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Beyond the surface – Microplastic hotspots in the water column of a top plastic-polluted deep lake

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ABSTRACT

Most research on microplastics in lakes has focused on particles floating on the surface, while little is known about microplastic occurrence in subsurface layers, even though these layers comprise most of the lake's volume. This knowledge gap is concerning because, without a deeper understanding of microplastic vertical occurrence, the full impact of microplastics on lake ecosystems cannot be accurately assessed. To fill this gap, this study investigated the seasonal variation in the concentration and composition (size, shape, and polymer type) of microplastics in different layers of the water column in Lake Lugano (Switzerland and Italy), a deep southern perialpine lake known for high microplastic pollution on the surface. Microplastic samples were collected seasonally from the water column, specifically from four layers representative of the surface (0–0.2 m), subsurface (0–10 m), middle (10–20 m), and bottom (20–80 m) layers of the lake. The highest microplastic concentrations were found in the upper three layers (surface: 18.9 particles m^{-3}; subsurface: 29.2 particles m^{-3}; middle: 30.9 particles m^{-3}), whereas the lowest concentrations were found in the bottom layer (4.3 particles m^{-3}). In addition, the layers showed differences in microplastic composition (size and shape) and intra-annual variation, suggesting that the interplay between seasonal environmental changes and hydrodynamic conditions may be a key driver of plastic distribution in deep lakes. The observed high concentration of microplastics between 0 m and 20 m depth, which encompass the euphotic zone, suggests a high risk of interactions between microplastics and freshwater organisms, warranting further investigation.

1. Introduction

Plastic has reached a yearly global production of more than 430 million tons (PlasticsEurope, 2025), and most of this massive production is destined to end up in the environment. According to the United Nations Environmental Program, only one fifth (21 %) of the plastic ever produced was incinerated or recycled, whereas the remainder ended up in landfills and the environment (UNEP, 2005). The large volumes of plastic dispersed, combined with the high chemical and mechanical resistance of this material, have made plastic one of the main sources of litter pollution in the world (Tian et al., 2023). For example, plastics are estimated to form approximately 80 % of all marine litter (Andrady, 2011). Such a high level of contamination poses risks to human and environmental health (Eerkes-Medrano et al., 2015). Moreover, these risks are growing because plastic production is still on the rise (PlasticsEurope, 2025), and so is probably plastic dispersion in the environment (Galloway and Lewis, 2016).

Microplastics (MPs), defined as plastic particles below 5 mm in length (Galgani et al., 2010), are of special ecological concern because they can harm organisms in many ways, for example through ingestion or exposure to MP-adsorbed chemicals (Bellasi et al., 2020; Nava and Leoni, 2021; Nava et al., 2024). MPs are found almost ubiquitously across land, water, and air environments (Akanyange et al., 2022). Part of the MPs dispersed from land sources (e.g., landfills, sewage systems) are carried to the oceans by river systems (Waldschläger et al., 2020), forming a global waterborne 'plastic pathway'. As the final destination

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of MPs originating from land-based sources, the oceans have long been perceived as the primary sink for MPs and therefore a strategic focus for research efforts (Chen et al., 2024). However, recent research indicates that lakes can act as important sites of accumulation along the plastic pathway (Alfonso et al., 2020; Boucher et al., 2019; Free et al., 2014; Nava et al., 2023). As a result, lakes can have MP concentrations that equal or even exceed the concentrations measured in marine litter hotspots (Nava et al., 2023).

Although research on MP pollution in lakes has been burgeoning over the past decade, the distribution of MPs within lake environments is poorly understood (D'Avignon et al., 2022). So far, most investigations have focused on measuring MP concentrations in the surface layer (e.g., Binelli et al., 2024; Cox et al., 2021; Dusaucy et al., 2021; Eriksen et al., 2013; Faure et al., 2015; Felismino et al., 2021; Mason et al., 2016; Mason et al., 2020; Nava et al., 2023; Pasquier et al., 2022; Sighicelli et al., 2018). However, as plastics can sink (Elagami et al., 2022; Elagami et al., 2023; Khatmullina and Chubarenko, 2021; Liu et al., 2020), plastic pollution is not confined to the lake surface. In large and deep lakes, the surface layer makes up only a small fraction of the lake's total volume. Additionally, these lakes typically undergo seasonal thermal stratification, which is likely to influence the distribution of MPs throughout the water column. Thus, for deep lakes, relying on surface sampling alone is a poor proxy for assessing the concentration of MPs across the entire lake volume. Nevertheless, only a small number of studies has addressed the vertical distribution of MP in lakes (Anagha et al., 2023; Chen et al., 2024; Fox et al., 2022; Lenaker et al., 2019; LfU, 2019; Tamminga et al., 2020; Tikhonova et al., 2024), with contrasting observations. As a result, numerous questions remain about the behavior and fate of MPs in deep lakes. For example, do MPs accumulate below the surface? If so, are they evenly distributed throughout the water column, or do they accumulate at specific depths? What factors, such as MP properties and lake hydrodynamics, influence their distribution in subsurface waters? Without extending the investigations to the whole water column, the full extent of lake MP contamination, the associated biological risks, and the role of lakes as MP hotspots along the plastic pathway cannot be fully understood.

In this study, the MP concentration was investigated between different layers (up to 80 m depth) of a deep natural lake located at the southern side of the Alps (Lake Lugano, Switzerland and Italy). A recent global survey spanning 38 lakes from 23 countries and all continents ranked Lake Lugano as one of the most polluted in terms of surface MP concentration (Nava et al., 2023). This research is the first to explore MP

