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Review

Microplastics' Impact on the Environment and the Challenging Selection of Reliable Key Biomonitors

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Abstract: Microplastics (MPs) persist for long periods in the environment, causing adverse effects on aquatic and terrestrial ecosystems. The accumulation of MPs in various trophic levels mostly depends on weathering phenomena, their reduced dimensions and the improved bioavailability; this ultimately causes their ingestion by organisms living in different niches. The modern concern about MPs toxicity collides with the current unavailability of standardized and reliable methodologies to assess the risks associated with the exposure of organisms from different habitats. Hence, the identification and selection of appropriate biomonitors for MPs pollution risk assessment should focus on the identification of easy-to-implement assays, rapidly interpretable results (e.g., based on the MPs bioaccumulation capabilities in their tissues) and standardizable methodologies. The present review analyzed some emerging biomonitors exploited for MPs evaluation, selected and examined according to their potential use as specific biological indicators for diverse environments. The research was focused on plants, as biological models for airborne microfibers toxicity evaluation; mussels, as key organisms for the establishment of MPs accumulation in marine environments; land snails, representing emerging organisms selected for studies of MPs' impact on soil. Furthermore, recent findings evidenced the influence of microplastics on the composition of environmental microbiota, enhancing pathogenic biofilms formation, leading to increased water, soil, food, crops and waste contamination. Disposing of harmonized and validated methods to study MPs' impact on the environment, integrated with promising machine learning tools, might sensibly support the risk management strategies protecting human and animal health.

Keywords: anthropogenic microfiber; environmental pollution; land snails; mussels; MPs; plants; antimicrobial resistance; biofilms

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1. Introduction

Human-derived fibers (MFs) are defined as synthetic fibers of petrochemical origin (i.e., polyester, polyamide, polypropylene, etc.); artificial fibers originating from artificial cellulose or silk (i.e., viscose and rayon); natural fibers (i.e., cotton and wool) [1]. Anthropogenic fibers are usually employed as raw materials in many applications, such as the textile and apparel industries, as well as in fishing nets and geo-textiles production. MFs are released into aquatic environments from untreated and treated domestic and industrial wastewater [1–4], from fishing net degradation in aquaculture and fishery activities [5,6] or

from textiles used for geotechnical, geo-environmental and civil engineering purposes [7,8]. Recently, bio-solids used as fertilizers in landfills were demonstrated to be a source of fibers leaching into surface- and ground-water sources [9].

Among anthropogenic fibers, microplastics (MPs) are attracting more attention, being the most widespread to which all organisms are continuously exposed. Global plastic production has increased to such an extent (from 1.5 to 335 million tons) that the current historical period has been referred to as the 'plastic era' [10]. In fact, the production of plastics has increased exponentially and is now the economically most important and leading industrial sector. This growth is mainly due to its specific properties, such as durability, flexibility, lightweight and finally, low cost, which have led to its use in many sectors. For example, plastics are widely used in many sectors ranging from the production of household essentials, to packaging materials in the food industry, agri-food industries such as fertilizers and pesticides, textile manufacturers, and personal care products [10]. MPs are plastic fragments whose dimensions range from 1 µm to 5 mm; they can be of primary origin (i.e., released into the environment in the same dimensions they are produced, e.g., microbeads) or secondary origin (deriving from the degradation of plastic materials and debris). Depending on their structure and shape, MPs can be grouped as fibers, foams, beads, fragments (including films) and pellets [11]. Plastic pollution affects different environments, particularly water bodies and seawater, inland waterways, wastewater outflows and the atmosphere [12]. Due to atmospheric dust resuspension and sea aerosols, as in the case of particulate matter (PM), MPs are exchanged among all environmental matrices. A substantial concern is, moreover, represented by the transportation, through plastic debris, of organic additives, which are usually detected in plastic in concentrations ranging from 0.1% to 1% and are not chemically bound to the polymers [13]. Apart from their intrinsic toxicity, MPs, due to their high surface area-to-volume ratio and hydrophobicity, can interact and absorb other molecules/particles present in the environment, acting as carriers of these chemicals, and release them into new sites, as well as in living beings. This effect is defined as the "Trojan horse effect" and it is based on MPs' features (composition, size, shape, color and functional groups). Sorption of organic compounds and heavy metals on MPs can effectively increase the exposure risk and bioavailability of these contaminants to organisms, leading to synergistic, antagonistic or potentiating effects [5].

Due to danger and widespread plastic pollution around the world, numerous alternative biodegradable materials have been introduced to make numerous everyday objects such as plastic bottles, shopping bags, food packaging, straws, etc. [14]. Today, these initiatives are still not enough, since MPs have been detected in all environmental compartments, whose networks are destabilized by these fibers, even in remote areas (e.g., the Mount Everest; [15]), as well as in many consumer products such as foods and beverages (i.e., edible seaweed and dried fish snacks) [16,17], and even in human blood and breast milk [18]. MPs mainly found in the environmental compartments include polyethylene terephthalate (PET), polystyrene (PS) and polypropylene (PP) [19]. Aquatic as well as terrestrial ecosystems are compartments principally destabilized by MPs. In watersheds, aquatic organisms known to ingest these fibers include amphipods, copepods, lugworms, barnacles, mussels, decapod crustaceans, seabirds, fish and turtles [20]. In the terrestrial environment, particularly in the soil, the main organisms directly exposed to microplates are plants, numerous invertebrates and some vertebrates living in fields [21]. The present review aims at highlighting the rapid spread of microplastics, focusing attention on the impact on organisms at multiple trophic levels by specific models, and at assessing the risk for the environmental matrices, living animals and, ultimately, for humans. The most recent data on the presence and effects of MPs on sessile or low-motile organisms living in aquatic and terrestrial environments were analyzed; additionally, we examined the potential of specific organisms to be exploited for biomonitoring study as potential bioindicators, supporting the health risk assessment strategies to mitigate the effects of MPs on human health. The growing concern about plastic pollution resulted in an increased number of scientific articles on the topic over the past decade. In nature, there are no clear separations

between the different environmental compartments: there is a continuous exchange of energy and matter among soil, air and water, and consequently also among the organisms that compose their biota. In this review, we examined the literature concerning specific biological tools employed to evaluate the environmental diffusion of MPs.

A total of 149 research articles were selected on Scholar, PubMed and Scopus search engines utilizing the following keywords: plants; biomonitor; bioaccumulation; mosses; lichens; microplastics; anthropogenic microfibers; land snails; mussels; MPs in mussels bioaccumulation; MPs and soil and gastropods; MPs pollution; AMR and MPs; antimicrobial resistance genes and MPs; Plastisphere. Articles were selected according to the following criteria: (i) focused on a panel of biomonitors for which the authors had direct research experience; (ii) focused on microplastics' diffusion and effects on the biota.

A crucial issue is linked to the extreme variety of MPs biomonitors suggested lately, posing difficulties in selecting a standardized and internationally recognized species' panel to be employed for the assessment of microplastics pollution in the environment. This review, therefore, primarily aimed to suggest a list of potential biological indicators to be employed for MPs pollution assessment in various habitats (i.e., air, water, soil, etc.) (Figure 1). The choice of the described species was operated based, according to the available bibliography, on the easiness of implementing the assays in laboratory environments and the capability of accumulating MPs or providing rapidly interpretable endpoints. The review process subsequently defined an overview of the methodologies employed for each identified biomonitor, and aimed to perform an MPs pollution risk assessment, outlining the critical issues, the current challenges, the limitations and the possible technical solutions, with the scope of directing and specifically addressing future research on microplastics, to adopt shared, robust and comparable methods.

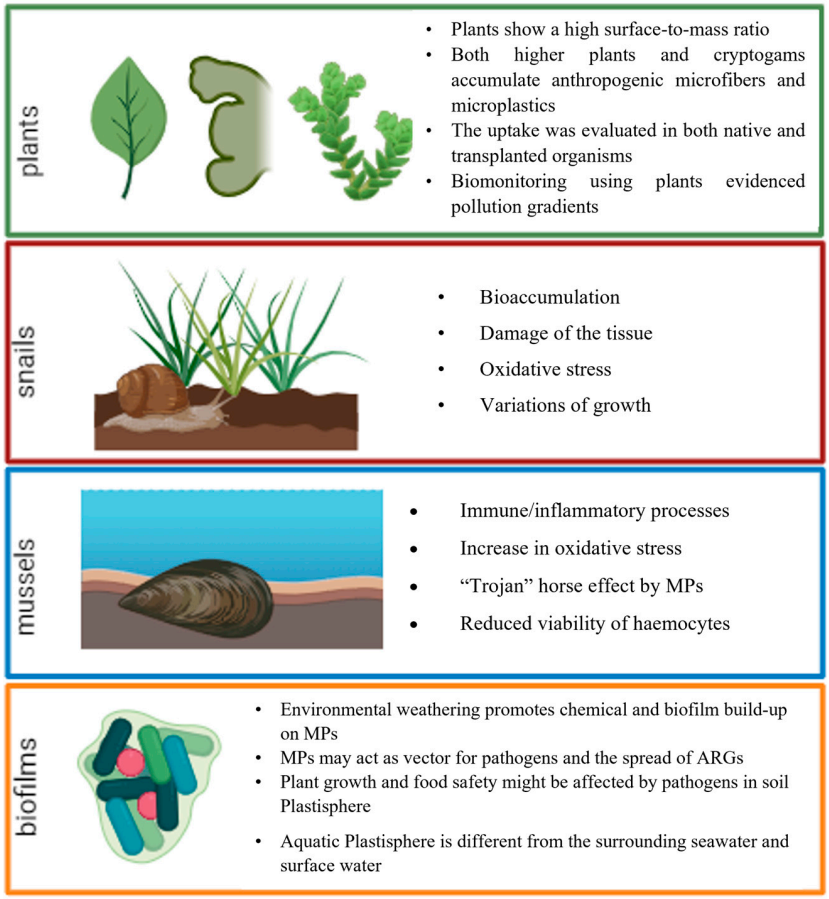

Figure 1. Summary scheme of microplastics effects on the potential biomonitors described in the present review.

