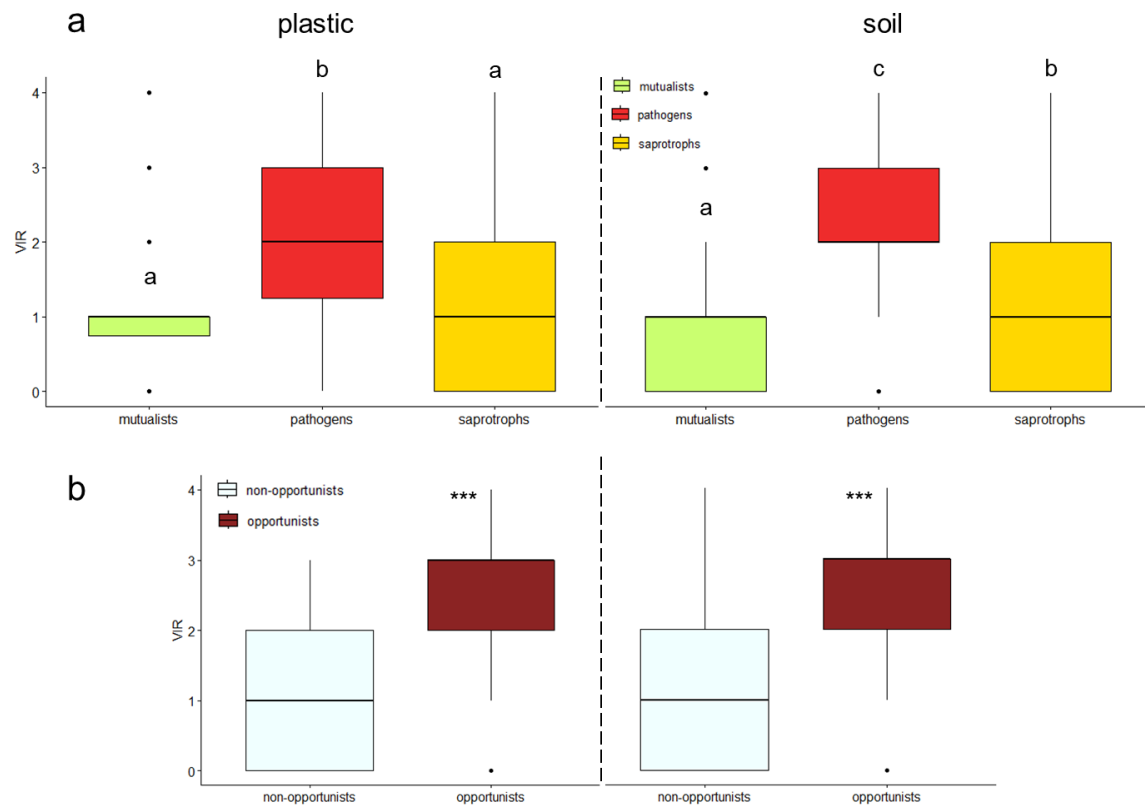


Supplementary Information

Gkoutselis et al. *Fungal Plastiphily and its Link to Generic Virulence Traits Makes Environmental Microplastics a Global Health Factor*



Supplementary Fig. 1 Virulence scores among ecological guilds and opportunists found on plastic and in the soil. VIR = virulence score. The lower-case letters indicate significant differences between groups ($p < 0.05$) via one-way ANOVA. (***) indicates highly significant differences according to one-way ANOVA.

Supplementary Table 1. One-way ANOVA test p values for significant differences in Shannon diversity among the most species-rich classes in the plastisphere metacommunity. Agar. = Agaricomycetes, Trem. = Tremellomycetes, Euro. = Eurotiomycetes, Sord. = Sordariomycetes and Doth. = Dothideomycetes. 'other' codes for all remaining identified and unassigned classes.

	Agar.	Trem.	Euro.	Sord.	Doth.
Agar.					
Trem.	0.0001				
Euro.	0.0506	0.0594			
Sord.	0.0000	0.0000	0.0000		
Doth.	0.0000	0.0000	0.0000	0.0005	
other	0.0000	0.0000	0.0000	0.4752	0.0600

Supplementary Table 2. One-way ANOVA test p values for significant differences in Shannon diversity among the most species-rich classes in the soil metacommunity. Agar. = Agaricomycetes, Trem. = Tremellomycetes, Euro. = Eurotiomycetes, Sord. = Sordariomycetes and Doth. = Dothideomycetes. 'other' codes for all remaining identified and unassigned classes.

