FINAL PROJECT REPORT

Project: “Fresh Project 4.0”: Life in plastic, it's fantastic: unravelling the microalgal community of plastisphere across European lentic systems (PhytoPlastic)

Project funded by European Federation for Freshwater Sciences (EFFS-EFYR)

The report was prepared by the PhytoPlastic project Principal Investigators (PIs):

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December 2024

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1. GENERAL INFORMATION AND FUNDING

This final report presents the outcomes of the project “Life in Plastic, It's Fantastic: Unraveling the Microalgal Community of the Plastisphere Across European Lentic Systems (PhytoPlastic)”. The document provides an overview of the project's implementation, achievements, preliminary scientific results, and related activities.

The PhytoPlastic project was the recipient of the 4th Collaborative European Freshwater Science Project for Young Researchers (“FreshProject”) call. Running from October 1, 2022, to September 30, 2024, the project successfully met its objectives. The Principal Investigators (PIs) leading the initiative were Veronica Nava (University of Milano-Bicocca, Department of Earth and Environmental Sciences, Italy) and Julia Gostyńska (Adam Mickiewicz University in Poznań, Institute of Environmental Biology, Department of Hydrobiology, Poland).

This project was awarded by the European Federation of Freshwater Sciences (EFFS) and the European Fresh and Young Researchers (EFYR) group. Funding was provided through contributions from the following EFFS member societies:

- Freshwater Biological Association (FBA)
- Deutsche Gesellschaft für Limnologie e.V. (DGL)
- Asociación Ibérica de Limnología (AIL)
- Association Française de Limnologie (AFL)
- Česká limnologická společnost (CLS)
- Swiss Society for Hydrology and Limnology (SSHL)
- Associazione Italiana di Oceanologia e Limnologia (AIOL)
- Magyar Hidrológiai Társaság (MHT)
- Irish Freshwater Sciences Association (IFSA)
- Hrvatsko Udruženje Slatkovodnih Ekologa (HUSEk)
- The Society of Austrian Limnologists (SIL-Austria)

2. PROJECT STRUCTURE

2.1. Project objectives

Among the multiple stressors that affect aquatic ecosystems, plastic pollution is deemed a widespread environmental issue. Since the ubiquitous presence of plastic debris in aquatic systems has been established, the focus of research has shifted towards assessing their impact on aquatic organisms and ecosystem functions. The interaction of plastics with aquatic biota starts from low trophic levels; indeed, plastics represent a new habitat for rafting organisms to the point that the term "plastisphere" was coined to define the diverse community growing on the surface of plastic debris. Even if heterotrophic bacteria tend to be the focus of plastisphere research, the presence of microalgae within the epiplastic biofilm has been repeatedly documented. However, further research is needed to explore the microalgae-plastic interactions, and several questions remain to be addressed, especially for freshwaters.

Given the widespread presence of plastic debris in freshwater systems and the lack of knowledge about the ecological implication of their presence, the present project is aimed at studying the temporal establishment and development of phytobenthos on different plastic polymers over a wide geographical scale to better characterize the interaction of plastics with key organisms of aquatic ecosystems, i.e. microalgae. The overreaching goal is to understand whether plastic debris can represent a new niche for the microalgal community in freshwater systems and determine whether substrate-specific properties or environmental factors prevail in shaping microalgal assemblages on plastic debris. In particular, the project aimed at:

1. evaluate whether different plastic polymers constitute suitable substrates for the development of microalgal communities;
2. quantify the microalgae biomass developed on microplastics with different polymeric composition and determine whether biomass vary significantly among substrates across a variety of aquatic systems;
3. identify the microalgae species that are able to develop on different substrates and understand whether plastics exert a strong enough selection to drive species sorting, overcoming other niche-defining factors;
4. evaluate the temporal and seasonal evolution of the epiplastic community of microalgae in relation to several environmental variables.

2.2. Methods of the project

Experiments were conducted in lentic systems with different features. Sheets of polyethylene, polypropylene, and glass (which serve as control being an inert substrate) were deployed in each system (Fig. 1). To assess the temporal and seasonal evolution of the colonization, samples were collected in succession after 3, 7, 15, and 30 days and the experiments were replicated in each season (Fig. 2). Several physical and chemical parameters was analyzed alongside (e.g., temperature, dissolved oxygen, nutrient concentration) to understand the relationship with the environmental variables. For each substrate and replicate, we assessed the phytobenthic biomass estimating the chlorophyll a, and the ash-free dry mass. Moreover, microalgae composition was determined on a subset of samples to understand the community composition colonizing the different substrates. This represents, at the best of our knowledge, the first coordinated experiment conducted at a large spatial scale to explore the plastisphere and, thereby, a unique dataset was generated that allow us to identify the key drivers of the process, leaving aside the site-specific and possibly confounding variables.

