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Evaluating polystyrene nanoparticle toxicity in eelgrass: impacts on photosynthetic efficiency, oxidative stress, and DNA integrity

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Abstract. Micro- and nanoplastic pollution is ubiquitous in marine and coastal areas with the potential to impact valuable ecosystems, such as seagrass meadows. One of the important questions in seagrass ecology today is whether micro- and nanoplastics could seriously threaten seagrass health, and which parameters would be the best to observe such impacts. As a part of this research necessity, we have explored the effect of pure polystyrene nanoparticles on the maximum quantum yield of PSII, oxidative stress parameters, and DNA integrity on leaves of *Zostera marina* L. fragments, under controlled conditions. The three different indicator types are not negatively impacted when eelgrass fragments are subjected to a short time exposure (> 10 days). To find the most responsive indicators for micro- and nanoplastics impacts, further studies on a variety of metrics, operating at different seagrass structure levels and time frames, are necessary.

Key words: Pure polystyrene, nanoparticles, eelgrass, short-term exposure, photosynthesis capacity, oxidative stress, DNA integrity.

Introduction

Plastic pollution has become a major environmental issue, due to the ubiquitous distribution of plastics in all types of living environment (water, terrestrial, soil, other organisms) (e.g., Barnes et al., 2009, Azeem et al., 2021; Jia et al., 2023; Jeong et al., 2024; Ali et al., 2024), their long-term persistence (Gallo et al., 2018; Chamas et al., 2020; Krause et al., 2020; Turner et al., 2020) and their adverse and toxic effects on living organisms (e.g., Alberghini et al., 2022; Pavičić-Hamer et al., 2022; Jia et al., 2023; Jeong et al., 2024; Zhang et al., 2024; Abbas et al., 2025; Chaudhary et al., 2025; Kovacs et al., 2025). During the past decades, the presence of plastic particles with microscopic dimensions – the micro- and nanoplastics has become a growing concern (Barnes et al., 2009). These particles have been detected in the human body (Kovacs et al., 2025), as well as in plant (Azeem et al., 2021; Jia et al., 2023; Chaudhary et al., 2025) and animal taxa (Alberghini et al., 2022). As a global contaminant, microplastics are ubiquitous in marine environments (Mutuku et al., 2024). Coastal waters are particularly vulnerable to microplastic (MP) and nanoplastic (NP) pollution due to their proximity to possible sources (Lebreton et al., 2017; Strokal et al., 2023). Seagrass meadows are emblematic coastal ecosystems lying at the foundation of the coastal food webs (Li et al., 2021; Lin et al., 2021; Li et al., 2022). Due to their fundamental role in sustaining ecological balance and supporting ecosystem services delivery, as well as their vulnerability to human-induced impacts, seagrass ecosystems have been continuously a research focus on different aspects of these im-

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pacts (e.g., Burkholder et al., 2007; Unsworth et al., 2017; Glasby & West, 2018; Sagerman et al., 2019; Unsworth et al., 2022). During the past decade, studies on plastic pollution within seagrass ecosystems have been constantly increasing (Suripatty et al., 2026) because of the growing concern about possible impacts they may have on seagrass meadows' health and service delivery.

While the role of seagrass to serve as sinks and traps for micro- and nanoplastic at a meadow level is relatively well documented (Suripatty et al., 2026), the effect of these particles on seagrass physiology and health has only recently started to gain attention. Three commonly encountered plastic types (polystyrene (PS), polyethylene (PE), and unspecified plastic polymer, used in gallon caps) have been investigated in experimental studies (Menicagli et al., 2022; Molin et al., 2023; Nugraha et al., 2025; Egea et al., 2026).