	Agar.	Trem.	Euro.	Sord.	Doth.
Agar.					
Trem.	0.8884				
Euro.	0.1229	0.0945			
Sord.	0.0000	0.0000	0.0000		
Doth.	0.0000	0.0000	0.0000	0.2387	
other	0.0000	0.0000	0.0000	0.0710	0.3366

Supplementary Table 3. One-way ANOVA test p values for significant differences in Shannon diversity among the most species-rich classes between plastisphere (rows) and soil metacommunity (columns). Agar. = Agaricomycetes, Trem. = Tremellomycetes, Euro. = Eurotiomycetes, Sord. = Sordariomycetes and Doth. = Dothideomycetes. 'other' codes for all remaining identified and unassigned classes.

		soil					
		Agar.	Trem.	Euro.	Sord.	Doth.	other
plastic	Agar.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Trem.	0.0433	0.0565	0.0015	0.0000	0.0000	0.0000
	Euro.	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
	Sord.	0.0029	0.0017	0.2754	0.0000	0.0000	0.0000
	Doth.	0.0000	0.0000	0.0001	0.0004	0.0000	0.0001
	other	0.0020	0.0014	0.1228	0.0000	0.0000	0.0000

Supplementary Table 4. One-way ANOVA test p values for significant differences in Shannon diversity among guilds in the plastisphere metacommunity.

	pathogens	saprotrophs	mutualists
pathogens			
saprotrophs	0.5114		
mutualists	0.0000	0.0000	
unidentified	0.0000	0.0000	1.0000

Supplementary Table 5. One-way ANOVA test p values for significant differences in Shannon diversity among guilds in the soil metacommunity.

	pathogens	saprotrophs	mutualists
pathogens			
saprotrophs	0.0051		
mutualists	0.0000	0.0000	
unidentified	0.0000	0.0000	1.0000

Supplementary Table 6. One-way ANOVA test p values for significant differences in Shannon diversity among guilds between plastisphere (rows) and the soil metacommunity (columns).

		soil			
		pathogens	saprotrophs	mutualists	unidentified
plastic	pathogens	0.0001	0.0000	0.0000	0.0000
	saprotrophs	0.0001	0.0000	0.0000	0.0000
	mutualists	0.0000	0.0000	0.0195	0.0195
	unidentified	0.0000	0.0000	0.0195	0.0195

Supplementary Table 7. One-way ANOVA test p values for significant differences in Shannon diversity among abundance fractions in the plastisphere metacommunity. AAT = always abundant taxa, CAT = conditionally abundant taxa; CRAT, conditionally rare and abundant taxa; MT, moderate taxa; CRT = conditionally rare taxa; ART = always rare taxa.

	AAT	CAT	CRAT	MT	CRT
AAT					
CAT	0.0000				
CRAT	0.4395	0.0000			
MT	0.0066	0.0000	0.0035		
CRT	0.0000	0.0000	0.0000	0.0000	
ART	0.0000	0.3954	0.0000	0.0000	0.0000

Supplementary Table 8. One-way ANOVA test p values for significant differences in Shannon diversity among abundance fractions in the soil metacommunity. AAT = always abundant taxa, CAT = conditionally abundant taxa; CRAT, conditionally rare and abundant taxa; MT, moderate taxa; CRT = conditionally rare taxa; ART = always rare taxa.

	AAT	CAT	CRAT	MT	CRT
AAT					
CAT	0.0000				
CRAT	0.0000	0.0000			
MT	0.0000	0.2875	0.0000		
CRT	0.0000	0.0000	0.0000	0.0000	
ART	0.0000	0.0007	0.0000	0.0087	0.0002

Supplementary Table 9. One-way ANOVA test p values for significant differences in Shannon diversity among abundance fractions between plastisphere (rows) and the soil metacommunity (columns). AAT = always abundant taxa, CAT = conditionally abundant taxa; CRAT, conditionally rare and abundant taxa; MT, moderate taxa; CRT = conditionally rare taxa; ART = always rare taxa.

		soil					
		AAT	CAT	CRAT	MT	CRT	ART
plastisphere	AAT	0.0000	0.0000	0.8127	0.0000	0.0000	0.0000
	CAT	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000
	CRAT	0.0000	0.0000	0.6621	0.0000	0.0000	0.0000
	MT	0.1272	0.0000	0.0096	0.0000	0.0000	0.0000
	CRT	0.0000	0.0000	0.0000	0.0030	0.0000	0.7935
	ART	0.0000	0.0884	0.0000	0.0251	0.0000	0.0001

Supplementary Table 10. ANOSIM tests of abundance fractions of the plastisphere and soil metacommunities based on Bray–Curtis similarity of OTUs. Tests were based on 999 permutations.

