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1       **Mechanical Weathering of Polystyrene Microplastics**  
2       **Intensifies Biological Stress but Enhances Ecosystem**  
3       **Multifunctionality in a Freshwater-Sediment Microcosm**  
4

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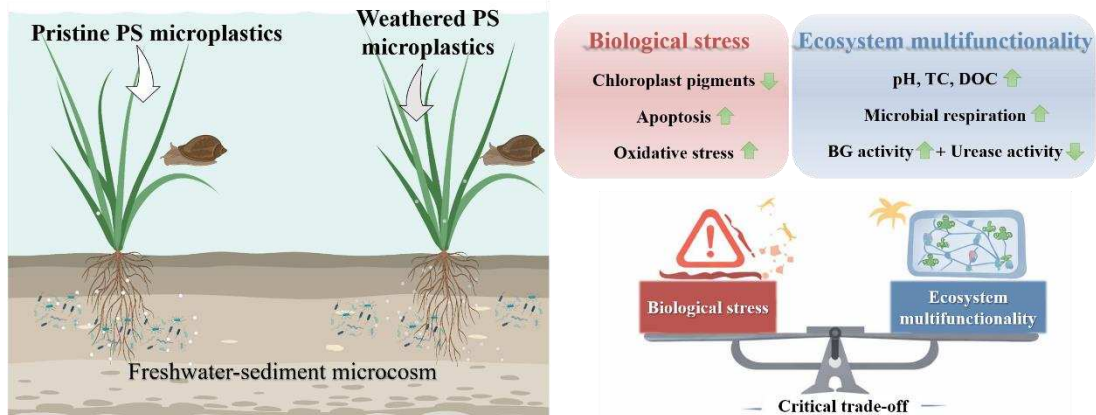
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13 **Graphic abstract**



15 **Abstract**

16 Microplastics are ubiquitous pollutants in freshwater ecosystems, yet their impacts  
 17 on freshwater-sediment ecosystems remain poorly understood. Here, we investigated  
 18 how pristine and mechanically weathered polystyrene (PS) microplastics influence  
 19 organisms, sediment properties, and overall ecosystem multifunctionality (EMF) within  
 20 an integrated freshwater-sediment microcosm containing the rooted macrophyte  
 21 *Vallisneria spiralis*, the benthic snail *Lymnaea stagnalis*, and sediments. Results  
 22 showed that mechanical weathering transformed the originally smooth spherical PS  
 23 microplastics into particles with rougher and more irregular surfaces. Weathered PS  
 24 microplastic induced stronger biological stress in both plants and snails, reflected in  
 25 higher integrated biomarker response (IBR) scores. Weathered PS microplastics  
 26 reduced chlorophyll a and carotenoid content and also altered plant nutrient content,  
 27 indicating impaired photosynthesis and altered carbon metabolism. In snails, weathered  
 28 PS microplastics triggered oxidative stress and significantly increased caspase-3  
 29 activity, suggesting elevated apoptotic responses and ionic regulation demands. Despite  
 30 organism-level impairment, both pristine and weathered PS microplastics enhanced

31 sediment EMF. This enhancement was primarily driven by increased dissolved organic  
32 carbon, elevated  $\beta$ -glucosidase activity, and intensified microbial respiration, reflecting  
33 stimulated carbon cycling. These results reveal ecological trade-off between organism  
34 health and ecosystem performance. Short-term functional stability was maintained  
35 through accelerated microbial metabolism, potentially masking underlying biological  
36 deterioration. Our findings demonstrate that microplastic weathering amplifies  
37 biological stress while simultaneously promoting ecosystem-level functional buffering,  
38 highlighting that apparent stability may entail long-term ecological risk. Integrating  
39 environmentally representative mechanically weathered microplastics with multi-  
40 species ecosystem assessments provides a more comprehensive framework for  
41 evaluating the ecological risks of microplastic contamination.