Although studies on the effect of PE microparticles on the widespread eelgrass are available (Molin et al., 2023; Egea et al., 2026), there is a need to expand the assessments of the potential effects of other commonly used plastic types, such as PS. PS is the sixth most produced fossil-based plastic in the EU for 2024, accounting for 5.1% of the total production (Plastic Europe, 2025). Studies on its phytotoxicity on aquatic plants have shown that MPs adhere to plant surfaces, reducing light and nutrient uptake, inducing oxidative stress in higher plants, and causing chloroplast deformation, suppressed biomass production, and downregulated pathways related to cellular processes, genetic information processing, and metabolism in algae (reviewed in Basu et al., 2025). In the seagrass *C. nodosa*, Menicagli et al. (2022) observed negative effects on photochemical efficiency in plants treated with 500 nm PS NP, after two days of exposure. In addition, PS NP can induce root degeneration, increased leaf mortality, and oxidative stress (Menicagli et al., 2022). Furthermore, microplastics can fracture and damage DNA through direct and indirect genotoxic mechanisms (Tang et al., 2025).

The present study aims to investigate whether virgin polystyrene nanoparticles less than 1 μm (GESAMP, 2019) induce detectable overall physiological and genetic stress in eelgrass under controlled laboratory conditions, measured by effecttive quantum yield of PS II, oxidative stress markers, and DNA integrity as an indicator of potential genotoxic effects.

Materials and methods

Design of experiment

This study employed a controlled laboratory experiment to investigate whether polystyrene nanoparticles affect the eelgrass at physiological, biochemical, and genetic levels. The shoots were field-collected, acclimated to laboratory conditions, and maintained under uniform environmental settings. Sixteen of them were exposed to polystyrene nanoparticles at a single concentration for a period of 12 days, while another 16 shoots were kept under identical conditions without exposure, serving as a control group. The effects were assessed by measuring photosynthetic efficiency, oxidative stress levels (lipid peroxidation and antioxidant capacity), and genotoxicity, including DNA integrity.

Sampling area and plant material collection

Eelgrass shoots were collected from approximately 2 m depth via SCUBA diving from a shallow-water eelgrass meadow in the Varna Bay, the Bulgarian Black Sea (43.22° N, 27.98° E) (Fig. 1) during August, 2023. At the time of sampling, water salinity in the location was 15, and water temperature was 24°C at the bottom.

Laboratory conditions

In the laboratory, a total of 32 test fragments were rinsed with artificial seawater (ASW) and put into glass beakers with a volume of 2 L, containing ASW with a salinity of 14. Each fragment consisted of one visibly healthy shoot (only vegetative ones were used) and a rhizome with at least four internodes. Fragments were kept without sediment and were attached to the bottoms of the beakers via silicone suction cups to prevent plant floating and exposure of leaves to air. Before putting them into the beakers, the ASW in each of them was oxygenated with a commercially available air pump for aquaria, until oxygen concentration reached at least 8 mgL^{-1}. The plant fragments were illuminated with artificial plant growth lights (PPFD ~ 100 μmol photons $m^{-2}s^{-1}$) and kept at a temperature of 26°C. Plants were visually inspected daily for signs of stress (lack of gas bubbles at the root tips and leaf edges, falling leaves, root necrosis). The level of oxygen saturation in the beakers was monitored daily with WTW Multi-line 3410 Portable Digital Multiparameter (conductivity sensor TetraCon 925 and oxygen sensor FDO

925) for signs of photosynthesis - respiration disbalance. Water loss due to evaporation during the experiment was compensated for with the addition of distilled water. Plants were left to acclimate to laboratory conditions for four days.

Nanoparticles

Commercially available virgin polystyrene particles (Sigma-Aldrich®, St. Louis, Missouri, USA) with nominal diameters of 0.5 µm were added, without further treatment, as a water suspension to 16 randomly selected beakers, to reach a concentration of 67 μgL^{-1} (Menicagli et al., 2022).

Maximum quantum yield of PSII measurements

The maximum quantum yield of PSII (Fv/Fm) provides a rapid, non-destructive way to monitor stress in plants (Baker, 2008). At the start of the experiment, the maximum quantum yield of PSII (Fv/Fm) was measured on the third youngest leaf (previously dark-adapted for 20 min) of each fragment using a DIVING-PAM-II (Walz©) fluorometer. On the 12th day of the test period, Fv/Fm was measured again to test for detectable signs of overall stress.