Fig. 1. *Photographs illustrating the structures used in experiments, with sheets of polyethylene, polypropylene, and glass attached.*

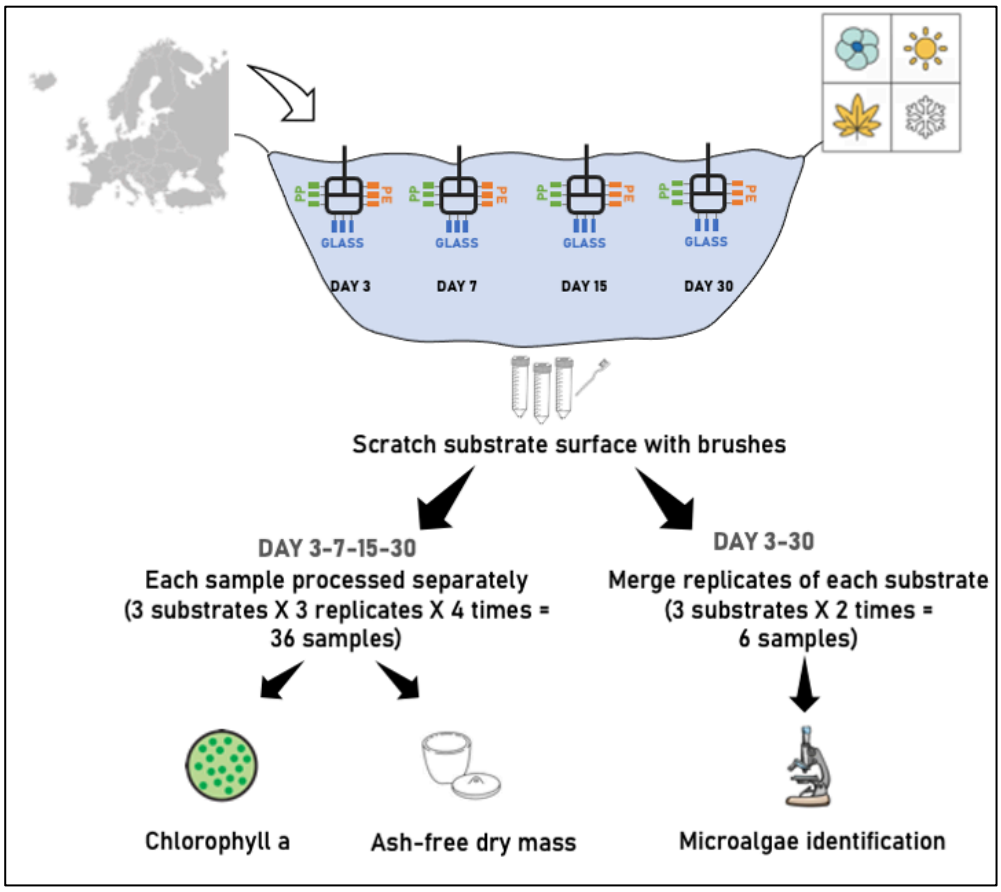


Fig. 2. *Schematic representation of the sampling design.*

2.3. Development of the project

The project was structured into four main tasks: the pre-experimental phase, the experimental and laboratory phase, data analysis and publication, and communication and dissemination, spanning from October 1, 2022, to September 30, 2024 (Fig. 3). Regular online meetings were conducted from the project's onset via Zoom or WebEx to update participants on progress, discuss tasks, and assign responsibilities. Agendas and recordings from these meetings were shared with all participants afterward.

Email served as the primary communication tool, supplemented by Slack for team discussions. Dropbox and Google Drive were utilized for organizing and sharing key materials, including environmental sampling and laboratory analysis protocols, meeting agendas and recordings, presentations, participant lists, experimental datasets, conference abstracts and presentations, photos, and other relevant documents. During manuscript preparation, Zotero was used to

compile a shared literature database. All project participants were granted access to these online resources.

