ACS ES&T Water

pubs.acs.org/estwater

Article

Microplastic Removal and Biodegradation by Native Mediterranean Fungus *Alternaria alternata*

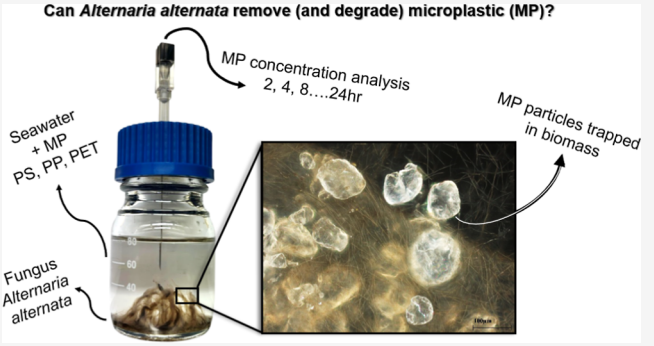
Yarden Schindler and Ines Zucker*

Cite This: *ACS EST Water* 2025, 5, 4079−4088

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The threat and predominance of microplastics (MPs) in marine environments has prompted a growing interest in their interactions with microorganisms that naturally colonize them (i.e., the plastisphere). This study investigates the interaction of *Alternaria alternata*—a fungus native to the Mediterranean Sea—with polystyrene (PS) MPs, focusing on the potential of the fungus to remove and degrade MPs in seawater. We first designed and constructed a custom laboratory setup in which an immobilized benthic fungal mat, contained in temperature-controlled glass vials, was exposed to ∼1.7 μm weathered MPs ranging in size from 0.45 to 30 μm. This scenario emulates environmental conditions occurring in the benthic layer of seagrass habitats. We observed a 96% reduction in PS MP particle concentration within 24 h in the presence of a live fungus, which was significantly higher than the removal of the MP from sedimentation or exposure to inactivated fungus. Micro- and nanoscale visualizations illustrate the capture and entrapment of MPs within the fungal biomass. The fungus displayed selectivity, favoring PS over polyethylene terephthalate (PET) and polypropylene (PP). Further analyses indicated the formation of a transformation product following interaction between the fungus and PS MPs, indicating that active fungal interaction, rather than gravitational settling, was the dominant driver of MP removal. In addition, thermogravimetric analysis revealed structural alterations within the fungal cell wall upon exposure to PS-based MPs, further supporting the hypothesis of PS utilization by the fungus. Overall, this study offers new insights into the use of the fungus *A. alternata* for the biological decomposition of PS MPs and serves as an effective natural method for removing MP from seawater without disrupting the ecological balance.



KEYWORDS: *microplastic, marine fungi, biodegradation, mycoremediation, Mediterranean Sea, polystyrene, fungal plastisphere, Alternaria alternata*

1. INTRODUCTION

Plastic, prized for its versatility, tunability, lightweight nature, durability, and affordability, has become ubiquitous across numerous applications.[1] However, these same advantageous traits also contribute to plastic's pervasive environmental accumulation due to widespread consumption paired with its slow decomposition.[2,3] The ocean serves as the principal global sink for plastic pollution. Plastic waste commonly enters water sources and accumulates in oceanic regions, especially in those with limited circulation and turnover.[4] In 2020, global oceanic plastic accumulation ranged from 18 to 385 million metric tonnes, with 9.8% of that accumulation settling to various depths, including the seabed.[5] The Mediterranean Sea is particularly vulnerable to plastic pollution due to its semi-enclosed nature, heavily populated and busy coastline, and limited outlets.[6] Annually, the Mediterranean accumulates up to 610,000 tonnes of plastic, with 94% sized within the micron range.[7] Among the main classes of plastic polymers identified in the Mediterranean Sea are polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET).[8] The substantial presence of plastic waste in the marine environment raises critical questions about plastic's fragmentation, transport, and overall ecological impact.[1,2]

In marine environments, plastic debris undergoes a range of protracted weathering processes, including ultraviolet (UV)-driven photodegradation, mechanical abrasions (e.g., wave-induced fragmentation), thermal degradation, and biodegradation.[9] The confluence of these weathering processes leads to the breakdown of larger plastic pieces into progressively smaller particles, ultimately yielding microplastics (MPs) and nanoplastics (NPs) within the micro- and nanosize ranges,

Received: March 13, 2025
Revised: June 7, 2025
Accepted: June 10, 2025
Published: June 20, 2025

respectively.[10] Environmental weathering alters plastic properties. Specifically, physical abrasion changes the plastic surface texture, resulting in increased roughness and cracking, while photodegradation results in oxidative surface functionalities. As a result, weathered MPs disperse more widely than non-weathered MP, facilitating the transport of these pollutants throughout aquatic environments.[11]

In seawater, MPs interact with a diverse range of marine organisms, from microorganisms to large fish and mammals, serving as a substrate for microorganism colonization and biofilm formation. This microbial community in close proximity to plastic particles, termed the "plastisphere", constitutes a unique ecosystem characterized by reciprocal interactions and influences on both plastic properties and microbial activity.[12] While fungal interactions with plastics in aquatic environments are of considerable interest, the intricacies of the interactions within the plastisphere remains understudied. However, limited field experiments have suggested the strong impact of the fungal phyla *Ascomycota* and *Basidiomycota* on PS and PE substrates in marine environments.[13,14] Once fungi colonize a plastic surface, both intracellular and extracellular fungal enzyme systems may aid in plastic breakdown.[15]

The fungus *Alternaria alternata*, belonging to the Ascomycota phylum (family Pleosporaceae), thrives in both terrestrial and aquatic environments and is commonly found in the plastisphere.[16] Notably, this fungus demonstrates remarkable adaptability to diverse conditions, including the Mediterranean Sea, where it has been identified as a significant component of the seagrass microbiome. Characterized by septate hyphae—thread-like structures divided by cross-walls—*A. alternata* plays a crucial role in microbial communities across various ecosystems.[17,18] Its adaptability enables it to survive and colonize on substrates like plastic debris[19] and studies have demonstrated its potential for degrading PE and polylactic acid (PLA) in marine settings.[20] Although its enzymatic capabilities suggest a potential to degrade a broader range of plastics, its efficacy on other plastics, particularly PS, remains unknown. Additionally, interactions of marine fungi in general—and *A. alternata* specifically—with MPs are rarely researched, despite the greater abundance and wider distribution of MPs in the marine environment compared to plastic waste. Such interactions may lead to both accelerated plastic degradation (due to the higher surface area-to-volume ratio of MPs) and the entrapment of these plastic particles within fungi networks. Interestingly, given *A. alternata*'s prevalence in seafloor sediments, its interactions with aged MPs are more likely than interactions with floating plastic debris,[21] because aged MPs are more readily dispersed throughout the water column due to their oxidative surface modifications and reduced hydrophobicity.[22]

This study investigated the potential of *A. alternata* in removing PS MPs from seawater with a strong emphasis on ensuring the environmental relevancy of the examined conditions. A novel, custom-designed laboratory setup was used, utilizing modified glass bottles with roughened bottoms to enhance fungal adhesion and simulate natural marine conditions. Weathered PS MPs were engineered using an accelerated weathering protocol to mimic the environmental properties of environmental MPs approximately one year old. Removal of PS MPs by *A. alternata* was measured over time, comparing results to removal by both sedimentation and inactivated fungus (to decouple physical and biological phenomena). Biodegradation potential was assessed using multiple indirect measures. Additionally, the fungus's selectivity for different plastic types was evaluated. Our findings highlight *A. alternata's* potential as an effective, natural agent for reducing MP concentrations and biodegrading PS MPs in aquatic environments.