	R ²	p (perm)
AAT	0.4944	0.0001
CAT	0.1572	0.0001
CRAT	0.2719	0.0001
MT	0.6025	0.0001
CRT	0.2729	0.0001
ART	0.4898	0.0001

Supplementary Table 11. Parameters and fits of the neutral community model as adapted to the plastisphere, soil and the combined (both) metacommunities. *m* is a single free parameter describing the migration rate, which is interpreted as the influence of drift and dispersal; *m.ci* and *m.mle* are the fitting parameters of *m*; Rsqr, Root Mean Square Error (RMSE), Akaike information criterion (AIC), and Bayesian Information Criterion (BIC) are used to assess the overall fit of the mode.

	<i>m</i>	sum Sq	<i>m.ci</i>	<i>m.mle</i>	Rsqr	RMSE	AIC	BIC
plastic	0.0079	6.792	0.0086	0.0856	0.6196	0.0856	−1923	−1913
soil	0.0092	12.85	0.0098	0.0803	0.6750	0.0803	−4387	−4376
both	0.0135	7.275	0.0142	0.0619	0.7202	0.0619	−5181	−5170

Supplementary Table 12. Parameters and fits of the binominal model as adapted to the plastisphere, soil and the combined (both) metacommunities. *mle* represents the fitting parameters of the binominal model; Rsqr, Root Mean Square Error (RMSE), Akaike information criterion (AIC), and Bayesian Information Criterion (BIC) are used to assess the overall fit of the mode.

	<i>mle</i>	Rsqr	RMSE	AIC	BIC
plastic	0.2744	−5.6920	0.3592	237	247
soil	0.2618	−5.1981	0.3509	317	328
both	0.2233	−4.2174	0.2672	−302	−291

Supplementary Table 13. Pairwise ANOSIM tests of fungal partitions of plastisphere and soil metacommunity, respectively, based on Hellinger distance of OTUs. Tests were based on 999 permutations.

	plastic		soil	
	R ²	p (perm)	R ²	P (perm)
above, below	0.631	0.001	0.804	0.001
above, neutral	0.773	0.001	0.962	0.001
below, neutral	0.886	0.001	0.845	0.001

Supplementary Table 14. Pairwise ANOSIM tests between sample partitions of plastisphere and soil metacommunity based on Hellinger distance of OTUs. Tests were based on 999 permutations.

soil	plastic	R ²	p (perm)
neutral	neutral	0.747	0.0001
neutral	below	0.949	0.0001
neutral	above	0.833	0.0001
below	neutral	0.774	0.0001
below	below	0.834	0.0001
below	above	0.787	0.0001
above	neutral	0.913	0.0001
above	below	0.920	0.0001
above	above	0.906	0.0001

Supplementary Table 15. PERMANOVA main test of fungal communities of the different sample partitions based on Hellinger distance of OTUs. p-values were obtained from type III sums and 999 permutations under the full model. d.f.: degrees of freedom. Sq: squares. perms: number of unique permutations per comparison.

		d.f.	sum Sq	mean Sq	Pseudo-F	p-value	perms
plastic	Location	2	42.607	21.304	39.071	0.001	998
	Residuals	134	73.063	0.5453			
	Total	136	115.67				
soil	Location	2	31.993	15.996	23.527	0.001	997
	Residuals	141	95.869	0.6799			
	Total	143	127.86				
combined	Location	5	81.149	16.23	26.42	0.001	997
	Residuals	275	168.93	0.6143			
	Total	280	250.08				

Supplementary Table 16. PERMANOVA pairwise tests of fungal communities of the different sample partitions based on Hellinger distance of OTUs. PERMANOVA P-values were obtained from type III sums and 999 permutations under the full model.

	comparison	t (perm)	p (perm)	perms
plastic	neutral, below	6.5565	0.001	997
	neutral, above	5.6152	0.001	997
	below, above	6.6195	0.001	998
soil	neutral, below	5.0115	0.001	997
	neutral, above	5.352	0.001	994
	below, above	4.1896	0.001	997
combined	neutral, below	5.9397	0.001	996
	neutral, above	5.645	0.001	998
	below, above	5.2974	0.001	999

