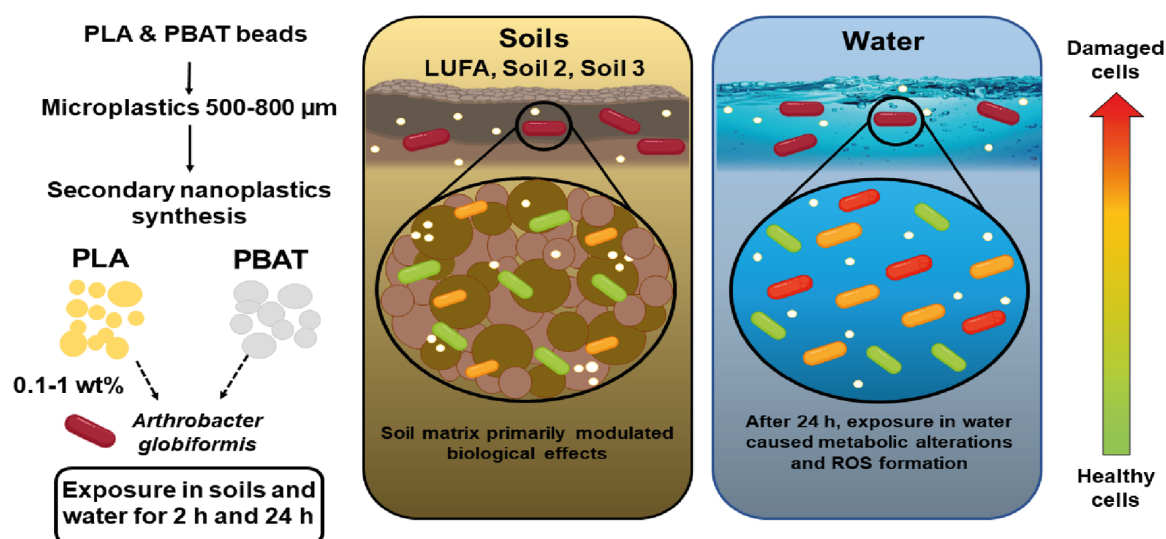


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Comparative ecotoxicity of well-defined biodegradable nanoplastics in soil and water using the environmentally relevant bacterium *Arthrobacter globiformis*

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Abstract

The acute toxicity of polylactic acid (PLA) and poly(butylene adipate-co-terephthalate) (PBAT) nanoplastics in soil and aquatic environments was assessed using as endpoints the inhibition of the dehydrogenase activity as well as reactive oxygen species (ROS) formation and membrane damage in *Arthrobacter globiformis*, a ubiquitous environmentally relevant microorganism. Nearly monodisperse nanoplastic particles (100–200 nm) were prepared using an oil-in-water emulsion technique. Dehydrogenase activity was monitored across three distinct soil types spiked with PLA or PBAT nanoplastics: the standard sandy soil LUFA 2.1 and two real agricultural soils. While significant time-dependent inhibition was observed in the standard soil, responses were markedly attenuated in the agricultural soils, where high organic matter and carbonate content likely reduced nanoplastic bioavailability. Conversely, exposure to the nanoplastics in water, lacking soil buffering, produced an initial metabolic stimulation followed by a marked decline after 24 h, accompanied by increased membrane permeability and suggesting altered cellular integrity under prolonged exposure conditions. This exposure scenario also revealed polymer-dependent differences, with PLA treatments showing higher membrane permeability responses than PBAT after prolonged exposure. These findings underscore that results obtained using standard reference soils may significantly differ from those in natural soils, and that in the absence of complex environmental matrices, nanoplastic toxicity appears to be influenced by exposure duration and the degradation behaviour of the polymer backbone. Although biodegradable plastics are increasingly promoted as sustainable alternatives, the nanoplastics generated from them may pose a risk, inducing biological damage to environmental organisms even after short-term exposure.

Introduction

Over the past few decades, plastics have become an integral part of modern life, offering versatility and durability at a relatively low cost. However, the same properties that make plastics attractive to industry and consumers also contribute significantly to their environmental persistence and widespread accumulation. Global plastic production now exceeds 400 million tonnes per year, yet only a small fraction is effectively recycled or reused (1). Most plastic waste ends up in landfills or is dispersed throughout ecosystems as environmental waste (2). The combination of mismanaged disposal and losses during

use and production has resulted in persistent entry of plastics into terrestrial and aquatic environments, where they undergo slow and uncontrolled degradation (3). Through processes such as mechanical abrasion, photo-oxidation, and chemical weathering, large plastic debris progressively fragment into microplastics (< 5 mm), which can further degrade into nanoplastics (< 1000 nm) (4, 5). These smaller particles are capable of dispersing widely and give them unique physicochemical properties that potentially enable deeper interactions with biological systems and environmental compartments (6).

Despite this growing recognition, the majority of available studies have focused on nanoplastics derived from traditional polymers, particularly polyethylene (7). However, in recent years, the market share of biodegradable plastics has expanded rapidly due to the urgent global need to address the pollution caused by traditional plastics and the subsequent demand for circular economy solutions (8). Biodegradable and compostable plastics offer

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a promising alternative to conventional plastics, although compostable plastics are a specific subset of biodegradable materials designed to degrade under defined composting conditions (9, 10). When properly managed at the end of their life, these materials may reduce long-term environmental persistence (11). Among the most widely used biodegradable polymers are polylactic acid (PLA) and poly(butylene adipate-co-terephthalate) (PBAT), which are increasingly employed in packaging, consumer goods, and agricultural applications such as mulch films. This shift raises important questions about the environmental fate and biological effects of biodegradable plastics, which remain far less well understood than those of conventional plastics (12, 13). PLA and PBAT were selected as representative biodegradable polyesters because they offer different chemical profiles that influence their environmental interactions. PLA is a biobased, aliphatic polymer, whereas PBAT is a fossil-based polymer with an aromatic-aliphatic structure. These chemical differences dictate their degradation kinetics driven by the rapid hydrolysis of PLA compared to the more stable PBAT. Despite these differences in origin and stability, both polymers contain abundant hydrolysable ester linkages that serve as the primary targets for enzymatic degradation, allowing for a direct comparison of how polymer backbone stability affects their toxicological profiles.

Although nanoplastic research has primarily focused on aquatic systems (14, 15), soils represent the ultimate sink for a significant proportion of plastic waste (16). Unlike in water, where nanoplastics are readily available to organisms, soils are a highly complex and heterogeneous environment in which interactions with mineral phases, organo-mineral complexes and pore water chemistry control particle aggregation, retention and mobility (17). These interactions could either buffer or modulate the potential toxicity of nanoplastics or lead to their long-term accumulation and have chronic effects on soil-dwelling organisms. Within this framework, *Arthrobacter globiformis* serves as a critical biological indicator, being a widespread and ecologically versatile bacterium predominantly associated with soil environments, yet frequently isolated from freshwater ecosystems (18). It belongs to the genus *Arthrobacter*, which comprises diverse aerobic, Gram-positive bacteria within the class Actinobacteria (order Actinomycetales, family Micrococcaceae). Members of this genus exhibit marked metabolic versatility and distinct morphological plasticity throughout their growth cycle, with cell size and shape strongly influenced by nutrient availability. As a key microbial decomposer whose metabolic impairment can have

cascading effects on higher trophic levels and overall ecosystem functioning, *A. globiformis* is widely utilized as a model organism for evaluating toxicity across both terrestrial and aquatic interfaces (19). By assessing specific endpoints such as dehydrogenase activity, a sensitive proxy for microbial metabolic health and soil quality, alongside membrane damage and oxidative stress (ROS), we can directly quantify how biodegradable nanoplastic accumulation disrupts the essential biochemical processes that maintain environmental productivity in both soil and water matrices.

Despite the rapid market expansion of biodegradable polymers like PLA and PBAT, our understanding of their environmental impact remains significantly behind that of traditional plastics. A critical knowledge gap exists due to the scarcity of reproducible, well-characterized nanoplastic test materials, which hinders the ability to conduct rigorous, controlled exposure experiments. Furthermore, while soils act as a primary sink for plastic waste, most research continues to focus on aquatic systems, leaving the comparative behaviour of biodegradable nanoplastics across different environmental interfaces largely unexplored. To address these limitations, this study presents the successful synthesis of well-defined PLA and PBAT nanoplastics using an emulsion-solvent evaporation technique. Using the ubiquitous bacterium *A. globiformis* as an ecologically relevant model, we provide the first simultaneous toxicological assessment of these biodegradable nanoplastics in both soil and water. This approach offers new insights into how complex environmental matrices modulate nanoplastic toxicity and clarifies the potential risks posed by sustainable plastic alternatives.

Experimental

Materials

PLA (Mw 60,000) pellets were obtained from Sigma-Aldrich (Spain), while PBAT (Mw 100,000, Ecoflex® F Blend C1200) pellets were supplied by BASF (Germany). *A. globiformis* (CECT 388 or ATCC 8010) was acquired from the Spanish Type Culture Collection (Spain). Ultrapure water was produced using a Milli-Q Q-POD® Ultrapure Water System (resistivity >18 MΩ cm). Liquid nitrogen was supplied by Linde (Spain). Whatman Grade GF/B 100% borosilicate glass microfiber filters, binder-free, 47 mm diameter, nominal particle retention 1 µm, were purchased from Fisher Scientific. The fluorescent probe 2',7'-dichlorofluorescein diacetate (H₂DCF-DA, CAS: 2044-85-1), sodium chocolate (CAS: 206986-87-0) and

dichloromethane (CAS: 75-09-2) were acquired from Sigma-Aldrich. AlamarBlue Cell Viability Reagent, resazurin sodium salt (CAS: 62758-13-8) and propidium iodide (PI) (CAS: 25535-16-4) were purchased from Fisher Scientific.

