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New ecological frontiers in the plastisphere: Diatoms and macroinvertebrates turnover assessment by a traits-based approach

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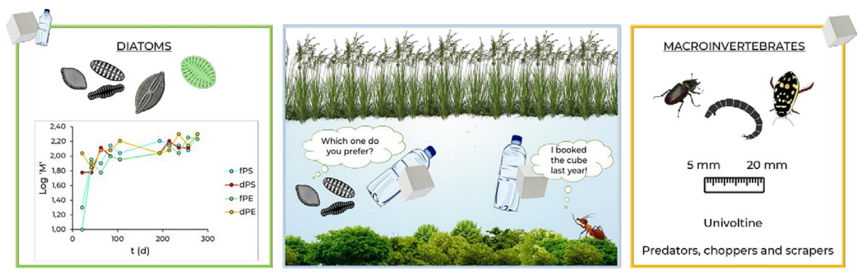
HIGHLIGHTS

- We adopted trait-based approach on diatom and macroinvertebrate colonizing plastic.
- We used virgin substrate of PS and PET located in a wetland.
- Diatoms belonging to mainly motile guild colonized both polymers.
- Predator, chopper and scraper macroinvertebrates preferred PS.

GRAPHICAL ABSTRACT



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ABSTRACT

To date, there are very few studies regarding the colonization of artificial substrates in wetlands by macroinvertebrates and diatoms and even fewer are the studies in Italy that take into consideration the diatomic guilds and the biological and ecological traits proposed in literature. Wetlands are at the forefront through the most delicate and threatened freshwater ecosystems. In this study, we want to evaluate the colonization capacity of plastics of diatoms and macroinvertebrates and characterize the diatomic and macroinvertebrate communities using a "traits-based" approach focusing on the colonization of virgin substrates made of polystyrene and polyethylene terephthalate. The study was conducted within the 'Torre Flavia wetland Special Protection Area' a protected wetland area in Central Italy. The study was conducted from November 2019 to August 2020. The results obtained in this study show a tendency of diatom species to colonize artificial plastic supports placed in lentic environments without differences related to the plastic type and water depth. There is also a greater number of species belonging to the "*Motile*" guild, endowed with a high motility that they exploit to search for more ecologically suitable habitats for settlement. Macroinvertebrates, prefer settlement on polystyrene supports, those on the surface, probably due to the anoxic conditions present on the bottom and the physical structure of the polystyrene that provides shelter to many animal taxa. The analysis on traits highlighted the establishment of an ecologically diverse community mainly formed by univoltine organisms, with dimensions between 5 and 20 mm, predators, choppers and scrapers feeding on plant organisms and animals, but without the formation of a clear ecological system, that is, without evidence of ecological relationships established between two or more taxa. Our research can contribute to underline the ecological complexity of biota inhabiting plastic litter in freshwaters and the implications for plastic-impacted ecosystems biodiversity enrichment.

1. Introduction

The first data regarding plastic waste in water reservoirs dates back to the 1970s, but only after half a century plastic pollution become one of the most important topics in the world of science (Kasavan et al., 2021). Plastic debris are hydrophobic and their surface can enhance the biofouling favouring the colonization by micro- and macro-organisms (Smith et al., 2021; Taurozzi et al., 2022). This unique community, inhabiting the plastic surfaces, is called "Plastisphere" (Amaral-Zettler et al., 2020; Barros and Seena, 2021; Zettler et al., 2013). Among this community it could be easy to find different organisms like diatoms (Cheng et al., 2021) and macroinvertebrates (Mghili et al., 2022).

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Diatoms are unicellular microalgae characterized by siliceous cell wall (frustule), inhabiting all the photic zones (Rabiee et al., 2021). These photosynthetic microalgal species along with other aquatic microbes are the primary colonizers that form biofilm and serve as trigger for other larger organisms to colonize on the surface (Taurozzi et al., 2022). It is estimated that they are responsible for 20 % of carbon fixation and can tolerate huge environmental gradients, such as variations in physic-chemical water parameters thank to their great adaptability (Marella et al., 2020). The use of diatoms as bioindicators is widely spread (Celekli et al., 2021; Della Bella et al., 2007): the analysis of the diatom assemblage structure, species' autecology, and biological traits allows to characterize a specific waterbody from an ecological and biological point of view (Minaoui et al., 2021). The interaction between algae and plastics has been poorly studied, but laboratory experiments show that plastics may cause negative impacts on these organisms (Wu et al., 2019).

Macroinvertebrates (invertebrates >0.5 mm) are a heterogeneous group of organisms which include an important variety of taxonomic groups like insects, molluscs, crustaceans and many others (Schmera et al., 2022), observed by naked eyes and ubiquitous, inhabiting many terrestrial and water ecosystems (Sumudumali and Jayawardana, 2021). Macroinvertebrates are excellent bioindicators (Torrisi et al., 2010), covering a range in sensitivity to a variety of stressors and are easy to sample (Marcheggiani et al., 2019; Eriksen et al., 2021), used since decades to assess the status of aquatic ecosystems (Bonacina et al., 2022). Macroinvertebrates usually spent their whole life in the same water basin integrating all environmental changes: for this reason, macroinvertebrates community structure, ecology and particular ecological traits have shown a potential for their use as ecological indicators of environmental stress (Costa et al., 2020).

The effects of plastics can be serious as regards physical, toxic, and behavioural impacts, but its association with other pollutants may enhance effects (Azevedo-Santos et al., 2021). There are many types of plastics, each formed by the assembly of different polymers (Cesarini et al., 2021), and each has its own specific purpose of use linked to the properties of the compound (van Emmerik and Schwarz, 2019). Moreover, additives can be added to the plastic polymers to enhance specific physical-chemical characteristics (make plastics more softer, prevent damage, prevent ignition, add fragrances, inhibit chemical degradation) (Sridharan et al., 2022). These functional additives play a major role in the toxicity of plastics, leading to a potential future peak release of toxic compounds (Rillig et al., 2021), related not only to the plastic in se, but also to the chemical additives added and released in the environment. The durability of plastic, that makes it such an attractive material to use, also makes it highly resistant to degradation: for this reason disposing of plastic waste is problematic (Bajt, 2021). Despite the approximately 30.000 different polymers registered for use in the European Union, the most common plastic materials are: polystyrene (PS), polyethylene terephthalate (PET), polyurethane (PUR), polyvinyl chloride (PVC), polyethylene (PE) and polypropylene (PP) (Cera et al., 2020; Strungaru et al., 2019). In particular, PS and PET are two of the most largely used plastics worldwide, in food and industry packaging, disposable cutlery, medical products and toys, and many other applications (González-Fernández et al., 2020).

