

Supplementary Materials for:

Microplastic exposure across trophic levels: effects on the host-microbiota of freshwater organisms

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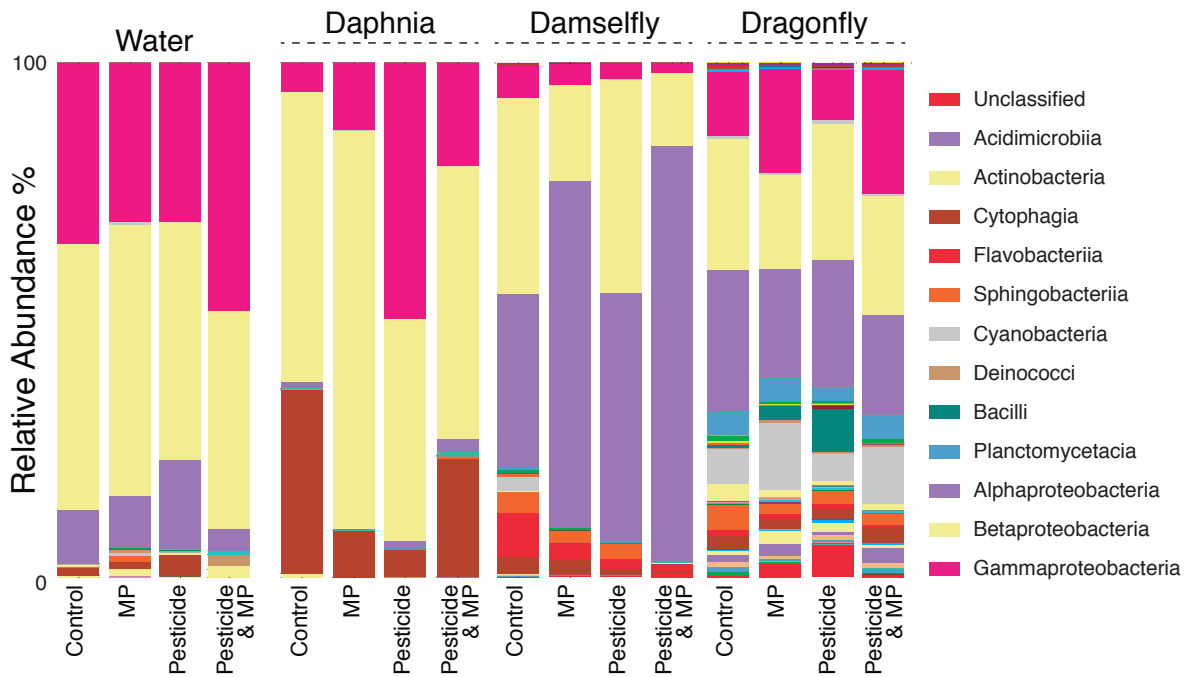


Figure S1. Class level taxa relative abundance of the microbiome of *Daphnia*, the damselfly and the dragonfly, including the relative abundance of the water microbiota extracted from the filters. The exposure treatments were: microplastics (MPs), the pesticide deltamethrin (DMT), a combination of MPs and DMT, and the Control group (no exposure to either MPs or DMT).

Table S1. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota phyla in the *Daphnia*. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Variable	MPs	DMT	MPs:DMT
<i>Daphnia</i>	MANOVA			
	Pillai	0.413	0.449	0.447
	Approx $F_{6,8}$	0.937	1.088	1.079
	p-value	0.518	0.443	0.447
	Proteobacteria			
	$\chi^2_{1,13}$	5.286	4.239	5.468
	p-value	0.021	0.039	0.019
	Bacteroidetes			
	$\chi^2_{1,13}$	7.399	5.81	8.009
	p-value	0.006	0.016	0.005
	Actinobacteria			
	$\chi^2_{1,13}$	6.519	4.738	5.403
	p-value	0.011	0.029	0.02
	Planctomycetes			
	$\chi^2_{1,13}$	0.075	3.997	0.101
	p-value	0.784	0.046	0.75
	Firmicutes			
	$\chi^2_{1,13}$	0.399	0.879	1.154
	p-value	0.528	0.348	0.283
	Cyanobacteria			
$\chi^2_{1,13}$	0.049	0.002	0.476	
p-value	0.824	0.963	0.49	