2. Plant Biomonitoring of Airborne Anthropogenic Microfibers (Including Microplastics)

Plants can be considered very good biomonitors, i.e., organisms that react to environmental changes, including pollution, either displaying measurable variations, or accumulating xenobiotics in their tissues in ways largely depending on specific macro- and micro-morphological traits (e.g., cell wall composition and structure, presence of trichomes, cuticle, waxes, etc.). Plants show a surface-to-mass ratio up to 100 times higher than animals, since their body mainly develops in a bi-dimensional mode (leaves, thalli), or even uni-dimensional if we consider hairy structures or filamentous organs (e.g., roots), attaining a large surface contact with pollutants. For these reasons, plants are considered good accumulators of pollutants also present in the air in the form of PM as metal(loid)s, PAH (Polycyclic aromatic hydrocarbon) (low to high molecular weight) and other POPs (Persistent Organic Pollutants) and microplastics [22]. Cryptogams, as mosses and lichens (which are not strictly plants, but a stable symbiosis between algae/cyanobacteria and fungi), lack leaves or roots; consequently, they ab/adsorb water and nutrients from the entire thallus surface. The absence of such organs determines the lack of any forms of nutrient selection, leading pollutants to easily enter cells in the same way as water and nutrients. As a result, such organisms show a chemical composition reflecting the inputs of the surrounding environment [23–26].

Cryptogams and vascular plants have been largely used in the biomonitoring of airborne pollutants linked to PM; plant organisms have only recently been tested in the biomonitoring of airborne MPs, which represent a fraction of PM [27]. In fact, it is not obvious that plants could intercept and accumulate MPs similarly to other pollutants dispersed as PM, due to specific properties of plastic polymers such as density, their larger span, shapes and different chemical-physical traits such as insolubility in water. For these reasons, the biomonitoring methods applied so far need to be customized considering the peculiarity of these pollutants and the specificity of plant traits.

2.1. Biomonitoring of Airborne Anthropogenic MFs by Cryptogams

The first evidence of the reliability of cryptogams as possible biomonitors of plastic particles was provided by Capozzi et al. [28], evaluating the possibility of using the moss *Sphagnum palustre* L. for the biomonitoring of NPs in aquatic environments. The moss, exposed to polystyrene nanoparticles in a laboratory trial, accumulated in its tissues the administered nanoparticles. The study also evidenced some critical points of the method, such as the importance of establishing an adequate exposure time to reach a significant accumulation. Since 2020, the possibility of biomonitoring airborne microplastics with cryptogams has grown further. Roblin and Aherne [29], following the guidelines of the ICP vegetation (2020), proved the feasibility of using the naturally growing *Hylocomium splendidens* (Hedw.) Schimp. for the biomonitoring of airborne anthropogenic MFs. This work, conducted in Ireland in sites far from pollution sources, highlighted how anthropogenic MFs (including microplastics) may undergo long-range atmospheric transport and can accumulate in moss carpets. This research represents a turning point for the methodology, since the experiment proved the capability of the moss to work well also in the real environment, and because the adopted approach could provide two types of data: (i) the number of microplastics and (ii) the length of microplastics, both useful parameters for the risk assessment of human exposure to microplastics. The adopted method is a three-step approach consisting of (i) extraction of plastics from the plant matrix with wet peroxide oxidation; (ii) visual identification, counting, morphological and dimensional characterization of the particles under a stereomicroscope; and (iii) qualitative characterization of the identified particles using approaches such as FT-IR or μRaman. Following the work by Roblin and Aherne [29], other studies evaluated cryptogams as possible candidates for the biomonitoring of MFs. Loppi et al. [30] tested the naturally growing lichen *Flavoparmelia caperata* (L.) Hale, to monitor MPs at different distances around a landfill dumping site. From the point of view of the effectiveness of the biomonitoring method, results reported by Loppi et al. [30] showed that the thalli of native *F. caperata* collected near the landfill

accumulated a greater number of MPs compared to those collected from more distant sites, indicating that the lichen was able to highlight a gradient in the MFs' fallout. The same approach was applied in Campania Region (southern Italy) by Capozzi et al. [31], who analyzed the microplastic accumulated on the native moss *Hypnum cupressiforme* Hedw. harvested by seven semi-natural and rural sites. The authors found a higher number of MPs and longer fibers accumulated in mosses collected from sites closer to urbanized areas, while small-sized MP classes and a lower level of MPs characterized the deposition on mosses collected from sites having a high altitude above sea level. This result strengthened the hypothesis that long-range transport is also a function of the dimensional characteristics of the particles (i.e., the smaller the particles, the longer and higher the transport distance).

The non-homogeneous distribution of the target species in the study area limits the use of passive biomonitoring (i.e., based on the harvesting of native material). To solve this problem, it is often used to harvest another species holding similar pollutant accumulation capability in the sites lacking the target species. To this aim, researchers usually compare the content of the target pollutant in two native species found in the same site, in a test of intercalibration. This type of study must be repeated each time a new pollutant is considered, since biomonitors have a specific pollutant-response behavior. Jafarova et al. [32] performed this type of comparison, evaluating the effectiveness of co-located lichen *Evernia prunastri* (L.) Ach. and moss *Pseudoscleropodium purum* (Limpr.) M. Fleisch. as biomonitors of the atmospheric deposition of MFs. Research outcomes suggest that the moss should be preferred over lichen due to its higher accumulation capacity. As stated by the authors, some aspects should be further considered to better interpret results: (i) the differences in the ecology of the two species (i.e., the lichen epiphytic and the moss epigeous); (ii) the structural characteristics of the two cryptogams and in particular the higher surface-to-mass ratio of the moss resulting in a more efficient intercepting and retaining capacity; (iii) the different ages of the analyzed tissues. Nevertheless, despite the different accumulation signal provided, it is important to highlight that both organisms utilized in the above-mentioned study clearly indicated that the deposition of atmospheric MPs also occurred in the remote areas investigated. Another comparison, based on the passive approaches, among nine lichen species and a moss, comes from Khodabakhshloo et al. [33], who evaluated their capacity as biomonitors of microplastics and microrubbers (MRs) across different altitudinal transects near Shiraz City (Iran). The authors confirmed the inverse relationship between particle size and distance travelled, with the longer particles found near Shiraz. Interestingly, from a methodological point of view, in this work it appears that among the tested lichens, those of the genus Acarospora showed the best bioaccumulation performance for MPs and MRs. While in overall terms, the moss *G. crinita* was suggested as the best choice in the studied environment, both for its wide distribution and for its ability to intercept and accumulate a broader range of MPs and MRs [33]. Taurozzi et al. [34] investigated the airborne microplastic accumulation potential of lichens from the genera *Cladonia* and *Xanthoria* along three sites characterized by different anthropic impacts. The authors found a gradient in the number of microplastic fibers accumulated in lichens' thalli across the sites, with an increasing accumulation from the natural to the urban sites, highlighting the direct relationship between the length and abundance of airborne microplastic fibers, with the shortest fibers found at natural sites and the longest near the center of Rome. According to the authors, no differences in the accumulation capacity emerged between the two genera [34].

In addition to the non-homogeneous spatial distribution of the studies based on the native entity, they suffer from the uncertainty associated with the age of the thalli, and, therefore, the exposure time of the analyzed material. Recently, the possibility of using transplants of cryptogams for the biomonitoring of MFs has been investigated. The transplants allow fixing a defined exposure time and offer the possibility of a rational exposure design not limited by the presence/absence of the target species. As a drawback, the transition from the use of native species to transplants must deal with the choice of an optimal exposure time, ensuring a clear signal; this aspect, especially in the first attempts,

proved to be a leap in the dark. Indeed, the accumulation in native species is evaluated in portion of the biomonitors that most often have an estimated age between 1 and 3 years; therefore, there is no information on the possibility that the accumulation could occur even in shorter exposure times. As for transplants of cryptogams, usually exposure periods from 3 to 12 weeks were applied; this variation depends on the sensitivity of the biomonitor, but also on the target pollutant (e.g., usually 3–6 weeks for elements and 6–12 weeks for PAHs) [32].

To date, three articles have investigated the potential use of transplants of cryptogams for the assessment of airborne MFs [35–37]. Jafarova et al. [35] transplanted the lichen *E. prunastri* in the urban area of Milan for a 3-month period. The researchers found an increasing gradient in MFs deposition from areas with low deposition (i.e., control site far from pollution sources, and urban parks) to areas with high deposition (i.e., urban center, semi-periphery and periphery). The transplants provided the possibility of organizing a monitoring net, following a rational scheme, capable of highlighting trends in microplastic deposition, which would have been impossible by using native lichens due to the lack of the target species in Milan's center and periphery area. This study indicates that 3 months of exposure are enough to provide a good bioaccumulation signal, allowing the repetition of multiple monitoring campaigns over a year. It is well known that the peculiar traits of mosses and lichens can favor or hamper the accumulation of specific pollutants; therefore, the evidence that a transplanted lichen is a valuable tool for MP biomonitoring does not mean that a moss is adequate as well. To fill this gap in knowledge, Bertrim et al. [36] assessed the efficacy of *Pleurozium schreberi* (Brid.) Mitt. transplanted in bags for the monitoring of atmospheric MFs. The authors demonstrated that the moss, exposed for 6 weeks, can accumulate airborne anthropogenic microfibers. They also confirmed that microplastic deposition increases with urban density.