Supplementary Table 17. One-way ANOVA test p values from pairwise comparisons for significant differences in species richness (observed), Shannon diversity and (species) evenness between sample partitions of the plastisphere and soil metacommunity, respectively.

		plastic	soil
	comparison	p (perm)	p (perm)
Observed	neutral, below	0.0000	0.0000
	neutral, above	0.0000	0.0000
	below, above	0.0000	0.0000
Shannon	neutral, below	0.0000	0.0000
	neutral, above	0.7637	0.0000
	below, above	0.0000	0.0000
Evenness	neutral, below	0.6322	0.5573
	neutral, above	0.0000	0.5017
	below, above	0.0005	0.3359

Supplementary Table 18. One-way ANOVA test p values from pairwise comparisons for significant differences in species richness (observed), Shannon diversity and (species) evenness of sample partitions between plastisphere and soil metacommunity.

			soil		
			neutral	below	above
Observed	plastic	neutral	0.0000	0.0000	0.0000
		below	0.0000	0.7754	0.0000
		above	0.0000	0.0000	0.0000
Shannon		neutral	0.0000	0.0000	0.0000
		below	0.0000	0.7067	0.0000
		above	0.0000	0.0000	0.0000
Evenness		neutral	0.0262	0.0715	0.4595
		below	0.0487	0.0668	0.3168
		above	0.0022	0.2326	0.0164

Supplementary Table 19. Key properties of co-occurrence networks. Average virulence scores were calculated from annotated phylotypes only.

	Nodes	Edges	Network diameter	Clustering coefficient	Modularity	Positive ratio	Average path length	Average degree	Average betweenness	Av. virulence score	Proportion of pathogens
plastic	97	166	9	0.41	0.63	1	3.73	3.42	66.3	1.88	0.41
soil	259	498	15	0.38	0.77	0.998	5.82	3.85	409.4	1.52	0.21

Supplementary Table 20. Keystone taxa as derived from the soil and plastisphere networks. Fungi with the highest degree in soil (≥ 8) and plastisphere (≥ 9) networks were defined as hub taxa. Fungi with the highest betweenness centrality (top 10 of each compartment) were declared gatekeeper taxa. Fungi that belonged to both categories were defined as keystone taxa. Doth. = Dothideomycetes, Sord. = Sordariomycetes, unid. = unidentified, Agar. = Agaricomycetes, F = false, T = true, sap = saprotroph, path = pathogen, mut = mutualist. (*) indicates unidentified species within the genus.

OTU ID	Taxon	Class	above-partition	Primary lifestyle	Plastic biodegradability	Virulence score	Degree	Betweenness centrality	Closeness centrality
plastic									
61d92067	* <i>Fusarium</i>	Sord.	F	path	T	2	9	255	0.34
65a5d2ed	unidentified	unid.	T	/	/	/	9	325	0.35
69a453f5	* <i>Stagonospora</i>	Doth.	T	path	F	1	13	442	0.39
189a431b	* <i>Pyrenochaetopsis</i>	Doth.	T	mut	T	1	10	564	0.40
3af3dcdd	<i>Phoma omnivirens</i>	Doth.	T	path	T	3	19	859	0.44
soil									
4c19d908	* <i>Subulicystidium</i>	Agar.	F	sap	F	0	17	3486	0.24
74944029	unidentified	unid.	F	/	/	/	18	4394	0.24
9e4abd9c	unidentified	unid.	F	/	/	/	15	4559	0.22
89b225ba	* <i>Phaeosphaeria</i>	Doth.	F	sap	F	0	8	6464	0.24

Supplementary Table 21. Taxonomic and ecological groups of pathogens tested for differential relative species richness, abundance, and selection between soil and plastisphere metacommunity. Selection here was approached using the across-metacommunity selection (AMS) as described in this study. Included filamentous fungi and yeasts were cosmopolitan adaptable pathogens (CAOs) as determined by our meta-analysis (Supplementary Data 2). doth. path. = dothideomycetous pathogens, doth. opp. = dothideomycetous opportunists, trem. path. = tremellomycetous pathogen, trem. opp. = tremellomycetous opportunists.

