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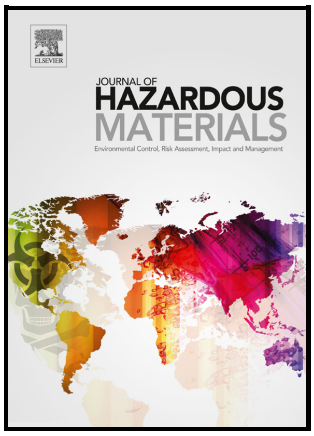

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Impacts of low concentrations of nanoplastics on leaf litter decomposition and food quality for detritivores in streams

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Abstract

In forested streams, leaf litter decomposition is a vital ecosystem process, governed primarily by aquatic hyphomycetes. These fungi are crucial mediators of nutrients and energy to invertebrates and higher trophic levels. Very little information is available on the impact of low concentrations of different sizes of nanoplastic particles (NPPs) on leaf litter decomposition and aquatic hyphomycetes communities. Besides, NPPs impact on leaf litter nutritional quality and invertebrate feeding behaviour is unknown. We conducted a microcosm assay with varying concentrations (0 to 25 μg L^{-1}) of small (100 nm; SNPPs) and large (1000 nm; LNPPs) plastic particles to assess their impact on leaf litter decomposition, sporulation rates and community structure of aquatic hyphomycetes. Furthermore, leaf litter was retrieved and fed to invertebrates to assess feeding rates. Our results indicated that leaf litter decomposition, fungal sporulation and abundance were significantly affected by NPPs

concentrations and sizes. By contrast, leaf litter nutritional quality was impacted only by sizes. NPPs, particularly SNPPs, augmented leaf litter polyunsaturated fatty acids (18 % to 31 %), consequently improving food quality; however, invertebrates' feeding rates were not impacted. Overall, our study provides novel insights on the risks posed by NPPs with pronounced impact at the basal trophic level.

Keywords: Aquatic hyphomycetes; invertebrates; polystyrenes; freshwaters; nutritional profile

Introduction

Plastic pollution is acknowledged as a global challenge with significant scientific and societal concern in aquatic systems worldwide [1, 2]. Plastics are used in various applications, from construction to medicine, due to their durability, corrosion resistance, and low production cost [3]. Globally, it is predicted that 1.15 to 2.41 million tonnes of plastic may enter the ocean annually via rivers [4]. With the rise in plastic litter, mainly single-use plastics during the COVID-19 pandemic, globally the plastic flow into the oceans is anticipated to triple by 2040 [5].

Nanoplastics are plastic particles of sizes ranging from 1 to 1000 nm [6]. They are released into the environment as a result of the fragmentation of bulk plastics or through the products containing nanoplastics, like paints, medicines, electronics, or as by-products of processes like 3D printing [7, 8]. Considering the nano-specific properties of nanoplastic particles

(NPPs), they are increasingly acknowledged as an emerging threat with particular concern to freshwater ecosystems [9-11]. As yet, research on the effect of NPPs on freshwater systems has been predominantly focussed on the large rivers and lakes, while streams have been disregarded [4, 12].

Globally, headwater streams comprise more than 98 % of the stream segments [13]. They are the primary interface between terrestrial (where plastics are utilized) and freshwater ecosystems. Stream ecosystems are vulnerable to many anthropogenic impacts that may impair and deteriorate the ecosystem functioning in varying magnitude, spanning from severe alterations with noticeable effects to inconspicuous and cryptic variations. In the forested headwater streams, the decomposition of allochthonous plant litter is a crucial ecosystem process [14] propelling energy and nutrients from one trophic level to another [15, 16]. Consequently, modulating the cycling of carbon and the efflux of carbon dioxide and methane (greenhouse gases), implying positive feedbacks to climate change [17, 18]. Among the microbes, fungi, especially aquatic hyphomycetes, are the indispensable players in litter decomposition. Aquatic hyphomycetes are potential sources of nutrients [19, 20] contributing to the enhancement of leaf litter nutritional quality and palatability [21, 22], which is pertinent to invertebrate's optimal growth and survival [23]. Thus, aquatic hyphomycetes are primarily responsible for channelizing the nutrients trapped in the leaf litter to higher trophic levels [24, 25]. The invertebrate, *Echinogammarus meridionalis* Pinkster (Amphipoda), is a shredder detritivore, predominant in the slow running freshwaters. They are common to the Mediterranean region and distributed throughout Europe [26]. *Echinogammarus meridionalis* primarily feed on coarse particulate organic matter, playing an essential role in detritus processing in the freshwaters. They form a crucial functional link in the food webs and also serve as prey for several predator fish species.

To date, there is relatively limited information available on the impact of environmentally realistic concentrations of NPPs on leaf litter decomposition [27, 28]. Likewise, little is known whether NPPs adversely interact with the leaf litter and aquatic hyphomycetes community [27]. Leaf litter as a food resource for shredders has emerged as a pertinent research topic focusing on trophic links in streams [24]; however, the knowledge about leaf litter lipid and carbohydrate profiles is exceptionally scarce [29, 30]. Therefore, exploring the impact of the NPPs modified leaf litter quality on the invertebrate's feeding behaviour will be worthwhile.

Polystyrenes are commonly used in single-use plastic products, medical applications, food packaging, and fast food containers [31]. They are acknowledged to significantly contribute to the pollution of aquatic ecosystems [11, 32]. It is demonstrated that the daily-use polystyrene products can break down into NPPs [33]. Predicting the risks of NPPs to aquatic ecosystems is challenging due to technological limitations in estimating their environmental concentrations. Most of the studies demonstrating the harmful effects of NPPs on aquatic organisms are based on concentrations that are several magnitudes higher (> ~0.5 mg L^{-1}) [10] than the concentrations predicted to be environmentally relevant (1 pg L^{-1} to 15 μg L^{-1} for ~50 nm) [34, 35]. A study using a range of NPPs concentrations (1.6 to 102.4 mg L^{-1}) evidenced a negative impact on leaf litter decomposition capability by five selected worldwide distributed species of aquatic hyphomycetes, namely *Anguillospora crassa*, *Tetracladium marchalianum*, *Tetrachaetum elegans*, *Articulospora tetracladia* and *Tricladium splendens*. In addition, aquatic hyphomycetes' tolerance towards NPPs was species-specific with *T. marchalianum* being the most sensitive [27]. Another recent study evidenced that leaf decomposition process in the streams was constrained by the altered aquatic fungal community structure and microbial metabolism after exposure to polystyrene NPPs (50-100 nm; 1 to 100 μg L^{-1}) [28]. However, it is unclear to what extent very low

concentrations of NPPs influence the leaf litter decomposition process by aquatic hyphomycetes and invertebrates.

The goal of this study was to use environmentally realistic concentrations (up to 25µg L^{-1}) of two sizes (100 and 1000 nm) of NPPs to assess their effects on 1) leaf litter decomposition, 2) aquatic hyphomycetes sporulation rates and community composition, 3) leaf litter nutritional profiles (fatty acids and carbohydrates) and, in addition, 4) invertebrate feeding behaviour. We hypothesized that the tested concentrations and sizes of NPPs would negatively impact litter decomposition and community composition of aquatic hyphomycetes, based on our prior inference on the adverse effect of NPPs on the functioning of selected species of aquatic hyphomycetes [27]. Furthermore, if the NPPs influence the performance of the aquatic hyphomycetes, it is speculated to compromise the leaf litter nutritional quality, which is the primary factor governing the feeding behaviour of the invertebrates [24]. Therefore, we predicted that NPPs would consequently influence the feeding behaviour of invertebrates as previously observed for nano copper oxide [36]. The experiments were executed in microcosms to mirror the natural stream environment. The endpoints determined were leaf litter mass loss, aquatic hyphomycetes sporulation including community composition, leaf litter nutritional quality, namely fatty acids and carbohydrates profiles, and the feeding rates of invertebrates. Overall, we predicted that SNPPs would elicit a more pronounced effect than the LNPPs for all the endpoints tested, as demonstrated previously for nano copper oxide [37].