42 **Keywords:** polystyrene microplastics, mechanical weathering process, microcosm,  
43 environmental multifunctionality

44

## 1. Introduction

Microplastics (particles < 5 mm) have emerged as persistent and pervasive contaminants within aquatic environments, including rivers, lakes and sediments [1]. High concentrations of microplastics have been extensively documented in aquatic systems globally with recent research focus shifting from oceans to freshwater and sediment environments [2, 3]. Sediments in particular act as both accumulation zones and secondary sources, mediating prolonged interactions between microplastics, organisms and geochemical processes [4]. Globally, reported microplastic abundances in freshwater sediments ranged from dozens to tens of thousands per kilogram. The highest recorded concentration was 335,714 items per kilogram, found in sediment from Lake Huron in Canada [5].

Microplastics in freshwater sediments can result in direct and indirect impacts on element cycling, ultimately cascading into a suite of negative effects on organisms in the freshwater-sediment ecosystem [6-8]. Furthermore, microplastics can alter physicochemical properties of sediments, the activities of microbes and benthic organisms, functional enzyme activities, and the expression of genes involved in nutrient metabolism, which may subsequently impact elemental cycling [9-11]. In addition, microplastics can have adverse impacts on the health of organisms in the freshwater-sediment ecosystem, including growth, reproduction, digestion, metabolism, survival, and the diversity of benthic fauna [12].

Despite growing awareness, major gaps remain in our understanding of how microplastics impact interlinked ecological compartments, not simply individual

species in isolation, but the combined dynamics of sediment, rooted aquatic plants, benthic invertebrates and overlying water. Submerged macrophytes and benthic invertebrates are key biological components linking sediment biogeochemistry with ecosystem functioning [13, 14]. *Vallisneria spiralis* is a widespread submerged macrophyte that contributes substantially to primary production, sediment stabilization, oxygen transport to the rhizosphere, nutrient cycling, and habitat provision for aquatic organisms. *Lymnaea stagnalis* is a representative benthic freshwater gastropod inhabiting the sediment-water interface. As an important grazer and detritivore, it participates in organic matter decomposition, nutrient recycling, and energy transfer across trophic levels. Together, these two species represent complementary functional groups occupying different trophic levels within freshwater sediment ecosystems. Their integration into a sediment microcosm enables simultaneous evaluation of organism-level physiological responses, sediment biogeochemical processes, and ecosystem multifunctionality under microplastic exposure.

Moreover, environmental weathering of microplastics is a continuous process involving mechanical abrasion, photochemical oxidation, chemical transformation, and biological degradation, which collectively modify particle morphology, surface chemistry, functional groups, hydrophobicity, and the release of polymer-derived constituents, thereby altering the environmental behavior and biological effects of microplastics [15-18]. While the weathering of microplastics through accelerated oxidation under laboratory conditions has been extensively investigated, mechanical abrasion remains comparatively understudied. In aquatic environments, mechanical

abrasion occurred through wind-driven motion, water turbulence (including waves and tidal actions), and abrasion from sand, particularly in sediment-rich area [19]. Weathered microplastics may exhibit different ecological effects relative to pristine microplastics, but few studies have compared their effects in structurally complex microcosms. From an ecosystem-function perspective, rather than focusing solely on mortality or individual biochemical endpoints, it is increasingly important to adopt ecosystem multifunctionality (EMF) paradigms that capture the simultaneous performance of multiple processes, such as nutrient cycling, organic matter decomposition and carbon sequestration [20]. Linking organism-level stress responses (e.g., oxidative stress, apoptosis) to sediment functionality and EMF provide a more holistic picture of ecosystem resilience or vulnerability under microplastic stress.

In this study, we established a freshwater-sediment microcosm that integrates sediment, water, the rooted macrophyte *Vallisneria spiralis* and the freshwater snail *Lymnaea stagnalis*. We compared the impacts of pristine and mechanically weathered polystyrene (PS) microplastics on these interconnected compartments. Our objectives were: (i) to evaluate how mechanical weathering modifies the biological (plants and snails) and sediment microbial/biogeochemical responses to PS microplastics; and (ii) to assess how these responses influence EMF.

## **2. Materials and methods**

### **2.1 Sediment preparation, test organisms, and microplastics**

The submerged plant *V. spiralis* was obtained from Biotope Aquatics Ltd. (United

Kingdom) and freshwater snails (*L. stagnalis*) were purchased from Blades Biological Ltd. (United Kingdom). PS is one of the most common types of microplastics found in freshwater sediments [21]. Pristine PS microplastics were sourced from Ningbo Chuangjin Import and Export Co., Ltd. Mechanically weathered PS microplastics were produced by mechanically grinding the pristine particles using a stainless steel grinder. Formulated sediment was prepared following the OECD Guideline 218 protocol [22].

