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BRIEF REPORT

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Microplastic fibres affect soil fungal communities depending on drought conditions with consequences for ecosystem functions

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Abstract

Microplastics affect soil functions depending on drought conditions. However, how their combined effect influences soil fungi and their linkages with ecosystem functions is still unknown. To address this, we used rhizosphere soil from a previous experiment in which we employed microplastic fibres addition and drought in a factorial design, and evaluated their effects on soil fungal communities. Microplastics decreased soil fungal richness under well-watered conditions, likely linked to microplastics leaching toxic substances into the soil, and microplastic effects on root fineness. Under drought, by contrast, microplastics increased pathogen and total fungal richness, likely related to microplastic positive effects on soil properties, such as water holding capacity, porosity or aggregation. Soil fungal richness was the attribute most affected by microplastics and drought. Microplastics altered the relationships between soil fungi and ecosystem functions to the point that many of them flipped from positive to negative or disappeared. The combined effect of microplastics and drought on fungal richness mitigated their individual negative effect (antagonism), suggesting that changes in soil water conditions may alter the action mode of microplastics in soil. Microplastic leaching of harmful substances can be mitigated under drought, while the improvement of soil properties by microplastics may alleviate such drought conditions.

INTRODUCTION

Microplastics are a global change factor affecting terrestrial ecosystems (Rillig, 2012; Rillig & Lehmann, 2020; Rochman et al., 2019). Microplastic particles (<5 mm) can enter the soil in a variety of shapes and polymer types through additions such as soil amendments, irrigation, flooding or atmospheric input (Bläsing & Amelung, 2018). Among these, fibres are one of the most abundant microplastic types, entering the ecosystem mainly through soil amendments and atmospheric deposition (Dris et al., 2015; Li et al., 2018). Their linear shape, chemical structure and physical properties contribute to explaining their effects on soil-water dynamics (de Souza Machado et al., 2019; Lozano, Aguilar-Trigueros, Onandia, et al., 2021). That is, fibre addition can increase soil water holding capacity and lead to differential retention of water, altering soil water conditions, with consequences for soil functionality and plant performance (de Souza Machado et al., 2019; Haque & Fan, 2023; Lozano, Aguilar-Trigueros, Onandia, et al., 2021). Previous research also showed that microplastic fibres affect soil aggregation, soil microbial activity (Boots et al., 2019; Liang et al., 2021), litter decomposition and soil respiration. Nonetheless, many of these effects highly depended on the soil water status (Lozano, Aguilar-Trigueros, Onandia, et al., 2021), for example,

litter decomposition increased with microplastic fibres under well-watered conditions, while decreasing under drought conditions. Microplastic fibres can cause effects of a similar magnitude as drought on soil ecosystem multifunctionality Lozano, Aguilar-Trigueros, Onandia, et al., 2021, which can propagate to the aboveground, as microplastics and drought alter the productivity and composition of plant communities most likely via effects on soil biota (Lozano & Rillig, 2020).

Soil biota have a key role in determining the ecological responses of terrestrial ecosystems to global change factors (Bardgett & van der Putten, 2014; Rillig et al., 2019). In particular, soil fungi are involved in a series of crucial soil processes such as litter decomposition, soil organic matter turnover, nutrient mineralization, symbiosis, pathogenesis and plant growth (Bardgett & van der Putten, 2014). Therefore, microplastics and drought effects on terrestrial systems are likely strongly modulated by soil biota. On the one hand, microplastics may affect the composition of soil bacterial communities by altering their abundance, diversity and activity with consequences for key processes such as nitrification (Fei et al., 2020; Huang et al., 2019; Seeley et al., 2020; Zhao et al., 2021), or potentially affect soil fungal communities as observed in aquatic systems (Kettner et al., 2017), with consequences on soil micro-food webs (Liu et al., 2023). Indeed, research has shown that microplastics could accumulate fungal pathogens on their surfaces (Gkoutselis et al., 2021), leading to pathogen transfer into the soil matrix (Yu et al., 2021). Likewise, previous studies showed that responses to microplastics diverge for different fungal taxa (Wang et al., 2020). However, despite these emerging results, our knowledge about microplastic effects on soil fungal communities is rather scarce, compared to data available for bacteria.

By contrast, the knowledge about the effects that drought may have on fungal communities is comparatively extensive (e.g., Lozano, Aguilar-Trigueros, Roy, & Rillig, 2021; Lozano et al., 2022; Meisner et al., 2018; Ochoa-Hueso et al., 2018). Drought may decrease or increase the relative abundance of pathogenic species belonging to *Chlamydiae* and *Tenericutes*; decrease mutualist abundance such as that of *Glomeromycetes* (Ochoa-Hueso et al., 2018); or increase the abundance and richness of saprotrophic communities Lozano, Aguilar-Trigueros, Roy, & Rillig, 2021). So far, the effects of these two global change factors have been evaluated in isolation, ignoring that global change factors act in concert (Rillig et al., 2019). Microplastics and drought have a clear combined effect on soil functionality and plant performance (Lozano, Aguilar-Trigueros, Onandia, et al., 2021; Lozano & Rillig, 2020) and therefore, a potential joint effect on soil fungal communities; therefore, their combined effect could be synergistic or antagonistic, exacerbating or mitigating the individual effects of each factor (Morris et al., 2022; Piggott et al., 2015).

Fungi are a heterogeneous kingdom that encompasses multiple taxa belonging to different functional (i.e., pathogens, saprotrophs or mutualists) (Madigan et al., 2006), or morphological groups (i.e., filamentous, yeast or zoosporic). Here, we aimed to determine the individual and interactive effects of microplastic fibre addition and drought on overall fungal communities, and on different functional, taxonomic and morphological groups within these communities. We additionally tested if the combined effect of drought and microplastics on soil fungal communities differs from what is predicted by an additive, multiplicative or dominance model, and thus may lead to antagonistic or synergistic interactions. In addition, by combining the present results with previous data Lozano, Aguilar-Trigueros, Onandia, et al., 2021), we were able to assess to what extent the linkages between fungal community attributes and ecosystem functions (e.g., plant productivity, soil aggregation, soil respiration, β-glucosidase and litter decomposition) deviate in the presence of microplastics. To achieve these goals, we used rhizosphere soil from a previous experiment where microcosms of plant communities grew in a factorial design that included microplastic fibre addition and drought treatment (Lozano & Rillig, 2020) and then characterized soil fungal communities using ITS amplicon Illumina sequencing. We expected that the effects of microplastic fibre addition on fungal abundance, richness and composition would be strongly dependent on soil water availability. We also expected an interactive effect of microplastic and drought (i.e., an antagonistic or synergistic effect), and that alterations in fungal communities would help explain the effects of microplastics and drought on ecosystem functions.