Nanoparticle synthesis

PLA and PBAT pellets were used as precursors for nanoplastic fabrication using the emulsion-solvent evaporation technique. Prior to use, commercial PLA and PBAT pellets were washed three times with Milli-Q water to ensure chemical purity and eliminate any potential artifacts. To accelerate polymer dissolution, PLA and PBAT pellets were subjected to cryogenic fragmentation prior to nanoparticle synthesis. The pellets were immersed in liquid nitrogen in a metal container until brittle and were then mechanically ground after nitrogen evaporation. The resulting material was collected and homogenized by sieving using a mechanical vibratory sieve shaker (Retsch) to obtain particles in the size range of 500–800 μm . PLA and PBAT were then dissolved in dichloromethane (DCM, 1 g in 20 mL) under continuous stirring until complete dissolution. The aqueous phase was prepared by dissolving 150 mg of sodium cholate in 150 mL of ultrapure water (1.0 g/L), stirring for 3 min to ensure homogenization. Sodium cholate, a bile salt and a comparatively mild surfactant relative to synthetic alternatives, was used to stabilize the oil-in-water emulsion by lowering the interfacial tension between the organic solvent and the aqueous phase, which prevents polymer droplets from coalescing (20). The organic phase was then added dropwise to the aqueous phase under constant stirring, forming an emulsion. To achieve fine droplet dispersion, the mixture was subjected to probe sonication at 40% amplitude (VCX 750, ultrasonic probe from Bioblock Scientific) for 5 min, while keeping the sample on an ice bath to prevent thermal degradation or premature solvent evaporation. The resulting milky-white appearance indicated the successful formation of a stable emulsion.

To concentrate the nanoparticle suspension and ensure the complete removal of residual DCM, the sample was processed via rotary evaporation (Laborota 4001, Heidolph). Prior to this step, the suspension was passed through a 1 μm glass fibre filter to eliminate larger particles and aggregates. Multiple batches of the same polymer were prepared in parallel and subsequently pooled to achieve a sufficient stock volume. To remove unwanted substances remaining from the synthesis, the suspension was purified through five consecutive centrifugation-wash cycles, during which the supernatant was discarded and replaced with ultrapure water. Finally, the stock

concentration was determined gravimetrically from the processed suspension. Briefly, known aliquots were dried to constant weight, and the remaining dry residue was used to calculate the mass concentration of the nanoparticles prior to use. The potential presence of residual DCM in the nanoparticle suspension was checked by gas chromatography (GC) and the results are provided in the Supplementary Information. The final nanoparticle suspensions were stored in ultrapure water at 4 °C in the dark until further use.

Analyses

The colloidal properties of the nanoplastics were assessed using a Zetasizer Nano ZS (Malvern Instruments). Prior to analysis, the suspensions were homogenized by probe sonication using an ultrasonic processor to minimize sedimentation and aggregation. The hydrodynamic diameter, polydispersity index (PDI), and size distributions were determined via Dynamic Light Scattering (DLS). Surface charge and colloidal stability were evaluated by measuring the ζ -potential through Electrophoretic Light Scattering (ELS). Four runs were analysed, corresponding to the two polymers (PLA and PBAT) tested at two concentrations (1 and 10 mg/mL). For each replicate, DLS measurements consisted of at least 10 individual runs, while ζ -potential was determined over 30 runs. Measurements were performed both in ultrapure water and in the working medium used for the bioassays. Additionally, the pH of each suspension was recorded to provide context for the electrokinetic measurements.

Soil sampling and characterisation

Three types of soil, each with distinct characteristics and origins, were used. The first type of soil, Soil 1, was a commercial reference sample from the LUFA collection (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, LUFA 2.1). Soil 2 was collected from an organic vegetable farm in Soto del Real (Madrid; 40°45'23.9"N, 3°46'49.7"W). Soil 3 was obtained from non-organic cereal farmland in the Mesa de Ocaña area of the Huerta de Valdecarábanos (Toledo; 39°52'12.7"N, 3°37'07.9"W). The soils were selected to represent matrices with contrasting physicochemical properties and agricultural management histories, including a standardised reference soil, an organically managed agricultural soil, and a conventionally managed agricultural soil.

Soil properties were determined using standard analytical techniques according to OECD protocols. Measurements included pH, salinity, organic matter content (by oxidation), total nitrogen, C/N ra-

tio, exchangeable cations (Na, K, Ca, Mg), available phosphorus (Olsen method), total carbonates and active lime (CaCO_3), and particle size distribution (Bouyoucos method). The properties of the soils are summarized in Table S1, Supplementary Information, SI.

Bioassays

As a ubiquitous soil and freshwater microorganism, *A. globiformis* plays a critical role in nutrient cycling and organic matter degradation, making it an ecologically relevant model for assessing microbial responses to environmental contaminants. Following ISO 18187:2024, *A. globiformis* was cultured in Medium B (Table S2, SI) (previously sterilized by autoclaving at $121 \pm 3^\circ\text{C}$ for 20 min) at 30°C for 16 ± 1 h in the dark with agitation at 150 rpm. Subsequently, 1 mL of this culture was transferred to fresh Medium B and incubated for a further 3 h under the same conditions. The inoculum was then adjusted to $\text{OD}_{600} = 0.4 \pm 0.1$, as specified by ISO 18187:2024, using a working medium corresponding to a 1:500 dilution of Medium B (Table S2, SI) (21–23), which was used in both water and soil assays to ensure comparable nutrient conditions between matrices. The growth kinetics of *A. globiformis* and the metabolic activity of the inoculum adjusted to $\text{OD}_{600} = 0.4 \pm 0.1$ were additionally confirmed by growth curve analysis and FDA hydrolysis measurements (Figure S1 and Figure S2, SI).

Prior to testing, soils were sieved through a 2 mm mesh to remove coarse debris and homogenize the samples (24, 25). The initial water content of each soil was determined gravimetrically. Subsequently, 50 g of each soil were transferred into 50 mL glass beakers for treatment preparation. For nanoplastic treatments, the volume of each PLA or PBAT stock suspension needed to obtain the target nominal concentrations (0.1 and 1% w/w, corresponding to 1 and 10 g kg^{-1} dry soil, respectively), selected to represent medium-term accumulation and environmental hotspot scenarios, respectively, was calculated individually, and Milli-Q water was added as needed to reach the final moisture content of 20%. The samples were then vigorously mixed with a stainless-steel spoon to ensure homogeneous distribution throughout the soil matrix (25). Negative controls were prepared by adjusting the soil to 20% moisture with Milli-Q water only, without nanoplastic addition. Positive controls were prepared in accordance with ISO 18187:2024 using the reference toxicant benzyldimethylhexadecylammonium chloride (C16-BAC). A stock solution of C16-BAC was first prepared in distilled water and then added to the soils to obtain a final concentration of 600 mg kg^{-1} dry soil. All samples were then equi-

librated for at least 24 h in the dark at $4 \pm 2^\circ\text{C}$ before further processing. All treatments and controls were performed in triplicate.

For water assays, experiments were conducted in 25 mL Erlenmeyer flasks with a final volume of 10 mL. As in soil assays, exposure concentrations ranged from 0.1 to 1% (w/w), corresponding to 1 and 10 g L^{-1} respectively. For each nanoplastic treatment, three biological replicates were performed, together with three replicates for each control. Negative controls were prepared following the same procedure but without nanoplastic addition, replacing the nanoplastic suspension volume with Milli-Q water. Positive controls were prepared using C16-BAC at the same concentration employed in soil assays, adapted to the water matrix. Additionally, to evaluate whether dissolved compounds remaining from the synthesis procedure could contribute to the observed biological responses, supplementary control assays were performed using PLA and PBAT suspensions at their highest tested concentrations after ultrafiltration through 50 kDa MWCO Vivaspin centrifugal filters. These ultrafiltrated samples were exposed to bacteria for 2 h and 24 h following the same experimental procedure used for the nanoplastic suspensions (Figure S3, SI). This ultrafiltration process removes particles larger than approximately 5 nm (26) and was intended to verify that no significant biological effects were associated with dissolved residual compounds potentially remaining from the synthesis procedure.

Upon reaching the exponential growth phase, cultures were subjected to 2-h and 24-h exposure assays under shaking conditions. The 2-h exposure was therefore selected as the standardized contact time defined by the method (ISO 18187:2024) to assess early metabolic responses. The 24-h exposure was included to evaluate more prolonged physiological responses over an extended exposure period (21, 27, 28). Horizontal shaking was applied throughout the assays to maintain an aerobic suspension and ensure reproducible fluorescence kinetics; in soil treatments, it also promoted consistent contact between *A. globiformis* and the solid soil matrix.

