

Journal Pre-proof

Microplastic contamination in terrestrial ecosystems: A study using barn owl (*Tyto alba*) pellets

Alessandro Nessi, Anna Winkler, Paolo Tremolada, Francesco Saliu, Marina Lasagni, Luca Luigi Mario Ghezzi, Alessandro Balestrieri



PII: S0045-6535(22)02774-6

DOI: <https://doi.org/10.1016/j.chemosphere.2022.136281>

Reference: CHEM 136281

To appear in: *ECSN*

Received Date: 5 July 2022

Revised Date: 24 August 2022

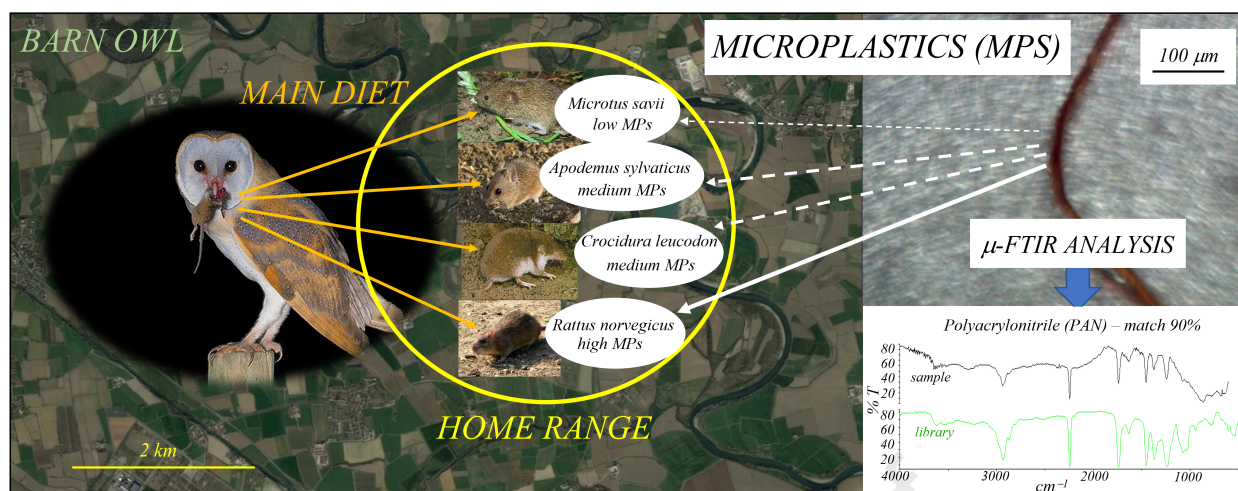
Accepted Date: 28 August 2022

Please cite this article as: Nessi, A., Winkler, A., Tremolada, P., Saliu, F., Lasagni, M., Ghezzi, L.L.M., Balestrieri, A., Microplastic contamination in terrestrial ecosystems: A study using barn owl (*Tyto alba*) pellets, *Chemosphere* (2022), doi: <https://doi.org/10.1016/j.chemosphere.2022.136281>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier Ltd.

Alessandro Nesi: Conceptualization, Investigation, Writing - original draft; A. Winkler: Investigation, Methodology; F. Saliu: Investigation, Methodology; M. Lasagni: Investigation; L. Ghezzi: Investigation; P. Tremolada: Project administration, Supervision, Writing - review & editing; A. Balestrieri: Data curation, Supervision, Writing - review & editing



Microplastic contamination in terrestrial ecosystems: a study using barn owl (*Tyto alba*) pellets

Alessandro Nessi^{1*}, Anna Winkler¹, Paolo Tremolada¹, Francesco Salvi², Marina Lasagni², Luca Luigi Mario Ghezzi¹, Alessandro Balestrieri¹

¹ Department of Environmental Science and Policy, University of Milan, 20133 Milan, Italy

² Department of Earth and Environmental Sciences, University of Milano-Bicocca, 20126 Milan, Italy

* Corresponding author, e-mail: alessandro.nessi93@gmail.com

Abstract

Microplastics (MPs) are recognised as an emerging environmental problem that needs to be carefully monitored. So far, MPs have been widely recorded in marine and freshwater ecosystems. Still, few studies have focused on MP occurrence in terrestrial ecosystems, although soils are suspected to be one of the main MP reservoirs. To test a non-invasive method for assessing MP contamination in terrestrial ecosystems, we analysed the pellets of a top terrestrial predator, the barn owl (*Tyto alba*). Sixty pellets were collected from three agricultural areas (20 pellets each) and analysed to assess both barn owl diet and MP content. Thirty-four MPs were confirmed by micro-Fourier Transform Infrared Spectroscopy (μ -FTIR) analysis in 33% of the pellets (min-max 1 – 5 MPs per pellet). Most of the detected items were microfibrils (88.2%). Polyethylene terephthalate, polyacrylonitrile and polyamide were the most abundant polymers. One of the three sites was significantly less contaminated. In the two sites with the highest MP occurrences, barn owl diet was characterised by predation on synanthropic rodents, particularly brown rats (*Rattus norvegicus*), which may indicate habitat degradation and increased exposure to MPs. Analyses also suggest that Savi's pine vole (*Microtus savii*) is the prey least at risk of MP contamination, probably due to its strictly herbivorous diet. We argue that the analysis of barn owl pellets may represent a cost-effective method for monitoring MP contamination in terrestrial ecosystems.