Project activities were disseminated using:

- Project website: https://phytoplastic.wixsite.com/my-site
- Instagram: https://www.instagram.com/phytoplastic
- X: https://x.com/PhytoPlastic

As part of the project, we organized two online seminars for both project participants and external attendees. In the first seminar, we invited Dr. Katrin Wendt-Potthoff from the Helmholtz Center for Environmental Research - UFZ in Germany, who delivered a presentation titled “Microplastics in Dams and Water Reservoirs: Distribution and Biogeochemical Interactions.” The second seminar featured PhD Silvia Galafassi from the Water Research Institute – CNR in Italy, who presented on “Microbial Fouling of Polymers: An Environmental Challenge of Microplastics.”

A GitHub workshop was also organized for project members to introduce the platform and its functionalities. This training proved to be highly beneficial, as the GitHub platform was later utilized for managing datasets, preparing statistical analyses, and storing related workflows.

Additionally, a side project focusing on plastisphere DNA was launched to expand the scope of the research.

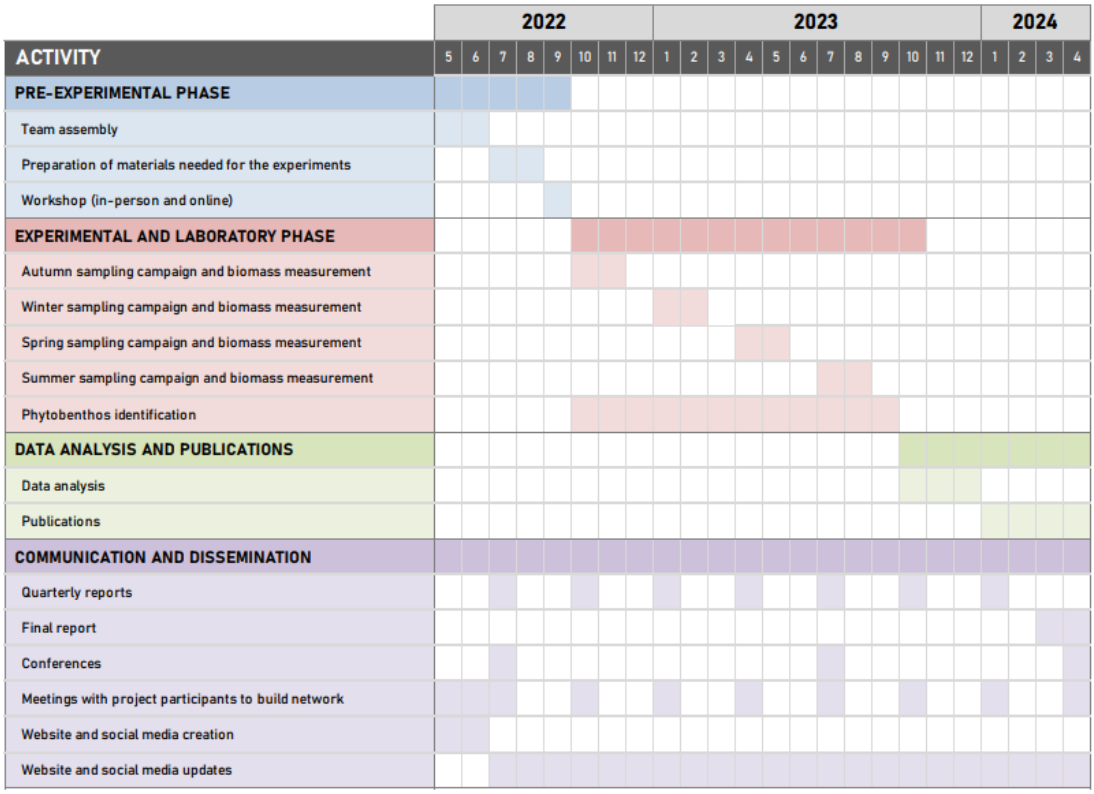
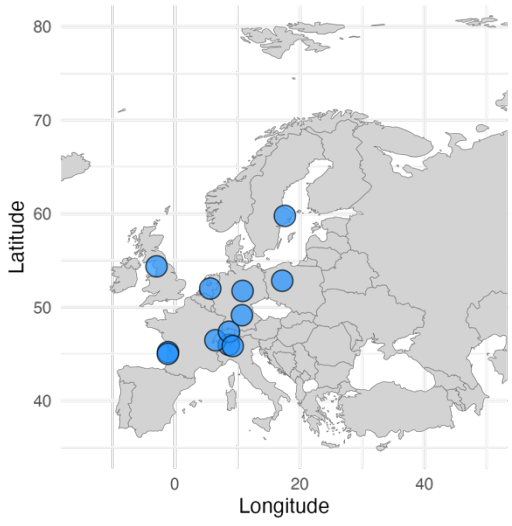