The accumulated plastics in waterbodies can be classified into different size classes: macroplastics (>5 cm), mesoplastics (5 mm < item < 5 cm), microplastics (0.1 μm < item <5 mm) and nanoplastics (<0.1 μm) (Thushari and Senevirathna, 2020). The impact of macroplastics can have on the marine environment has long been the subject of environmental research, but fewer studies focused on the impacts on freshwaters and wetlands (Gallitelli and Scalici, 2022; Cesarini et al., 2023). The environmental impact of macroplastics include particularly: the injury and death of marine birds (Battisti et al., 2019), mammals (Poeta et al., 2017), fishes, and reptiles (Staffieri et al., 2019) resulting from plastic entanglement and ingestion (Barboza et al., 2019) and the transport of non-native species to new habitats on floating plastic debris (Amaral-Zettler et al., 2020). Moreover, Cesarini and Scalici (2022) demonstrated that also the riparian vegetation can be affected by macroplastics, acting as a trap for plastic debris that can impact seriously these ecosystems.

Despite the increasing recent concern about the plastic impacts on freshwaters, and in particular wetlands (Cera et al., 2022; Matamoros et al., 2020), studies focusing on the ecological characterization of inland freshwaters through a traits-based approach using diatomic guilds and macroinvertebrate traits are in an early stage, most of these focusing on rivers. Mediterranean wetlands often suffer natural or anthropic disturbances, like water stress and droughts: the effects of these disturbances can affect in many ways different species assemblages, like diatoms and macroinvertebrates (Causarano et al., 2009). Moreover, the communities that form on plastic waste are, to date, poorly investigated.

The objective of this study is to analyse the colonization capacity of two biological indicators, diatoms and macroinvertebrates, on plastic support artificially settled in situ through an ecological traits-based approach. In particular, the hypothesis are:

- Diatoms belonging to the “Motile” guild are more abundant respect to high profile and low profile ones;
- The number of species of diatoms show an increase along a time gradient ranging from the first to the tenth sampling;
- PS results better than PET to provide a suitable substrate for the colonization of macroinvertebrates;
- Among macroinvertebrates communities, the various modalities are equally represented, without a temporal trend.

This study could pose an important step to the knowledge of plastisphere communities and raise awareness about coastal ecosystems; therefore, it is necessary to identify targeted strategies for the management of coastal wetlands.

2. Material and methods

2.1. Study area

The study area is the ‘Torre Flavia wetland Special Protection Area’ (sensu 147/2009 EU Directive) (41°57′43″N 12°2′49″E), a protected area that extends for about 40 ha and is part of the municipality of Ladispoli and Cerveteri, located along the Tyrrhenian coast (Fig. 1). The “Torre Flavia natural monument” consists of a beach and a wetland situated behind a system of dunes. The dunes are very important area for biodiversity because of the presence of many plant species, like *Elymus farctus* (Runemark e Melderis, 1978), *Cakile maritima* (Scopoli, 1772), *Pancratium maritimum* (Linnaeus, 1753). The wetland instead represents a hotspot of animal biodiversity with many species of birds, as well as amphibians, reptiles, mammals, and fishes like the little ringed plover (*Charadrius dubius*, Scopoli, 1786), the Kentish plover (*Charadrius alexandrinus*, Linnaeus, 1758), the common toad (*Bufo bufo*, Linnaeus, 1758), the European pond turtle (*Emys orbicularis*, Linnaeus, 1758), the fox (*Vulpes vulpes*, Linnaeus, 1758), the porcupine (*Hystrix cristata*, Linnaeus, 1758), the mullet (*Mugil cephalus*, Linnaeus, 1758) and the eel (*Anguilla anguilla*, Linnaeus, 1758) (Battisti et al., 2020).

2.2. Experimental design

The experimental protocol provided for the identification of one sampling site within one of the swamp canals. Ten supports were inserted in this canal with an approximate distance of about 3 m from each other and 1 m from the banks of the canal. Entrance to the study area is forbidden to the public. For the purpose of the study, 10 supports were created consisting of two PS cubes of the size 20 × 20 cm and two PET bottles (32.6 × 8.2 cm). The PS cube and the PET bottle were connected by a rope and one of the two bottles was filled with sand to make possible to go to the bottom (the rope was not used as a substrate for the research). Therefore, four typologies of epiplastic microhabitats were evaluated: floating and dipped PS (fPS and dPS, respectively) and floating and dipped polyethylene (fPE and dPE, respectively). Each support was also found to be in the same lighting and ventilation conditions as the others, in order to create as little variability as