Since the peculiar traits of lichens and mosses make them very different in their ability to accumulate pollutants, the protocols adopted for their exposures could follow different rules and precautions. The optimization of the exposure protocol of moss and lichen transplants in the biomonitoring of airborne MFs was recently investigated by Capozzi et al. [37]. The authors evaluated, in a parallel transplant experiment lasting 6 weeks, (i) the different behavior of the moss *H. cupressiforme* and the lichen *Pseudevernia furfuracea* (L.) Zopf var. furfuracea in the accumulation of MFs, and (ii) the effect on the uptake of MFs due to the bag used for transplants. This research indicates the moss had an overall greater accumulation capacity, while the lichen had less retention capacity, especially of the smaller MFs. Moreover, the authors found that transplants without a covering net provided greater sensitivity, yielding a higher accumulation in both species.

2.2. Biomonitoring of Airborne Anthropogenic MFs by Higher Plants

To date, the biomonitoring of airborne MFs using higher plants has been evaluated exclusively through a passive method applied to native species, crops or rows of ornamental trees.

One of the first pieces of evidence indirectly evaluating the capacity of higher plants as biomonitors of airborne MPs came from Liu et al. [38]. The research, indeed, focused on the ability of plants to determine the decrease in atmospheric MPs during their transport (i.e., through their entrapment). The authors sampled green leaves of vascular plants in two regions (i.e., the mega city of Shanghai and the scarcely populated oceanic island of Liandao, Lianyungang) to determine the trends of MPs depositions. In general, a certain portion of atmospheric MPs was found retained on plant leaves, which act as a temporary sink, indicating a decreasing trend in atmospheric MPs deposition from the terrestrial to the ocean environment.

The role of plants in intercepting atmospheric particles, providing a natural filter for these pollutants, stimulated the work of Liu et al. [39], who evaluated the pine needles as biomonitors of airborne MPs in Shihezi City. Liu et al. [39] found a widespread presence of MPs on the surface of pine needles, evidencing the traffic as the main source. Interestingly,

the characterization of intercepted particles and source analysis allowed the authors to demonstrate that industrial activities mainly generate debris and pellets, while traffic flows films and fibers.

To better understand the relationship between microplastic accumulation, the leaf properties and their position on trees, Leonard et al. [40] quantified the contribution to the variability of microplastic concentrations of different land uses, the height of the leaf and leaf surface hydrophilicity. The study was conducted in Los Angeles (USA), by sampling leaves of several plant species from 19 sites and varying the heights of collection. The authors discovered that the leaf's position above the ground influenced data variability and some leaf surface properties. According to the authors' conclusions, the high uncertainty in predicting microplastic content on the leaves might limit their use as biomonitors in urban areas.

The work of Jafarova et al. [41], employing leaflets of *Robinia pseudoacacia* L, arrived at different conclusions. In this case, the plants evidenced a different behavior in the uptake of plastic debris according to their nature; in fact, tire wear particles accumulated in leaflets facing the rural roadside, while a higher number of microfibers was found in leaflets from urban parks. The widespread availability of *R. pseudoacacia* and its MPs retention capacity suggest this species is a valuable resource for investigating airborne microplastics deposition.

An interesting perspective emerges from the work by Canha et al. [42], who investigated the contamination by microplastics of lettuce plants cultivated in urban gardens in Lisbon (Portugal) in view of the possible human risk assessment due to exposure to microplastics or the ingestion of contaminated crops. The authors compared the MPs content in lettuce cultivated in urban gardens with lettuce cultivated in rural sites and lettuce purchased in supermarkets. The research indicates that plants from urban gardens and rural areas hold comparable MPs contamination, mainly deriving from road traffic, and this contamination was 70% higher than the content found in lettuce purchased from the market.

2.3. Critical Issues and Future Perspectives in Biomonitoring of Airborne MPs by Plants

The interest in the biomonitoring of previously non-investigated pollutants is leading to the development of a series of methods and protocols which initially produce data that are not directly comparable. However, this exploratory phase, often guided by the expertise of individual researchers, and the availability of tools, is essential for acquiring new knowledge, and fine-tuning the methodology to produce protocols that work in the different experimental conditions and are customized to the research purposes. As a successive step, the comparison of different studies focused on the biomonitoring of airborne MPs could profitably address the main concerns and provide solutions.

In the works published so far, there are many points of convergence, but unfortunately also many points of divergence, reducing data comparability. Table 1 puts in evidence that the biomonitoring of MPs by plants is still in an explorative phase; several biomonitors and protocols have been adopted but there is a lack of any type of calibration among the approaches. It is necessary to carry out further experiments to identify the best performing species and, even more, their fundamental traits, microstructures and chemical properties that allow MPs uptake and retention. Furthermore, the times and conditions of exposure and a way to accomplish adequate quality control should be considered in active biomonitoring. However, at this stage, all scientific contributions based on robust experimental designs are useful to set the ground for a more mature and standardized methodology. We consider it appropriate to underline some of the weaknesses to better address future research work, in view of finding a shared method allowing data comparability.

Table 1. Overview of the articles published on biomonitoring of airborne microfibers by plants.

Authors	Species/Biomonitoring Method	Exposure Time	Extraction	Height of Exposure or Collection Above the Ground Level	Size of Smallest Particles	MP Categories	Visual Identification Criteria	Qualitative Analyses of MPs	Data Provided
Bertrim and Aherne [36]	*Pleurozium schreberi* (Active)	6 weeks	wet peroxide oxidation (WPO)	not specified	50 µm	fibers, films and fragments	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	-	Number of MPs g^{-1}; daily microplastic deposition: MP m^{-2} day^{-1} (based on the surface area of the moss bag); microplastic particle volume (mm^3 g^{-1})
Capozzi et al. [28]	*Sphagnum palustre* (Active, administred nanoplastics)	7, 14, 21 days	not appliable	not appliable	not appliable	nanoparticles	Fluorescent microscopy equipped with a TX2 fluorescence filter	not appliable	not appliable
Capozzi et al. [31]	*Hypnum cupressiforme* (Passive)	~2–3 years	wet peroxide oxidation (WPO)	epilithic (<1 m above the ground)	200 µm	fibers	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	FT-IR analyses	N of MFs g^{-1}; length (mm) class distribution of MPs
Capozzi et al. [37]	*Pseudevernia furfuracea*; *Hypnum cupressiforme* (Active)	6 weeks	wet peroxide oxidation (WPO)	4 m	200 µm	fibers	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	µRaman	Number of MPs g^{-1}; daily microplastic deposition: MP m^{-2} day^{-1} (based on the moss SLA); length (mm) and class distribution of MPs
Khodabakhshloo et al. [33]	*Grimmia critina, Acarospora bullata, A. cervina, Anaptychia bryorum, Candelariella rhodax Poelt and Vězda, Calogaya biatorina, Collema polycarpon, Dermatocarpon miniatum, Lobothallia sp., Placidium squamulosum (Ach.) Breuss, Squamarina lentigera.*	not specified	wet peroxide oxidation (WPO) followed by density separation with Zn Cl_2	not appliable	not specified	fibers, films, microrubbers	Stereomicroscope following Hidalgo-Ruz et al. [45] and Abbasi et al. [46].	µRaman	N of MPs g^{-1} and cm^2; length (µm) class distribution of MPs
Jafarova et al. [35]	*Evernia prunastri* (Active)	12 weeks	wet peroxide oxidation (WPO)	1.5–2 m	83 µm	microfibers and fragments	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	-	Number of MPs g^{-1}; daily microplastic deposition: MP m^{-2} day^{-1} (based on mass/area ratio of lichen); length of MPs (µm)
Jafarova et al. [32]	*Evernia prunastri, Pseudoscleropodium purum* (Passive)	~2–3 years	wet peroxide oxidation (WPO)	epiphytic lichen and epigeic moss (height not specified)	147 µm	fibers and fragments	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	-	Number of MPs g^{-1}; length of MPs (µm) and their proportion (%)

