ORIGINAL ARTICLE

The effects of microplastics size and type on entrapment by freshwater macrophytes under vertical and lateral deposition

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ABSTRACT

Marine and freshwater macrophytes are known to filter off microplastics from the water column; however, the effects of microplastic size and type on their retention by different macrophytes species have yet to be investigated. Here we tested the retention of different sizes and types of microplastics, introduced under two flow regimes (vertical deposition in still water and lateral deposition in a unidirectional current), by two submerged macrophyte species, *Hydrilla verticillata* and *Mayaca fluviatilis*, using ex-situ experiments. Microplastics entrapment efficiency in macrophytes was determined by calibrating the dry weight (DW) of the plant and analysing the characteristics of each macrophyte species via a comparison of their leaf outer-edge perimeter-to-area ratio (P:A). The entrapment efficiency of macrophytes was higher in still water than in moving water. *Hydrilla verticillata* had greater average leaf surface area and retained the most polyethylene terephthalate (PET) of size 800-1000 µm under both lateral deposition in moving water (1.75±0.11 g) and vertical deposition in still water (2.85±0.24 g). Conversely, *M. fluviatilis* had greater P:A, surface area, and high surface cellulose and retained the most PET of size 600-800 µm in both moving (0.73±0.07 g) and still (0.92±0.159 g) water. Our findings highlight the influence of microplastic size and material type, macrophyte morphology and surface area, and water flow conditions in determining the entrapment rate of microplastics by macrophytes.

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INTRODUCTION

Microplastics (MPs) are small-sized (<5 mm) plastic particles that can be fragmented from plastic items by mechanical stresses, such as abrasion, as well as various environmental processes, including weathering and hydrodynamic forces (Espinosa *et al*., 2016; Law and Thompson, 2014; Singh and Sharma, 2008). MP materials are introduced predominantly into aquatic ecosystems through natural mechanisms such as surface runoff (Browne, 2015; Murphy *et al*., 2017) and anthropogenic activities such as discharge from sewage treatment plants and release of agricultural waste.

Once introduced into freshwater ecosystems, MPs undergo various transformations and interactions that contribute to their environmental impact. Freshwater ecosystems such as oligotrophic lakes have high ultraviolet penetration that can lead to increased plastic fragmentation (Free *et al*., 2014). In water bodies, MPs can adsorb organic pollutants and heavy metals (Bakir *et al*., 2014; Thompson *et al*., 2008) while biofilms on the surface of MPs can adsorb nitrogen and phosphorus (Kong and Koelmans, 2019; Yang *et al*., 2020).

MPs can have many negative effects when ingested by an organism including toxic effects to respiratory, digestive, and nervous systems (Wright *et al*., 2013). MPs suspended within the water column and adhering to the surface of macrophytes not only affect plant photosynthesis but also pose a threat to the extended food web, including human beings, through their uptake by higher trophic levels (Saley *et al*., 2019; Feng *et al*., 2020). Studies have revealed an increased abundance of larger-sized MP particles in sub-millimeter size classes in freshwater bodies (Cózar *et al*., 2014; Lenz *et al*., 2016). The vertical distribution of MPs in freshwater bodies reflects their presence floating on or just below the surface, in the water column, and settling in the sediment (Lenaker *et al*., 2019; Qiu *et al*., 2015; Turra *et al*., 2014). MPs that accumulate at the water's surface (Waldschläger and Schüttrumpf, 2019) can be trapped by free-floating macrophytes (Mateos-Cárdenas *et al*., 2019). In contrast, submerged macrophytes such as *Vallisneria natans* (Lour.) H.Hara and *Ceratophyllum demersum* L. also play a crucial role in the capture and accumulation of MPs below the water's surface, where these particles can settle or be swept into the dense underwater plant structures (Wang *et al*. 2021; Wang *et al*. 2023). Emergent macrophytes such as *Iris pseudacorus* and *Nelumbo nucifera* intercept MPs at and above the water line, often capturing debris trapped among their stems and leaves (Esterhuizen and Kim, 2022; Huang *et al*., 2023; Rozman *et al*., 2023).

Macrophytes can impede water flow and trap sediments, reducing the chance of resuspension and maintaining water clarity (Gacia *et al*., 1999; Jeppesen *et al*., 2007). However, this characteristic may also facilitate MPs entrapment and deposition on and near macrophytes (Ng *et al*., 2022). The mechanisms of trapping of MPs by macrophytes can be via physical adhesion mediated by the morphological features of the plants, adsorption by the surface biofilms of macrophytes, and/or by cellulose walls of plant cells under the action of electrostatic forces (Feng *et al*., 2020; Kalčíková, 2020). There is, however, a limited number of studies on freshwater macrophytes trapping MPs, for example, Mateos-Cárdenas *et al*. (2019) showed that small-sized polyethylene (PE) MPs can strongly adsorb to all surfaces of *Lemna minor*. Another study examined nanoscale plastic bead attachment to a prototypical cellulose substrate and the interactions involving two prevalent algal species, *Chlorella* and *Scenedesmus*, elucidating the electrostatic force between MPs and cellulose components in living organisms (Bhattacharya *et al*., 2010). A study by Tan *et al*. (2023) revealed the physical trapping mechanism of MPs by macrophytes whereby MPs were immobilized through a process facilitated by the clamping action of the different leaf morphologies. Additionally, recent work has shown that micro- and nanoplastics negatively affect the growth, chlorophyll content, and microbial community of *Hydrilla verticillata* (Yu *et al*., 2022). These studies collectively highlight the range and importance of possible interactions between macrophytes and MPs, and their potential ecological consequences.