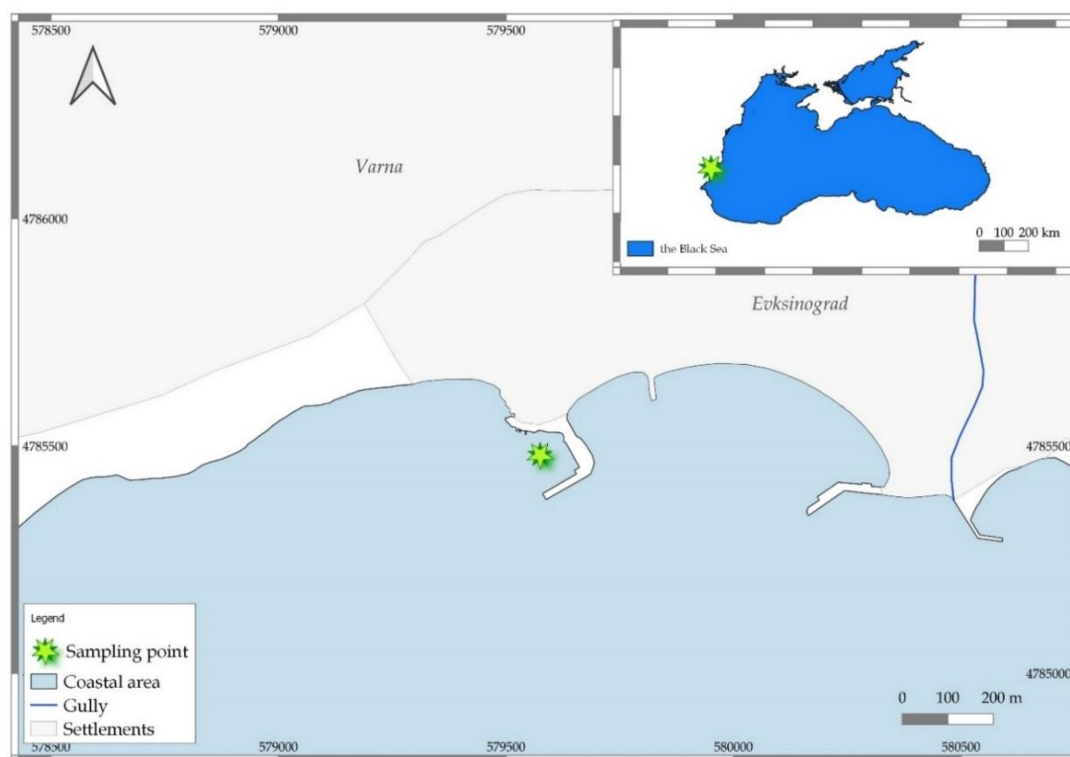

Fig. 1. Sampling station in the Varna Bay, the Black Sea.

Assessment of lipid peroxidation and antioxidant capacity of eelgrass leaves.

Lipid peroxidation

Lipid peroxidation (LPO) was determined according to the method of Hodges et al. (1999). Single fresh leaves of eelgrass (n = 6 for the control group and n = 6 for the group treated with PS particles (the PS group)) were homogenized in 50 mM potassium phosphate buffer. The homogenates were centrifuged at 5000 rpm for 15 min, and the supernatants were collected. Aliquots of each sample (analyzed in triplicate) were mixed with a solution containing 0.5% thiobarbituric acid (TBA) and 5% trichloroacetic acid (TCA), and the mixtures were heated in a boiling water bath for 30 min. After cooling to room temperature, the samples were centrifuged again at 5000 rpm for 15 min. Absorbance was recorded at 450, 532, and 600 nm to separate the true malondialdehyde (MDA) signal from interfering compounds. LPO levels were calculated according to the formula:

$$\mathbf{MDA = (6.45\times(A532-A600))-(0.56\times A450)}$$

where A532 is the absorbance of the MDA–TBA complex; A600 is the correction for non-specific turbidity, and A450 is the correction for sugar–TBA and other interfering compounds.