EXPERIMENTAL PROCEDURES

Experimental setup

In May 2019, we established a fully crossed orthogonal design ($n = 5$) where a plant community consisting of seven grassland species (i.e., *Festuca brevipila, Holcus lanatus, Calamagrostis epigejos, Achillea millefolium, Hieracium pilosella, Plantago lanceolata and Potentilla argentea*) grew under the effect of microplastics (absent, present) and soil water conditions (drought and well-watered) (see details on Lozano & Rillig, 2020). Sandy loam soil (0.07% N, 0.77% C, pH 6.66) was collected from Dedelow, Brandenburg, Germany (53° 37′ N, 13° 77′ W) where such plant species naturally grow. The soil was dry when collected, and then it was sieved (4 mm mesh size), homogenized and mixed with microplastic fibres at a

concentration of 0.4% w/w or received no microplastic (Polyester fibres of ~30 μm diameter manually cut to 1.28 ± 0.03 mm length; Rope Paraloc Mamutec polyester white, item number, 8442172, Hornbach.de). The soil was subjected to 70% or 30% of water holding capacity, the latter representing drought conditions. Two months later, at harvest time, shoot mass, root mass, soil aggregation, litter decomposition, β-glucosidase and soil respiration were measured Lozano, Aguilar-Trigueros, Onandia, et al., 2021), and rhizosphere soil, which is the soil that remained attached to the roots after gentle movement, were sampled in different parts of the pot and kept at −80°C for the molecular analyses on which we report here.

Molecular analyses of fungal communities

We extracted DNA from 0.25 g of homogenized soil from each of the 20 soil samples using the DNeasy Power Soil Pro Kit (Qiagen, GmbH, Germany), following the manufacturer's directions. Then, fungal sequences were amplified from soil DNA extracts using the fITS7 and ITS4 primers, which in combination yield amplicons that span the fungal ITS2 region (Ihrmark et al., 2012). This primer set is highly specific for fungi and outperforms alternative combinations in terms of preserving the richness and composition of fungal communities. We added 1 μL DNA to 25 μL reaction mixture (7 μL buffer (10 mM magnesium chloride), 1 mL deoxynucleoside triphosphate (10 mM), 1 μL fITS7 (10 μM), 0.5 μL ITS4 (10 μM) and 0.5 μL DNA polymerase (1 U μL-1) (Kapa Biosystems Wilmington, MA). PCR consisted of initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 98°C for 20 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s and then final elongation at 72°C for 5 min. PCR products were purified using magnetic beads (CleanNA; GC Biotech B.V., Waddinxveen, the Netherlands) following the manufacturer's directions. Purified products were indexed for multiplexing following the same protocol as before, but using 12 cycles. DNA concentration of purified amplicons was measured using Quant-IT PicoGreen dsDNA Reagent (Invitrogen) to ensure an equimolar pooling. Afterward, amplicons were sequenced on an Illumina MiSeq instrument using v.3 2 × 300 bp cycles chemistry at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv).

Bioinformatics

We resolved raw sequences to amplicon sequence variants (ASVs) using the dada2 pipeline (Callahan et al., 2016), customizing the ITS workflow version 1.8 (https://benjjneb.github.io/dada2/ITS_workflow.html). We removed undefined base calls (Ns) and trimmed the adapters and primer oligos using Cutadapt v. 3.4 (Martin, 2011). Since the quality of forward reads was generally good, while that of reverse reads rapidly declined after the 150th base pair (bp), merging was avoided and only forward reads were used to define ASVs. This approach is more accurate than the alternatives in terms of representing the diversity and composition of mock communities (Pauvert et al., 2019). Forward reads were subsequently filtered based on the following parameters: (i) Only reads with lengths larger than 50 base pairs were allowed, (ii) the ends of each read were trimmed once quality scores fell below 10 and (iii) the maximum expected error rate was set to 2. A machine-learning algorithm was used to model the error frequency patterns of the filtered reads, while a de-replication function was used to find unique variants among those. Based on the error frequency patterns previously learned, the sample inference algorithm native to Dada 2 was then used to define ASVs. Chimeras were removed. Raw reads were subsequently mapped back to the newly defined ASVs, to obtain a read abundance table. Taxonomic identity was assigned using the RDP Bayesian classifier algorithm available in dada2 (Wang et al., 2007) against UNITE database 8.3 (Abarenkov et al., 2010). The minimum number of matches required to assign a taxonomic identity was set to 50 out of 100 tries. Fungal taxa were assigned to one of three functional groups: pathogens, saprotrophs and mutualists, and one of three morphological groups: filamentous, yeast and zoosporic, based on functional guild and growth form data present in the 'FungalTraits' database (Põlme et al., 2020), Briefly, we loaded the database into R version 4.1.2 (R Core Team, 2021) and merged it with the taxonomy table using join functions from the package collection tidyverse (Wickham et al., 2019). Thus, ASVs that had a genus identity were supplemented with information on functional groups and growth forms based on the 'primary_lifestyle' and 'Growth_form_template' columns of the original trait database. Taxa were assigned to each of the three fungal functional groups according to their primary lifestyle. Dimorphic yeasts were included as 'yeast'.

Normalization of reads to calculate richness

To account for an unequal number of reads across samples, the ASV abundance table was sub-sampled without replacement to a minimum depth of 26,572 reads per sample, which corresponds to the median library size across samples. Sub-sampling without replacement remains a widely used normalization technique and it is valid when measuring diversity and community composition using presence-absence data

(Weiss et al., 2017), but see McMurdie and Holmes (2014) for a different view. Normalization of abundance across samples eliminated one ASV and discarded 170,102 reads (29% of the total reads). Since our chosen normalization technique discarded a small portion of reads, relative abundance changes presented in subsequent sections should be regarded with caution. One replicate of the combined microplastic and drought treatment, only had 270 reads, thus considered defective and excluded from the analyses. A rarefaction curve indicated that at the chosen normalization depth all samples had reached saturation in terms of ASV richness (Figure S1).

Statistical analysis

We evaluated the effect of microplastics and drought on the abundance, richness and composition of functional (pathogens, saprotrophs and mutualists), taxonomic (main phyla and classes), morphological groups (filamentous, yeasts and zoosporic fungi) and overall fungal communities. Sequences reported as ectomycorrhizal were not included in the functional group analysis (i.e., mutualists), given ectomycorrhizal fungi are not the main mutualist associated with herbaceous plants and their ecology fundamentally differs from that of arbuscular mycorrhizal fungi. We only considered fungal groups with read abundances greater than 1% of the total reads before normalization.

Microplastic, drought and their interaction were considered explanatory variables. Fungal richness and abundance were analysed using linear models. When the assumptions of normality and variance homoscedasticity were not fulfilled, we implemented the 'varIdent' function from the 'nlme' R package (Pinheiro et al., 2021) and as suggested by Zuur et al. (2009). That is, we accounted for heterogeneity in the drought treatment for certain variables: *Basidiomycota* and filamentous abundance and *Chytridiomycota* richness. Similarly, we accounted for heterogeneity in the microplastic treatment for these variables: mutualist abundance, *Ascomycota* and *Dothideomycetes* richness. Since after this procedure data, some fungal groups were not suited for linear models, we implemented generalized linear models with a quasibinomial distribution and a logit link function to avoid overdispersion for *Chytridiomycota*, *Agaricomycetes, Dothideomycetes, Sordariomycetes, Spizellomycetes* and zoosporic abundance. Likewise, we used a Poisson distribution, detected overdispersion and corrected the standard errors using a quasi model (also called quasipoisson) (Zuur et al., 2009) with a logit link function for mutualists, *Leotiomycetes, Pezizomycetes* and yeast fungal richness, as this distribution best fits this type of data (Zuur et al., 2009). After this, the models were ready and we were able to perform multiple comparisons to assess whether fungal attributes (i.e., abundance and richness) differed within each microplastic or drought treatment. For that, we used the function 'glht' from the 'Multcomp' R package, along with the Tukey test and the function 'sandwich' for the eponymous R package (Hothorn et al., 2008). This last function provided a heteroscedasticity-consistent estimate of the covariance matrix (Bretz et al., 2011; Zeileis, 2006). Fungal abundance (i.e., relative abundance) was calculated based on the total number of reads within each sample divided by the total number of sequences.