The interaction between MPs and macrophytes is governed by intricate environmental variables and the combinations of macrophyte species and microplastic types encountered within aquatic ecosystems (Bhattacharya *et al*., 2010; Goss *et al*., 2018; Kalčíková, 2020). Duckweed (subfamily Lemnoideae), particularly species within the genus *Lemna*, have been intensively studied among freshwater macrophytes. For example, Ceschin *et al*. (2023) revealed the dose and exposure time of MPs dependent factors on the absorption of 1-5 μm MPs on *Lemna minuta* in the laboratory environment. Rozman *et al*. (2023) investigated the adhesion of MPs on the root surface biofilm of free-floating macrophytes, *Lemna minor*. Under the effect of slow-moving water over a 7-day duration, the number of adhered MPs increased compared to the static regime. Gallitelli *et al*. (2023) further examined the adhesion of PE MPs on macrophytes structure under the condition of horizontal hydrological flow, *Myriophyllum spicatum* having a more branched and 3D-structure than *Potamogeton crispus*, acting as a "net" to trap more MPs. By testing different types of macrophytes, Tan *et al*. (2023) concluded that significant differences exist between three freshwater macrophytes' capability of retaining 800-1000 μm polyamide (PA) fragments. The architectural complexity described as mesh-like in *Cabomba caroliniana,* and leaves arranged in whorls in *Egeria densa* capture more MPs than the simple leave structure of *Hygrophila polysperma*. Although different factors were tested in these studies, gaps in understanding the multifaceted mechanisms of microplastic interactions with macrophytes persist, particularly when considering combined factors.

In this study, we investigated the factors influencing the deposition of MPs on two freshwater submerged macrophytes, *Hydrilla verticillata* and *Mayaca fluviatilis*, under contrasting regimes. Specifically, we asked how different flow-deposition regimes (vertical and lateral) and how different MP types (polyamide and polyethylene terephthalate) and sizes (smaller and larger) affect the deposition of MPs in these two macrophytes species. We constructed a simplified model of microplastic migration with water and buoyancy by setting up two experimental setups to simulate vertical mixing and lateral flow in freshwater bodies. A combination of macrophytes and MPs was replicated in the two experimental setups for vertical deposition in still water and lateral deposition in unidirectional flow experiments.

METHODS

Macrophyte species

The submerged macrophytes *Hydrilla verticillata* and *Mayaca fluviatilis* (Fig. 1) are both common introduced species in Singapore (Sim *et al*., 2022). They are adapted to various water conditions and possess rapid reproductive capabilities (Yakandawala and Dissanayake, 2010). They are known to become dominant in many freshwater environments (Langeland, 1996; Murphy, 1988), forming dense semi-floating mats with intertwined vegetation (Gordon-Bradley *et al*., 2014; Madigan *et al*., 1975). *Hydrilla verticillata* exhibits a highly branched structure, with narrowly serrate leaves arranged in whorls (Cook and Lüönd, 1982). *Mayaca fluviatilis* possesses simple needle-shaped leaves arranged spirally (Su *et al*., 2020). In Singapore, these non-native macrophytes are found in Lower Seletar Reservoir (*H. verticillata*) and MacRitchie Reservoir (*M. fluviatilis*), and represent the only two species that form substantial populations locally (Sim *et al*., 2022).

For the experiments, both species were purchased from a local aquarium vendor, thoroughly rinsed with deionized water to remove surface biofilms and placed in a 5-L tank at room temperature (~26°C). An aquarium LED light (RS-H90, 7W) was used as a light source for 12 h d^{-1} and aeration was provided constantly *via* an aquarium pump. Standardization for the macrophytes was achieved by pruning the branches to a 10-cm length from the apex, discarding surplus branches. Ten treated branches were prepared for each experiment.

Microplastics

Four categories of MPs were used for the experiments: polyamide 6 (PA) of sizes 600-800 μm and 800-1000 μm, and polyethylene terephthalate (PET) of similar size ranges (Fig. 2 a,b). PA and PET MPs are widely used in commodities and account for the largest portion of global synthetic fibres (Textile Exchange, 2022). PA (nylon) is the main component of textiles, and PET is mainly used as packaging material (Issac and Kandasubramanian, 2021). The two size ranges (600-800 μm, 800-1000 μm) of MPs tested in this study are highly susceptible to accumulation in organisms (Wang F *et al*., 2021). All MPs were purchased from an industrial supplier and sorted through a sieve shaker (ELF, 2000) for 5 min to ensure consistent size categorization. MPs were rinsed with deionized water and dried overnight in an oven at 70 °C to ensure accurate initial weight and to remove potential contaminants.

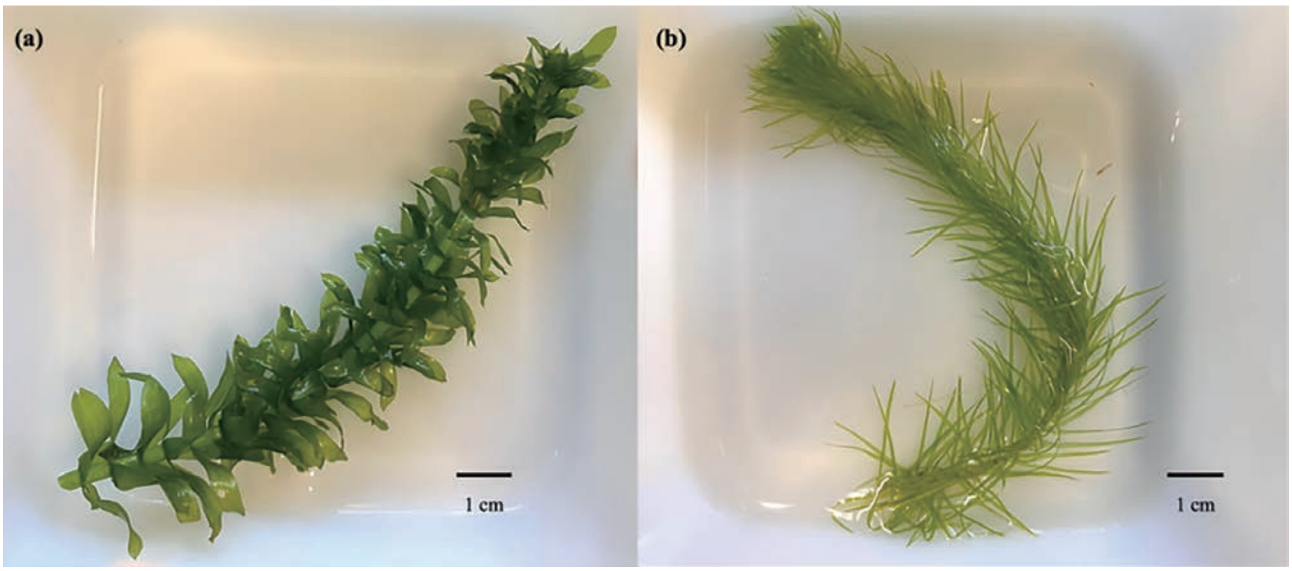

Fig. 1. Non-native macrophyte species in Singapore Reservoir. a) *Hydrilla verticillata* with whorled leaves. b) *Mayaca fluviatilis* with spirally arranged leaves.