Materials and methods

Physico-chemical characteristics of the stream water

Conductivity, dissolved oxygen, pH and temperature in the stream waters [Lousã (N 40º 5′ 59″, W 8° 14′ 2″) and Redinha stream (N 39º 58′ 43.48″, W 8º 34′ 23.87″); central Portugal] were recorded *in situ* (WTW, Weilheim, Germany). Four aliquots of stream water samples

were collected, pooled and mixed thoroughly. These water samples were filtered using glass fiber filters (0.5 µm porosity) and stored at -20 °C until analysis. The concentration of total nitrogen (TN), ammonia (NH_3), and silicon (Si) was measured using a Skalar San^{++} Autoanalyser (Netherlands) by adapting and optimizing the methodologies for TN [38], NH_3 [39] and Si [40]. Calibration curves were performed for each parameter, namely: 140 to 2100 µg N L^{-1}, 40 to 1000 µg N-NH_3 L^{-1} and 100 to 5000 µg Si L^{-1}. The limit of quantification of the methods was 140 µg N L^{-1}, 40 µg N-NH_3 L^{-1} and 100 µg Si L^{-1}. The limits of detection were 42 µg N L^{-1}, 12 µg N-NH_3 L^{-1} and 30 µg Si L^{-1}. Total dissolved carbon (TDC) in the water samples were measured [41] using LiquiTOC analyser (Skalar San^{++}, Netherlands).

Suspension of nanoplastics

Nanoplastic particles of 100 nm and 1000 nm polystyrene were used (Sigma-Aldrich; 100,000 mg L^{-1} aqueous suspensions); they were devoid of stabilizers or additives. NPPs have sulphate groups on their surface and have a zeta potential of -50 to -60 mV. Plastic aqueous suspensions were sonicated (42 kHz, 100 W, Branson 2510, USA) for a minute before use. The nominal exposure concentrations of plastics were 0 (control), 0.25, 2.5 and 25 µg L^{-1} (10-fold increase at each step). The range of concentrations used in the experiments was chosen to approximately mirror the current low (0.25 µg L^{-1}) and high (2.5 µg L^{-1}) values, and 25 µg L^{-1} was selected based on the prediction that global plastic waste input will increase 10-fold by 2025 [1, 42]. A 100 mg L^{-1} of plastics stock suspension was prepared from 100,000 mg L^{-1} aqueous suspension in filtered (5 µm porosity, Millipore, USA) sterile (autoclaved, 120 ºC, 20 min) stream water from Lousã. The stock suspensions were diluted with filtered sterile stream water to obtain the required exposure concentrations.

Scanning electron microscopy (SEM) was used to characterize the nano polystyrene surface topography of the stock solution. SEM was performed on a Hitachi TM-1000 tabletop microscope, operating at 5 kV and on a VEGA3 SBH from TESCAN, 15 kV. Plastic samples

were sputter-coated with platinum. Plastics size in the stock suspension was measured by dynamic light scattering (DLS) via a Zeta PALS Zeta Potential Analyzer (Brookhaven Instruments Corporation, USA).

Leaf litter colonization

The leaf litter experiments were performed with *Alnus glutinosa* (Black alder: Betulaceae), a riparian species, widespread in the Holarctic and occurring in the Neotropics [15]. Freshly fallen leaves were hand-picked at a single site at Parque Verde do Mondego Coimbra, Portugal (N 40º 11′ 21″, W 8º 25′ 30″), air-dried and stored at room temperature until use. Leaves were leached in distilled water for 24 h and 12 mm discs were punched out using a cork borer. Each ten sets of leaf discs were allocated to 61 fine mesh bags (0.5-mm mesh size; Fig. S1). A 0.5 mm mesh size was chosen to prevent colonization by macroinvertebrates and deployed in the Lousã stream in the autumn season for 7 days [43] to allow colonization by microbes (Fig. S2a). The streams' physicochemical characteristics were measured at the time of deployment of the leaf litter (Table 1). The wet mass of the colonised leaf discs in sets of 10 each used in the microcosm (n=56) assay was estimated by gently drying the surface of the leaf discs with sterile filter paper. Initial wet mass [44] of each set of leaf discs was used to account for the mass loss that might have incurred due to leaching (7 days) per leaf litter bag. The initial dry mass of each set of leaf discs was estimated by multiplying the wet mass of leaf discs of each set by a conversion factor. The conversion factor was calculated as DM /WM, where WM is the average wet mass of a set of 10 discs taken from five-leaf litter bags after colonization and DM is the average oven (105 ºC for 24 h) dry mass of the same discs.

Experimental setup

To examine the impact of NPPs, ten leaf discs from the 56 leaf litter bags were rinsed with distilled water and added to Erlenmeyer flasks (leaf litter mass loss and sporulation, n=4 and

nutritional profiles and animal feeding experiments, n=3) containing 25 mL sterile (120 °C for 20 min) stream water suspended with nominal concentrations of plastics (Fig. S2b). Microcosms were performed for 26 days in an orbital shaker (115 rpm) at 16 °C under a 12 h^L: 12 h^D photoperiod. The NPPs suspensions in stream water were renewed every five days (6 days for the last change). Suspensions from the Erlenmeyer flasks (assigned for sporulation analyses) were pooled and preserved with 2 ml of 2.22 % formaldehyde (Sigma) for assessing the fungal sporulation rates and community composition. From the microcosms comprising leaf discs assigned for evaluating the nutritional profiles and animal feeding assay, one set of randomly selected leaf discs per microcosm was weighed to achieve constant weights for all the replicates (n=3) and frozen (-80 ºC) for evaluating the carbohydrates and fatty acids profiles. Furthermore, three-leaf discs from each microcosm were randomly selected, cut into half, lyophilized (-50 ºC, 12 h, Lablyo mini, UK) and used to feed the invertebrates (Fig. S2c). After completing the NPPs exposure assays (litter decomposition and animal feeding experiments), the suspensions containing the fine particulate organic matter (FPOM) and NPPs were collected, 5 ml from the replicate samples were mixed and subjected to Fourier-Transform Infrared (FTIR) spectroscopy. FTIR spectral analysis was conducted to detect the diverse functional groups in the suspensions; the analysis was carried out using a FTIR- 4100 (Jasco, USA) with an attenuated total reflectance (ATR) plate. In addition, the surface morphology of the particulate matter and NPPs in the suspensions were recorded by high resolution-field emission-scanning electron microscopy (HR-FE-SEM, SU8010, Hitachi, Japan). HR-FE-SEM was attached to an energy dispersive X-ray microanalysis (EDS) setup for performing elemental image analysis. Sputter coating was performed with platinum.

Leaf litter mass loss

The leaf discs were oven-dried (105 °C for 24 h, Thermo Scientific Heratherm, USA) and

weighed in sets to the nearest 0.001 mg (AS 220/C/2, Radwag, Poland) to assess mass loss during the 26 days of exposure to NPPs in the laboratory.

Fungal sporulation rates and community composition

To the pooled suspensions consisting of spores from each microcosm, 0.5 % Triton X-100 (Sigma-Aldrich, Germany) was added to disperse conidia in the solution. Appropriate volumes of each sample depending on the spore density were filtered through a 5 µm pore size cellulose nitrate filter (Sartorius Stedium Biotech GmbH, Germany). Conidia were stained with 0.05 % cotton blue in lactic acid (Sigma-Aldrich, Germany) and were identified using taxonomic keys [45, 46] and counted under a light microscope (100×; Diaplan, Leica, Germany) [47]. At least 200 conidia and ten microscopic fields were counted and the results were expressed as Conidia g^{-1} DM day^{-1}.