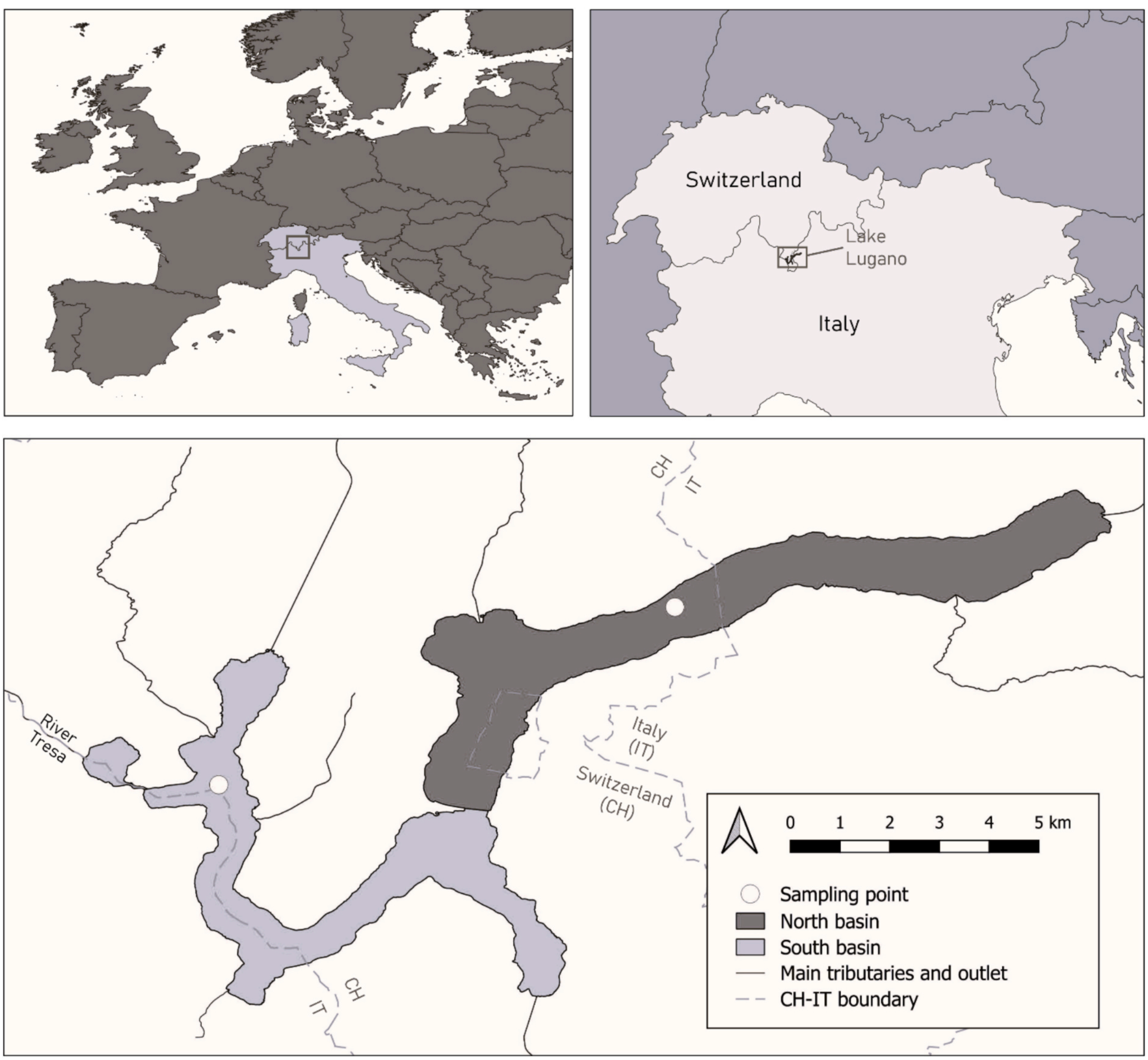

Fig. 1. Geographical location of Lake Lugano, a deep natural lake located south of the Alps, on the border between Switzerland and Italy (45°59′0″ N, 8°58′0″ E). The white dots indicate the position of the two pelagic sampling stations, which are located near the deepest points of the North (maximum depth 288 m) and South (maximum depth 95 m) basins.

concentrations in the layers below this highly contaminated surface. MP concentration and composition (by size, shape and polymer type) were measured within different layers of the water column (surface, subsurface, middle, and bottom layer) and across seasons. The results will provide insights into the drivers shaping MP vertical distribution in deep lakes, offering crucial evidence to identify related ecological risks.

2. Methods

2.1. Study area

Lake Lugano is a deep lake located south of the Alps, across the border between Switzerland and Italy (45°59′0″ N, 8°58′0″ E; Fig. 1). Despite Lake Lugano having a relatively small surface area (48.9 km^2), its marked depth, pronounced thermal stratification, and long residence time are characteristics shared with large lakes (Boyce et al., 1989), suggesting comparable vertical patterns of suspended particles, including MPs. The lake is divided into three basins connected by narrow channels. The main basins are the North basin (27.5 km^2) and the South basin (20.3 km^2), which are separated by a causeway. The smaller Ponte Tresa basin (1.1 km^2) was not included in this study. The two larger basins have different hydrodynamic characteristics. The northern basin, which is deeper (maximum depth 288 m) and has a long water theoretical residence time (12.3 years), is nearly meromictic; it turned over only twice since systematic monitoring began in the early 1980 s. The southern basin is shallower (maximum depth 95 m), has a shorter theoretical residence time (1.4 years), and is nearly holomictic, although in recent years it skipped turnovers after unusually mild winters (Lepori et al., 2018). In each basin, turnover occurs once a year in late winter or early spring, usually between January and March. After the turnover, the lake enters a stratification period, which lasts until the fall, when cooling leads to a progressive deepening of the thermocline (Barbieri and Polli, 1992).

The lake has several small-to-medium tributaries, most of which flow into the South basin. As a result, the South basin receives the greatest river discharge, compared to the northern one (mean daily discharge, North basin: 2.7 $m^3 s^{-1}$; South basin: 7.8 $m^3 s^{-1}$). The only outlet (River Tresa; Fig. 1) drains away from the Ponte Tresa basin (mean daily discharge: 9.2 $m^3 s^{-1}$). Almost the entire population of the watershed is served by a combined sewer system (which combines wastewater and surface waters). Most sewage treatment plants, including the one serving the city of Lugano, are located in the South basin watershed. Sewer overflows, which release untreated wastewater directly into the lake or its tributaries during heavy rainfall events, are scattered throughout the watershed.

In 2003, the population in the Lake Lugano watershed included 195,443 residents and 70,616 tourists. A large part of the population lives in the city of Lugano and its suburban surroundings (62,315 residents), located on the shore of the North basin. Other settlements range from villages to small towns. The lake provides the resident population and tourists with drinking water (e.g., the main water supplier obtains 20 % of the water from the lake), thermal energy, and recreational opportunities. The predominant land uses in the watershed are woodland (67 % of the area), urban development (11 %), and summering pastures (9 %; Ferrario, 2009).

Lake Lugano was originally oligotrophic (Niessen et al., 1992), but during the second half of the 20th century, the lake became eutrophic due to increased loadings of untreated or poorly treated wastewater. A restoration program, started in the 1970s, has significantly improved the lake conditions, although the trophic state has only partially recovered (Lepori et al., 2023). Other environmental pressures include global warming (Lepori and Roberts, 2015), biological invasions (Capelli et al., 2023), and widespread alteration of lake shores (artificial shores: 65 % of the area).