To evaluate microplastic and drought effects on fungal community composition at the functional, taxonomic and morphological levels, ASVs abundance tables were transformed to presence-absence data using the function 'decostand' from the 'vegan' R package (Oksanen et al., 2021). Then, this table was used as the response variable in a constrained redundancy analysis by using the function 'rda' from the same R package (Borcard et al., 2018). The constraints for each RDA were specified as follows: microplastics + drought + microplastics:drought. The significance of each constraint was tested using 9999 permutations of the presence-absence table using the function 'anova.cca' from the 'vegan' R package. Finally, to visualize the compositional distance between communities, non-metric multidimensional scaling was employed using the function 'metaMDS' from the same R package. To do so, the distance was estimated using the binary version of the Jaccard dissimilarity index by using the function 'vegdist'. Then, the global NMDS algorithm was run to find a stable solution in 4 dimensions with a minimum number of 200 random starts. A bidimensional plot was selected.

In addition, we calculated the predicted combined effect of microplastics and drought, using three contrasting null models initially proposed by Folt et al. (1999): the simple additive, simple multiplicative and dominance models. These null models predicted the joint impact of drought and microplastics based on their individual responses relative to the control. Following Morris et al. (2022), we calculated the additive: $S_A + S_B - C$; multiplicative: $S_A S_B/C$ and dominance model: (S_A, $|S_A - C| > |S_B - C|$, otherwise S_B), where the fungal response to the individual effect of drought, microplastics and control corresponds to S_A, S_B and C, respectively. Effect sizes were calculated using Hedge's g as a standardized mean difference, which considers sample size when calculating the overall effect size between the observed response and the predicted model. Each model calculation was repeated 99 times by bootstrap sampling with replacement (Carvalho et al., 2010).

Lastly, we assessed the relative importance of fungal communities to ecosystem functions in soils with or without added microplastics, by using the metric 'pmvd' from the 'Relaimpo' R package (Grömping, 2006),

which is based on sequential R^2 values, that takes care of the dependence on orderings by weighted averages with data-dependent weights and also guarantees that a regressor with a coefficient of zero is assigned a relative importance of zero (Grömping, 2006). For this analysis, richness, abundance of pathogen, saprotroph and mutualist and total fungal richness were selected, while data on ecosystem functions were taken from Lozano, Aguilar-Trigueros, Onandia, et al. (2021). In addition, we performed linear regressions by using the function 'lmodel2' from the eponymous R package (Legendre, 2018). When needed, variables were log-transformed to meet normality and heteroscedasticity assumptions. The abundance and richness of mutualists were log+2 transformed. Statistical analyses were done with R version 4.1.2 (R Core Team, 2021). Data selection and organization were achieved by employing package phyloseq (McMurdie & Holmes, 2013). All figures were produced by using ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2020) and the drawing editor Inkscape (Inkscape Project, 2022).

RESULTS

Sequencing performance

We obtained a total of 696,535 forward reads, which were resolved to 1137 ASV. Pathogens, saprotrophs and mutualists were represented by 108, 440 and 24 ASVs, respectively. *Ascomycota* was represented by 451 ASVs, *Basidiomycota* by 258 ASVs and *Chytridiomycota* by 98 ASVs, which were classified depending on their function as pathogens, saprotrophs and mutualists or had an unknown function. Some were classified as ectomycorrhiza (mainly belonging to the genera *Cortinarius* which is found to be associated with deciduous trees) (Borovička et al., 2017) (Figure S2). The most abundant classes (with a total relative abundance higher than 1%), within each main phylum were the *Ascomycota: Dothideomycetes, Leotiomycetes, Pezizomycetes and Sordariomycetes*; the *Basidiomycota: Agaricomycetes* and *Tremellomycetes*; and the *Chytridiomycota: Spizellomycetes* (Figure S2). Other minor phyla (with relative abundance lower than 1%) were *Mortierellomycota, Glomeromycota, Mucoromycota, Rozellomycota* and *Zoopagomycota*.

Microplastics decrease the richness of pathogens, saprotrophs, mutualists and the whole fungal community under well-watered conditions while having the opposite effect under drought

We found that microplastics influenced the richness of the different functional groups and the whole fungal community, in a pattern that depended on the soil water status (Figure 1A–E). That is, under well-watered conditions, pathogens, saprotrophs, mutualists and the whole fungal community decreased in richness with microplastics by 47%, 33%, 45% and 40%, respectively, ($p < 0.02$, Table 1), while under drought conditions, although all the fungal functional groups tended to have an opposite pattern increasing in richness with microplastics, the statistical evidence was only clear for pathogens and the whole fungal community, whose richness increased by 55% and 57%, respectively ($p < 0.01$, Figure 1A,C). The abundance of functional groups was not affected by microplastics or drought, in contrast to composition (Table 1). The whole fungal community composition differed in both well-watered and droughted soils depending on the presence or absence of microplastics. Saprotroph community composition in each soil water treatment differed when the microplastics were present versus absent while for mutualists this difference was only evident in droughted soils. Pathogen assemblage composition was not affected by microplastics or drought (Figure 2B). Drought and microplastics in isolation each decreased fungal community richness in comparison with the control. However, when both factors were present, total fungal richness was similar to that of control soils. Likewise, the whole fungal community composition differed in relation to the control in both droughted and microplastic-polluted soils, but again, this pattern disappeared when both factors were present. Such responses were consistent across the different fungal functional, taxonomical and morphological groups (Figure 2A–J).

Microplastics decrease the richness of the main phyla and classes under well-watered conditions while having the opposite effect under drought

Microplastic and drought effects on the richness and composition of the most abundant fungal phyla were similar to those observed for fungal functional groups. (Figure 1F–H, Table 2). That is, under well-watered conditions, *Ascomycota, Basidiomycota* and *Chytridiomycota* decreased in richness with microplastics by 43%, 37% and 36%, respectively ($p < 0.03$; Table 2), while under drought conditions, although fungal richness tended to increase when microplastics were present, the evidence was not sufficient. Likewise, when both factors were present, phylum richness was similar to that observed in control samples (i.e., well-watered without microplastics added). Such strong dependence of microplastic effects on soil water status was also found when assessing the effect on richness at the class level (Figure S3a–g, Table S1). In terms of abundance, the most abundant phyla and classes were not

