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Microplastics in the Amur tiger's habitat: Occurrence, characteristics, and risk assessment

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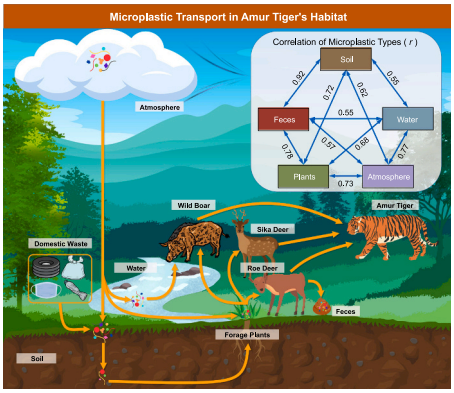
HIGHLIGHTS

- With increasing soil sampling depth, the particle size of microplastics (MPs) significantly decreases.
- Polyvinyl chloride was the most common type of MPs found in wildlife feces.
- Certain sample types exhibited strong correlations in MP polymer type distributions.
- Pollution Load Index indicates that the MP pollution is generally at a low level, but exhibits spatial heterogeneity.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) are emerging environmental pollutants that pose a significant threat to wildlife within forest ecosystems. However, the quantity and types of MPs in wildlife forest habitats remain unclear. This study is the first to assess the distribution of MPs in the Amur tiger habitat of northeast China. Our results showed that MPs were detected in soil, water, atmosphere, forage plants, and ungulate and top predator feces within the forest ecosystem, respectively. The average diameter of all detected MPs was 44.99 ± 34.80μm. The predominant polymers found in the samples were polyamide, polyvinyl chloride, and polyurethane. Certain sample types shared similar MP polymer type distributions, indicating potential links in their sources and transfer pathways.

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Consequently, these findings provide some new insights on the new pollution problem in Amur tiger forest habitats and prompt us to consider how to control and manage the MPs pollution sources in the tiger conservation.

1. Introduction

The widespread use of plastic products is a hallmark of modern convenience, although improper use and disposal of plastics have evolved into a formidable pollution crisis [26]. Plastic waste discharged into the environment is broken down into tiny fragments by various physical, chemical, and biological processes. These plastic particles, known as microplastics (MPs) [87], are emerging pollutants and particularly problematic due to wide dispersion, persistence, and accumulation in the environment, in addition to resistance to degradation [86]. Over the past decade, there have been increasing reports of the detection of MPs in the soil, water, and atmosphere with dispersion to remote islands, plateaus, seabeds, and even polar regions [10,107,36,47,5,59,88,91].

The widespread distribution of MPs in the environment is concerning, but equally alarming is the growing body of evidence indicating that MP exposure can have a range of negative effects on organisms, such as cardiac toxicity [96], endocrine disruption [4], gastrointestinal inflammation [119], reproductive disorders [70], and even death [2]. The hydrophobicity, small particle size, and high surface area of MPs make them more likely to adsorb organic pollutants (e.g., Polychlorinated biphenyls, malachite green, etc.) [23,50,82], inorganic pollutants (e.g., Fe, Al, Cr^{6+}, etc.) [78,81,90], or release additives from the plastic itself [25]. The combined toxicity of MPs and chemical pollutants may pose even more severe adverse effects on organisms [15].

However, the harmful effects of MPs are not limited to individual organisms; they can also migrate and accumulate across different trophic levels through the food chain, posing a threat to entire ecosystems. Studies have shown that MPs can be absorbed by crops and enter their edible parts, thereby entering animals' bodies through the food chain [42,58]. Consequently, the transfer of MPs along terrestrial food chains is crucial to clarify the exposure routes of MPs. For instance, MPs in garden soil can transfer along the soil-earthworm-chicken food chain and accumulate in chicken manure and gizzards [57]. In marine ecosystems, some researchers have also reported empirical evidence of the nutritional transfer of MPs from fish to marine top predators [67]. Although the potential threat of MPs to marine top predators has been confirmed, this danger is not limited to marine ecosystems. Top predators in forests may also face similar environmental risks, particularly when their habitats are potentially contaminated by MPs.

The Amur tiger (*Panthera tigris altaica*) is global endangered top predators in northeast forests, playing a crucial role in maintaining food web structure and ecosystem stability [73]. As a flagship and umbrella species in its community, it is often one of the most focused groups in biodiversity conservation [49]. The Amur tiger has long been a hotspot species in wildlife ecology research and serves as an important indicator of forest ecosystem quality and service functions [94]. The population status of large carnivores and related prey in this area is continuously monitored and improved land management strategies and conservation efforts [39,68,97]. At the same time, the health status of the Amur tiger, including factors such as viral infections and the intake of environmental pollutants, has also attracted the attention of many researchers [93,99]. Nevertheless, to date, no studies have systematically explored the impact of MPs on the ecological environment of this region, nor assessed the potential threats to the Amur tiger. There is limited research on MPs in forest ecosystems or wildlife habitats [13,43]. Some researchers have detected MPs in sediments from wildlife reserves in Jakarta, Indonesia [14] and in animal feces from the Qinling Nature Reserve in Shaanxi Province, China [98], but no comprehensive reports on MP pollution across various environmental media and food chain nodes have been published. As a top predator at the top of the food chain, the Amur tiger is a flagship species that represents biological diversity, genetic diversity, and ecosystem diversity, making it of significant conservation and research value. This study aims to provide a comprehensive analysis of MP levels in various environmental media, plants, and animal feces within the Amur tiger's habitat, offering new perspectives for MP investigations in forest ecosystems.

Therefore, the aims of the present study were to 1) clarify the abundances, types, and spatial distribution of MPs in different environmental media within the forest ecological system of the tiger habitats; 2) exploring potential evidence of MP flow across the food chains, and 3) assess current levels of MPs in the tiger habitats. The results of this study will provide crucial data to clarify the distribution of MPs in forest ecosystems and aid local authorities to formulate strategies to limit the effects of MPs and develop appropriate conservation policies for the Amur tiger habitats.

2. Methods and materials

2.1. Study site

This study was conducted in the Amur tiger habitat of northeast forest habitat (42°38'–44°14'N, 129°05'–131°18'E), located in northeastern Asia (Fig. 1). The terrain of the area generally slopes from west to east. The average annual precipitation is 400–500 mm, with 70 % occurring between June and August. Autumn experiences less rainfall, and spring and winter are even drier. The climate becomes increasingly arid from east to west, as the eastern region has a temperate monsoon climate with hot, rainy summers and cold, dry winters, while the western region has a temperate continental climate characterized by cold winters, hot summers, large annual temperature variations, concentrated rainfall, and distinct seasonal temperature changes, but less overall annual precipitation and stronger continentality. Major rivers include the Hunchun, Tumen, Gaya, and Suifen. The zonal soil is primarily dark brown, with intrazonal varieties, including marsh, alluvial, albic, meadow, and peat.

The study area is predominantly forested, covering an area of 13,600 km^2, with a timber volume of 200 million m^3 and a forest coverage rate of 96.6 %. The main tree species include Mongolian oak (*Quercus mongolica*), fir (*Abies* spp.), Korean pine (*Pinus koraiensis*), birch (*Betula* spp.), larch (*Larix* spp.), spruce (*Picea* spp.), and poplar (*Populus* spp.), forming primarily secondary forests. Deciduous, coniferous, and mixed coniferous-deciduous forests account for 76.1 %, 16.6 %, and 7.3 %, respectively, of the forest area.

We conducted field surveys and, based on the analysis of infrared camera data, identified 10 sampling points with high frequencies of Amur tiger activity. At these sampling points, samples of soil, water, atmosphere, forage plants and wildlife feces, including tigers and ungulate prey, were collected from designated sampling points in the spring (March) and autumn (September) of 2023 (Fig. 1). It should be noted that in the spring, we collected soil samples at the 10 sampling points. Considering the uneven distribution of water bodies, we temporarily increased the number of water sampling points to 19 in the spring. Due to the limited span of the study area, air sampling was conducted only at 4 sampling points in the spring. As there was no vegetation growth in the study area during the spring, plant samples were only collected in the autumn. Due to flooding in the study area during the autumn, some roads were destroyed, and as a result, samples from all types could only be collected from 6 out of the 10 sampling points. Animal feces were collected during surveys, and specific

sampling points were not designated for this purpose. Details of the samples are shown in Table S1.

2.2. Sample collection and pretreatment

2.2.1. Soil

Surface (0–5 cm) and deep (25–30 cm) soil samples (200 g per sample) were collected using a stainless steel shovel. The collected soil was sieved through a 5-mm stainless steel standard sieve to remove branches, stones, and other debris larger than 5 mm. Each soil sample was thoroughly mixed, and dried at 60°C in an electric blast drying oven for 24 h. The dried soil was mixed uniformly, and 10 g was accurately weighed into a beaker, mixed with 30 mL of $ZnCl_2$ (1.7–1.8 kg/L) solution for 10 min by stirring [75], and left to stand for 24 h to allow the supernatant to naturally separate from the precipitate. Transfer the supernatant to a clean glass beaker. To thoroughly extract MPs, repeat this extraction process three times. Mix the supernatant with 30 mL of H_2O_2 (30 %) to digest impurities [52]. After standing for 24 h, the supernatant was collected [108].