Extract preparation

Eelgrass leaves from the control group and the PS group were air-dried at room temperature for 10 days. The dried material was subsequently macerated in 96% ethanol for 48 h at a solid-to-solvent ratio of 1:30 (w/v). The extracts were then centrifuged at 5000 rpm for 20 min, and the resulting supernatants were collected and used for the determination of DPPH, ABTS, FRAP, and CUPRAC activities.

DPPH assay

The free radical scavenging activity of the extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the method of Brand-Williams et al. (1995), with minor modifications. The assay is based on the reduction of the stable DPPH• radical in the presence of antioxidant compounds, resulting in a decrease in absorbance. A freshly prepared DPPH solution in methanol (0.1 mM) was used. An aliquot of the sample extract (previously diluted 1:5 with methanol) was mixed with the DPPH solution in a 1:1 (v/v) ratio. The reaction mixtures were incubated in the dark at room temperature for 30 min. Methanol mixed with the sample extract served as a blank, while methanol with DPPH solution served as a control. After incubation, absorbance was measured at 517 nm using a spectrophotometer. The radical scavenging activity was calculated as a percentage inhibition of DPPH• according to the equation:

Inhibition=((Acontrol−Asample)/Acontrol)×100

All measurements were performed in triplicate.

ABTS assay

The ABTS radical cation (ABTS$\bullet^+$) scavenging activity was determined according to the method of Re et al. (1999), as modified by Raynova et al. (2015). The assay is based on the ability of antioxidants to quench the ABTS$\bullet^+$ radical, resulting in a decrease in absorbance. The ABTS$\bullet^+$ radical cation was generated by reacting ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Before analysis, the ABTS$\bullet^+$ solution was diluted with ethanol to obtain an absorbance of about 0.700 at 734 nm. An aliquot of the sample extract (diluted 1:10) was added to the ABTS$\bullet^+$ working solution, and the mixture was incubated at room temperature for 6 min. Absorbance was measured at 734 nm against an appropriate blank. The radical scavenging activity was expressed as a percentage inhibition of the ABTS$\bullet^+$ radical. All measurements were performed in triplicate.

CUPRAC assay

The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed according to the method described by Apak et al. (2004), with minor modifications. The method is based on the reduction of Cu(II) to Cu(I) by antioxidants present in the sample, in the presence of neocuproine, forming a colored Cu(I)–neocuproine complex. The reaction mixture consisted of 1 mL of 10 mM copper(II) chloride ($CuCl_2$), 1 mL of 7.5 mM neocuproine (in ethanol), and 1 mL of 1 M ammonium acetate buffer (pH 7.0). Subsequently, an appropriate volume of the sample extract (previously diluted 1:1 with solvent) was added, and the total volume was adjusted to 4.1 mL with distilled water. The mixtures were incubated at room temperature for 30 min to allow complete color development. Absorbance was then measured at 450 nm against a reagent blank using a spectrophotometer. A calibration curve was prepared using Trolox as a standard, and the results were expressed as Trolox equivalents (μM TE). All measurements were performed in triplicate.

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie & Strain (1996). The assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of antioxidants, forming a blue Fe^{2+}–2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) complex. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a ratio of 10:1:1 (v/v/v). The reagent was pre-warmed to 37°C before use. An aliquot of the sample extract was added to the FRAP reagent, and the reaction mixture was incubated at 37°C for 4 min. Absorbance was measured at 593 nm against a reagent blank using a spectrophotometer. A calibration curve was prepared using Trolox as a standard, and the results were expressed as Trolox equivalents (μM TE). All measurements were performed in triplicate.

