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Supplementary Material

Leaf morphology affects microplastic entrapment efficiency in freshwater macrophytes

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Supplementary figures for methods

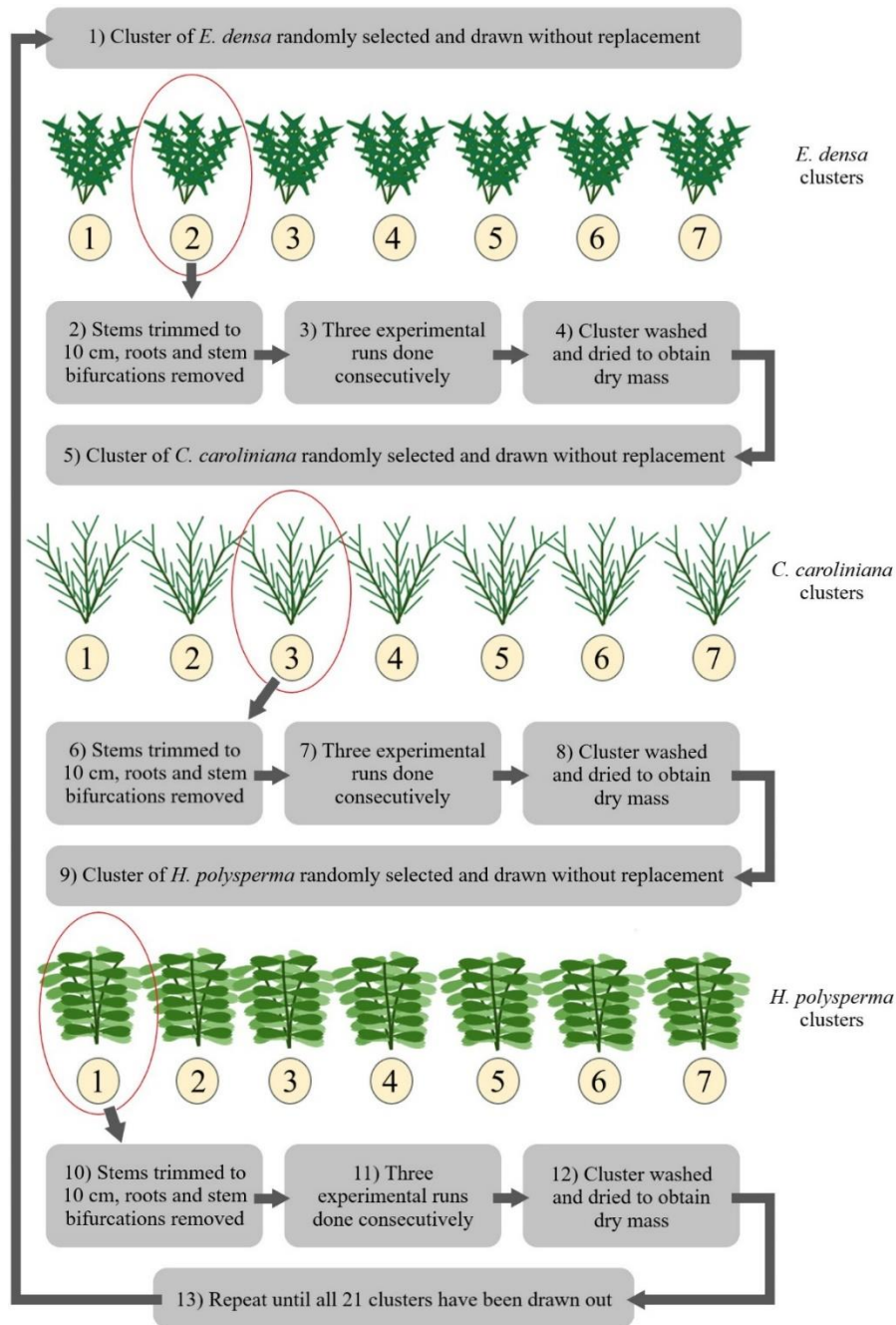


Fig. S1. Diagram showing random sampling of clusters, workflow and systematic alternation between the three plant species for the selection of the subsequent cluster. The red circle indicates cluster that has been randomly selected for a particular round.