Keywords Barn owl; Microplastic; Bird pellets; Small mammals; Plastic ingestion

29 Introduction

30 Microplastics (MPs), synthetic polymer particles < 5 mm in size (Pico et al., 2019; Sorensen and Jovanović,
31 2021) are considered persistent pollutants of increasing concern, showing complex movements between
32 abiotic and biotic ecosystem compartments through different pathways (e.g., atmospheric, trophic, or water
33 transport; Zhou et al., 2020; Hashmi, 2022). Based on their origin, MPs within the environment can be divided
34 into primary and secondary. Primary MPs are specifically manufactured to be added to products (cleaning
35 products, personal care products) to improve their functionality (Hernandez et al., 2017), while secondary
36 MPs derive from the fragmentation of larger plastic materials (e.g., fibres from synthetic clothes, tyre wear,
37 etc.) via biological, physical, and chemical processes (Carr et al., 2016; Napper and Thompson, 2016; Kole et
38 al., 2017; Almroth et al., 2018; So et al., 2022).

39 The small size of MPs makes them likely to be ingested by organisms at the base of the food chain (Iannilli
40 et al., 2019; Silva-Cavalcanti et al., 2017; Long et al., 2017; Guilhermino et al., 2018). In the marine
41 environment, MP uptake has been recorded in a wide variety of taxa (Cole et al., 2011), including fish
42 (Boerger et al., 2010; Choy and Drazen, 2013; Lusher et al., 2013; Avio et al., 2015; Alomar and Deudero,
43 2017), aquatic mammals (Donohue et al., 2019), and ca. 50% of seabirds (Lusher et al., 2018).

44 In comparison, MPs in terrestrial taxa have been less investigated, although soils may represent a large
45 plastic reservoir (Bläsing and Amelung, 2018; Hurley and Nizzetto, 2018; Kawecki and Nowack, 2019), and
46 MP contamination in terrestrial environments is expected to be 4 to 23 times larger than that in the ocean
47 (Horton et al., 2017). Due to human activities, urban and agricultural soils are the most exposed to MP
48 pollution (Chae and An, 2018; Moller et al., 2020). The main MP pathways include atmospheric deposition
49 (Evangelidou et al., 2020), accidental release (Geyer et al., 2017) and agricultural practices such as the use of
50 plastic mulching, fertilisers, pesticides and irrigation by surface waters, potentially contaminated by MPs
51 (Bläsing and Amelung, 2018; Liu et al., 2018; Lv et al., 2019; Corradini et al., 2019; Büks and Kaupenjohann,
52 2020; Chen et al., 2020; Crossman et al., 2020). Among all these sources, plastic mulching and compost seem
53 to play a major role in the contamination of agricultural soil (Xu et al., 2020; Huang et al., 2020; Van
54 Schothorst et al., 2021; Wang et al., 2021). Throughout Europe, 44,000 – 430,000 t of MPs are added annually
55 in sewage-sludge amended agricultural soils (Nizzetto et al., 2016), determining contamination hot-spots. As
56 for aquatic environments, most MPs are fragments and fibres of polyethylene (PE) and polypropylene (PP)
57 (Yang et al., 2021).

58 Microplastics can affect both the biophysical properties of soils (de Souza Machado et al., 2019) and soil
59 organisms, causing oxidative stress and cytotoxicity and disrupting energy homeostasis (Prata et al., 2020).
60 In earthworms, toxicity studies have shown a significant decrease in growth and reproductive rates,
61 inflammation, tissue damage and oxidative stress (Lahive et al., 2019; Huerta Lwanga et al., 2016; Rodriguez-
62 Seijo et al., 2017; Wang et al., 2019). MPs have been shown to accumulate in mice's liver, intestine and
63 kidneys, with negative effects on growth rates, energy and lipid metabolism, and oxidative stress (Deng et
64 al., 2017; Lu et al., 2018; Yang et al., 2019). A field simulation study has confirmed that MPs can be transferred
65 from prey to predator species (earthworm to chicken) through the terrestrial food chain, posing a possible
66 risk also for human health (Lwanga et al., 2017).