Analysis of DNA integrity

A total of 32 samples were used for the analysis of DNA fragmentation by agarose gel electrophoresis (16 in each group: control and NP-exposed). Genomic DNA was extracted from the eelgrass using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany) according to the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop Lite Plus Spectrophotometer (Thermo Fisher Scientific, USA). From each DNA sample, subsamples with equal concentrations (10 ng μL^{-1}) were prepared. A volume of 6.7 µL (corresponding to 67 ng DNA) from each sample was used for visualization on a 1.3% agarose gel (run at 80 mV for 1 hour) to assess DNA degradation. GeneRuler Express DNA Ladder (Thermo Scientific™) was used as a marker. Gel was stained with GelRed® Nucleic Acid Gel Stain (Biotium), and DNA fragments were visualized under UV light and photographed (GelDoc Go Imaging System, Bio-Rad). Samples were categorized on a scale from 1 to 4 according to DNA degradation level, following the criteria described in Quinet et al. (2016) as applied by Masiá et al. (2021).

Statistics

Statistical analysis for plant stress indicators was done in MS Excel, using the add-on Real Statistic Resource Pack (Release 9) (Zaiontz, 2023). Assessments were done at the significance level of 0.05.

Map creation

Sampling station map was produced with QGIS v. 3.44.8. Shape-files for urban areas, coastal waters, and streams were obtained from the Black Sea Basin Directorate (https://www.bsbd.bg/) and for the Black Sea – from the European Environmental Agency website (EEA, 2022). Map CRS: EPSG - WGS 84/UTM 35 N.

Results

Maximum quantum yield of PSII

At the end of the experiment, two fragments lacking gas bubbles at the edges of leaves and root tips, and exhibiting pale green, falling leaves, brown root tips, and a lower level of oxygen saturation in the beakers were considered outliers (Fv/Fm = 0.571 and 0.599, indicating significant stress). These fragments were not included in the analysis. All the other plant fragments were alive at the end of the experimental period. The maximum quantum yield of PSII of the untreated group was 0.706 ±0.006 (95% confidence interval) and of the treated group was 0.704 ± 0.005 (95% confidence interval). The data for Fv/Fm were normally distributed (untreated: Shapiro-Wilk w = 0.98, p = 0.93, d'Agostino-Pearson DA = 0.20, p = 0.91; treated: Shapiro-Wilk w =0.97, p = 0.81; d'Agostino-Pearson DA = 0.52, p = 0.77) and variations were homogenous (F-test, p = 0.42). Our results show that PS NP (500 nm) does not cause a detectable impact on the maximum quantum yield of the PS II, in eelgrass fragments after 12 days of exposure (Student's t-test: t (28) = 0.18, p = 0.86), Fig. 2.

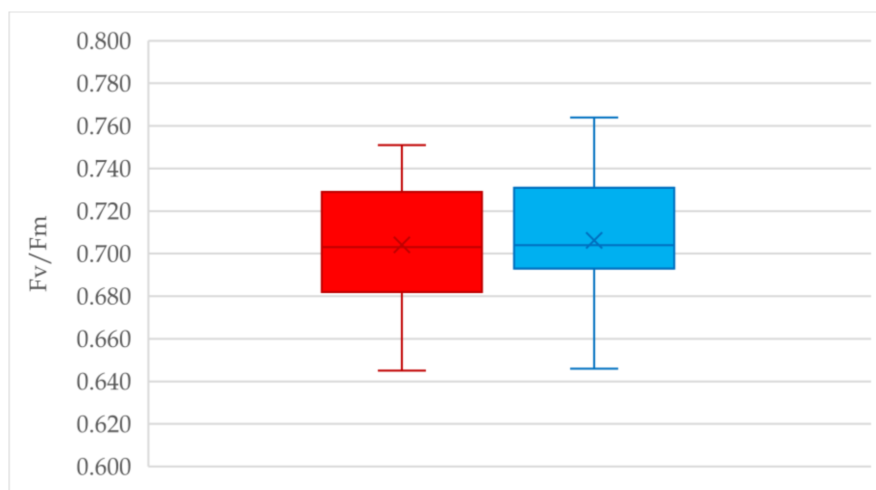

Fig. 2. Box plot of NP-exposed (red) and control (blue) eelgrass fragments at the 12th day of exposure.