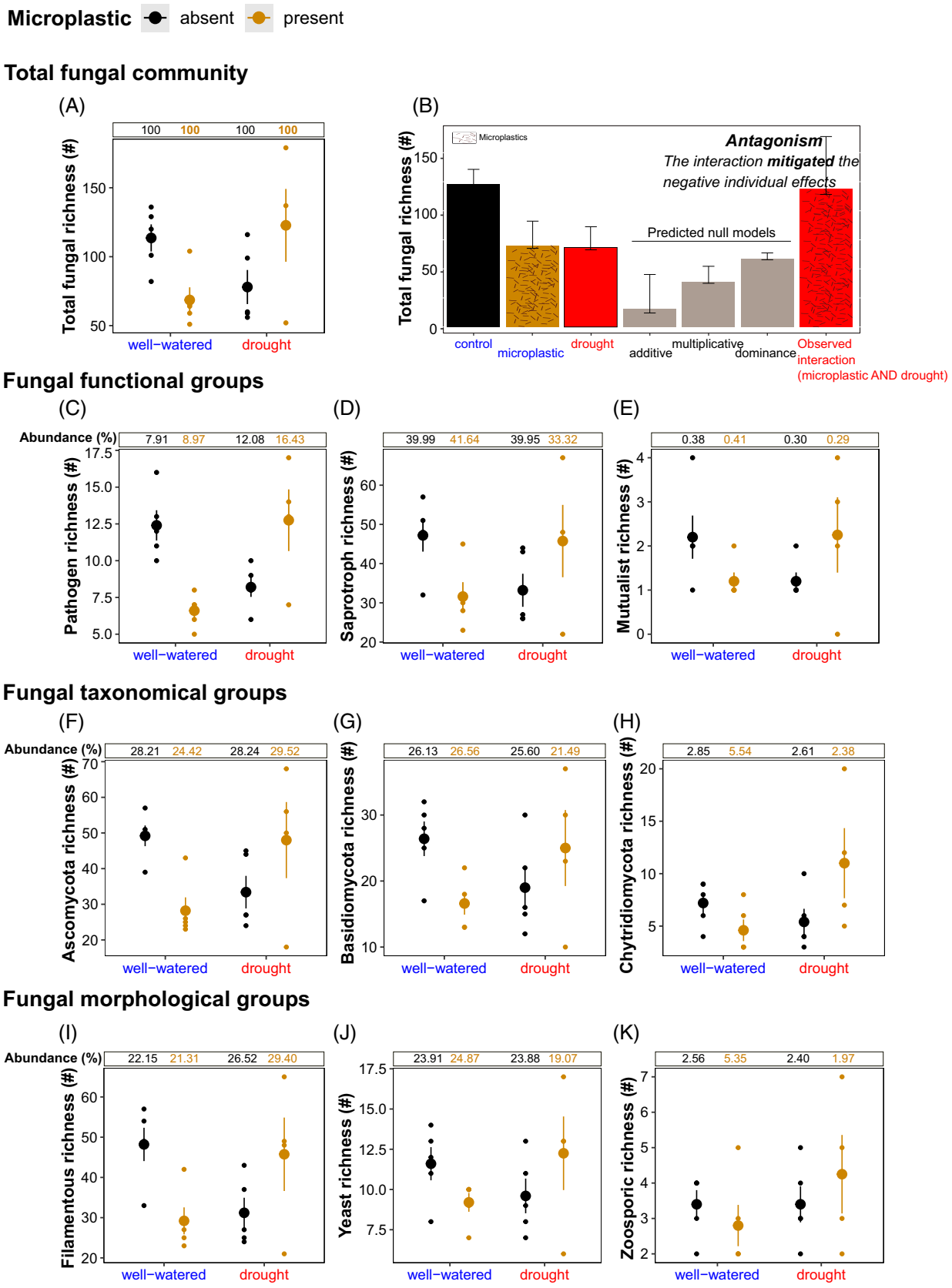

FIGURE 1 Fungal richness and abundance. Microplastic and drought effects on the richness of (A) total fungal community and richness and abundance of (C–E) fungal functional groups, (F–H) fungal taxonomical groups and, (I–K) fungal morphological groups. Panel (B) shows the individual effect of drought and microplastics and their combined effect (observed interaction) on total fungal richness. The predicted null model results are also included. Richness is represented by their mean and standard error. Data points are shown as circles. The numbers above the bars show the mean relative abundance (percentage calculated based on the total number of sequences). See *p* values in Tables 1 and 2; $n = 5$.

TABLE 1 Microplastics and drought effects on attributes of fungal functional groups.

Functional group	Fungal attribute	Microplastics (*M*)	Drought (*D*)	*M* × *D*
Pathogens	Abundance	0.25 (0.62)	1.58 (0.22)	0.05 (0.81)
	Richness	0.75 (0.39)	0.36 (0.55)	**21.49 (<0.01)**
	Composition	1.05 (0.21)	1.04 (0.28)	*1.12 (0.06)*
Saprotrophs	Abundance	0.27 (0.61)	1.03 (0.32)	1.13 (0.30)
	Richness	0.19 (0.66)	0.02 (0.88)	**7.03 (0.01)**
	Composition	1.00 (0.43)	1.00 (0.42)	**1.19 (<0.01)**
Mutualists	Abundance	0.05 (0.81)	2.34 (0.14)	0.05 (0.81)
	Richness	0.006 (0.93)	0.07 (0.93)	**5.97 (0.02)**
	Composition	0.82 (0.96)	0.92 (0.70)	*1.15 (0.10)*
Overall fungal community	Richness	0.04 (0.83)	0.20 (0.65)	**9.44 (<0.01)**
	Composition	1.01 (0.32)	1.02 (0.20)	**1.21 (<0.01)**

Note: Relative abundance, richness, and composition of pathogens, saprotrophs, mutualists and the total community. Microplastic (*M*), drought (*D*) and their interaction were considered as explanatory variables. Fungal composition (NMDS) was analysed through permutational multivariate ANOVA (PERMANOVA). *F* and *p* values (in parentheses) are shown. $p < 0.05$ in bold; $p < 0.1$ in *italic*. df = 1, $n = 5$.

affected by either microplastics, drought or a combination of both (Table 2). A notable exception was the class *Leotiomycetes* (*Ascomycota*) which decreased by 19% with drought (Figure S3c, Table S1). The composition of fungal phyla was also affected by the combined effect of microplastics and drought (Table 2). *Ascomycota* and *Chytridiomycota* composition differed in well-watered soils when the microplastics were present, an effect that was not observed in droughted soils (Figure 1F,H).

Microplastics decrease the richness of filamentous fungi and yeasts under well-watered conditions while having the opposite effect under drought

Filamentous fungi and yeasts, represented by 451 and 104 ASVs, were affected by microplastics and drought. That is, under well-watered conditions, filamentous fungi and yeasts decreased in richness by 39% and 21%, respectively, with microplastics in soil (Figure 1I,J Table 2). By contrast, under drought conditions, both morphological groups tended to increase in richness with microplastics, although only slightly. Likewise, when microplastics and drought conditions were present, both morphological groups maintained their richness in relation to control soils. Zoosporic fungi, represented by 41 ASVs were not influenced by microplastic, drought or the combination of them (Figure 1K), as observed for all morphological groups in terms of abundance. Community composition was affected by the combined effect of our factors (Table 2). Filamentous and zoosporic fungal communities differed in well-watered soils when the microplastics were present, an effect that was not observed in droughted soils (Table 2, Figure 2H,J).