Leaf litter fatty acids composition

Fatty acids were analysed on the leaf litter after exposure of colonised leaf discs to NPPs concentrations; total lipids and fatty acid methyl esters (FAMEs) were measured [48]. An internal standard methyl nonadecanoate (C19:0, Fluka, Germany) was added to the samples to identify methyl esters. The organic phase of the leaves was extracted with hexane and, after centrifugation, the supernatants were collected in the vials kept at -80 ºC until analyses by gas chromatography-mass spectrometry (GC-MS). The analyses were performed in a Trace 1310 Thermo Scientific gas chromatograph. This equipment has a TR-FFAP column with 0.32 mm internal diameter, 0.25 µm film thickness and 30 m length. The injector port was lined with a splitless glass liner. A Thermo Scientific ISQ 7000 Network Mass Selective Detector at scanning *m*/*z* ranges specific for fatty acids in Selected Ion Monitoring (SIM) mode acquisition was used. The initial oven temperature was 80 °C, following a linear temperature increase of 25 °C min^{-1} to 160 °C, followed by another temperature ramp of 2 °C min^{-1} to 190 °C and ultimately an increase of 40 °C min^{-1} until a final temperature of 230 °C,

which was maintained for 5 min. The carrier gas was Helium maintained at a flow rate of 1.4 mL min^{-1} column head pressure. Identification of each peak as a FAME was achieved by retention time and mass spectrum by comparing with database (NIST) and standards, Supelco® 37 component FAME mix (Sigma-Aldrich, Germany). The FAMEs concentrations were then calculated using the integrated peak areas identified in the samples as described earlier [48].

Leaf litter carbohydrates composition

Carbohydrates were extracted from leaf litter samples following a modified protocol [49]. The leaf litter samples were boiled in ethanol at a final 85 % (v/v) concentration for 10 min. The mixture was cooled and centrifuged for 1 min at 2000 rpm (Hyper-VC220) and the supernatant was removed by decantation and the sugars soluble in alcohol (SA) were recovered using a centrifuge evaporator and then dried at room temperature. The remaining alcohol insoluble residue (AIR) was dried overnight to remove ethanol at room temperature. SA and AIR fractions were subjected to a pre-hydrolysis with 72 % H_2SO_4 at room temperature for 3 h, followed by hydrolysis using 1 M H_2SO_4.

The obtained neutral sugars were converted to their alditol acetates [50] and analysed by gas chromatography through a Thermo Scientific (USA) Trace 1310 Network, equipped with a flame ionization detector (GC-FID). A TG-WAXMS A (30 m length, 0.32 mm i.d., 0.25 µm film thickness) gas chromatography column was used and the oven was programmed to an initial temperature of 180 ºC, following a linear temperature increase of 5 ºC min^{-1} until the final temperature of 230 ºC, maintaining this temperature for 12 min. The carrier gas was helium at a flow rate of 2.5 mL min^{-1}. The monosaccharides were identified by retention time comparison with standards. Sugar quantifications were obtained by comparison of the chromatographic peaks with an internal standard (2-deoxyglucose).

Leaf litter feeding by invertebrates

Echinogammarus meridionalis were collected from the Redinha stream (N 39º 58′ 43.48″, W 8º 34′ 23.87″). The physico-chemical characteristics of the stream water are given in Table 1. The invertebrates were transferred to the laboratory on ice and separated (by naked eye) according to size. Fifteen litres of stream water were filtered by using filter paper (5 µm porosity; Millipore, USA) to separate the debris and fungal spores in the stream water. Individual animals of similar size (7.4 mm ± 3.6) were acclimatized in the filtered stream water, aerated with aquarium pumps for four days. Individuals were fed *ad libitum* with lyophilized (-50 ºC, 12 h, Lablyo mini, UK) alder leaf litter previously conditioned in the stream for 26 days. The animals were starved for 24 hrs before the feeding experiment. Animals were distributed individually to glass bottles (55 mm diameter and 85 mm height) with 80 mL stream water (n=10 per treatment; Fig. S2c); the glass bottles were provided with half of the lyophilized pre-weighed leaf disc reserved for animal feeding experiments. Additionally, control microcosms (n=4 per treatment) were maintained without the animals to assess the leaf litter mass loss due to microbial activity. Microcosms were constantly aerated with aquarium pumps (Fig. S2c). When the overall 50 % [51] of the leaf disc's surface area remained in most of the microcosms, the experiment was terminated; stream water from the microcosms was subjected for FTIR analysis to confirm the presence or absence of NPPs in the suspensions. Furthermore, the remaining leaf discs and the animals retrieved were lyophilized (-50 ºC, 12 h, Lablyo mini, UK) and weighed (d = 0.1 µg, UMX2 Mettler Toledo, USA) to attain the final leaf dry mass (mg) and invertebrate dry mass (mg). Dry mass (DM, mg) of leaf discs consumed by the *E. meridionalis* was calculated as $(L_i - L_f) - (C_i - C_f) / (I_f \times \text{time})$. L_i is the initial weight (mg) of the leaves fed to the animals and L_f is the final weight (mg) of the leaf disc after consumption and I_f is the animal dry mass (mg) time t (1.5 days). C_i and $C_{f,}$ respectively, represent the initial and final dry mass (mg) of the control leaf discs

(without animals) (Fig. S2c). The feeding rate was expressed in mg leaf DM mg^{-1} animal DM d^{-1}.

Statistical analyses

D'Agostino & Pearson omnibus normality test for normality and Barlett's tests for homogeneity of variances were performed for the leaf litter mass loss, fungal sporulation rates and animal feeding rates. Two-way univariate Permutational Analysis of Variance (PERMANOVA) was used to assess the impact concentrations and sizes of plastic particles on leaf mass loss as the data did not fulfil the ANOVA assumption (homoscedasticity) after transformation. Unrestricted permutation of the raw data (9999 permutations) was used for PERMANOVA. Posteriori pairwise comparisons were used to determine the significant differences ($p<0.05$) between the tested NPPs concentrations. Two-way ANOVAs followed by Tukey's post hoc test were used to test the significant effects ($p<0.05$) of sizes (100 and 1000 nm) of and concentrations (0. 0.25, 2.5 and 25 µg L^{-1}) of NPPs on fungal sporulation rates and animal feeding rates.

Multidimensional scaling (MDS) after log (x+1) transformation was based on the Bray-Curtis similarity matrix to visualize the impact of sizes and concentrations of the plastic fraction on aquatic hyphomycetes community structure (abundance), fatty acids and carbohydrates composition. PERMANOVA was used to test the significant effects ($p<0.05$) of sizes (100 and 1000 nm) and concentrations (0, 0.25, 2.5 and 25 µg L^{-1}) on fatty acids and carbohydrates profiles following similar parameters as stated above for PERMANOVA. ANOVA was performed using Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA). MDS and PERMANOVA analyses were performed using PRIMER 6 (Primer-E Ltd., U.K.) [52].

Results

Characterization of plastic fractions

The average diameter of SNPPs and LNPPs in the stock suspension, assessed by SEM, was

147.71 ± 6.12 nm (Fig. 1a) and 1030 ± 0.02 nm (Fig. 1b). These observations were congruent with SNPPs (148 nm) and LNPPs (1000 nm) DLS data, revealing that plastic particles were spherical and the suspension was well dispersed with little or no agglomeration.

The FTIR spectra of the suspensions after leaf litter decomposition assay represent the asymmetric and symmetric stretching bands at 2925 - 2852 cm^{-1}, confirming the presence of methylene groups ($-CH_2$). The FTIR spectra of NPPs showed spectral changes corresponding to the formation of new bands at 3360 - 3240 cm^{-1} (hydroxyl group), 1641 cm^{-1} (double bond or C=O groups), and 1031 cm^{-1} (C–O bonds) [53]. The broad peak around 3336 cm^{-1} represents the stretching bands of the hydroxyl groups (-OH) (Fig. 2a,b). Therefore, it can be concluded that when the NPPs concentration increased, the peaks in the suspensions decreased (Fig. 2a,b), which may indicate the adsorption of NPPs on the leaf surface [54]. The HR-FE-SEM image of the suspensions also evidenced agglomeration and adsorption with the increase in concentration for both sizes (100 and 1000 nm) of NPPs (Fig. 3a-g). In addition, EDS analysis of the suspension also revealed the distribution of major elements (carbon > oxygen > silicon > aluminium) (Table 2); carbon composition increases along with the rise in the NPPs exposure concentrations. The elemental maps of suspensions (0 and 25µg L^{-1} NPPs) denote the distribution of carbon, oxygen, aluminium and silicon (Fig. S3a-c). No distinct effects of NPPs size and concentration in these suspensions were visualized using DLS, probably due to interference of the FPOM originating from leaf litter during the decomposition process.

Leaf litter mass loss

The leaf litter mass loss decreased with an increase in NPPs exposure concentrations (Fig. 4a). Among the plastic particles, the inhibitory effect of SNPPs (42 % to 53 %) was more pronounced than LNPPs (49 % to 54 %) (Fig. 4a). The mass loss was 60 % in the control microcosm and reduced to the maximum (by ~20 %) when exposed to 25 µg L^{-1} of SNPPs.

