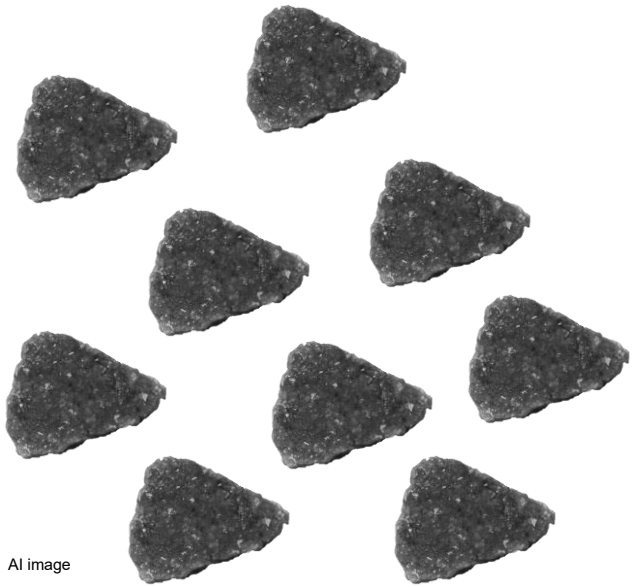

AI image

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1 µm → >5 mm

Morfologia:

- Fibre
- Frammenti
- Foglietti

MP-E

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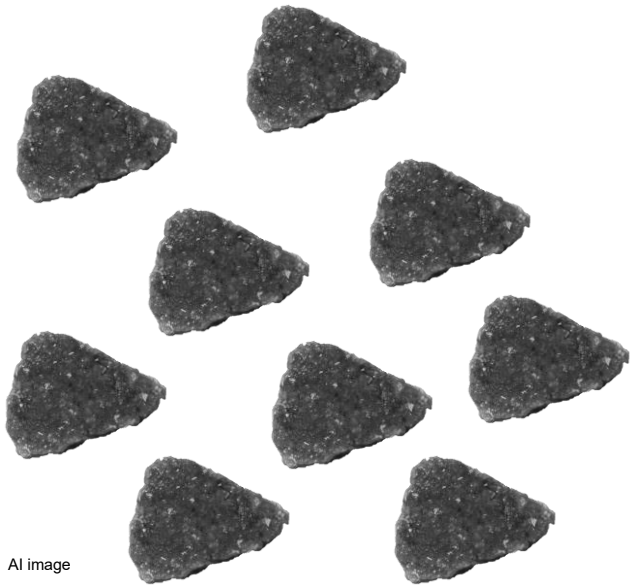