Table S2. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota genera (g_) or microbiota families (f_), if genus was not possible to be assigned in the *Daphnia* microbiome. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Variable	MPs	DMT	MPs:DMT
<i>Daphnia</i>	MANOVA			
	Pillai	0.338	0.561	0.637
	Approx $F_{6,8}$	0.682	1.705	2.337
	p-value	0.670	0.237	0.132
	f_ <i>Comamonadaceae</i>			
	$\chi^2_{1,13}$	0.664	0.012	0.186
	p-value	0.415	0.914	0.666
	g_ <i>Leadbetterella</i>			
	$\chi^2_{1,13}$	7.159	5.612	7.746
	p-value	0.007	0.018	0.005
	g_ <i>Aeromonas</i>			
	$\chi^2_{1,13}$	2.210	4.934	5.07
	p-value	0.137	0.0263	0.024
	g_ <i>Pseudomonas</i>			
	$\chi^2_{1,13}$	0.054	0.595	1.134
	p-value	0.815	0.44	0.287
	g_ <i>Curvibacter</i>			
$\chi^2_{1,13}$	3.769	3.014	0.733	
p-value	0.052	0.083	0.392	
g_ <i>Limnobacter</i>				
$\chi^2_{1,13}$	5.596	3.099	3.224	
p-value	0.018	0.078	0.072	

Table S3. Results for the univariate models testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance on low abundant genera (g__) or families (f__), if genus was not possible to be assigned, of the main phyla that constitute more than 0.5% of the total relative abundance in the *Daphnia* microbiome. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Phyla	Variable	MPs	DMT	MPs:DMT
<i>Daphnia</i>	Bacteroidetes	f__<i>Sphingomonadaceae</i>			
		$\chi^2_{1,13}$	8.793	2.912	4.849
		p-value	0.003	0.088	0.028
		g__<i>Acidovorax</i>			
		$\chi^2_{1,13}$	3.069	2.316	0.364
		p-value	0.08	0.128	0.546
		o__<i>Burkholderiales</i>			
		$\chi^2_{1,13}$	4.736	2.812	4.425
		p-value	0.029	0.094	0.035
	Actinobacteria	f__<i>Microbacteriaceae</i>			
		$\chi^2_{1,13}$	3.314	3.510	<0.001
		p-value	0.069	0.061	0.999
		g__<i>Salinibacterium</i>			
		$\chi^2_{1,13}$	3.672	3.135	<0.001
		p-value	0.055	0.077	0.999
Planctomycetes	g__<i>Microbacterium</i>				
	$\chi^2_{1,13}$	6.721	7.185	<0.001	
	p-value	0.009	0.007	0.999	
	g__<i>Pirellula</i>				
	$\chi^2_{1,13}$	0.031	1.921	0.132	
	p-value	0.861	0.166	0.716	
Unclassified	g__<i>Planctomyces</i>				
	$\chi^2_{1,13}$	0.103	5.206	0.092	
	p-value	0.748	0.022	0.762	
Unclassified					
$\chi^2_{1,13}$	0.011	0.635	0.594		
p-value	0.916	0.425	0.441		

Table S4. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota phyla in the Damselfly. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Variable	MPs	DMT	MPs:DMT
Damselfly	MANOVA			
	Pillai	0.479	0.341	0.202
	Approx $F_{6,15}$	2.303	1.292	0.634
	p-value	0.089	0.319	0.701
	Proteobacteria			
	$\chi^2_{1,20}$	5.161	3.301	0.458
	p-value	0.023	0.069	0.499
	Bacteroidetes			
	$\chi^2_{1,20}$	2.07	1.519	0.332
	p-value	0.15	0.218	0.564
	Cyanobacteria			
	$\chi^2_{1,20}$	7.343	3.851	0.164
	p-value	0.007	0.050	0.685
	Planctomycetes			
	$\chi^2_{1,20}$	5.341	1.746	0.104
	p-value	0.021	0.186	0.747
	Unclassified			
	$\chi^2_{1,20}$	8.057	2.208	0.216
p-value	0.004	0.137	0.642	
Gemmatimonadetes				
$\chi^2_{1,20}$	4.413	1.674	0.09	
p-value	0.036	0.196	0.764	