2. METHODS

2.1. Engineered Microplastic—Weathering Process and Characterization. Polystyrene (PS)-based microplastics (MP)—which will be named in short PS MP—were generated following a top-down protocol outlined by Sarkar et al.[23] PS single-use forks were frozen overnight at −80 °C, mechanically ground (Ika Werke M20, Germany), and sieved through a 100 μm stainless steel mesh. To simulate thermal effects, the particles were heated to 70 °C for 24 h in a lab oven (Carbolite, MRC, China). To induce photodegradation, batches of 100 mg of MP powder were treated in a UV−ozone chamber (ProCleaner, BioForce Nanosciences, USA) for 5 h, with the powder mixed hourly to ensure uniform oxidation across all particles. Lastly, the oxidized particles were placed in a glass bottle containing 50 mL of deionized distilled water (DDW) from Milli-Q Direct (Merck Millipore, Germany) and subjected to probe sonication (QSonica, LLC Q 125, USA) in an ice bath for 1 h, alternating 7 s on (70% amplitude) and 3 s off. The same protocol was also applied to polypropylene (PP)-based plastic and polyethylene terephthalate (PET)-based plastic for the generation of PP MPs and PET MPs (Figure S1 in the Supporting Information).

The resulting particles were size fractionated by sequential filtration using 30 μm (Whatman, UK) and 0.45 μm nylon filters (Merck KGaA, Germany). This procedure removed particles larger than 30 μm and particles smaller than 0.45 μm, including nanoparticles, which fall below the particle counter's lower detection limit of 1 μm. The resultant three size-differentiated MP populations were disinfected in 100% ethanol for 1 h, rinsed in DDW to remove any residual ethanol, and then suspended in filter-sterilized artificial seawater (ASW). ASW was made by mixing DDW with living reef Red Sea salt, autoclaving the solution, and filtering through a 0.22 μm filter (Corning, USA). The resulting particle solution was stored at 4 °C until used.

Surface oxidation was assessed using Fourier transform infrared (FT-IR) attenuated total reflectance (ATR) spectroscopy (Tensor 27, Bruker, USA). Spectra were recorded for the particles in the range of 400−4000 cm^{-1} with a spectral resolution of 4 cm^{-1} over 16 scans. For each measurement, 10 mg of oxidized particles were placed and compressed on the ATR diamond, and the spectrum was normalized to the background correlation spectrum. The degree of surface oxidation was evaluated by calculating the carbonyl index (CI), defined as the ratio of the absorbance of the carbonyl group (1730 cm^{-1}) to that of the methylene group (1450 cm^{-1}), following the method of Halle et al.[24]

Particle morphology was assessed across multiple characterization methods. Changes in surface morphology were examined using high-resolution scanning electron microscopy (HR-SEM) (ZEISS, Gemini SEM 300, Germany). Prior to HR-SEM imaging, the particles were placed on carbon tape and sputter-coated with chromium (Quorum-150TS plus; UK) for 5 min. To determine the surface charge of the particles, the zeta potential of each sample was measured twice at 25 °C using a zeta-sizer system (Malvern Instruments, UK). Particle

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

size distribution and concentration were determined using a Multisizer 4e particle sizer and counter (Beckman Coulter, USA) equipped with a 50 μm aperture. To do so, a 500 μL particle suspension was first diluted with 9.5 mL of ISOTON II (Beckman Coulter, USA) to fit within the range of quantification. Contact angle was measured by placing a water droplet on the macroplastic surface and analyzing the left and right contact angles from digital images.

2.2. Fungus Isolation and Identification. The fungus was first observed and successfully isolated following a one-year exposure of a PS MP slurry—originating from the same ground-fork stock described above—in a seawater solution subjected to natural environmental conditions on a rooftop. The seawater was neither sterilized nor filtered, and the samples were sealed. Therefore, the most likely source of the fungus is the unprocessed sea salt used to prepare the seawater solution. Significant growth of suspected filamentous fungus was observed, and genomic sequencing using Operational Taxonomic Unit (OTU) clustering against the SILVA database (18S) identified the microorganism as *A. alternata* with 97% sequence similarity. As plastic was the only carbon source during the one year of environmental exposure, it is hypothesized that the fungus used the plastic for energy. Fungal isolates were purified through successive subcultures on potato dextrose agar (PDA) supplemented with 0.5 mg/mL chloramphenicol to inhibit bacterial growth. The fungus was stored in 40% glycerol and 60% phosphate buffer at −80 °C. The fungus's zeta potential was determined from streaming potential measurements with the analyzer SurPASS 3 electrokinetic (Anton Paar GmbH, Austria) equipped with a conductivity probe and a pH electrode. Images of the fungus were captured using an optical microscope (Eclipse LV100ND, Nikon, Japan).

2.3. Experimental System. To achieve experimental conditions that closely simulate the fungus's natural habitat in seagrass on the seabed and to facilitate its attachment to the surface, a unique custom-made sterile bottle system was developed. We modified 100 mL borosilicate glass bottles by fusing 5 g of borosilicate glass shards onto the bottles' inner bottom surface using a technique known as tack fusing, conducted at approximately 900 °C. This was followed by slow annealing in a controlled oven environment, with a gradual temperature decrease from 560 to 60 °C over a total duration of 3 h. After removal of excess material, approximately 2.5 g (±1.1) of fused glass remained in each bottle (Figure S3). This system facilitated the repeatability of experiments and ensured consistent conditions throughout the study. Additionally, a 3.5-in., 22 G needle was embedded in the bottle's cork, securely positioned at a uniform height in the center, to enable consistent sampling of the solution from the bottles while incurring limited disturbance to the experimental environment.

2.4. Fungal Culture Conditions. Fungus retrieved from −80 °C freezer storage was cultured for 10−14 days on Petri dishes containing 15 mL of PDA and supplemented with 0.5 mg/mL chloramphenicol to prevent bacterial contamination. Once full growth was achieved, the fungal biomass was collected using a scalpel, then vortexed in DDW and filtered through a 100 μm filter. A mixture comprising 3 mL of the fungal solution (0.335 g/L), 2 mL of potato dextrose broth (PDB), and 15 mL of ASW (40 g/L salt) was prepared. This mixture was placed in the custom-made sterile bottle system and placed on a shaker incubator (LOM-80, mrc, UK) at 140 rpm at a controlled temperature of 25 °C. Within 10 days, the fungal filaments adhered to the bottom surfaces of the bottle system, forming a biofilm and reaching an average mass of 2.11 ± 0.19 g per bottle. An illustration of the conditions of the fungal culture is provided in Figure S4.

2.5. Microplastic Removal Experiments. Two experimental groups were conducted in triplicate for testing removal of each MP type: one group consisted of live fungus and the other consisted of inactivated fungus. Inactivation of the fungus was accomplished by immersing fungus within the bottle system in 1 mL of bleach and 20 mL of DDW water overnight, followed by thorough washing with sterile DDW to remove any residual bleach and biomass. The bleach treatment denatures cellular proteins and degrades extracellular polymeric substances through oxidative damage, effectively eliminating fungal viability and associated organic matter.[25] Additionally, three control groups were established in duplicate: one with live fungus without MPs (to evaluate leaching of particulate matter by the fungi), another with inactivated fungus without MPs, and a third with PS MPs alone (to assess gravitational settling). Before the onset of the experiment, all bottles were rinsed with 20 mL of ASW. Each experimental group received 70 mL of an ASW solution containing 1.1×10^6 particles/mL of PS MPs, while control groups without MPs were filled with 70 mL of sterile ASW.

Five sampling points were established at various time intervals to assess particle removal and removal rates. A 500 μL sample was extracted using the needle fixed within the system (described above), diluted with 9.5 mL of ISOTON II (Beckman Coulter, USA), and analyzed for particle size distribution and concentration utilizing a Multisizer 4e particle sizer and counter (Beckman Coulter, USA) equipped with a 50 μm aperture. Particle concentration was also measured indirectly by a turbidity meter (2100Q, HACH, USA). A 10 mL sample was taken from each bottle for turbidity measurements and subsequently returned to its original container. A calibration curve was generated to establish the correlation between turbidity (in NTU) and concentration (mL^{-1}) as measured by the particle counter, revealing a proportional and linear relationship between turbidity and the solution's concentration (Figure S7).