67 Knowledge about the ingestion of MPs by terrestrial birds is scarce. Zhao et al. (2016) found a total of 364
68 anthropogenic materials in the gastrointestinal tract of birds from Shanghai (mostly common buzzard *Buteo*
69 *buteo* and large hawk-cuckoo *Cuculus sparveroides*), of which 7.7% were MPs, while in central Florida Carlin
70 et al. (2020) recorded MPs in all examined specimens (n = 63) of 8 species of birds of prey. The red-shouldered
71 buzzard (*Buteo lineatus*) showed a significantly higher abundance of MPs than typical fish eaters, such as the
72 osprey (*Pandion haliaetus*), suggesting that terrestrial birds of prey may be more exposed to bioaccumulation
73 than aquatic predators (Carlin et al. 2020). Recently, MPs have been found in nearly all tree swallow
74 (*Tachycineta bicolor*) chicks analysed in southern Ontario (Canada; Sherlock et al. 2022).

75 While plastic ingestion is usually assessed by analysing the stomach content of dead animals, minimally
76 invasive methodologies, such as the analysis of pellets or faeces, may provide useful indications on the

exposure to which individuals are subject during their life (Acampora et al., 2017a). To date, MPs in bird pellets have been investigated mainly in seabirds, such as the great skua (*Stercorarius skua*) (Ryan and Fraser, 1988; Hammer et al., 2016), fulmar (*Fulmarus glacialis*), black-legged kittiwake (*Rissa tridactyla*) and cormorant (*Phalacrocorax carbo*) (Acampora et al., 2017 a, b). Studies on freshwater birds mostly concerned herbivorous species, for which the main sources of MP are sediments and water rather than food (English et al., 2015; Holland et al., 2016; Gil-Delgado et al., 2017; Reynolds and Ryan, 2018). Only two recent works (D'Souza et al., 2020; Winkler et al., 2020) have investigated the presence of MPs in freshwater predators' pellets: the kingfisher (*Alcedo atthis*) and the dipper (*Cinclus cinclus*).

In terrestrial ecosystems, owls (Strigiformes) usually expel the undigested remains of their prey as conspicuous pellets, which can be easily found in great numbers under resting sites and can often be easily assigned to species-level. While pellets have been largely used to assess owl feeding habits (e.g. Janžekovič and Klenovšek, 2020) and the diversity of small mammal communities within their home range (Heisler et al. 2016; Roulin, 2016), to our knowledge, their MP content has never been investigated.

Aiming to gain insights into MPs in terrestrial food webs, we chose the barn owl (*Tyto alba*) as an indicator species to assess the occurrence of MPs in an agricultural landscape. Barn owls are generalist, nocturnal predators which feed opportunistically on available prey (Love et al., 2000; Meek et al., 2012). We chose the barn owl for three reasons. First, barn owls are strongly associated with agricultural environments throughout their wide distribution range, and we expected their synanthropic habits to make them effective indicators of MP pollution caused by human activities. Cultivated areas are estimated to cover over 42% of EU land area. As they are expected to further expand in the next decade (Perpiña Castillo et al., 2018), MP contamination needs to be monitored effectively. Second, barn owls are territorial and often keep their home ranges and breeding sites over several years (Roulin, 2020), allowing prey items and MP pollution to be related to a well-defined area. Barn owls are very faithful to their preferred roosts, usually farmhouses and siloes, allowing the collection of most of their ejections in one or a few sites. Third, small rodents and insectivores form the bulk of barn owl diet (Taylor, 1994; Roulin, 2020) throughout its wide distribution range, including the intensively cultivated lowlands of northern Italy (Bosè and Guidali, 2001; Balestrieri et al., 2019). In human-altered ecosystems, small mammals, particularly generalist and synanthropic rodents, have been demonstrated to bioaccumulate soil pollutants and transfer them to higher trophic levels (e.g., trace metals; Rogival et al., 2007; Smith et al., 2007). As MPs are ubiquitous and long-lasting with respect to most soil pollutants (de Souza Machado et al., 2018), small mammals can be expected to ingest and accumulate them directly from the environment or preying on arthropods and earthworms and transfer them to barn owls. Differently from most soil pollutants, MPs are particulate matter. Thus, their bioaccumulation and transfer along the food chain are expected to occur differently. This issue has been poorly investigated in terrestrial environments, while more data are currently available for aquatic ecosystems (e.g., Walkinshaw et al., 2020).