The combined effect of microplastics and drought on total fungal richness was opposite to what was predicted by null models

We found that the individual effect of drought and microplastics decreased total fungal richness while by contrast, their combined effect tended to increase it in comparison with control soils (Figure 1B). Such a combined effect was not predicted by any of our null models. The observed combined effect was the opposite and deviated from what was predicted (Figure 1B and Figure S4). That is, the additive model predicted that such a combined effect would decrease total fungal richness by 87%, while the multiplicative model predicted a decrease of 68%, and the dominance model a decrease of 52%, in comparison with the control (Figure 1B). However, by contrast, the observed combined effect tended to increase soil fungal richness in comparison with control soils (Figure 1B).

The relative contribution of soil fungal community attributes to ecosystem functions is affected by the presence of microplastics

The relative importance of fungal attributes to ecosystem functions showed that the relationships between soil fungal community attributes and ecosystem functions were strongly altered by the presence of microplastics in soil (Figure 3). For instance, in soils without added microplastics, pathogen richness was the fungal attribute that explained shoot mass ($\beta = 63.89\%$), saprotroph richness better explained root mass ($\beta = 45.68\%$), while mutualist richness most explained

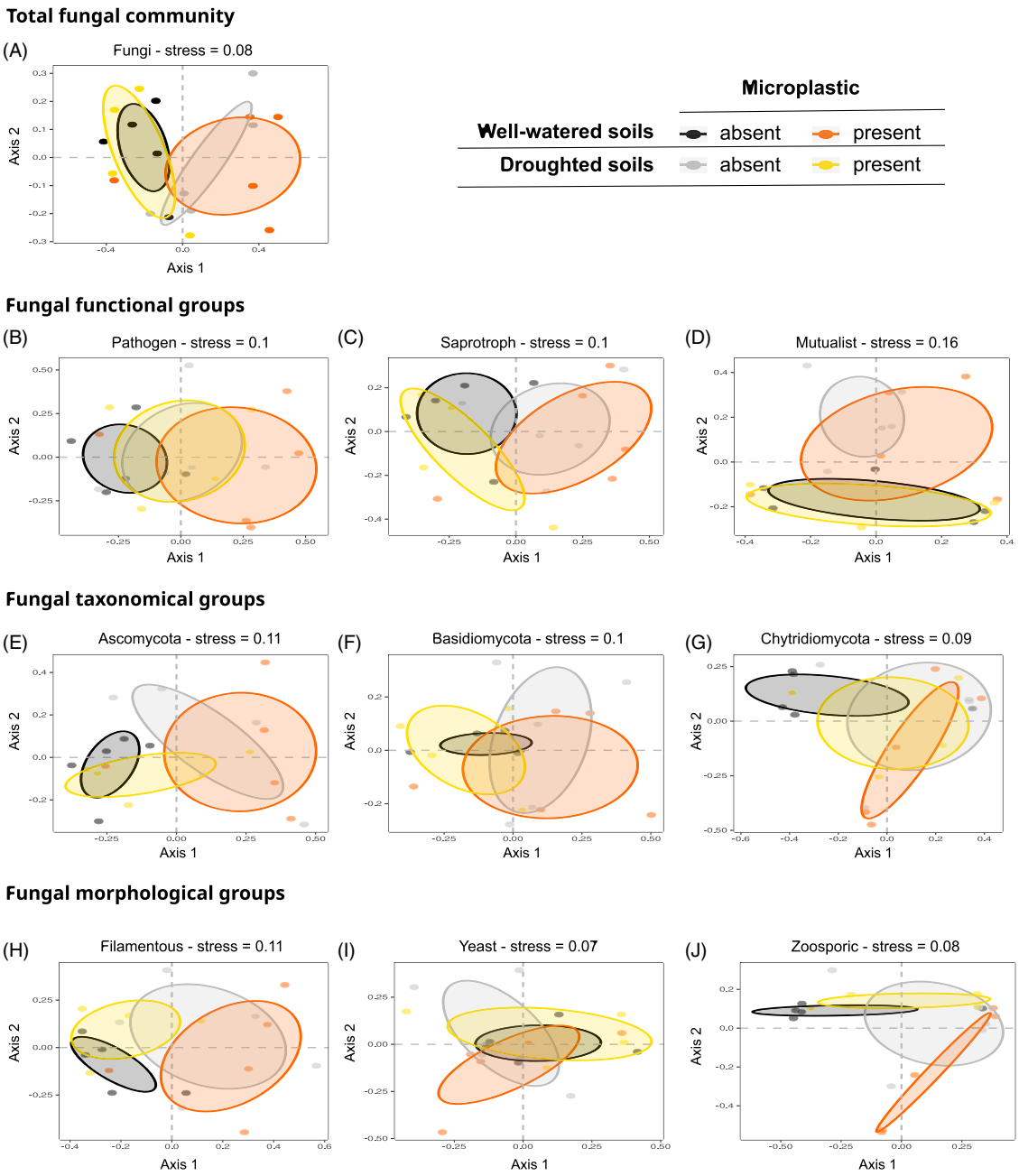

FIGURE 2 Fungal community composition. Non-metric multidimensional scaling (NMDS) of Jaccard dissimilarities between samples belonging to (A) total fungal community, (B–D) fungal functional groups, (E–G) fungal taxonomical groups and (H–J) fungal morphological groups. The first two axes of each ordination are shown. Each point represents a sample in the bi-dimensional space. The coloured ellipses represent one standard deviation of the group centroid.

soil aggregation ($\beta = 30.96\%$) (Figure 3A,B,C). However, the presence of microplastics in soil altered or weakened such relationships. As such, in soils with microplastics, pathogen richness contribution to shoot mass decreased almost three times ($\beta = 22.03\%$), root mass was better explained by mutualist richness ($\beta = 39.84\%$), while the relationship between the latter and soil aggregation was weaker ($\beta = 17.54\%$) compared to that in soils without microplastics. See additional relationships in Figure 3.

Relationships between soil fungal community attributes and ecosystem functions are affected by the presence of microplastics in soil

We observed that in soils without added microplastics, total fungal richness and saprotroph richness were positively correlated with shoot mass and root mass, respectively ($R^2 = 0.39$, $p = 0.04$; $R^2 = 0.48$, $p = 0.02$). By contrast, these relationships flipped to

TABLE 2 Microplastics and drought effects on fungal taxonomical and morphological group attributes (relative abundance, richness and composition).