Table S5. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota genera (g_) or microbiota families (f_), if genus was not possible to be assigned in the Damselfly microbiome. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Variable	MPs	DMT	MPs:DMT
MANOVA				
	Pillai	0.65	0.291	0.454
	Approx $F_{6,15}$	4.641	1.024	2.081
	p-value	0.007	0.447	0.117
g_ <i>Rickettsia</i>				
	$\chi^2_{1,20}$	15.214	3.204	1.513
	p-value	<0.001	0.073	0.219
f_ <i>Comamonadaceae</i>				
Damselfly	$\chi^2_{1,20}$	0.989	0.098	<0.001
	p-value	0.320	0.754	0.976
g_ <i>Flavobacterium</i>				
	$\chi^2_{1,20}$	5.833	4.644	0.223
	p-value	0.016	0.031	0.637
g_ <i>Methylophilus</i>				
	$\chi^2_{1,20}$	0.013	0.014	3.427
	p-value	0.914	0.907	0.064
f_ <i>Sphingomonadaceae</i>				
	$\chi^2_{1,20}$	5.823	2.474	0.546
	p-value	0.0158	0.116	0.46
g_ <i>Rhizobacter</i>				
	$\chi^2_{1,20}$	10.263	1.593	0.163
	p-value	<0.001	0.207	0.686

Table S6. Post hoc contrasts on the univariate relative abundances of the main six phyla of the microbiome of *Daphnia*, damselflies and dragonflies, testing differences between treatments: Control, exposure to microplastics (MPs), exposure to deltamethrin (DMT), and the combined exposure to MPs and DMT. Only significant and marginally non-significant p-values are shown.

	Variable	contrast	p-value
<i>Daphnia</i>	Proteobacteria	Control – MPs	0.098
	Bacteroidetes	Control – MPs	0.075
	Proteobacteria	Control – MPs & DMT	0.044
Damselfly	Unclassified	Control – MPs	0.047
		Control – MPs & DMT	0.001
Dragonfly	Bacteroidetes	Control – MPs	0.036
	Actinobacteria	Control – MPs	0.045

Table S7. Results for the univariate models testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance on low abundant genera (g__) or families (f__), if genus was not possible to be assigned, of the main phyla that constitute more than 0.5% of the total relative abundance in the Damselfly microbiome. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Phyla	Variable	MPs	DMT	MPs:DMT
Damselfly	Bacteroidetes	g__<i>Sediminibacterium</i>			
		$\chi^2_{1,13}$	0.006	0.222	2.523
		p-value	0.939	0.637	0.112
		g__<i>Leadbetterella</i>			
		$\chi^2_{1,13}$	0.474	0.011	1.279
		p-value	0.491	0.917	0.258
	Cyanobacteria	g__<i>Emticicia</i>			
		$\chi^2_{1,13}$	0.419	2.620	0.134
		p-value	0.517	0.105	0.714
		g__<i>Leptolyngbya</i>			
		$\chi^2_{1,13}$	7.414	3.876	0.015
		p-value	0.006	0.049	0.902
	Unclassified	f__<i>Sphingomonadaceae</i>			
		$\chi^2_{1,13}$	5.823	2.474	0.546
p-value		0.016	0.116	0.46	
Unclassified					
		$\chi^2_{1,13}$	8.63	2.412	0.293
		p-value	0.003	0.120	0.588

Table S8. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota phyla in the Dragonfly. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold

Organism	Variable	MPs	DMT	MPs:DMT
Dragonfly	MANOVA			
	Pillai	0.39	0.084	0.302
	Approx $F_{6,27}$	2.877	0.411	1.949
	p-value	0.027	0.865	0.109
	Proteobacteria			
	$\chi^2_{1,32}$	0.865	0.811	1.116
	p-value	0.352	0.368	0.291
	Cyanobacteria			
	$\chi^2_{1,32}$	0.431	1.293	0.742
	p-value	0.511	0.255	0.389
	Bacteroidetes			
	$\chi^2_{1,32}$	8.057	2.644	3.018
	p-value	0.004	0.104	0.082
	Planctomycetes			
	$\chi^2_{1,32}$	1.125	3.868	3.955
	p-value	0.289	0.049	0.047
	Actinobacteria			
	$\chi^2_{1,32}$	7.438	0.003	0.129
p-value	0.006	0.957	0.72	
Acidobacteria				
$\chi^2_{1,32}$	4.07	2.288	3.207	
p-value	0.044	0.13	0.073	