Group	richness		abundance		selection	
	plastic	soil	plastic	soil	plastic	soil
filamentous fungi						
<i>Acremonium</i>	0.0054	0.0040	0.0025	0.0004	0.0098	0.0053
<i>Alternaria</i>	0.0054	0.0020	0.0812	0.0624	0.0098	0
<i>Aspergillus</i>	0.0119	0.0095	0.0051	0.0066	0.0294	0.0159
<i>Bipolaris</i>	0.0043	0.0025	0.0039	0.0100	0.0196	0.0053
<i>Chaetomium</i>	0.0032	0.0020	0.0035	0.0138	0.0098	0.0106
<i>Cladosporium</i>	0.0043	0.0035	0.0308	0.0239	0	0
<i>Curvularia</i>	0.0151	0.0075	0.0248	0.0502	0.0686	0.0053
<i>Didymella</i>	0.0022	0.0015	0.0004	0.0008	0.0098	0
<i>Epicoccum</i>	0.0032	0.0015	0.0122	0.0101	0.0098	0
<i>Fusarium</i>	0.0162	0.0100	0.1211	0.1833	0.0294	0.0159
<i>Penicillium</i>	0.0032	0.0085	0.0003	0.0008	0	0.0212
<i>Phoma</i>	0.0022	0.0015	0.0074	0.0059	0.0098	0
yeasts						
<i>Aureobasidium</i>	0.0022	0.0015	0.0023	0.0005	0	0.0053
<i>Candida</i>	0.0011	0.0015	0	0.0001	0	0
<i>Cryptococcus</i>	0.0097	0.0035	0.0010	0.0008	0.0490	0
<i>Exophiala</i>	0.0076	0.0040	0.0002	0.0003	0	0.0053
<i>Hannaella</i>	0.0065	0.0025	0.0052	0.0045	0.0490	0
<i>Knufia</i>	0.0011	0.0005	0	0	0	0
<i>Malassezia</i>	0.0032	0.0015	0.0026	0.0003	0	0
<i>Papiliotrema</i>	0.0043	0.0015	0.0010	0.0034	0.0196	0
<i>Rhodotorula</i>	0.0065	0.0035	0.0130	0.0088	0.0098	0
ecological groups						
pathogens	0.2006	0.1371	0.5017	0.4495	0.4804	0.1323
opportunists	0.2082	0.1482	0.5284	0.5168	0.4804	0.1746
CAOs	0.1230	0.0784	0.3185	0.3903	0.3235	0.0952
doth. path.	0.0755	0.0427	0.3425	0.2110	0.1961	0.0265
doth. opp.	0.0647	0.0427	0.2988	0.1965	0.1863	0.0423
trem. path.	0.0227	0.0095	0.0075	0.0145	0.1176	0
trem. opp.	0.0356	0.0176	0.0376	0.0306	0.1176	0.0053
hidden killers	0.0227	0.0146	0.0061	0.0075	0.0784	0.0159

Supplementary Table 22. Values of selection indices and virulence scores of taxonomic and ecological groups of pathogens. WGS = within-group selection, TAS = times average selection, SI = selection index and VIR = virulence score. Included filamentous fungi and yeasts were cosmopolitan adaptable pathogens (CAOs) as determined by our meta-analysis (Supplementary Data 2). VIR of CAO genera was calculated at genus level. VIR of ecological groups is the unweighted average VIR of all included genera. doth. path. = dothideomycetous pathogens, doth. opp. = dothideomycetous opportunists, trem. path. = tremellomycetous pathogen, trem. opp. = tremellomycetous opportunists.