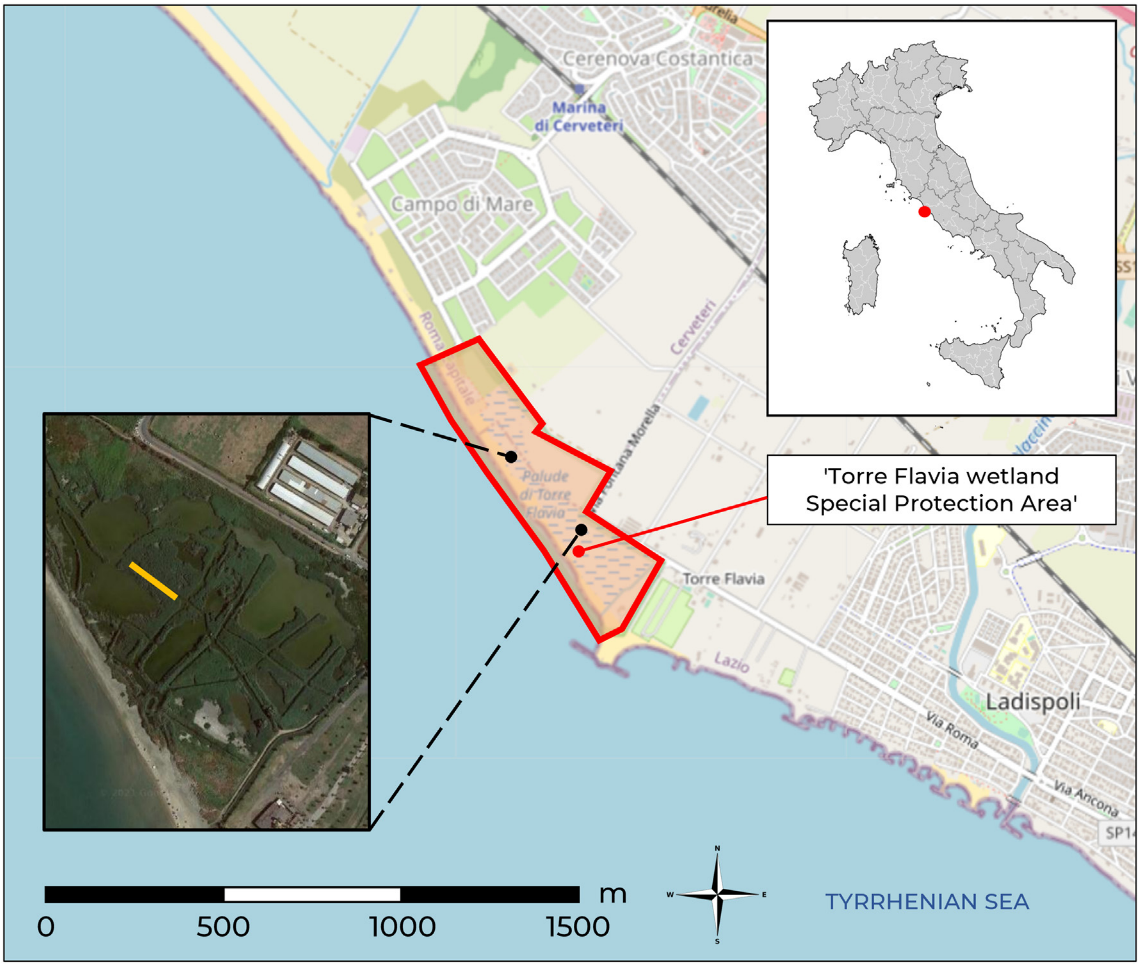

Fig. 1. The study area located in a protected wetland in Latium Region, Central Italy. In red the edges of the 'Torre Flavia wetland Special Protection Area' (sensu 147/2009 EU Directive), in yellow the canal.© OpenStreetMap contributors.

possible within the experimental design (Fig. 2). The supports were assembled in the laboratory and then transported to the study area.

The study was conducted for 10 months from November 2019 to August 2020, sampling every 20 days, with an interruption of sampling for 3 months from March to May 2020 due to the COVID-19 pandemic.

After being collected, the supports were brought to the bank and here, after cutting the rope that connected the dipped supports with the floating ones with a blade, they were divided and placed in two different plastic bags to avoid contamination of material between one and the other. Then we proceeded with a preliminary visual analysis of the surfaces of the supports to verify the possible presence of macroinvertebrates that, where present, were taken with tweezers, and each placed in a 50 ml Falcon with ethanol (70 %) for the storage of samples, waiting to be transferred to the laboratory for further analysis.

The same supports were then used to obtain the vegetal component useful for our research: using a commercial toothbrush the surface of each support was scratched (taking care to use a different brush for each of the four supports), on each side of the cube. Then, each toothbrush was immersed in a 50 ml Falcon containing 70 % diluted ethanol and canal water taken at the same time as the toothbrush cleaning operations: in this way the diatomic component was also made suitable to be carried out in the laboratory and for oxidation operations.

Samples were transported to the laboratory where oxidation of diatoms was carried out following the standardized protocol by ISPRA (2014a, 2014b) and the permanent slides were prepared. For the identification of diatom species, the morphological analysis was conducted with a Leica microscope with 100× magnification in immersion and using taxonomic guides (Taylor et al., 2007; Ector and Hlúbiková, 2010; ISPRA, 2014a, 2014b; Bahls et al., 2018; DREAL Languedoc Roussillon, 2021). For each slide, the analysis was considered completed when identification at the species level of at least 400 valves was achieved. The diatomic species were then classified threw guilds, based on their ecological characteristics.

The analysis on macroinvertebrates were carried out using a stereomicroscope (Nikon C-LEDS with 4.0× magnification) for the correct and detailed observation of samples larger than 1 mm. The morphological identification of the taxa present was carried out using specific taxonomic guides (Sansoni, 1998; Campaioli et al., 1994; Chinery, 2010), also considering the possible presence of species belonging to terrestrial taxa and not necessarily related to the aquatic environment. Using dichotomous keys, it was possible to identify each individual at least at the level of the family they belong to. The individuals properly identified were then conserved in Eppendorf with an ethanol solution (80 % v/v). Aquatic macroinvertebrates were then classified through 9 traits (7 biotic and 2 ecological) using literature source by Tachet et al. (2010).

2.3. Diatom guilds and macroinvertebrate traits

Diatoms were classified according to the guilds proposed by Passy (2007), dividing them into the three guilds: "High profile", "Low profile" and "Motile" (Table 1).

Macroinvertebrate communities were analysed by considering seven biological traits (Tachet et al., 2010), in order to analyse and summarize the most common used biological information on macroinvertebrates