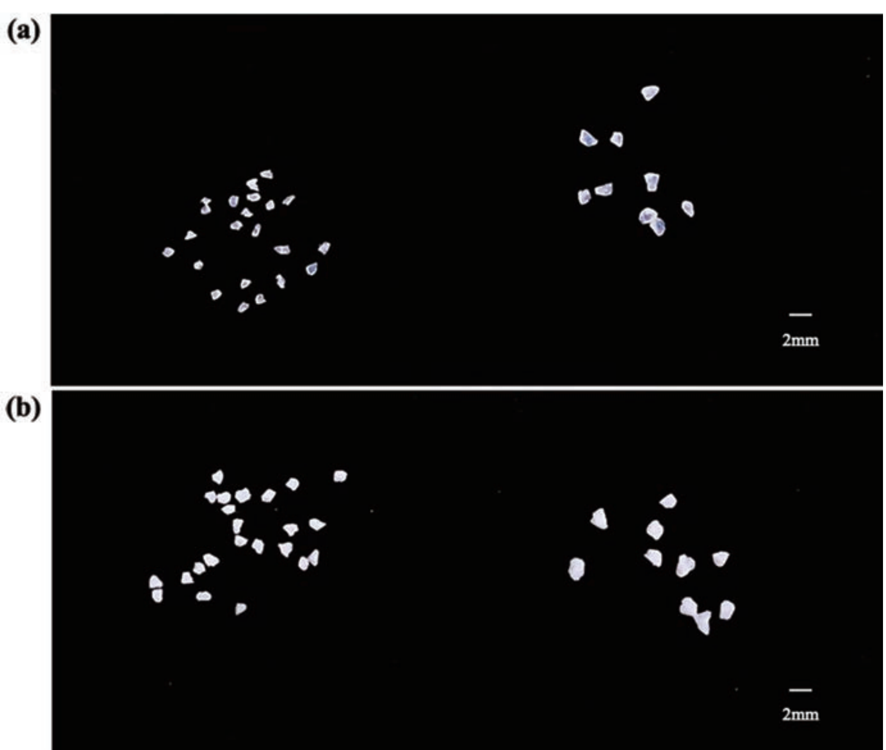

Fig. 2. Microplastics groups used in the experiment. a) Translucent 600-800 μm (left) and 800-1000 μm (right) polyamide 6. b) Solid white 600-800 μm (left) and 800-1000 μm (right) polyethylene terephthalate.

Experimental design: vertical deposition in still water

The vertical deposition model was designed to replicate the initial introduction and downwelling of MPs in water bodies. The experimental set-up was adapted from Tan *et al*. (2023) where an inverted plastic cylinder was securely connected to a 250 mL conical flask *via* rubber tubing. The apparatus was supported by a modified plastic bottle that allowed a sealing clip to be clamped to the rubber tubing at the end of each experimental run (Fig. 3).

For each trial, the experimental setup was filled with water to a fixed point and pre-prepared clusters of 10 plants each were randomly placed in the setup and allowed to disperse (Fig. 3a). Separately, 5 g of MPs and 200 mL of deionized water were mixed in a beaker, shaken until the MPs were scattered evenly in the water and then quickly poured into the funnel. To ensure that there was no residual MPs in the beaker, 100 mL of deionized water was added to the beaker and used to flush the funnel again. A plastic sheet of a similar diameter to the plastic cylinder was used to disrupt water surface tension. This device forced floating MPs below the water surface, ensuring their submersion (Fig. 3b).

After waiting for 20 s, the sealing clip was clamped on the valve tubing (Fig. 3c). The MPs that were not entrapped on the macrophytes, i.e., those collected in the conical flask, were kept for counting. The experiment was repeated four times (n=4) for each treatment group of MPs and each species of macrophyte. The control was the same procedure but without any macrophytes.

After each trial, most of the water in the conical flask (~230 mL) was removed using a syringe. The flask, plus the remaining water and all the MPs, was then dried in an oven at 70°C for 24 h (*Fig. S1*). The conical flask was also dried and weighed with precision to four decimal places before the start of the experiment. The mass of the MPs retained by the macrophytes was calculated using the following equation:

$$M_{MP_1} = M_{MP_0} - M_{MP_{cf}} \qquad \text{(Eq. 1)}$$

Where $M_{MP_{cf}}$ represents the mass of dried MPs in the conical flask after the experiment, and M_{MP_0} and M_{MP_1} represent the mass of initial MPs and the mass of MPs retained in the macrophytes, respectively.