## 2.2 Overall experimental design

A 5-week freshwater-sediment microcosm experiment was established, containing a sediment layer, overlying deionized water, freshwater plant *V. spiralis* and snail *L. stagnalis*. Microcosms were constructed in 3 L glass beakers and included 800 g of dried sediment in each glass beaker. Pristine and weathered PS microplastics were added into the sediment to obtain a concentration of 0.1% of the sediment dry weight (DW) by thorough manual homogenization. This concentration is commonly used in sediment microcosm studies to evaluate biological and ecosystem-level responses under relatively high contamination scenarios [23-25]. The plants and snails were introduced into the system, and 2.8 L of deionized water was slowly added to each replicate.

Treatments included control, pristine PS microplastic, and weathered PS microplastic groups. Each treatment consisted of five independent replicate glass beakers (n = 5). Each beaker contained approximately 800 g of artificial sediment, five healthy *Vallisneria spiralis*, and three *Lymnaea stagnalis* individuals. Each beaker was

considered one independent experimental replicate for all subsequent analyses. After exposure, sediment and water samples were collected for physicochemical and enzymatic analyses, while plant and snail tissues were analyzed using a suite of biochemical assays.

## 2.3 Water and sediment properties

Dissolved oxygen, pH and conductivity of the overlying water were measured every five days using a HACH meter. Sediment pH was measured using a pH meter with a sediment-to-water ratio of 1:2.5 (w/v). Dissolved organic carbon (DOC), total carbon (TC) and total nitrogen (TN) content in sediment and TC, total organic carbon (TOC), total inorganic carbon (TIC) content in water were determined with TOC analyzer (Elementar vario MICRO cube).  $\beta$ -1,4-glucosidase (BG), a key enzyme involved in sediment carbon cycling, and urease, commonly used as an indicator of nitrogen cycling [26], were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. BG activity was determined based on the hydrolysis of a chromogenic substrate, whereas urease activity was quantified by measuring ammonia released following urea hydrolysis using a colorimetric assay. The respiration rate of sediment was determined following established protocols described in reference [27].

## 2.4 Plant analysis

### 2.4.1 Chloroplast pigments

The chlorophyll contents of plants were extracted from 0.2 g of leaf using 15 mL of 95 % alcohol at room temperature. The concentrations of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), and carotenoids were determined spectrophotometrically at 665 nm, 649 nm, and 470 nm, respectively, and calculated according to standard equations [28].

#### 2.4.2 Scanning electron microscopy (SEM) observation

For SEM observation, one representative *Vallisneria spiralis* plant was randomly selected from each treatment after completion of the exposure experiment. The leaf and root samples were fixed in 2.5% glutaraldehyde, dehydrated in gradient concentrations of ethanol, coated with a layer of gold, and then the images were observed using SEM (Carl Zeiss EVO MA 15). Multiple fields of view were examined for each tissue, and representative images are presented in the Results.

#### 2.4.3 Oxidative stress markers

Leaves were homogenized in 0.1 mol L<sup>-1</sup> phosphate-buffered saline (PBS, pH 7.4), and the homogenates were centrifuged at 10,000 rpm for 15 minutes at 4 °C. The resulting supernatants were used to measure total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content using assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. T-AOC was determined based on the ferric ion reducing antioxidant power (FRAP) assay. SOD activity was measured using the xanthine–

xanthine oxidase method by quantifying the inhibition of superoxide anion-mediated nitrite formation. MDA content was determined using the thiobarbituric acid (TBA) method as an indicator of lipid peroxidation.

#### 2.4.4 Nutrient content

Leaf soluble sugar, starch, and free amino acid contents were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's protocols. Briefly, approximately 0.1 g fresh leaf tissue was homogenized with the corresponding extraction solution, followed by centrifugation to obtain the supernatant for colorimetric determination. Soluble sugar and starch contents were determined by the anthrone colorimetric method [29], while free amino acid content was quantified using the copper-ninhydrin colorimetric method [30].