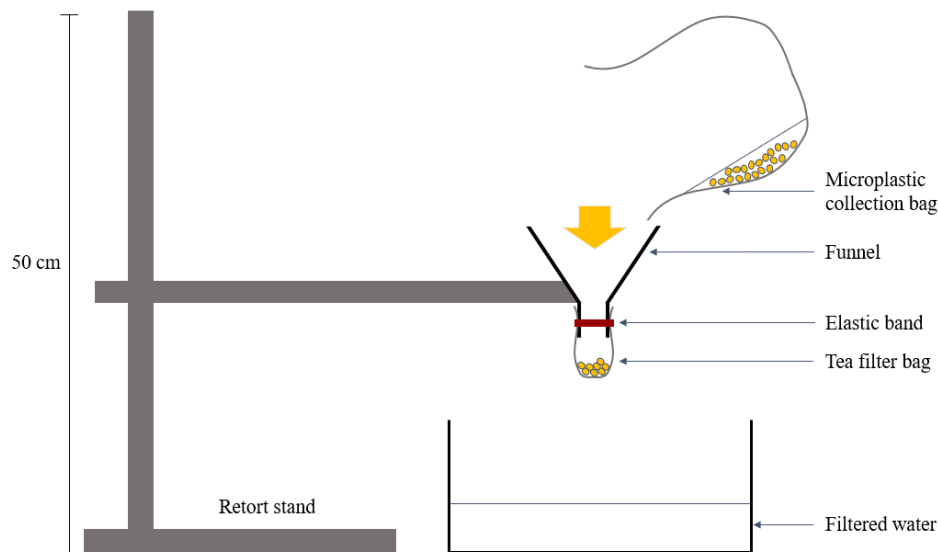


Fig. S2. Collection setup to filter out the microplastics in the collection bag.

Statistical tests for standardised microplastic retention and standardised leaf surface area

Table S1. Shapiro–Wilk and Levene Test *P*-values for standardised retention by species was conducted in R (ver. 4.0.3) using methods specified in the “car” package.

Species	Shapiro–Wilk Test	Levene Test
<i>C. caroliniana</i>	0.4832	0.1468
<i>E. densa</i>	0.5369	
<i>H. polysperma</i>	0.1361	

Table S2. Shapiro–Wilk and Levene Test *p*-values for dry mass (g) by species was conducted in R (ver. 4.0.3) using methods specified in the “car” package.

Species	Shapiro–Wilk test <i>P</i> -value	Levene Test <i>P</i> -value
<i>C. caroliniana</i>	0.6962	0.5381
<i>E. densa</i>	0.4402	
<i>H. polysperma</i>	0.5314	

Table S3. One-way ANOVA results of the plant species, *C. caroliniana*, *E. densa* and *H. polysperma*, for absolute mass of microplastics trapped.

	Degrees of freedom	Sum of squares	Mean sum of squares	<i>F</i> value	<i>P</i> -value
Species	2	3.933	1.9667	14.49	0.000178
Residuals	18	2.443	0.1357	-	-

Table S4. Estimates of one-way ANOVA for absolute microplastic mass of *C. caroliniana*, *E. densa* and *H. polysperma*, compared with absolute microplastic mass of *C. caroliniana* as baseline.

Plant species	Estimate	s.e.	<i>t</i> value	<i>P</i> -value
<i>Cabomba caroliniana</i>	1.9137	0.1392	13.745	5.51e-11
<i>Egeria densa</i>	-0.5010	0.1969	-2.544	0.0203
<i>Hygrophila polysperma</i>	-1.0596	0.1969	-5.381	4.10e-05

Table S5. Tukey multiple comparisons test of means for absolute microplastic mass of *C. caroliniana*, *E. densa* and *H. polysperma*.