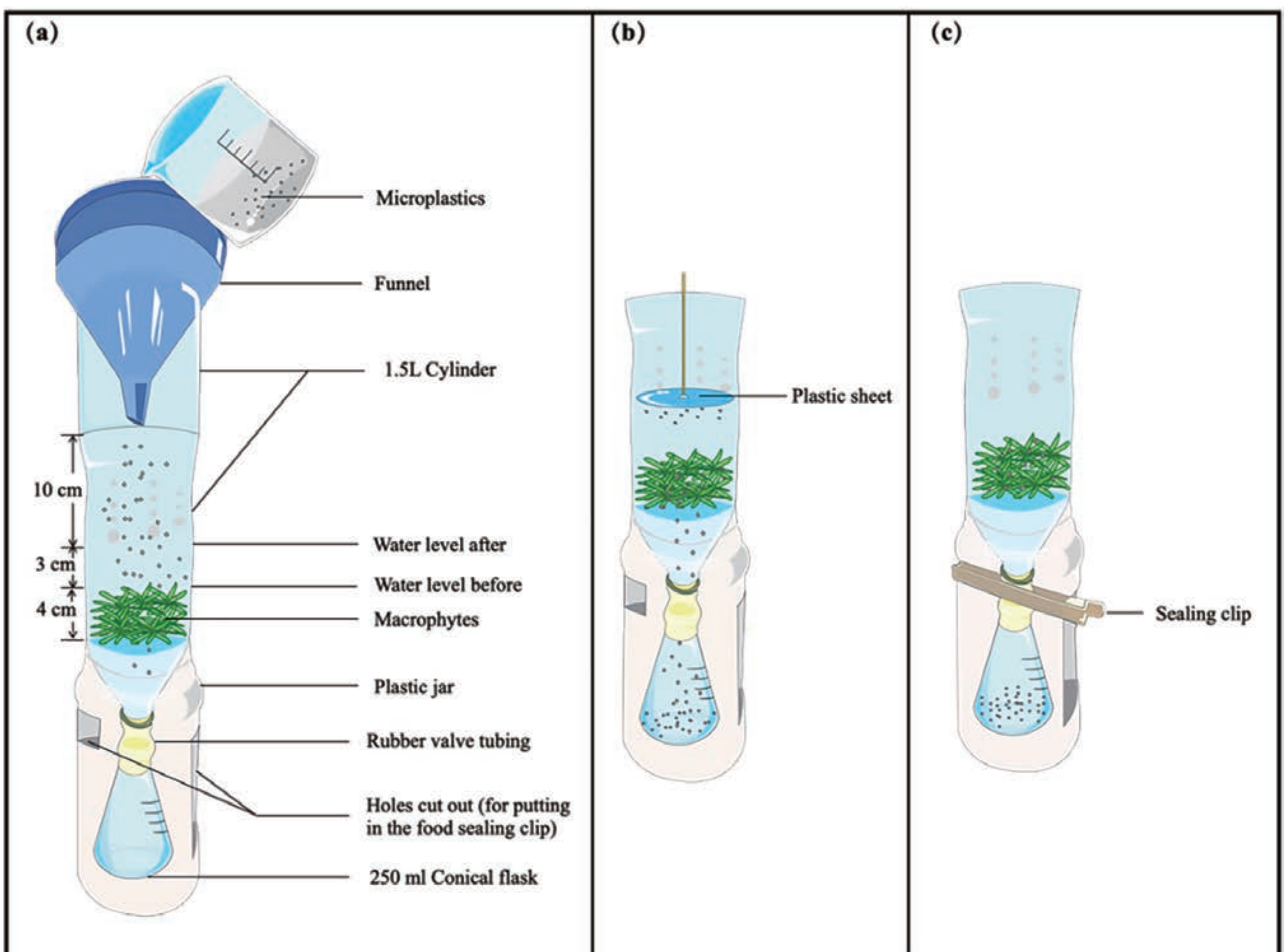

Fig. 3. Experimental setup for vertical drop. a) The initial height of the water surface was 4 cm above the funnel of the inverted 1.5 L bottle, and the mixture of microplastics and deionized water was poured from the funnel. b) When the microplastics were dropped, the small amount of microplastics floating on the water surface was gently tapped into the water using a chopstick with plastic sheet stuck to it. c) All microplastics were dropped and then waited for 20 s before clamping the food sealing clip at the balloon.

Experimental design: lateral deposition in a unidirectional current

The experimental setup for the horizontal flow model is shown in Fig. 4. The experiments were performed using a flume (Fig. 4a). The flume was filled with water at a constant depth of 17 cm and the propeller generated a continuous flow of water to simulate horizontal hydrodynamics in the freshwater bodies (Fig. 4a) (Eq. 2). The input device for MPs consisted of a funnel attached above water to one end of a glass tube with the other end submerged and secured to the sink by a wooden bar (Fig. 4a). Ten branches of the treated 10-cm macrophytes were gathered into a bundle, and secured loosely by an elastic band (Fig. 4b). A weighted iron ring was tightened in the elastic band to equilibrium buoyancy thereby enabling the macrophytes bundle to be suspended in the water (Fig. 4b). To control the depth of the MP release in water, the input device level was kept fixed, and the depth of the clusters in the water was controlled by adjusting the length of the line holding the plant clusters in place to accommodate the trajectory of the different densities and types of MPs in the water used in the experiments. In the direction of water flow, the cross-section behind the macrophytes were provided with a box-shaped filter mesh (250 μm pore size) MPs collection bag (dimensions 20 cm × 24 cm × 15 cm) (Fig. 4c, *Fig. S2a*). The open side of this collection bag facing the macrophytes were tightly attached to the bottom of the flume with a weighted iron sheet and secured to the inside wall of the sink on both sides using clear tape (Fig. 4c).

In each trial, the propeller was adjusted and flow rate measured with a flow meter to stabilize at 6 km h^{-1} for 5 min. The flow rate was calculated by:

$$Q = v \times A = 0.08\ \mathrm{m^3\,h^{-1}} \qquad \text{(Eq. 2)}$$

where A is the cross-sectional area of the flume channel, as calculated to be 0.048 m^2, and v is the speed of the fluid, measured at 1.67 m s^{-1} (equivalent to 6 km h^{-1}).

To introduce the MPs into the flume, 5 g of MPs and 200 ml of deionized water were mixed in a beaker, the same as the vertical deposition model. The MPs-water mixture was then added to the flume through the funnel. To ensure no residual MPs were left behind, the funnel, glass tube, and inner walls of