2.6. Indications for Dual Fungus-Microplastic Interactions. Capture and entrapment of MPs by the fungus was imaged using HR-SEM. Before imaging, the fungus was thoroughly washed multiple times with DDW to remove any residual salt. The samples were then placed on a carbon tape and sputter-coated with chromium prior to HR-SEM analysis. Thermogravimetric analysis (TGA) was performed in a temperature range from 40 to 650 °C at a heating rate of 5 °C min^{-1} in a nitrogen atmosphere (Q5000 TA Instruments) to analyze thermal changes that may occur to the fungus in the presence of MPs.

Supernatants from a two-month experiment with PS MPs and fungus (as well as control experiments of fungus alone and PS MPs alone at similar conditions) were analyzed to account for the generation of any MP degradation products. First, the samples were diluted and examined with a UV−visible spectrophotometer (Shimazu 2600) in the 185−400 nm range. Then, the same samples were analyzed using high-performance liquid chromatography (HPLC, Agilent 1260 Infinity Series) coupled with a photodiode array UV−VIS detector. A sample volume of 100 μL was injected into a Poroshell 120 EC C18 (250 mm × 4.6 mm, 4 μm) column (Agilent Technologies, USA) at 30 °C. HPLC-grade MeOH

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

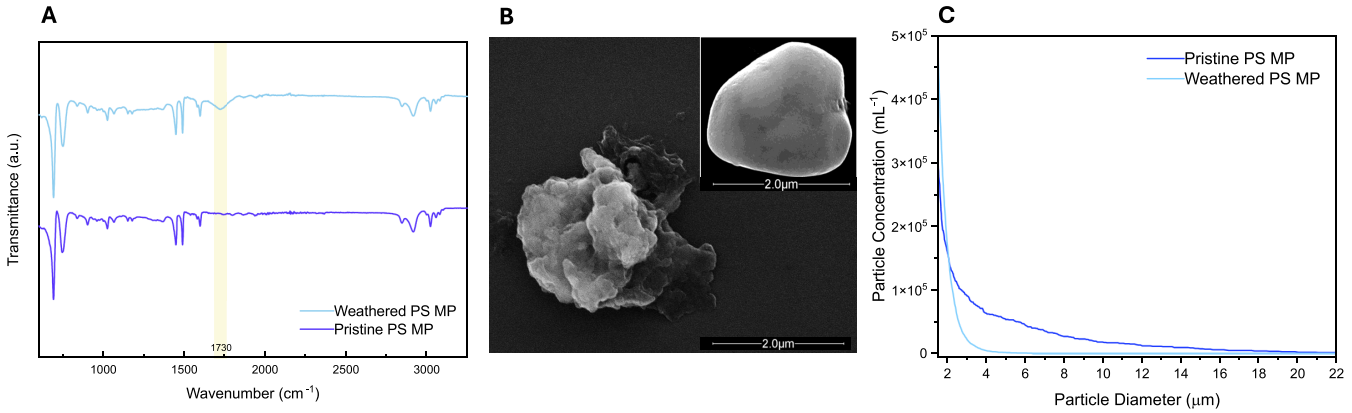


Figure 1. (A) Fourier transform-infrared (FT-IR) spectroscopy of both weathered and pristine polystyrene (PS) microplastic (MP). (B) Scanning electron microscope micrographs of weathered and pristine (inset) PS MP. (C) Particle size distribution of weathered and pristine PS MP populations.

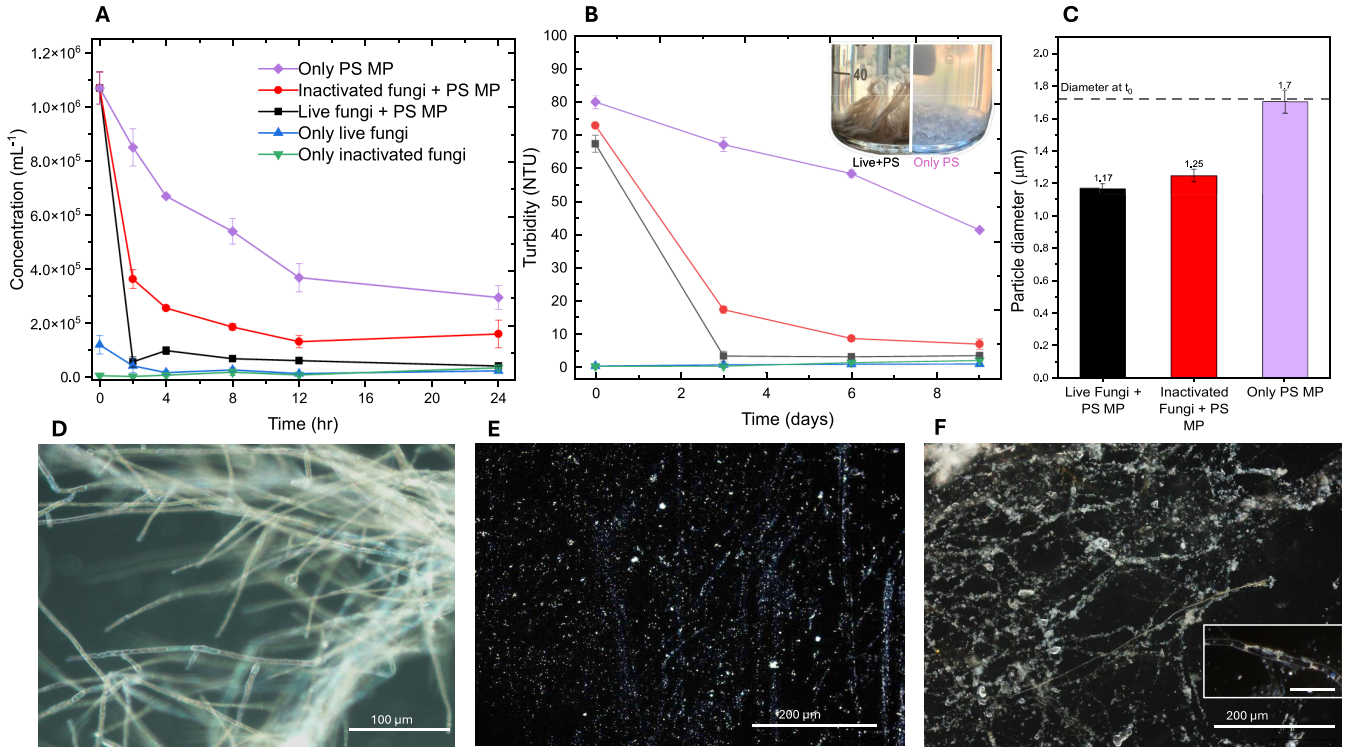


Figure 2. (A) Removal kinetics of polystyrene (PS) microplastic (MP) from simulated seawater in isolation (purple) and in the presence of inactivated fungi (red) and live fungi (black). Two additional control experiments were conducted to assess the particulate biomass contribution of live (blue) and inactivated (green) fungi. (B) Removal kinetics measured indirectly via turbidity measurements. Inset provides a visual indication of turbidity loss in the presence of the fungi compared to control sample of PS MPs only. (C) PS particle mean diameter at the onset and after 24 h of interaction with a live fungus (black) and inactivated fungus (red) as well as in isolation (purple). (D) Light microscopy image of live fungus, (E) PS MPs suspended in seawater solution, and (F) fungus with PS MPs in seawater (inset: close-up on fungus hyphae with adhered microplastic). Removal kinetic experiments were conducted in triplicates, and error bars represent the standard deviation of particle count (or turbidity) results.

(Bio-Lab Ltd., Israel) and DDW with 0.1% acetic acid (Merck KGaA, Germany) were used as the mobile phase. The elution program started at 70% MeOH for 1 min, at which point the 70% MeOH decreased linearly to 50% MeOH over 9 min. The flow rate was 1 mL min^{-1}. The absorption wavelength was set to 270 nm.