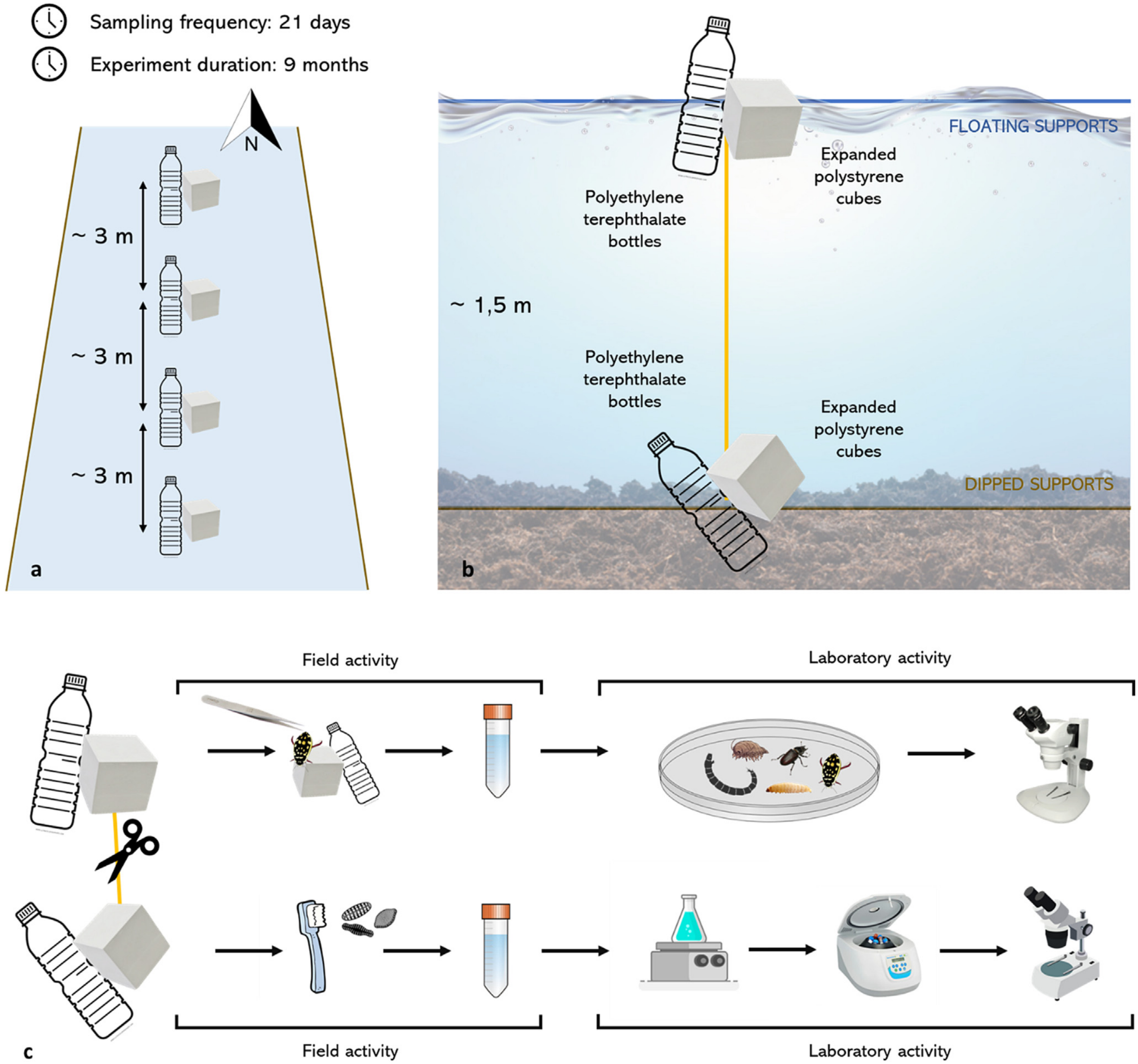

Fig. 2. Experimental design: (a) the canal chosen for the support release and following sampling activities; (b) the plastic supports composed by expanded PS cubes and polyethylene terephthalate bottles both floating on the water surface and dipped on the bottom; (c) the analysis and identification methods of macroinvertebrates and diatoms, showing the field and laboratory activities (Power Point).

concerning the life cycle of taxa ("Maximal size", "Life cycle duration", "Potential number of reproduction cycles per year"), the ability of resistance or resilience ("Dispersal", "Resistance form") and behavioural aspects of nutrition ("Food", "Feeding habits") (Usseglio-Polatera et al., 2000) (Table 2).

Table 1
Ecological guilds of diatoms used in this study and description of correspondence ecological profile (Passy, 2007).

Guilds	Ecological profile
High profile	Species favoured by a high presence of nutrients and low disturbance levels
Low profile	Species present in nutrient-poor habitats with high disturbance levels
Motile	Species whose distribution increases with increasing nutrients and decreases with increasing disturbance. These species are endowed with a high mobility and are able to move to more favourable habitats in case of need

Regarding ecological traits (Usseglio-Polatera et al., 2000; Tachet et al., 2010), the "temperature" and the "trophic status" of the waters were considered, providing the ecological optimum of the species found (Table 2). A value defined based on the affinity of that taxa to that subtrait or modality (Tachet et al., 2010) was assigned. The values were 0 = No affinity; 1 = Weak affinity; 2 = Average affinity; 3 = Strong affinity; 4 = Very strong affinity.

2.4. Data analysis

Morphological and ecological data were digitized and divided according to sampling, the nature of the substrate and the position of the substrates (floating or dipped).

Statistical analyses were carried out independently for diatom and macroinvertebrate components.

The analyses included the relation of a taxa to the respective guild or trait, and bivariate linear models were then applied to relate the number

Table 2
Biological and ecological traits and subtraits (= modalities) of aquatic macroinvertebrates used in this study (Usseglio-Polatera et al., 2000). No. = number of modalities for each trait.

Biological traits	No.	Modalities
Maximal size	1	≤0.25 cm
	2	>0.25–0.5 cm
	3	>0.5–1 cm
	4	>1–2 cm
	5	>2–4 cm
	6	>4–8 cm
	7	>8 cm
Life cycle duration	1	≤1 year
	2	>1 year
Potential number of reproduction cycles per year	1	<1
	2	=1
	3	>1
Dispersal	1	Aquatic passive
	2	Aquatic active
	3	Aerial passive
	4	Aerial active
Resistance form	1	Eggs, statoblasts, gemmules
	2	Cocoons
	3	Cells against desiccation
	4	Diapause or dormancy
	5	None
Food	1	Fine sediment + microorganisms
	2	Detritus <1 mm
	3	Plant detritus ≥1 mm
	4	Living microphytes
	5	Living macrophytes
	6	Dead animal ≥1 mm
	7	Living microinvertebrates
	8	Living macroinvertebrates
	9	Vertebrates
Feeding habits	1	Absorber
	2	Deposit feeder
	3	Shredder
	4	Scraper
	5	Filter-feeder
	6	Piercer
	7	Predator
	8	Parasite/parasitoid
Ecological traits	**No.**	**Modalities**
Temperature	1	Cold (<15 °C)
	2	Warm (>15 °C)
	3	Eurythermic
Trophic status	1	Oligotrophic
	2	Mesotrophic
	3	Eutrophic

of species belonging to a specific guild (in the case of diatoms) or trait (in the case of macroinvertebrates) found in each of the four cases with time, in order to search for any significant temporal trends. The diatomic species were classified through the guilds proposed by Passy (2007) and Rimet and Bouchez (2012).