Fig. 3. *Diagram showing project tasks from October 1, 2022 to September 30, 2024.*

2.4. Study sites

Environmental experiments were conducted in 15 bodies of water in 8 European countries (Fig. 4).



Site	Country	Maximum depth (m)	Waterbody area (km²)	Waterbody volume (km³)
Ekoln	Sweden	50	30	0.46
Tête d'or	France	3.8	0.17	0.00034
Segrino	Italy	8.6	0.38	0.0012
Durowskie	Poland	14.6	1.437	0.0113229
Greifensee	Switzerland	32.3	8.45	0.1485
Altmühlsee	Germany	2.5	4.5	0.0138
Aurora	Netherlands	1.3	0.006	0.000006
Maggiore	Italy/Switzerland	370	212	37
Rappbode	Germany	89	3.9	0.11
Geneva	Switzerland/France	310	580.03	89
Windermere	UK	66	14.73	0.56
Hourtin	France	13.65	56.67	0.21
Lacanau	France	7	19.85	0.053
Stechlin	Germany	69.5	4.3	0.0969
Lugano	Switzerland/Italy	288	48.9	6.5

Fig. 4. Map illustrating the locations of the sampling sites, accompanied by a table summarizing key information about the water bodies.

3. PROJECT TEAM

The project involved 45 people who worked on various aspects of the project, including experimental and laboratory work, statistical analyses, manuscript writing, and other project-related activities. The project participants included: Master student, PhD student, and early Post-Doc. Below is the list of participants:

No.	Surname and name	Country
1.	Abbasi Mona	Sweden
2.	Aurich Patrick	Germany
3.	Barral-Fraga Laura	France
4.	Barthélémy Nans	France
5.	Bick Berenike	Sweden
6.	Boateng Charles Mario	Ghana
7.	Bottone Anna	Sweden
8.	Burri Bryan	Switzerland
9.	Cabrerizo Marco J.	Spain
10.	Cairola Geoffrey	France
11.	Chevalier Manon	France
12.	Chonova Teofana	Switzerland
13.	Cour Mathilde	France
14.	De Santis Vanessa	Italy
15.	Dory Flavia	France
16.	Drost Annemieke	Netherlands
17.	Elster Josef	Czech Republic
18.	Farez Valeria	Germany
19.	Fatras Baptiste	France
20.	Fehlinger Lena	Spain
21.	Figler Aida	Hungary
22.	Gionchetta Giulia	Switzerland
23.	Gostyńska Julia	Poland
24.	Gray Emma	Ireland
25.	Halabowski Dariusz	Poland
26.	Harvey Daniel	England
27.	Heinrich Lena	Germany
28.	Jaffer Yousuf Dar	Germany
29.	Jakobsson Ellinor	Sweden

30.	Merkli Stefanie	Switzerland
31.	Misteli Benjamin	France
32.	Mo Yuanyuan	Germany
33.	Mori-Bazzano Laureen	Switzerland
34.	Moser Valentin	Switzerland
35.	Nava Veronica	Italy
36.	Nowakowski Kyra	Germany
37.	Oloyede Adekolurejo	England
38.	Orlandi Valentina	Italy
39.	Pasqualini Julia	Germany
40.	Rotta Federica	Switzerland
41.	Schmid-Paech Bianca	Germany
42.	Touchet Camille	France
43.	Vaziourakis Konstantinos-Marios	Sweden
44.	Vázquez Victor	Spain
45.	Yousefi Azadeh	Italy

4. PRELIMINARY RESULTS

We collected 2,160 individual observations for chlorophyll a and ash-free dry mass. However, some samples were excluded due to issues encountered during analysis, and additional data gaps exist due to adverse environmental conditions that impacted some of the deployed structures. All collected data underwent a thorough review to ensure accuracy, including verification of calculations. The dataset is now complete and prepared, with data analysis currently underway.