Pairwise comparisons		Mean difference	Lower bound	Upper bound	Adjusted <i>P</i> -value
<i>E. densa</i>	<i>C. caroliniana</i>	-0.5009595	-1.003496	0.00157675	0.0507999
<i>H. polysperma</i>	<i>C. caroliniana</i>	-1.0595738	-1.562110	-0.55703755	0.0001161
<i>H. polysperma</i>	<i>E. densa</i>	-0.5586143	-1.061151	-0.05607802	0.0280830

Table S6. One-way ANOVA results of the plant species, *C. caroliniana*, *E. densa* and *H. polysperma*, for percentage microplastics mass trapped.

	Degrees of freedom	Sum of squares	Mean sum of squares	<i>F</i> value	<i>P</i> -value
Species	2	1652.9	826.4	16.62	8.13e-05
Residuals	18	894.8	49.7	-	-

Table S7. Estimates of one-way ANOVA for percentage microplastic mass retained for *C. caroliniana*, *E. densa* and *H. polysperma*, compared with percentage microplastic mass retained for *C. caroliniana* as baseline.

Plant species	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
<i>Cabomba caroliniana</i>	39.333	2.665	14.760	1.69e-11
<i>Egeria densa</i>	-10.535	3.769	-2.796	0.012
<i>Hygrophila polysperma</i>	-21.728	3.769	-5.765	1.83e-05

Table S8. Tukey multiple comparisons test of means for percentage microplastic mass retained for *C. caroliniana*, *E. densa* and *H. polysperma*.

Pairwise comparisons		Mean difference	Lower bound	Upper bound	Adjusted <i>P</i> -value
<i>E. densa</i>	<i>C. caroliniana</i>	-10.53538	-20.15354	-0.917234	0.0305820
<i>H. polysperma</i>	<i>C. caroliniana</i>	-21.72785	-31.34600	-12.109695	0.0000520
<i>H. polysperma</i>	<i>E. densa</i>	-11.19246	-20.81061	-1.574310	0.0213014

Table S9. One-way ANOVA results of the plant species, *C. caroliniana*, *E. densa* and *H. polysperma*, for microplastic mass retained per leaf area.

	Degrees of freedom	Sum of squares	Mean sum of squares	<i>F</i> -value	<i>P</i> -value
Species	2	0.0008256	0.0004128	12.36	0.000419
Residuals	18	0.0006012	0.0000334	-	-

Table S10. Estimates of one-way ANOVA for microplastic mass retained per leaf area for *C. caroliniana*, *E. densa* and *H. polysperma*, compared with *C. caroliniana* as baseline.

Plant species	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
<i>Cabomba caroliniana</i>	0.0321136	0.0021843	14.702	1.8e-11
<i>Egeria densa</i>	-0.0136596	0.0030890	-4.422	0.000329
<i>Hygrophila polysperma</i>	-0.0007494	0.0030890	-0.243	0.811062

Table S11. Tukey multiple comparisons test of means for microplastic mass retained per leaf for *C. caroliniana*, *E. densa* and *H. polysperma*.

Pairwise comparisons		Mean difference	Lower bound	Upper bound	Adjusted <i>P</i> -value
<i>E. densa</i>	<i>C. caroliniana</i>	-0.0136596	-0.0215433	-0.0057758	0.0009142
<i>H. polysperma</i>	<i>C. caroliniana</i>	-0.0007494	-0.0086331	0.0071344	0.9681345
<i>H. polysperma</i>	<i>E. densa</i>	0.0129102	0.0050264	0.0207939	0.0015543

Table S12. One-way ANOVA results of the plant species, *C. caroliniana*, *E. densa* and *H. polysperma*, for standardised microplastic mass trapped.