Table 1. *Cont.*

Authors	Species/Biomonitoring Method	Exposure Time	Extraction	Height of Exposure or Collection Above the Ground Level	Size of Smallest Particles	MP Categories	Visual Identification Criteria	Qualitative Analyses of MPs	Data Provided
Loppi et al. [30]	*Flavoparmelia caperata* (Passive)	~1 year	wet peroxide oxidation (WPO)	1–2 m	27 µm	fibers and fragments	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	µRaman	Number of MPs g^{-1}; length of MPs (µm)
Roblin and Aherne [29]	*Hylocomium splendens* (Passive)	~2–3 years	wet peroxide oxidation (WPO)	epigeic moss (height not specified)	300 µm	microfibers	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	µRaman	Number of MPs g^{-1}; daily microplastic deposition: MP m^{-2} day^{-1} (based on the biomass of moss, i.e., 2 kg dry weight m^{-2}); length (mm) and class distribution of MPs
Jafarova et al. [41]	*Robinia pseudoacacia* (Passive)	~8 months	water washing	not specified	10 µm	fiber, fragment, and tire wear particles	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	FT-IR	Number of MPs g^{-1}; daily microplastic deposition: MP m^{-2} day^{-1} (based on leaflets mass/ surface ratio); length (mm) and class distribution of MPs
Canha et al. [42]	*Lactuca sativa* (Passive)	not specified	wet peroxide oxidation (WPO)	not specified	336 µm	microfiber or fragment	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	-	Number of MPs g^{-1}
Taurozzi et al. [34]	*Cladonia and Xanthoria*	not specified	wet peroxide oxidation (WPO)	1.5–2 m	100 µm	fibers and fragments	Stereomicroscope following Noren et al. [43] and Windsor et al. [44]	FT-IR	Number of MPs * number of $lichens^{-1}$; length of MPs (mm)
Liu et al. [39]	*Pittosporum tobira, Camellia japonica, Pittosporum tobira; Aucuba japonica; Buxus sinica, Pittosporum tobira and Trachelospermum jasminoides* (Passive)	not specified	leaves flushed with filtrated Milli-Q water	not specified	16 µm	fibrous and fragments	Stereomicroscope	µRaman and FT-IT	Number of MPs cm^{-2} (based on leaves surface); length (mm) and shape, polymer and size class distribution %
Liu et al. [38]	*pine needles* (Passive)	~1 year	Method follows: Liu et al. (2019c)	2.2 m	500 µm	fibers, fragments, films and pellets	not specified	µRaman	Number of MPs g^{-1}; length (mm) and shape, polymer and size class distribution %
Leonard et al. [40]	*Acer saccharum, Rhus ovata, Buxus sempervirens, Leymus condensatus, and Chamaerops humili* (Passive)	-	Method follows: Koutnik et al., 2022	<0.6 m, 0.6–1.2 m, and >1.2 m	10 µm	fibers	Smartphone method (Leonard et al. [47])	FT-IR	n cm^{-2}

From the published works, we could distinguish two groups of issues: a first group (A) includes problems strictly linked to the methodology for the evaluation of the airborne microplastics, and a second group (B), which we could define as intrinsic to biomonitoring methods using plants, including issues that are independent from the target pollutant. As for group A, many of the obstacles to the use of plants as biomonitors of microplastics are linked to the scarce knowledge of their capacity to capture and retain these pollutants. In fact, each plant species is characterized by different ecological needs that are reflected in a wide range of morphologies and consequently in a different accumulation potential. In addition to this, it appears clear that neither the sampling nor the identification methods of microplastics are currently based on a shared and standardized protocol. For both passive and active approaches, the collection and post-harvest phases must also be coordinated, defining for instance the amount of biomonitor tissue to be evaluated. Also, the pre-washing of samples should be properly evaluated; this practice, in fact, could remove those particles lightly adhered to the tissues, which can be a source of data variability. Furthermore, the data reporting should be uniform, including the way to report the number of MPs found on the surface of each biomonitor. As regards the extraction and identification, it would be necessary to understand whether the protocols and criteria borrowed for other matrices are also the best choice for plants. The most used extraction protocol involves the use of wet peroxide oxidation that might not always be the best choice, since plants by their nature can present fibrous structures very similar to anthropogenic microfibers that are hardly oxidable, and hence undistinguishable during the identification phase. All the above can thus determine biases in the method. As a final aspect, it would be necessary to define the minimum size of particles on which it is possible to perform visual characterization without major ambiguities, and define the minimum number of particles on which to perform polymeric characterization. Currently, these two aspects are discretionary and, therefore, a source of data variability.

The problems we gather in group B could be partly solved by adopting the standards previously validated for other pollutants and through sharing protocols by researchers. As for the passive biomonitoring approach, it would be appropriate to define the right collection protocol: (i) to prefer the collection of leaves, lichens or mosses not subjected to surface washing; (ii) to define the height of collection (for passive approaches) or exposure (for active approaches); (iii) to define the biomonitor amount for the analyses. Another aspect to focus on is the correct age attribution to the leaves and to the portions of the thallus to be collected, preferring portions for which this attribution is not ambiguous. As for the experimental design, for both the passive and active approaches, two critical points are the definition of the sampling density and the number of replicates for each sampling area, and the frequency of repetition over time of the monitoring campaign. In this regard, according to some authors, the minimum number of sampling sites must be calculated based on the equations reported by Elzinga et al., 2001 [48]. As for the number of replicas for each site, a minimum number of three bags per sampling site placed at the center of an area with a radius of 50 m can be considered adequate (ISPRA, Manuals and Guidelines [49]). None of the works produced so far investigated the repetition of the monitoring campaign over time. As a general suggestion, studies should be carried out to evaluate the best temporal frequency, which should always be calibrated, from time to time, to the specific needs of the survey.

3. Land Snails for Environmental Monitoring of Microplastics

The study of soil pollution is of high priority, not only in urban and industrial areas, but also in agriculture. In recent years, soil ecosystems have been exposed to an increasing accumulation of pollutants. The use of some mollusks as sentinel organisms represents an interesting model for testing environmental health and the terrestrial gastropods, particularly snails, might be considered as relevant bioindicators for testing soil quality and monitoring pollution [50,51]. Snails effectively show a wide distribution, limited mobility and ease of sampling. They are important components of herbivore and detritivore fauna

in many ecosystems and live in close contact with the upper layers of the soil [50,52]. Their foot is in contact with soil particles and tends to accumulate many compounds. Terrestrial snails are also exposed to pollution by digestive and pulmonary systems. Isopods have similar characteristics and are used as bioindicators of soil contamination, but they occupy other habitats and are hard-bodied, soil-dwelling invertebrates, compared to terrestrial snails that have a soft body that allows greater exchange by the cutaneous route of exposure [53]. Gastropods can also be employed in laboratories, to predict effects in natural populations, by exposure to pollution, and examine the cause-and-effect relationships [54,55]. Wild snail species, studied in their natural environment, can be excellent bioindicators to monitor the impact of various pollutants on soil ecosystem quality based also on biomarker responses [56] and important wildlife sentinels providing early warning signs of environmental risk to humans [57–59]. Plastic fibers are undoubtedly disseminated, posing an emerging threat to terrestrial ecosystems [59]. Compared to microplastic pollution in the marine and freshwater ecosystems, the soil ecosystem has been little studied, and only in recent years has the attention shifted to it [59,60]. Soil effectively is a large repository for plastics, as the sea, and the spread of MPs in it, is a new global change factor which the international scientific community is taking an interest in. The soil environment is affected by MP fibers from various sources. The largest sources are agriculture and urban activities by residual plastic, plastic coat fertilizers and sewage irrigation, but also by atmospheric deposition [61–63]. Plastic residues found in the soil are transformed into MPs by degradation due to environmental action over time. MP fibers result from the gradual transformation of plastic waste into tiny particles by the action of physical, chemical and biological processes such as ultraviolet radiation, water or air erosion, photo-oxidative degradation or earthworms [59]. The resulting MPs dispersed in the soil can mix with other pollutants such as pesticides, heavy metals and organic pollutants, producing deleterious effects on soil fauna and flora [64,65], and impacting the soil's physical properties [60,66]. To assess the effect of MP pollutants on the environment, chemical measurements are excellent but not sufficient. However, despite the wide diversity and spread of the terrestrial gastropod groups in the world, these animals are little considered to test microplastics and nanoplastics bioaccumulation and their action mechanisms and ecotoxicity. In the last few years, some terrestrial gastropod species have been employed to isolate and identify different MP/NP types to understand the distribution of these pollutants in the gastropod fauna and their usefulness as bioindicators.

3.1. Effects of Microplastics on Land Snails

In a study in 2019, Panebianco et al. [67] showed for the first time the presence of microplastics in three different species of edible snails of the genus Helix (*H. aperta*, *H. aspersa* and *H. pomatia*). The research was carried out on a total of 425 snails found either directly in nature, in trade (samples taken in nature) and from breeding, from different areas of Italy (provinces of Reggio Calabria, Messina, Catanzaro and Bari) and Tunisia, divided into 85 samples (five snails for each sample) and analyzed by preliminary tissue digestion and subsequent microscopic observation. The authors examined the presence of MPs by analyzing their different shapes (fragments, foam, film, pellet resin and fibers). They revealed only fibers and fragments of MPs, and the first was the predominant shape (56.41%) present in the foot but also in the visceral sack. The finding of MPs also in farm snails confirmed the notable diffusion of MPs, not excluding the possible role of irrigation water, from aquifers, used in such plant's snail breeding and the consequent possibility of also finding MPs in terrestrial gastropods destined for human consumption. The accumulation of these MPs could be trapped by the cutaneous mucus or even ingested from contaminated vegetation [67]. Land snails can distinguish the indigestible particles using olfactory and taste receptors located on their lips [68]. For this reason, the authors have hypothesized that because of their small sizes and/or the formation of MPs agglomerates, these are not detected by the snails and are consequently ingested with food or by the fragmentation of larger debris due to the activity of the radula when the snail starts to