AI image

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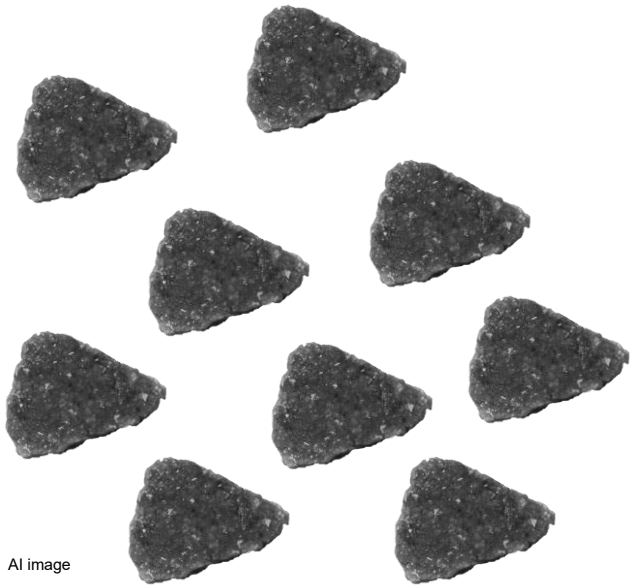
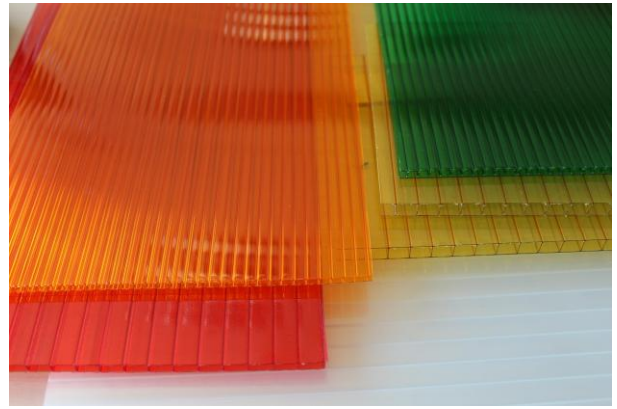
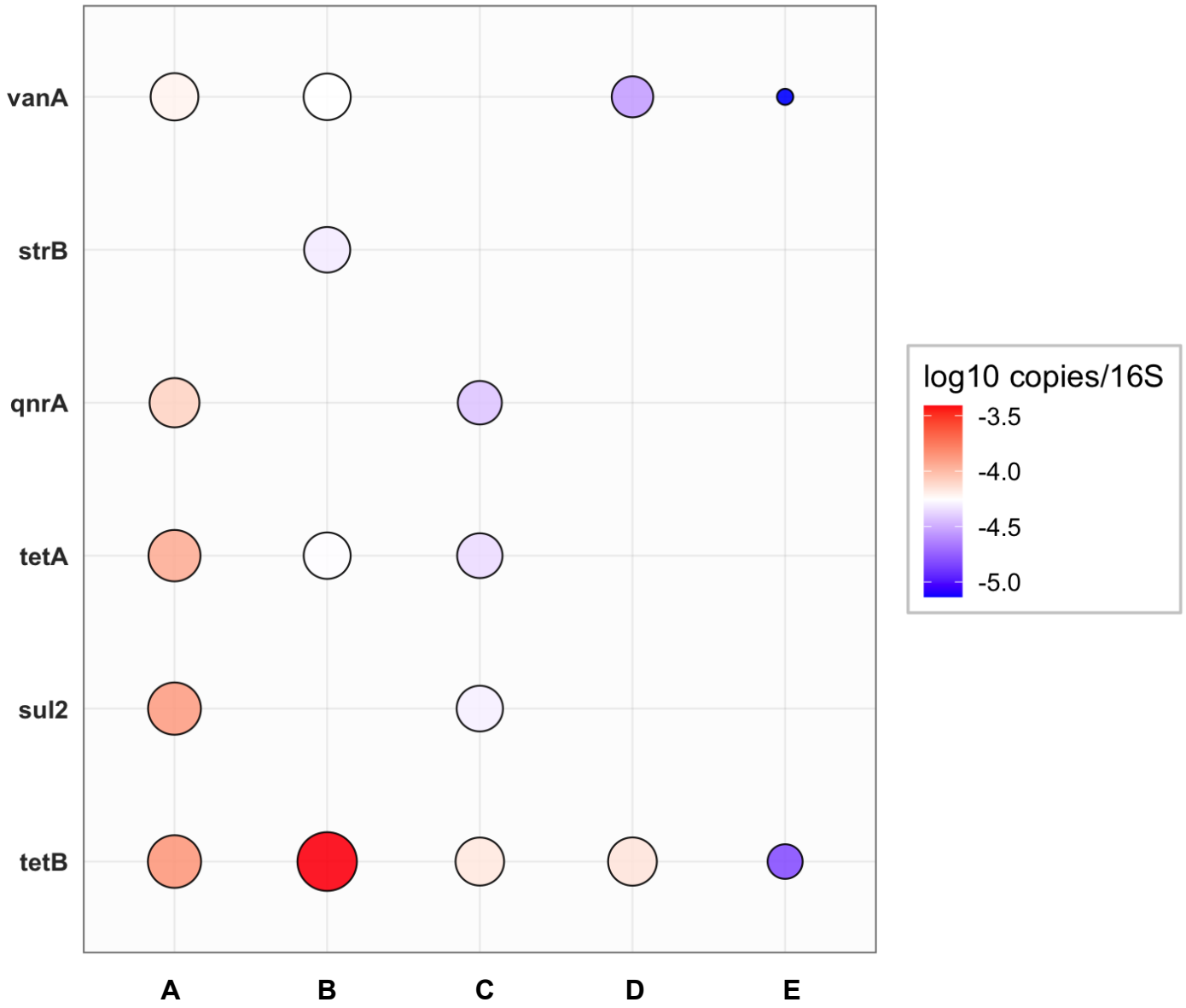