A gas chromatography−mass spectrometry (GC−MS) system (Agilent 7890B GC coupled with 5977A MSD) equipped with a 7693 automatic liquid sampler was used to investigate the potential emergence of new molecules in the solution resulting from interaction between the fungus and PS MPs. The inlet temperature was set to 250 °C with a splitless injection for 1 min. A DB-5 ms UI column (Agilent), measuring 30 m × 250 μm × 0.25 μm film thickness, was employed with a constant helium flow rate of 1.2 mL/min. The column oven temperature program began at 60 °C for 2 min, followed by linearly increasing the temperature by 10 °C/min to 70 °C (maintained for 0 min) and then linearly increasing by 20 °C/min to 320 °C (maintained for 0 min). The mass spectrometer operated in the scan mode from 45 to 450 amu at a rate of 3.5 scans per second, with an ion source temperature of 250 °C. Extraction was performed by mixing the sample

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

with hexane at a ratio of 5 mL of sample to 0.5 mL of hexane. The mixture was vortexed for 30 s and subsequently centrifuged at 4000 rpm for 5 min.

3. RESULTS AND DISCUSSION

3.1. Microplastic Characteristics. Polystyrene (PS)-based plastic sourced from disposable plastic forks was processed into environmentally relevant microplastic (MP) particles using an accelerated weathering protocol which utilizes laboratory instrumentation to apply mechanical, thermal, and oxidative degradation mechanisms, based on Sarkar et al.[23] Figure 1 illustrates the surface physicochemical characteristics of PS particles before and after weathering. The Fourier-transform infrared (FT-IR) spectra (Figure 1A) of the weathered particles illustrate a peak at wavelength 1730 cm^{-1}, which is not present in the spectrum for the untreated particles. This peak signifies the formation of carbonyl functional groups in the polymer during the particle oxidation within the weathering process.[26] The carbonyl index (CI) is used to evaluate the extent of polymer photodegradation and aging and is determined by calculating the ratio between the absorbance area of the 1730 cm^{-1} wavelength peak and that of the reference peak at 1450 cm^{-1} associated with CO_2. Here, the CI showed an increase of 0.43 after weathering, which signifies advanced stages of oxidation, slightly lower than that of semienvironmental PS plastic which has degraded for 11 months, as showed in other studies.[23,24] In addition, weathered PS was more hydrophilic compared to pristine PS (Figure S2) which stands in line with the oxidation effect on the MP physicochemical characteristics.

Figure 1B illustrates a scanning electron microscopy (SEM) micrograph of a treated MP particle, characterized by a rough and cracked surface from the weathering protocol. In contrast, the inset depicts a pristine, smooth-surface particle derived from ground polystyrene forks (no weathering protocol). The formation of microcracks and fragmentation found on the treated MP particles increased their surface area, which—combined with the presence of functional groups such as carbonyls—closely resembles characteristics of MPs in marine environments. This similarity enhances the relevance of this research to environmental studies.[11] Furthermore, these characteristics increase the susceptibility of the particles to further degradation mechanisms and creates favorable conditions for the attachment of microorganisms and subsequent potential biological decomposition.[27] Size distribution analysis (Figure 1C) indicates a mean particle diameter of 1.75 μm of the weathered PS MP, confirming that the particles were sieved properly, as this study focuses on the interaction between fungi and microparticles within the targeted size range of 0.45 to 30 μm.

3.2. PS Microplastic Removal Experiments. The efficacy of MP removal from water by the fungus *A. alternata* was examined kinetically. Figure 2A shows a 96% reduction in PS particle concentration within 2 h of interaction with live fungus, decreasing to 0.44×10^5 particles per mL after 24 h (a kinetic constant of 2.14 h^{-1}, Figure S5 and Table S1). The control groups, on the other hand, had much lower kinetic rates; the fungus alone incurred a negligible contribution of particles at the size range measured, while PS MPs alone resulted in 36.8% removal of particles within 24 h probably due to gravitational sedimentation (kinetic constant of 0.22 h^{-1}, Figure S5 and Table S1). This MP sedimentation rate exceeds the theoretical prediction by Stokes' Law, likely due to non-spherical shapes and heterogeneous size distribution. Conversely, an 85% decrease in particle concentration was observed in the presence of inactivated fungus (0.66 h^{-1}, Figure S5 and Table S1), likely reflecting passive physical interactions such as surface entrapment or adhesion to residual cell structures. Since the inactivation process included oxidative bleaching, which removes both fungal viability and extracellular organic matter, this condition serves as a stringent control for nonbiological MP removal, allowing clear evidence for the active biological removal obtained with the live fungus treatment.

Not surprisingly, the mean size of MPs remaining in the supernatant (~1.7 μm) did not decrease following the control experiments (i.e., in the presence of PS MPs alone, Figures 2C and S6). The presence of fungus (live or inactivated) resulted in a decrease in mean size diameter in the supernatant (1.17 and 1.26 μm, respectively), which suggests preferential interaction with a larger MP population. This comparative analysis of the groups suggests that genuine biological processes are occurring, and the fungi are actively removing particles from the water by attachment to the fungal biomass.

Quantifying particle concentration to evaluate MP removal might often be a challenging task outside of a laboratory setting. Therefore, we explored indirect turbidity measurements as an indicator of MP concentration over longer time frames. The calibration curve correlating concentration values to turbidity measurements is presented in the Supporting Information (Figure S7). The presence of live fungus resulted in a 95.2% reduction in turbidity after 6 days (Figure 2B), corroborating the decreases in concentration observed in the removal experiments. In contrast, existence of particles alone (no fungus) exhibited only a 27% decrease in turbidity over the same period, due to gravitational settling. The inactive fungus also drove a decrease in turbidity, though at a slower rate, achieving 88% removal of the PS MPs. Even after 9 days, a substantial disparity in turbidity was observed between the sample containing inactive fungus and the one with live fungus, highlighting the superior particle removal capabilities of the living fungal biomass. Notably, the solutions that included fungus and PS MPs were markedly clear, as visually depicted in the inset of Figure 2B. One should note that while the turbidity measurements offer a faster and simpler technique than quantifying concentrations directly, technical aspects of measuring turbidity (such as the need for large volumes of solution) may require adjustments in the setup and online sampling. Moreover, turbidity can also result from non-MP sources such as spores, bacterial cells, or other particulates, and should not be overinterpreted as a direct measure of MP concentration without proper validation. While this study accounted for such variables, future applications should include complementary methods to ensure specificity.

Qualitative analysis using an optical microscope visualizes the MP-capturing phenomenon. The fungus in isolation displays typical branched filamentous hyphae (Figure 2C). The PS MP population suspended in the solution (Figure 2D) illustrates a size range between 0.45 and 30 μm and a mean particle diameter of 1.9 μm, similar to the particle distribution in Figure 1C. Following a 24 h experiment interacting the fungus with the PS MPs, there is clear evidence of the presence of particles along the fungus filamentous hyphae (Figure 2F), which effectively capture and retain the scattered particles, as illustrated in the high-magnification inset image.