Assessment of physiological parameters

The dehydrogenase activity of *A. globiformis* was determined according to ISO 18187:2024 as a proxy for microbial metabolic activity and soil quality (19). For soil assays, a resazurin solution was prepared and applied according to ISO 18187:2024, whereas the commercial AlamarBlue reagent was used for water assays. Both assays use resazurin as the active compound, but AlamarBlue is optimized for free-living bacterial cells in liquid media. Following ISO 18187:2024, soil dehydrogenase assays were

conducted in transparent 24-well microplates. To isolate the activity of the inoculated *A. globiformis*, indigenous soil enzymes were inactivated by dispensing 600 mg of soil and 600 μ L of Milli-Q water per well, using three replicate wells per treatment, followed by two cycles of pasteurization at 80 °C for 10 min and cooling for 15 min in an ice bath. Although some alteration of soil physicochemical properties by the pasteurization step cannot be fully excluded, this pre-treatment was applied uniformly to all controls and treatments within each soil in order to suppress indigenous dehydrogenase activity and allow comparison under consistent experimental conditions. Following deactivation, wells were inoculated with 400 μ L of *A. globiformis* suspension adjusted to OD₆₀₀ = 0.4 $\pm$ 0.1. The plates were incubated for 2 h at 30 °C and 150 rpm in the dark. After 2 h, 800 μ L of a resazurin solution (45 μ g/mL) were added and fluorescence was measured every 15 min for 1 h. This procedure was repeated after 24 h of exposure, with fluorescence recorded every three minutes. Due to the higher bacterial density after 24 h, the conversion of resazurin occurred rapidly; therefore, only the initial 20 min of the fluorescence curve were analysed to avoid signal saturation and maintain sensitivity.

To ensure reliable interpretation of fluorescence responses and to account for potential background signals associated with the soil matrix and nanoplastic suspensions, several experimental controls were included following ISO 18187:2024. Blank A, prepared without bacterial inoculation, was used to correct for background fluorescence after deactivation. In addition, for nanoplastic-amended soils, specific Blank A controls were prepared at 0.1 and 1 wt% without bacterial inoculation in order to account for background fluorescence in the presence of nanoplastics. Blank B, prepared by pasteurizing 600 μ L of Milli-Q water alone followed by the addition of 600 mg of soil, was used to assess the natural dehydrogenase activity of the substrate and the effectiveness of the pasteurization step. The negative control consisted of uncontaminated soil inoculated with *A. globiformis*, while the positive control consisted of soil amended with benzyldimethylhexadecylammonium chloride and inoculated with bacteria.

The same protocol was applied to the assays in water (working medium) at both 2 and 24 h to ensure comparability across systems and exposure times. Fluorescence measurements were performed in 96-well plates. To evaluate the physiological effects of PLA and PBAT nanoplastics on *A. globiformis*, membrane integrity and ROS formation as an indicator of oxidative stress were assessed in water after 24 h of exposure. Intracellular reactive oxygen species

(ROS) were quantified using H₂DCF-DA at a final concentration of 100 μ M, with excitation/emission wavelengths of 480 $\pm$ 10 nm and 550 $\pm$ 10 nm, respectively. Membrane integrity was assessed using 3 μ M PI, with excitation/emission at 535 $\pm$ 10 nm and 617 $\pm$ 10 nm. Bacterial suspensions were incubated with the fluorochromes at room temperature in the dark for 30 min (ROS) and 15 min (PI) prior to fluorescence measurement.

Additional abiotic and biological controls were included in both ROS and membrane permeability assays to assess potential background fluorescence and verify the correct performance of the fluorescent probes. Controls consisting of medium alone and nanoplastics suspended in medium without bacterial inoculation were prepared at both tested concentrations (0.1 and 1%) in order to evaluate possible abiotic fluorescence contributions associated with the exposure media and nanoplastic suspensions. In addition, bacteria incubated in medium without nanoplastics were used as negative controls. For membrane permeability assays, bacteria exposed to 70% ethanol were used as positive controls for propidium iodide staining, whereas for ROS assays, bacteria exposed to 3 μ L of 30% hydrogen peroxide were used as positive oxidative stress controls. The fluorescence values obtained from the abiotic controls were subtracted from the corresponding treatments where appropriate.

Quality assurance and quality control

Quality assurance and quality control (QA/QC) procedures were performed following the guidelines described elsewhere (29). To minimize plastic contamination, glassware was used whenever possible throughout the study, and cotton laboratory coats were worn during all procedures. All experimental preparations were conducted under sterile conditions within a laminar flow hood equipped with UV light (TelstarTM AV-30/70). To minimize microbial contamination, nanoplastic suspensions were exposed to UV irradiation prior to use; autoclaving of the nanoplastics was not applied as it may alter their physicochemical properties. Erlenmeyer flasks, Milli-Q water, pipette tips, culture media, and all other autoclavable materials used during bacterial handling and bioassay preparation were sterilized prior to use in a vertical autoclave (Med 12 Selecta, ref. 4001756) at 121 °C. Non-spiked controls were processed in parallel with spiked treatments to monitor for potential cross-contamination. To prepare the stock suspensions and verify the exposure concentrations in the bioassays, the materials were weighed on an analytical balance to determine the exact mass recovered after preparation. Immediately before testing, all stock suspensions were ultrasonicated for 1 min to

ensure homogeneity. Each assay was performed with a minimum of three biological replicates.

Statistical Analysis

All statistical analyses were performed using R software (version 4.5.1). The analyses were conducted using the packages *readxl*, *dplyr*, *car*, *emmeans*, *ggplot2* and *effectsize*, together with base R statistical functions. Data were analysed using two-way analysis of variance (ANOVA) to detect significant differences between treatments, considering polymer type (PLA and PBAT), exposure concentration (0.1 and 1 wt%), and incubation time as fixed factors. For soil assays, soil type was also considered as a factor to evaluate the influence of the matrix. When appropriate, post hoc comparisons were conducted using Dunnett's or Tukey's tests. Results are presented as mean $\pm$ standard deviation ($n = 3$), and statistical significance was accepted at $p < 0.05$.

Results and discussion

Physicochemical characterization

The ATR-FTIR analyses of the particles in powder form confirmed the chemical structure of the original polymers. As shown in Figure 1, the IR spectra of PLA (red line) and PBAT (blue line) exhibited the characteristic absorption bands of each polymer. For PLA, characteristic peaks included asymmetric -CH_3 stretching at 2948 cm^{-1} and a sharp C=O ester vibration at 1748 cm^{-1} . Asymmetric and symmetric -CH_3 bending were observed at 1447 and 1382 cm^{-1} , respectively, with C-O stretching at 1186 and 1082 cm^{-1} . Peaks at 862 and 756 cm^{-1} corresponded to C-COO stretching and C-O bending (21). The PBAT spectrum reflected its aromatic-aliphatic structure: CH_2 stretching at 2957 cm^{-1} , a strong ester C=O peak at 1717 cm^{-1} , and a benzene ring skeletal vibration at 1557 cm^{-1} . Additional bands included CH_2 in-plane bending (1407 cm^{-1}), C-O stretching (957 , 1267 and 1107 cm^{-1}). The peak at 726 cm^{-1} (benzene ring CH-plane bending) confirms the PBAT structure (30).

ATR-FTIR spectra did not reveal any characteristic C-Cl stretching vibrations (typically observed near $700\text{--}740\text{ cm}^{-1}$), suggesting that residual DCM was below the technique's detection limit. However, given the high absorption of the polymer matrix in the fingerprint region, which can mask trace solvent signals, further validation was performed. Hexane extracts of the nanoplastic suspensions were analysed via GC-FID. Under the selected chromatographic conditions, dichloromethane (DCM) eluted at 1.02 min , while

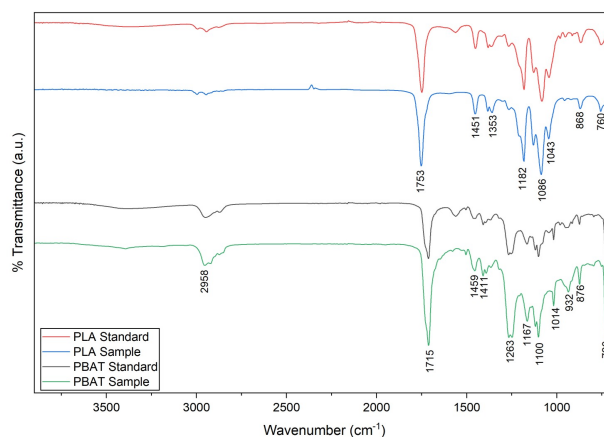


Figure 1: ATR-FTIR spectra of PLA and PBAT nanoplastics prepared in this work.

n-hexane showed a distinct peak at 1.24 min (Figure S4, SI). Both PLA and PBAT extracts exhibited only the hexane peak ($t_R \approx 1.24\text{ min}$), with no detectable signal at the DCM retention time (Figure S5, SI). In contrast, the DCM extraction control showed a clear DCM peak at 1.02 min together with the hexane signal (Figure S3, SI). Separate injections of pure DCM and hexane confirmed retention-time assignments and absence of co-elution. These results demonstrate that DCM was not detected in the nanoplastic suspensions under the applied analytical conditions.