Fungal group	Phyla/growth form	Fungal attribute	Microplastics (*M*)	Drought (*D*)	*M* × *D*
Taxonomical group	Ascomycota	Abundance	0.14 (0.70)	0.36 (0.55)	0.40 (0.53)
		Richness	0.55 (0.46)	2.84 (0.11)	**9.37 (<0.01)**
		Composition	0.95 (0.79)	0.98 (0.54)	**1.23 (<0.01)**
	Basidiomycota	Abundance	0.01 (0.89)	1.39 (0.25)	1.06 (0.31)
		Richness	0.49 (0.49)	0.00 (0.99)	**5.53 (0.03)**
		Composition	*1.05 (0.09)*	0.99 (0.51)	**1.09 (0.02)**
	Chytridiomycota	Abundance	1.61 (0.22)	2.15 (0.16)	1.19 (0.29)
		Richness	1.09 (0.31)	1.17 (0.29)	**5.32 (0.03)**
		Composition	1.07 (0.10)	*1.09 (0.08)*	**1.21 (<0.02)**
Morphological group	Filamentous	Abundance	0.01 (0.90)	1.91 (0.18)	0.17 (0.67)
		Richness	0.38 (0.54)	0.05 (0.81)	**10.75 (<0.01)**
		Composition	1.01 (0.36)	0.98 (0.64)	**1.17 (0.01)**
	Yeast	Abundance	0.79 (0.38)	2.33 (0.14)	2.56 (0.13)
		Richness	0.00 (0.97)	0.09 (0.76)	**4.16 (0.05)**
		Composition	0.96 (0.68)	1.04 (0.23)	1.06 (0.16)
	Zoosporic	Abundance	1.57 (0.22)	2.42 (0.14)	1.55 (0.23)
		Richness	0.00 (0.94)	1.10 (0.31)	1.23 (0.28)
		Composition	1.02 (0.34)	1.10 (0.16)	**1.33 (<0.01)**

Note: Microplastic (*M*), drought (*D*) and their interaction were considered as explanatory variables. Fungal composition was analysed through permutational multivariate ANOVA (PERMANOVA). *F* and *p* values (in parentheses) are shown. $p < 0.05$ in bold. df = 1, $n = 5$.

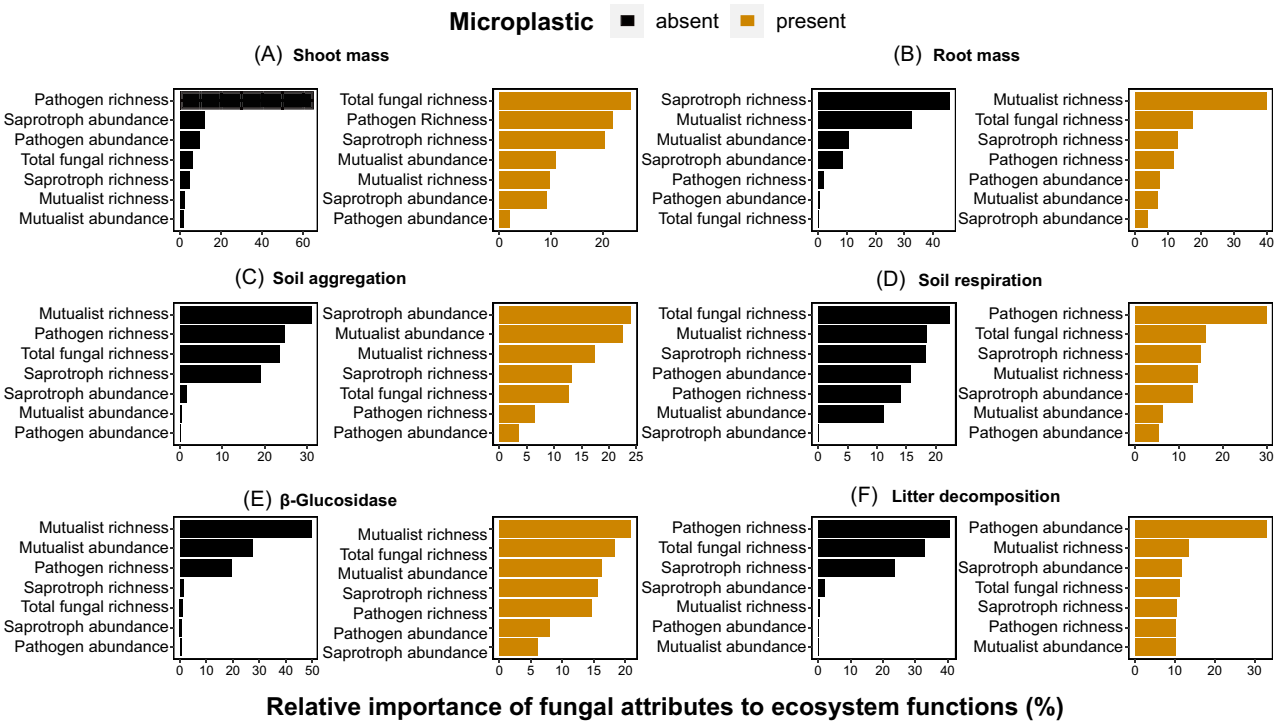

FIGURE 3 The relative importance of fungal attributes (total richness, mutualist, pathogen and saprotroph abundance and richness) to ecosystem functions ((A) shoot mass, (B) root mass, (C) soil aggregation, (D) soil respiration, (E) β-glucosidase and (F) litter decomposition) in soils without and with added microplastics fibers (absent, present). Data on ecosystem functions were extracted from Lozano, Aguilar-Trigueros, Onandia, et al. (2021). The proportionate contribution of each attribute considered both its direct effect (i.e., its correlation with each function) and its effect when combined with the other variables in the regression equation. The metrics 'pmvd' was used for the calculation and the down-weighting via the cluster was taken into account.