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

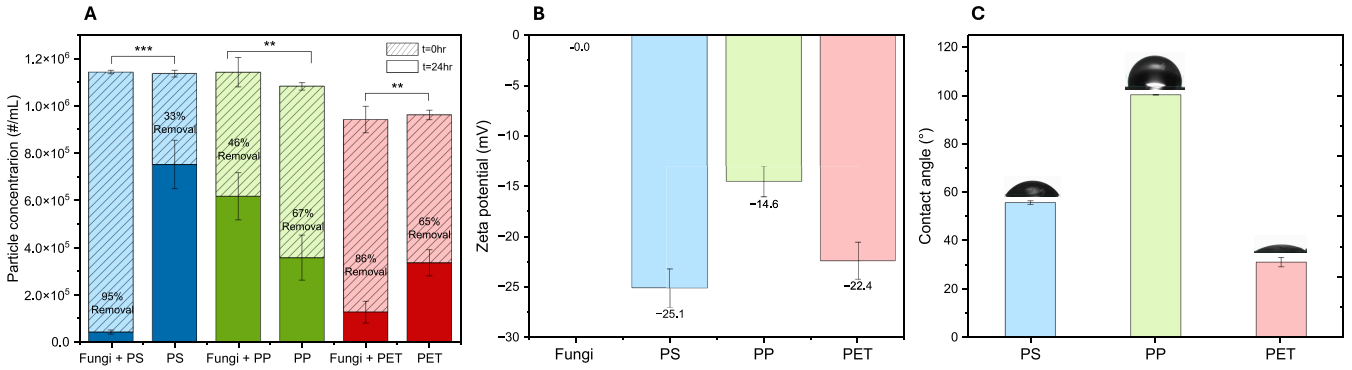


Figure 3. (A) Comparison of weathered microplastic (MP) removal across common plastic types, including polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET), by measuring MP concentrations at the onset of experimentation and again after 24 h. (B) Zeta potential of a live fungus, as well as weathered PS MP, weathered PP MP, and weathered PET MP. (C) Contact angle of weathered PS (blue), PP (green) and PET (red) macroplastics.

3.3. Plastic-Type Selectivity. The selectivity of the fungus toward various plastic types was investigated in a comparative experiment involving three types of polymeric bases commonly found in the marine environment: PS, polypropylene (PP), and polyethylene terephthalate (PET). Each type of plastic was subjected to the same accelerated weathering protocol used for PS MPs (Figures S1 and S8 illustrates PP and PET MP characteristics before and after weathering). Figure 3A illustrates the concentration of remaining particles in a seawater solution after 24 h, both in the presence of the fungus and in its absence. The results, as shown in Figure 3A, indicate that PS MPs exhibit the highest removal rate of 95%, similar to the kinetics experiment, followed by PET MPs with 86% removal, and PP MPs with 46% removal.

To assess the influence of gravitational sedimentation or flotation on these results in the absence of fungal activity, control groups containing only particles were also tested for particle removal. In the control groups, PS, PET, and PP particle removal rates were 33%, 65%, and 67%, respectively. The high removal rate observed for PP MPs is primarily attributed to their low density (0.83 to 0.85 g/cm^3), which causes them to float on the water surface rather than settle. This buoyancy minimizes their interaction with the fungus residing at the bottom. Thus, only 46% removal was observed for PP MPs in the live fungi condition, reflecting the two competing mechanisms—fungal activity and flotation—and the dominance of the latter. In contrast, while PET MPs exhibit a relatively high gravitational settling rate (due to their high specific density, 1.37−1.45 g/cm^3), the enhanced likelihood of passive interaction with the fungal biomass was not expressed in removal rates by the fungus (86% compared to 95% for PS MPs). PS MPs—with a density of 1.04−1.08 g/cm^3, which closely matches that of seawater (1.03 g/cm^3)[28]—were in fact the ones that were most effectively removed by the live fungus. These findings underscore the specificity of fungal-mediated MP removal toward PS MPs.

Zeta potential analysis (Figure 3B) was conducted to investigate potential electrostatic interactions between the fungus and the three types of plastics examined, aiming to further explain the observed selectivity in the experiment. The results indicated that PS and PET MPs carry a significantly more negative charge compared to PP MPs. While the neutral charge of the fungus suggest that electrostatic interactions are not the main drivers for the selectivity phenomena, literature suggest that such neutral zeta potential on the fungus reflects a balance of positively and negatively charged surface components, allowing localized cationic regions (e.g., proteins or amine-rich molecules) to possibly engage in microscale electrostatic interactions with negatively charged plastics.[29−31]

Hydrophobic interactions were also hypothesized as a possible factor in fungal−MP interactions. Contact angle analysis shows that PP MPs are more hydrophobic compared to PS and PET MPs. However, while this trend follows the settling trends observed, it cannot explain the (hydrophobic) fungus selectivity. Since neither surface hydrophobicity nor overall zeta potential fully accounts for the preferential removal of PS MPs, we propose that the experimental setup—which mimics natural and realistic conditions in the ocean—is driving selectivity, limiting effective contact between buoyant particles and the benthic fungal biomass, thereby restricting bioactive removal.

3.4. Microplastic Removal Mechanism. The removal of PS MPs by the fungus was found to be affected by the fungus viability (i.e., lower removal rates with inactivated fungus as compared to active fungus), but the removal mechanism should be further explored. As the PS MPs removal was most significant as compared to the other plastics, the mechanistic study was conducted with PS. The first visual indication by SEM micrographs (Figure 4) substantiates the conclusions drawn from the removal experiments, demonstrating that the fungus actively captures PS MPs by trapping them within the fungal biomass, comprising both the fungal body and its secretions. In Figure 4A, the fungal filaments envelop the particle, while Figure 4C presents a close-up view of PS MP particles embedded and entirely encapsulated by a proteinaceous substance secreted by the fungus, which is most likely part of the extracellular enzymatic system.[32] *A. alternata* is known to produce a complex array of extracellular enzymes, including laccases and peroxidases, which have been suggested to be involved in the degradation of synthetic polymers such as polyethylene.[33,34] These oxidative enzymes are suggested to initiate the breakdown of the polymer chains and facilitate further degradation.[35] Additionally, *A. alternata* secretes hydrolytic enzymes like esterases and hydroxylases, which play roles in the depolymerization and assimilation of plastic degradation products.[36]

Beyond enzymatic activity, the fungus produces extracellular polymeric substances (EPS), comprising polysaccharides, proteins, lipids, and nucleic acids. These EPS components contribute to biofilm formation and enhance the fungus's

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

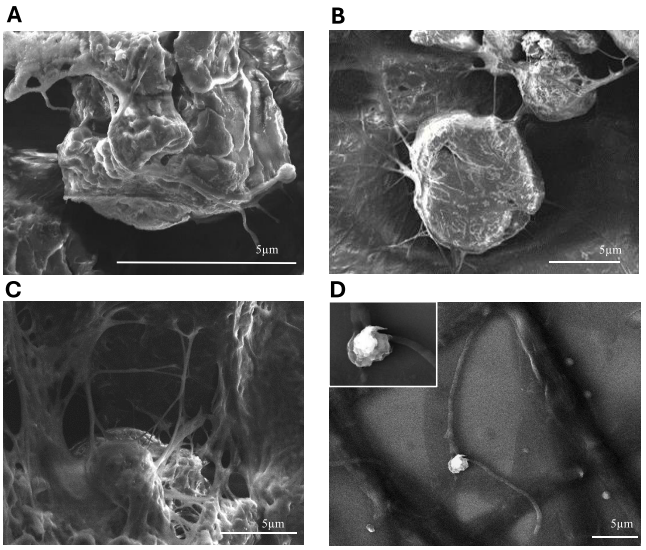


Figure 4. Scanning electron microscope (SEM) micrographs of a typical interaction between polystyrene (PS) microplastic and live fungi (A–C) and inactivated fungi (D).

ability to adhere to and interact with hydrophobic plastic surfaces.[37] The EPS matrix not only facilitates the physical entrapment of MPs but also creates a microenvironment conducive to enzymatic degradation.[38]

Figure 4D show a representative SEM image of a PS MPs particle lying on the surface of an inactivated fungus. The particle appears to rest arbitrarily on the fungal body structure and is not encased in a network of proteins or enzymes. This observation supports the assertion that the living fungus traps particles within its biomass, facilitating MP decomposition. It should be noted that in order to capture SEM images of the samples, numerous washings with distilled water were performed to eliminate salt. Despite extensive washing, the persistence of particles within the matrix of live fungus indicates strong adhesion. Conversely, after washing the inactivated fungus, it was challenging to identify particles that remained attached.