Both concentrations (Two-way PERMANOVA, $F_{3,24}$=97.22, p=0.0001) and sizes of plastic particles ($F_{1,24}$=20.10, p=0.0003) had a significant impact on the leaf litter mass. Interactions were observed between the concentrations and sizes of plastic fractions ($F_{3,24}$=6.36, p=0.002). All the concentrations tested were significantly different from each other (pairwise test, p=0.0001-0.0016).

Fungal sporulation rates

The fungal sporulation rates were strongly impacted by SNPPs than by LNPPs (Fig. 4b) and decreased with an increase in NPPs exposure concentrations. In control microcosms, the fungal sporulation rate of aquatic hyphomycetes attained 1.1×10^6 conidia g^{-1} leaf dry mass day^{-1} and was inhibited up to 41 % to 72 % and 14 % to 56 %, respectively, by SNPPs and LNPPs (Fig. 4b). The sporulation rates were significantly affected by exposure concentrations (Two-way ANOVA, $F_{3,24}$=22.37, p=0.00008) and sizes ($F_{1,24}$=74.26, p=0.00000) of plastic particles. Interactions between concentrations and sizes of plastic fractions were significant ($F_{1,24}$=3.04, p=0.0486). All the tested concentrations inhibitory effects were significantly different from each other (Tukey's Test, p=0.00016 to 0.00342).

Aquatic hyphomycetes community structure

Twenty-six species of aquatic hyphomycetes have been observed in our study. *Articulospora tetracladia* was the most abundant species in control (0 µg L^{-1}) treatment, whereas *Flagellospora curvula* was the dominant species in all the exposure concentrations (except for 2.5 µg L^{-1} of SNPPs) and sizes of plastic particles. There were no apparent differences in species richness among NPPs concentrations or sizes (Table 3). The MDS ordination discriminated aquatic hyphomycetes community structure (based on abundance) both by concentrations (PERMANOVA $F_{3,24}$=2.42, p=0.002) and sizes (PERMANOVA $F_{3,24}$=3.93, p=0.0001) of NPPs (Fig. 5a). Significant differences were observed between all the tested

concentrations (pairwise test, p=0.0003 to 0.037) except for between 2.5 and 25 µg L^{-1} of plastic particles.

Leaf litter fatty acids composition

In general, leaf litter exposed to SNPPs showed lower saturated fatty acids (SFA) and higher polyunsaturated fatty acids (PUFA) content than leaves exposed to LNPPs (Table 4). The diversity (total number) of fatty acids is similar under stress induced by different sizes of NPPs (Table 4). The most abundant PUFA measured were α-linolenic acid (C18:3n3) and linoleic acid (C18:2n6c), with high concentrations, especially after exposure to SNPPs. SFA and monounsaturated fatty acids (MUFA) were more abundant than PUFA in leaf litter, with palmitic acid (C16:0) and stearic acid (C18:0) being the main fatty acids. Palmitoleic acid (C16:1), a bacterial marker, increased notably when exposed to SNPPs, specifically at the highest concentration. Also abundant were the long-chain SFA, such as docosanoic acid (C22:0) and lignoceric acid (C24:0), especially in the presence of LNNPs. The MDS ordination (Fig. 5b) grouped the fatty acids by sizes (PERMANOVA $F_{3,21}$=3.49, p=0.026) of plastic fractions but not by concentrations ($F_{3,21}$=1.05, p=0.3905).

Leaf litter carbohydrates composition

The AIR fraction obtained was 25 % of the total leaf litter biomass analysed, and 17 % was constituted by polysaccharides (Table 5). The mean sugar content in the SA fraction of the leaf litter samples was 0.3 %. The sugar composition of the SA fraction was similar to the AIR fraction composition in terms of diversity, containing arabinose (Ara), glucose (Glc), galactose (Gal) and xylose (Xyl). Glucose dominates the sugar profile in the AIR fraction, except for treatments with 0.25 and 2.5 µg L^{-1} of SNPPs, where Xyl became the most dominant sugar. In the SA fraction, Xyl dominates the sugar profile followed closely by Glu, except for the treatment with 25 µg $L^{-1}$of SNPPs where the Glu level increased. Sugar profiles of the AIR fractions were discriminated only by sizes (PERMANOVA $F_{3,13}$=9.00,

p=0.0029) of plastic fraction through the MDS ordination and not by plastic particle concentrations (Fig. 5c). The SA fractions were not sensitive to concentrations or sizes of plastic particles (Figure not shown).

Invertebrate feeding rates

Invertebrate feeding rates were not significantly impacted by NPPs concentration (Two-way ANOVA, $F_{3,72}$=1.33, p=0.27) and sizes ($F_{3,72}$=1.12, p=0.29) (Fig. S4). However, a decrease in feeding rates along with an increase in concentration was observed when LNPPs treated leaf discs were fed to animals. No clear trend was observed after feeding the animals with the leaf discs exposed to SNPPs. Nonetheless, the feeding rates of the *E. meridionalis* were reduced by 50 % when fed with leaves treated with 25 µg L^{-1} of SNPPs. FTIR analyses of the suspension confirmed that the stream water from the exposure assay did not contain any NPPs. This implies that the NPPs adsorbed or agglomerated on the leaf discs were intact even when they were added to stream water to feed the animals.

Discussion

Leaf litter decomposition is a vital ecosystem process governing the nutrient cycling and transfer of energy to higher trophic levels [23]. Aquatic hyphomycetes are capable of transforming recalcitrant polymers in the leaf litter into more labile molecules subsequently enhancing the palatability and nutritional quality of the leaf litter for invertebrate consumption. This leaf litter transformation is critical for invertebrates, as they require an adequate balance of organic and inorganic molecules for growth [24]. Moreover, aquatic hyphomycetes release copious number of spores, which are key constituent of fine particulate organic matter, serving as a food source for filter feeders and collectors [16]. Anthropogenic stressors may induce variations in the fungal community composition and leaf litter decomposition thereby influencing food webs and stream ecosystem functioning [27, 28]. NPPs are emerging environmental concern and there is a gap in the potential impact of

realistic environmental concentrations of NPPs on litter decomposition [27, 28]. This hampers the clarity on the magnitude of NPPs pollution on freshwater ecosystem functioning and health. To date, only a single study, each targeting the NPPs' impacts on aquatic hyphomycetes [27] and litter associated aquatic fungi [28] is available. Our experiments reflected a range (0 - 25 μg L^{-1}) of environmentally realistic concentrations, revealing that not only NPPs concentrations but also sizes (100 and 1000 nm) had an impact on leaf litter decomposition and aquatic hyphomycetes. Besides, only NPPs size influenced the leaf litter nutritional quality. In contrast to our predictions, the NPPs did not elicit any adverse impact on the invertebrate feeding behaviour.

Previously, a decrease in average litter decomposition by aquatic hyphomycetes isolates was evidenced after exposure to 102.4 mg L^{-1} NPPs (100 nm, polystyrene); however, the concentrations used were not environmentally realistic [27]. Our study revealed that litter decomposition was more impacted by 100 nm than 1000 nm. An earlier study [37] also demonstrated a similar tendency: the smaller the nanoparticles (copper oxide), the more intense their impact on leaf litter decomposition. It is well acknowledged that the plastic particles' surface area increases with a decrease in size, thereby offering more biological contact and increasing the reactivity in aquatic systems [55]. The small size of SNPPs may justify the exacerbated toxicity of SNPPs on leaf litter decomposition and aquatic hyphomycetes communities.

The physico-chemical conditions of stream waters also impact the adsorption and agglomeration capacity of NPPs to particulate organic matter (POM) [56]. It is evidenced that nano polystyrenes interact with dissolved organic matter (DOM), accelerating their aggregation rate due to the intense hydrophobic interactions between NPPs-DOM, which enhances the vertical carbon flux [57]. In addition, it was recently demonstrated that 25 nm polystyrene NPPs in freshwaters facilitate POM formation and enhance its transition from

DOM [58]. The interaction between NPPs and DOM may also determine the fate of NPPs in the freshwaters [59], potentially affecting the aquatic communities and ecosystem functioning [60].