The preliminary findings are illustrated in Fig. 5, which presents boxplots of chlorophyll a concentrations (Fig. 5a) and ash-free dry mass (AFDM) (Fig. 5b) across the different lakes. These data are organized to emphasize variations among seasons, substrates, and collection times, providing an overview of how environmental and experimental factors influence biofilm development.

An overall increase in microalgal biomass, as indicated by chlorophyll a levels, was observed over the colonization period. This trend highlights a progressive accumulation of autotrophic

organisms, such as algae, over time. Notable differences among the substrates were detected. For instance, while glass (used as a control substrate) and plastic substrates exhibited comparable biomass in most cases, the glass substrate occasionally supported greater growth than LDPE (low-density polyethylene). This suggests that algae may opportunistically colonize plastics, but their growth is not necessarily enhanced compared to non-plastic surfaces. Instead, the findings imply that the physical or chemical properties of the substrate, rather than its composition as plastic, likely play a key role in colonization dynamics.

AFDM measurements, which provide a more comprehensive assessment of the organic biomass on substrates (including autotrophs, heterotrophs, and other organic material), revealed distinct seasonal and temporal patterns. Higher colonization was observed during Spring and Summer, with lower average values in Winter, reflecting the influence of temperature and other seasonal factors on biofilm development. Over time, the organic matter content increased steadily, reaching its maximum after 30 days of colonization. By the end of the experimental period, LDPE substrates consistently supported lower organic biomass on average compared to glass and PET (polyethylene terephthalate). Interestingly, glass and PET substrates exhibited similar colonization levels, suggesting that PET may offer a surface conducive to biofilm formation comparable to that of glass.

These findings collectively indicate that substrate type, seasonality, and time of colonization are crucial factors influencing both microalgal and overall organic biomass accumulation. While plastic surfaces are colonized, their role as a substrate for biofilm formation appears to be complex and context-dependent, warranting further investigation to elucidate potential ecological implications.