2.2. Sampling activities

Seasonal sampling (January/winter, April/spring, July/summer, November/autumn; Electronic Supplementary Material (ESM) Table S1) was replicated at two pelagic sampling sites (Fig. 1) in 2022, one located near the deepest point of the northern basin (46°00′38.818″ N, 9°01′14.343″ E) and one near the deepest point of the southern basin (45°57′31.381″ N, 8°53′37.492″ E), to give a better overall representation of MP contamination in the lake. Sampling was conducted in good weather conditions, at least 5 days after any previous rainstorm (Hitchcock, 2020).

The vertical profile of the lake was divided into four layers. As a preliminary study, sampling was conducted at the surface (0–0.2 m depth) and in three layers of the water column (subsurface layer: 0–10 m, middle layer: 10–20 m, bottom layer: 20–80 m depth). The boundaries between these layers (hereafter referred to as ‘subsurface,’ ‘middle,’ and ‘bottom’) were defined based on historical trends in the lake’s thermal stratification (DACD-SUPSI, 2022), and were subsequently confirmed using data from 2022 (DACD-SUPSI, 2023; ESM Fig. S1), in order to reflect the average depths of the epi-, meta- and hypolimnetic layers. Samples were collected using plankton nets (Liu et al., 2020). Although MPs are often sampled using a mesh size of 300 μm (Pasquier et al., 2022), in this study, a 100 μm mesh was used to include smaller MPs, which may be particularly numerous (Hidalgo-Ruz et al., 2012) and can be ingested by freshwater organisms, such as small fish and mussels (Kaba et al., 2025; Kankiliç et al., 2023). The surface layer was sampled by towing a plankton net (23.020 KcDenmark; diameter: 0.5 m) horizontally alongside the boat (boat speed approximately 3–7 km h^{-1}) along linear transects of 150–500 m each, depending on the abundance of plankton and floating debris, to avoid net clogging. During the tows, only the bottom one third of the net diameter was immersed in the water (Nava et al., 2023). The subsurface was sampled by hauling the same plankton net (23.020 Kc-Denmark; diameter: 0.5 m) vertically from the depth of 10 m to the surface. It should be noted that the volume of the surface layer included in the 0–10 m samples represents about 1 % of the total volume filtered by the vertical haul. For that reason, it is assumed that the 0–10 m layer predominantly represents the subsurface layer, and that MPs belonging to the surface are a negligible proportion. The middle (10–20 m) and bottom (20–80 m) layers were sampled by vertical hauls, using a plankton net fitted with a closing mechanism (CP3–110 Aquatic Biotechnology; diameter: 0.4 m). Although no flowmeter was employed during the sampling, high filtration efficiency was ensured for both surface and water column sampling. The nets were designed with a high filtering-area-to-mouth-area ratio ($R > 6$) to promote consistent water inflow and minimize the formation of standing waves that could bias volume estimation (Harris et al., 2000).

After sampling, the net content was collected into a clean glass jar from the cod end, using a squeeze bottle filled with lake water to help rinse it out. As the volume of lake water used for rinsing accounted for only 0.01 % of the total water filtered during each trawl, any contamination from this water to the recovered MPs is assumed to be negligible. Three replicate samples (i.e., three horizontal tows for the surface layer and three vertical hauls for each water column layer) were collected on each sampling. The replicates collected within the same layer of the water column (i.e., subsurface, middle, and bottom) were pooled into a single sample, while the surface replicates were stored separately due to the larger amount of coarse material collected during each transect. In the laboratory, the samples were stored at room temperature in the dark until further analysis.

2.3. Sample processing

Samples were processed following a protocol developed from published recommendations and guidelines (Masura et al., 2015; Monteiro and da Costa, 2022; MSFD, 2023). First, all samples were reduced in volume by wet sieving, to separate MPs (size < 5 mm) from organic coarse material and macroplastics, which were not considered further. Additionally, to gain more information on MP size distribution, MPs were separated into different operational size categories (large MPs: 5–1 mm, medium MPs: 1–0.3 mm, small MPs: 0.3–0.1 mm) using three stainless steel nested sieves (VWR; mesh size 1.0, 0.3, and 0.1 mm). To maximize recovery efficiency, the glass containers in which the samples were stored were thoroughly rinsed with filtered deionized water. Next, the organic material occurring in the samples was oxidized using hydrogen peroxide (30 % H_2O_2; sample volume ratio 30–50 % depending on organic content) catalyzed with iron solution [Fe(II) 0.05 M], as proposed by Tagg et al. (2017). After overnight oxidation, samples were filtered onto gridded nitrate cellulose filters (Sartorius; 1.2 μm pore size) using a glass filtration device and dried for 1 h at 50 °C.

2.4. Plastic quantification and characterization

MPs were analyzed under a dissecting microscope (Wild M5; magnification 25–50x). Each MP item was counted and assigned to one of six shape categories (fragment, film, fiber, foam, pellet, and bead; ESM Table S2) following criteria proposed by Lusher et al. (2020). MP concentrations (MP m^{-3}) were calculated by dividing the number of MPs by the lake water volume filtered within each layer. The filtered water volume was estimated by multiplying the immersed (surface) or total (subsurface, middle, bottom) area of the net mouth by the trawl length (total filtered volume, surface: 39.8 m^3; subsurface: 5.9 m^3; middle: 3.8 m^3; bottom: 22.6 m^3).

2.5. Quality assurance: Sampling equipment and laboratory contamination

Quality assurance measures were taken to prevent unintentional contamination (Gao et al., 2023). To minimize contamination from the laboratory environment, all samples were kept in closed glass containers until processing. Wet sieving and other sample manipulations were performed in a fume cupboard, while drying samples were covered with aluminum foil. Whenever possible, glass and stainless-steel materials were used instead of plastic. To reduce cross-contamination, stainless-steel sieves were thoroughly washed before and after each sample following a strict triple-rinsing protocol, which has been shown to remove over 90 % of retained particles (Song et al., 2021).