#### 2.5 Oxidative stress, enzyme activities in snails

The soft tissues of three snails were collected and homogenized in ice cold PBS solution using a glass homogenizer. T-AOC, SOD activity, MDA content, ATPase, alkaline phosphatase (AKP), acetylcholinesterase (AChE) and caspase-3 activities were measured by assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. ATPase activity was determined by measuring the inorganic phosphate released during ATP hydrolysis. AKP activity was measured colorimetrically based on the hydrolysis of disodium phenyl phosphate. AChE activity

was determined using a colorimetric method based on the hydrolysis of acetylthiocholine. Caspase-3 activity was determined using a colorimetric assay based on the cleavage of the chromogenic substrate Ac-DEVD-p-nitroanilide (Ac-DEVD-pNA), with the released p-nitroaniline quantified at 405 nm.

## 2.6 Integrated biomarker response version 2 (IBRv2) analysis and EMF

IBRv2 analysis was conducted to integrate the indicator responses of plants and snails into composite indices, calculated separately for each treatment group [30]. The plant-related indicators included Chl-a, carotenoids, SOD, MDA, soluble sugars, amino acids and starch, while snail related indicators included SOD, MDA, AChE, AKP, ATPase, and caspase-3 (Text S1).

Freshwater-sediment EMF was characterized using sediment basic properties (pH, DOC, TC and TN), C-related enzyme activity (BG), N-related enzyme activity (urease), and microbial decomposition (respiration rate). Z-scores were used to standardize each variable following the approach of Delgado-Baquerizo et al. [20]. Then the multifunctionality index was calculated as the average of the standardized values for all variables [26].

## 2.7 Data analysis

The data are expressed as mean  $\pm$  SD. One-way analysis of variance (ANOVA) with IBM SPSS Statistics 22 was used to evaluate the differences among different groups and  $p < 0.05$  considered statistically significant.

### 3. Results

#### 3.1 Characterization of pristine and weathered PS microplastics

SEM images of pristine microplastics showed smooth surfaces, whereas mechanically weathered microplastics exhibited rougher surfaces and more irregular morphologies (Fig. S1). SEM-based particle size analysis showed that pristine and mechanically weathered PS-MPs had comparable particle size distributions, suggesting that mechanical weathering primarily altered surface morphology rather than causing substantial particle-size reduction (Table S1). FTIR spectra showed that the characteristic absorption bands of PS remained unchanged after mechanical weathering, with no detectable new oxygen-containing functional groups (e.g., carbonyl or hydroxyl bands), indicating that the weathering process primarily modified particle surface morphology rather than polymer chemical composition (Fig. S1). These changes demonstrate that mechanical weathering altered the surface structure of PS microplastics, increasing surface roughness.

#### 3.2 Impact of PS microplastics on water and sediment properties

The pH and dissolved oxygen levels in all groups increased over time, although no significant difference was observed between the control and microplastic treatments after exposure (Fig. S2). A significant increase ( $p < 0.05$ ) in conductivity was found in both pristine and weathered microplastic treatments compared to the control. Similarly, both TC and TOC concentrations in the overlying water increased significantly after PS

microplastic exposure (Fig. S3).

PS microplastics significantly altered sediment properties with pH, TC and DOC in both pristine and weathered microplastic groups being higher than that of the control (Fig. 1A-C). Urease activity decreased significantly in the weathered PS microplastic group, whereas BG activity increased in both pristine and weathered PS groups (Fig. 1D and 1E). Moreover, both pristine and weathered microplastics enhanced sediment respiration rates.

### 3.3 Impact of PS microplastics on plant *V. spiralis* and snail *L. stagnalis*

Pristine and weathered PS microplastics did not result in any visible plant damage or affect plant fresh weight, although weathered PS microplastics significantly reduced Chl-a and carotenoid content, while pristine particles decreased carotenoid content (Fig. 2). SEM images revealed that weathered microplastics adhered to both leaves and roots of *V. spiralis* (Fig. 3), confirming direct physical interaction between microplastics and plant tissues.