Table S9. Results for the MANOVA testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance of the six main microbiota genera (g_) or microbiota families (f_), if genus was not possible to be assigned in the Dragonfly microbiome. The univariate models testing the effects of exposure to MPs and DMT on the relative abundance of the six main microbiota phyla are also included. Significant and marginally non-significant p-values are highlighted in bold

Organism	Variable	MPs	DMT	MPs:DMT	
Dragonfly	MANOVA				
		Pillai	0.367	0.119	0.324
		Approx $F_{6,27}$	2.609	0.612	2.153
		p-value	0.04	0.719	0.08
		f_ <i>Comamonadaceae</i>			
		$\chi^2_{1,32}$	12.227	5.470	7.028
		p-value	<0.001	0.019	0.008
		g_ <i>Ralstonia</i>			
		$\chi^2_{1,32}$	3.199	3.623	2.451
		p-value	0.074	0.057	0.117
		g_ <i>Chamaesiphon</i>			
		$\chi^2_{1,32}$	3.640	0.043	0.073
		p-value	0.056	0.835	0.787
		g_ <i>Aeromonas</i>			
		$\chi^2_{1,32}$	0.058	0.462	3.851
		p-value	0.809	0.497	0.05
	g_ <i>Rhodobacter</i>				
	$\chi^2_{1,32}$	10.236	0.003	0.129	
	p-value	0.006	0.957	0.72	
	g_ <i>Acinetobacter</i>				
	$\chi^2_{1,32}$	4.07	2.048	1.6	
	p-value	0.001	0.152	0.207	

Table S10. Results for the univariate models testing the effects of exposure to microplastics (MPs) and deltamethrin (DMT) on the relative abundance on low abundant genera (g__) or microbial taxonomic rank, if genus was not possible to be assigned, of the main phyla that constitute more than 0.5% of the total relative abundance in the Dragonfly microbiome. Significant and marginally non-significant p-values are highlighted in bold.

Organism	Phyla	Variable	MPs	DMT	MPs:DMT	
Dragonfly	Cyanobacteria	c__Chloroplast				
		$\chi^2_{1,13}$	7.281	7.106	3.574	
		p-value	0.007	0.008	0.06	
		f__FamilyI				
		$\chi^2_{1,13}$	0.652	4.026	3.376	
		p-value	0.419	0.045	0.066	
	Bacteroidetes	g__Leptolyngbya	$\chi^2_{1,13}$	0.096	2.003	0.012
			p-value	0.757	0.157	0.912
		f__Saprospiraceae	$\chi^2_{1,13}$	8.781	3.033	1.691
			p-value	0.003	0.082	0.193
		g__Emticicia	$\chi^2_{1,13}$	4.054	0.004	3.229
			p-value	0.044	0.948	0.072
		Unclassified	$\chi^2_{1,13}$	5.902	15.090	13.292
			p-value	0.015	<0.001	<0.001
Planctomycetes	g__Pirellula	$\chi^2_{1,13}$	<0.001	3.8	1.798	
		p-value	0.993	0.051	0.18	
	g__Planctomyces	$\chi^2_{1,13}$	5.077	3.376	6.532	
		p-value	0.024	0.066	0.011	
	g__Gemmata	$\chi^2_{1,13}$	0.382	1.322	0.038	
		p-value	0.536	0.250	0.845	

Table S11. Results of the GLMM testing the effects of exposure to microplastics (MPs), deltamethrin (DMT) and their interaction on damselfly survival. Significant and marginally non-significant p-values are highlighted in bold.

Survival	MPs	DMT	Time	MPs:DMT	MPs:Time	DMT:Time	MPs:DMT:Time
Damselfly							
F _{1,532}	0.363	7.987	212.814	0.019	0.4433	2.353	0.654
p-value	0.547	0.005	<0.001	0.889	0.506	0.126	0.419