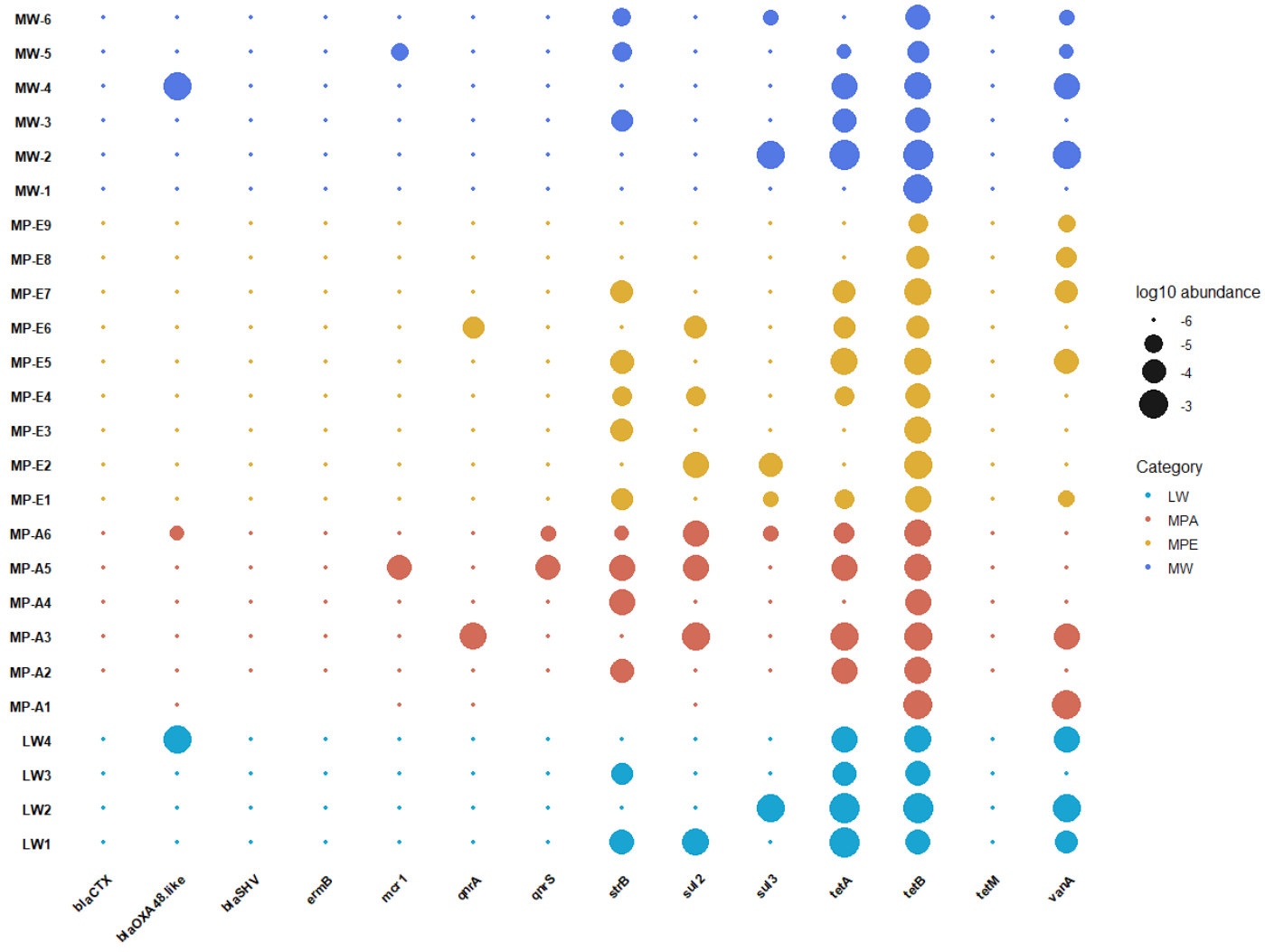
AI image