feed [67]. Comparing the bioaccumulation results of this study (0.07 ± 0.01 MPs/g) with those (0.36 ± 0.01 MPs/g) obtained with the same protocol on mussels [69] more frequently consumed by humans, the authors suggest the low contribution of edible snails to the overall exposure of humans to MPs. However, these data are indicative of the importance of using land snails to study the impact of MPs on the terrestrial environment and the health of soil organisms. Song et al. [70], in a study on land snail *Achatina fulica*, have revealed for the first time that microplastic pollutants in soil are taken up by this animal, showing physiological effects. In this research, snails were exposed to polyethylene terephthalate microfibers at concentrations of 0.01–0.71 g kg $^{-1}$ (dry soil weight) for 28 and 40 days. PET microfibers were shown to be ingested and eliminated by the digestive system of the animal. The digestion of snails caused cracks and deterioration of the surface of polyethylene terephthalate microfibers, which was mostly dependent on the action of the intestine. Furthermore, prolonged exposure to MFs had negative impacts on the feeding and excretion of snails. MFs induced significant villi damage in the gastrointestinal walls of 40% of the snails but did not influence the histology of the liver and kidney. Moreover, exposure to 0.71 g kg^{-1} MFs reduced glutathione peroxidase (59.3 ± 13.8%) and total antioxidant capacity (36.7 ± 8.5%) but elevated the malondialdehyde level (58.0 ± 6.4%) in the liver, which indicates oxidative stress involved in the toxic mechanism. These data showed the adverse impact of MFs on the fitness of soil organisms and highlighted the ecological risks of microplastic pollution in terrestrial ecosystems. Subsequently, Song et al. [71] showed also that *A. fulica* might depolymerize and biodegrade polystyrene into microplastics, suggesting their use for plastic biodegradation in soil environments. The African giant snail *Achatina fulica* has been also used in a study that has shown the transfer of plastic debris from the soil to a plant (the mung bean, *Vigna radiata* L.), and then to the snail [66]. The mung bean plants were directly exposed to polystyrene NPs by adding them to the soil for 10 days. The snails were indirectly exposed to plastic by feeding for 14 days on the leaves of mung bean plants that had internalized NPs. NPs transferred in *A. fulica* inhibited the activities of the snails, altered the tissue of their digestive gland, proventriculus and stomach and decreased the gut microbiome calculated in the feces of the snails exposed especially to 10 mg kg^{-1} NPs [72]. More recently, Li et al. [73] have also shown in *Achatina fulica* the transfer of polystyrene nanoplastics in the lettuce–snail food chain, suggesting the evaluation regard the risk of NPs in terrestrial ecosystems.

In 2021, De Felice et al. [74] reported that the snail *Achatina reticulate*, after exposure through diet for 40 days to polyethylene terephthalate microplastics with irregular shapes and at two concentrations (1% and 10% *w*/*w*; i.e., g PET-MPs), was able to efficiently ingest irregularly shaped and sized PET-MPs. However, they did not observe mortality and oxidative stress. This evidence of oxidative stress contrasts with the result in *Achatina fulica* mentioned earlier [71]. The authors have assumed that in *A. reticulata* the mucus might act as a protector for the gastrointestinal tract, avoiding the direct contact of the intestine with PET-MPs and helping the healing if needed. However, in treated *A. reticulata*, PET-MPs ingestion caused significant variation in the growth trajectories of snails, whereby treated specimens grew more than control ones [74].

However, the *Cantareus aspersus* snail, alimented for 28 days with low-density polyethylene (LDPE) particles dispersed in the food at different concentrations and sizes, has shown that the ingestion and digestion processes along the snail's digestive tract do not lead to a measurable fragmentation of the MP particles [75]. In this experiment, the big-sized particles improved growth at the lowest exposure concentration tested. Instead, at the molecular level, only small-sized particles triggered oxidative stress, but without causing quantifiable cytotoxic or genotoxic effects, which are important toxic endpoints because a wide variety of environmental contaminants directly or indirectly affect DNA. The authors have concluded that it is important to know the contribution of the polymer and the associated additives in plastic compound toxicity but that the snail *C. aspersus* is a good bioindicator of MPs toxicity in terrestrial environments [75].

3.2. Critical Issues in Biomonitoring of MPs by Land Snails

Although gastropods play essential roles in aquatic and terrestrial environments, little attention has been given to MPs/NPs' harmful effects on land snails, which are also potentially valid bioindicators to test soil pollution. These few studies already show the harmful action of microplastics that would be ingested from snails, accumulated and degraded with consequences on behavior, histological alterations an increase in oxidative stress and the potential to increase the toxicity of other chemicals and impair ecological levels. The data collected so far confirm how real the presence of microplastics and nanoplastics in soil is and how this can interfere with the life of organisms inhabiting these polluted environments. Further research is required since the toxicity of these microparticles may depend on their size, material and concentration. In addition, their toxicity may also vary depending on their binding to other pollutants in the soil. Certainly, land snails are excellent organisms for assessing the presence of microplastics in soil and knowing their toxicity to animals and humans. Future research is recommended to use this organism model and analyze whole soft tissues such as pedal mucus, foot, but also their feces; and the respiratory system is emerging to test atmospheric pollution and is of great interest.

4. Mussels in Aquatic Environmental Monitoring

The abundance of anthropogenic fibers in aquatic environments may generate negative environmental impacts on the ecosystem and biota. Various aquatic organisms mistake anthropogenic fibers for food and ingest them, resulting in negative health impacts due to the fibers themselves and the toxic pollutants present in the environment (i.e., heavy metals, persistent organic pollutants) adsorbed on the surface of the fibers [76]. Among various aquatic organisms, bivalves play an important role in both freshwater and marine ecosystems due to their contribution to nutrient recycling, thus creating and modifying the habitat as well as affecting food webs. Furthermore, it should be considered that bivalves are an important part of the global food source. Due to anthropogenic activities, bivalve abundance and species richness are declining [77]; the presence of anthropogenic fibers could be one causative factor. Worldwide, studies have been carried out to assess the accumulation of microplastics or anthropogenic fibers in several marine and freshwater bivalve species. The results have shown that MPs and fibers are prevalent in all the tested species [78–84]. Among these, mussels, sessile filter-feeding animals widespread in many geographical areas, are the most studied in water biomonitoring activities [85–88]. In fact, for these characteristics, mussels are historically considered excellent sentinel organisms for water biomonitoring. In detail, it has been shown that the exposure of mussels to various pollutants induces significant morphological and molecular alterations, as well as increased oxidative stress [88]. Regarding the most common mussel of the Mediterranean Sea, *Mytilus galloprovincialis*, it was shown that specimens living in natural sites (Bay of Castel dell'Ovo, Naples) contaminated by an Endocrine Disrupting Chemical (EDC) such as Bisphenol A (BPA) (a substance released by plastics) presented worse health conditions than animals from unpolluted sites. In particular, the authors recorded in BPA-contaminated mussels a reduction in the gonadosomatic index (growth parameter of the animal), as well as alterations in the organization of the gonads due to an increase in apoptosis. In addition, structural alterations of the ovarian follicles with oocyte degeneration and altered expression of estrogen receptors (ER1 and ER2) were found [88]. In a subsequent study, conducted on male samples, it was shown that treating animals in tanks with different concentrations of heavy metals (contaminants that can be adsorbed by MPs), alone and in mixtures, leads to alterations in the spermatogenetic process. The authors found tissue alterations in the testis, in which metals, particularly cadmium, caused the disconnection and disorganization of germ cells within the spermatic cyst, also impairing the normal chromatin compaction of the spermatozoa, an essential condition for preserving the fertilizing capacity of these cells [89].

4.1. Effects of Microplastics on Mussels

From the published works on mussels, two types of studies can be distinguished: some scientific articles analyzed the effects caused by accidental exposure to microplastics in natural habitats as a result of human activities; many other studies were conducted on mussels experimentally exposed to microplastics.

4.1.1. Environmental Exposure

Studies on the presence and effects of MPs in mussels have been conducted in different geographical areas worldwide. In a recent study performed on the widespread mussel species *Mytilus edulis* collected from natural banks in Portugal, the ability of mussels to bioaccumulate environmental microplastics dispersed in water was evaluated. Specifically, the amount, shape, color and chemical properties of MPs and MP-like particles in whole soft tissues collected in January and February 2019 from four natural banks of the Portuguese coast were assessed in vivo. Three sites were located in estuarine areas influenced by anthropogenic pressures and freshwater discharges, and one was located on a pristine coastline far from urbanized areas. The recorded MP and MP-like concentrations ranged from 0.54 to 3.0 g^{-1} with no significant differences between sites. Particle sizes ranged from 36 to 4439 µm, and fibers were the most abundant form (50%), followed by films (22%) and spherules (18%). Using mid-infrared spectroscopy (FT-MIR), the authors found that 69% of the particles analyzed were plastics, as six polymers and two polymer blends were identified, and 32% were cellulose-based materials. Finally, the fibers identified in the mussel tissues were mainly composed of cotton and viscose (77%) [90]. In a further study conducted on *Mytilus edulis*, the presence of microplastics and other debris of anthropogenic origin was assessed in specimens from the coastal waters of the United Kingdom, as well as from supermarkets [81]. Fibers were detected in all samples from all sites, with quantitative differences depending on the area. Seawater samples from six locations showed 3.5 ± 2.0 debris/L on average (range: 1.5–6.7 items/L). In wild mussels sampled from eight locations in the UK coastal environment, the total number of detrital elements ranged from 0.7 to 2.9 items/g tissue and 1.1 to 6 items/g tissue. In the case of supermarket-purchased mussels, the microplastic abundance was significantly higher in pre-cooked mussels (1.4 pieces/g) than in those supplied live (0.9 pieces/g). In addition, micro-FT-IR spectroscopy conducted on 136 randomly selected samples showed that 50% of the characterized debris was microplastics, while the remaining percentage was rayon and cotton. The levels of microplastics detected in supermarket-bought mussels represent, according to the authors, a route of exposure for humans; therefore, they suggest that among the many parameters assessed before the sale of these animals, the quantification of toxic pollutant fibers is also very important in food safety management and environmental monitoring measures [81,91]. The worrying impact of microplastics on marine species and trophic nets has also been assessed on mussels from the Tyrrhenian Sea. A study conducted in 2021 evaluated the potential contamination by plastic microfibers in *M. galloprovincialis* from the Tyrrhenian Sea for human consumption. Anthropogenic debris was extracted from the digestive tract of mussels and quantified under an optical microscope. Preliminary results showed the presence of potential plastic and natural microfibers in 73% of the samples. Mussels contained an average of 1.33 microfibers/g and 7.66 pieces/individual. Considering that mussels are consumed whole, contamination with microfibers may move up the trophic chain and reach humans [92].