Due to the small, microscopic particle size (mean of 1.75 μm), direct separation of particles from fungal biomass presented significant challenges. Therefore, plastic decomposition and its use by fungus as a carbon source were investigated through indirect analyses. In particular, we investigated the potential emergence of new molecules in the seawater solution resulting from fungal exposure to PS MPs and analyzed the changes in fungal composition.

Gas chromatography–mass spectrometry (GC–MS) analysis revealed the presence of dissolved organic molecules in a fungal solution spiked with PS MPs. As illustrated in the chromatogram in Figure S9, two peaks emerged following the interaction between the plastic and the fungus, which were absent in solutions containing either PS MPs or fungus alone. The first peak at 5.1 min (Figure S10) could not be unambiguously identified and was not matched to a specific molecule. However, it can be asserted with high confidence that its molecular peak is at 176 m/z, and it likely contains one

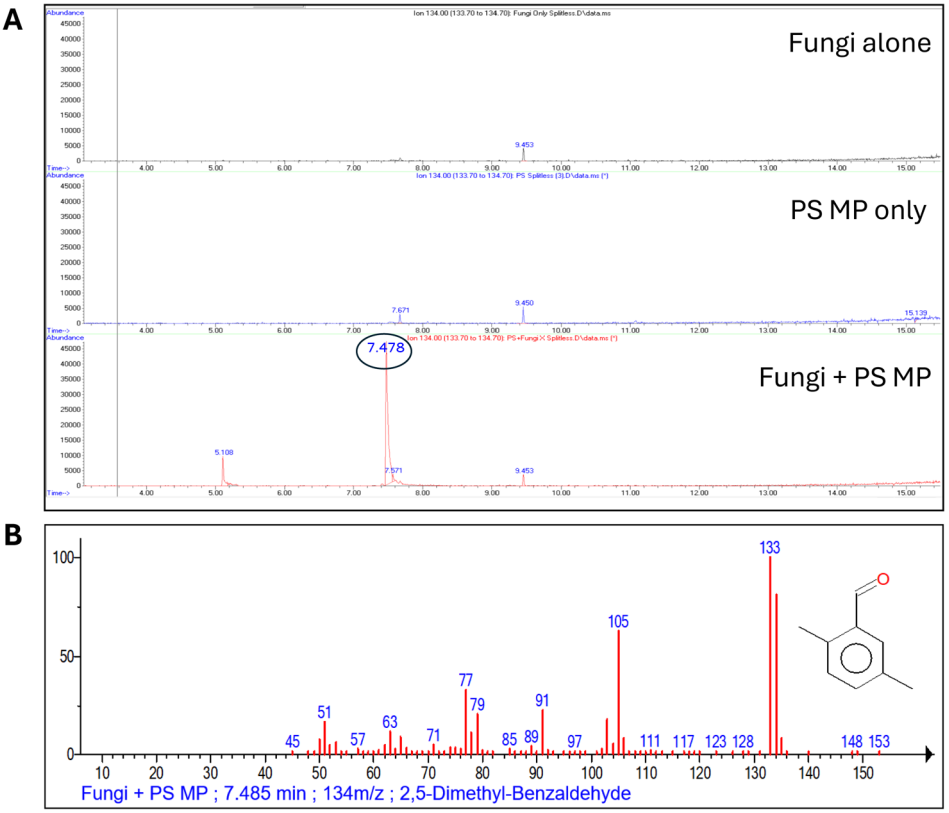


Figure 5. (A) Gas chromatography–mass spectrometry (GC–MS) chromatogram of extracted ion m/z = 134 with a peak at 7.48 min observed only in samples with fungus and polystyrene (PS) microparticles (MPs) together. (B) Electron ionization mass spectrometry (EIMS) spectrum of the peak at 7.48 min for the fungus with PS MPs sample shows a 90% match with the NIST library, identifying the compound as dimethylbenzaldehyde.

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079–4088

or two silicon atoms, which may have originated from the borosilicate bottles. The peak at a retention time of 7.485 min (Figure 5A) was identified (as a 90% match) as dimethylbenzaldehyde with 134 m/z as the molecular peak (Figure 5B). While benzaldehyde is a well-established oxidative degradation product of polystyrene, typically formed via thermo-oxidation or, its methylated derivative—dimethylbenzaldehyde—result from a biologically mediated transformation.

The methylation reaction of benzaldehyde requires very specific conditions such as enzymatic mediation, and it is possible that benzaldehyde could function as a starting point in a complex multistep biosynthetic pathway within the fungus. This pathway may involve various enzymatic transformations leading to the formation of dimethylbenzaldehyde,[39] which is observed following the fungal interaction with PS MPs.

Certain fungal species within the *Ascomycota* phylum have demonstrated the ability to metabolize aromatic aldehydes, utilizing them as carbon sources and energy. This metabolic capability is especially prominent in fungi isolated from environments subjected to hydrocarbon pollution.[40] Numerous aromatic aldehydes, such as dimethylbenzaldehydes, are involved in various biological processes, functioning as signaling molecules, intermediaries in metabolic pathways, or constituents of more complex molecules with biological functions. Consequently, the emergence of this molecule upon fungal exposure to PS MPs likely represents a partial step in a more extensive biological process. This suggests a chemical interaction between the fungus and the particles, indicating the potential for further biodegradation.

Spectrophotometric analysis provided additional evidence of the existence of dissolved organic molecules in a fungus solution spiked with PS MPs, as an adsorption peak appeared around 270 nm following the interaction of the fungus with PS MPs, in contrast to solutions containing PS MP alone or fungus alone (Figure S11). High-performance liquid chromatography (HPLC) analysis also revealed a prominent absorptive peak at 270 nm at a retention time of 6 min that was exclusively observed in the water sample containing *A. alternata* fungus and PS MPs together (Figure S12). In contrast, this peak was absent in samples containing either the fungus or the PS MPs alone. This peak suggests the presence of molecules potentially released following a chemical interaction between the fungus and the plastic, providing a preliminary indication of decomposition products in the solution.

Thermogravimetric analysis (TGA) was used to show variations (measured as a function of increasing temperature) in the physical and chemical properties of the fungus in the presence of MPs. In a separate experiment, the fungus was exposed to a higher concentration of PS MPs (6×10^6 particles per mL) over a duration of two months to allow for measurable thermal analysis. Calculations (eq S1) estimate that PS MPs composed approximately 0.04% of the treated fungal sample. This negligible mass contribution of the MPs, together with the plastic degradation at relatively high temperatures (~300 °C, blue curve in Figure 6), allows us to refer to the thermal changes occurring at lower temperatures as changes within the fungal biomass rather than additive thermal effects.

As illustrated in Figure 6, the highlighted temperature range exhibits a notable difference between the fungus treated with PS MPs and the untreated fungus. The decrease in mass in the 25−120 °C range is mostly related to the release of adsorbed

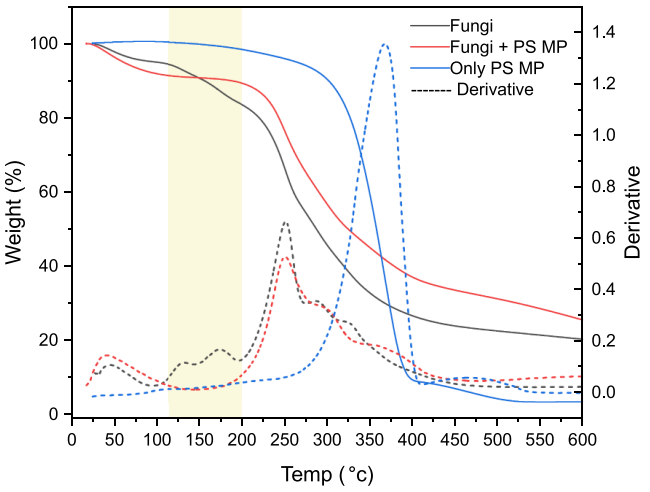


Figure 6. Thermogravimetric analysis (TGA) of the fungus alone (black), fungus that has interacted with polystyrene (PS) microplastics (MPs, red), and PS MPs only (blue).

water that is present even in dried samples. The second decrease in mass within the 120−250 °C range is related to the degradation of polysaccharide side chains alone (such as a-glucans) or in glycoproteins, mainly branched glucomannan.[41] The changes observed suggest an alteration in the cellular structure of the fungus following PS exposure, indicating a potential active interaction with the particles and the possibility of their assimilation by the organism. These components are critical for various functions, contributing to the overall strength and flexibility of the fungal cell wall, and are essential for fungal survival, environmental interactions, adhesion, and biofilm formation.[42] A reduction in the thermal stability of polysaccharide side chains within a fungal sample may indicate changes in its structural characteristics and chemical composition.