In the current study, sporulation rates were more sensitive to SNPPs as reported for nanoparticles [37], probably due to higher surface contact between fungi and NPPs. The fungal sporulation rates were the most sensitive parameter to NPPs exposure and corroborated our previous studies on nanoparticle exposure [37, 61]. The suppression of fungal sporulation is a fundamental response against stress for optimal energy management. Moreover, the sensitivity of fungal sporulation rates to nanoparticles is impacted by concentrations [61] and sizes [37]. In addition, our study revealed that, NPPs concentration and size influenced the aquatic hyphomycetes species abundance but not the species richness. Generally, filamentous fungi secrete hydrophobins, which are cysteine-rich proteins characterized by hydrophobicity and are known to protect them against NPPs [62]. Therefore, toxicity was not observed to be uniform [62]. Previously, it was demonstrated that aquatic hyphomycetes species differed in their sensitivity to NPPs [27]. In addition, nanoparticles were found to alter aquatic hyphomycetes' community structure [43, 61]. This indicates that NPPs can exert selective pressure in aquatic hyphomycetes species influencing their activities with consequences on biodiversity and, subsequently, ecosystem functioning.

Here, leaf litter was used to assess the nutritional profiles as plant litter is the fundamental energy supplier, sustaining stream detrital food webs [63, 65]. It has been suggested that the nutrient content of leaf litter may be ameliorated mainly by aquatic hyphomycetes and also by bacteria (to a lesser degree) that colonize the leaves in the stream. Furthermore, it has been demonstrated that microbial colonized detritus is more readily consumed and assimilated by stream invertebrates than sterile (autoclaved) leaves [65]. The lipids belonging mainly to aquatic hyphomycetes in the colonised leaves were proposed to be responsible for this

preference [66, 67]. This hypothesis is also supported by the high PUFA content in aquatic hyphomycetes [19]. The supply of PUFA to aquatic invertebrates is particularly important because it compromises the consumers' secondary production. Since these molecules cannot be synthesized by the consumers or are synthesized at a meagre amount with high-energy demand, aquatic invertebrates may acquire them mainly through their diet [68]. Given that PUFA contributes to food quality, these lipids could affect stream detritivores' optimal functioning [66, 67].

Interestingly, SNNPs had a pronounced impact on the leaf litter's nutritional quality by enhancing the PUFA content, which increased with the decrease in SNPPs exposure concentrations, suggesting fungal metabolic responses in the PUFA synthesis pathways. LNNPs improved the leaf litter PUFA content, but no specific trend was evident in our study with respect to NPPs concentration. The overall increase in leaf litter PUFA content after NPPs exposure coincided with the decrease in leaf litter decomposition. Long-chain SFA (C>20) such as docosanoic acid and lignoceric acid, whose elevated levels were observed especially in the leaf litter after exposure to LNPPs, are components of cuticular waxes which serve as markers of leaf litter input into freshwater from terrestrial systems. During the litter decomposition process, linoleic acid (C18:2n6c) is reported to decline rapidly compared to other fatty acids [69], which explains the lowest levels of linoleic acid in our control microcosms. The fatty acids, linoleic acid and palmitoleic acid (C16:1) are considered, respectively, as fungal and bacterial markers [69]. Linoleic acid and palmitoleic acid are also abundant in other living organisms, but since in our study, the fatty acid measurements were conducted on dead leaves which had lost most of their fatty acid content prior to leaf fall and in addition the usage of meshes to prevent the leaves from invertebrate colonialization, ascertain that these fatty acids belong mainly to the fungal decomposers. Furthermore, although linoleic acid is also a marker of green plants and diatoms [70], other diatom markers

(eicosapentaenoic acid) were not detected.

High amounts of carbohydrates have been reported in aquatic hyphomycetes, with the alcohol insoluble fraction (AIR) proportionally higher than the alcohol-soluble fraction [20], which corroborates our study. Notably, Glc content in AIR sugars in the leaf litter was lower when exposed to low SNPPs concentrations (0.25 and 2.5 µg L^{-1}) but was markedly increased upon exposure to the highest concentration (25 µg L^{-1}). This tendency could be due to the fungal response to stress at the highest SNPPs concentration promoting the degradation of the polysaccharides in the leaf litter. Probably, high-stress conditions (25 µg L^{-1} SNPPs) would have induced fatigue-like behaviour in the invertebrates and this observation in our study is consistent with other organism's responses to pollutants [71]. Further research is needed to understand how the NPPs might affect the fungal ability to utilize leaf litter carbohydrates. It is evidenced that nanoplastic surface properties strongly dictate their behaviour in freshwaters, consequently shaping its environmental identity and availability to interact with the DOM closely [59].

In general, carbohydrates and fatty acids are readily metabolised during litter decomposition in streams and tend to decline as decomposition progresses [69, 72]. In our study, after exposure to plastics, an improvement in leaf nutritional quality was noted. As a result, the leaves may appear more appealing to invertebrates, thus posing a potential risk for easily transferring plastic particles through the stream food chain.

The animal experiment was terminated after one and a half days when an overall 50 % of the leaf discs were consumed by the invertebrates. In our study, a visible decrease in invertebrate swimming activity was noted (by the naked eye) after 12-15 hrs of feeding on leaves pre-exposed to 25 µg/L NPPs (highest) concentrations. Increasing evidence indicate that NPPs may induce toxicity in invertebrates by altering the expression of genes pertaining to physiological activities, oxidative stress or damage and neurological functions [73].

However, the impact of exposure time on these toxicity parameters needs to be explored further. In addition, it is challenging to fully address how and to what extent the structural properties and concentration of NPPs contribute to their toxicity and related mechanisms.

Our study (Fig. S4) may also indicate the need for a considerably larger replication and more prolonged exposure periods. Here, invertebrates' feeding rates were not enhanced by the improved nutritional quality of the SNPPs pre-exposed leaf litter. This might be due to their inability to assimilate the nutrient locked in the leaf litter efficiently. In another study, when the *Echinogammarus marinus* were fed with algal feed spiked with a low dose of polystyrene microbeads (8 µm, ~0.9, 9 and 99 micro-plastics g^{-1}) for 35 days, the feeding rates of the animals were not impacted [74] corroborating our findings. In addition, the feeding behaviour, mortality, or mobility of *Gammarus duebeni* (freshwater Amphipod) were not affected when fed with plants (*Lemna minor*) grown in a suspension containing polyethylene microplastics (10-45µm; 50,000 microplastics mL^{-1}); nonetheless, plastic particles were found in the animals' gut [75]. In a recent field study, the authors demonstrated the presence of microplastic particles (up to 0.14 mg $tissue^{-1}$) inside the stream invertebrates (Baetidae, Heptageniidae and Hyderopsychidae) guts and tissues [76], confirming that plastics are likely to be transferred across the freshwater food webs. The transfer of NPPs between trophic levels has been verified mainly in marine ecosystems [77] but is still poorly understood in freshwaters.

The interplay between invertebrate NPPs stress response and functions is still in its infancy; therefore, further studies are critical for deepening our understanding of stress-coping mechanisms. There is a growing body of evidence suggesting that the animals fed with NPPs are able to elicit strong cellular responses without impacting their feeding behaviour. For instance, when the marine Branchiopoda *Artemia fransciscana* was exposed to amino-modified polystyrene (0-10 µg mL^{-1}; 50 nm), oxidative stress was triggered after 48 h [78].

Similarly, oxidative stress-induced damages were observed in the marine rotifer *Brachionus koreanus* when exposed to polystyrene (50 and 500 nm) for 24 hrs without impacting the feeding behaviour [79].

Conclusions

Overall our study provides novel information that environmentally relevant concentrations of nanoplastics may pose a risk to basal trophic levels of brown food webs, mainly aquatic hyphomycetes and their functioning in the stream by affecting sporulation, abundance and litter decomposition ability. Our investigation also implies that aquatic fungal sporulation could be used as an indicator in assessing the impact of nanoplastics in freshwaters. Furthermore, NPPs size (100 and 1000 nm) affected the leaf litter's nutritional profiles. However, these pre-exposed leaves did not impact the feeding behaviour of invertebrates (*E. meridionalis*). Given the underlying complexity of NPPs interactions within the ecosystems, future animal feeding experiments should consider longer exposure periods and diverse functional feeding groups of invertebrates. Moreover, in-depth studies should be directed to understand the mechanism of nanoplastics interaction with aquatic decomposers and natural organic matter in freshwaters environments.