In *V. spiralis*, weathered PS significantly increased SOD activity and MDA content, whereas T-AOC remained unchanged (Fig. 4). Weathered PS microplastics also increased soluble sugar and free amino acid levels while decreased starch content in the leaves (Fig. 5).

In *L. stagnalis*, weathered PS microplastics significantly increased SOD activity, while pristine PS microplastics had no significant effects (Fig. S4). No significant change in T-AOC and MDA levels was found in all microplastic treatments. Caspase-3

activity was significantly promoted in the weathered PS microplastic group, whereas AChE and AKP activities remained unaffected. Weathered PS significantly increased ATPase activity (Fig. S5).

### 3.4 Impact of pristine and weathered PS microplastics on the whole microcosm (IBR and EMF analysis)

IBR analysis revealed higher IBR values for both *V. spiralis* and *L. stagnalis* in the weathered PS microplastic group compared to the pristine PS microplastic group (Fig. 6), confirming that weathered PS microplastics induced stronger biological impacts on organisms within the microcosm. The EMF of the freshwater-sediment microcosm was significantly higher in both pristine and weathered PS microplastic treatments compared to the control (Fig. 6C), indicating that this freshwater-sediment microcosm had the ability to tolerate the PS microplastic pollution without a decline in sediment functions and multifunctionality. Carbon-related sediment enzymes and microbial respiration were the dominant contributors to EMF.

## 4. Discussion

### 4.1 EMF as an integrative indicator of microplastic effects

EMF is used to evaluate ecosystem functions and services under environmental stress and pollution, integrating various biogeochemical pathways, such as nutrient cycling, organic matter decomposition and productivity [26]. Despite its growing use in terrestrial microplastic studies, its application to microplastic-contaminated

freshwater sediments remains limited [20].

In our study, both pristine and mechanically weathered PS microplastics significantly increased EMF despite inducing measurable physiological impairment in *V. spiralis* and *L. stagnalis*, including reduced photosynthetic pigments, oxidative stress, and apoptosis (Fig. 7). This divergence indicates that ecosystem-level functional performance does not necessarily decline in parallel with organism-level impairment. The increased EMF in our study aligns with previous research demonstrating that a relatively low concentration of PS nanoplastics in soils (0.3 g/kg) had a positive impact on EMF [31]. Liu et al. [26] reported 1% PE microplastics significantly improved soil EMF by influencing the activity of  $\alpha/\beta$ -1, 4-glucosidase, urease and protease, and ammonium-N content. Such similar increases in soil EMF under microplastic exposure have been attributed to stimulated microbial activity and altered nutrient cycling.

Mechanistically, EMF enhancement in our microcosm was primarily driven by carbon-related processes, including elevated BG activity, increased microbial respiration, and higher DOC concentrations (Fig. 1), which reflected intensified microbial carbon cycling and greater substrate turnover. Microplastic-induced stimulation of soil respiration and carbon flux has been reported in several studies [32, 33], suggesting that altered sediment physicochemical properties and additional carbon inputs can promote microbial metabolic activity. Such responses suggest a compensatory mechanism whereby microbial communities accelerate metabolic activity to maintain sediment functioning under disturbance. Thus, microplastic-induced increases in sediment carbon availability and physicochemical alteration may

stimulate microbial processes that temporarily sustain ecosystem output.

However, accelerated microbial metabolism requires increased metabolic energy expenditure, faster enzyme turnover, and accelerated consumption of available carbon substrates. While this metabolic intensification can preserve short-term functional stability, it may progressively deplete organic carbon reserves, alter redox conditions, and shift microbial community composition [34, 35]. Thus, the observed increase in EMF may reflect short-term functional buffering that temporarily masks physiological impairment at the organism level. Such functional stability should not be interpreted as evidence of sustained ecosystem health, because prolonged metabolic compensation may ultimately deplete energy reserves, alter community structure, and reduce ecosystem resilience. However, longer-term studies are required to determine whether the observed response represents transient functional compensation, ecosystem rebalancing, or eventual functional deterioration.

Collectively, these findings demonstrate that EMF is a valuable integrative metric for assessing ecosystem-scale responses to microplastic pollution. Integrating organism-level stress indicators with multifunctionality metrics is essential to distinguish genuine resilience from transient compensation driven by elevated metabolic demand.