Strictly aquatic taxa have been classified through traits theorized by Tachet et al. (2010), using seven biological traits and two ecological traits: “Maximal size”; “Life cycle duration”; “Potential number of reproduction cycles per year”; “Dispersal”; “Resistance form”; “Food”; “Feeding habits”; “Temperature”; “Trophic status”. The values of the subclasses were transformed (Logx +1) before proceeding with the correlation analysis. For the analysis of temporal trends, traits with a number of modalities >4 were chosen in order to include only traits with greater diversity. The number of total subclasses was also calculated considering all nine traits.

All the statistical analysis were carried out using the software “Paleontological Statistics” (PAST) (Version 4.12) (Hammer et al., 2001), considering significative p-values those with values lower than 0.05.

3. Results

3.1. Diatoms

A total of 97 diatom species on the 39 supports recovered (a PE support placed on the bottom was not found in the tenth sample) were identified. The complete list of diatoms frequencies and abundances and analyses of diversity indices can be found in Taurozzi et al. (2022).

25 among the 97 different species identified belong to the “High profile” guild, 21 to the “Low profile” guild and 51 to the “Motile” guild (Fig. 3).

A significant correlation between species distributions and temporal trends was found in all cases of exposure conditions only for the “Motile” guild (fPS, $p = 0.021$, $R^2 = 0.50$; dPS, $p = 0.016$, $R^2 = 0.584$; fPE, $p = 0.013$, $R^2 = 0.55$; dPE, $p = 0.037$, $R^2 = 0.43$) (Fig. 4).

Other significant correlations were obtained for the “High profile” guild on fPS ($p = 0.021$, $R^2 = 0.50$) and for the “Low profile” guild on fPE ($p = 0.019$, $R^2 = 0.51$) (Fig. 5). No significant results emerged about the “Low profile” guild on fPS, dPS and dPE (p = ns) and for the “High profile” guild on dPS, fPE and dPE (p = ns).

3.2. Macroinvertebrates

As regards the animal component, 36 different taxa within the 39 samples (one PS cube has been lost) were found. The classes represented were: Diptera, Coleoptera, Ephemeral, Heteroptera, Hymenoptera, Annelid, Arachnid, Colembola, Oligochaeta, Trichoptera, Tisanoptera, Gastropod and Crustacean. The complete list of macroinvertebrates frequencies and abundances and analyses of diversity indices can be found in Taurozzi et al. (2022).

For the biological traits, as regards the “Maximal size” trait, 33 % of animal taxa were found in the subclasses “3” and “4”, in which there are taxa with individuals ranging in size between 5–10 mm and 10–20 mm. As regards the “Life cycle duration” trait, emerged that 64 % of taxa had a life duration less or equal than one year. As regards the “Potential number of reproduction cycles per year” trait, a 58 % of univoltine taxa emerged, with only one annual generation. As regards the “Dispersal” trait, there is a uniform distribution in the four subclasses, with animal taxa characterized by active, passive, aquatic and aerial dispersion. As regards the “Resistance form” trait, animal taxa without any form of resistance are predominant (61 %). As regards the “Food” trait, emerge that most animal taxa (21 %) feed on detritus smaller than a millimetre, like live macrophytes, live micro and macro invertebrates; As regards the “Feeding habits” trait, 74 % of animal taxa were choppers, scrapers and predators were found. For ecological traits, as regards the “Temperature” trait, the presence of eurythermic organisms emerges. As regards the “Trophic status” trait, mainly mesotrophic and eutrophic organisms are present. For all the traits considered the analysis did not show significant temporal trends (MS: $R2 = 0.02$, $p > 0.05$; RF: $R2 = 0.2$, $p > 0.05$; F: $R2 = 0.004$, $p > 0.05$; FH: $R2 = 0.003$, $p > 0.05$) (Fig. 6).

4. Discussion

This study represents the first coupled research of diatomic guilds and macroinvertebrate traits on plastic substrates in Italian wetlands. Here we showed that the hypothesized surface availability for settlement among different plastic substrates generated also significant shifts in the composition of the diatom ecological guilds and macroinvertebrates traits.

Patchiness of coastal ecosystems, like coastal wetlands, make important to study diatoms variability from a functional perspective (Heine-Fuster et al., 2021). Diatoms are responsible for about 20–25 % of the Earth's global primary production (De Tommasi, 2016) and their photosynthetic activity accounts for 40 % of the marine primary production. It's known that diatom species tend to inhabit the sunlit plastisphere (Amaral-Zettler et al., 2020) but here we provide the evidence that also plastics supports in the dark can favour diatom settlement. The use of diatom guilds allows