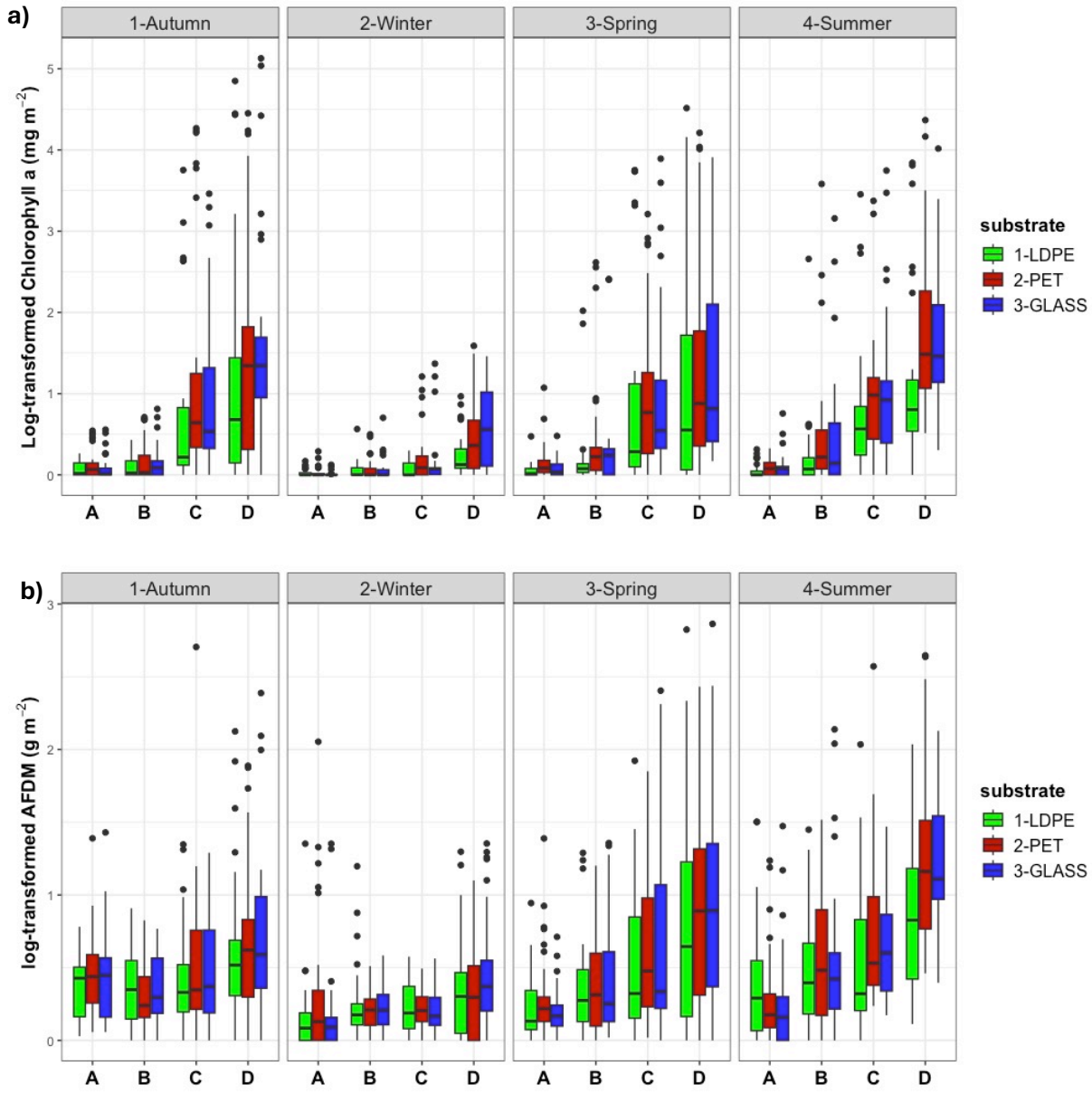
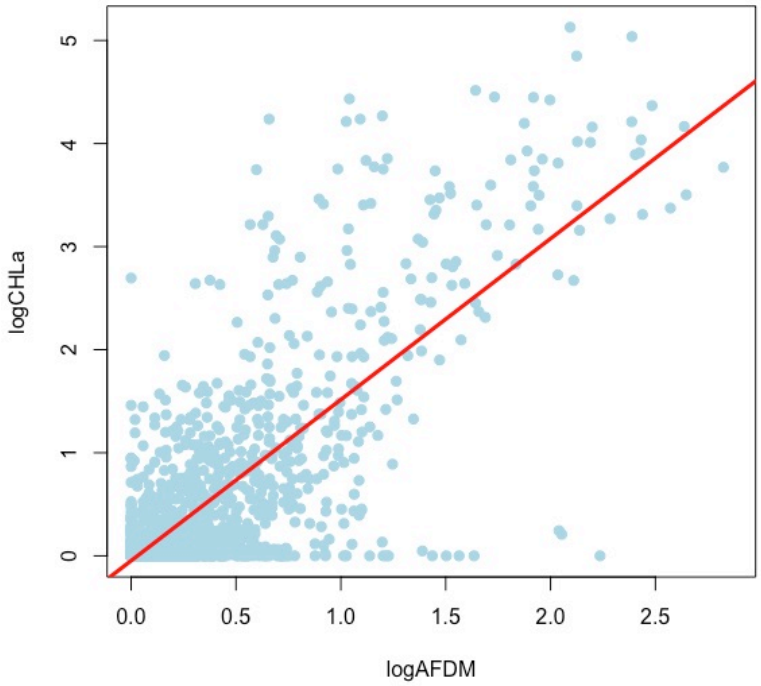


Fig. 5. *Preliminary results of (a) Chlorophyll a and (b) Ash-Free Dry Mass (AFDM), aggregated across different lakes, seasons, substrates, and sampling times (A = 3 days, B = 7 days, C = 15 days, D = 30 days).*

Figure 6 illustrates the correlation between the two key parameters considered in this study: chlorophyll *a* and ash-free dry mass (AFDM). A generally strong correlation was observed between these parameters, indicating that increases in autotrophic biomass (as measured by chlorophyll a) are often accompanied by increases in overall organic biomass (AFDM). However, certain outliers or deviations from this trend were identified, suggesting that specific conditions or factors may affect this relationship. These cases warrant further

investigation to identify potential causes, such as variability in substrate composition, seasonal effects, or microbial community dynamics.