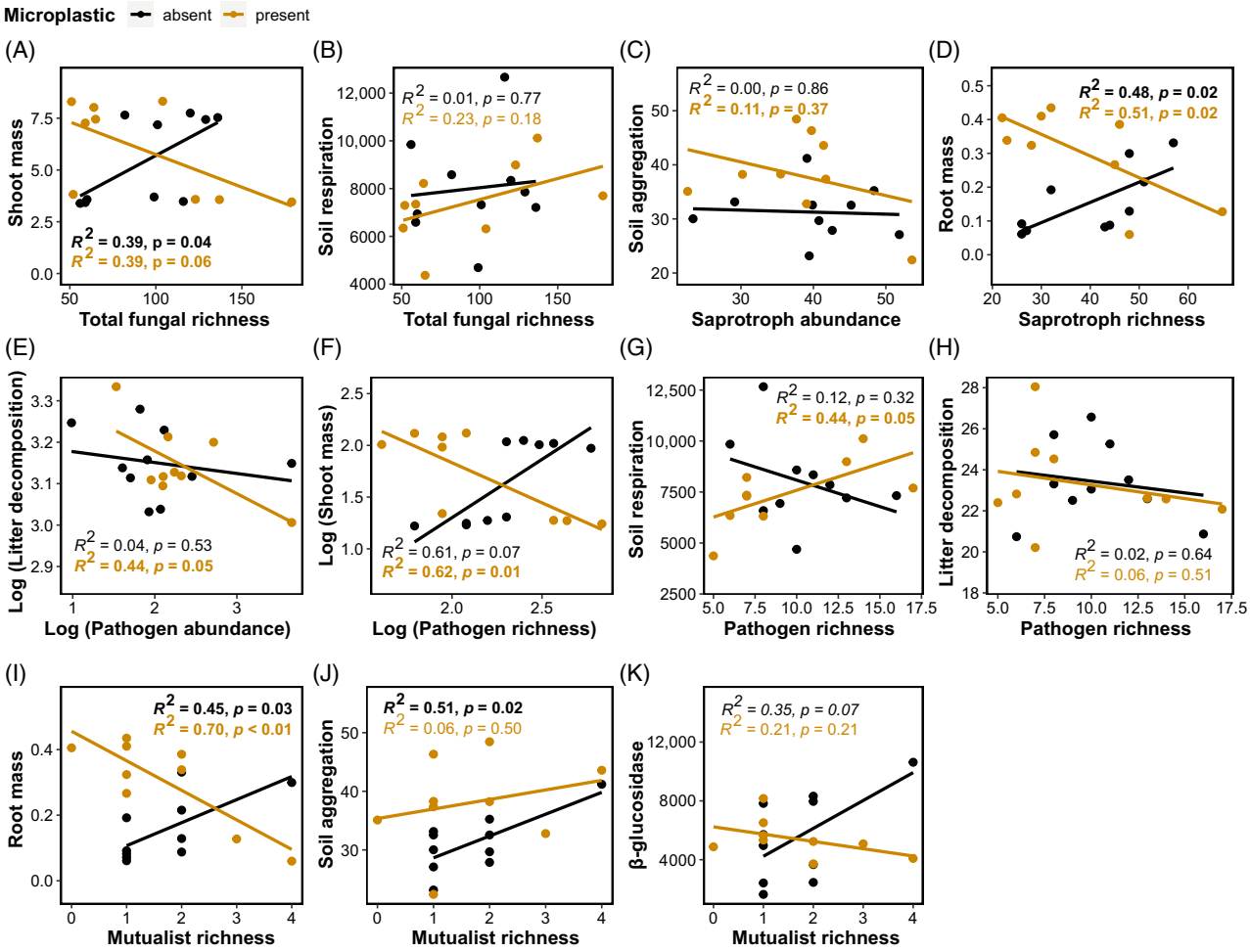

FIGURE 4 Relationships between the fungal functional attributes and ecosystem functions ((A,F) shoot mass, (B,G) soil respiration, (C,J) soil aggregation, (D,I) root mass, (E,H) litter decomposition, (K) β-glucosidase, in soils without and with added microplastic fibres (absent, present). These fungal attributes were those that better explained the different ecosystem functions (see Figure 3).

negative when microplastics were added into the soil ($R^2 = 0.39$, $p = 0.06$; $R^2 = 0.51$, $p = 0.02$) (Figure 4A,D). In soils without microplastics, pathogen richness was positively correlated with shoot mass ($R^2 = 0.61$, $p = 0.07$) while the opposite was true in soils with microplastics ($R^2 = 0.62$, $p = 0.01$) (Figure 4F). Likewise, in soils without microplastics mutualist richness was positively correlated with root mass ($R^2 = 0.45$, $p = 0.03$), soil aggregation ($R^2 = 0.51$, $p = 0.02$) and β-glucosidase ($R^2 = 0.35$, $p = 0.07$) (Figure 4I–K). However, these relationships disappeared ($R^2 = 0.06$, $p = 0.50$; $R^2 = 0.21$, $p = 0.21$) or changed their direction (negative correlation with root mass [$R^2 = 0.70$, $p < 0.01$]) with microplastics added. Pathogen abundance was negatively correlated with litter decomposition in soils with microplastics ($R^2 = 0.44$, $p = 0.05$), while such a relationship was absent in soils without microplastics ($R^2 = 0.04$, $p = 0.53$) (Figure 4E). See the additional relationships between fungal attributes and ecosystem functions in Figure S5.

DISCUSSION

Our fully factorial experiment revealed that the negative effects of microplastic pollution on rhizosphere fungal communities are strongly modulated by soil moisture availability. The individual negative effect of microplastics on soil fungal richness and community composition was mitigated when acting in combination with the additional stress factor of drought. Such a pattern was consistent when partitioning the assessment across different functional, taxonomic or morphological groups, suggesting that microplastics exert different modes of action when acting in isolation or in combination with drought. In isolation (under well-watered conditions), microplastics chemical toxic substances can be leached into the soil matrix, especially those chemicals of lower partition that are easily extractable by water, which may hinder soil fungal richness. By contrast, the presence of drought limits this mode of action of microplastics. That is, under drought conditions, microplastic fibres indirectly help

hold water for longer and contribute to soil aggregation, mitigating drought effects.

Microplastic fibres decrease the richness of all soil fungal groups under well-watered conditions

Under well-watered conditions, the presence of microplastic fibres in the soil decreased the richness of functional, taxonomical and morphological groups, and of the whole fungal community. This can be caused by microplastic leaching toxic chemicals into the soil, and with microplastic effects on root morphological traits.

Microplastic fibres can contain hazardous monomers (Lithner et al., 2011) and a wide range of additives are used to enhance fibre properties such as flexibility, colour or durability (Hahladakis et al., 2018). Added to this, their high surface area facilitates the absorption of different pollutants such as polycyclic aromatic hydrocarbons (Wiśniowska & Włodarczyk-Makuła, 2022). Such toxic substances can be extractable by water (Kim et al., 2020), which favours their leaching into well-watered soils, perhaps explaining the decrease in soil fungal richness. The toxic effect of microplastic fibres has been also observed in planktonic crustaceans such as *Daphnia magna* (Jemec et al., 2016) or soil nematodes (Kim et al., 2020), but see Oono et al. (2020) for a contrary view. Likewise, the extra carbon provided by microplastic fibres (Rillig, 2018), could also favour soil saprotrophs with rapid proliferation (r-strategists), which may prompt the observed increase in saprotrophs abundance, which in the end may negatively affect fungal diversity. Previous research has observed such trends in some bacterial groups exposed to microplastics (Wei et al., 2022). In addition, microplastic fibres have been observed to decrease root fineness (Lozano & Rillig, 2020), which may change the quality and quantity of root exudates (Warembourg et al., 2003; Williams & Vries, 2020), likely explaining the decrease not only in saprotrophs richness, a fungal group known to establish positive associations with fine roots (Lozano, Aguilar-Trigueros, Roy, & Rillig, 2021; Semchenko et al., 2018) but also in pathogen richness, which is related to this root trait (Lozano, Aguilar-Trigueros, Roy, & Rillig, 2021). The possible formation of anaerobic compartments in the proximity of microplastics in soil could have negatively affected soil fungal diversity.

Overall, microplastics in soil could affect root trait expression through a physical or chemical mechanism. Microplastic effects on soil water dynamics (e.g., increase of soil aeration and water retention) (Lozano & Rillig, 2020), would modify resource availability altering the water-seeking strategies of plants (root trait expression, de Vries et al., 2016, Lozano et al., 2020). Such effects can be also triggered by the legacy effect of microplastics in soil, since plants may have reduced root diameters for diminishing pathogenic infection associated with this legacy (Lozano & Rillig, 2020), which helps explain our observed decrease in pathogen richness. Also, hazardous chemical substances could stress the plant to the point of altering root trait expression, as observed with other stressors (Lozano et al., 2020). Nonetheless, further research is needed to support these ideas.

Under drought conditions, the addition of microplastic fibres increases pathogen and total fungal richness

Contrary to the patterns described above, under drought conditions, microplastics in soil increased the richness of functional, taxonomical and morphological groups, and of the whole fungal community, which could be explained by microplastic fibre effects on soil properties and thus on plant communities.