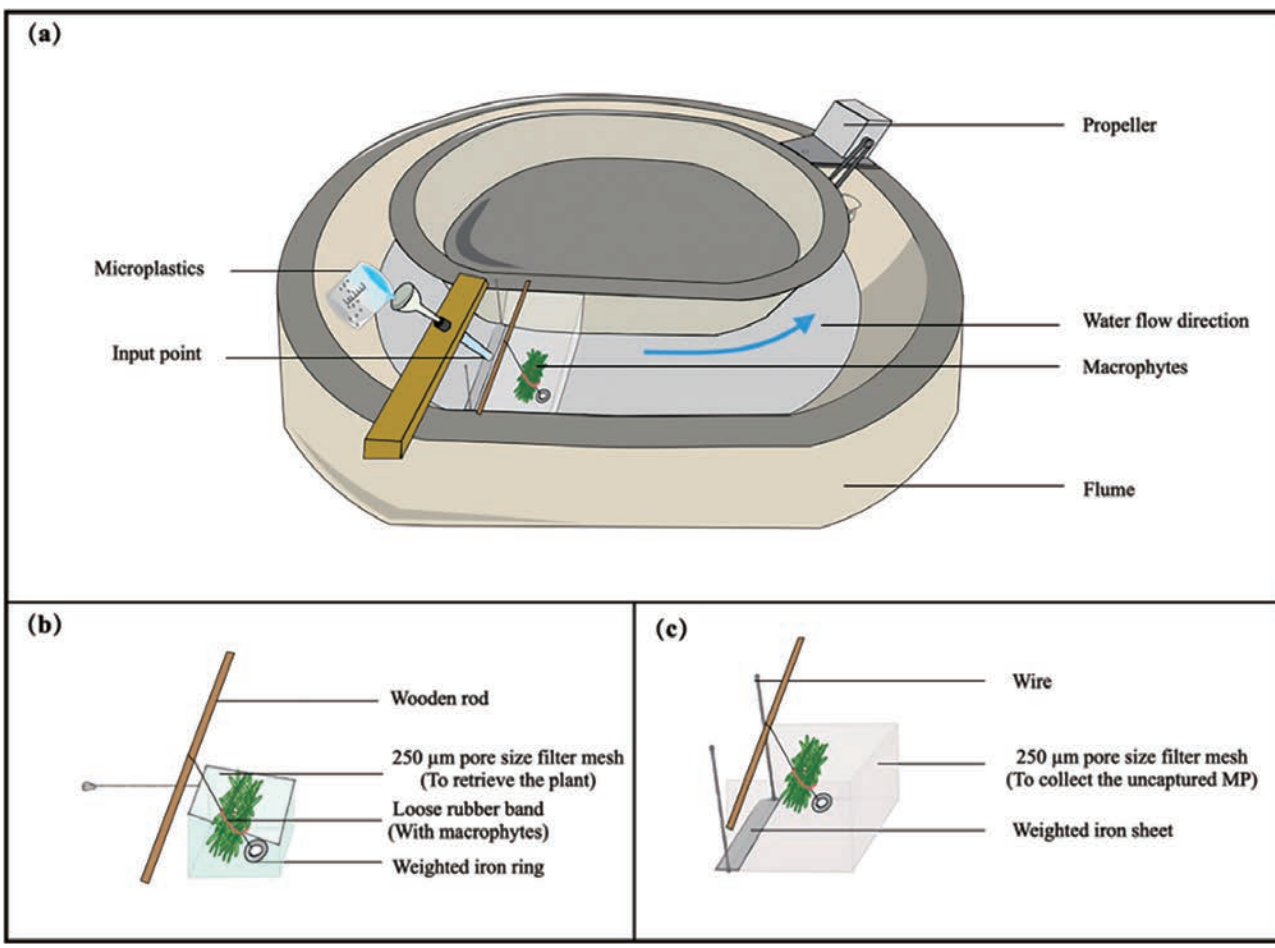

Fig. 4. a) The horizontal drop setup showing the flume that is used to control the flow rate of 6 km h^{-1} and the plants being held in place floating in a rubber band; microplastics were dropped in from a glass tube connected with a funnel of 5 cm diameter. b) The 250 μm filter mesh knitted into a bag-shaped was used to retrieve the rubber band with plant clusters after the experiment to gain trapping weight of microplastics by the macrophytes. c) A 250 μm pore size box-shaped with two open filer mesh sides was used to collect uncaptured microplastics.

the beaker were thoroughly flushed. A period of 20 s was allowed for all MPs to be either entrapped by the macrophytes or the filter mesh.

At the end of each trial, a separate 250-µm pore size filter mesh was used as a net to retrieve the plant cluster (Fig. 4b) and the elastic band was carefully removed. The macrophytes and MPs were dried in an oven at 70°C for 24 h. Subsequently, the macrophytes were removed and the MPs entrapped on the macrophytes were then weighed, yielding the entrapped mass (). Standardization of macrophyte biomass followed the approach used in the vertical deposition experiment.

Measuring the entrapment efficiency of MPs after vertical deposition in still water and lateral deposition in a unidirectional current

The macrophytes were carefully removed from the setup and soaked in a beaker containing water for 10 min before rinsing thoroughly with deionized water to remove the MPs after the respective experiments. The treated plant clusters were dried in the oven at 70°C for 24 h and weighed to obtain the dry weight (DW) (*Fig. S2b*). In the horizontal experiment, the removed plant clusters from the dried filter mesh bags were also weighed to obtain the DW. The retention capacity of the macrophytes was calibrated by calculating the entrapped mass per dry weight ratio of each macrophyte species:

$$R' = \frac{DW_{Ave}}{DW} \times M_{MP_1} \qquad \text{(Eq. 3)}$$

Where R represents the retained mass per dry weight ratio of each macrophyte species for 5 g of MPs. M_{MP1} represents the mass of MPs entrapped in the macrophytes. is the average dry weight of each macrophytes species that were used in all the experiments while DW is the dry weight of macrophytes in each experiment run.

Quality assurance: negative control

For the vertical drop experiment, a negative control without plants was set up to test the experimental setup on the resultant MPs remaining in the conical flask. The experiment was repeated for each size and type of MPs (n=4) and all MP particles trapped in other parts of the experimental setup that did not fall into the conical flask were counted (Koelmans *et al*., 2019).

Macrophyte morphology

To calculate the surface area of the leaves and the complexity of plant morphology, four of the 10 plant branches used in each group of experiments were selected randomly, and a single leaf from the middle of each branch (at 5 cm of the branch) were selected randomly and digitally scanned after being fixed in position. Subsequently, each of these four plants was also placed in a small white dish filled with water and photographed vertically to replicate the morphology of the macrophyte in water (Ng *et al*., 2022). The surface area of a single leaf and the area and perimeter of the outer edge of each naturally placed plant were measured using ImageJ (v. 1.53 J8; Fig. 5; Warman *et al*., 2011). The macrophyte complexity for each species was calculated by using the perimeter of the plant outer edge perimeter-to-area ratio (P:A) (Levi *et al*., 2015). The obtained results were then averaged separately.

Statistical analysis

All data were checked using Shapiro-Wilk's test for normality and Levene's test for homogeneity of variances prior to the following statistical analysis. Independent Samples t-test was used to compare the morphological characteristics represented by leaf area and the plant outer edge perimeter-to-area ratio (P:A) of the two macrophytes species, *Hydrilla verticillata,* and *Mayaca fluviatilis*. To correct for potential Type I errors resulting from multiple comparisons, we applied the Bonferroni correction method using the "effsize" package (R-project, 2016). Analysis of Variance (ANOVA) followed by post hoc pairwise comparisons with the Benjamini-Hochberg (BH) test was used to determine whether there were any differences in the MPs retention ability of different macrophyte species and the different groups (i.e. material type and size) of MPs within each macrophytes species. For data sets that deviated from normality, Kruskal-Wallis test was applied to evaluate statistical disparities among groups. The statistical analysis above was repeated for both vertical deposition in still water and lateral deposition in a unidirectional current experiments and was conducted using R (v. 4.2.1).