2.2.2. Water

At each sampling site, 1 L of surface water from the river was collected using a clean 1 L aluminum bottle and filtered through a 5 mm stainless steel standard sieve to remove non-plastic impurities. The aluminum bottles were gently shaken to distribute MPs evenly within the sample. All water samples were transferred to glass beakers. Ultrapure water was used to rinse the sample bottles to ensure no MPs adhered to the container walls, and repeat this process three times. An equal amount of H_2O_2 was then added to the glass beakers to digest impurities. After standing for 24 h, the supernatant was collected [60, 84].

2.2.3. Atmosphere

MPs in the atmosphere were collected using a portable negative pressure automatic gas sampler (Model CFZ22A, Huakuang Machinery Equipment Co., Ltd., Jining, Shandong, China), a replaceable membrane filter (Model DLJ-S50mm, Delvstlab Co., Ltd., Haining, Zhejiang, China), and 0.22 μm cellulose acetate filters (Model 50 mm, Xingya Purification Materials Factory, Shanghai, China). Sampling was conducted at each site with a flow rate of 22 L/min for 1 h, resulting in an atmospheric flux of 1.32 m^3 per sample. A clean replaceable membrane filter was used for each sampling to prevent sample cross-contamination. After sampling, the filter was carefully removed from the replaceable membrane filter holder and fully immersed in 30 mL of H_2O_2 (30 %) for ultrasonic treatment for 30 min, ensuring the MPs were fully transferred to the H_2O_2 solution. The filter was then removed and rinsed with 10 mL of H_2O_2 to prevent MPs from adhering to the filter surface, thereby minimizing sample loss. The sample solution containing MPs was left to stand for 24 h to fully digest organic matter before proceeding to the next stage of detection and analysis [123,20,38].

2.2.4. Feces

Fresh feces of Amur tigers, sika deer (*Cervus nippon*), roe deer (*Capreolus pygargus*), and wild boar (*Sus scrofa*) were collected using clean stainless steel tweezers and metal spoons (50 g per sample). During sample collection, the samplers stood downwind to avoid contamination from atmospheric deposition. All feces were species-identified and matched to the corresponding animals (the species identification process is detailed in Text S1). To eliminate the effect of moisture content on the fecal weight, we dried the feces at 50°C for 72 h until a constant weight was achieved. To avoid further fragmentation of MPs due to physical factors such as grinding and mixing, we selected feces with intact shapes for weighing (e.g., for sika deer, whole spherical feces with regular shapes were chosen). Approximately 4 g of fecal sample (details of the samples are shown in Table S1) was weighed and placed into a glass beaker. Concentrated HNO_3 (68 %) in three times the weight of the sample was used for digestion at room temperature to initially remove animal proteins and residual plant fibers from the feces. After standing for 24 h, the beaker was heated to 85°C and digested for 2 h to maximize the removal of substances that could interfere with MP detection, and

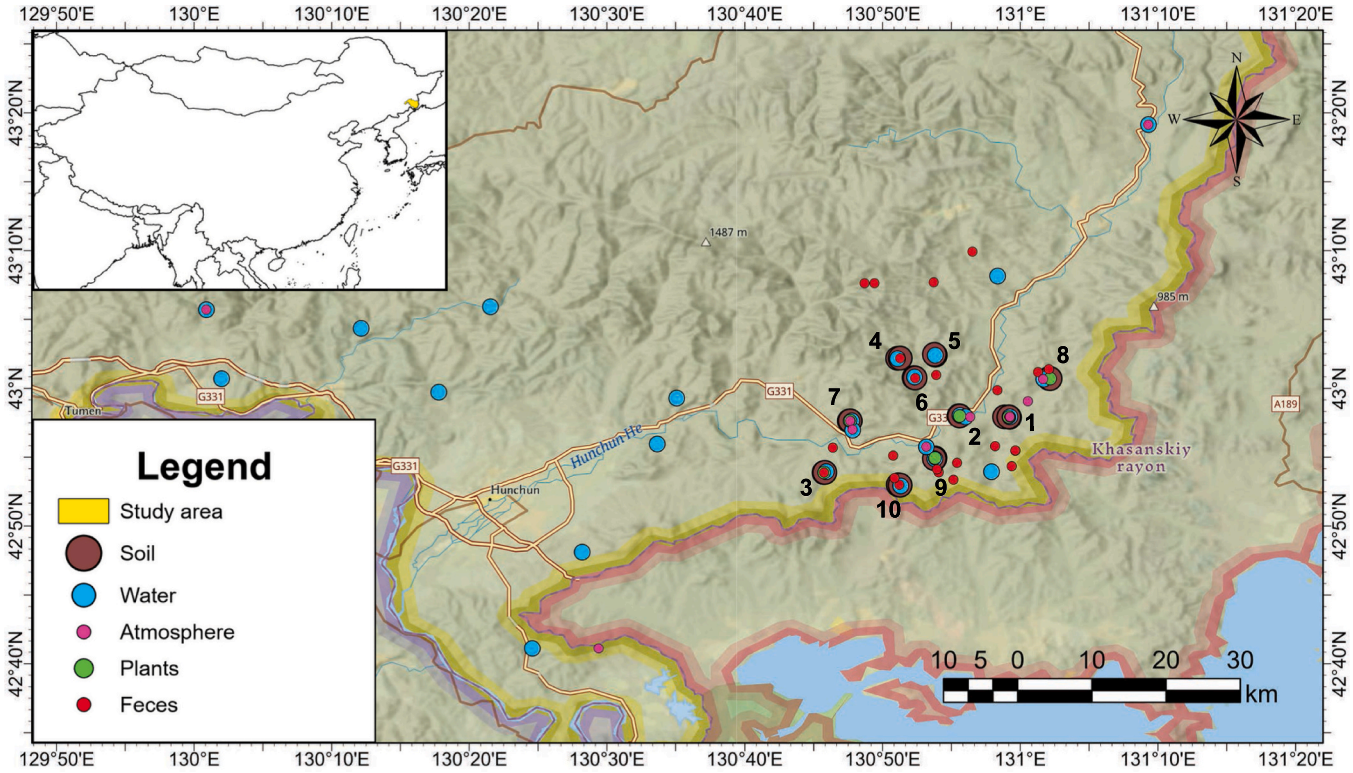

Fig. 1. Sampling sites of different media in tiger habitat.

then cooled to room temperature [53,55,80].

In the preliminary trial of this study, we found that plants and animal feces were difficult to completely digest with H_2O_2 or KOH. However, heated HNO_3 was able to fully dissolve all visible organic matter, significantly improving the efficiency of sample processing and the accuracy of detection. However, the use of HNO_3 for digestion also has certain limitations. While HNO_3 effectively digests organic matter at both room and elevated temperatures, some studies have reported that it may dissolve PA[65], potentially leading to an underestimation of MP levels.

2.2.5. Forage plants

For the forage plants, we selected three representative plant species preferred by herbivores in the reserve, based on field observations (evidence of grazing), imagery data (infrared cameras), and related dietary studies [120,44,64]. These plants were categorized into two groups based on their relative height: Sedge grass (*Carex meyeriana*) (low--layer), Amur lilac (*Syringa reticulata var. amurensis*) (high-layer), and Manchurian striped maple (*Acer tegmentosum*) (high-layer). At each sampling site, we collected one sample (50 g per sample) each from the low and high-layer plants. Due to the absence of Amur lilac at some sites, we substituted it with Manchurian striped maple.

During the collection of forage plant samples, for high-layer plants, we sampled the leaves that herbivores preferred to eat; for low-layer plants, both leaves and roots were collected (soil attached to the roots was carefully cleaned to avoid interference with MP testing). These two parts are both likely to be consumed by herbivores. We minimized the shaking of plant leaves during sampling to avoid losing MPs from the surface. The drying and digestion processes for the forage plants samples were the same as for the animal feces samples. During digestion, the entire leaf, including the surface MPs, was selected to represent the level of MPs herbivores might ingest through plants consumption. Each digested sample weighs about 3.3 g. It is important to note that, in the study area, no leaves were growing during the spring sampling, so forage plant samples were only collected in the autumn (details of the samples are shown in Table S1).

2.2.6. Sample preservation

All samples were wrapped in aluminum foil, sealed in resealable bags, and frozen at −18°C until laboratory processing. After processing, all liquid samples containing MPs were vacuum filtered onto 0.22-μm cellulose acetate filters, which were then sealed for analysis.

2.3. Sample detection and analysis

The filters were placed in coarse glass tube containing anhydrous ethanol solution and subjected to ultrasonic treatment for 2 h. After removing the filter membrane, nitrogen gas was used to purge the ethanol. Once the ethanol solution was concentrated to approximately 200 μL, the sample was dropped onto a Kevley slide. After the ethanol evaporated, the samples were scanned for MPs using a laser direct infrared (LDIR) imaging system (Model 8700; Agilent Technologies, Inc., Santa Clara, CA, USA) with the following parameters: spectral resolution, 4 cm^{-1}; number of scans, 64; wavenumber range, 1000–1800 cm^{-1}; and particle size, 20–500 μm [55,85]. The LDIR imaging system was calibrated before use to ensure accurate detection of all types of MPs included in the spectral database. The results were compared against a reference spectral library, and matches with a similarity of ≥ 0.65 were identified as MPs[12,16].