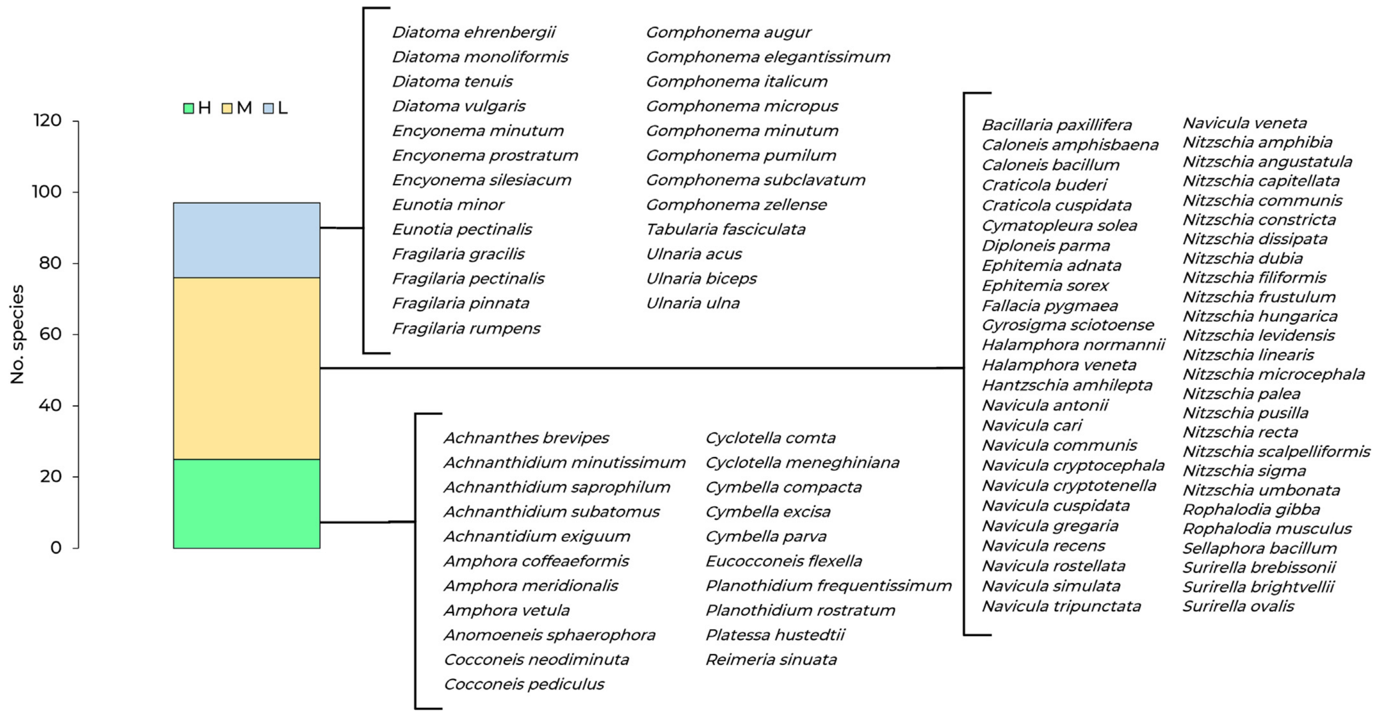

Fig. 3. Number of species (No. species) belonging to "High profile" guild (H), "Low profile" guild (L) and "Motile" guild (M) and relative species found.

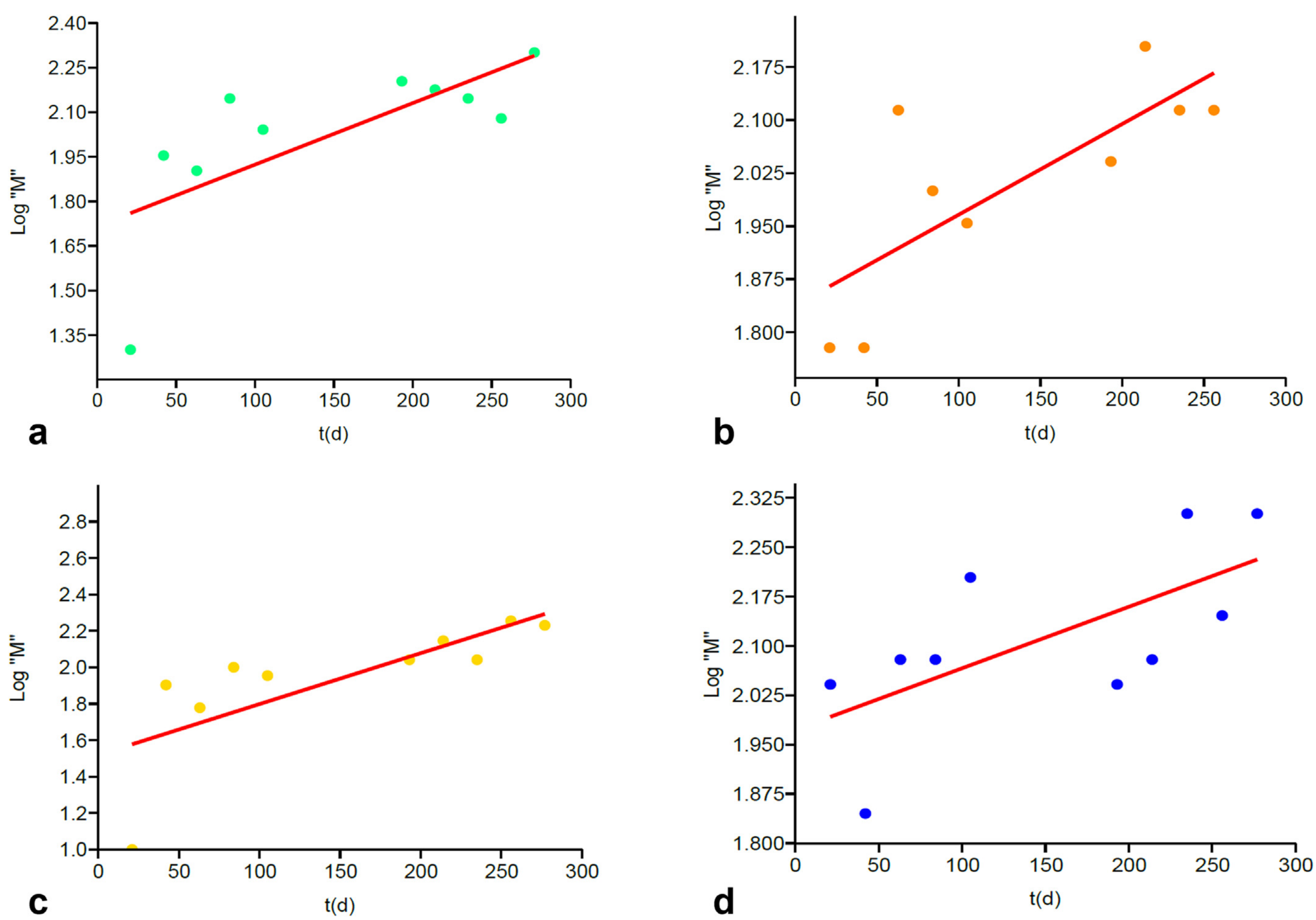

Fig. 4. Plot of log transformed data showing the regression line as regards the number of species belonging to the "Motile guild" calculated for fPS (a), dPS (b), fPE (c) and dPE (d). Log "M": Logarithm of "Motile" guild values; Time is expressed in days (PAST).