The colloidal properties of generated PLA and PBAT nanoplastics were evaluated by dynamic light scattering (DLS) and electrophoretic light scattering (ELS) to determine hydrodynamic diameter and surface charge (ζ -potential), respectively. Measurements were carried out at $25\text{ }^\circ\text{C}$ and $\text{pH } 7$ in both ultrapure water and the contact medium employed in the bioassays. The results are presented in Table 1. In ultrapure water, all samples exhibited negative ζ -potential values ranging from -23 to -30 mV , consistent with good colloidal stability and attributed to the carboxyl groups of sodium cholate. Values in parentheses represent the standard deviation of the size distribution (peak width).

Hydrodynamic size distributions in ultrapure water revealed moderately polydisperse populations, with intensity-weighted peaks centred in the $119\text{--}193\text{ nm}$ range. Within this range, PLA nanoplastics tended to exhibit slightly larger hydrodynamic size and slightly more negative ζ -potential values than PBAT. These values are consistent with the size range reported for nanoplastics generated under environmentally relevant conditions (31). Slightly higher hydrodynamic diameters observed in the more concentrated samples can be attributed to a greater propensity for particle-particle interactions and aggregation of the smaller particles (32). DLS size distributions for

Table 1. Physicochemical characterization of PLA and PBAT nanoplastics at two concentrations (0.1 and 1 wt%) in ultrapure water and contact medium. Hydrodynamic size (by intensity), polydispersity index (PDI) and ζ -potential were determined by dynamic light scattering (DLS) and electrophoretic light scattering. ζ -potential values are given as mean $\pm$ standard deviation.

	Ultrapure water			Contact medium		
	Size (nm)	PdI	ζ -potential (mV)	Size (nm)	PdI	ζ -potential (mV)
PLA 0.1 wt%	174 (42)	0.16	-25 ± 8	195 (82)	0.18	-52 ± 11
PLA 1 wt%	193 (55)	0.25	-30 ± 11	206 (90)	0.34	-52 ± 4
PBAT 0.1 wt%	119 (70)	0.21	-23 ± 9	282 (94)	0.29	-38 ± 1
PBAT 1 wt%	139 (81)	0.32	-22 ± 7	296 (103)	0.37	-46 ± 2
No nanoplastics	n.a.	n.a.	n.a.	728 $\pm$ 154	1	-8 ± 1

particles in water (0.1 wt%), combined with scanning electron micrographs of PLA and PBAT nanoplastics, revealed low-polydispersity samples composed of nearly spherical particles (Figure 2).

In contact medium, both PLA and PBAT nanoplastics exhibited an increase in hydrodynamic size compared to ultrapure water, indicating the formation of assemblies in the presence of medium constituents. For PLA, a moderate increase in hydrodynamic diameter was observed at both concentrations together with a slight increase of the polydispersity index, which may be associated with interactions with ionic components of the culture medium rather than extensive aggregation. PBAT nanoparticles exhibited a higher increase in hydrodynamic size, likely driven by interactions with ionic species in the medium. This hypothesis is supported by the more negative zeta potential values observed for all particles in the contact medium, indicating an overall increase in electrostatic stabilization, consistent with previous observations for nanoplastics exposed to complex aqueous matrices (33).

Exposure of *Arthrobacter globiformis* to nanoplastics in soils

The biological effects of PLA and PBAT nanoplastics were assessed using *A. globiformis*, a ubiquitous bacterium that plays a fundamental role in organic matter mineralization and biogeochemical nutrient cycling. Its application in ecotoxicological assays, such as the dehydrogenase activity test, provides a rapid and sensitive proxy for microbial metabolic health upon contact with environmental contaminants (19).

The impact of nanoplastic exposure on *A. globiformis* was first evaluated in soil microcosms after 2 h exposure, following the ISO 18187:2024 standard. In LUFA 2.1 soil, results revealed that dehydrogenase activity (used as a proxy for microbial metabolic activity (19)) responded to PLA nanoplastic exposure in a time-dependent manner. Significant effects were found for nanoplastic dose ($p < 0.001$), exposure time ($p < 0.001$), and their interaction ($p < 0.01$). Application of PLA nanoplastics at 0.1 wt% resulted in significant differences relative to the control from 30 min onwards ($p < 0.001$), reflecting an inhibition of dehydrogenase activity, while increasing the concentration to 1 wt% led to a significant but transient effect at 45 min ($p = 0.047$), which was no longer evident at 60 min (Figure 3). Regarding PBAT, significant effects were similarly detected for nanoplastic concentration ($p < 0.001$), incubation time ($p < 0.001$), and their interaction ($p < 0.001$). Significant differences relative to the control appeared from 15 min onwards and persisted throughout the entire incubation period ($p < 0.001$), again indicating an inhibition of dehydrogenase activity. LUFA standard sandy soil is characterized by the most acidic pH among the three tested soils and by a relatively low organic matter content compared with Soil 2 (Table S1, SI). Although statistically significant differences relative to the control were detected, the magnitude of these effects remained moderate and did not result in

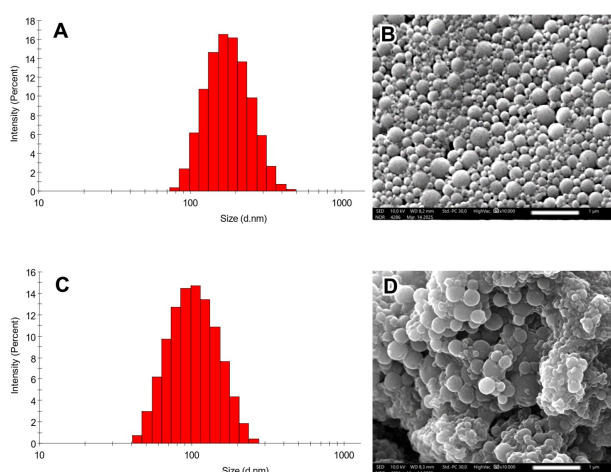


Figure 2: DLS size distribution (in water, 0.1 wt%) and SEM images of (A, B) PLA and (C, D) PBAT nanoplastics.

pronounced alterations of dehydrogenase activity in line with the findings from the 24 h exposure analyses (Figure S6, SI). Similar results have been reported in previous soil studies in which microbial enzymatic activity exhibited limited sensitivity to short-term incubations (34).

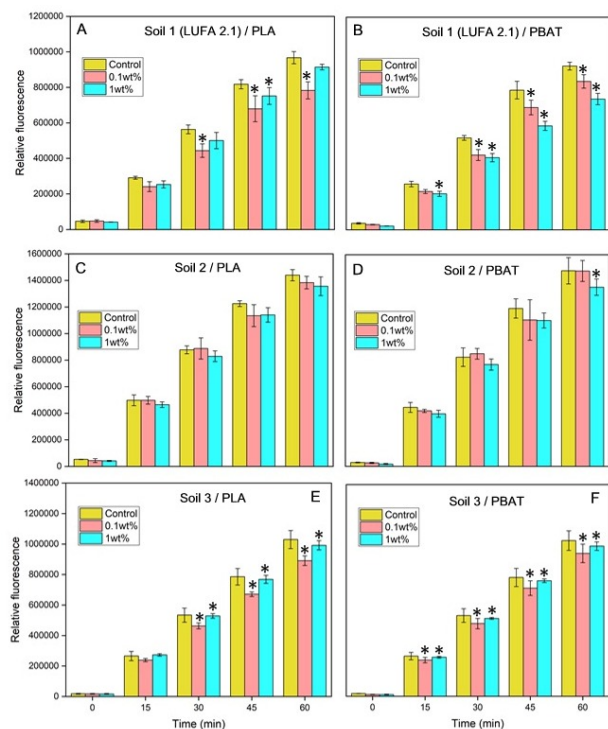


Figure 3: Dehydrogenase activity of *A. globiformis* exposed to PLA and PBAT nanoplastics (0.1 and 1 wt%) in three soil types. Activity was measured over a 60-min period and expressed as relative fluorescence units (RFU) after subtraction of the corresponding Blank A controls. Asterisks denote significant differences from the control (Dunnett's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

In contrast to LUFA, exposure to PLA and PBAT nanoplastics in Soil 2 generally did not induce significant inhibition in dehydrogenase activity (Figure 3). No time-dependent treatment effects were observed, except for a single significant inhibition of dehydrogenase activity in the 1 wt% PBAT treatment after 60 min ($p < 0.05$). This attenuated response was also maintained after 24 h exposure (Figure S6, SI), where no consistent inhibition trends relative to the corresponding controls were observed. The limited response observed in this soil can be attributed to its physicochemical properties (Table S1, SI). Soil buffering capacity is a key property governing the stability of soil ecosystems and the bioavailability of contaminants (35). It regulates microbial activity and limits physicochemical changes induced by external stressors (36). Compared with the other soils tested, Soil 2 exhibited a higher organic matter content, which generally confers enhanced buffering capacity, pro-

motes the adsorption of particulate contaminants, and facilitates the formation of heteroaggregates (37, 38). Collectively, these processes reduce direct interactions between nanoplastics and microbial cells (39).

In Soil 3, exposure to PLA induced a significant time-dependent inhibition of dehydrogenase activity ($p < 0.01$). Post hoc analyses revealed significant differences relative to the control for both PLA concentrations from 15 min onwards ($p < 0.01$). A comparable response was observed following PBAT exposure, with significant differences relative to the control detected from 15 min and persisting up to 60 min for both concentrations ($p < 0.01$). Although statistically significant, these differences corresponded to relatively small shifts in activity rather than marked changes in the overall temporal pattern (Figure 3 and Figure S6, SI), indicating a limited functional impact even after prolonged exposure.