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Legend to Figures:

Fig. 1 SEM images of 100 nm (a) and 1000 nm (b) plastic fractions at 100 mg L^{-1} in the stock suspension.

Fig. 2 Fourier-Transform Infrared (FTIR) spectroscopy of the suspensions comprising increasing concentrations (0 to 25 µg L^{-1}) of small sized nanoplastics (a) and large sized nanoplastics (b) and fine particulate organic matter (FPOM) after leaf litter decomposition assay.

Fig. 3 High resolution-field emission-scanning electron microscope (HR-FE-SEM) of the suspensions comprising NPPs at various concentrations and sizes and fine particulate organic matter (FPOM) after leaf litter decomposition assay. The HR-FE-SEM image at 0 µg L^{-1} (a); small sized nanoplastics at 0.25 (b) 2.5 (c), 25 µg L^{-1} (d) and large sized nanoplastics at 0.25 (e) 2.5 (f), 25 µg L^{-1} (g). The arrows indicate agglomeration.

Fig. 4 Leaf mass loss (a) and fungal sporulation (b) when exposed to different concentrations and sizes of nanoplastic particles; all the concentrations and sizes were significantly different ($p<0.05$). Different letters indicate significant differences between nanoparticle concentrations. (mean ± standard error; n = 4)

Fig. 5 Multidimensional scaling (MDS) plots of the abundance of aquatic hyphomycetes (a) and profiles of fatty acids (b) and carbohydrates (alcohol insoluble fraction; AIR) (c) in the control microcosm and after exposures to nanoplastic particle concentration and sizes. The stress was < 0.20 for the plot. Small nanoplastic particles (SNPPs) and large nanoplastic particles (LNPPs).