	Degrees of freedom	Sum of squares	Mean sum of squares	<i>F</i> -value	<i>P</i> -value
Species	2	45.02	22.510	10.53	0.000936
Residuals	18	38.47	2.137	-	-

Table S13. Estimates of one-way ANOVA for Standardised MP Mass of *C. caroliniana*, *E. densa* and *H. polysperma*, compared with Standardised MP Mass of *C. caroliniana* as baseline.

Plant species	Estimate	s.e.	<i>t</i> -value	<i>P</i> -value
<i>Cabomba caroliniana</i>	7.9059	0.5526	14.307	2.84e-11
<i>Egeria densa</i>	-2.6100	0.7815	-3.340	0.003646
<i>Hygrophila polysperma</i>	-3.4353	0.7815	-4.396	0.000348

Table S14. Tukey multiple comparisons test of means for standardised microplastic mass of *C. caroliniana*, *E. densa* and *H. polysperma*.

Pairwise comparisons		Mean difference	Lower bound	Upper bound	Adjusted <i>P</i> -value
<i>E. densa</i>	<i>C. caroliniana</i>	-2.6100	-4.6044	-0.6156	0.0097009
<i>H. polysperma</i>	<i>C. caroliniana</i>	-3.4354	-5.4298	-1.4410	0.0009672
<i>H. polysperma</i>	<i>E. densa</i>	-0.8254	-2.8198	1.1690	0.5523536

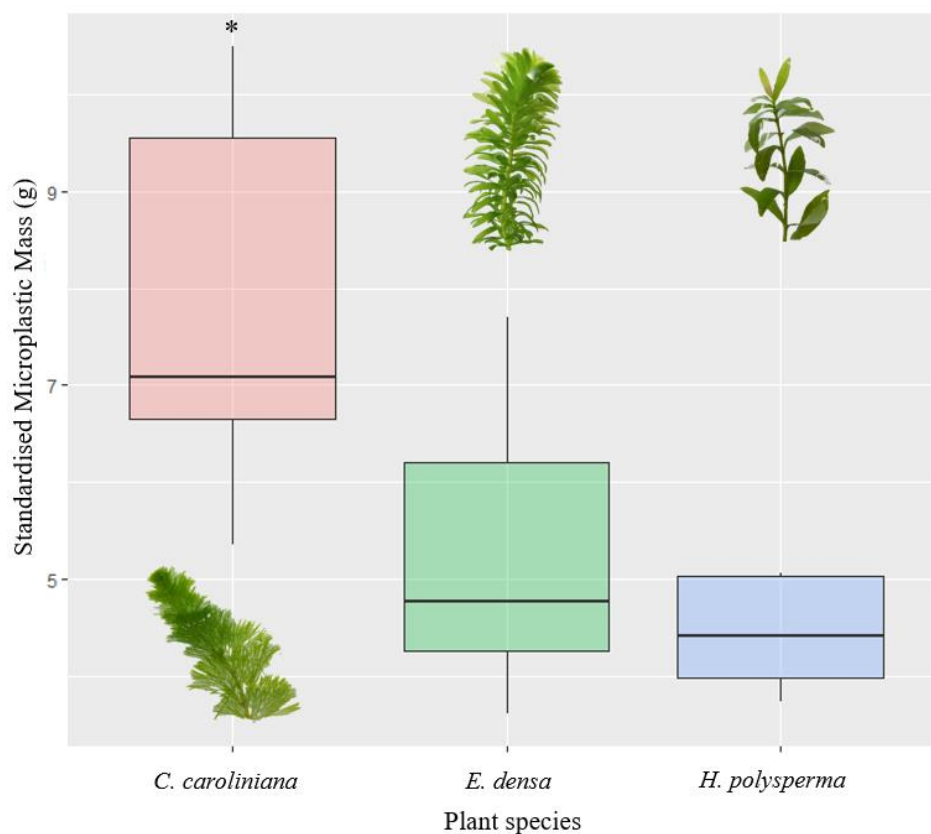


Fig. S3. Mass of microplastics trapped among the three plant species, *C. caroliniana*, *E. densa* and *H. polysperma*. Asterisk indicates species with significant difference in standardised microplastic mass retained as compared to other species.