Assessments of the presence in mussel tissues of pasty fibers were also conducted in specimens from the Adriatic Sea, a semi-enclosed basin with a low water recirculation rate and high anthropogenic pressures associated with unsustainable fishing and inputs of contaminants. Specimens of native blue mussels (*M. galloprovincialis*) collected in the open sea were compared with those collected in coastal areas. Microplastics were recovered from the soft tissues of all mussels analyzed. The coastal organisms showed a load of 1.06–1.33 fragments g^{-1} (wet weight) and 0.62–0.63 fibers g^{-1} (wet weight), while the offshore organisms showed an accumulation of 0.65–0.66 fragments g^{-1} (wet weight)

and 0.24–0.35 fibers g^{-1} (wet weight). The size class distribution revealed a marked prevalence of smaller particles (20 µm to 40 µm) and the most recurrent polymer type in the organisms analyzed was polyethylene (PE), followed by polypropylene (PP), polyethylene terephthalate (PET) and equal amounts of polystyrene (PS), polyamide (PLY) and polyvinyl chloride (PVC). From the data analyzed in this study, the authors observed a different distribution of microplastics concerning the different sites where various anthropogenic activities affect the water quality [93].

Contamination by microplastics is not only a problem in marine environments, but also in freshwater basins. In this regard, the presence of microplastics has been demonstrated in the duck mussel, *Anodonta anatina*, which lives in the Swedish river (Höje), where the number of microplastic debris found in mussels increased with their size [94]. Furthermore, it was shown that microplastic concentrations are higher near urban areas with wastewater treatment plants than rural areas. According to the authors, a large number of the microplastics found in the study are probably secondary microplastics that originate from textiles. Indeed, Napper and Thompson [14] estimated that 700,000 fibers can be released from a single wash with about six kilograms of synthetic material, and most end up in rivers such as the Höje [94]. The distribution, persistence, availability and biological uptake of microplastics in freshwater systems were also evaluated in a large river system such as the Colorado River, which crosses several states through a predominantly rural and agricultural territory. In detail, a study characterized the quantities and morphology of microplastics in different environmental compartments of two large basins along the Colorado River: Lakes Mead and Mohave, within the Lake Mead National Recreation Area. To assess the presence of microplastics, surface water and surface sediments were sampled at a total of nine locations. The sampling points were targeted at different subbasins with varying levels of anthropogenic impact. Quagga mussels (*Dreissena bugensis*) were sampled at a subset of locations to assess the biological uptake of microplastics. Microplastic concentrations were 0.44–9.7 particles/cubic meter at the water surface and 87.5–1010 particles/kilogram dry weight (kg/dw) at the sediment surface. Sediment core concentrations were 220–2040 particles/kg/dw, with no clear upward or downward trend over time. Microplastic concentrations in mollusks ranged from 2.7 to 105 particles/body. Fibers were the most abundant particulate type found in all different samples. Despite the low number of samples, microplastic concentrations are reported to be highest in areas with the greatest anthropogenic impact [95].

4.1.2. Laboratory Exposure

Environmental exposure studies have only shown the bioaccumulation of microplastics in the tissues of marine mussels; in fact, the cellular effects of these pollutants have been demonstrated through laboratory studies. In particular, in a study carried out in 2023, the effects of polyethylene terephthalate fibers were observed in the laboratory in various organs of *Mytilus galloprovincialis*, collected in the Ligurian lagoon (La Spezia, Italy); these fibers were similar in size to the MFs released by tissue washing and were therefore an ingestible material for mussels. The effects of animals' exposure were evaluated for 96 h at the concentrations of 10 and 100 µg/L, corresponding to approximately 150 and 1500 MF/mussel/L, respectively. The authors demonstrated that MF exposure stimulated extracellular immune responses both in vitro and in vivo, given the presence of hemolymph cells in the tissue, indicating the induction of immune/inflammatory processes. In both gills and the digestive gland, stimulation of antioxidant enzyme activities was recorded, suggesting an increase in oxidative stress, together with dose-dependent histological changes. Furthermore, the most significant accumulation of microplastics was recorded in the digestive gland compared to the gills. Taken together, the evidence shown in this paper suggests that at environmental exposure levels, PET-MFs have a significant impact on mussel physiology, impairing the function of two important organs, the digestive gland and the gills [91]. In contrast, damage to the gonads and gills of *M. galloprovincialis* was demonstrated for polystyrene MPs. Indeed, in both males and females, dose-dependent

effects such as the disorganization and degeneration of germ cells and proliferation of the gill epithelium following exposure in a tank to 5 µm polystyrene at different concentrations (0.5 and 1 µg/mL) [96]. In both gonads and gills [97], infiltrates of immune cells (markers of ongoing inflammation) and increased activity of the cellular antioxidant system (marker of increased oxidative stress) were found [96,97]. Specifically, among the antioxidant systems, biochemical investigations have shown that MPs cause an increase in the enzymatic activity of glutathione-S-transferase, catalase and superoxide dismutase [96]. In addition, MPs have also been shown to decrease metabolic efficiency by switching metabolism from aerobic to anaerobic; in particular, a reduction in the efficiency of the electron transport chain was found in animals exposed to MPs alone [96]. The reduction in metabolic efficiency resulted in a 36% reduction in mussel growth. All these effects were exacerbated when these substances were conjugated to MPs. Thus, the effects were amplified when MPs of PS were conjugated with BPA and cadmium (Cd); although these are preliminary data, a synergistic effect of these substances was thus demonstrated, confirming that MPs act like a "Trojan horse" [96]. The influence of microplastics on the metabolic pathways of *M. galloprovincialis* was further investigated in the digestive gland, the main site of pollutant storage, and thus deputed for detoxification. In particular, modern metabolomics investigations based on proton nuclear magnetic resonance (1H NMR), combined with chemometrics, have made it possible to clarify that exposure at different times to MPs polystyrene of 3 µm alters numerous metabolites present in the digestive gland including amino acids, osmolytes and molecules involved in energy metabolism and antioxidants [98]. Further tank experiments conducted in *M. galloprovincalis* also evaluated the acute toxicity of polystyrene on hemolymph cells. In detail, haemocytes of *M. galloprovincialis* were exposed to 10 µg/L polystyrene nanoparticles (PS-NPs; 50 nm) for 24 h. From these analyses, the authors showed that the cell viability of mussel haemocytes decreased significantly after 24 h exposure and the LC50 of PS-NPs ranged from 180 to 217 µg/L [99].

Considering both environmental MP exposure studies and laboratory exposure of mussels, all the data described in this section show that pollution by anthropogenic fibers such as microplastics is a global problem affecting water quality, not only in the seas but also in freshwater basins. Furthermore, the finding of microplastics in various mussel tissues suggests that the survival of these species could be seriously compromised, as these substances alter the cellular structure and activities of the organs in which they bioaccumulate. Finally, mussels, regardless of the damage they have suffered, are an excellent sentinel species, as they have an ability to bioaccumulate microplastics and can gather information on the state of water pollution.

5. Environmental Weathering on MPs and the Phenomenon of Microbial Biofilm Formation

As highlighted in the previous sections, many plant, terrestrial and marine organisms can be used as bioindicators for the assessment of plastic materials toxicity. Beyond issues related to the impacts of large plastic debris and microplastics (MPs) on aquatic and terrestrial species [100,101], ranging from entanglement and ingestion to suffocation and death, one of the most important challenges consists of the so-called "environmental weathering" on MPs and its consequences. Weathering phenomena strongly affect MPs, resulting in modifications of their physicochemical properties (such as the crystallinity, the pollutants absorption potential, the size, the mechanical properties, etc.) and the release of chemical additives, secondary metabolites (i.e., oligomers) and leachates, which may represent a source of potential toxicity for the surrounding environmental niches [102]. Weathering of plastic wastes might also promote the accumulation of polycyclic aromatic hydrocarbons (PAHs), metals and per- and polyfluoroalkyl substances (PFAS) [103,104]. In this section, a particular focus is given to the state of the art of the complex mechanisms between microplastics (under the influence of different weather conditions) and the colonizing bionts, in particular the adhering microorganisms (often forming biofilm on plastic debris), in different ecosystems, considering, from a One Health perspective, the impact not only on the environment, but also on animals and humans.

Several studies reported that the microbial biofilm established on MPs dispersed in the environment differs from the typical microbiota of surrounding habitats [105]. In this context, the term "Plastisphere" was coined to describe the specific microbial community of heterotrophs, autotrophs, predators and symbionts, established and influenced by different plastic surfaces [106,107]. During the last decade, various research has been conducted to fill the knowledge gap on the composition of such relatively novel habitats in marine and freshwater environments [108,109]; light is also being shed on the Plastisphere microbiota in terrestrial ecosystems, particularly in soil [110]. The formation of biofilms on microplastics is strongly influenced by a variety of environmental and biogeographical factors, such as particle size, substrate type, surface properties, location of the sample and the concentration of nutrients in the surrounding environment. In addition, as a function of their long residence time in the environment, many MPs have been deteriorated, namely aged (e.g., solar radiation leads to the depolymerization of MPs and alteration of their physical and chemical properties), thus exhibiting augmented degradation and increasing their exchange surface area; the phenomenon in turn promotes the attachment of biological and chemical contaminants to MPs [111]. Biofilm formation on plastics starts with the "conditioning film" (CF) process, involving the deposition of biomolecules, including glycine, glycoproteins, lipids, nucleic acids, ions, polysaccharides, proteins and aromatic amino acids. The above-mentioned biomolecules act through the modification of the surface and of the chemical and physical properties of the substrate, beyond behaving as chemo-attractants for microorganisms. The latter stages of the formation of a mature biofilm are due to many concomitant chemical, physical and biological factors, such as the conditions of the surrounding medium (or environment), the intrinsic factors of the bacterial cells involved and the surface of the substrates [112].