This response is consistent with the physicochemical characteristics of Soil 3 (Table S1, SI), which is expected to confer substantial buffering capacity and consequently attenuate the biological effects of nanoplastics. Soil 3 is characterized by an alkaline pH and elevated concentrations of calcium, magnesium, and carbonates (including active lime). These properties promote particle aggregation through cation bridging and carbonate-mediated stabilization mechanisms, thereby reducing nanoplastic mobility and bioavailability (36). Similar attenuated responses have been reported for other contaminants in carbonate-rich soils, supporting the interpretation of a limited biological impact under these conditions (34, 39). The consistency of responses across treatments may indicate that nanoplastic addition did not substantially alter soil redox conditions or oxygen dynamics during the experimental period, in line with previous findings highlighting the dominant role of physicochemical controls over dehydrogenase activity (40).

The influence of polymer type (PLA and PBAT) on the dehydrogenase activity of *A. globiformis* was assessed using two-way ANOVA with polymer type and exposure time as fixed factors. The results, normalized to their respective controls, are presented in Figure 4. and show no significant polymer-dependent differences except for LUFA soil at the initial measurement ($p < 0.01$). However, this divergence likely reflects transient experimental variability rather than a sustained polymer-specific effect. Focusing on the stabilized reaction phase (≥ 15 min), PLA and PBAT displayed overlapping trends. Apart from the buffering effect of soil, another reason for not finding differences between PLA and PBAT is most probably the presence in both polyesters of abundant

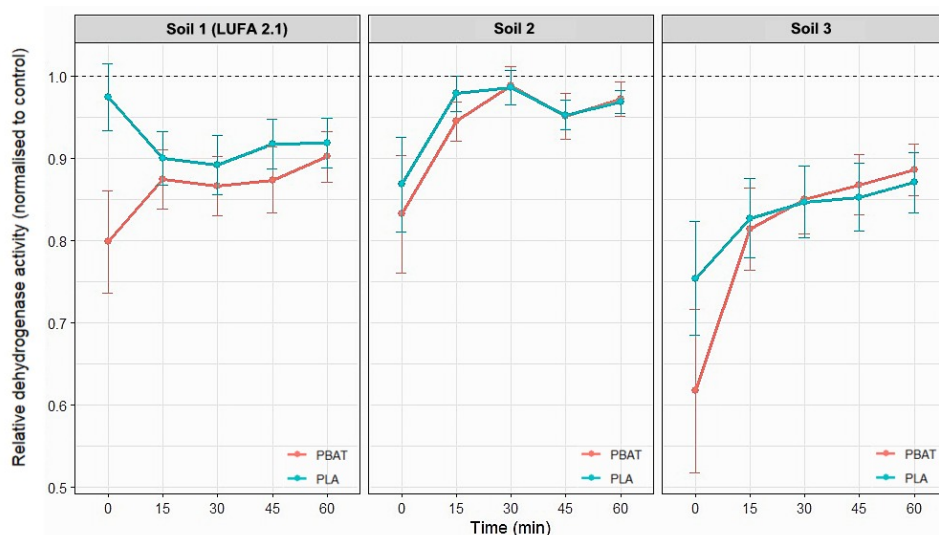


Figure 4: Normalized dehydrogenase activity of *A. globiformis* after 2 h exposure to PLA and PBAT nanoplastics in Soil 1 (LUFA 2.1), Soil 2, and Soil 3. Activity was measured over 60 min. The dashed line represents the control level (1.0). Error bars represent the standard deviation ($n=3$).

hydrolysable ester linkages in their backbones, which serve as the primary targets for enzymatic degradation (41). Similarly, in Soil 2 and Soil 3, no statistically significant differences between polymer types were detected at any time ($p > 0.05$), indicating comparable short-term effects across soils with contrasting physicochemical properties. Relative dehydrogenase activity remained close to the control level for Soil 2, indicating minor differences between treatments and controls. However, the values recorded for Soil 3 indicated a certain short-term functional impact with relative dehydrogenase activity < 0.9 , which indicated a moderate deviation from the controls.

The effect of nanoplastics exposure on *A. globiformis* was also studied in the absence of interaction with the soil matrix. The results of the short-term exposure are shown in Figure 5. Two-way ANOVA revealed that both PLA ($p < 0.001$) and PBAT ($p < 0.001$) significantly stimulated dehydrogenase activity compared to the control. However, there were differences in their behavior with exposure time. While PLA followed a temporal trend comparable to the control (no interaction, $p = 0.102$), PBAT displayed a significant treatment-time interaction ($p = 0.039$), indicating that PBAT-induced metabolic activity stimulation became more pronounced over time. Post-hoc comparisons (Figure 5) showed no significant differences from the control for both nanoplastics during the initial 15 min of exposure. Thereafter, a progressive increase in dehydrogenase activity emerged, reaching statistical significance at longer contact times. For PLA, the 1 wt% treatment induced a significant increase in dehydrogenase activity from 30 min onwards ($p = 0.041$), which persisted through the 60-min assay (p

$= 0.011$). In contrast, the 0.1 wt% PLA treatment only reached statistical significance at 60 min ($p < 0.001$). PBAT exhibited a stronger metabolic response as dehydrogenase activity significantly increased with the 0.1 wt% treatment becoming significant from 30 min onwards and both concentrations showing significant effects after 45 min ($p > 0.0001$).

Consistent with the observations in soil, the response in water occurred rapidly, showing an increase in dehydrogenase activity within the first 135–150 min after exposure (Figure 5). The observed increase in dehydrogenase activity may reflect an early physiological response to nanoplastic exposure, potentially associated with short-term metabolic adjustment under stress conditions (42). Importantly, this increase should not be interpreted as enhanced bacterial viability or fitness; rather, it may indicate an immediate metabolic response as *A. globiformis* attempts to maintain cellular homeostasis in the presence of PLA and PBAT nanoplastics (40). This metabolic burst is often an adaptive mechanism to counteract early-stage oxidative stress or membrane destabilization. However, such heightened activity is typically transient and comes at a significant energetic cost, which may eventually lead to metabolic exhaustion and a subsequent decline in viability as the protective capacity of the cell is overwhelmed (43).

To clarify the mechanism and extent of the metabolic responses observed after exposure to nanoplastics, we performed an additional set of experiments conducted after 24 h of exposure. This approach enabled the assessment of both early (+2 h) and prolonged (+24 h) bacterial responses following exposure to PLA and PBAT nanoplastics, in line with exposure durations commonly applied in ecotoxic-

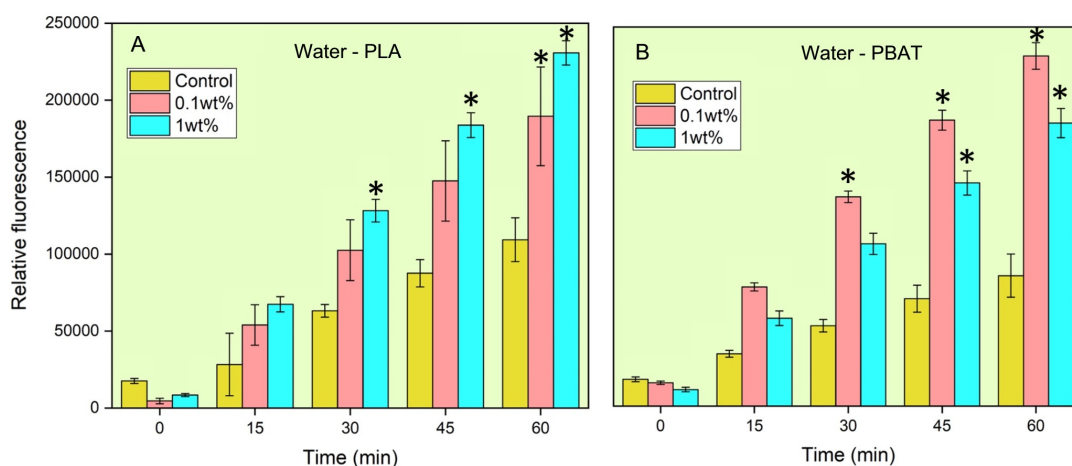


Figure 5: Results of dehydrogenase activity of *A. globiformis* after 2 h exposure to PLA (A) and PBAT (B) nanoplastics in water. After the initial 2 h exposure period, enzyme activity was measured kinetically over an additional 60 min (representing total time from 120 to 180 min). Testing was conducted at two concentrations (0.1 and 1 wt%). Results are expressed as relative fluorescence units (RFU) after subtraction of the corresponding Blank A controls. Asterisks denote significant differences from the control (Dunnett's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

logical studies (21, 44-46). In addition to dehydrogenase activity, ROS formation and membrane permeability were also evaluated and the results shown in Figure 6. Importantly, the dehydrogenase kinetics recorded after 24 h of exposure are not directly comparable, time point by time point, with those shown in Figure 5 after 2 h exposure, because after 24 h the bacterial density was higher and resazurin conversion occurred more rapidly.