Table S15. Table of leaf surface area, average leaf surface area, and standardised leaf surface area for *C. caroliniana*, *E. densa* and *H. polysperma*

Plant species	Leaf surface area (cm ²)			Average Leaf Surface Area (cm ²)	Standardised Leaf Surface Area (cm ² g ⁻¹)
	Stem 1	Stem 2	Stem 3		
<i>C. caroliniana</i>	47.892	69.265	61.621	59.593	247.360
<i>E. densa</i>	119.621	58.33	51.72	76.557	279.187
<i>H. polysperma</i>	27.281	15.263	39.159	27.234	137.161

Supplementary figures for results and discussion

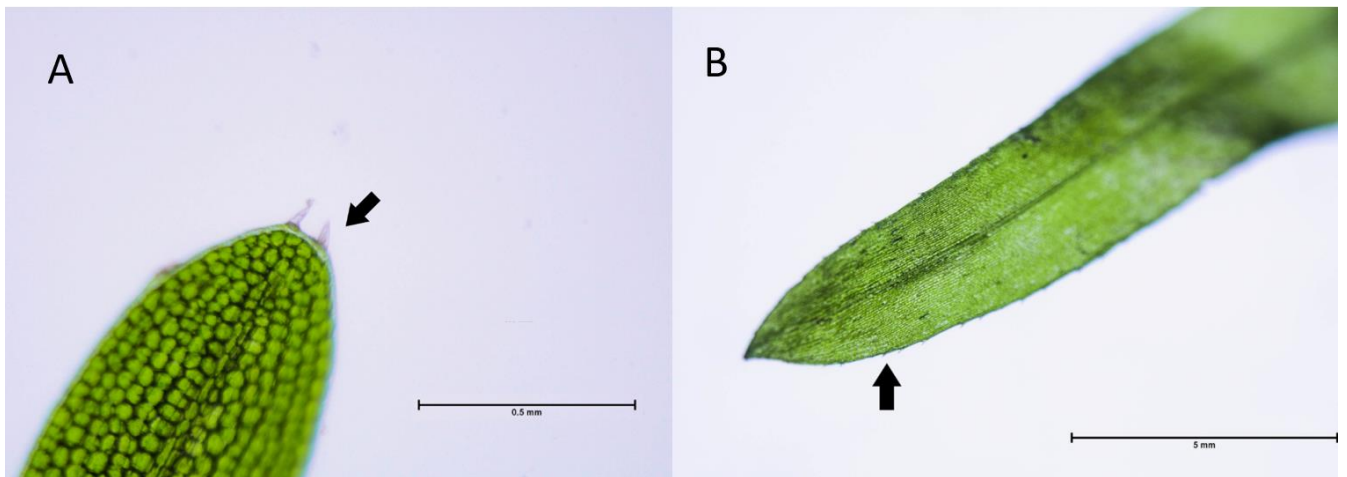


Fig. S4. (A) Terminal of a branch from *C. caroliniana* bearing two trichrome structures. (B) Leaf from *E. densa*, showing the dentate leaf margin. Scale bars: (A) 0.5 mm; (B) 5 mm.

Differences in distribution of dry mass

The relationship between average mass of microplastics retained and dry mass of plant samples differed between the species examined. Line of best fit showed very weak negative correlation to dry mass in *C. caroliniana*, but dry mass was positively correlated in *E. densa* and *H. polysperma* (Fig. A5). Furthermore, the range of dry mass for each species is distinct. The lack of an overall clear trend between dry mass and average mass of microplastic retention indicates that dry mass is not the only explanatory variable influencing retention. This is supported as linear models for average mass of microplastics against dry mass did not show significant correlations for any of the three species.