To build on these preliminary findings, future analyses will aim to:

a) **Identify environmental drivers**: The data will be analyzed to determine how specific characteristics of the lakes influence the relationship between chlorophyll a and AFDM. For example, the role of the trophic status (e.g., oligotrophic, mesotrophic, or eutrophic conditions) in shaping biofilm growth and organic matter accumulation will be explored. Differences in nutrient availability, light penetration, and water chemistry are expected to be significant contributors to the observed patterns.

b) **Integrate with additional parameters**: The correlation between chlorophyll a and AFDM will be assessed in the context of other measured parameters, such as nutrient concentrations (e.g., nitrogen and phosphorus), pH, temperature, and oxygen levels. This comprehensive approach will help identify the drivers of variability and provide a deeper understanding of how environmental and substrate-specific factors interact.

c) **Examine substrate-specific dynamics**: A deeper investigation into the role of substrate types (e.g., glass, LDPE, PET) will be conducted to determine whether material properties influence deviations from the observed correlation. This will include examining surface roughness, hydrophobicity, and biofilm composition to understand substrate-driven effects.

d) **Seasonal and temporal trends**: Future analyses will explore how the correlation between chlorophyll *a* and AFDM evolves over different seasons and time points. Seasonal variations in biofilm community composition and environmental conditions may influence the strength and consistency of the relationship.

5. PROJECT BUDGET

We received a total budget of €9,150 from the funding societies. To date, the majority of expenses have been allocated to conference participation, amounting to €3,176.38 (34.7% of the total budget). Additional expenditures included reimbursements for individuals involved in constructing scientific structures, fuel for field experiments, and payments for analyses. Many laboratory facilities contributed by covering some additional costs, helping to reduce overall expenses. These combined expenditures totaled €647.79 (7.1% of the project budget).

At the end of the project, we have a remaining budget of €5,325.83. This will be allocated to:

a) Participation in the 14th Symposium for European Freshwater Sciences in Turkey in 2025, where the project's results will be presented.
b) Covering the open access (OA) publication costs for the forthcoming PhytoPlastic project article.

6. PROJECT ACHIEVEMENTS

I. <u>CONFERENCES (oral presentations)</u>

- 13th Symposium for European Freshwater Sciences in Newcastle Upon Tyne, England, June 18-23, 2023, *Unravelling the microalgal community in the plastisphere: preliminary results from the PhytoPlastic project*

II. CONFERENCES (posters)

- LXIV. Hydrobiologist Days in Tihany, Hungary, October 4-6, 2023, *Unraveling the microalgal community in the plastisphere: PhytoPlastic, the new international project*
- XXII Congress of the Iberian Association Limnology in Vigo, Spain, June 3-28, 2024, *Exploring the plastisphere: Colonization dynamics of microalgae on plastics in European freshwater ecosystems*
- XXVIII Congresso Associazione Italiana di Oceanologia e Limnologia in Lecco, Italy, June 24-28, 2024, *The PhytoPlastic project: Large-sclae collaboration exploring the plastisphere across European lakes*
- XXXIII Congresso Società Italiana di Ecologia (SItE) in Rome, Italy, September 23-26, 2024. *PhytoPlastic project: exploring the plastisphere community in European lentic systems*

III. SCIENTIFIC ARTICLES

- Nava V., Dar J., Y., De Santis V., Fehlinger L., Pasqualini J. Adekolurejo O. A., Burri B., Cabrerizo M., J., Chonova T., Cour M., Dory F., Drost A. M., Figler A., Gionchetta G., Halabowski D., Harvey D. R., Manzanares- Vázquez V., Misteli B., Mori-Bazzano L., Moser V., Rotta F., Schmid-Paech B., Touchet C. M., Gostyńska J. 2024. Zooming in the plastisphere: the ecological interface for phytoplankton–plastic interactions in aquatic ecosystems. Biological Reviews Cambridge Philosophical Society. https://doi.org/10.1111/brv.13164

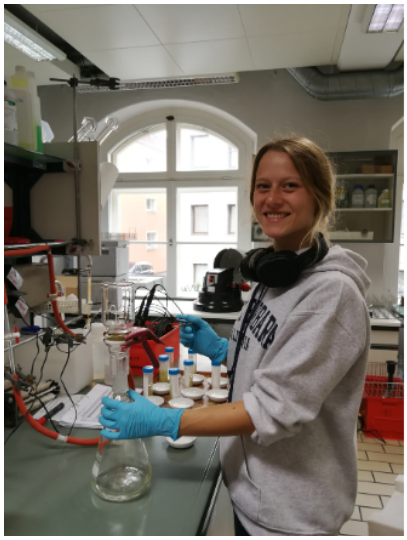
Although the PhytoPlastic project has officially finished, we are going to publish a paper with the results in 2025, which we would like to present at the 14th Symposium for European Freshwater Sciences in Turkey. Moreover, we are planning to attend further scientific conferences to discuss the results obtained.