The results showed that after 24 h of exposure to nanoplastics, the dehydrogenase activity was still above controls at the lowest concentration (0.1 wt%) but declined when cells were exposed to 1 wt% concentration of nanoplastics, especially in the case of PLA, indicating a substantial reduction in bacterial metabolic activity. Post hoc comparisons against the control showed significant effects only for the 1 wt% treatment at 6 and 18 min ($p < 0.001$). By contrast, dehydrogenase activity remained detectable following PBAT exposure. Post hoc comparisons revealed a significant increase relative to the control only at the highest concentration after 18 min ($p < 0.001$). Comparison between concentrations, independently of polymer type, revealed a significant concentration-dependent effect, with statistically significant differences between the 0.1 and 1 wt% treatments only at 18 min ($p < 0.001$).

Panels C and D of Figure 6 showed a slight decrease of intracellular ROS levels in PLA-exposed cells. Although the reduction observed at 1 wt% did not reach statistical significance ($p = 0.068$), the value approached the threshold, suggesting a trend toward impaired cellular function. Since intracellular ROS production can only be quantified in metabolically active cells, this decrease is likely due to a loss of cell

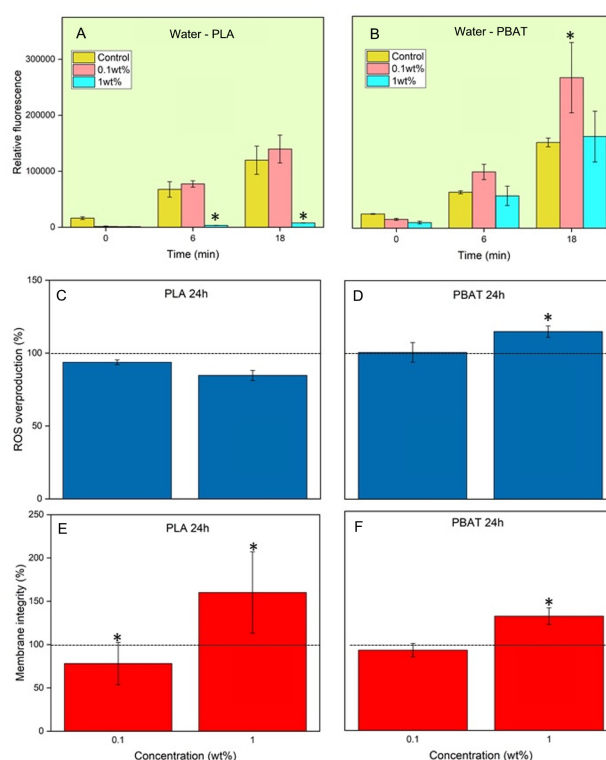


Figure 6: Physiological responses of *A. globiformis* after 24 h exposure to PLA (A, C, E) and PBAT (B, D, F) nanoplastics in water. Dehydrogenase activity, ROS formation and membrane permeability were evaluated at two concentrations (0.1 and 1 wt%). Dehydrogenase activity is expressed as relative fluorescence units (RFU) after subtraction of the corresponding Blank A controls. ROS formation and membrane permeability are expressed as percentages relative to the control, which was set at 100% and is represented by the dashed line. Treatments marked with an asterisk differ significantly from the control (Dunnett's test, $p < 0.05$). Error bars represent the standard deviation ($n = 3$).

viability, which reduces the population of bacteria capable of generating detectable ROS formation (47). Similar patterns have been reported in recent studies where exposure to high concentrations of PLA-derived oligomers resulted in decreased ROS levels due to extensive cell death (21). This interpretation is supported by the significant increase in membrane permeability ($p = 0.0014$) shown by propidium iodide staining, which reflects severe membrane disruption and loss of cellular integrity (Figure 6E and F).

These differences between polymers are more plausibly explained by the higher susceptibility of PLA to hydrolytic degradation, which leads to the early formation of low-molecular-weight oligomers that are more bioavailable and biologically active (21), thereby inducing stronger metabolic impairment than PBAT, which exhibits greater chemical stability and slower degradation kinetics. Consequently, PLA induces stronger metabolic impairment compared to PBAT, a polymer with greater chemical stability and slower degradation kinetics (30). Polymer-specific effects were statistically confirmed by direct comparisons between PLA and PBAT at 6 and 18 min ($p = 0.0485$ and $p = 0.0136$, respectively), indicating that each polymer elicited different effects on dehydrogenase activity at these time points. In addition, at the highest PBAT concentration, ROS formation ($p = 0.00195$) and membrane permeability increased markedly ($p = 0.0054$), indicating that prolonged exposure to elevated concentrations can still result in substantial cellular damage. Overall, prolonged exposure revealed polymer-specific responses that were not detected in short-term assays, suggesting that exposure duration may play an important role in nanoplastic toxicity, and raising the possibility that polymer-specific degradation behaviour may also contribute to these differences.

Other studies report that biodegradable plastic residues significantly impact soil properties, biota, and plant life, particularly at concentrations at or above 1% w/w (48). Research indicates that commercial biodegradable polymer residues can alter the soil C/N ratio and shift microbial diversity by favoring specific taxa, while also compromising physical soil structure by interfering with aggregate formation (49). Because these plastics fragment more easily than conventional counterparts, they readily release nanoplastics and oligomers; for instance, PLA at concentrations at or below 1% w/w has been shown to increase the bioavailability of heavy metals in soil (50). In aquatic environments, biodegradable plastics persist and pose ecological risks similar to traditional plastics, as their breakdown into mobile, toxic secondary nanoplastics threatens aquatic organisms (51). Toxic effects have been documented across

nearly all marketed polymers, including polycaprolactone and polyhydroxyalkanoates, in addition to PLA and PBAT (21, 52, 53). However, the reported concentrations vary significantly, ranging up to thousands of particles per litre or kilogram of sediment, and a frequent lack of detailed characterization regarding particle size, shape, and additives makes it difficult to isolate the specific properties driving these harmful effects. It is important to highlight that our study used nanoplastics generated from commercially available biodegradable materials, free of interfering chemicals and plastic additives. This approach allows the evaluation of how the specific features of these materials influence environmental microorganisms and differs from most nanoplastic toxicity studies, which predominantly rely on standardized suspensions potentially conveying surfactants of stabilizers (54). The exposure levels selected for this study, 0.1 wt% and 1 wt%, were designed to represent medium-term accumulation and environmental 'hot-spot' scenarios, respectively. To contextualize these concentrations relative to agricultural practices, the 0.1 wt% dose corresponds to approximately 200 g/m² of nanoplastics. This value was calculated assuming a standard topsoil plow layer depth of 15 cm and an average soil density of 1.33 g/cm³. For comparison, a typical single-year application of plastic mulch is approximately 25 g/m²; thus, our lower test concentration represents roughly one order of magnitude higher than a single application, simulating the cumulative effect of several years of biodegradable plastic use. It is important to note that these concentrations were selected to evaluate acute, short-term responses rather than long-term or chronic effects.

The contrasting responses observed between water and soil matrices highlight the critical role of the exposure medium in modulating nanoplastic effects. While short-term exposures in soil did not induce severe metabolic disruption in *A. globiformis*, likely due to physicochemical stabilization by soil components, the absence of this matrix effect in water resulted in a clear biphasic response: an initial metabolic burst followed by clear enzymatic inhibition and loss of cell viability, with these adverse effects being more pronounced for PLA than for PBAT. These results underscore the growing consensus that ecotoxicological assessments must encompass diverse environmental matrices (55).

Our findings are consistent with the principle that toxicity is governed by bioavailability rather than total concentration, a concept especially relevant in complex matrices like soil where contaminant mobility is significantly restricted (56). In contrast, in water, a higher fraction of nanoplastics remains bioaccessible, which may enhance their interaction with

cells. This fundamental difference explains the more pronounced biological effects observed in water compared to soil.

Conclusions

We successfully synthesized monodisperse, spherical PLA and PBAT nanoplastics via emulsion-solvent evaporation, using sodium cholate as surfactant, selected for its reported biocompatibility, while avoiding the use of additional reagents known to exert antimicrobial or cytotoxic effects in bacterial assays. Their biological effects were then evaluated by monitoring *A. globiformis* dehydrogenase activity as a proxy of metabolic activity across soil and water.

Soil matrix, rather than polymer type or concentration, primarily modulated biological impacts. The pronounced inhibition in standard sandy soil (LUFA 2.1) compared to attenuated responses in agricultural soils suggests that ecotoxicological data from reference soils should be interpreted with caution.

In water, *A. globiformis* exhibited an initial increase in dehydrogenase activity after 2 h exposure to both polymers, followed by a reduction in activity after 24 h exposure. This temporal response pattern may reflect an early metabolic adjustment followed by a later decrease in metabolic activity under prolonged exposure conditions. The reduction observed after 24 h was more pronounced for PLA than for PBAT.

After 24 h of exposure in water, the inhibition of *A. globiformis* dehydrogenase activity, particularly in PLA treatments, was accompanied by membrane disruption and altered ROS overproduction. While PBAT-exposed cells retained higher dehydrogenase activity and showed clear ROS overproduction, the rapid viability loss observed in PLA-exposed cells appeared to suppress ROS generation. These polymer-specific responses suggest that nanoplastic toxicity is influenced by the material's intrinsic properties such as their potential release of bioavailable degradation products.

The comparison between water and soil matrices revealed that nanoplastics induced significant microbial impairment even during short-term exposure. However, soil physicochemical properties act as natural buffers, masking this toxicity by limiting nanoplastic bioavailability through matrix interactions.