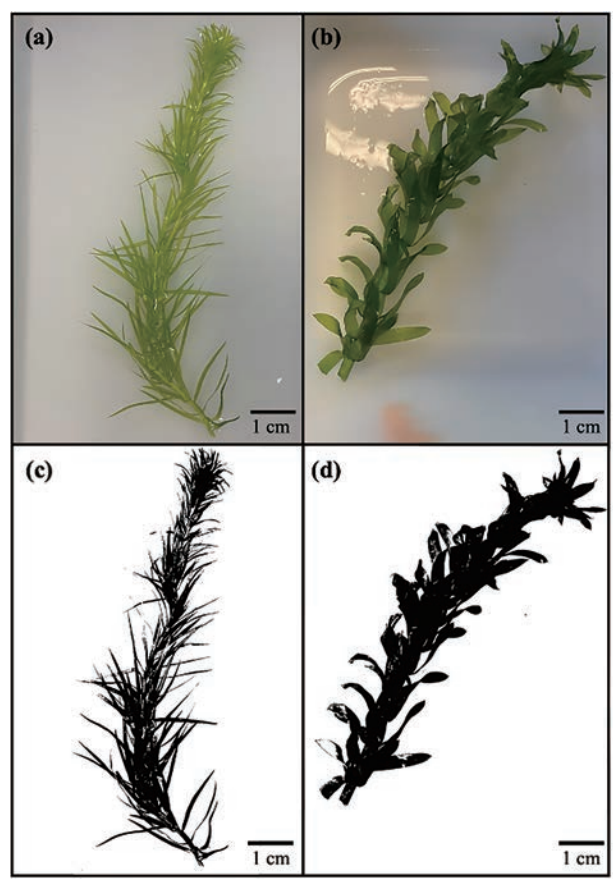

Fig. 5. The outer edge of macrophytes species *Mayaca fluviatilis*, (a) and *Hydrilla verticillata* (b) were transferred to ImageJ (c) and (d), respectively, to measure the perimeter of outer edge.

RESULTS

Entrapment ability of *Mayaca fluviatilis* and *Hydrilla verticillata* for microplastics

The results for both horizontal and vertical experiments revealed a significant difference (p<0.001) in microplastic trapping between the treatment groups containing macrophytes and the control groups without macrophytes (Fig. 6 a,b). For the vertical experiment, microplastic retention was significantly higher for *Hydrilla verticillata* compared to *Mayaca fluviatilis* across all tested microplastic types (p<0.05, *Tab. S1*). No significant difference was found between the two species in the horizontal experiment (p=0.29, *Tab. S1*).

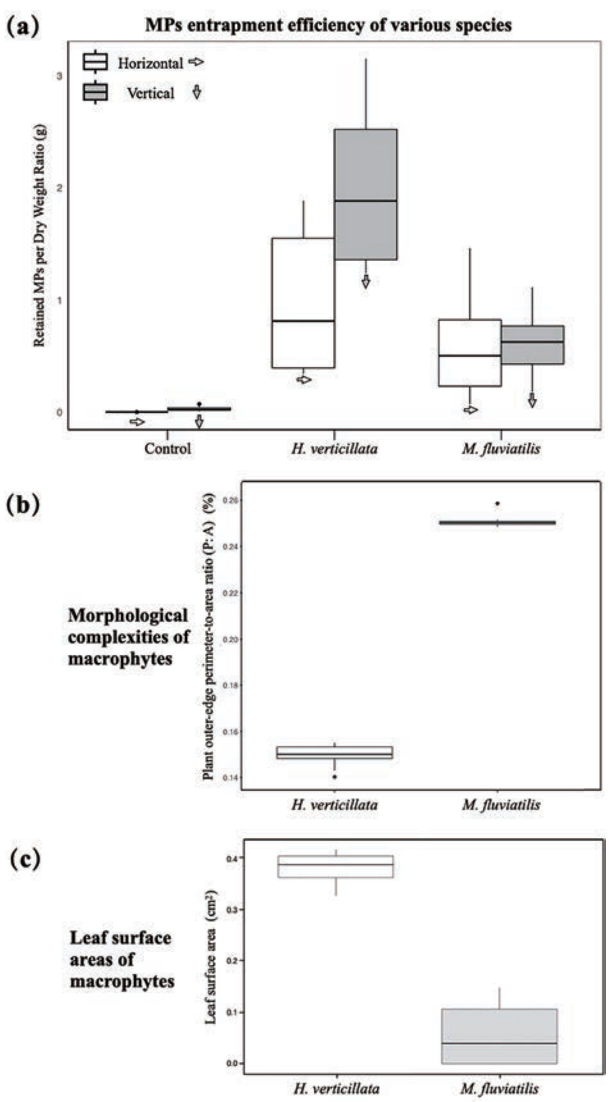

Fig. 6. a) Calibrated mass of microplastics trapped by the macrophytes species *Hydrilla verticillata* and *Mayaca fluviatilis* of horizontal experiment (white boxes) and vertical experiment (grey boxes) of all microplastics groups. The errors represent for the experimental methods of microplastics input directions. b) Morphological complexities of macrophytes compared by the plant outer-edge perimeter-to-ratio (P:A). c) Leaf surface areas of randomly selected leaves macrophytes.

Microplastic entrapment between *Hydrilla verticillata* and *Mayaca fluviatilis* was significantly different for all four microplastic groups (types and size). *Post-hoc* pairwise comparisons (*Tab. S2*) revealed that *Hydrilla verticillata* exhibited a significantly higher entrapment ability of smaller-sized MPs (600-800 µm) for both PA and PET when compared to *Mayaca fluviatilis* (p<0.001, Fig. 7). This pattern of higher retention by *Hydrilla verticillata* was consistent with larger MPs (800-1000 µm), where it also retained both PA and PET types more effectively than *Mayaca fluviatilis* (p<0.001, Fig. 7). In the vertical deposition (still water) experiment, *Hydrilla verticillata* trapped more MPs than *Mayaca fluviatilis* (ANOVA, p<0.001, *Tab. S3*), especially for smaller PET MPs. However, there was no significant difference in retention between the two larger PA and PET microplastic types for *Mayaca fluviatilis* (p=0.139, Fig. 7). *Hydrilla verticillata* had a similar retention rate for different-sized PA MPs (p=0.615, Fig. 7), but significantly lower retention of smaller PET MPs as compared to the larger sized PET.