2.4. Morphologies of MPs

Based on the LDIR imaging system data, MPs were categorized according to the roundness ratio as particles (pellet) (≥0.6) or non-particles (<0.6). Non-particle MPs were further classified as fibers (≥3) or fragments (<3) based on the aspect ratio. In this classification scheme, fragments encompass all irregular shapes other than particle-like and fibrous forms. [101].

2.5. Quality control

To minimize contamination, cotton clothing, latex gloves, and cotton masks were worn during field sampling, analysis, and solution preparation. These measures help reduce MP contamination from the operators and the environment. All distilled water and chemical reagents were filtered through 0.22-μm cellulose acetate filters to remove any potential fine particles and contaminants. Containers and equipment made of stainless steel, aluminum, or glass were washed with filtered ultrapure water three times before use to prevent cross-contamination. Samples were covered with aluminum foil and sealed during processing and standing, with exposure to air limited to less than 10 min to reduce contamination from airborne MPs. Testing of blank controls showed no MP contamination, confirming the reliability of the results. We measured the spiking recovery rates for three representative samples under two main treatment methods: H_2O_2 digestion (for soil samples) and HNO_3 digestion (for plant and fecal samples). The spiking recovery rate tests included six common types of MPs: polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyamide (PA). The detailed testing process is provided in Text S2. The recovery rate results showed that under different digestion methods, the recovery rates for most MPs were above 80 %, except for PP in plants (74.29 %) and PE in feces (78.13 %). In addition, 30 mL of pure water was used as a blank control and processed using the same treatment method as for the soil and feces, with three repetitions to detect potential MP contamination in the laboratory. No MP contamination was found on the filter membrane of the blank control samples (average MP count < 1 per filter membrane), confirming the reliability of the experiment.

2.6. Calculation of the MP abundance ratio between different media

Since the MP abundance units in water bodies and the atmosphere cannot be directly compared with those in plant and fecal samples, we selected the average MP abundance in soil as the representative of environmental MP abundance for the calculation of the MP abundance ratio.

To calculate the MP abundance ratio between plant samples and soil, we used the following formula:

$$CR_{ps} = \frac{C_p}{C_s} \quad (1)$$

Where CR_{ps} is the ratio of MP abundance in plant samples to that in soil, C_p is the MP abundance in plant samples, and C_s is the MP abundance in soil.

For the MP abundance ratio between fecal samples and soil, we used the following formula:

$$CR_{fs} = \frac{C_f}{C_s} \quad (2)$$

Where CR_{fs} is the ratio of MP abundance in fecal samples to that in soil, and C_f is the MP abundance in fecal samples.

The calculation of the plastic abundance ratio is based on the study by Lwanga et al. [57] on MP transfer in terrestrial food chains[57], and also partially references the calculation methods for BAF (Bioaccumulation Factor) and BMF (Biomagnification Factor)[6,62].

2.7. Calculation of pollution load index (PLI)

The PLI method not only evaluates the pollution level at a specific point but also assesses the overall pollution level of a region. It is widely used to evaluate MP pollution in soil or sediments[102]. PLI is

calculated using the following formulas:

$$CF_i = \frac{C_i}{C_{oi}} \quad (3)$$

$$PLI_i = \sqrt{CF_i} \quad (4)$$

$$PLI_{Zone} = \sqrt[n]{PLI_1 \times PLI_2 \times PLI_3 \cdots \times PLI_n} \quad (5)$$

where CF_i is the concentration factor of MP at sampling point i, C_i is the MP concentration at sampling point i, and C_{oi} is the baseline MP abundance, which theoretically refers to MP concentration in the early stages of the plastic industry. In this study, C_{oi} is defined as the lowest MP concentration among all sampling points.

PLI_i is the PLI at sampling point i, and PLI_{Zone} represents the PLI for the entire study area, taking into account the number of sampling points. The pollution assessment levels are categorized as follows based on the PLI values: Level I (<10), Level II (10−20), Level III (20−30), and Level IV (>30)[76].

2.8. Statistical analysis

Data were expressed as mean ± standard deviation (SD). The MP abundance in soil, plant, and animal feces samples is expressed as number per gram (n/g), in water samples as number per liter (n/L), and in air samples as number per cubic meter (n/m^3). The calculations and graphical representations of MP pollution characteristics were performed using GraphPad Prism. The Kolmogorov-Smirnov test was used to determine whether the dataset follows a normal distribution. One-way analysis of variance (ANOVA) was used to determine differences in MP abundance or characteristics among different sample categories. A value of $p < 0.05$ was considered statistically significant, while a value of $p < 0.01$ was considered highly significant. Post-hoc multiple comparisons were conducted using the Scheffé method. To evaluate the consistency of MP polymer type distributions among different sample types, Spearman correlation analysis was performed, and the results were presented in the form of a heatmap. ArcGIS Pro is used for spatial analysis and visualization. The basemap resources include Esri, USGS, OpenStreetMap contributors, TomTom, Garmin, FAO, NOAA, and USGS.

3. Results

3.1. Abundance of MPs in different media within tiger forest systems

The average abundance of MPs in the surface and deep soil samples collected in the spring and autumn were 9.58 ± 7.66 n/g, 9.18 ± 7.30 n/g, 10.72 ± 9.75 n/g, and 8.35 ± 3.79 n/g, respectively (Fig. 2b). The average abundance of MPs in spring and autumn soil samples were 9.39 ± 7.38 n/g and 9.53 ± 7.33 n/g, respectively (Fig. 2c). The average abundance of MPs in the surface and deep soil samples were 10.04 ± 8.41 n/g and 8.84 ± 6.03 n/g, respectively (Fig. 2c). The abundance of MPs in all soil samples ($n = 59$) ranged from 1.3 to 39.7 n/g, with an average abundance of 9.45 ± 7.3 n/g (Fig. 2g).

The average abundance of MPs in spring and autumn water samples were 9.84 ± 7.65 n/L and 12.33 ± 17.18 n/L, respectively (Fig. 2a). The abundance of MPs in all water samples ($n = 25$) ranged from 1 to 44 n/L, with an average abundance of 10.44 ± 10.32 n/L (Fig. 2g). One of the samples did not detect any MPs.

The average abundance of MPs in spring and autumn atmospheric samples were 11.17 ± 10.19 n/m^3 and 12.25 ± 15.07 n/m^3, respectively (Fig. 2a). The abundance of MPs in all atmospheric samples ($n = 10$) ranged from 0.76 to 33.33 n/m^3, with an average abundance of 11.82 ± 12.69 n/m^3 (Fig. 2g). One of the samples did not detect any MPs.

The average abundance of MPs in low-layer and high-layer plant samples were 11.31 ± 8.33 n/g and 21.00 ± 18.68 n/g, respectively (Fig. 2d). The abundance of MPs in all plant samples ($n = 12$) ranged from 0.61 to 45.35 n/g, with an average abundance of 16.16 ± 14.69 n/g (Fig. 2g).

In spring, the average abundances of MPs in the fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 12.02 ± 12.05 n/g, 17.50 ± 8.21 n/g, 24.32 ± 18.55 n/g, and 28.38 ± 23.36 n/g, respectively. In autumn, the average abundance of MPs in the fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 18.43 ± 16.18 n/g, 17.34 ± 14.41 n/g, 17.65 ± 15.60 n/g, and 16.11 ± 9.39 n/g, respectively (Fig. 2a). The average abundances of MPs in the fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 15.68 ± 13.82 n/g, 17.41 ± 11.24 n/g, 20.51 ± 15.78 n/g, and 21.37 ± 16.40 n/g, respectively (Fig. 2e). In spring and autumn, the average abundances of MPs in all animal fecal samples were 20.55 ± 15.60 n/g and 17.38 ± 12.69 n/g, respectively (Fig. 2f). The abundance of MPs in all fecal samples ($n = 28$) ranged from 0.74 to 55.13 n/g, with an average abundance of 18.74 ± 13.83 n/g (Fig. 2g).

Analysis of variance revealed that the abundance of MPs was significantly higher in animal fecal samples than soil samples ($p < 0.01$), and significantly higher in plant samples than soil samples ($p < 0.05$) (Fig. 2g).

In the calculation of the MP abundance ratio between different samples, we ranked the sample types from low to high based on their position in the forest ecosystem, as follows: environmental samples (soil, water, air), plants, prey (wild boar, sika deer, roe deer), and top predator (Amur tiger). We used the MP abundance in soil as a background value and compared it with the MP abundance in plants and animal feces. The MP abundance ratio of forage plants relative to soil was 1.71; the ratios of wild boar, sika deer, and roe deer feces relative to soil were 1.66, 1.84, and 2.17, respectively; the overall average MP abundance ratio of prey feces relative to soil was 1.89; and for the top predator, the Amur tiger, the MP abundance ratio in its feces relative to soil was 2.26.