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Data availability

Raw data for this article are available at Zenodo at <https://doi.org/10.5281/zenodo.20288699>. Additional data supporting this article have been included as part of the Supplementary Information.

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Supplementary Information

Comparative ecotoxicity of well-defined biodegradable nanoplastics in soil and water using the environmentally relevant bacterium *Arthrobacter globiformis*

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Contents:

Table S1. Physicochemical characterization of the soils used in this study. Soil 1 corresponds to the LUFA reference soil (LUFA 2.1), Soil 2 to the Soto del Real agricultural soil (Madrid, Spain), and Soil 3 to the Valdecarábanos agricultural soil (Toledo, Spain). Analyses were performed following standard procedures (OECD-based protocols) and included pH, salinity ($\mu\text{S}/\text{cm}$), organic matter (oxidation method, %), total nitrogen (%), C/N ratio, exchangeable cations (Na, K, Ca and Mg; ppm), available phosphorus (Olsen; ppm), total carbonates and active lime (% CaCO_3), and particle-size distribution (Bouyoucos/hydrometer method; clay, silt and sand, %). “-”: not determined.

Table S2. Information on culture medium (Medium B) and working medium composition and growth conditions.

Figure S1. Growth curve of *A. globiformis* based on $\ln(\text{OD}_{600})$ measurements over time. The highlighted region indicates the exponential growth phase used for bacterial inoculum preparation in the bioassays.

Figure S2. Metabolic activity of the initial *A. globiformis* culture adjusted to $\text{OD}_{600} = 0.4 \pm 0.1$, assessed by fluorescein diacetate (FDA). The Control showed a markedly higher fluorescence signal than the positive inactivation control (Control+; 3 μL of 70% ethanol added to 180 μL of bacterial suspension). Data are expressed as relative fluorescence units (RFU) after background correction. Error bars represent standard deviation ($n = 6$).

Figure S3. Dehydrogenase activity of *Arthrobacter globiformis* exposed to ultrafiltrated PLA and PBAT suspensions at the highest tested concentration, used as negative controls to evaluate potential effects associated with dissolved compounds remaining from nanoplastic synthesis. Activity was measured after 2 h and 24 h of exposure following the same experimental procedure used for nanoplastic suspensions and expressed as relative fluorescence units (RFU). Error bars represent standard deviation ($n = 3$).

Figure S4. GC-FID control chromatograms for peak identification of hexane and dichloromethane (DCM). (A) Hexane control, showing the hexane peak (RT $\approx$ 1.23 min). (B) DCM control, confirming the DCM peak (RT $\approx$ 1.02 min). (C) Mixed control (hexane + DCM), showing both peaks at their respective retention times, used to verify peak assignment by retention-time matching.

Figure S5. GC-FID chromatograms of hexane extracts of nanoplastic suspensions (PLA and PBAT). The chromatograms show the hexane peak (RT $\approx$ 1.23–1.24 min), while no detectable peak was observed at the DCM retention time (RT $\approx$ 1.02 min), indicating negligible DCM residues in the analyzed suspensions and no interference with subsequent bioassays.

Figure S6. Dehydrogenase activity of *A. globiformis* exposed to PLA and PBAT nanoplastics (0.1 and 1 wt%) in three soil types after 24 h of contact. Activity was measured over a 60-min period and expressed in relative fluorescence units. Asterisks denote significant differences from the control (Dunnett's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

Table S1. Physicochemical characterization of the soils used in this study. Soil 1 corresponds to the LUFA reference soil (LUFA 2.1), Soil 2 to the Soto del Real agricultural soil (Madrid, Spain), and Soil 3 to the Valdecarábanos agricultural soil (Toledo, Spain). Analyses were performed following standard procedures (OECD-based protocols) and included pH, salinity ($\mu\text{S}/\text{cm}$), organic matter (oxidation method, %), total nitrogen (%), C/N ratio, exchangeable cations (Na, K, Ca and Mg; ppm), available phosphorus (Olsen; ppm), total carbonates and active lime (% CaCO_3), and particle-size distribution (Bouyoucos/hydrometer method; clay, silt and sand, %). “-”: not determined.

Properties	Soil 1 (LUFA)	Soil #2	Soil #3
pH	5.6	6.4	8.1
Salinity ($\mu\text{S}/\text{cm}$)	90.3	83.4	199
Organic matter by oxidation (%)	2.2	3.8	1.4
Total nitrogen (%)	0.159	0.271	0.114
C/N	8.1	8.1	6.9
Sodium (ppm)	57.0	70.8	72.0
Potassium (ppm)	60.2	247.0	870.0
Calcium (ppm)	1778	2676	9442
Magnesium (ppm)	57.2	157	371
Available phosphorus (Olsen) (ppm)	22.0	35.5	15.6
Total carbonates (% CaCO_3)	2.1	2.9	9.8
Active lime (% CaCO_3)	–	–	2.3
Texture (Bouyoucos Method)			
Clay	3.1	0.6	6.3
Silt	21.1	21.1	28.0
Sand	75.8	78.3	65.7

Table S2. Information on culture medium (Medium B) and working medium composition and growth conditions.

Chemical compounds	Medium B (g/L)	Working medium (mg/L)
Casein peptone	3,33	6.66
Yeast extract	1.67	3.34
D(+)-glucose	1.67	3.34
NaCl	1.67	3.34

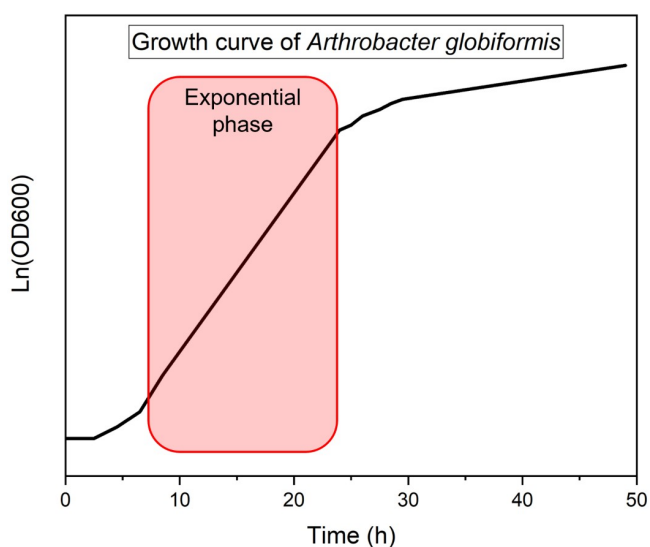


Figure S1: Growth curve of *A. globiformis* based on $\ln(\text{OD}_{600})$ measurements over time. The highlighted region indicates the exponential growth phase used for bacterial inoculum preparation in the bioassays.

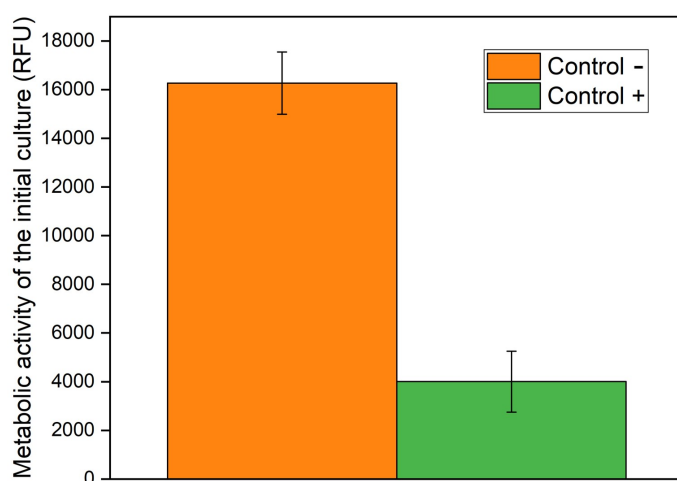


Figure S2: Metabolic activity of the initial *A. globiformis* culture adjusted to $OD_{600} = 0.4 \pm 0.1$, assessed by fluorescein diacetate (FDA). The Control showed a markedly higher fluorescence signal than the positive inactivation control (Control+; 3 μ L of 70% ethanol added to 180 μ L of bacterial suspension). Data are expressed as relative fluorescence units (RFU) after background correction. Error bars represent standard deviation ($n = 6$).