The formation of a mature biofilm on microplastics has been observed in different ecological niches; it may represent a vector for pathogens, and scientific evidence is accumulating on the risk of horizontal gene transfer (HGT) in the exopolysaccharide matrix and thus the migration, propagation and spread of antimicrobial resistance genes (i.e., ARGs) mediated by mobile genetic elements (i.e., MGEs) through plasmid-mediated conjugation, phage transduction and natural transformation by extracellular DNA [113]. Many reports mentioned this association and considered MPs as the "Trojan horse" in the transmission of ARGs and thus of antimicrobial resistance (AMR) [111].

5.1. Influence of MPs in the Propagation of AMR in Terrestrial Ecosystems

The soil Plastisphere has been substantially less investigated than the aquatic Plastisphere, and there are still many gaps in our knowledge of this ecological niche [110]. Studies have so far evidenced different properties compared to aquatic systems; firstly, the soil Plastisphere constitutes a less mobile system, as it is not subject to the transport of the water column, capable of increasing the interactions between the biotic substances of the bacterial biofilm and the abiotic factors typical of the soil environments. Consequently, the ecological behavior and composition of soil microbial communities are also closely regulated by the type of soil (which is extremely heterogeneous and depends on the parent rock), its different mineral composition and the different effects that other environmental components may have on the different plastic materials.

Indeed, several studies [114–116] have shown that different soil factors, such as pH, texture, organic matter and even soil edaphic fauna and microfauna, significantly influence the distribution of microplastics. For example, different soil textures have been shown to influence the ability of plastics to move through the soil (e.g., in a clay soil, the stickiness of the particles may prevent plastics from moving into deeper layers, whereas in a sandy soil, they are more likely to move) [117]. A recent study found a significantly higher number of microplastics in a sandy soil by comparing it with soils of diverse types [118], as also shown by variations in the numbers of isolated particles in different geographic regions [119,120]. Furthermore, soil texture has been shown to represent a relevant factor in mediating the impact of microplastics on soil hydraulic properties. Plastics indeed significantly reduce

infiltration and retention, affecting water movement differently in the different soils studied (loam, clay and sand), with a greater impact on soils with higher clay content and organic matter [121]. In addition, acidic soil has a lower adsorption capacity compared to neutral soil, because pH changes its surface potential and adsorbtion sites [117]. The presence of edaphic fauna (such as earthworms, collembola and mites) also affects the migration of soil microparticles from surface layers to deeper layers through the ingestion, expulsion or movement of microparticles through their body surface [122,123].

Further research is undoubtedly needed on the ecological risk of antibiotic resistance genes potentially transferred to the soil–plant interface, and whether potential pathogens in the Plastisphere could affect plant growth and food safety [124]. Among the most advanced techniques for studying the behavior of antibiotic-sensitive and antibiotic-tolerant cells, the most promising results have come from the single-cell Raman spectroscopy coupled with stable isotope labeling (such as 2D,15N and 13C), which is also applied to Plastisphere organisms. The methodology allows the trajectory of physiological evolution towards resistance to be followed in situ [125]. To study the diversity, composition and function of microbial communities, the most widely employed techniques remain metagenomic and metatranscriptomic sequencing, which have been recently integrated with machine learning, artificial intelligence and big data analysis methodologies. Wang et al. [126] exploited such techniques to assess how the presence of tetracycline (a largely employed antibiotic), polyethylene (PE) and copper affects the presence of AMR genes in the soil microbial community; the researchers reproduced in vitro the typical condition of soils amended with animal manure, which is naturally rich in heavy metals and antibiotics. Study findings highlighted the close correlation between the number of MPs and the increase in ARGs in examined soils, highlighting the potential of the investigated pollutant to increase the risk associated with one of most widely employed agricultural amendment means [126]. The transfer of ARGs from soil microbial communities to plants was also assessed using different concentrations of polyethylene, based on the premise that leafy vegetables have been identified as potential vectors for the transfer of ARGs from the environment to the human diet [127]. The study either monitored the evolutions in microbial communities or quantified eight common resistance-conferring genes. The results indeed evidenced the increase in ARGs and mobile genetic elements (MGEs) in lettuce tissue, roots and leaves compared to the untreated group, and a very noticeable change in the soil microbiota diversity and relative abundance in function by the addition of 0.5%, 1% and 2% polyethylene to the soil [128].

Metagenomics was also used in a study conducted in 2023, assessing the impact of plastic mulching (i.e., the practice of covering soil with plastic layers to provide more favorable conditions for plant development, resulting in a more efficient crop production) on soil, and studying the distribution patterns of ARG and MGE in eight Chinese provinces, based on the distribution of major cotton producing areas. To investigate the distribution patterns of microorganisms in soils exposed to long-term plastic mulch stress, the characterization of the microbial community structure of each site was performed. The genera *Gossypium*, *Pseudomonas*, *Nitrospira*, *Sphingomonas*, *Arthrobacter*, *Nitrososphaera*, *Streptomyces*, *Nocardioides*, *Steroidobacter* and *Chitinophaga* represented 25.9% of the detected genera in the metagenomic analyses. Most interestingly, the genera *Mesorhizobium, Nocardioides*, *Pelagibius*, *Arthrobacter*, *Sphingosinicella*, *Streptomyces*, *Luteimonas*, *Lysobacter*, *Pontibacter*, *Nitrospira*, *Actinophytocola*, *Chitinophaga* and *Gossypium* were selected as potential hosts of ARG and MGEs by comparative analysis with peer-review studies available in the literature. Results showed that soils holding a longer history of plastic mulching, and thus long-term plastic film contamination, brought out a higher relative abundance of antibiotic-resistant bacteria, MGE and ARG types, mostly associated with multiple drug resistance genes, compared to the microplastic-free soil controls or water [129]. A further study published in 2020 focused attention on the detection of ARGs from MPs, using high-throughput quantitative fluorescence polymerase chain reaction in samples collected from vegetable soils exploited for three and ten years, mulched with plastic films and fertilized with manure. Outcomes showed a

different abundance and type of ARGs and heavy metals as a function of MPs' particle size; in particular, resistance genes for macrolide-lincosamide-streptogramin B (MLSB) and vancomycin detected in larger MPs decreased, while resistance genes for fluoroquinolones, quinolones, florfenicol, chloramphenicol and amphenicol increased. Study results suggest that the likelihood of transferring mobile genetic elements is higher when plastic particles are larger, and/or have been exposed to more erosion and/or come from soils that have been cultivated for longer [130]. Yang et al. [131] performed soil DNA extraction followed by high-throughput sequencing of an in vitro microcosm (using a soil with a history of more than 5 years of rice–cereal rotation, without film mulching, manure fertilization or pesticide application), to which polyethylene, polypropylene and polystyrene were experimentally added; the findings evidenced a significantly higher number of total ARGs in the Plastisphere than in the surrounding soil for all MP types and sizes considered in the study [131]. Therefore, the potential risk posed by microplastics to the spread of antibiotic resistance highlights the need for more in-depth monitoring of the Plastisphere in the soil aimed at protecting human health.

5.2. Influence of MPs in the Propagation of AMR in Aquatic Ecosystems

The presence of microplastics in aquatic ecosystems has been documented for decades, while considering the widespread detection of plastic debris in rivers, lakes, coastal and offshore water and wastewater [112]. Many anthropogenic activities strongly affect aquatic environments: aquatic pollution by MPs and its relatives is closely related to different eco-geographical conditions, sea tides, wind direction and hydrodynamics [132]. As previously described, the formation of microbial biofilms on MPs in diverse environments has also attracted the attention of many scientists because of the risk of gene transfer, which is increased by the mobility of MPs–biotic component complexes and the potential of transporting pathogenic microorganisms and ARGs even from remote environments [133]. Municipal and hospital wastewater represents the preferred vehicle of antibiotic resistance genes into aquatic ecosystems, especially because most wastewater treatment plants are not designed to specifically remove MPs or AMRs; indeed, only tertiary treatment is able to remove up to 99% of MPs or AMRs, but most of the plants do not foresee tertiary treatment phases. It has indeed been estimated that the release of MPs from the different wastewater treatment plants (WWTPs) in Germany ranges up to 7.3×10^{12} particles/year [134], while the estimated global concentration of AMRs exceeds 104 gene copies each mL (GC/mL) for the most common AMR genes (tetracycline or sulfonamides) [116], whose concentration results are significantly higher than the starting concentration measured upstream of WWTPs.

Many studies report the presence of MPs and ARGs in sludge or wastewater [135–137]; nonetheless, research demonstrating the association between microplastics and resistance genes in aquatic environments, especially in hospital wastewater, is still lacking [105]. Bydalek et al. [132] monitored the formation and evolution of biofilms associated with experimentally added microplastics (in particular, HDPE, PVC, PET, PS) over time in a full-scale conventional wastewater treatment system covering 2100 population equivalents combined with a wetland. Utilizing bacterial 16S rRNA gene sequencing and qPCR for quantitative analysis of AMR genes (sul1, ermB, tetW, intiI1), the authors observed the predominance of species belonging to the genera *Aeromonas*, *Klebsiella* and *Streptococcus* as the main AMR-related pathogenic genera in microplastic-associated biofilms [138].