3.2. Size distribution of MPs in different media within forest systems

The average sizes of MPs in the surface and deep soil samples in spring and autumn were 44.42 ± 36.94 μm, 41.72 ± 29.53 μm, 48.63 ± 35.94 μm, and 43.48 ± 33.48 μm, respectively (Fig. 3a). The average sizes of MPs in spring, autumn, surface, and deep soil samples were 43.01 ± 33.28 μm, 46.37 ± 34.97 μm, 46.42 ± 36.52 μm, and 42.41 ± 31.14 μm, respectively (Figs. 3b and 3c).

The average sizes of MPs in spring water, autumn water, spring atmosphere, autumn atmosphere, low-layer plants, and high-layer plants were 48.83 ± 34.34μm, 42.47 ± 35.93 μm, 51.03 ± 30.74 μm, 39.68 ± 41.88 μm, 35.42 ± 23.12 μm, and 41.26 ± 31.75 μm, respectively (Figs. 3d, 3e and 3f).

In the spring, the average sizes of MPs in the fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 47.29 ± 25.74 μm, 50.01 ± 36.19 μm, 41.95 ± 24.16 μm, and 45.57 ± 41.38 μm, respectively. In autumn, the average sizes of MPs in the fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 47.36 ± 32.94 μm, 52.62 ± 46.38 μm, 52.13 ± 34.25 μm, and 56.43 ± 46.62 μm, respectively (Fig. 3g). The average sizes of MPs in all fecal samples of wild boars, sika deer, roe deer, and Amur tiger were 47.33 ± 29.67 μm, 50.96 ± 40.18 μm, 45.96 ± 28.96 μm, and 47.82 ± 42.71 μm, respectively (Fig. 3h). In spring and autumn, the average sizes of MPs in fecal samples were 46.09 ± 36.07 μm and 52.01 ± 40.53 μm, respectively (Fig. 3i).

The average sizes of MPs in soil, water, atmosphere, plants, and fecal samples were 44.47 ± 34.06 μm, 47.02 ± 34.85 μm, 43.97 ± 38.35 μm, 39.13 ± 29.02 μm, and 48.07 ± 37.71 μm, respectively (Fig. 3j).

Significant differences in MP sizes were observed (Fig. 3j). MPs were significantly larger in surface vs. deep soil samples in spring ($p < 0.05$), surface vs. deep soil samples in autumn, and all surface vs. all deep soil samples ($p < 0.01$), whereas the MPs were significantly smaller in spring vs. autumn soil samples ($p < 0.01$), low-layer vs. high-layer plant

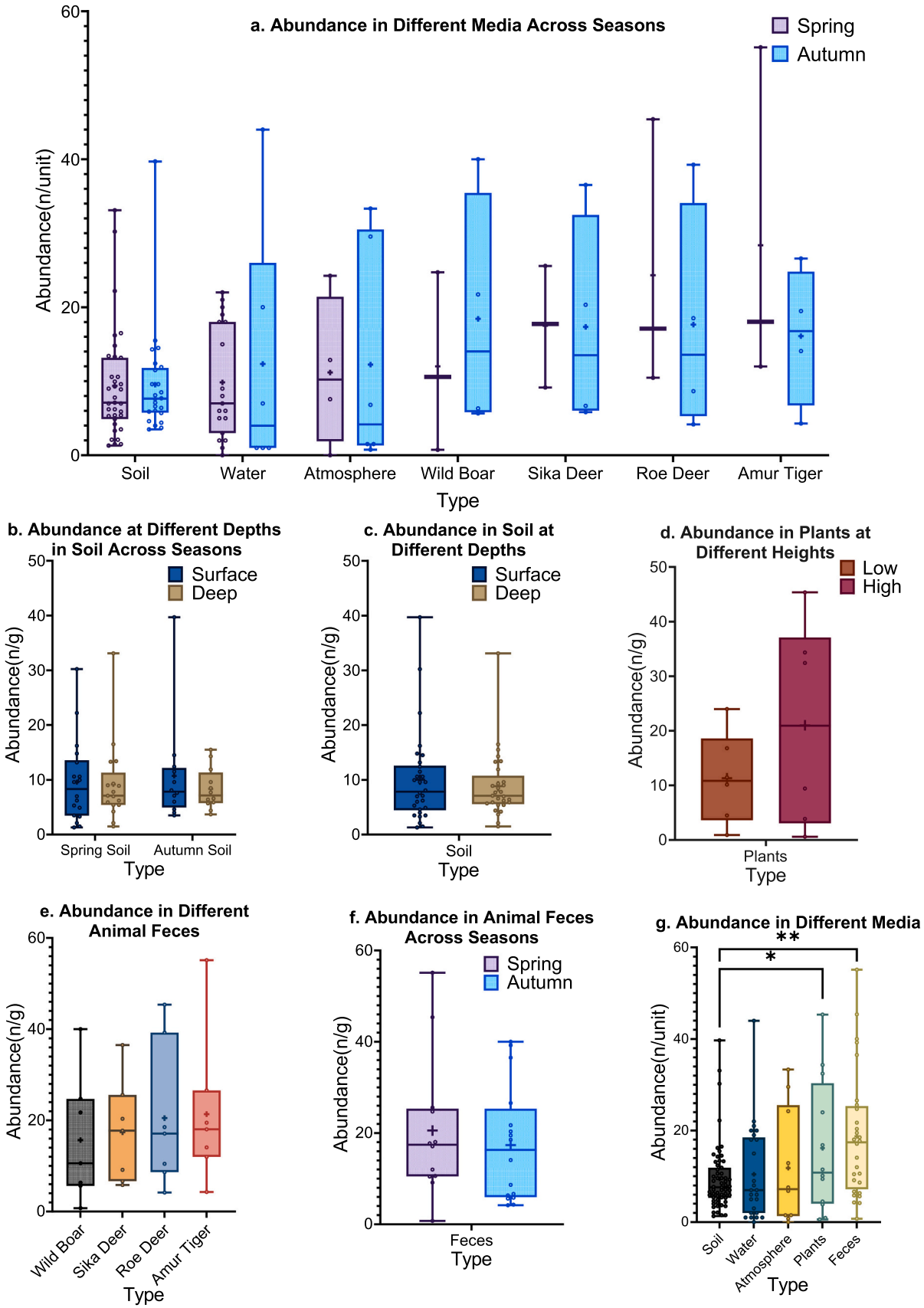

Fig. 2. Abundance of MPs in different media types. In panel a, the Y-axis units for soil and animal feces are n/g, for water it is n/L, and for atmosphere it is n/m^3 . "*" and "**" indicate the significance level of differences in MP abundance between different sample types: * ($p < 0.05$); ** ($p < 0.01$), while absence of annotation indicates non-significance. In a box plot, the central line represents the median, and the "+ " symbol represents the mean.