#### 4.2 Weathering process alters property of PS microplastics and exacerbates their ecotoxicological impacts

SEM observations revealed roughened and irregular particle surfaces after

mechanical weathering, whereas FTIR spectra remained nearly identical between pristine and weathered PS microplastics, indicating that mechanical weathering mainly altered the physical surface characteristics of the particles without substantially changing the chemical structure of the PS polymer. Yan et al. [36] demonstrated that mechanical weathering substantially altered the surface morphology and physicochemical characteristics of PS, PP, and polyethylene terephthalic microplastics through free radical processes, increasing surface roughness and reactivity. In our study, weathered PS microplastics induced higher oxidative and metabolic disturbances in organisms, underscoring the potential biological risks of weathered microplastics to ecosystem organisms. These findings align with previous study, reporting that weathering process could enhance interactions with living organisms and increase bioavailability and toxicity [37, 38]. Moreover, these results indicate that mechanical fragmentation, though less studied than photochemical or oxidative weathering, critically shapes microplastic toxicity in aquatic systems.

Moreover, although mechanical weathering is often reported to generate smaller plastic fragments through fragmentation [39, 40], no significant reduction in particle size was observed in the present study. Instead, mechanical weathering primarily altered the surface morphology of PS microspheres, producing rougher surfaces and irregular edges while maintaining a similar particle size distribution. This discrepancy is likely attributable to the relatively short weathering duration and the intrinsic properties of the commercial PS microspheres used in this study. Thus, changes in surface properties, including increased roughness, irregular morphology, and potentially altered surface

chemistry, are more likely responsible for the increased toxicity of weathered PS microplastics in this study.

#### 4.3 Biological responses across trophic levels

Biological responses in *V. spiralis* and *L. stagnalis* revealed adverse impacts induced by microplastics. In *V. spiralis*, exposure to weathered PS microplastics significantly reduced Chl-a and carotenoid levels (Fig. 2), indicating impaired photosynthetic efficiency. This result is in alignment with several studies which have demonstrated that microplastics can impair photosynthetic pigment content and impact photosynthetic efficiency through physical adherence and physiological interference [41-43]. Microplastics can adhere to plant surfaces via electrostatic interaction, hydrophobic interaction, and surface roughness, leading to a shadowing effect that reduces the light-receiving area and, consequently, photosynthesis [43]. The SEM results in our study confirmed such adhesion, particularly in the weathered microplastic group (Fig. 3). Increased SOD and MDA levels in the weathered PS microplastic group confirmed oxidative stress and membrane lipid peroxidation (Fig. 4). Hu et al. [44] also found that UV-aged polyvinyl chloride (PVC) microplastics induced more adverse impacts on the submerged aquatic species, *Vallisneria natans*, than pristine PVC. Elevated soluble sugars and amino acids, together with decreased levels of starch, was suggested to show metabolic reallocation under stress (Fig. 5). The increased soluble sugar level in our study may be a stress resistance mechanism to assist in eliminating ROS and an antiphytotoxic mechanism that compensates for reduced protein content,

which was also found in a previous study [45]. As the main product of carbon metabolism, the decrease of starch level in the weathered microplastic group indicated impaired carbon fixation and altered carbohydrate metabolism [46]. Decreased starch level is regarded as a stress tolerance mechanism in plants, which can enhance hydrolysis and reduce synthesis, leading to the breakdown of starch and proteins into small molecules like amino acids or soluble sugars and the release of energy [47, 48]. Consistent with these findings, our results imply that weathered PS microplastics triggered plant defense responses and interfered with carbohydrate metabolism, energy balance, and carbon fixation to a greater extent than pristine microplastics.