Replicate control samples were prepared to account for potential contamination due to sampling equipment (i.e., field blanks) and laboratory environment (i.e., laboratory blanks). Field controls (n = 3 for each net) were obtained by rinsing nets cod end with distilled water (100 mL) to quantify potential contamination from the net (e.g., particles released from the nets' material or residual MPs from previous hauls). Blanks were processed by rinsing the net from the outside, mimicking the way the net content was collected in the field. Laboratory blanks (n = 8) were obtained by directly filtering 100 mL of distilled water on gridded cellulose filters (Sartorius; 1.2 μm pore size) and exposing them uncovered to the lab environment alongside sample processing (approximately 24 h). Each control sample was analyzed under the microscope, and MPs were counted, classified according to their shape, and assigned to the respective size category (i.e., 5–1 mm, 1–0.3 mm, 0.3–0.1 mm) using maximum Feret's diameter (Hartmann et al., 2019). Based on blank analysis, the field and laboratory limit of detection [LOD: mean + 3 * standard deviation of particle number; MacDougall et al. (1980)] were estimated to be 15 and 34 MPs, respectively. The number of MPs counted in lake samples was above those limits (average 188 MPs sample^{-1}), except for four samples whose particle count was below the laboratory LOD (ESM Fig. S2). No correction was applied to the results in accordance with MSFD (2023). Because data were analyzed only within averaged estimates rather than individually, the potential error introduced by samples below LOD is expected to be minimal.

2.6. Quality control: Visual identification accuracy

To ensure the absence of false positives during visual identification, visual identification and micro-Raman spectroscopy analysis were compared for a subsample of particles (Cowger et al., 2024; De Frond et al., 2023). A subsample of 10 % of the particles/fibers visually-identified as potential MPs [minimum 30 MPs and maximum 50 MPs; all particles were included if fewer than 30; De Frond et al. (2023), MSFD (2023)] was randomly selected among all the layers of the summer samples, and analyzed by micro-Raman spectroscopy, to determine if the particles/fibers had a polymeric nature and, if so, what type of polymer they were. A total number of 139 potential MPs were manually removed from the filters with tweezers, placed on microscope slides and held in place with a coverslip to avoid environmental contamination, and without using any additives or adhesives that might alter polymer identification (Nava et al., 2023). Particles were characterized by shape and color, photographed and measured using ImageJ software (version 1.53e). Next, all potential MPs were analyzed by micro-Raman spectroscopy (in–Via Qontor micro-Raman Spectroscope, Renishaw). The laser sources (532 nm and 785 nm), focused by a 50x objective, were switched depending on sample response, and the acquisition parameters were optimized to maximize the signal-to-noise ratio. MP identification was performed by matching the Raman spectra of the particles analyzed with reference libraries (Anger et al., 2018; Lo Bue et al., 2023; Nava et al., 2021), enriched by a set of known polymer sample spectra acquired by the authors as standards. When the quality of spectra acquired allowed, both the fingerprint and the functional groups stretching spectroscopic regions were considered. Based on the acquired spectra, particles were classified as plastic (i.e., consistent with a specific polymeric class of materials), anthropogenic (i.e., particles/fibers clearly identified as anthropogenic but not confirmed as a specific polymer, such as cotton or particles/fibers dyed with synthetic pigments), and unidentified (i.e., inconclusive identification due to lack of diagnostic spectroscopic features). In this study, micro-Raman spectroscopy was used as a qualitative diagnostic support to the quantification protocol. For this reason, no specific statistical interpretation (e.g., by season or water layer) was applied to the spectroscopy data.

2.7. Statistical analysis

Statistical analysis was performed using the software Minitab® (version 20.3). Analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) were used to compare MP concentrations and composition including size (categories: 5–1 mm, 1–0.3 mm, 0.3–0.1 mm) and shape (categories: fragment, fiber, 'other shapes' including film, foam, pellet, and bead), among lake layers (surface, subsurface, middle, and bottom) and sampling season. To avoid pseudoreplication (Hurlbert, 1984), the samples collected within each combination of sampling station, season, and layer, which are not statistically independent, were pooled. Differences in MP concentrations were tested using a two-way ANOVA followed by Tukey's post-hoc tests. Differences in size composition (categories: 5–1 mm, 1–0.3 mm, 0.3–0.1 mm) and shape composition (categories: fragment, fiber, 'other shapes') were analyzed using MANOVA followed by pairwise comparisons. Data for these analyses were square-rooted to meet parametric test assumptions.

Variation in MP composition was further investigated using Principal Component Analysis (PCA). PCA was performed with RStudio package factoextra (version 2024.12.1) on a matrix of correlation coefficients after taking the square root of MP concentrations to reduce data skewness. MPs were categorized into nine groups representing all combinations of size and shape (3 size categories x 3 shape categories).

3. Results

3.1. MP occurrence in Lake Lugano water column

A total number of 9043 plastic particles were identified across all samples, with fragments (total number: 3063) and fibers (total number: 5800) being the dominant shapes. Other shapes (including film, foam, pellet, and bead) were rare, representing less than 1 % of all MP counted. MP concentrations differed among layers and seasons. In general, MP were consistently lower in the bottom layer (average across sampling stations and season: 4.33 ± 2.81 MP m^{-3}; Fig. 2) than in all other layers (surface: 18.86 ± 9.55 MP m^{-3}; subsurface: 29.20 ± 23.10 MP m^{-3}; middle: 30.87 ± 28.72 MP m^{-3}; Fig. 2, ESM Table S3). MP concentrations in the subsurface and middle layer were higher than in the surface layer in the first two sampling seasons, whereas they were similar in the second half of the year (Fig. 2). This result was further supported by the ANOVA (Table 1) and post-hoc tests (ESM Table S4), which indicated a 'layer' effect, reflecting the significantly lower MP concentration in the bottom layer compared to the three layers above. Additionally, a significant 'season' effect indicated a difference between the first half of the study period (January/winter and April/spring), characterized by higher MP concentrations (especially in the 0–20 m layer), and the second half (July/summer and September/autumn) which had lower densities overall (ESM Table S4).

3.2. MP qualitative characterization: size, shape and polymer composition

MP size composition differed significantly among layers, mainly because larger MPs in the 5–1 mm and 1–0.3 mm categories decreased with increasing depth (Fig. 3). In particular, MPs in the 5–1 mm category were several times more abundant in the surface layer than in the other layers. MP composition in the bottom layer showed a greater relative abundance of smaller MPs (MPs in the 0.3–0.1 mm size range were particularly abundant, averaging > 50 % of the MPs counted in this layer; Fig. 3). The observed differences in MP size composition between layers were associated with changes in shape composition, with fragments and other shapes almost disappearing below the 20 m depth (Fig. 3).