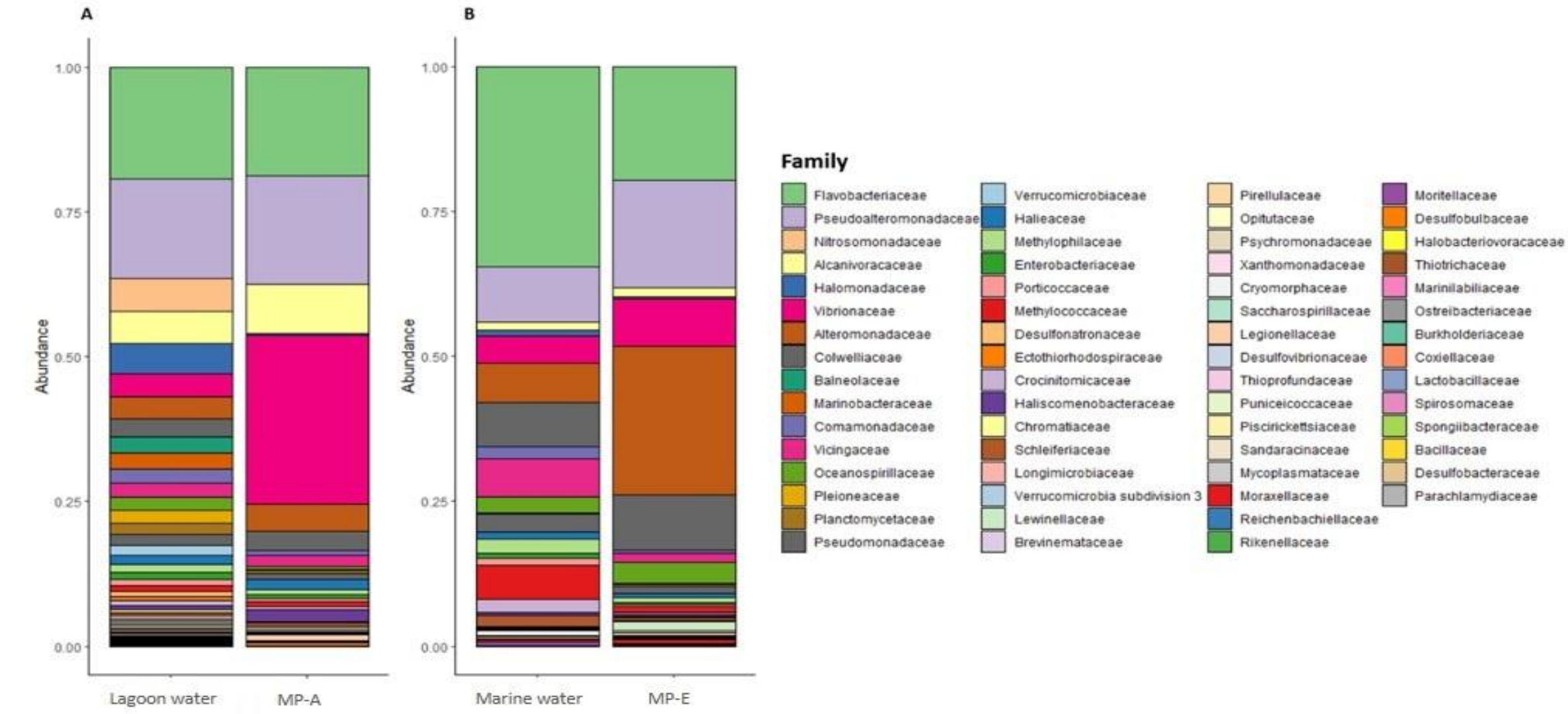
ARGs quantification using digital droplet PCR