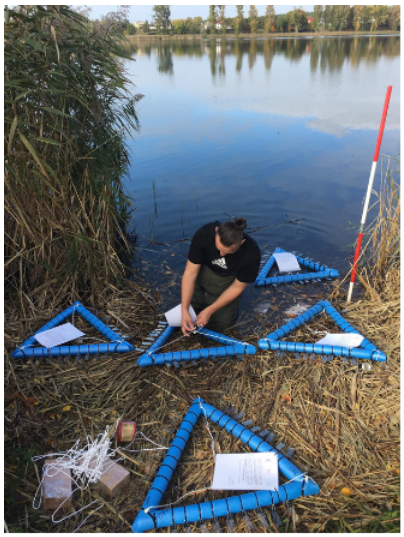
7. ACKNOWLEDGEMENTS

We would like to express our gratitude to all participants of the PhytoPlastic project for their enthusiasm and cooperation during the project development. Their great commitment in conducting experiments, performing laboratory analyses and contributing to data analysis was invaluable. We express our appreciation to their supervisors and research institutions for providing the necessary facilities and support to carry out the research. Special thanks are due to the European Federation of Freshwater Sciences (EFFS) and the European Young Scientists (EFYR) for granting the PhytoPlastic project the possibility to carry out the project, providing financial and organizational support. Finally, we would like to thank all those who provided us with substantive, organizational and general support throughout the project, contributing to its successful completion.

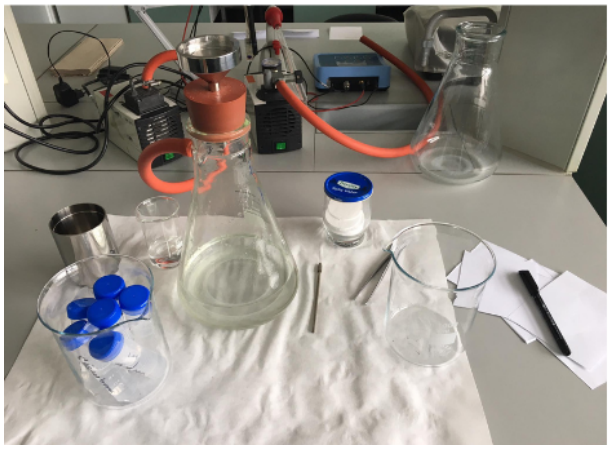
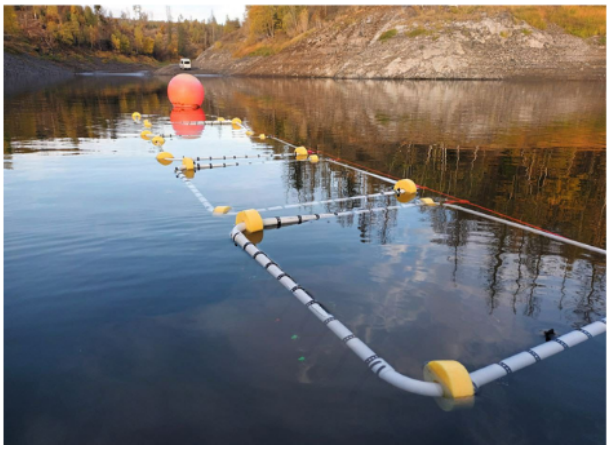
8. SUPPLEMENTARY MATERIALS

A collection of photographs showcasing team members engaged in field activities and laboratory analyses.

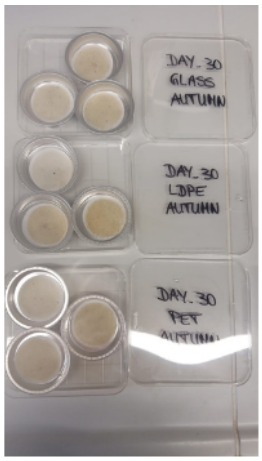






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