Nowadays, many rivers flow through highly polluted urban areas; such habitats are additionally affected by the discharge of treated sewage. The increased load of primary and secondary microplastics in river systems especially depends on industrial emissions, agricultural run-off, wind and soil erosion. The contamination of river habitats by sewage sludges has been widely documented by monitoring urban river sediments and surface waters [139,140]. In the estuary of Xinglin Bay (China), a study of the aquatic environment was conducted, targeting the presence of AMR genes in biofilms attached to polyethylene (PE), polystyrene, polypropylene and polyvinyl chloride (PVC), which were kept in sus-

pension for 30 days. A total of 251 ARGs and 46 MGEs were detected, predominantly in the Plastisphere on PP and PVC, and less on PE and PS. Higher relative abundances were found in PVC for beta-lactams, fluoroquinolones, phenicols, tetracyclines and trimethoprim, and significantly higher abundances were found in PVC and PP for MLSB and sulphonamide. Moreover, polystyrene was found to be the preferred polymer for many pathogens such as *Giardia lamblia*, *Klebsiella pneumoniae* and *Legionella* spp. [141].

Metagenomic and metatranscriptomic sequencing analyses were also performed to profile the presence, abundance and transcriptional levels of antibiotic resistance genes (ARGs) in the PLA- and PVC-bound Plastisphere of urban river waters of the Haihe River, the largest river basin in northern China, using bioreactors. Also in this case, 173 ARGs conferring resistance to 24 classes of antibiotics commonly used in humans and animals were detected, of which 75 had evidenced transcriptional activity. In particular, the Enterobacteriaceae family was identified as the preferred host of ARGs, and *Enterobacter cloacae* actively expressed the tetG gene [142].

Furthermore, in 2020, a study was carried out to correlate the Plastisphere on samples collected from three freshwater sites and three seawater sites in Shandong Province (China); employing a random forest machine learning model, differences in the microbial and fungal compositions of the Plastisphere were accurately distinguished from surrounding seawater and surface water; the results allow us to hypothesize that microplastics act as a filter for microorganisms in the environment, selecting only those that are capable of tolerating the chemicals on the plastics, exploiting such polymers as the preferred carbon source, and that microplastics could represent a shelter for the microbiota already present on their surface, transporting microorganisms from the source of release into the ecosystem [143].

With regards to seawater, many field and laboratory studies on Plastispheres allowed the detection of pathogenic microorganism genera associated with plastic debris, such as *Vibrio* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Mycobacterium* spp. [144,145]; nonetheless, the above-mentioned microorganisms were not directly associated with AMR genes.

Investigations on the association between the increase in MPs in aquatic environments and the augmentation of AMR have been spreading in the last year, though further research is substantially needed to assess the real risk to human health associated with antimicrobial resistance genes, and directly to the presence of pathogens, spreading in the environment.

Undoubtedly, multidisciplinary studies involving not only biotic but also abiotic aspects should be carried out in the future. Besides the simultaneous and increasingly accurate characterization and quantification of microplastics and microfibers, using more narrow filters (e.g., those employed for μFTIR technologies) [146], aquatic ecosystem studies should also consider the prediction of the behavior and movement of particles using standardized machine learning models. Thereby, potential accumulation points, massive sources and their pathways might be detected in advance [147], and might rely on increasingly specific remote sensing (i.e., satellite imagery, aerial surveys, hyperspectral sensors) and imaging technologies integrated with AI algorithms, proven essential over the past few years as identifying hotspots and targeted clean-up actions [148].

Although the aforementioned metagenomics approaches have advanced knowledge in several fields over the last decades, with applications in biotechnology, ecology and the environment, they still have several limitations, such as the high cost and complexity associated with the sequencing and analysis of massive amounts of data. In some cases, these data are also lacking and incomplete, and there is high variability in the sequenced microorganisms, which are often unknown and therefore lack genetic references, making an integrated approach to identifying genes and functional pathways difficult. Sample contamination during collection and analysis, which can affect the accuracy and interpretation of results, represents another limitation. An increasingly relevant challenge is to combine metagenomics with other omics studies, such as metatranscriptomics, metaproteomics and metabolomics, to obtain an ever more complete understanding of the composition and

functionality of microbial communities in different environments; the continuous progress in sequencing technologies and bioinformatics will help with this [149].

6. Conclusions

The biomonitoring and the evaluation of the impact of MPs on the environment are the object of an increasing number of studies, many of which compare different biomonitors to develop reliable methodologies for studying MPs diffusion and the associated exposure risk. Regardless of the habitat and cellular complexity of organisms, this review shows that exposure to microplastics determines the appearance of a variety of interactions and alterations that must be considered with attention for a comprehensive understanding of MPs' associated risks.

The attention was focused on plant and animal species specifically demonstrated to accumulate MPs in a massive quantity; the analyzed studies, therefore, consider the described species as potential biomonitors for assessing MPs' related risks.

The use of plants in the biomonitoring of airborne MPs, although discussed in a limited number of studies and lacking standardization, clearly shows both points of convergence and divergence. The selection of the most promising plant species employed for passive or active biomonitoring is of primary importance, because the micro-morphological traits can greatly affect MPs uptake and retention. Moreover, the microplastic extraction methodology should be implemented and customized to the biomonitor characteristics and always complemented by qualitative characterization of the extracted MPs. In addition, three main points should be considered for the proper selection of trustworthy plant biomonitors: (i) selection of species: for the so-called "passive" biomonitoring, it is necessary to focus on native and ubiquitous species; alternatively, the choice has to focus on a panel of plants showing similar uptake ability. As for the "active" method, several exposure times and conditions should be tested, aiming to select the best biomonitor for MPs accumulation. (ii) MPs extraction techniques: most protocols for MPs extraction from plants have been adapted by other matrices, and, therefore, it is not certain that these are the most suitable, not considering the specific characteristics of the plant biomonitor. (iii) Visual identification, data provided and qualitative analyses: to dispose of comparable data, it is advisable to establish the same methods of observation and visual characterization of MPs and always associate the latter with a qualitative analysis using the best available technique (e.g., FT-IR or μRaman). To date, approximately 30% of the reviewed works have not provided these data, while this characterization is essential in defining the origin of the particulate matter under investigation. Also, units of measurement based on the same reference criteria should be adopted (e.g., the way data of MPs deposition are reported, basing deposition on different surfaces, with difficulties in data comparison reducing the method's repeatability).

Additionally, although gastropod communities play an important role in terrestrial environments, studies employing land snails to assess the impact of microplastics on soil are still few compared to other animal models such as the example of mussels in aquatic environments. Instead, these animals are model organisms of great interest for this area of research. Their characteristics are very attractive for their use as MPs bioindicators in soil. They are diffuse, nonetheless exhibiting limited mobility, showing the ability to accumulate the microplastics through their tissues. The data collected by some studies in laboratories and someone in the field encourage future studies to use this organism as an integrated approach to biomonitoring the soil and to analyze the potential negative effects of MPs, also in combination with other chemicals, on biota. Land snails must be considered as relevant bioindicators for testing soil quality and monitoring pollution and for combining the information of isopods and earthworms.

As regards the importance of mussels in assessing MPs contamination in marine environments, these animal models are increasingly being employed, due to their ability to be sessile and their excellent filter feeders, which make them ideal organisms to study microplastics pollution. Various studies have shown that mussels are ideal for conducting investigations on both the bioaccumulation of MPs dispersed in the water and for detecting

the induced molecular and cellular damage. Hence, the bioaccumulation exerted by mussels is of great importance because it also makes it possible to identify the type of MPs dispersed and thus to determine the area-specific type of anthropogenic activity. However, to date, no MPs-specific molecular markers capable of showing indirect correlations between cellular damage and MPs have been identified in this model, since the molecular and cellular parameters altered by MPs are the same as those altered by other pollutants.

More interestingly, the recent cutting-edge findings evidencing the capability of MPs to enhance the pathogenic components in the biofilm formation process led to the need to enlarge the risk assessment to the evaluation of the spreading of antibiotic resistance in environments where high concentrations of MPs are detected. A more in-depth monitoring of the Plastisphere is indeed essential, to further protect the environment, animals and humans' health. This approach represents the real challenge on which several scientists working on MPs are focusing; it thus represents innovative insights in the field of microplastics monitoring.

From a One Health perspective, although the identification and definition of plant and animal models are essential to assess the chemical risk to humans and animals, the metagenomic study of aquatic and terrestrial environments' microbiota might constitute the most efficient means to evaluate the microbiological risks connected to the presence of the pathogens on MPs surfaces, aided by the extreme persistence of MPs in the above-mentioned habitats. At microorganisms' level, the outcomes coming from the microplastics' biofilm composition might constitute a biomonitor of the potential hazards related to the persistence of plastic pollutants in the described habitats.

In conclusion, this review evidenced the urgent need to employ reliable and, at the same time, easy to assess biomonitors for the various environments affected by MPs pollution. Most importantly, once identifying valid and unified biomonitors, the set-up of harmonized methodologies to conduct a proper and reliable assessment of MPs abundance in diverse environmental compartments is crucial. The setting up of standardized protocols fine-tuned for the different environmental matrices and ecosystems might represent the starting point to acknowledge the source, diffusion and fate of MPs, implementing mitigation strategies to reduce their impact.

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