Lipid peroxidation and antioxidant activity of eelgrass leaves

Lipid peroxidation, expressed as MDA concentration, was lower in the PS group compared to the control; however, this difference was not statistically significant (Fig. 3A). Antioxidant activity exhibited a similar or slightly decreased trend in PS-treated leaves. Specifically, DPPH radical scavenging (Fig. 3B) and ABTS assay results (Fig. 3C) showed a slight reduction in the PS group, but these differences were not statistically significant. In contrast, the CUPRAC assay demonstrated a modest increase in antioxidant capacity in PS-treated leaves relative to controls, although this change was also not statistically significant (Fig. 3D). No FRAP activity was detected in the samples in the conducted experiments. Overall, PS particles' treatment did not induce significant alterations in LPO or antioxidant activity of eelgrass leaves.

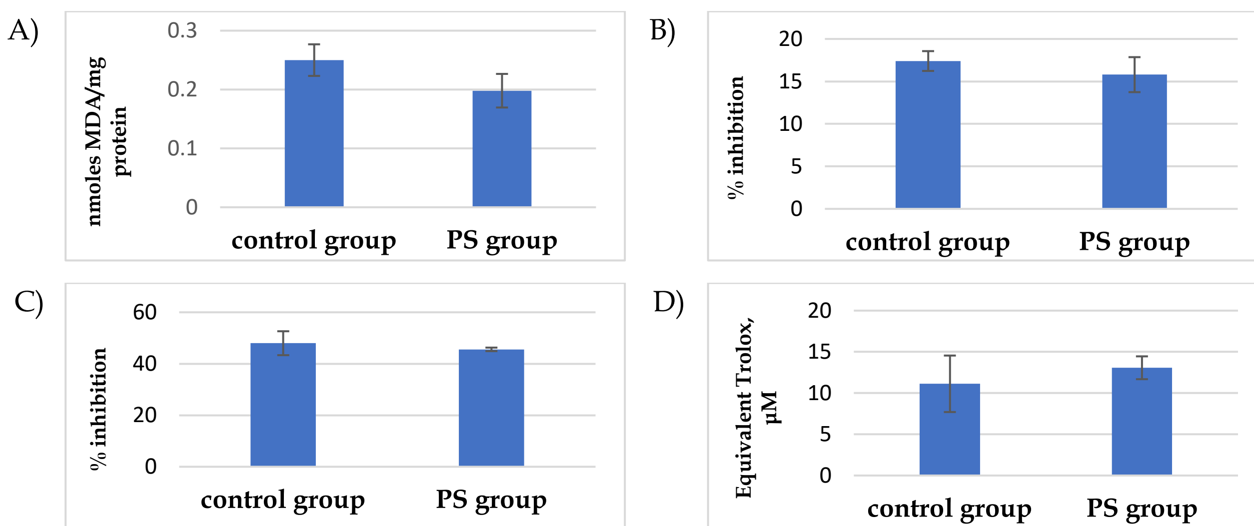

Fig. 3. Effects of polystyrene nanoplastics on lipid peroxidation and antioxidant responses of eelgrass leaves: (A) lipid peroxidation, (B) DPPH radicals scavenging activity, (C) ABTS radical scavenging activity, and (D) cupric ion reducing antioxidant capacity (CUPRAC).

DNA integrity

DNA integrity, assessed based on the migration patterns of DNA fragments using agarose gel electrophoresis, showed that all DNA samples from both groups were classified into integrity category 1, as indicated by compact genomic bands without observable smearing (Fig. 4).

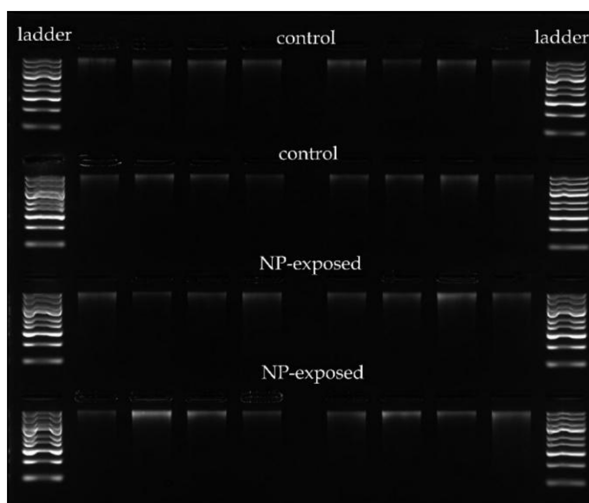

Fig. 4. Agarose gel electrophoresis of DNA samples from control and NP-exposed eelgrass fragments.