The MANOVA results indicate a significant 'layer' effect (Table 2), mainly because MPs larger than 0.3 mm sharply decreased from the surface (mean: 10.4 ± 6.75 MP m^{-3}) to the bottom (mean: 1.51 ± 0.86 MP m^{-3}). This difference was caused mainly by disproportionately lower concentrations of fragments (mean: 1.06 ± 0.72 MP m^{-3}) and 'other shapes' (mean: 0.01 ± 0.01 MP m^{-3}) in the bottom layer relative to the upper layers. Consequently, the 20–80 m layer had a higher relative abundance of smaller filamentous MPs (on average > 75 % of the MPs counted) compared to the layers above it (Fig. 3). In addition, 'season' had a marginally significant effect on MP shape composition (Table 2), although post-hoc tests revealed no significant pairwise differences (ESM Table S5).

The first two Principal Components (PC1 and PC2) extracted 75 % of the variation in the data matrix (PC1 = 49 %, PC2 = 26 %; Fig. 4). Based on PC loadings (ESM Table S6), PC1 reflected the overall concentration of MPs, separating samples with higher concentrations (i.e., surface, subsurface, middle) from samples with lower concentrations (i.e., bottom) across all shape and size categories. In addition, samples from the surface, the subsurface, and the middle layers showed greater scatter along PC1 compared to bottom layer samples, which indicates greater temporal (within year) variation in overall MP concentration. PC2

Table 1
Results of a two-way ANOVA testing for differences in MP total concentration among layers [surface (0–0.2 m), subsurface (0–10 m), middle (10–20 m), and bottom (20–80 m) layers] and sampling season. Bold font highlights statistically significant results.

	Sum of squares	df	Mean square	F	p (same)
Layer	48.672	3	16.224	10.380	**< 0.001**
Season	45.661	3	15.220	9.741	**< 0.001**
Interaction	21.866	9	2.430	1.555	0.211
Within	24.999	16	1.562		
Total	141.198	31			

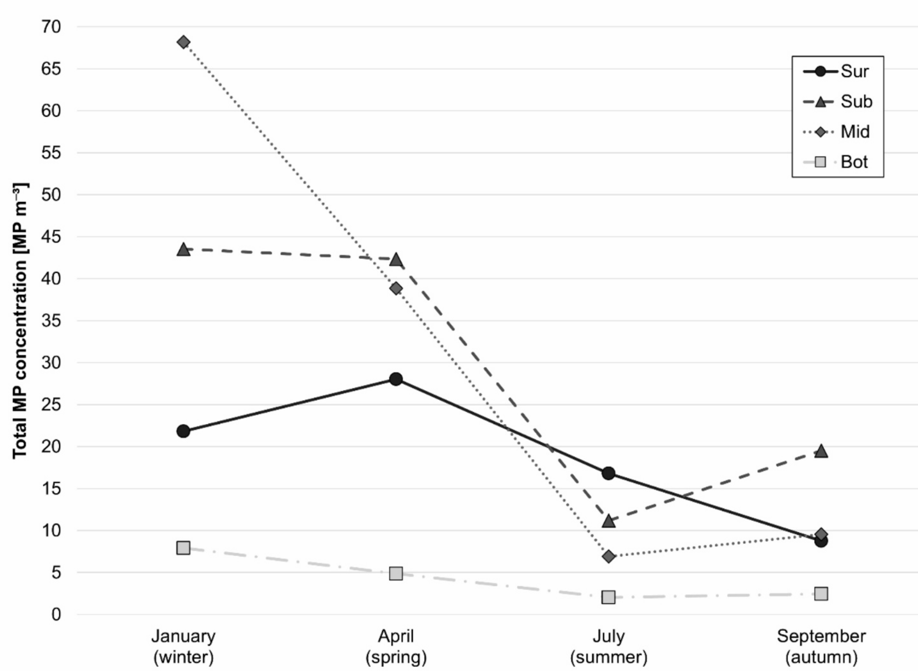

Fig. 2. Average MP concentrations (all shape and size combined) by sampling season and lake layer (for further details see ESM Table S3). Sur: surface layer (0–0.2 m); Sub: subsurface layer (0–10 m); Mid: middle layer (10–20 m); Bot: bottom layer (20–80 m).

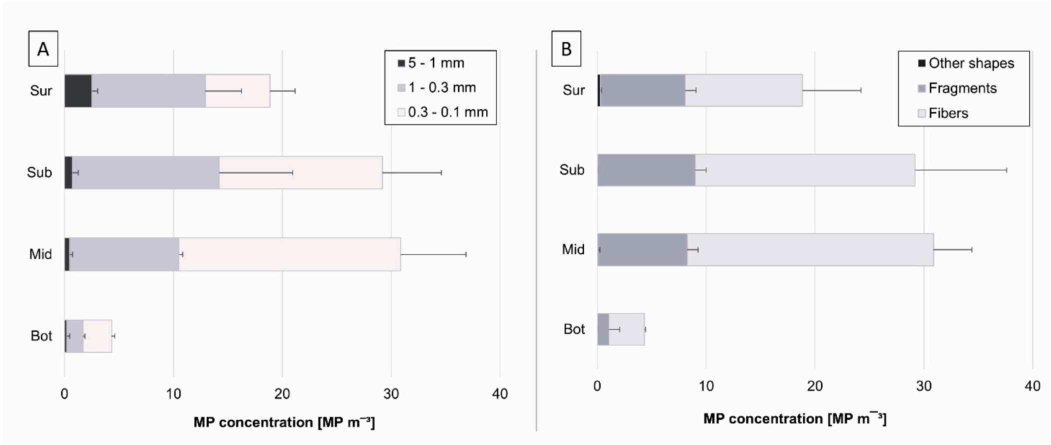

Fig. 3. Total MP concentration (all seasons combined) in the surface and water column layers of Lake Lugano characterized by size range (A) and morphology (B). Sur: surface layer (0–0.2 m); Sub: subsurface layer (0–10 m); Mid: middle layer (10–20 m); Bot: bottom layer (20–80 m).

Table 2
Results of MANOVA testing for differences in MP size composition (A), and shape composition (B). Bold font highlights statistically significant results.

(A)	Layer	Season
Wilkś lambda	0.260	0.605
F	5.206	1.614
p (same)	**< 0.001**	0.130
(B)	Layer	Season
Wilkś lambda	0.424	0.523
F	2.983	2.148
p (same)	**0.005**	**0.038**

reflected a compositional shift from higher concentrations of larger MPs in surface layer (high scores, indicating higher concentrations of fragments and 'other shapes' in the 5–1 mm category; ESM Table S6), from all other samples characterized by higher concentrations of smaller MPs (higher concentrations of fragments and filaments in the 0.3–0.1 mm size category; Fig. 4).