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Fig 1

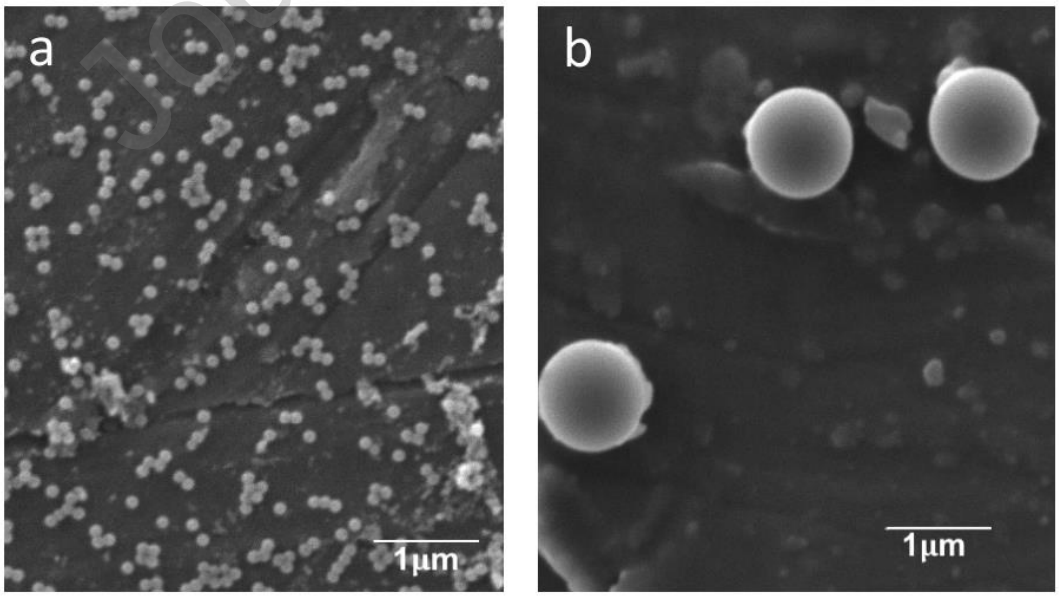


Fig 2

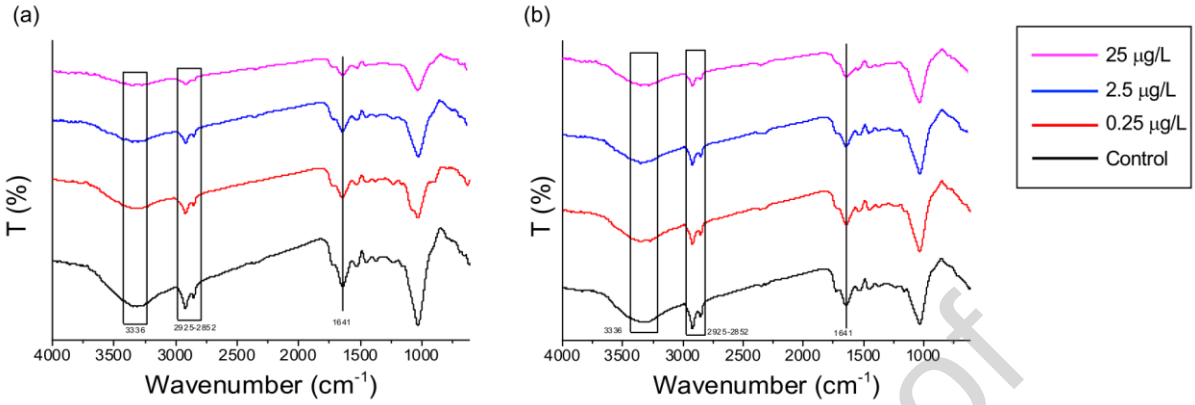


Fig 3

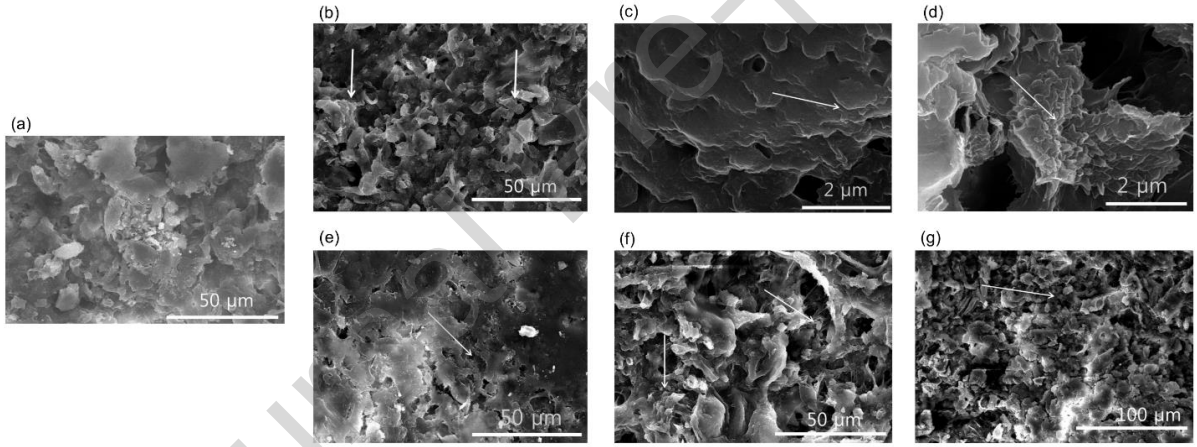


Fig 4

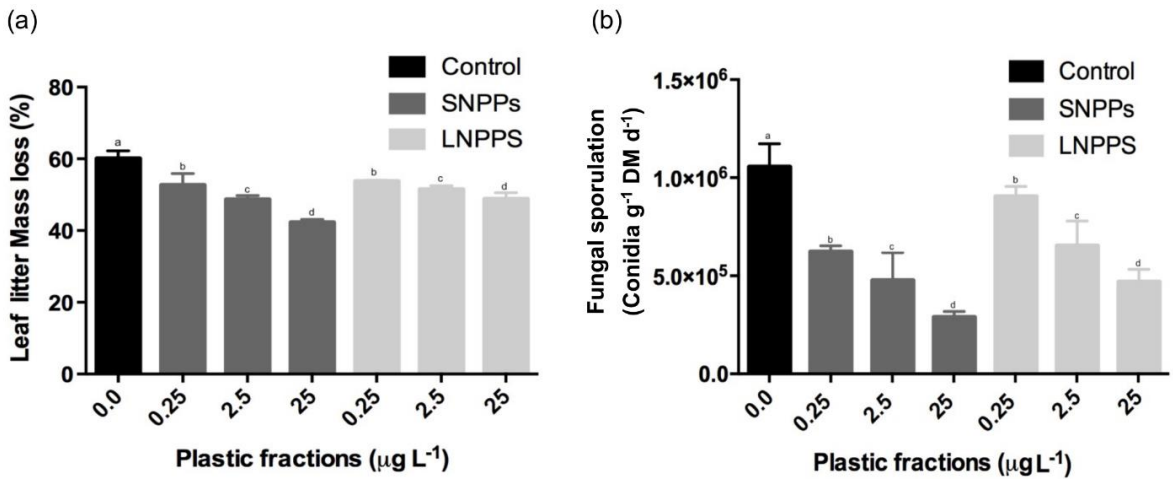
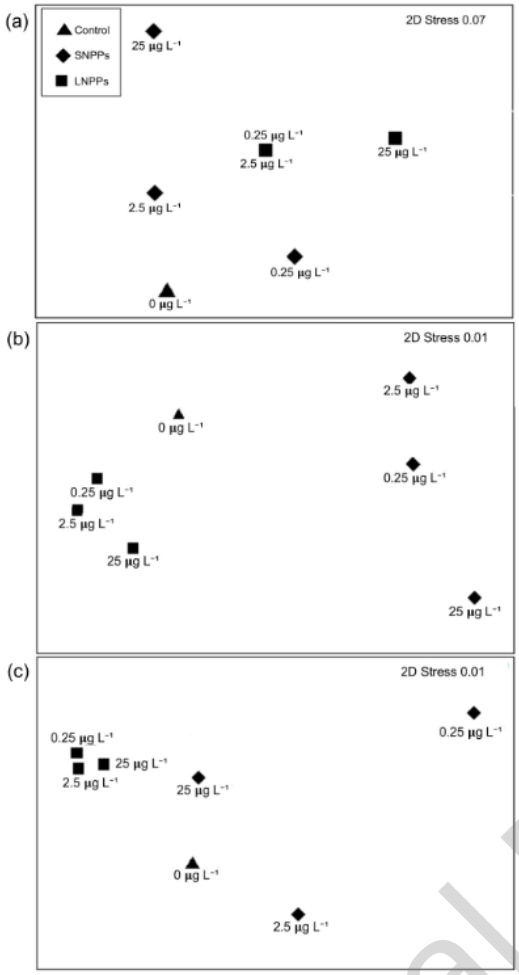


Fig 5



Graphical abstract

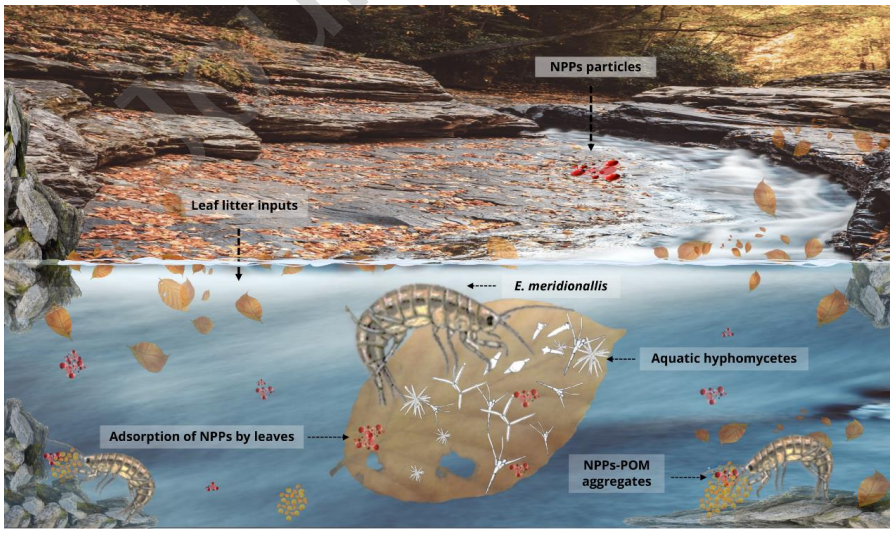


Table 1: Physico-chemical characteristics of the stream water from Lousã and Redinha

Physical characteristics	Lousã	Redinha
Conductivity (µs/cm)	34.1	311
Dissolved oxygen (mg/L)	10.4	5.91
pH	7.0	6.79
Temperature (ºC)	9.2	16.3
Chemical characteristics		
Silicon (µg/L)	455.8	1464.5
Ammonia (µg N-NH_3/L)	68.8	16.7
Total nitrogen (µg/L)	348.8	1818.9
Total dissolved carbon (mg/L)	5.565	35.272

Table 2: Elemental composition of the suspensions after leaf litter decomposition assay

	Control	SNPPs			LNPPs		
Concentrations (µg/L)	0.00	0.25	2.5	25	0.25	2.5	25
Elements Wt (%)							
Carbon	36.57	61.89	70.6	83.53	63.02	68.36	69.31
Oxygen	24.78	17.52	23.87	13.82	34.69	29.71	28.33
Silicon	25.15	12.21	2.51	0.68	1.77	0.59	1.10
Aluminium	1.44	4.25	0.90	0.48	0.53	0.44	0.59

Table 3. Mean species abundance and species richness of aquatic hyphomycetes community (mean ± SE, n=4)

Species	Control (µg L^{-1})	SNNPs (µg L^{-1})			LNNPs (µg L^{-1})		
	0.00	0.25	2.5	25	0.25	2.5	25
Alatospora acuminata Ingold	4.00±2.40	10.75±1.89	10.50±3.38	2.75±1.49	8.50±4.29	8.50±4.29	1.50±1.19
Alatospora pulchella Marvanová	15.00±4.92	7.50±2.06	13.75±7.23	2.50±1.89	12.00±3.03	12.00±3.01	7.75±2.46
Anguillospora filiformis Greath	2.00±0.82	3.75±1.11	2.00±0.91	0.25±0.25	6.25±2.72	6.25±2.72	5.25±2.01
Anguillospora longissima (Sacc. & Syd.) Ingold	0	0.50±0.50	0	1.00±0.71	0.25±0.25	0.25±0.25	0.25±0.25
Articulospora tetracladia Ingold	98.50±3.66	63.5±4.73	51.5±5.75	38.25±8.98	53.5±6.66	45.50±6.76	49.00±17.44
Campylospora chaetocladia Ranzoni	0	1.00±0.71	0.75±0.75	2.00±0.91	2.00±1.15	2.00±1.15	0
Clavariopsis aquatica De Wild	0.50±0.29	0.25±0.25	0.25±0.25	0.75±0.75	1.00±1.00	1.00±1.00	3.5±1.66
Clavatospora longibrachiata (Ingold) Sv. Nilsson	0	0.75±0.25	1.00±0.41	0.25±0.75	0	0	0
Culicidospora aquatica R.H Petersen	0.25±0.25	0	0	0	1.25±0.75	1.25±0.75	1.00±0.58
Dimorphospora foliicola Tubaki	48.75±8.11	36.25±3.90	57.25±11.83	46.25±9.01	52.00±14.18	46.75±14.04	27.00±5.28
Flagellospora curvula Ingold	22.00±22.27	66.00±6.28	55.50±6.60	92.00±9.89	67.25±6.49	63.00±4.32	79.75±5.85
Heliscella stellata Ingold *& V.J. Cox*	0.25±0.25	0	0	0	0	0	0
Lemonneira aquatic De Wild	0.25±0.25	0	0	0	0	0	0
Lemonneira terrestris	0.50±0.50	1.50±0.70	0	0	1.00±0.41	1.00±0.41	1.00±0.70