Discussion

Maximum quantum yield of PSII

The decrease in maximum quantum yield of PS II is related to abiotic and biotic stress in plants and is widely used as a measure of this (Baker, 2008). Our results are consistent with findings of other studies on a different seagrass species (*C. nodosa*) under the same exposure period and NP type (Menicagli et al., 2022), as well as studies on the same species using different MP types at higher concentrations (Molin et al., 2024). Menicagli et al. (2022) did not find a statistically significant difference between control and treated groups as concerns the Fv/Fm indicator, but reported lower quantum yield of electron flux to PSI end electron acceptors and increased proportion of dissipated energy when all reaction centers are open. They observed increased leaf chlorophyll content, which they interpreted as a compensatory adaptation to the shadowing effect of plastic particles adhered to the leaf surface. Similarly, studies on *Z. marina* exposed to larger and preconditioned PE and PP particles at much higher concentrations (25, 250, and 1000 mg L^{-1}) did not show a negative impact on the maximum quantum yield of the PS II in a short-term (14 days) experiment (Molin et al., 2024). A significant difference between the control and treated group was observed only in fragments with intact epiphytes and only at the 25 mg L^{-1} treatment. Unlike the results reported for exposure periods longer than 10 days (Menicagli et al., 2022; Molin et al., 2024; and the present study), Menicagli et al. (2022) observed differences in several photosynthesis efficiency indicators, including the maximum quantum yield of PS II, on the second day of exposure, indicating increased plant stress. They suggested that this effect could be attributed to lower light reaching photosynthetic pigments due to the particles' adherence to the leaf surface. Molin et al. (2024) also speculated that microparticles could promote stress due to shading and inhibitory effects of plastic additives such as bisphenol A or leachates and contaminants, originating from plastic particles. Overall, available data suggest that during the initial stages of MP exposure, seagrass photosynthesis performance may experience negative impacts, which are shortly mitigated through compensatory adaptation mechanisms (Menicagli et al., 2022), which could explain the lack of significant impact on the Fv/Fm indicator in the present experiment. In addition to photosynthesis responses, seagrass may react to micro- and nanoplastic contamination with other responses, including altered growth patterns (negative net change in shoot number, lower total number of leaves per shoot, greater number of dead leaves per shoot, lower net change of leaf number per shoot, root degeneration) (Menicagli et al., 2022), decrease in leaf growth rate, total root length, rhizome elongation rate, resulting in lower belowground biomass, when particles enter plants through the root system (Egea et al., 2026). Increased oxidative stress, as indicated by hydrogen peroxide content, level of lipid peroxidation, and antioxidants content (Menicagli et al., 2022), and decreased non-structural carbohydrates content (Egea et al., 2026), and reduced dark respiration rate (Molin et al., 2024) are also reported to be induced by microparticle loading.