The analysis of the subsample of 139 potential MPs by micro-Raman spectroscopy showed that, except for two cotton fibers, 65 % of the particles isolated via the visual approach were correctly identified as plastics. When the composition of the remainder could not be identified due to a lack of diagnostic vibrational features detected on the spectra, the particles were classified as anthropogenic but were not attributed to a specific class (15 %). An example of this second group is reported in Fig. 5B, where the band patterns registered on the Raman spectrum, acquired on a red fiber, were ascribable mainly to the red pigment, such as PR242 (Soprano Spectral Library database), commonly used for plastic materials dyeing. Thus, the particle is probably an artificial fiber, although no polymeric diagnostic bands were detected, possibly due to overlap with the artificial pigment vibrational resonance. Overall, the spectroscopic analysis clearly identified seven different types of plastic polymers classes within the subsample of particles analyzed, across all layers (Fig. 5). The polymer composition varied with shape, as most fragments were composed of polypropylene (PP; 57.9 %) and polyethylene (PE; 31.6 %; Fig. 5), whereas most fibers were composed of polyethylene terephthalate (PET; 62.3 %), polyamide (PA; 17.0 %) or PP (11.3 %).

4. Discussion

In lakes, MP surveys and monitoring programs have largely focused on floating MPs (D'Avignon et al., 2022; Dusaucy et al., 2021; Nava et al., 2023; Pasquier et al., 2022), presumably based on the assumption that the surface is the most contaminated layer, as it is the first recipient of floating plastics originating from land sources. This study showed that in Lake Lugano, a deep stratified lake highly contaminated with MPs in its surface waters (Nava et al., 2023), the high concentrations of MPs extended well into the mid depths. In fact, the subsurface (0–10 m) and middle (10–20 m) layers had similar or higher MP concentrations than the surface layer (0–0.2 m), while only the bottom layer (20–80 m) had lower MP concentrations. This vertical distribution is consistent with research findings from both freshwater and marine environments, which estimated that floating plastics account for less than 1 % of total plastic waste entering the environment (Boucher et al., 2019; Egger et al., 2020). Although a negative relationship between sampled water volume and MP concentration has frequently been reported, this is not expected for the concentrations observed in the present study, as this pattern is far less pronounced in net-based sampling. This is because nets do not retain very small particles (i.e., those smaller than the mesh size) which, in contrast, are efficiently captured even at low sample volumes by grab and pump filtration methods (Cross et al., 2025). By limiting collection to a larger size range, nets reduce the influence of highly abundant small particles on concentration estimates, thereby minimizing the effect of sampling volume.

The presence of relevant concentrations of MPs has been well documented throughout the water column in marine ecosystems (Zhao et al., 2025) but is rarely reported in lakes. Despite this, a few studies from lakes have reported the presence of MPs across multiple depths of the water column (Anagha et al., 2023; Chen et al., 2024; Fox et al., 2022; Lenaker et al., 2019; Tamminga et al., 2020; Tikhonova et al., 2024). The results of the present study are consistent with these previous observations, supporting the idea that the water column can act as a major sink of MPs in aquatic ecosystems. These observations suggest that future monitoring strategies should take vertical variability into account to obtain unbiased estimates of contamination.

Different hypotheses have been proposed to describe the vertical distribution of MP particles in the water column of lakes (Liu et al., 2024). However, the identification of the key drivers of this distribution is proving difficult, due to local-context factors such as lake location, hydrological conditions, and/or particles properties (Elagami et al., 2023). One of these hypotheses emphasizes the role of vertical changes in water density between layers (Elagami et al., 2023; Gunaalan et al., 2024; Uurasjärvi et al., 2021). According to this hypothesis, in thermally stratified lakes, such as Lake Lugano, the transition in water density between the warm epilimnion to the cold hypolimnetic waters (i.e., bottom layer in the present study) is expected to slow down the sinking of plastic particles, causing MPs to remain longer in the less dense waters above the thermocline (Elagami et al., 2023). Indeed, this study, which