Resistoma MP-E

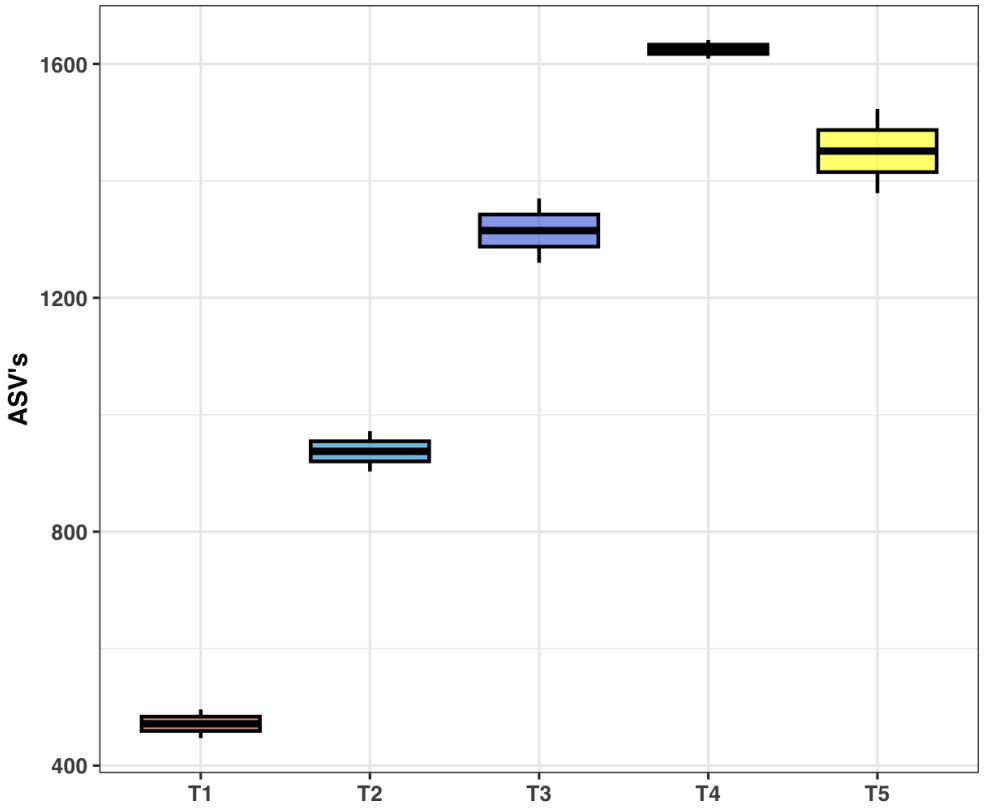


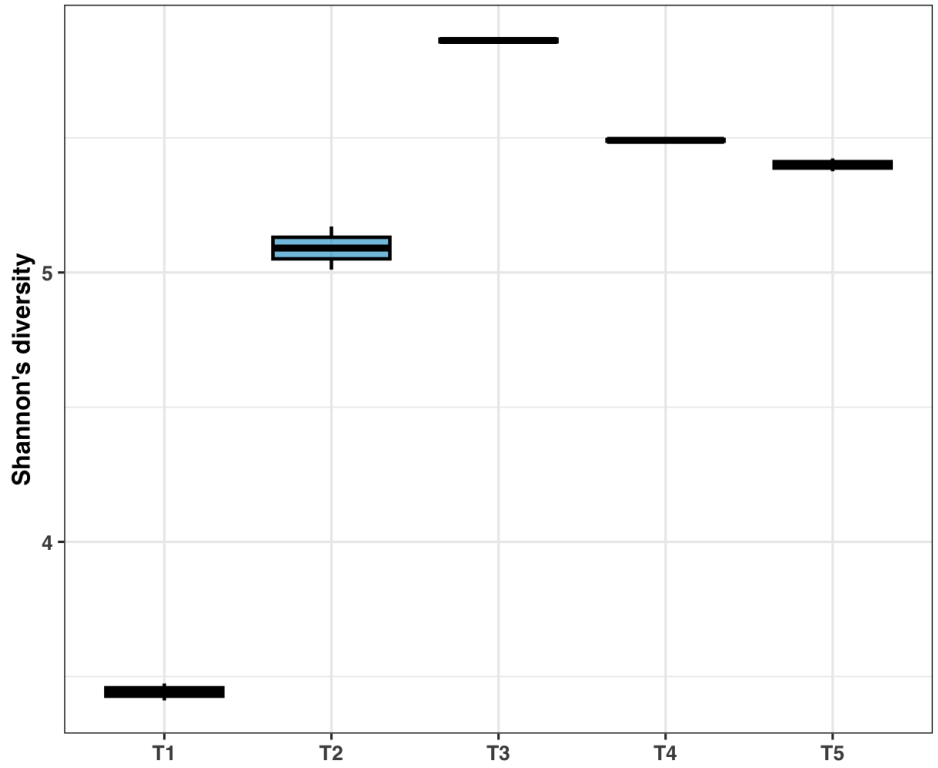
Comunità diverse tra loro e con quelle ambientali