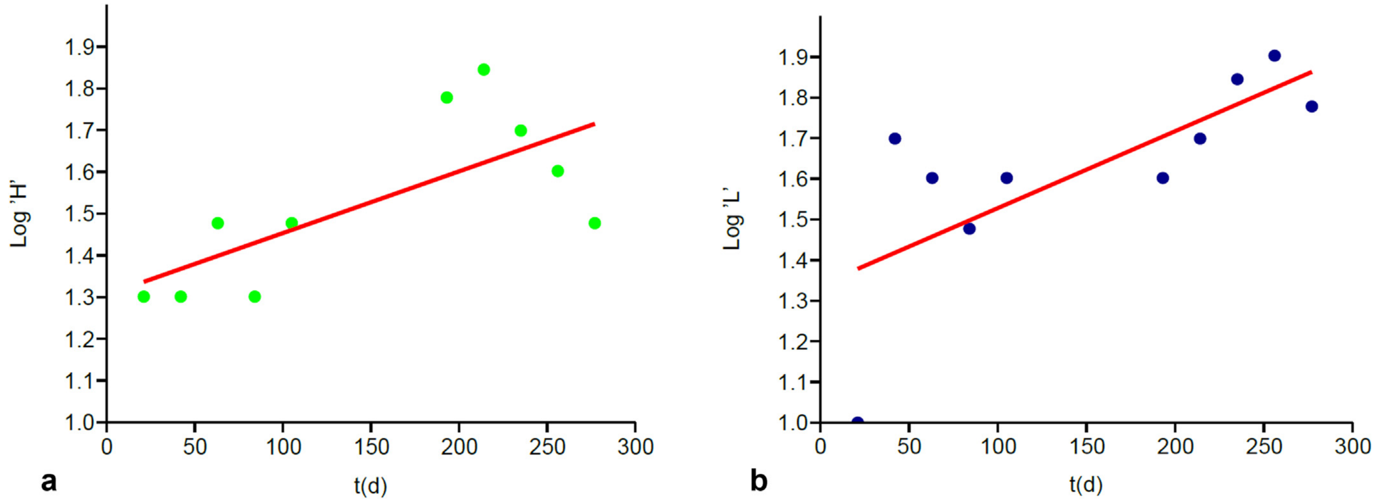

Fig. 5. Plot of log transformed data showing the regression line as regards the number of species belonging to the "High profile" guild calculated for fPS (a), and the "Low profile" guild calculated for dPE (b). Time is expressed in days (PAST).

to identify community responses to changes in the ecosystem (Wang et al., 2022). However, the relationship between diatom ecological guilds and anthropogenic pollution is poorly understood (Mbao et al., 2020) and few studies focused on the importance of ecological guilds to be descriptors to assess the ecological status of salt- and freshwaters (Gelis et al., 2020). Although the presence of this important gap, it is widely accepted that diatom guilds are suitable to be used to highlight the responses of diatoms to environmental changes and reflect the dispersal ability of the species (Guo et al., 2020).

On the other hand, analyses of macroinvertebrates traits have long been applied since the early 1900s (Kefford et al., 2020), especially because they represent a low-cost method to investigate macroinvertebrates dispersal in function of ecosystem structure (Sarremejane et al., 2020). Furthermore, macroinvertebrates represent an important link between the vegetal component and vertebrate fauna of aquatic ecosystems (Gleason et al., 2018). Although many studies focused on the response of macroinvertebrates to different pollutants, like pesticides, to assess the ecological status of a specific waterbody (Sumudumali and Jayawardana, 2021; Yadamsuren et al., 2020), our study contributes to reduction of the knowledge gap about responses of macroinvertebrate communities to plastic pollution in wetlands.

Most of the diatom species found belong to the "Motile" guild, followed by the "High profile" guild and the "Low profile" guild. In each of the four cases examined, the number of species belonging to the "Motile" guild was always more than double compared to those belonging to the other two guilds. Species belonging to the "Low profile" guild are favoured species

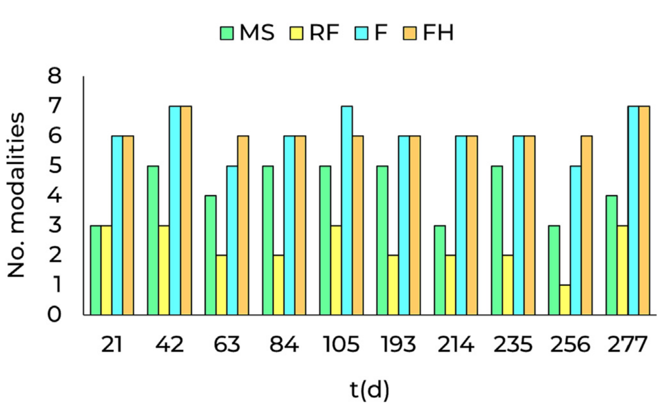

Fig. 6. Number of subtraits (modalities) present in each sampling for the traits examined. MS = "Maximal size"; RF = "Resistance form"; F = "Food"; FM = "Feeding habits"; t(d) = time is expressed in days.

in nutrient poor and disturbed habitats, those belonging to the "High profile" guild are favoured in nutrient rich and little disturbed habitats, while species belonging to the "Motile" guild increase following the nutritional gradient and decrease along the disturbance gradient but having the ability to move easily to search for the required ecological conditions (Heine-Fuster et al., 2021). The "Motile" guild includes the largest number of species that are tolerant of eutrophication and pollution situations (Peszek et al., 2021): the largest number of species belonging to the latter guild complies with the ecological characteristics of the study area, which favours their development.

The study also highlights a temporal trend for the number of species belonging to the "Motile" guild: in all four cases analysed there was a significant increase in the number of species along a time gradient ranging from the first to the tenth sampling. This data can be explained thanks to the high mobility capacity of the species belonging to this guild, able to dominate in many ecological conditions compared to the "High profile" and "Low profile" guilds, linked to more restrictive conditions of nutrient availability and disturbances (Gelis et al., 2020). Regarding the PS supports on the surface, a significant temporal trend of colonization was highlighted for the species belonging to the "High profile" guild and this result can be explained through the physical structure of the PS: the rough surface of the PS, in addition to the various empty spaces present, can allow the adhesion and accumulation of floating nutrients (McArtor et al., 2021), favouring those diatomic species whose presence is linked by a high availability of nutrients. At the same time, a significant temporal trend emerged for the species belonging to the "Low profile" guild in the PET supports placed on the surface: in this case, contrary to what was said for the PS supports, we hypothesise that the smooth surface of the PET could disfavour adhesion (Ganesan et al., 2022; Krsmanovic et al., 2021) of nutrients and therefore favours the presence of diatomic species adapted to settle in nutrient-poor habitats.