Group	WGS		TAS		SI		VIR	
	plastic	soil	plastic	soil	plastic	soil	plastic	soil
filamentous fungi								
<i>Acremonium</i>	0.2	0.13	1.82	1.32	0.0020	0.0007	2	2
<i>Alternaria</i>	0.2	0	1.82	0	0.0020	0	3	3
<i>Aspergillus</i>	0.27	0.16	2.48	1.66	0.0080	0.0025	2	2
<i>Bipolaris</i>	0.5	0.2	4.54	2.11	0.0098	0.0011	3	3
<i>Chaetomium</i>	0.33	0.5	3.03	5.27	0.0033	0.0053	3	3
<i>Cladosporium</i>	0	0	0	0	0	0	3	3
<i>Curvularia</i>	0.5	0.07	4.54	0.70	0.0343	0.0004	3	3
<i>Didymella</i>	0.5	0	4.54	0.00	0.0049	0	3	3
<i>Epicoccum</i>	0.33	0	3.03	0.00	0.0033	0	3	3
<i>Fusarium</i>	0.2	0.15	1.82	1.58	0.0059	0.0024	2	2
<i>Penicillium</i>	0	0.24	0	2.48	0	0.0050	2	2
<i>Phoma</i>	0.5	0	4.54	0	0.0049	0	3	3
yeasts								
<i>Aureobasidium</i>	0	0.33	0	3.51	0	0.0018	4	4
<i>Candida</i>	0	0	0	0	0	0	4	4
<i>Cryptococcus</i>	0.56	0	5.05	0	0.0272	0	4	4
<i>Exophiala</i>	0	0.13	0	1.32	0	0.0007	4	4
<i>Hannaella</i>	0.83	0	7.57	0	0.0408	0	3	3
<i>Knufia</i>	0	0	0	0	0	0	4	4
<i>Malassezia</i>	0	0	0	0	0	0	4	4
<i>Papiliotrema</i>	0.5	0	4.54	0	0.0098	0	4	4
<i>Rhodotorula</i>	0.17	0	1.51	0	0.0016	0	3	3
ecological groups								
pathogens	0.26	0.09	2.39	0.96	0.1266	0.0126	2.30	2.21
opportunists	0.25	0.11	2.31	1.18	0.1220	0.0195	2.60	2.43
CAOs	0.29	0.12	2.63	1.22	0.0937	0.0110	2.91	2.63
doth. path.	0.29	0.06	2.60	0.62	0.0568	0.0016	2.25	2.55
doth. opp.	0.32	0.09	2.88	0.99	0.0590	0.0040	3.00	2.00
trem. path.	0.57	0.00	5.19	0.000	0.0672	0.0000	3.60	3.32
trem. opp.	0.35	0.03	3.30	0.30	0.0415	0.0002	3.00	3.06
hidden killers	0.38	0.10	3.46	1.09	0.0299	0.0016	2.95	2.69

Supplementary Table 23. Values of selection indices and virulence scores of different guilds of fungi. WGS = within-group selection, AMS = across-metacommunity selection, TAS = times average selection, SI = selection index and VIR = virulence score. VIR is the unweighted average VIR of all included genera.

Group	# species	WGS	AMS	TAS	SI	VIR
plastic						
pathogens	186	0.2634	0.4804	2.3942	0.1266	0.000
saprotrophs	225	0.1378	0.3039	1.2522	0.0419	
mutualists	28	0.1786	0.0490	1.6229	0.0088	
unidentified	488	0.0348	0.1667	0.3166	0.0058	n. a.
soil						
pathogens	273	0.0923	0.1323	0.9647	0.0126	
saprotrophs	414	0.1208	0.2646	1.2723	0.0320	
mutualists	55	0.0364	0.0106	0.3831	0.0004	
unidentified	1259	0.0890	0.5926	0.9371	0.0527	n. a.

Supplementary Table 24. Spearman correlation values between different combinations of traits in the plastisphere (bottom left) and soil MC (top right).

	Primary lifestyle	opportunism	extremotolerance	oligotrophy	melanisation	polymorphism	virulence score	
primary lifestyle		0.39	0.30	0.27	0.27	0.21	0.39	soil
opportunism	0.40		0.55	0.57	0.14	0.31	0.59	
extremotolerance	0.29	0.60		0.65	0.17	0.24	0.82	
oligotrophy	0.29	0.61	0.73		0.11	0.25	0.79	
melanisation	0.24	0.19	0.17	0.10		0.14	0.56	
polymorphism	0.13	0.39	0.31	0.36	0.13		0.44	
virulence score	0.36	0.65	0.84	0.82	0.53	0.53		
	plastic							

Supplementary Table 25. One-way ANOVA test p values from pairwise comparisons for significant differences in virulence score of ecological guilds in plastisphere and soil metacommunity.

	mutualists	pathogens	saprotrophs	
mutualists		0.0000	0.0019	soil
pathogens	0.0000		0.0000	
saprotrophs	0.2416	0.0000		
	plastic			

Supplementary Table 26. One-way ANOVA test p values from pairwise comparisons for significant differences in virulence score of ecological guilds between plastisphere and soil metacommunity.

		soil		
		mutualists	pathogens	saprotrophs
mutualists	plastic	0.3401	0.0000	0.1603
pathogens		0.0000	0.4364	0.0000
saprotrophs		0.0069	0.0000	0.6872