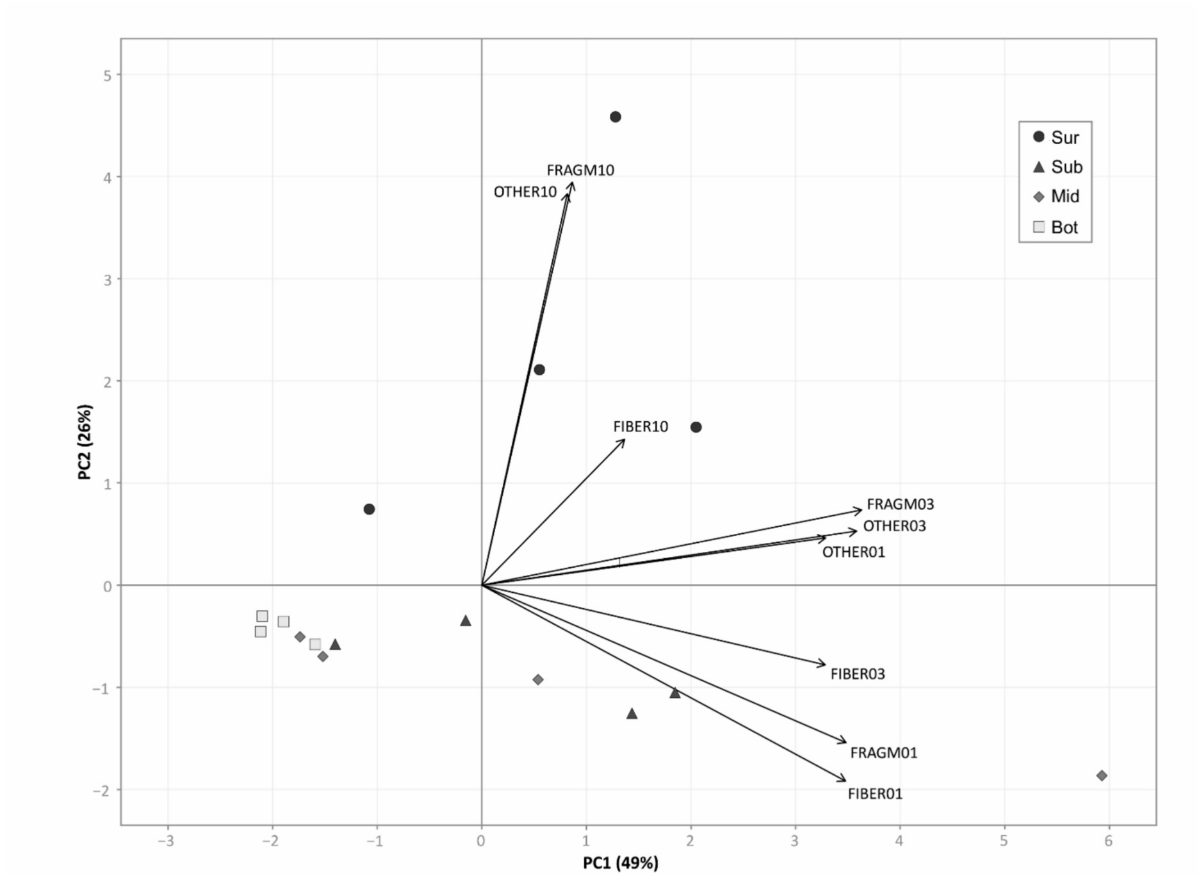

Fig. 4. Ordination of the samples (4 sampling dates x 4 layers) by PCA analysis (see ESM Table S6). Sur: surface layer (0–0.2 m); Sub: subsurface layer (0–10 m); Mid: middle layer (10–20 m); Bot: bottom layer (20–80 m); FRAGM10: Fragment 5–1 mm; FRAGM03: fragment: 1–0.3 mm; FRAGM01: fragment 0.3–0.1 mm; FIBER10: fiber 5–1 mm; FIBER03: fiber 1–0.3 mm; FIBER01: fiber 0.3–0.1 mm; OTHER10: other shapes 5–1 mm; OTHER03: other shapes 1–0.3 mm; OTHER01: other shapes 0.3–0.1 mm.

showed a constantly high concentration of MPs in the subsurface and middle layers (i.e., epilimnion and metalimnion) throughout the prolonged stratification period (i.e., April/spring, July/summer, and September/autumn sampling) strongly supports this hypothesis. Similarly, Tikhonova et al. (2024) observed a sharp decrease in MP concentration below the thermocline (depth: 5–15 m) in Lake Ladoga, Russia. Furthermore, Elagami et al. (2023) reported that water column instability during lake turnovers results in increased vertical transport of MPs due to water convection movements. This is in accordance with the results observed in Lake Lugano during water column mixing (i.e., January/winter sampling), when the middle and bottom layers exhibited the highest concentrations of MP, compared to the values observed at the same depths during the stratification. These results suggest that, regardless of their surface area, in deep lakes where climate change is expected to strengthen thermal stratification and weaken vertical mixing (Lepori and Roberts; 2015; Woolway et al., 2021), MPs could be retained above the thermocline for extended periods. This could result in higher concentration in the upper layers, although microplastics are flushed out of the lake by river outflow. However, more research is needed to allow reliable predictions about the effects of seasonal hydrodynamic changes on MP settling velocity and residence time in lakes.

Density gradients are probably not the only factor involved in determining vertical MP distribution patterns. According to a second hypothesis, MP vertical distribution and settling in the water column is mainly influenced by the physical and chemical properties of MP particles (Elagami et al., 2022; Khatmullina and Chubarenko, 2021; Nguyen et al., 2020). Specifically, particle size and shape are thought to be the most relevant parameter determining MP residence time, while polymer type seems to contribute to a lesser extent (Elagami et al, 2022). Positively and neutrally buoyant MPs with low surface-to-volume ratio, such as fragments found in the subsurface and middle layers, could be easily entrained by wind-induced surface water turbulence (Elagami et al., 2022; Kukulka et al., 2012; Reisser et al., 2015), resulting in a relatively homogeneous concentration of these particles in the top lake layers (i.e., above the thermocline). Conversely, negatively and neutrally buoyant particles of small size, such as fibers, could sink more easily into the hypolimnion (Khatmullina and Chubarenko, 2021). The pattern predicted by this hypothesis closely matches the results of the present study where larger fragments were mostly found in the upper layers of the water column, whereas smaller fibers were more evenly distributed and occurred at greater depths. Moreover, MP distribution in Lake Lugano echoes other findings from both marine (Chen et al., 2024; Reisser et al., 2015) and freshwater (Lenaker et al., 2019; Tamminga et al., 2020; Tikhonova et al., 2024) ecosystems, which found that the concentration of fragments tends to decrease with depth, while the proportion of fibers tends to increase. However, contrary to this explanation, laboratory experiments have shown that, within a single polymer type, larger MPs have faster settling velocity than smaller ones (Elagami et al., 2022) and therefore may be expected to sink faster into the deeper layer. The seemingly contradictory evidence between the patterns observed in lakes and experimental results warrants further investigation. A comprehensive study integrating particle shape and size distribution, polymer identification, and detailed analysis of density changes induced by biofouling and degradation (Alimi et al., 2022; Elagami et al., 2022; Munkhbat et al., 2024) in water ecosystems would provide a clearer understanding of the mechanisms controlling the vertical distribution of