Microplastic fibres affect different soil physical properties, especially those linked to soil-water dynamics. They can hold water for longer, increasing soil water holding capacity, which may favour soil fungal richness under drought conditions. In addition, microplastic fibres can help entangle soil particles, with positive effects on soil aggregation (Lozano, Aguilar-Trigueros, Onandia, et al., 2021), which may directly and indirectly (as it contributes to water retention) promote soil fungal richness (Bronick & Lal, 2005). In addition, as different plant species tend to enrich a certain community of soil microorganisms in their rhizosphere (Hortal et al., 2017), shifts in plant species composition could also contribute to any of the observed shifts. Also, drought conditions may favour fungal spore density (Escudero & Mendoza, 2005), which coupled with the fact that plastic is a suitable ecological habitat for a variety of fungal organisms (Gkoutselis et al., 2021), helps to explain the increase of soil fungal richness under such conditions.

Microplastic fibres alter fungal community composition depending on the soil water status

Our results showed that fungal composition was affected by microplastic presence either under well-watered or drought conditions. Saprotrophic fungi composition was affected by microplastics irrespective of drought conditions, while mutualists were affected by microplastics only under drought conditions. Also, fungal composition was similar in the combined effect of microplastic and drought in comparison to the control soil (well-watered without microplastics). Our results

suggest that such responses are driven by microplastic effects on root traits to which fungi are strongly linked, and by microplastic effects on fungal richness.

In comparison with the saprotrophic and pathogenic fungal species, which show some degree of independence from the host, mutualist response to microplastics and drought, can be tightly coupled with the plant root trait responses to such stressors. Mutualist composition and richness are strongly determined by root: shoot ratios Lozano, Aguilar-Trigueros, Roy, & Rillig, 2021), which is a root trait that increases with microplastic fibres in soil (Lozano & Rillig, 2020). Thus, the presence of microplastics under drought conditions may favour carbon allocation to the roots, which may ultimately be transferred to mycorrhizal fungi (Pickles et al., 2017; Verbruggen et al., 2017) as such symbiotic associations are particularly needed to promote drought resistance (Hartmann et al., 2020), and alleviate drought stress (Ruiz-Lozano et al., 2012).

In addition, the similar fungal composition under the combined effect of microplastics and drought in comparison with control soils may be due to several reasons. For instance, an increase in soil porosity driven by the addition of microplastic fibres (de Souza Machado et al., 2019; Lozano & Rillig, 2020) may directly favour hyphal growth and thus facilitate the exploitation of resources by fungi. This could lead to the maintenance of fungal richness under drought conditions in comparison with control soils, given there are additional nutritional niches to exploit. On the other hand, *Chytridiomycota*, an abundant group in our dataset, which is composed of mostly water-film inhabiting organisms that depend on their zoospores for dispersal and site selection (Volk, 2013) could have also maintained their richness and abundance when microplastics and drought were present due to microplastics positive effects on soil water retention.

Microplastics in soil alter the relationships between soil fungal communities and ecosystem functions

Multiple relationships between soil fungal communities and ecosystem functions were affected in sign and strength by the presence of microplastics in soil. For instance, in soil without microplastics, we observed that total fungal and pathogen richness were positively correlated with shoot mass, which can be expected as soil fungal diversity is considered an important regulator of plant growth (Mommer et al., 2018; Semchenko et al., 2018). Indeed, this may have occurred because the decline in biomass of an infected plant species could have been compensated by the increased biomass production of the other species (Van Ruijven et al., 2020), which aligns with the observed decrease in plant community evenness, where some plant species dominated over others (Lozano & Rillig, 2020), Likewise, root mass of dominant species could have acted as a barrier obstructing fungal pathogens from reaching other plant species (Yang et al., 2015), ultimately favouring their growth and thus the increase in community shoot mass. However, such correlations between shoot mass and pathogen richness were inverted when microplastic fibres were added to the soil. Similarly, in soils without microplastics, root mass was positively correlated with saprotroph and mutualist richness which turned out to be negative when microplastics were added into the soil. Saprotroph richness was the fungal attribute that most explained root mass ($\beta = 45.68\%$) in soils without microplastics, which suggests an intensive interaction between saprotrophs and root mass, especially with fine roots that release easily degradable carbohydrates and nutrients held in soil organic matter (Kuzyakov et al., 2000). Likewise, in soils without microplastics, mutualist richness was positively correlated with root mass as most carbon can be allocated belowground to acquire nutrients and water through mycotrophy (Brundrett, 2002; Comas et al., 2012), but again, these positive relationships were flipped in the presence of microplastics. Soil aggregation and microbial activity (β-glucosidase) in soils without microplastics were positively correlated with mutualist richness, as observed elsewhere (Hernández-Hernández et al., 2017; Rillig & Mummey, 2006), were also negatively affected by the presence of microplastics, as such relationships disappeared when microplastics were added into the soil. In soils without microplastics, litter decomposition and soil respiration were not related to pathogens. However, when microplastics were added to the soil, litter decomposition was negatively correlated with pathogen abundance, which can be linked to a negative effect of pathogens on saprotrophs activity through competition, antagonism or parasitism (Crowther et al., 2012; Williams et al., 2003). Soil respiration relationship with pathogens was also altered by the presence of microplastics in soil.

The combined effect of microplastic and drought mitigates their individual negative effects on fungal richness

Our results showed that the negative effects on soil fungal richness that microplastics and drought exert in isolation are mitigated when both factors act in concert. This antagonistic effect suggests that the modes of action of the individual factors either eliminate or surpass a critical threshold, such that the mechanisms underpinning the combined effect are opposite to that of the individual factors (Piggott et al., 2015). We observed that when acting in concert, drought is mitigated by microplastics likely due to their positive effect

on water retention, while microplastic toxicity is mitigated as under drought conditions the leaching of water-extractable chemicals into the soil is less pronounced. Thus, such an antagonistic effect arises because the mode of action of microplastics is affected by the soil water status. This antagonistic effect (Piggott et al., 2015), deviated from the predicted additive, multiplicative or dominant model, could be perceived as a desirable scenario from the perspective of land managers. However, this is not necessarily the case as any interaction different from what is directly predicted from the individual factor effects (i.e., synergism or antagonism), implies complex challenges for management. Our results highlight that just knowing the independent effects of each factor is no longer sufficient. As such, the effects of microplastics should be considered together with those of other global change factors such as plant invasiveness, warming or agrochemicals. In addition, further research aimed at better understanding the concurrent effects of multiple global change factors acting in concert is needed.

AUTHOR CONTRIBUTIONS

Y. M. Lozano: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (equal); investigation (lead); methodology (lead); writing – original draft (lead). **J. F. Dueñas:** Methodology (supporting); writing – review and editing (supporting). **C. Zordick:** Methodology (supporting). **M. C. Rillig:** Conceptualization (supporting); funding acquisition (equal); writing – review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All sequences recovered in this study are available at the European Nucleotide Archive (ENA) under project PRJEB56344: https://www.ebi.ac.uk/ena/browser/view/PRJEB56344. Raw reads are available with accessions ERR10302011-ERR10302030. Sample metadata is available with accessions ERS13513706-ERS13513725.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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