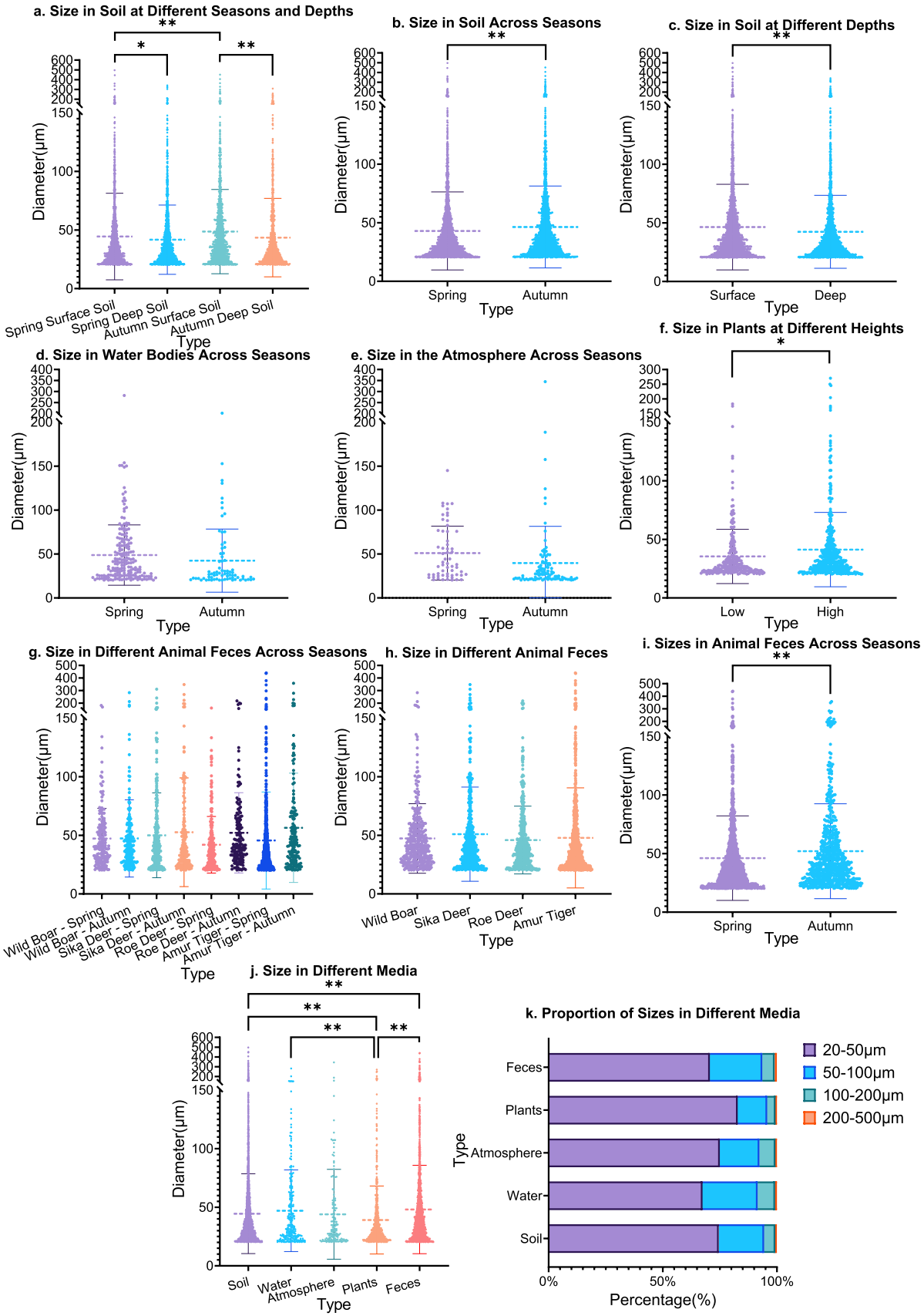

Fig. 3. Size (diameter) of MPs in different media types. "*" and "**" denote the significance level of differences in MP particle size between different sample types: * ($p < 0.05$); ** ($p < 0.01$), while absence of annotation indicates non-significance. In the scatter plot, the mean and standard deviation are labeled accordingly.

samples ($p < 0.05$), and spring vs. autumn fecal samples ($p < 0.01$). The MPs were significantly larger in animal fecal samples than soil and plant samples ($p < 0.01$), but significantly smaller in plant samples than water and soil samples ($p < 0.01$).

For statistical purposes, the MPs in the soil samples were classified by size as 20–50μm, 50–100μm, 100–200μm, and 200–500μm. MPs in the range of 20–50μm were the most common in the soil, water, atmosphere, plant, and fecal samples (74.52 %, 67.43 %, 75 %, 82.85 %, and 70.63 %, respectively), while MPs in the range of 200–500μm were the least common (0.68 %, 0.77 %, 0.64 %, and 0.99 %, respectively) (Fig. 3k). Overall, the abundance of MPs increased as the particle size decreased across all media.

3.3. Types of MPs in different media within forest systems

Among all samples, 32 types of MPs were detected (Fig. 4). The predominant MP compositions were PA (24.35 %), PVC (12.85 %), polyurethane (PU) (10.03 %), polyethylene terephthalate (7.36 %), and acrylates (6.34 %). PA content was highest in soil, water, atmosphere, and plant samples (25.65 %, 55.56 %, 62.82 %, and 40.36 %, respectively), while PVC content was highest in animal fecal samples (16.83 %).

To further investigate the correlation in MP type distributions among different environmental media, we calculated the Spearman correlation coefficients of MP polymer types across five sample categories: soil, water, atmosphere, plants, and feces. The results are presented as a heatmap (Fig. 5). Overall, the distributions of MP types showed varying degrees of positive correlation among different media, all of which were statistically highly significant ($p < 0.01$). Notably, fecal samples exhibited a strong correlation with soil ($r = 0.92$) and plants ($r = 0.78$), while a similarly high correlation was observed between atmosphere and water ($r = 0.77$). Additionally, strong correlations were also found between plants and atmosphere ($r = 0.73$), as well as between plants and soil ($r = 0.72$). In contrast, the correlations between water and feces ($r = 0.55$), and between atmosphere and feces ($r = 0.57$), were moderate.

The LDIR system not only accurately identifies types of MPs by comparing spectral data to a reference database, but also measures the width, height, particle size, and area (Fig. 6). Particles with a matching rate > 0.65 were identified as MPs.

3.4. Morphology of MPs in different media within forest systems

Particles and fragments were the most common morphological types, accounting for 56.35 % and 40.01 % of all MPs, while fiber-shaped MPs were the least common, accounting for only 3.63 %. Among the soil, plant, and fecal samples, particles were the predominant form of MPs, comprising 56.53 %, 88.62 %, and 49.41 %, respectively, while

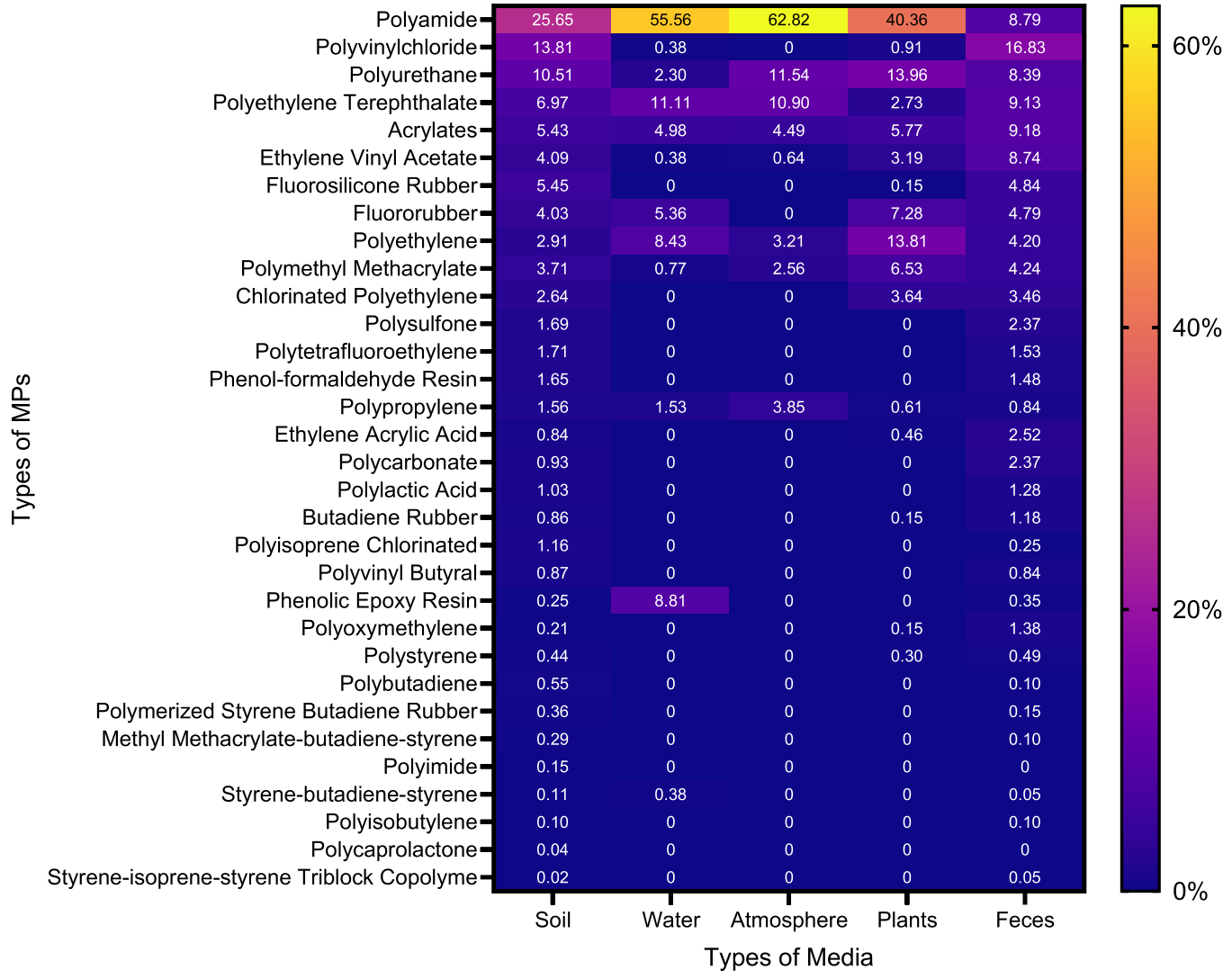

Fig. 4. Proportion of different types of MPs in various media in Amur tiger forest habitats.

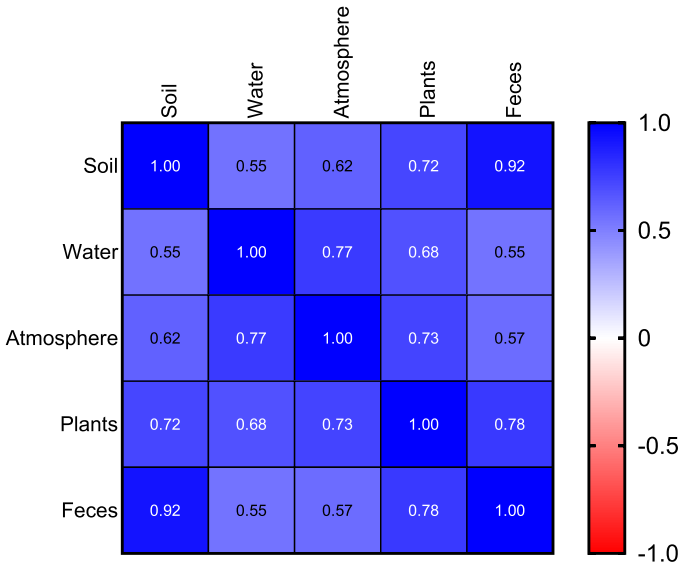

Fig. 5. Correlation heatmap of MP polymer type distributions among different sample types. All correlations were positive and significant at $p < 0.01$.