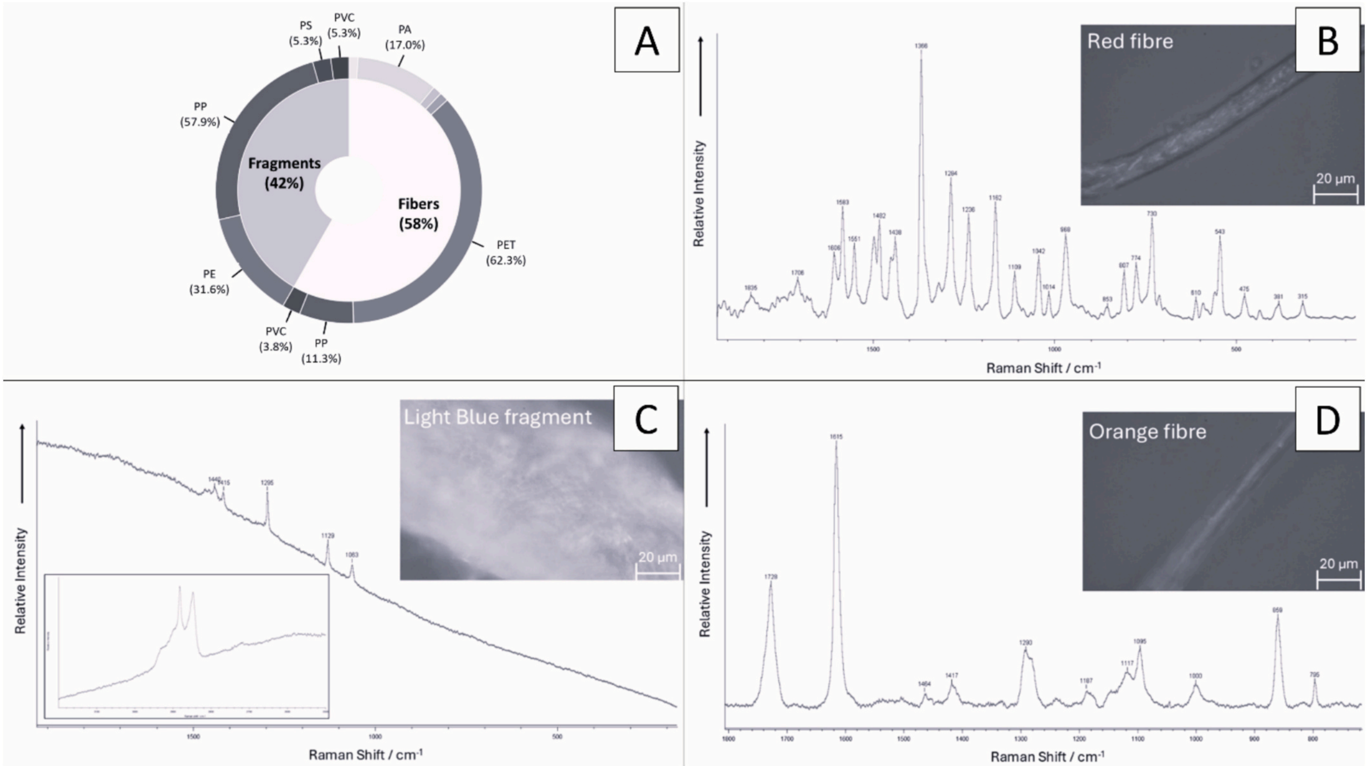

Fig. 5. (A) Total percentage of plastic polymer (all layers combined) identified by micro-Raman spectroscopy performed on a subset (10 %) of potential MPs, represented as function of dominant morphologies (i.e., fragments and fibers). PA: polyamide; PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene; PS: polystyrene; PVC: polyvinylchloride. (B) Microscope image (50x magnification) and Raman spectrum of red fiber classified as 'anthropogenic' due to the presence of bands ascribed to synthetic organic pigment. (C) Microscope image (50x magnification) and Raman spectrum of a light blue fragment consistent with PE; the box reports characteristic spectroscopic bands pattern at about 3000 cm^{-1} (Anger et al., 2018; Nava et al., 2021). (D) Microscope image (50x magnification) and Raman spectrum of an orange fiber identified as PET. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MPs in stratified aquatic environments.

MP concentration and composition in Lake Lugano varied not only vertically between layers but also temporally between sampling seasons, although mainly in the upper layers above the 20–80 m layer. Winter and spring samples had greater concentration than summer and autumn samples. This temporal difference suggests that the lake experienced pulsed inputs of MPs, possibly due to rainfall events. Intense rain would cause urban runoff and sewer overflows, with subsequent spikes in MP concentration in the near-surface waters, which are the direct recipients of this polluting load (Xia et al., 2020). A similar temporal trend has been observed in the surface water of Lake Maggiore, Italy, another lake with an urbanized basin, where MP concentrations were found to be significantly higher in winter compared to late summer and early autumn samples (Binelli et al., 2024). While most MPs are resuspended in the turbulent upper mixed layer because thermal stratification slows their vertical transport (Elagami et al., 2023), those that pass the thermocline steadily sink due to the calm, low-turbulence conditions (Khatmullina and Chubarenko, 2021), characteristics of the hypolimnion. As a result, the effect of pulsed inputs on hypolimnetic MP concentration and composition could be relatively dampened compared to the upper layers, as most MPs entering the hypolimnion ultimately accumulate in deep sediments (Lenaker et al., 2020; Quintana et al., 2025). More data on seasonal variation and vertical sedimentation dynamics are needed to identify the mechanisms behind these temporal changes.

The shape and polymer composition of the MPs observed in the lake provide insights into the pollution sources (Tian et al., 2023). Fragments generally originate from macroplastics (e.g., bottles, household items, packages) dispersed in the environment, whereas fibers originate predominantly from the wear and tear of synthetic textiles during washing, although fishing and boating equipment represents an additional source (Galgani et al., 2010). Because fragments and fibers were dominant in this study, the results support the idea that MP pollution in Lake Lugano was largely caused by secondary MPs (Boucher et al., 2019; Egger et al., 2020; Galgani et al., 2010). In addition, consistent with other studies, PP, PE and PET were the most abundant plastic polymers in Lake Lugano across water layers (Binelli et al., 2024; Lenaker et al., 2019; Mason et al., 2016; Tamminga et al., 2020). This result is not surprising because, according to global plastic production data, PP, PE, and PET are used in a wide range of applications such as packaging, clothing, buildings, and agriculture (PlasticsEurope, 2025). Therefore, the main sources of MP pollution to the lake probably included littering in nearshore environments (PP and PE fragments) and domestic waste (PET fibers) probably entering the lake through sewer overflows.

5. Conclusions

The results of this study emphasize the potential role of deep stratified lakes as hotspots of MP pollution, collecting high MP concentrations along the global plastic pathway. For example, the average MP concentrations found in the subsurface and middle layers in the present study in Lake Lugano were in the region of 30 MP m^{-3}, whereas MP concentrations in the North Atlantic Ocean do not usually exceed 4.98 MP m^{-3} (Mutuku et al., 2024). Given this, it is strongly recommended to permanently integrate water column sampling into MP monitoring programs, especially in deep lakes where subsurface waters account for most of the lake volume. Furthermore, in a changing climate scenario, the role of prolonged thermal stratification in determining transport dynamics of MPs should be clarified. From an ecological point of view, the constant presence of high MP concentrations in the upper 20 m, which encompass the euphotic zone where most organisms live, is concerning, because it increases the risk of potentially harmful interactions between MPs and lake organisms, for example through ingestion or exposure to harmful leachates (Bellasi et al., 2020; Nava and Leoni, 2021; Nava et al., 2024). Ignoring the persistence of this vertical distribution could cause misjudgments of the ecological risks linked to this contaminant. Additional research carried out in other large and highly urbanized lakes is urgently needed to raise awareness of the vulnerability of these ecosystems to plastic pollution, and to set new management goals to address MP environmental impacts.

CRediT authorship contribution statement

Federica Rotta: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Camilla Capelli:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Agnese Marchini:** Writing – review & editing, Conceptualization. **Barbara Leoni:** Writing – review & editing, Conceptualization. **Giusto Lo Bue:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maya Musa:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maria Pia Riccardi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Fabio Lepori:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization.

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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2025.102740.

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