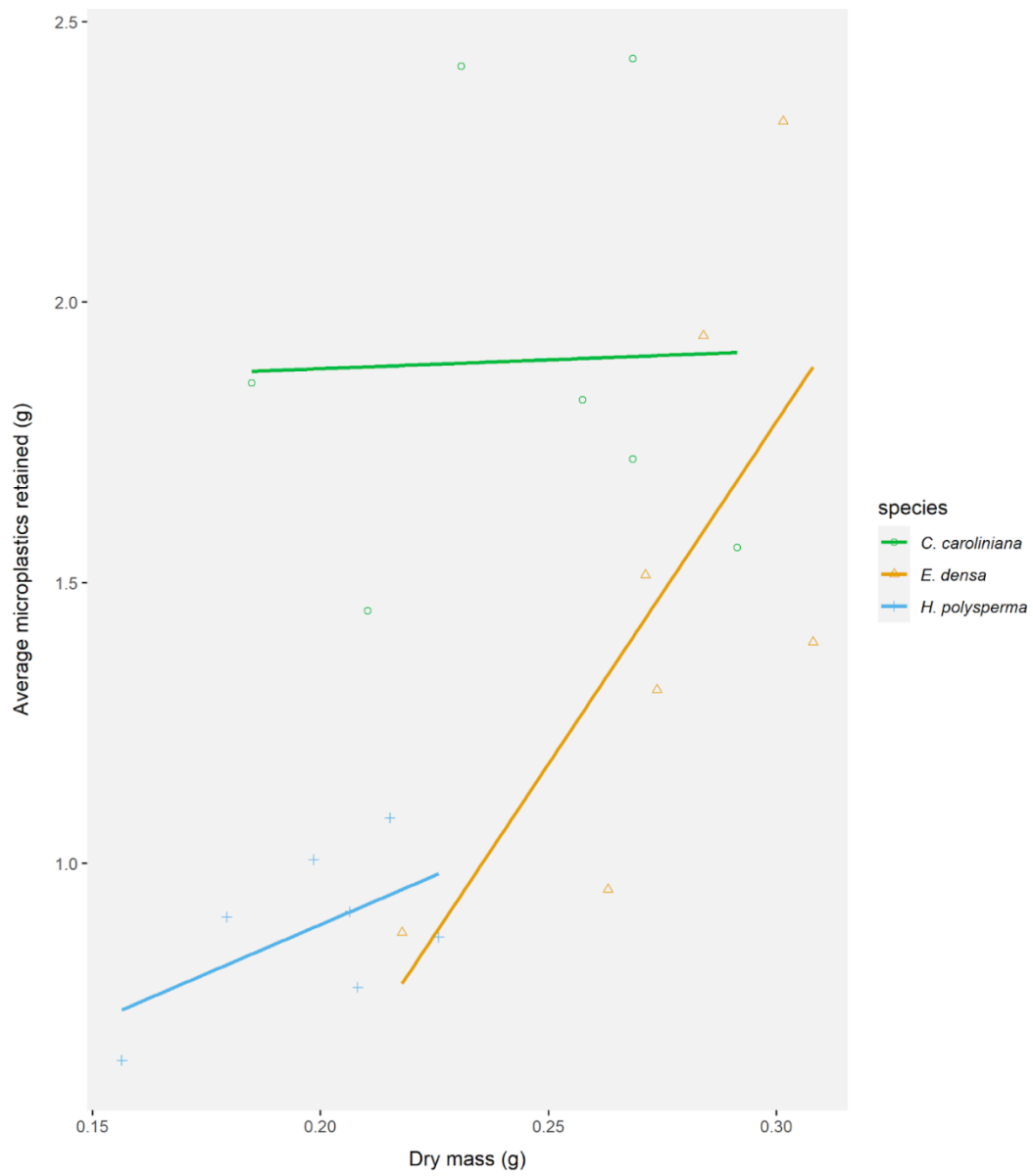


Fig. S5. Correlation between average microplastic retained and dry mass of plant by species