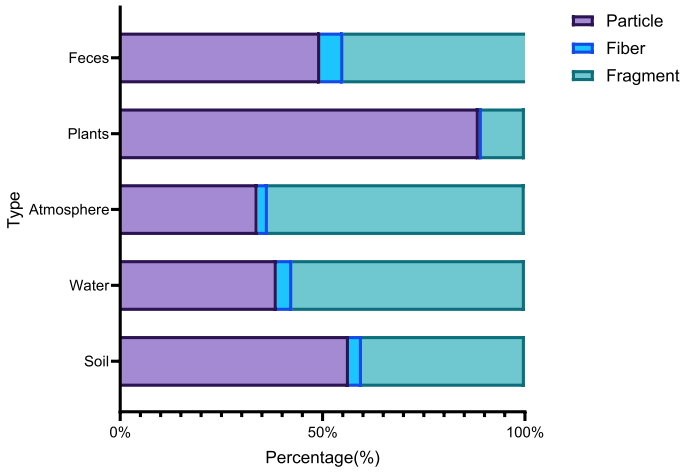

Fig. 7. Proportion of different MP shapes in various media in Amur tiger forest habitat.

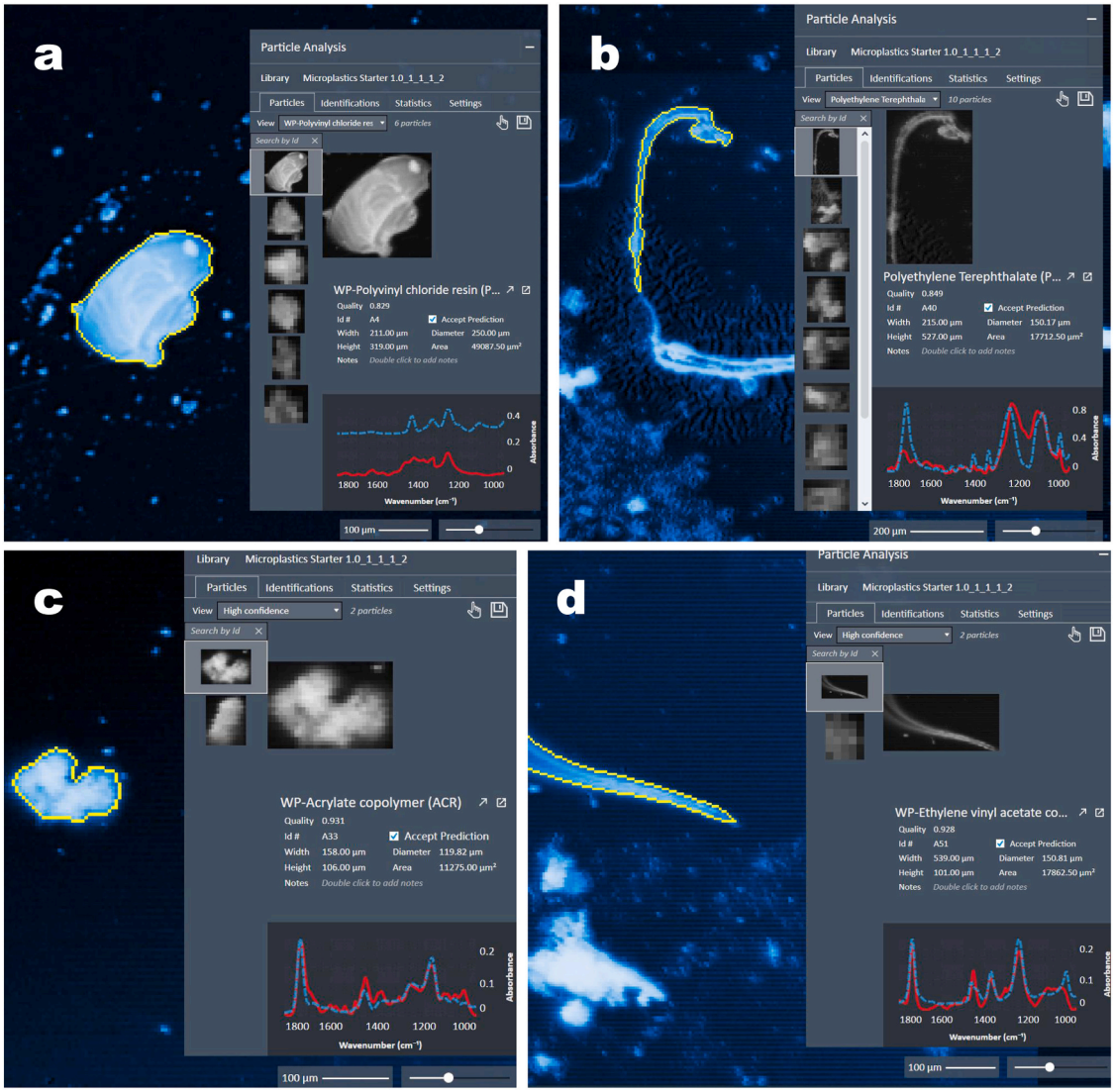

Fig. 6. MPs identified through LDIR system scanning. a: Polyvinyl chloride, b: Polyethylene terephthalate, c: Acrylate, d: Ethylene-vinyl acetate.

fragments were the most common form in the water and atmospheric samples, accounting for 57.47 % and 63.46 %, respectively (Fig. 7).

3.5. Evaluation of MPs as pollutants in forest systems

Among the 10 sampling points, Points 10, 3, and 7, which are located in the southwestern part of the study area closest to the industrial city of Hunchun, had the highest PLI values (4.82, 4.13, and 3.37, respectively) (Fig. 8). Point 10 had the highest PLI, likely due to frequent agricultural activities in the vicinity. The use of plastic films in greenhouses, which degrade over time, may release substantial amounts of MPs into the surrounding environment. Points 3 and 7 are approximately 30 km from Hunchun, thus MPs are likely transported via the atmosphere, resulting in higher PLI values.

Points 9, 6, and 4, situated in the central part of the study area, had moderate PLI values of 2.77, 2.32, and 2.02, respectively. In contrast, Points 5, 1, and 2 in the northeastern part of the study area had the lowest PLI values (1.64, 1.59, and 1.07, respectively). These points are relatively distant from densely populated areas and industrial production facilities, which likely contributed to the lower pollution levels.

Notably, Point 8, located in the easternmost part of the study area, had an anomalously high PLI value (2.86), likely due to the presence of roads and evidence of vehicular and construction machinery activity near this sampling point.

The overall PLI for the study area (PLI_{Zone}) was 2.42 (Fig. 8), indicating slight pollution (Level I) according to pollution evaluation standards. The PLI values across the sampling points decreased from southwest to northeast, exhibiting an inverse correlation with the distance from the industrial city in the southwest. This trend suggests an association between atmospheric transport of MPs and the observed pollution level.

4. Discussion

Analyses of soil, water, atmosphere, plant, and animal fecal samples from the Amur tiger forest habitats were conducted to determine the presence of MPs. To the best of our knowledge, this is the first investigation of MPs in the tiger habitat. Of the 134 collected samples, only two (1.49 %) had no MPs, resulting in a positivity rate of 98.51 %, indicating infiltration of this forest ecosystem by MPs, thereby confirming the study hypothesis.

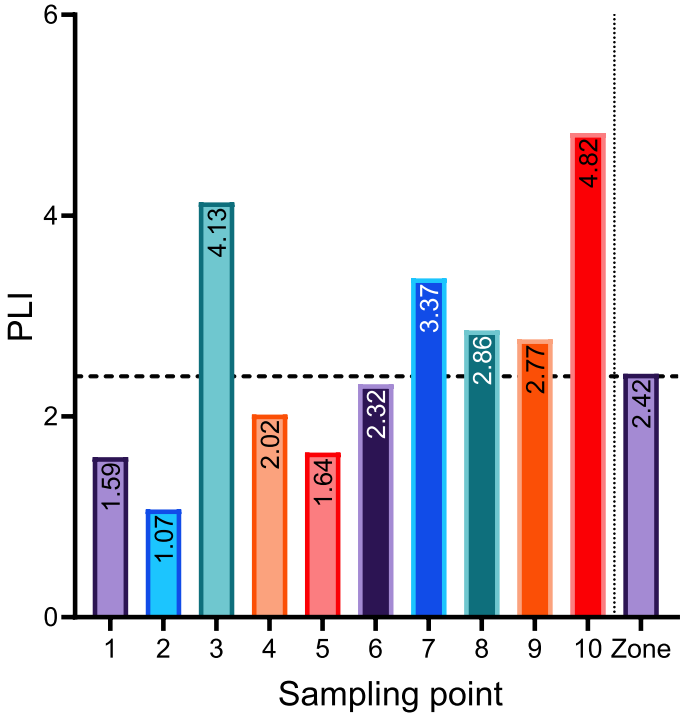

Fig. 8. MP PLI values across different sampling points and the entire study area. "Zone" represents the overall PLI value of the entire study area.