In *L. stagnalis*, weathered PS microplastic exposure increased SOD, caspase-3, and ATPase activities, while AChE and AKP remained unchanged (Fig. S4-S5). Elevated SOD activity indicated activation of antioxidant defenses against ROS, reflecting oxidative stress induced by weathered PS exposure [49]. The increase in caspase-3 suggested that oxidative damage might trigger apoptosis, consistent with previous observations in freshwater snails exposed to nanoparticles [49-51]. Programmed cell death may also be a result of oxidative stress and part of immune defense mechanisms of gastropods against pathogenic or environmental stressors [52]. Increased  $\text{Na}^+/\text{K}^+$ -ATPase activity likely reflected compensatory ion transport to restore gradients disrupted by oxidative membrane injury [53, 54]. Similar responses have been reported in zebrafish exposed to polyethylene microplastics, where ATPase activity was enhanced to maintain ionic balance and mitigate lipid peroxidation [55]. Collectively, these enzyme responses demonstrate that weathered PS microplastics impose oxidative

and energetic burdens on *L. stagnalis*, with apoptosis and ion-regulation pathways acting as compensatory defense mechanisms. Although no mortality occurred, these changes reveal physiological strain.

The IBR analysis quantitatively supported these results by summarizing multiple biochemical endpoints into a single stress index [56, 57]. Higher IBR scores under weathered PS microplastic exposure reflected that weathered microplastics exert greater biological stress on *V. spiralis* and *L. stagnalis* in the microcosm (Fig. 6).

#### 4.4 Alterations in sediment property and microbial dynamics

Both pristine and weathered PS microplastics significantly altered sediment properties and microbial processes (Fig. 1). Previous studies showed that microplastics caused different impacts on soil/sediment pH, depending on polymer type, concentration, shape, and size [58, 59]. For example, Liu et al. [60] found that polyamide (PA) microplastics increased sediment pH at 0.1% and 1% concentrations (w/w), potentially due to their weathering and degradation. Li et al. [61] found that microplastics reduced pH in acid soils but increased pH in alkaline soils, suggesting that microplastic accumulation would induce deterioration of pH in soils. Microplastics can also indirectly influence sediment pH by affecting microbial activity [62]. As pH plays a key role in nutrient bioavailability and plant performance, such shifts may have broader ecosystem implications [63]. Given that PS microplastics are primarily composed of carbon, their addition directly increased sediment TC content in this study. Generally, the carbon present in non-degradable microplastics consists of polymers that

are difficult to degrade, however, previous studies have shown that although the impact on DOC content is smaller than that of degradable microplastics, non-degradable microplastics could still increase DOC content in soil/sediment, as observed in this study [64, 65]. Furthermore, as the addition of microplastics has the potential to stimulate microbial activity, thereby promoting DOC accumulation in soil/sediment [66, 67]. In our study, the concurrent increase in TC and DOC (Fig. 1B-C) indicates greater carbon availability and accelerated microbial carbon processing, which is consistent with the elevated BG activity and respiration rates observed (Fig. 1E-F). These carbon-related processes were among the strongest positive contributors to EMF (Fig. 6), demonstrating that microplastic-driven shifts in sediment carbon turnover played a central role in the overall enhancement of ecosystem multifunctionality.

Enzymatic responses further demonstrated these alterations. Elevated BG activity and increased sediment respiration rates indicated enhanced microbial decomposition and carbon cycling (Fig. 1) [33, 68]. Several studies have shown that the addition of microplastics can increase soil respiration rate by stimulating microbial and enzymatic activities [33, 69, 70]. In addition, another study reported that the addition of microplastics increased respiration rate and microbial growth by increasing the pH and DOC content, with DOC being considered the source of soil microbial energy and nutrients [32]. While most studies have focused on soils, relatively little is known about microplastic effects on respiration in freshwater sediments. In contrast, urease activity was inhibited in the weathered PS microplastic group (Fig. 1D), suggesting an inhibition of nitrogen transformation processes by weathered PS microplastics. The

decreased urease activity was potentially related to reduced sediment TN content [11, 26]. These contrasting effects imply that microplastics can shift sedimentary metabolic balance toward carbon cycling dominance. In addition, more attention should also be directed toward microbial processes in the sediment, including microbial community structure, gene expression, and nutrient cycling pathways, to better understand the underlying mechanisms of microplastic toxicity.

The observed enhancement in EMF under microplastic exposure likely reflects the combined effects of increased microbial activity and carbon substrate availability. Such short-term stimulation of biogeochemical functions can elevate multifunctionality indices even under biological stress.