These alterations may reflect a biological response to MP exposure—such as stress-induced remodeling of the cell wall or metabolic adaptation—rather than direct degradation of the MPs themselves. However, we acknowledge that TGA alone cannot fully resolve this distinction. Therefore, we interpret these results as complementary to the other mechanistic evidence presented in this study (e.g., surface interaction imaging, leachate profiling, and particle size reduction), which collectively support the biological interaction between *A. alternata* and PS MPs.

4. CONCLUSIONS

In this study, we investigated the capability of *A. alternata* fungus to entrap, remove, and biodegrade microplastics (MPs) in seawater, with emphasis on polystyrene (PS) MPs. The experimental conditions were designed to reflect oceanic environmental scenarios, including fungal fixation, wave-like conditions, elevated salt concentration, and the presence of weathered MPs. In our experiments, living fungus in 24 h achieved 96% PS MP removal, which was higher than both natural gravitational sedimentation of the particles and physical removal by inactivated fungus.

Results from this study indicate that biological activity plays a crucial role in enhancing MP removal. Microscopic examination revealed that the MPs were trapped and bonded to the fungal biomass. Furthermore, we examined the selectivity of the fungus toward three different types of

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088

common plastic in marine plastic debris: PS, polypropylene (PP), and polyethylene terephthalate (PET). PS MPs had the highest removal percentage compared to PP and PET MPs, which was mostly attributed to the environmental conditions which allow interaction of the benthic fungus with suspended particles rather than settled or floated ones. Given its hyphal and fibrous morphology, *A. alternata* may function effectively as a trap for MP particles in aquatic environments. Moreover, advanced characterization provided a preliminary indication of the interaction mechanism between the fungus and PS MPs, suggesting a potential bioremediation process. Gas chromatography−mass spectrometry (GC−MS) analysis detected the emergence of molecules likely arising from the chemical interaction between the fungus and the particles, suggesting the potential for enhanced biodegradation processes of the PS MPs by the fungus. Furthermore, thermogravimetric analysis (TGA) demonstrated structural modifications within the fungal cell wall following exposure to PS MPs, providing preliminary support for the hypothesis regarding the utilization of PS MP by the fungus.

This study presents novel findings in both subject matter and experimental approach, offering valuable insight into the behavior of marine fungal biomass—specifically the native Mediterranean fungus *A. alternata*—within the dynamic and microbially rich environments of the plastisphere. The results suggest that benthic fungi like *A. alternata* may play an underexplored ecological role in capturing, retaining, and potentially transforming MPs within sediment layers at the seafloor, where long-term environmental effects are likely to manifest. Overall, the study is laying the groundwork for further research into natural, fungi-based bioremediation strategies. One such avenue includes deploying fungal cages in MP-rich marine zones, proposing an innovative, ecologically grounded method to mitigate plastic pollution in open marine environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.5c00299.

Schematics of microplastic weathering procedure (Figure S1), contact angle of PS microplastics (Figure S2), images of the experimental setup (Figure S3), fungal growth protocols (Figure S4), kinetic fit (Figure S5) and constants (Table S1) of microplastic removal, mean size of microplastics remaining (Figure S6), calibration curve correlating turbidity measurements with particle concentration (Figure S7), Fourier transform-infrared spectra of PET and PP microplastics (Figure S8), GC−MS spectra (Figures S9 and S10), absorption spectra of fungus solutions (Figure S11), HPLC-UV chromatograms (Figure S12) and one equation used for mass calculations (eq S1) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Ines Zucker − *School of Mechanical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel; Porter School of Earth and Environmental Studies, Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel;* orcid.org/0000-0001-7408-6757; Phone: (+972) 36408227; Email: ineszucker@tauex.tau.ac.il

Author

Yarden Schindler − *School of Mechanical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsestwater.5c00299

Author Contributions

CRediT: **Yarden Schindler** investigation, methodology, validation, writing - original draft; **Ines Zucker** conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing - review & editing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Y.S. expresses gratitude for the scholarship provided by Israel's Ministry of Environmental Protection. This research was partly supported by the Ministry of Innovation, Science & Technology, Israel (Grant No. 1001695288), and the National Key Research and Development Program of China (Grant No. 2023YFE0113800). Special thanks are extended to Dr. Yinon Yecheskel for his technical and scientific assistance, and to Dr. Igal Gozlan for his analytical consulting. We also thank David Gabbay, the professional glassblower from the University's Glass Blowing Workshop, for his contributions to constructing the experimental system. Acknowledgment is given to Prof. Diana Golodnitsky and Olga Guchok for their assistance with TGA analysis, to Dr. Alex Gordin from the Adama Center, and to Dr. Shay Covo for his support with fungal growth techniques. Additional thanks go to Shira Gavriely and all the members of the Zucker Lab for their ongoing support throughout the research process. Finally, the authors wish to acknowledge Dr. Andrey Ethan Rubin for initiating the project, assisting with polymer analysis, and providing valuable guidance.

■ REFERENCES

(1) Mong, G. R.; et al. A review on plastic waste valorisation to advanced materials: Solutions and technologies to curb plastic waste pollution. *J. Clean. Prod.* **2024**, *434*, 140180.

(2) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3* (7), No. e1700782.

(3) Andrady, A. L.; Neal, M. A. Applications and societal benefits of plastics. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 1977−1984.

(4) Papadimitriu, M.; Allinson, G. Microplastics in the Mediterranean marine environment: a combined bibliometric and systematic analysis to identify current trends and challenges. *Microplast. Nanoplast.* **2022**, *2* (1), 8.

(5) Cordier, M.; Uehara, T.; Jorgensen, B.; Baztan, J. Reducing plastic production: Economic loss or environmental gain? *Camb. Prisms. Plast.* **2024**, *2*, No. e2.

(6) Porter, A.; Godbold, J. A.; Lewis, C. N.; Savage, G.; Solan, M.; Galloway, T. S. Microplastic burden in marine benthic invertebrates depends on species traits and feeding ecology within biogeographical provinces. *Nat. Commun.* **2023**, *14* (1), 8023.

(7) Boucher, J.; Billard, G. *The Mediterranean: Mare Plasticum*; International Union for Conservation of Nature (IUCN): Gland, Switzerland, 2020; pp x+62. https://portals.iucn.org/library/node/49124.

(8) Llorca, M.; Álvarez-Muñoz, D.; Ábalos, M.; Rodríguez-Mozaz, S.; Santos, L. H.; León, V. M.; Campillo, J. A.; Martínez-Gómez, C.; Abad, E.; Farré, M. Microplastics in Mediterranean coastal area: toxicity and impact for the environment and human health. *Trends Environ. Anal. Chem.* **2020**, *27*, No. e00090.