In soil samples, the average abundance of MPs was 9.45 ± 7.3 n/g, which is significantly lower than the MP abundance of 49.6 n/g found in sediment from the East Frisian Islands in the Netherlands [48], 18.76 n/g found in soil from the forest buffer zone of the Chaobai River Basin in China [113], 3.88 ± 2.36 n/g in soil along the Yangtze River [121], and 2.80 n/g in soil from the Hehuang Valley on the Qinghai-Tibet Plateau [114], which is slightly lower than our findings but still within the same order of magnitude. Overall, the MP abundance in soil from the study area falls within a moderate range.

MPs may enter soil through surface deposition from the atmosphere and undergo vertical movement, rendering soil a sink for MPs [8]. In this study, the abundance of MPs tended to decrease with increasing soil depth, accompanied by a significant reduction in MP size [111,115], consistent with the findings of similar studies, suggesting that larger MPs tend to accumulate in surface soil layers, while smaller MPs migrate towards deeper soil layers. This trend may be related to soil filtration processes, as soil pores provide pathways for smaller MPs to enter deeper soil layers [45,8], as confirmed by simulation experiments [21]. Additionally, soil organisms play a crucial role in the vertical movement of MPs, as earthworms and ants can create channels within the soil [112,9], as well as bioturbation by plant roots, potentially facilitating the migration of smaller MPs into deeper soil layers [24].

In the water bodies of the study area, the average abundance of MPs was 10.44 ± 10.32 n/L, which is generally similar to that of the Wei and Chishui Rivers [118,46], but lower than the Pearl River in Guangzhou (19.86 n/L) and slightly higher than at the mouth of the Pearl River and surface water of the Three Gorges Reservoir (8.90 and 4.70 ± 2.81 n/L, respectively) [103,17]. In natural reserves with characteristics similar to our study area, the average MP abundance in water bodies in southern China ranges from 0.54 to 5.5 n/L [28]. Prior surveys found that the abundances of MPs in Chagan Lake and the Xianghai Nature Reserve were 3.61 ± 2.23 and 0.29 ± 0.11 n/L, respectively [109]. Notably, the abundances of MPs in water bodies of the study area were lower in spring than autumn, similar to the Antu River in Portugal, which had a higher abundance in October than March [77], in addition to the Pacific Ocean and Pearl River Delta [22,27]. The higher MP abundance in autumn (rainy season) may be due to increased water flow and velocity, improper plastic waste disposal, and human activities.

To date, most investigations of atmospheric MPs have focused on atmospheric deposition by collection of sediments at fixed locations [31,72,92], while relatively few studies included real-time quantification of atmospheric MPs, particularly within forest ecosystems. In the present study, the average abundance of MPs in atmospheric samples was 11.82 ± 12.69 n/m^3 , similar to the French Atlantic coast (9.6 n/m^3) [3], but significantly lower than atmospheric samples collected from Surabaya, Indonesia (55.93 – 174.97 n/m^3) and higher than the indoor and outdoor concentrations in Paris (5.4 and 0.9 n/m^3, respectively) [19]. The average atmospheric MP level in the present study was slightly lower than the atmosphere of the Qinghai-Tibet Plateau (19.0 ± 3.0 n/m^3) [56], but overall within the same order of magnitude. The average MP abundance of the plant samples in this study was 16.16 ± 14.69 n/g, which is very close to the average concentration of MPs in pine needles in Shihezi City [54]. Reportedly, the MP abundance in reeds around Dongting Lake and in nature reserves is 4.9 ± 2.6 n/g [110]. Two wetland plants (Australian poplar and reed grass) collected from the Dafeng Elk National Nature Reserve in China had MP abundances of 5.39 ± 6.58 and 2.97 ± 2.73 n/g, respectively [117], which were both lower than the results of the present study.

MPs primarily enter forest ecosystems through atmospheric deposition [95], thus the abundance of atmospheric MPs is likely influenced by rainfall, humidity, wind speed, and wind direction, as well as particle

size and shape. In addition, the higher atmospheric MP abundance in the study area could also be related to human activities, such as agriculture and industry. Plants serve as potential temporary sinks for atmospheric MPs [51]. Besides gravity, atmospheric deposition on leaves causes accumulation of MPs, as the rough surfaces, abundant stomata, and complex folds and creases of plant leaves may further enhance capture of atmospheric MPs [34]. Two surveys of urban tree leaves conducted in Los Angeles found that the abundance of MPs ranged from 0.14 to 25 n/cm^2 and was greater at heights of 0.6–1.2 m, consistent with the trends found in the present study, suggesting that the relative height of plants impacts capture of MPs. Also, the density, size, and shape of MPs influence transport and retention [32]. The density of MPs determines vertical distribution in the atmosphere and the subsequent abundance of MPs on plant leaves at different heights. In forest environments, taller plants may shade lower plants and intercept MPs deposited by gravity, resulting in higher MP abundances in taller than shorter plants. Additionally, considering the process utilized by plants to transport nutrients from soil to the tissues, this process may decrease the size of MPs detected within plants through intercellular transport and penetration.

Until now, reports on MP levels in natural reserves and wildlife feces have been exceedingly rare. MP concentrations in fecal samples collected from wild boars, sika deer, roe deer, and Amur tigers in the study area were 15.68 ± 13.82 n/g, 17.41 ± 11.24 n/g, 20.51 ± 15.78 n/g, and 21.37 ± 16.40 n/g, respectively, with an average concentration of 18.74 ± 13.83 n/g. The concentration of MPs in fecal samples of Tibetan wild ass (*Equus kiang*) is reportedly 140 n/g [55], which is significantly higher than in the present study. In the Qinling Nature Reserve, the MP abundances in fecal samples of leopard cats (*Prionailurus bengalensis*), golden snub-nosed monkeys (*Rhinopithecus roxellana*), and takins (*Budorcas taxicolor*) are reportedly 67.06, 27.69, and 20.53 n/g [98], respectively. A survey conducted in the western Antarctic Peninsula found that the MP abundances in fecal samples of the crabeater (*Lobodon carcinophaga*), Weddell (*Leptonychotes weddellii*), and leopard seals (*Hydrurga leptonyx*) were 15.8 ± 5.93, 14.0 ± 11.91, and 13.4 ± 6.37 n/g, respectively [11]. Although the MP abundances varies between these two studies, these measurements are generally comparable to the findings of the present study. In the Dafeng Elk National Nature Reserve, the MP abundance in fecal samples of elks (*Elaphurus davidianus*) was 0.21 ± 0.05 n/g [117], much lower than in the fecal samples of sika and roe deer in the present study. Notably, the MPs in spring soil were significantly smaller than in autumn soil, possibly because the frequent monsoons and precipitation in autumn can transport larger MPs through the atmosphere and other pathways over greater distances, leading to a significant increase in the size of MPs in autumn soil. Similarly, the MPs were smaller in spring vs. autumn fecal samples, which may be related to the food chain, as MPs in soil are transferred to animals through direct ingestion or indirectly through plants, resulting in the same seasonal changes to the sizes of MPs in soil and fecal samples. Digestion by animals may significantly increase the size of MPs in feces than soil and plants because smaller MPs can accumulate in the digestive tract. This may be especially true for herbivores, which have stomachs with four different chambers with specialized folds and grooves that can trap smaller MPs and the gastric fluid of herbivores may affect the digestion of MPs [117,74].

To further explore the relative abundance of MPs at different nodes of the food web in the Amur tiger's habitat, we calculated the MP abundance ratios in plants and wildlife feces based on the method proposed by Lwanga et al. [57] for studying MP transfer in terrestrial food chains. Based on the results, we speculate that wild animals in forest ecosystems may be facing potential risks of MP exposure. Taking the Amur tiger as an example, in addition to ingesting and absorbing MPs directly from the environment, the Amur tiger may also indirectly ingest MPs through predation. In the study area, the Amur tiger primarily preys on herbivorous roe deer and sika deer, as well as omnivorous wild boar, which mainly feed on plants. These prey animals could ingest MPs through plants consumption, leading to the transfer and accumulation of MPs across trophic levels, ultimately threatening the health of the Amur tiger. In marine food webs, some studies have already observed that MP abundance increases with trophic level [40], and evidence of MP transfer from fish to marine top predators has been found, with the hypothesis that trophic transfer may be an indirect but primary pathway for MP ingestion [67]. As another top predator, the Amur tiger may be more susceptible to the threat of MP accumulation. The toxicity of MPs is not limited to mechanical damage and inflammation; it can also reduce reproductive ability by inhibiting embryo development [1], which could further jeopardize species like the Amur tiger, as strong reproductive ability is crucial for expanding their population. Due to considerations of animal protection and ethical standards, there are significant limitations in sample collection. We more often collect animal feces rather than animal tissues, making it difficult to track the trend of MP flow along trophic levels through the calculation of BAF and BMF. Current research indicates that chemical pollutants (such as Polychlorinated Biphenyls and organochlorine pesticides) bioaccumulate and biomagnify at higher trophic levels in food chains [37,89]. Whether similar mechanisms occur for MPs, as for other pollutants, remains uncertain, and further studies on the transmission mechanisms of MPs in terrestrial food chains are needed. It should be noted that the MP abundance ratio calculation method used in this study has significant limitations. This method does not directly reflect the specific accumulation of MPs in animals, nor does it account for all potential influencing factors (such as MP degradation, fragmentation, and changes in characteristics). We chose this method because there is currently no unified and mature system available to assess the MP exposure levels in animals within terrestrial ecosystems. Therefore, the MP abundance ratio provides an effective starting point, allowing us to conduct preliminary quantitative analysis within the existing research framework and offering a reference for future, more precise assessment methods.