Morphological characteristics of *Hydrilla verticillata* and *Mayaca fluviatilis*

The plant outer edge perimeter-to-area ratio (P:A) (independent Samples t-test, p<0.001; Fig. 6b) of both macrophytes species were significantly different. *Hydrilla verticillata* (mean ±SD: 0.38±0.03 cm^2) had a larger single-leaf surface area (independent Samples t-test, p<0.001; Fig. 6c) than *Mayaca fluviatilis* (mean ±SD: 0.12±0.05 cm^2) while *Mayaca fluviatilis* (mean ±SD: 0.25±0.00%) had higher plant outer edge perimeter-to-area ratio (P:A) than *Hydrilla verticillata* (mean ±SD: 0.15±0.01%).

DISCUSSION

In experiments with vertical deposition in still water, macrophytes were placed in a stationary water column. MPs, once breaking surface tension, displayed settling behaviors influenced by their density and morphology. These physical properties dictate the settling rates of MPs in such environments, as discussed by Wang Z *et al*. (2021), who explored the relationship between particle characteristics and their behavior in water.

In static environments, MPs settle based on these inherent physical properties. Aquatic plants in these settings act as natural filters, capturing MPs through physical interception and surface adsorption. This interaction is influenced by the plants' morphological and chemical properties, enhancing their potential to retain MPs. Rolland *et al*. (2015), demonstrated that macrophytes can alter flow conditions, affecting sediment and particle deposition dynamics. Similarly, in our lateral deposition experiments under unidirectional flow, the presence of plants not only filtered MPs but also introduced hydraulic resistance that moderated the flow. According to Le Bouteiller and Venditti (2015) and Berger and Wells (2008), macrophytes influence flow characteristics, increasing hydraulic resistance and modifying shear stresses in their surroundings.

In addition, structurally complex plants have a profound impact on MPs transport and deposition. By altering water flow velocity and patterns, these plants create zones of reduced flow and increased turbulence (Uzun *et a*l., 2022). In our study, the intro-

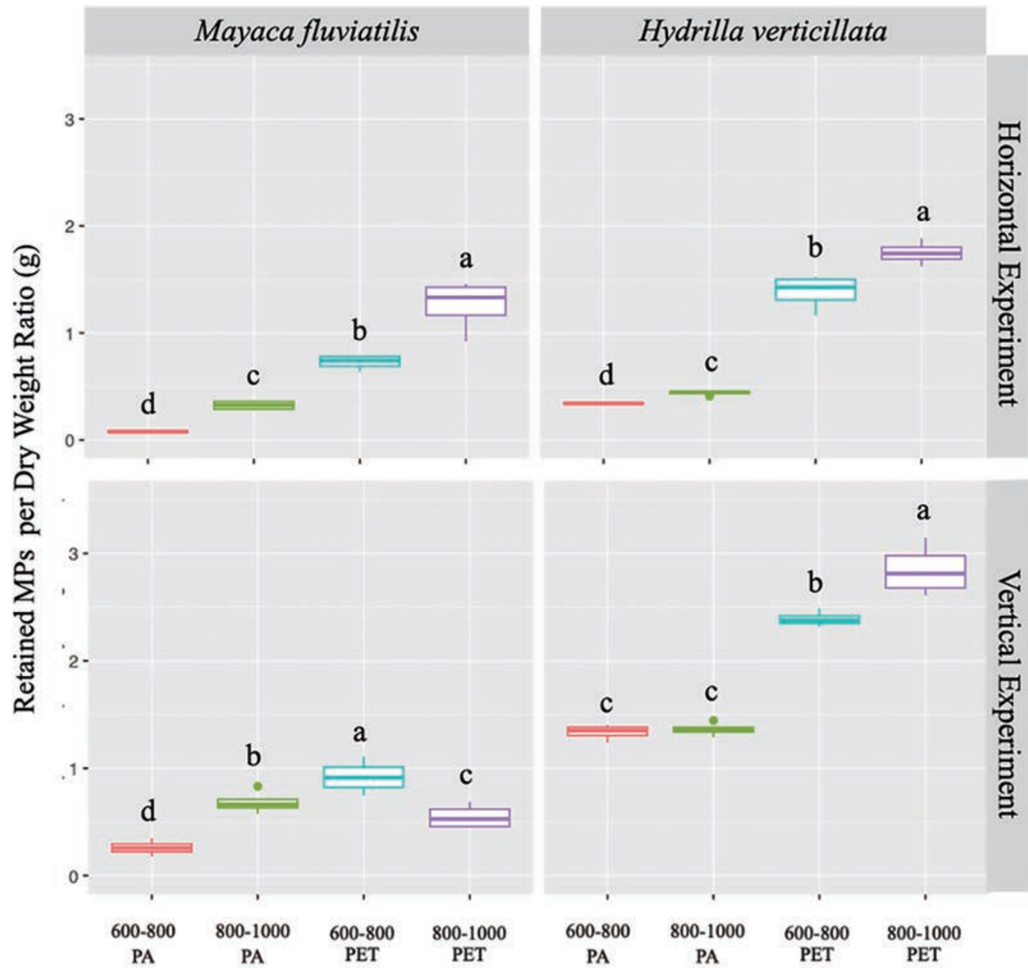

Fig. 7. Boxplot of different treatments for significant differences in retention between different groups of microplastics within macrophytes species in horizontal and vertical experiment. The letters a-d, assigned to plots, denote statistically significant differences in mean retention rates, where a indicates the highest and d the lowest mean, with no shared letters between groups of significantly different means.

duced concept of the plant outer-edge perimeter-to-ratio (P:A) also brought the insight of the larger complexity of the macrophytes which denotes the higher possibility of attaching smaller sized and low-density MPs.