Tubaki							
Lunulospora curvula Ingold	3.75±0.95	2.50±0.29	2.00±0.58	2.25±0.75	3.00±0.91	3.00±0.91	4.50±0.96
Margaritospora aquatica Ingold / *Goniopila monticola (Dyko) Marvanová & Descals*	1.00±0.41	3.00±2.35	0	0.75±0.48	1.50±0.65	1.50±0.65	4.25±0.48
Neonectria lugdunensis Sacc. & Therry	1.75±0.25	1.75±1.03	0.50±0.5	0.75±0.25	0.75±0.25	0.75±0.25	0.75±0.48
Taeniospora gracilis Marvanová	0	0	0	0	0.25±0.25	0.25±0.25	0
Tetrachaetum elegans Ingold	5.00±2.04	4.50±0.29	3.5±1.85	6.00±0.91	5.25±2.34	5.25±2.29	6.00±1.78
Tetracladium marchalianum De Wild	0	0	0	0	0.75±0.75	0.75±0.75	0
Tricelosporous acuminatus Nawawi	0	1.50±1.50	0.50±0.5	0	0	0	0
Tricelosporous monosporous Ingold	0.75±0.75	0.75±0.75	1.75±1.75	5.00±1.29	0	0	0
Tricladium chaetocladium Ingold	3.00±0.41	1.25±0.49	2.75±1.03	5.25±0.85	7.00±2.35	7.00±2.35	10.00±1.78
Tricladium splendens Ingold	0	1.50±0.87	1.50±0.65	0	0.50±0.29	0.50±0.29	0.75±0.25
Tricladium terrestre Ingold	0.75±0.75	0	0.25±0.25	1.00±0.41	0	0	0
Varicosporium elodeae W. Kegel	0.75±0.75	0	0	0	0	0	0.50±0.29
Species Richness	19	19	17	17	19	17	17

Table 4. Leaf litter fatty acid profiles after exposures to nanoplastic particles concentration and size types in µg of fatty acids per mg of wet weight (mean ± SE, n=3).

	Control			SNNPs			LNNPs	
	0.00 µg/L		0.25 µg/L	2.5 µg/L	25 µg/L	0.25 µg/L	2.5 µg/L	25 µg/L
C12:0	3.05±0.74		6.88±0.96	11.79±7.23	9.09±0.71	2.57±1.86	2.99±1.76	3.99±1.99
C14:0	32.77±22.86		22.74±4.13	26.13±13.67	78.96±55.63	11.95±6.08	12.19±3.45	16.60±6.16
C15:0	0.73±0.23		1.40±0.55	2.39±1.67	1.79±0.62	1.44±0.39	1.63±0.37	1.88±0.06
C16:0	112.60±52.86		139.86±24.94	129.97±57.43	175.96±39.95	156.80±84.03	164.29±99.01	130.40±52.31
C17:0	1.99±0.45		2.59±0.37	2.06±0.44	2.34±0.45	1.82±0.61	1.60±0.55	2.53±0.91
C18:0	37.44±26.81		17.80±7.62	20.09±11.02	62.39±18.55	62.65±47.31	102.63±53.58	161.50±37.81
C20:0	24.33±6.89		24.16±9.79	20.54±11.81	20.32±6.42	31.13±9.25	29.88±6.40	31.75±9.92
C22:0	55.36±19.61		27.26±12.16	25.85±13.70	33.64±13.22	85.96±26.09	86.77±27.29	64.55±19.83
C23:0	35.02±12.73		11.03±1.99	10.97±3.87	14.30±5.40	46.34±8.65	57.54±14.40	51.33±13.13
C24:0	47.39±18.75		21.33±3.25	22.68±11.13	24.62±10.04	57.11±14.66	93.77±26.95	63.82±15.32
SFA	**350.68**		**275.03**	**272.47**	**423.41**	**457.76**	**553.28**	**528.35**
C14:1	2.88±0.60		5.86±2.74	12.84±10.53	ND	3.67±1.77	4.08±1.29	4.41±1.41
C16:1	2.62±0.58		5.57±3.47	2.65±0.71	32.37±3.34	2.65±1.26	2.86±1.67	3.42±1.04
MUFA	**5.51**		**11.43**	**15.49**	**32.37**	**6.33**	**6.94**	**7.83**

C18:2n6c	10.59±1.98			32.70±14.86	21.86±10.90	21.92±7.89		13.74±5.29	9.75±3.13	17.93±6.61
C18:3n3	6.66±1.60			19.16±1.77	19.49±9.88	15.88±5.39		6.43±2.85	6.76±2.76	9.91±5.53
C20:2	6.60±2.23			3.37±1.19	4.39±2.37	4.22±0.64		9.61±2.03	9.31±3.03	9.57±3.04
PUFA	**23.86**			**55.23**	**45.75**	**42.01**		**29.78**	**25.82**	**37.41**
N	15			15	15	14		15	15	15
Wet weight (mg)	48.55±0.23			49.00±0.00	48.07±0.26	48.67±0.44		48.90±0.52	48.07±0.03	48.10±1.07

Small nanoplastic particles (SNPPs), large nanoplastic particles (LNPPs), Not detected (ND) saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).

Table 5. Leaf litter carbohydrate profiles after exposures to nanoplastic particles concentration and size types in µg of carbohydrates per mg of wet weight (mean ± SE, n=3).

Carbohydrates	Control	SNPPs			LNPPs		
	0.00 µg/L	0.25 µg/L	2.5 µg/L	25 µg/L	0.25 µg/L	2.5 µg/L	25 µg/L
Wet weight (mg)	46.48±1.60	48.57±0.52	48.93±0.82	48.73±0.15	46.18±2.84	48.87±0.59	49.10±0.06
AIR yield (µg AIR/mg ww)	248.82	191.74	248.80	269.34	242.45	250.88	224.75
% AIR in ww	24.98	19.17	24.82	26.94	24.20	25.07	22.48
AIR sugars (µg/mg ww)							
Ara	10.30±0.95	8.24±0.28	7.63±3.81	9.91±1.38	4.81±2.63	8.35±0.63	6.37±3.35
Gal	2.16±0.16	0.46±0.77	1.36±0.74	1.53±0.18	1.56±0.89	2.53±0.22	1.68±0.86
Glc	19.12±3.88	8.22±3.20	6.29±5.54	21.83±2.83	21.90±0.96	31.47±0.96	18.49±9.90
Xyl	16.07±1.44	11.90±2.78	14.39±6.97	16.31±1.30	10.36±5.33	15.26±1.37	9.02±4.68
Total sugars (µg/mg ww)	47.66	28.82	29.67	49.59	38.63	57.61	35.56
SA sugars (µg/mg ww)							
Ara	0.04±0.01	0.05±0.01	0.03±0.01	0.09±0.05	0.04±0.02	0.06±0.01	0.05±0.03
Gal	0.08±0.02	0.10±0.02	0.07±0.04	0.16±0.10	0.06±0.04	0.09±0.02	0.16±0.01
Glc	0.22±0.06	0.23±0.04	0.16±0.09	0.42±0.32	0.14±0.07	0.05±0.03	0.23±0.05
Xyl	0.29±0.06	0.24±0.03	0.18±0.0	0.35±0.18	0.26±0.17	0.36±0.04	0.34±0.04
Total sugars (µg/mg ww)	0.63	0.62	0.44	1.01	0.50	0.56	0.79

Small nanoplastic particles (SNPPs), large nanoplastic particles (LNPPs), alcohol insoluble residue (AIR), arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), soluble in alcohol (SA)

CRediT authorship contribution statement

Sahadevan Seena: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Irene B. Gutiérrez:** Methodology, Investigation, Formal analysis, Writing - review and editing. **Juliana Barros:** Methodology, Investigation, Formal analysis, Writing - review and editing. **Cláudia Nunes:** Methodology, Investigation,

Formal analysis, review and editing. **João Carlos Marques:** Writing - review and editing. **Santosh Kumar:** Methodology, Investigation, Formal analysis, Writing - review and editing. **Ana M.M. Gonçalves:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Impacts of nanoplastics (NPPs) on leaf decomposers and food quality were assessed
- NPPs (0-25 µg L^{-1}) impacted leaf mass loss, fungal sporulation and abundance
- NPPs (100 nm) had a pronounced impact on food quality
- Food quality did not impact the feeding behaviour of invertebrates
- Basal trophic levels are more impacted by low concentrations of nanoplastics