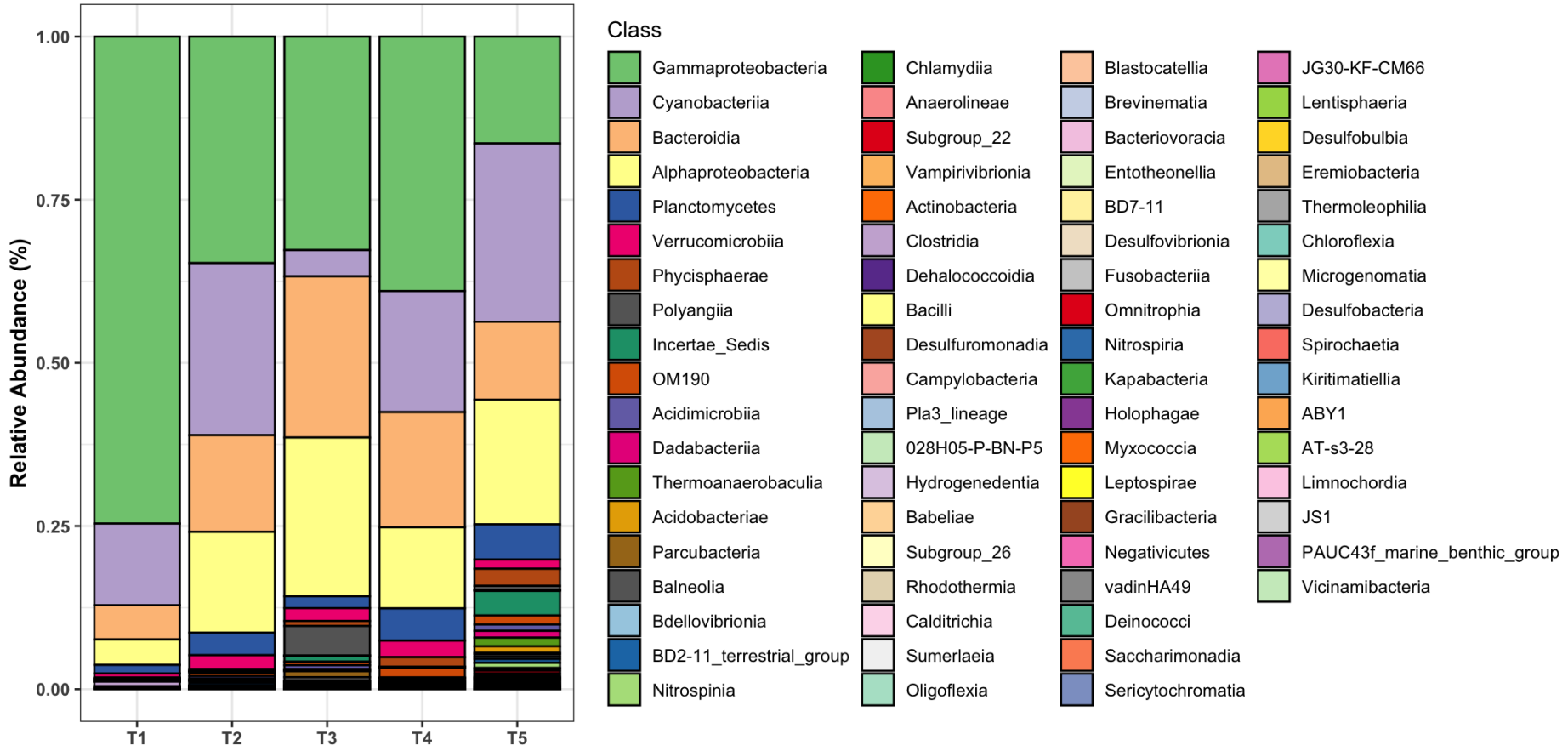
Aging sperimentale: andamento della biodiversità microbica

Monitoraggio dell'invecchiamento:
T1 – T2 – T3 – T4 – T5





Aging sperimentale: andamento della biodiversità microbica



Aging sperimentale: andamento della biodiversità microbica

- ***Pseudoalteromonas*** rappresenta il genere più abbondante al T1, ma la sua presenza diminuisce drasticamente al T5. Questo comportamento fa di Pseudoalteromonas un colonizzatore precoce, probabilmente capace di sfruttare rapidamente le superfici nuove delle microplastiche e le risorse facilmente degradabili, ma viene progressivamente sostituito da generi più adatti a un biofilm maturo.

- Allo stesso gruppo appartengono ***Aliivibrio*** e ***Marinicella***, che mostrano picchi precoci (T1–T2) seguiti da un forte declino

- I generi ***Colwellia***, ***Methylotenera***, ***Halomonas*** e ***Aliikangiella*** emergono in fasi intermedie (T3-T4) della successione del biofilm sono probabilmente adattati a utilizzare nutrienti derivati dai metaboliti dei colonizzatori iniziali o substrati complessi rilasciati dalle microplastiche degradate. Questi generi mostrano un aumento significativo a partire da T3, raggiungendo il massimo in T4

- ***Moritella***, ***Aliiglaciecola***, ***Woeseia*** emergono principalmente in T5. La presenza di Moritella esclusivamente al T4 indica un adattamento a condizioni specifiche del biofilm maturo.