(9) Cózar, A.; Echevarría, F.; González-Gordillo, J. I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á. T.; Navarro, S.; García-de-Lomas, J.; Ruiz, A.; et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111* (28), 10239−10244.
(10) Napper, I. E.; Thompson, R. C. Plastic Debris in the Marine Environment: History and Future Challenges. *Global Challenges* **2020**, *4*, 1900081.
(11) Sharma, S.; Bhardwaj, A.; Thakur, M.; Saini, A. Understanding microplastic pollution of marine ecosystem: a review. *Environ. Sci. Pollut. Res.* **2024**, *31*, 41402−41445.
(12) Amaral-Zettler, L. A.; Zettler, E. R.; Mincer, T. J. Ecology of the plastisphere. *Nat. Rev. Microbiol.* **2020**, *18*, 139−151.
(13) Kettner, M. T.; Oberbeckmann, S.; Labrenz, M.; Grossart, H. P. The eukaryotic life on microplastics in brackish ecosystems. *Front. Microbiol.* **2019**, *10*, 538.
(14) Kettner, M. T.; Rojas-Jimenez, K.; Oberbeckmann, S.; Labrenz, M.; Grossart, H.-P. Microplastics alter composition of fungal communities in aquatic ecosystems. *Environ. Microbiol.* **2017**, *19* (11), 4447−4459.
(15) Sánchez, C. Fungal potential for the degradation of petroleum-based polymers: An overview of macro- and microplastics biodegradation. *Biotechnol. Adv.* **2020**, *40*, 107501.
(16) Sun, Y.; Xie, S.; Zang, J.; Wu, M.; Tao, J.; Li, S.; Du, X.; Wang, J. Terrestrial plastisphere as unique niches for fungal communities. *Commun. Earth Environ.* **2024**, *5* (1), 483.
(17) Shaaban, M.; Shaaban, K. A.; Abdel-Aziz, M. S. Seven naphtho-γ-pyrones from the marine-derived fungus Alternaria alternata: structure elucidation and biological properties. *Org. Med. Chem. Lett.* **2012**, *2* (1), 6.
(18) Nakbi, A.; Banaoues, R. C.; Ben Dhiab, R.; Ghozzi, K.; Belgacem, S.; Challouf, R.; Haouas, N.; Babba, H. Marine fungal community composition and diversity across a polluted site in the south Mediterranean coast: the Monastir Bay, Tunisia. *Water Sci. Technol.* **2024**, *89* (2), 319−332.
(19) Philippe, A.; Salaun, M.; Quemener, M.; Noël, C.; Tallec, K.; Lacroix, C.; Coton, E.; Burgaud, G. Colonization and Biodegradation Potential of Fungal Communities on Immersed Polystyrene vs Biodegradable Plastics: A Time Series Study in a Marina Environment. *J. Fungi* **2024**, *10* (6), 428.
(20) Gao, R.; Liu, R.; Sun, C. A marine fungus Alternaria alternata FB1 efficiently degrades polyethylene. *J. Hazard. Mater.* **2022**, *431*, 128617.
(21) Baudena, A.; Kiko, R.; Jalón-Rojas, I.; Pedrotti, M. L. Low-Density Plastic Debris Dispersion beneath the Mediterranean Sea Surface. *Environ. Sci. Technol.* **2023**, *57* (19), 7503−7515.
(22) Yang, X.; Huang, G.; Chen, Z.; Feng, Q.; An, C.; Lyu, L.; Bi, H.; Zhou, S. Spotlight on the vertical migration of aged microplastics in coastal waters. *J. Hazard. Mater.* **2024**, *469*, 134040.
(23) Sarkar, A. K.; Rubin, A. E.; Zucker, I. Engineered Polystyrene-Based Microplastics of High Environmental Relevance. *Environ. Sci. Technol.* **2021**, *55* (15), 10491−10501.
(24) Ter Halle, A.; Ladirat, L.; Gendre, X.; Goudouneche, D.; Pusineri, C.; Routaboul, C.; Tenailleau, C.; Duployer, B.; Perez, E. Understanding the Fragmentation Pattern of Marine Plastic Debris. *Environ. Sci. Technol.* **2016**, *50* (11), 5668−5675.
(25) da Cruz Nizer, W. S.; Adams, M. E.; Allison, K. N.; Montgomery, M. C.; Mosher, H.; Cassol, E.; Overhage, J. Oxidative stress responses in biofilms. *Biofilm* **2024**, *7*, 100203.
(26) Yousif, E.; Haddad, R. Photodegradation and Photostabilization of Polymers, Especially Polystyrene: Review. *SpringerPlus* **2013**, *2*, 398.
(27) Ho, B. T.; Roberts, T. K.; Lucas, S. An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach. *Crit. Rev. Biotechnol.* **2018**, *38*, 308−320.
(28) Chubarenko, I.; Bagaev, A.; Zobkov, M.; Esiukova, E. On some physical and dynamical properties of microplastic particles in marine environment. *Mar. Pollut. Bull.* **2016**, *108* (1−2), 105−112.
(29) Ntow-Boahene, W.; Papandronicou, I.; Miculob, J.; Good, L. Fungal cell barriers and organelles are disrupted by polyhexamethylene biguanide (PHMB). *Sci. Rep.* **2023**, *13* (1), 2790.
(30) Malvern Instruments Ltd.. Zeta Potential—An Introduction in 30 Minutes. *Technical Note TN101104*; Malvern Instruments Ltd.: Worcestershire, U.K., 2015, p 14. https://www.malvern.com.
(31) Klauer, R. R.; Silvestri, R.; White, H.; Hayes, R. D.; Riley, R.; Lipzen, A.; Barry, K.; Grigoriev, I. V.; Talag, J.; Bunting, V.; et al. Hydrophobins from *Aspergillus* mediate fungal interactions with microplastics. *bioRxiv* **2024**.
(32) Faten, A. M.; Abeer, A. A. Enzyme Activities of the Marine-Derived Fungus *Alternaria alternata* Cultivated on Selected Agricultural Wastes. *J. Appl. Biol. Sci.* **2013**, *7*, 39−46.
(33) Gao, R.; Liu, R.; Sun, C. A marine fungus efficiently degrades polyethylene. *bioRxiv* **2021**.
(34) Gao, R.; Liu, R.; Sun, C. A marine fungus Alternaria alternata FB1 efficiently degrades polyethylene. *J. Hazard. Mater.* **2022**, *431*, 128617.
(35) Okal, E. J.; Heng, G.; Magige, E. A.; Khan, S.; Wu, S.; Ge, Z.; Zhang, T.; Mortimer, P. E.; Xu, J. Insights into the mechanisms involved in the fungal degradation of plastics. *Ecotoxicol. Environ. Saf.* **2023**, *262*, 115202.
(36) Sánchez, C. Fungal Potential for the Degradation of Petroleum-Based Polymers: An Overview of Macro- and Microplastics Biodegradation. *Biotechnol. Adv.* **2020**, *40*, 107501.
(37) Gkoutselis, G.; Rohrbach, S.; Harjes, J.; et al. Microplastics Accumulate Fungal Pathogens in Terrestrial Ecosystems. *Sci. Rep.* **2021**, *11*, 13214.
(38) Macris, B. J. Production and Characterization of Cellulase and β-Glucosidase from a Mutant of *Alternaria alternata*. *Appl. Environ. Microbiol.* **1984**, *47* (3), 560−565.
(39) Wang, M.; Beissner, M.; Zhao, H. Aryl-aldehyde formation in fungal polyketides: Discovery and characterization of a distinct biosynthetic mechanism. *Chem. Biol.* **2014**, *21* (2), 257−263.
(40) Prenafeta-Boldú, F. X.; Summerbell, R.; Sybren De Hoog, G. Fungi growing on aromatic hydrocarbons: Biotechnology's unexpected encounter with biohazard? *FEMS Microbiol. Rev.* **2006**, *30*, 109−130.
(41) Girometta, C.; Dondi, D.; Baiguera, R. M.; Bracco, F.; Branciforti, D. S.; Buratti, S.; Lazzaroni, S.; Savino, E. Characterization of mycelia from wood-decay species by TGA and IR spectroscopy. *Cellulose* **2020**, *27* (11), 6133−6148.
(42) Gow, N. A. R.; Latge, J.-P.; Munro, C. A. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol. Spectr.* **2017**, *5* (3), funk-0035-2016.

https://doi.org/10.1021/acsestwater.5c00299
ACS EST Water 2025, 5, 4079−4088