We performed linear regression analysis of the distribution of MP types between feces and biological samples (such as muscle samples from sika deer, roe deer, wild boar, and blood samples from the Amur tiger). The results showed a high correlation of 0.94 between the MP type distribution in feces and biological samples (Text S3). This supports the potential of feces as an indicator of animal MP exposure levels. In a study on birds, scholars suggested that the crop could serve as a non-lethal indicator to identify whether an organism has been exposed to and ingested MP particles [83]. Similarly, many wildlife studies have used feces as an indicator of animal MP exposure levels, including studies on northern fur seals (*Callorhinus ursinus*) [18], Baird's tapirs (*Tapirus bairdii*) [71], polar bears (*Ursus maritimus*) [35], Eurasian otters (*Lutra lutra*) [69], and beluga whales (*Delphinapterus leucas*) [63]. Prospective medical studies have indicated that human feces is a significant indicator of MP pollution in the human body [79], and some medical research has confirmed the correlation between plastic exposure and fecal MP abundance [116,30]. One study found that patients with inflammatory bowel disease had significantly higher levels of fecal MPs (41.8 n/g) compared to healthy individuals (28.0 n/g) [105], further confirming our concern: is the health of top predators also at risk from MP exposure? Some researchers have already established models of human gut MP exposure based on feces [104], providing a new direction for predicting health risks through the concentration of MPs in animal feces. However, more empirical research is needed to develop evaluation models for environmental MP levels, animal exposure, and health risks, which will be a key focus of future research.

The results of the present study found that PA was the main component of the collected MPs, suggesting a potential association with the production and washing of textiles [101], which can breakdown textile fibers into MPs that are subsequently released into the environment and transported to remote wildlife reserves through atmospheric deposition or surface runoff. During the coronavirus pandemic in 2019, indiscriminate disposal of commonly used medical masks that contained PA may have contributed to the higher detection levels of PA [7]. Among various samples, particularly animal feces, significant amounts of PU

were detected, consistent with the findings of previous studies on MPs in animal feces [55,98]. PU is commonly used in the construction and garment industries, suggesting potential as an important indicator of pollution levels in protected areas and wildlife. Additionally, PVC was the most prevalent MP type detected in animal feces. Studies on MPs in humans have reported that PVC has the highest polymer hazard index and risk level, and tends to accumulate in human tissues [122]. The high detection levels of PVC in feces may indicate that wildlife ingest more PVC through the diet, thereby posing greater threats of toxicity.

To further understand the interrelationships of MP pollution among various environmental and biological media, we conducted a Spearman correlation analysis based on the distribution of MP polymer types across different sample categories. The results revealed significant positive correlations ($p < 0.01$) between all pairwise media combinations, suggesting that in forest ecosystems, MP type distribution may be influenced by shared pollution sources or environmentally mediated interactions, leading to consistent contamination patterns across compartments. The highest correlation was observed between feces and soil ($r = 0.92$), indicating that a substantial portion of MPs found in wildlife feces may originate from direct contact with or unintentional ingestion of contaminated soil. This finding aligns with previous studies identifying soil as a major reservoir and exposure source of MPs in terrestrial ecosystems [100,106]. A similarly strong correlation was found between feces and plants ($r = 0.78$), implying that vegetation may act as an important intermediate carrier in the trophic transfer of MPs. Herbivorous animals may ingest MPs adhered to or absorbed by plants, which in turn may be transferred to top predators such as the Amur tiger through predation. The significant correlation between atmosphere and water ($r = 0.77$) suggests that these two environmental media may share common MP input pathways, such as atmospheric deposition and surface runoff, which can concurrently introduce MPs from agricultural, industrial, or peri-urban sources into the forest ecosystem [29,61]. The strong associations between plants and atmosphere ($r = 0.73$), as well as between plants and soil ($r = 0.72$), further reflect the integrative nature of MP transport in terrestrial systems. Vegetation may intercept MPs from the air via foliar surfaces or accumulate MPs from soil through root contact or surface adhesion [33,42]. By contrast, the relatively lower correlations between water and feces ($r = 0.55$), and between atmosphere and feces ($r = 0.57$), suggest that direct exposure routes such as drinking or inhalation may not represent the primary pathways of MP accumulation in wildlife inhabiting this forest region. Instead, indirect exposure through ingestion of contaminated plants and soil is more likely to play a dominant role in MP transfer into animal bodies.

The average PLI in the study area was 2.42, which is lower than that of the Chagan Lake (3.21) in Jilin Province (Yin, K. et al., 2021) and lake sediments from the Tibetan Plateau protected areas (2.87) [66], but higher than that of the Xianghai Lake (2.16) and Helan Mountain (1.76) [16]. It is also similar to the MP levels found in typical southern Chinese nature reserves (Class I risk) [28]and the Qilian Mountain area (1.60–2.62) [41].Overall, the MP levels in the Amur tiger habitat are considered moderate compared to various protected areas, with a relatively low risk rating (Class I risk). This suggests that the study area has a relatively mild pollution load of MPs. Notably, the PLI values of MPs tended to decrease from southwest to northeast within the study area, indicating that the level of MP pollution decreases with increasing distances from industrial regions. This trend is likely related to atmospheric transport of MPs, as dispersion in forest ecosystems is attenuated over greater distances. The dense foliage of plants likely acts as a barrier that intercepts MPs in the atmosphere and prevents further dissemination.

The results of this study revealed extensive MP pollution in the study area, as demonstrated by comprehensive sampling of soil, water, atmosphere, plants, and animal feces, thereby expanding single-media sampling in the environment for comprehensive assessment of MP levels. Horizontal comparisons with other studies found that although the PLI in the study area was relatively low, the diversity of MP types was remarkably high as compared to other nature reserves and remote areas. This implies that the majority of MP types can infiltrate wildlife conservation areas far from human activities through various pathways.

5. Conclusion

This study is the first to investigate the impact of MPs on the Amur tiger forest habitats. MPs were detected in soil, water, atmosphere, plants, and animal feces. The highest MP abundance found in Amur tiger feces. Seasonal variations and spatial distributions influenced the abundance and size of MPs, at least to some extent, as accumulation was higher in autumn. Smaller MPs (20–50μm) were most commonly detected, with PA, PVC, and PU as the most common types of MPs. The distribution of MP polymer types showed strong consistency among different sample types. Also, MPs were predominantly observed in the form of particles and fragments. Currently, the PLI of MPs is relatively low in the study area.

Although this study provides a preliminary quantitative analysis of MP contamination in the Amur tiger's habitat, there are still certain limitations, particularly in the evaluation methods of MP exposure and the consideration of influencing factors. Future research should further improve the evaluation methods and frameworks, especially by developing a more precise and unified system to comprehensively reflect the accumulation of MPs in different organisms. Additionally, the long-term biological health impacts of MPs need to be tracked to better understand their potential ecological risks.

As research progresses, we hope to offer a unique perspective to raise awareness of the importance of protecting the natural habitats of wildlife for biodiversity conservation. Protecting wildlife habitats not only helps mitigate the impact of pollutants such as MPs but is also crucial for maintaining ecological balance and biodiversity.

Environmental implication

The widespread presence of microplastic pollution and its potential toxicity have raised concerns about ecosystem safety. Reports on microplastic pollution in remote wildlife reserves are scarce. This study provides the first comprehensive characterization of microplastic pollution across various environmental media within a forest ecosystem. We also preliminarily investigate the pathways of microplastic transfer along the terrestrial food chain and assess the levels of microplastic contamination in the Amur tiger habitats. The aim is to elucidate the potential threats posed by microplastics to wildlife. We anticipate that this research will contribute to the monitoring of microplastic pollution and the protection of biodiversity.

CRediT authorship contribution statement

Zhang Yuanyuan: Writing – review & editing, Supervision, Funding acquisition. **Huang Baoxiang:** Investigation. **Guo Shuhao:** Validation. **Zhao Bitian:** Formal analysis. **Wang Yihan:** Visualization. **Liu Dongqi:** Writing – review & editing, Data curation. **Cheng Wannian:** Project administration. **Zhang Wentao:** Resources. **He Zhijian:** Resources. **Jiang Guangshun:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Huang Zekai:** Writing – original draft, Formal analysis, Conceptualization.

Ethics approval

The sampling process was to take fresh feces without disturbing wild animals. Following legal requirements, all experimental procedures were approved by Northeast Forestry University's Animal Care and Use Committee.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.138380.

Data availability

Data will be made available on request.

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