#### 4.5 Environmental implications

This study indicated mechanically weathered PS microplastics induced intensified biological stress (high IBR value), stimulated microbial metabolism, and further enhanced EMF. While increased microbial activity and carbon cycling maintained sediment multifunctionality, elevated IBR indices revealed significant physiological costs at the organismal level that may threaten biodiversity and energy flow over time. These findings emphasize the importance of integrating microplastic weathering and multifunctionality frameworks into microplastic risk assessments. Evaluations focused solely on risks to single biochemical endpoints or single species might underestimate ecological consequences [26, 31, 33]. Future studies should integrate biochemical, microbial, and multifunctionality indicators in long-term and field-based experiments,

while also incorporating quantitative assessments of microplastic recovery, spatial distribution, and stability within sediments. Such approaches would improve exposure characterization and help determine whether the observed enhancement of EMF reflects genuine ecosystem resilience, transient functional compensation, or the onset of long-term functional deterioration.

## 5. Conclusions

This study demonstrated that mechanical weathering amplifies the ecological effects of PS microplastics in freshwater-sediment ecosystems. Mechanical weathering increased the surface roughness and reactivity of PS microplastics. Elevated IBR values confirmed that weathered PS microplastics induced stronger oxidative and metabolic disturbances than pristine microplastics. In contrast, enhanced BG activity, microbial respiration, and DOC content under PS microplastic exposure contributed to higher EMF.

Together, these results revealed a tradeoff between organism health and ecosystem performance. Enhanced microbial carbon cycling buffered sediment functions, maintaining short-term functional stability despite biological stress. However, such stability appears to be sustained through elevated metabolic effort and accelerated energy turnover.

Overall, our findings suggest that short-term functional stability under microplastic exposure may entail long-term ecological risk. Our study provides an integrative framework linking microplastic weathering, organismal stress (IBR), and EMF. These

findings underscore the importance of incorporating microplastic weathering and multifunctionality concepts into risk assessments, and highlight the need for more complex, environmentally realistic experimental designs that move beyond single-organism exposures to capture cross-compartment interactions and ecosystem-level responses.

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### Figure captions

**Fig. 1.** Alteration of sediment properties after exposure to pristine and weathered PS microplastics. A: pH; B: TC content; C: DOC content; D: urease activity; E: BG activity; F: respiration rate. P-MPs represent pristine microplastics and W-MPs represent weathered microplastics. Data represent mean  $\pm$  SD. Different letters indicate significant differences between treatments ( $p < 0.05$ ).

**Fig. 2.** Chloroplast pigment level in plant leaves after exposure to pristine and weathered PS microplastics. A: Chlorophyll-a; B: Chlorophyll-b; C: carotenoids. P-MPs represent pristine microplastics and W-MPs represent weathered microplastics. Data represent mean  $\pm$  SD. Different letters indicate significant differences between treatments ( $p < 0.05$ ).

**Fig. 3.** SEM images of plant leaves and roots after exposure of pristine and weathered microplastics. A: leaves of the control group; B: roots of the control group; C: leaves of the pristine PS microplastic group; D: roots of the pristine PS microplastic group; E: leaves of the weathered PS microplastic group; F: roots of the weathered PS microplastic group.

**Fig. 4.** Oxidative stress in plant leaves after exposure to pristine and weathered PS microplastics. A: T-AOC level; B: SOD activity; C: MDA content. P-MPs represent pristine microplastics and W-MPs represent weathered microplastics. Data represent mean  $\pm$  SD. Different letters indicate significant differences between treatments ( $p < 0.05$ ).

**Fig. 5.** Nutrient content in plant leaves after exposure to pristine and weathered PS

microplastics. A: soluble sugar; B: free amino acids; C: starch. P-MPs represent pristine microplastics and W-MPs represent weathered microplastics. Data represent mean  $\pm$  SD. Different letters indicate significant differences between treatments ( $p < 0.05$ ).

**Fig. 6.** IBR analysis and EMF of freshwater-sediment microcosm after exposure to pristine and weathered PS microplastics. A: IBR analysis of plant *V. spiralis*; B: IBR analysis of snail *L. stagnalis*; C: EMF of freshwater-sediment microcosm. P-MPs represent pristine microplastics and W-MPs represent weathered microplastics.

**Fig. 7.** Overall effects of PS microplastics on freshwater-sediment microcosm.