Taking into consideration the different traits of macroinvertebrates, the analyses do not show a significant temporal trend in the number of subtraits, that is, for each trait the various modalities are equally represented starting from the first sampling up to the last. These results could indicate both a high colonizing capacity by different taxa, and a particular ability of the PS to provide the availability of a surface with physical characteristics particularly suitable for colonization (McArtor et al., 2021). Furthermore, the distribution of taxa within the traits used shows the presence of an ecologically diversified community, in which there are mostly univoltine organisms, with dimensions between 5 and 20 mm, predators, choppers and scrapers that feed on plant organisms and animals.

The presence of macroinvertebrates with different ecological characteristics can be indicative of a large number of ecological niches that can be

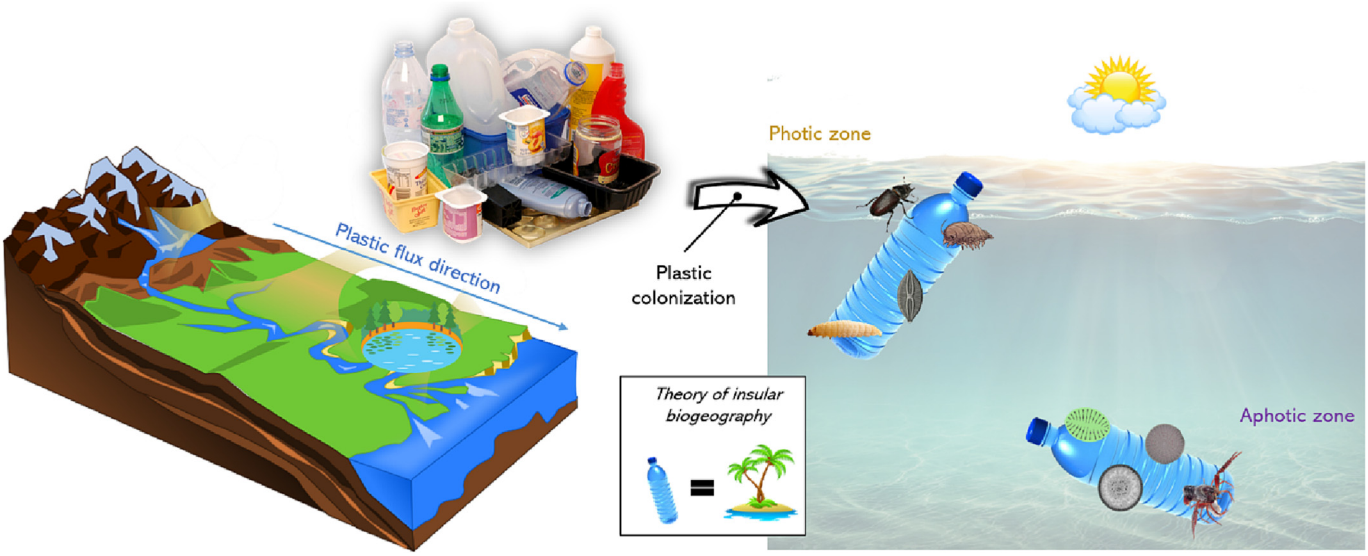

Fig. 7. Scheme of plastic fate on coastal wetlands and sea, and plastic colonization scheme followed by diatoms and macroinvertebrates (PowerPoint).

exploited within an artificial support (Bowley et al., 2022; Gallitelli et al., 2023; Ye et al., 2021) such as PS. At the same time, there was a colonization of the supports by different taxa, without this corresponding to the formation of a clear ecological pattern. The experimental protocol adopted in this study call to mind the MacArthur and Wilson (2016) research: the plastic supports used can be considered as 'islands' within a small system like that of a canal (Fig. 7).

However, unlike this theory which hypothesizes that the pioneer species after a certain period of time will move, expand or contract its realized niche, also due to too many or too few predators on the island, the results instead show a succession of taxa not expressed in ecological terms. The species present in the first samplings are largely traced also in the last samplings and the number of species tends to be constant. We hypothesise that colonization of virgin plastic substrates was carried on without the preliminary activity of pioneer species. Furthermore, as evidenced by the "Theory of insular biogeography", the proximity of the supports to the banks of the canal could have played a central role in the immigration of taxa (including terrestrial ones).Here taxa found different ecological niches to exploit and large surface area to colonize due to the large size and availability of nutrients we can consider the supports used as an "island" suitable for hosting numerous animal taxa without creating mutual-exclusive relationships. It is also possible to hypothesise that the presence of diatoms on plastics allows the presence of macroinvertebrates, but this aspect should be clarified with additional studies on this topic. However, further studies are needed especially regarding the chemical additives of plastics, which can represent an important discriminant in the impact caused to the biota colonizing plastics.

5. Conclusions

The use of diatom guilds to evaluate the ecological impact of anthropogenic pollutants on waterbodies is relatively recent but most of the studies on plastics pollution focus on marine waters. Although the use of macroinvertebrates traits is considered one of the most powerful tools for the analysis of the ecological status and human impact on freshwater ecosystems, wetland ecosystems are understudied from this point of view, lacking studies with this trait-based approach.

This study represents the first research in the Italian peninsula on this topic: the analysis of diatoms and macroinvertebrates represents an important starting point for the ecological and biotic characterization of the impacts of anthropogenic pollutants in wetlands. The study of diatomic guilds can provide with certainty the chemical-physical conditions of the analysed site. On the other hand, the analysis of the traits of macroinvertebrates allows us to understand which taxa are most impacted and which are most benefited by the presence of plastic in wetlands.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Davide Taurozzi: Investigation, Visualization, Writing – original draft, Writing – review & editing. **Giulia Cesarini:** Validation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Massimiliano Scalici:** Validation, Conceptualization, Supervision, Resources, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164186.

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