The retention rates of both macrophytes towards P:A and PET particles varied, whereas *Hydrilla verticillata* retained less P:A than PET in both vertical and horizontal experiments. This could be potentially attributed to the hydrophilic nature of P:A which reduces the electrostatic forces after forming hydrogen bonds with hydrogen ions in water (Wang Z *et al*., 2021), consequently reducing the adhesion of MPs to the macrophyte. Our results demonstrated a size-dependent variation in the retention of MPs, with *Hydrilla verticillata* having higher retention of larger-sized MPs, as compared to *Mayaca fluviatilis*. This may be attributed to its larger leaf surface area and higher stiffness, facilitating the formation of a denser mat that aids in microplastic capture (Frantz *et al*., 2015; Ng *et al*., 2022). *Mayaca fluviatilis* despite having a smaller leaf area and softer texture, showed an affinity towards adsorbing smaller, more hydrostatic PET particles possibly due to a higher concentration of surface cellulose in its cell wall (Little *et al*., 2018; Ng *et al*., 2022; Peller *et al*., 2021).

Our findings reveal the capacity of *Hydrilla verticillata* and *Mayaca fluviatilis* to trap MPs, highlighting a potential entry point for these particles into freshwater food webs. Macrophytes serve as resources for a wide range of organisms, possibly enabling MPs' ingestion and subsequent trophic transfer, as demonstrated by Setälä *et al*. (2014) and Canniff and Hoang (2018). Moreover, the ecological implications extend beyond the food web. MPs can adsorb toxic pollutants from the environment (Liu *et al*., 2023; Ashton *et al*., 2010; Rodrigues *et al*., 2019). Consequently, macrophytes may unintentionally become sinks for these substances, posing risks to herbivorous aquatic organisms. Furthermore, MPs' deposition into sediments may be enhanced by macrophytes, due to their known role in reducing water flow and promoting sediment particle settlement (Huang *et al*., 2020). This may increase the exposure of sediment-dwelling benthic organisms to MPs and associated pollutants (Teuten *et al*., 2007), while potentially transforming the sediment into a long-term storage sinks for MPs (Nizzetto *et al*., 2016; Schulz *et al*., 2003; Waldschläger and Schüttrumpf, 2019). Investigations of microplastic content in sediments directly beneath plants and in sediments surrounding plants have been conducted in the ocean (Egea *et al*., 2023; Ng *et al*., 2022). Our two *ex situ* experimental models also provide some ideas for *in situ* sampling studies, i.e., detecting the content of sediments below macrophytes as well as sediments behind macrophytes along the current direction in freshwater.

For this study, the MPs and macrophytes used in the experiment were biofilm free. However, biofilms are prevalent on the surfaces of both macrophytes and MPs in natural ecosystems. It has been demonstrated that these biofilms can considerably influence the retention mechanism (Suteja and Purwiyanto, 2022; Feng *et al*., 2020; Lin *et al*., 2021). The degree in which biofilms on both the microplastic and the macrophyte affects microplastic entrapment on macrophytes may prove to be a key area for future exploration. Another aspect that should be examined further is the effect of the shape of MPs on entrapment on macrophytes. Our study, which utilized approximately spherical shaped MPs (Hidalgo-Ruz *et al*., 2012), did not account for firstly, the variability in microplastic shape that may occur in the natural environment due to the fragmentation process such as degradation and erosion and secondly, the different settling rates of different shapes of MPs (Wang F *et al*., 2021). The inclusion of more *in situ* experiments and studies on different shapes of MPs, specifically fibres, being mostly recorded as the dominant shape type in freshwater bodies (Rebelein *et al*., 2021; Sarijan *et al*., 2021), in future investigations could provide a holistic understanding of the microplastic capture mechanism by macrophytes in the environment.

Similar to another flume experiment by Gallitelli *et al*. (2023), which used *Potamogeton crispus* and *Myriophyllum spicatum* as experimental macrophyte species to trap plastic particles, the highly proliferate nature of *H. verticillata* and *M. fluviatilis* also cause dense mats on/just below the water surface which brings high potential of trapping the MPs within the size range we used in the experiment. As such, removal of *H. verticillata* and *M. fluviatilis*, by employing a fine-mesh netting may enhance the elimination of MPs from the aquatic environment (and the associated food web) and mitigate the dispersion of macrophyte fragments (Chadwell and Engelhardt, 2008; Canfield and Hoyer, 1992). In addition, this approach may also be employed for evaluating the extent of environmental microplastic pollution in the freshwater body (Gallitelli *et al*., 2023). Macrophytes, specifically *H. verticillata*, given their high microplastic entrapment capacity, could be used as bioindicators to provide insight into the ecological quality of freshwater ecosystems (Kohler and Schneider, 2003). This dual-function application, microplastic extraction and microplastic pollution assessment, during macrophyte removal could bring about comprehensive improvements in both environmental quality and scientific understanding. The exact practical aspects, however, have not been fully explored or implemented yet.

CONCLUSIONS

In freshwater ecosystems, the interactions between macrophytes, microplastics (MPs), and hydrodynamic forces are highly complex and influenced by plant morphology, flow dynamics, and the physicochemical properties of MPs. This study highlights the role of varying water flow regimes in MP entrapment, demonstrating how macrophytes function as passive filters in dynamic aquatic environments (Kaiser *et al*., 2019; Kowalski *et al*., 2016). By incorporating different water flow models, we also examined the often-overlooked factors of MP size and material type in relation to their entrapment by *Hydrilla verticillata* and *Mayaca fluviatilis*. The findings revealed that entrapment efficiency varied between PA and PET MPs, likely due to differences in macrophyte morphology and the surface properties of the MPs. These results emphasize the need for a deeper understanding of species-specific macrophyte interactions with MPs under varying hydrodynamic conditions and highlight their potential role in mitigating MP pollution. Furthermore, bridging the gap between in-situ and ex-situ research is essential to translating controlled experimental findings into real-world applications, enabling more effective strategies for managing MP contamination in freshwater systems. Future research should focus on integrating laboratory and field-based approaches to better predict the ecological consequences of MP accumulation and develop targeted mitigation efforts within natural aquatic environments.

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Online supplementary material:

Fig. S1. Vertical experimental process for obtaining the uncaptured microplastics weight.

Fig. S2. Horizontal experimental process after each run of the macrophytes capturing microplastics.

Tab. S1. Post-hoc pairwise comparison of the retained MPs of different experimental treatment groups.

Tab. S2. Post-hoc pair wise comparison of the retained MPs of the experimental macrophytes species M. fluviatilis and H. verticillate.

Tab. S3. ANOVA of the retained MPs of the experimental macrophytes species M. fluviatilis and H. verticillate.