Oxidative stress indicators

Regarding oxidative stress, the results obtainned in this study did not show statistically significant differences in lipid peroxidation in eelgrass leaves treated with PS-NP, nor in the antioxidant activity of their ethanol extracts, compared to untreated controls (Fig. 3). It has been suggested that NP, depending on their size, shape, concentration, chemical composition, charge, and adsorbed toxins or pathogens, may influence nutrient uptake, disrupt metabolism, or induce oxidative stress and associated damage in plants (Li et al., 2021; Wang et al., 2022; Ekner-Grzyb et al., 2022). On the other hand, moderate stress induced by NP may trigger changes in gene expression and activate antioxidant defense pathways (Zhou et al., 2021; Wang et al., 2022; Spanò et al., 2022; Ekner-Grzyb et al., 2022). Accordingly, the slight reduction in LPO observed herein in the PS group compared to controls (Fig. 3A) likely reflects activation of antioxidant mechanisms that compensate (or even overcompensate) for the oxidative membrane damage caused by the PS-NP. This lack of a significant increase in LPO is consistent with previous observations in some plants, where low-level exposure to pollutants (Gill & Tuteja, 2010; Sharma et al., 2012), including PS-NP (Spanò et al., 2022), fails to induce substantial oxidative damage. In this study, the antioxidant responses measured by DPPH and ABTS radical scavenging assays showed modest, non-significant reductions in PS-treated samples (Fig. 3 B and Fig. C). Both DPPH

and ABTS methods are widely used to evaluate overall free radical scavenging potential and reflect the cumulative activity of various antioxidant molecules, including phenolics, flavonoids, and ascorbate (Apak et al., 2016; Re et al., 1999). The slight reduction observed here may indicate subtle changes in antioxidant metabolite pools, but the lack of statistical significance suggests that the basal antioxidant defense mechanisms in eelgrass leaves were largely sufficient to maintain redox balance under the applied PS-NP exposure. Conversely, the CUPRAC assay indicated a moderate increase in reducing capacity in PS-treated leaves (Fig. 3D), although this change was also not significant. CUPRAC measures the ability of antioxidants to reduce cupric (Cu^{2+}) to cuprous (Cu^{+}) ions, capturing a different aspect of electron-donating antioxidant activity (Apak et al., 2004). Notably, ferric reducing antioxidant power (FRAP) activity was below the detection limit in both control and PS-treated plants. Some studies have shown that aquatic plants, especially seagrasses, often exhibit lower reducing capacity than terrestrial species, possibly due to differences in phenolic composition or adaptive acclimation to the marine redox environment (Papenbrock, 2012; Astudillo-Pascual et al., 2021; Grignon-Dubois & Rezzonico, 2023). Therefore, the absence of a FRAP signal likely reflects the inherent biochemical characteristics of eelgrass in addition to the effect of PS-NP. Overall, the effects of NP on plant antioxidant systems appear to be complex and multifactorial, encompassing both direct and indirect mechanisms. Further research is needed to clarify the specific interactions between NP and plant antioxidant defenses and to determine the consequences for plant health and ecosystem functioning.

DNA integrity

Consistent with the absence of alterations in the effective quantum yield of PS II, no detectable differences in DNA integrity were observed between control and PS-exposed eelgrass fragments. This indicates that no measurable genotoxic effects were induced under the tested exposure regime. This may be attributed to cellular defense mechanisms that may have mitigated potential DNA damage. For example, recovery from DNA damage caused by MP exposure has been reported after a period without treatment (Jiang et al., 2025). However, it should be noted that, although successfully utilized in other studies (Masiá et al., 2021), agarose gel electrophoresis primarily detects extensive DNA fragmentation and may not reveal more subtle genotoxic effects, such as single-strand breaks or oxidative DNA lesions, which have been reported using more sensitive methods (Harshavarthini et al., 2025; Jiang et al., 2025). Furthermore, more pronounced toxic effects of aged microplastics compared to virgin particles have been demonstrated (Michailidou et al., 2024). Further studies incorporating a range of exposure periods, varying concentrations, the use of aged micro- and nanoplastics, and more sensitive genotoxicity assays are needed to fully assess their potential risks to seagrass species.

Conclusions

Results from our experiment have shown that three different stress indicator types (photosynthesis capacity, oxidative stress, and DNA integrity) are not negatively impacted when eelgrass fragments are subjected to a short time (> 10 days) pure PS nanoparticle exposure. Further studies are necessary to investigate the effects of PS and other plastic types on common eelgrass for longer exposure periods, higher concentrations, and additional stress indicators. The possibility micro- and nanoplastic to serve as concentration spots and a vector for marine pollutants transfer to seagrass should also be examined.

Acknowledgments

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