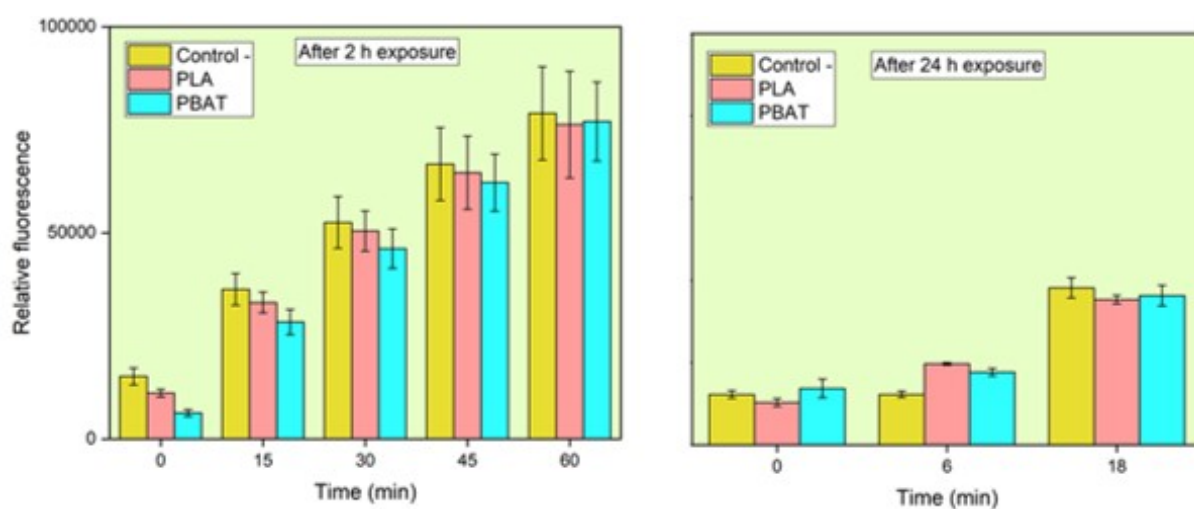


Figure S3: Dehydrogenase activity of *Arthrobacter globiformis* exposed to ultrafiltrated PLA and PBAT suspensions at the highest tested concentration, used as negative controls to evaluate potential effects associated with dissolved compounds remaining from nanoplastic synthesis. Activity was measured after 2 h and 24 h of exposure following the same experimental procedure used for nanoplastic suspensions and expressed as relative fluorescence units (RFU). Error bars represent standard deviation ($n = 3$).

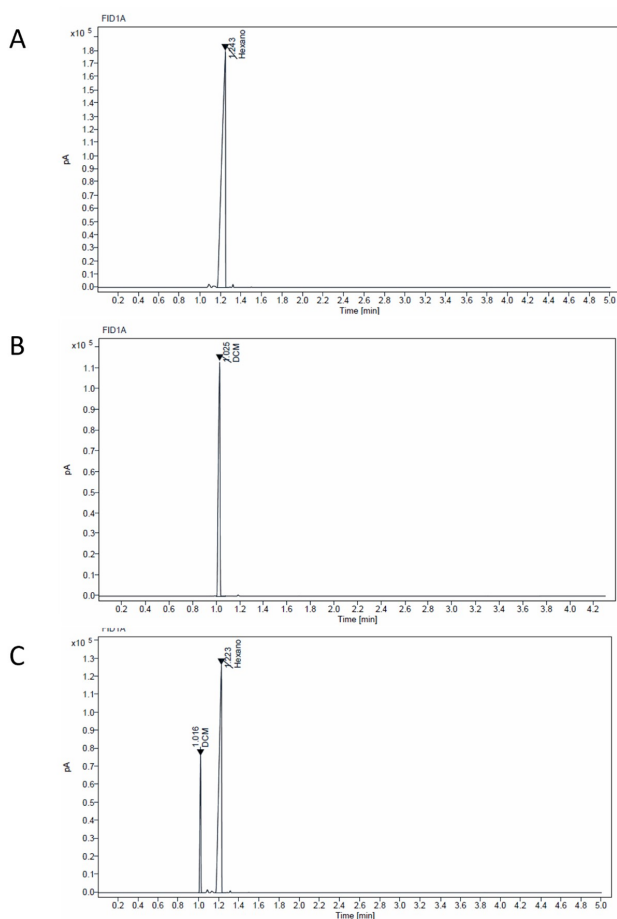


Figure S4: GC-FID control chromatograms for peak identification of hexane and dichloromethane (DCM). (A) Hexane control, showing the hexane peak ($RT \approx 1.23$ min). (B) DCM control, confirming the DCM peak ($RT \approx 1.02$ min). (C) Mixed control (hexane + DCM), showing both peaks at their respective retention times, used to verify peak assignment by retention-time matching.

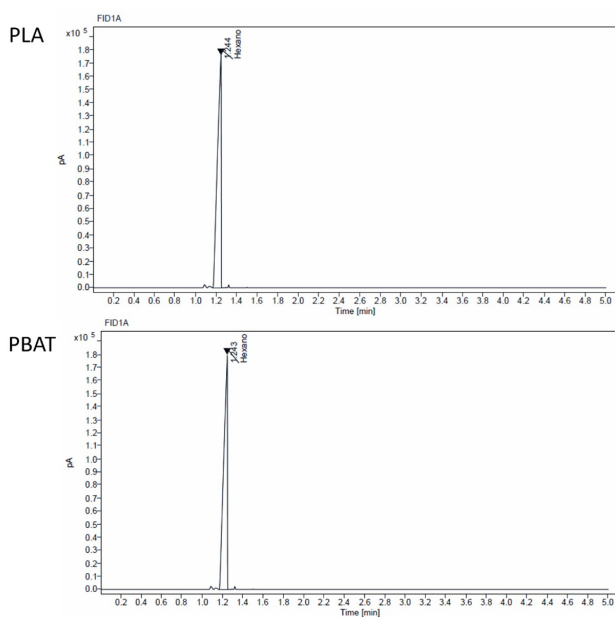


Figure S5: GC-FID chromatograms of hexane extracts of nanoplastic suspensions (PLA and PBAT). The chromatograms show the hexane peak ($RT \approx 1.23$ – 1.24 min), while no detectable peak was observed at the DCM retention time ($RT \approx 1.02$ min), indicating negligible DCM residues in the analyzed suspensions and no interference with subsequent bioassays.

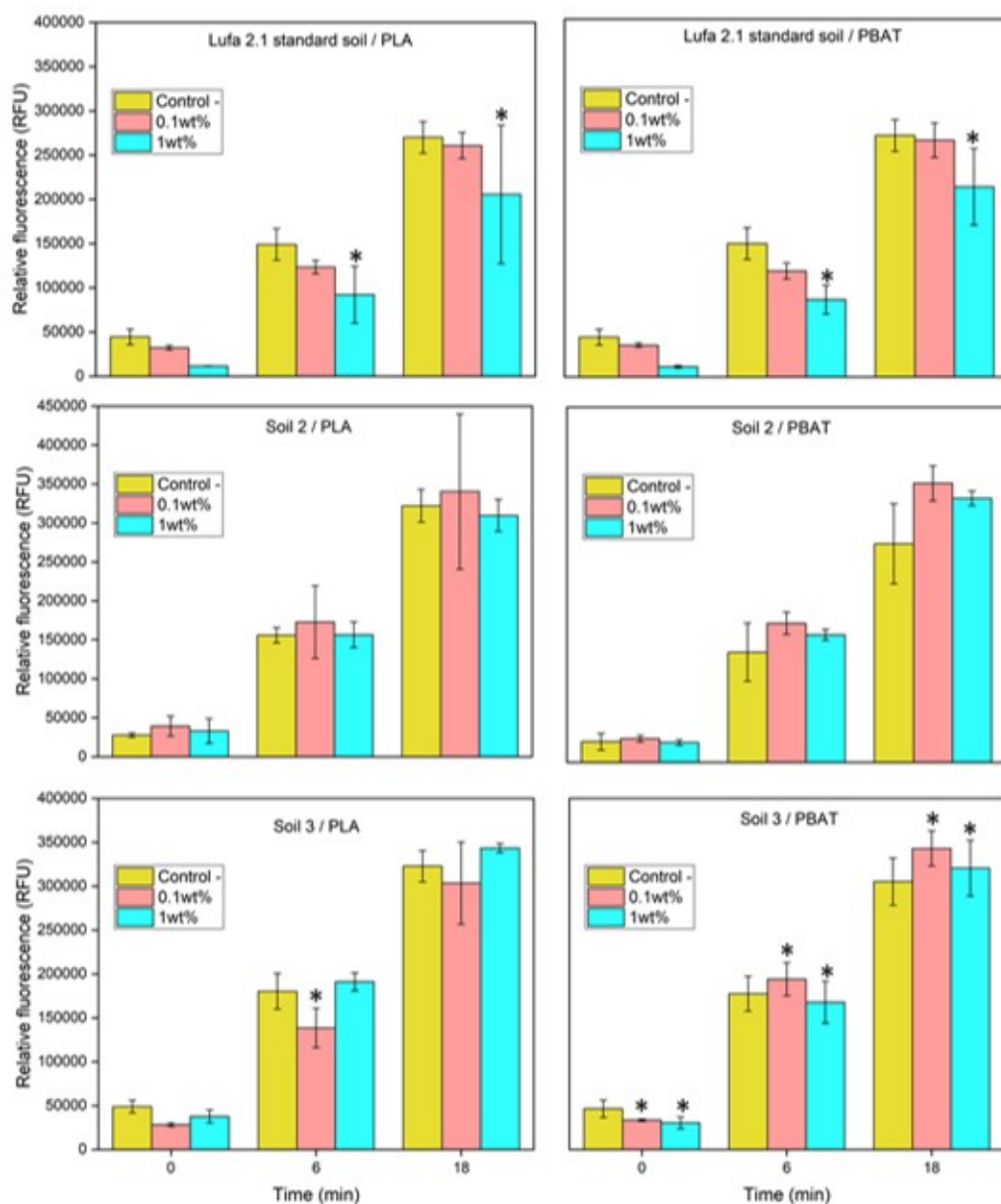


Figure S6: Dehydrogenase activity of *A. globiformis* exposed to PLA and PBAT nanoplastics (0.1 and 1 wt%) in three soil types after 24 h of contact. Activity was measured over a 60-min period and expressed in relative fluorescence units. Asterisks denote significant differences from the control (Dunnett's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).