Moreover, terrestrial food chains are not as well defined as aquatic ones, most terrestrial carnivores being opportunistic predators. With respect to previously investigated generalist predator birds, the specialist barn owl occupies the top of a trophic web with comparatively f

ew levels, allowing a more straightforward relationship with soil contamination. Moreover, the proportion of commensal rodents (*Rattus* spp.) in the diet increases with urbanisation (Hindmarch and Elliott, 2015), exposing barn owls to anticoagulant rodenticides, heavy metals and pesticides (Newton et al., 1990; Esselink et al., 1995), as well as to the ingestion of industrial products such as plastics, textiles and insulation materials (Roulin, 2020). Hence, our main aim was to assess the effectiveness of owl pellet analysis to highlight MP contamination in terrestrial ecosystems. We expected i) our target terrestrial top predator to ingest MPs directly or through its prey, and ii) MP concentration to vary according to barn owls' feeding grounds and habits.

Materials and methods

Study area and sample collection

The study was carried out in the lower half of the valley of the River Adda (North Italy, Lombardy region; mean altitude: 70 m a.s.l.) included in the Adda Sud Natural Park (Fig. 1). This area is mainly agricultural land, mostly consisting of crops (maize, wheat, soy and barley), hayfields and poplar plantations. Pellets were collected between 23/12/2020 and 03/02/2021 in three known barn owl resting/reproductive sites (A, B and C). During the sampling period, each site was used by 1 – 2 individuals. Sites A and B were unused farm silos next to active farms, while site C was an abandoned monastery. Pellets were collected using gloves and wearing cotton clothes (see below for a detailed description of the procedures adopted to prevent MP contamination). Land use in the three sampling sites was analysed, using digitalised land cover maps ("Dusaf 6.0 - Land use" updated to 2021), in a 2 km radius buffer around each roost (Fig. 1), corresponding to the average home range of barn owls (Frey et al. 2011; Massa et al., 2015; Roulin, 2020).

All sites mostly consisted of arable land areas (79.2% site A; 59.9% site B; 76.8% site C). In site B, the lower percent cover of cropland depended on the greater spread of poplar plantations (16.6 %, Tab. 1).

For each site, the twenty most recent pellets (damp and brilliant dark) were collected using latex gloves and stored individually in aluminium foil until analysis.

MP extraction and analysis

In the laboratory, pellets were transferred into glass Petri dishes and gently stirred using metal tweezers. Under a stereo microscope (Wild M3B Heerbrugg Switzerland, 6.4 X – 40 X), diagnostic undigested remains (i.e. skulls and jaws) were separated from the matrix for the identification of prey items using tweezers and needles. For the extraction of MPs, the matrix, including hair and residual bone remains, was chemically degraded using Fenton's reagent, a solution composed of hydrogen peroxide (H_2O_2 , 30%) and a ferrous sulphate (FeSO_4) solution (0.05 M). The reagent was prepared by dissolving 1.39 g of FeSO_4 granules (CARLO ERBA Reagents, France) in 100 mL of filtered Milli-Q water and adding 0.6 mL of concentrated sulphuric acid (H_2SO_4). Each sample was added with 20 mL of ferrous sulphate solution and 20 mL of H_2O_2 . When the reaction slowed down, further 20 mL of H_2O_2 were added, and samples were heated in an oven at 50°C for one hour. Temperature plays a major role in increasing the efficiency of the digestive action of H_2O_2 (Avio et al., 2015). Nonetheless, to preserve plastic materials, temperature must not exceed 60°C (Cowger et al., 2020; Lusher et al., 2020). When the solutions cooled down, 40 mL of H_2O_2 were added, and the solutions were kept at room temperature for a minimum of 48 hours. Then, MPs were separated from undigested remains by density separation, using a saturated sodium chloride solution (7.2 g of NaCl - Sigma Aldrich, Germany - per 20 mL of solution; 1.2 g/cm³), and following the method proposed by Thompson et al. (2004), with some minor modifications (classical setup; Crawford and Quinn, 2017). The process aims to make particles less dense than the solution, including MPs, to float, as to collect them by decanting the liquid above the layer of denser sediment (supernatant). Briefly, solutions were heated up at 50°C and stirred with a magnet for ca. 3 minutes, until the salt dissolved completely. Samples were left at room temperature for three hours, to promote density separation. During both stirring and settling flasks were covered with aluminium foils to prevent contamination. Successively, the supernatant was poured and filtered on cellulose membranes with a pore diameter of 20 µm (StonyLab, Cina), using an in-house manufactured glass filtration apparatus. We chose cellulose membranes as they were successfully used in previous studies for different matrices (Bakir et al., 2020; Bour et al., 2018; Wiggin and Holland, 2019; Han et al., 2020), while pore size prevented the clogging of the filter. Finally, cellulose filters were visually inspected using the stereo microscope, and potential plastic items were transferred using metal tweezers onto silver membrane filters (Sterlitech, 0.8-µm pore size, 13-mm diameter), which were stored in glass Petri dishes until analysis.

M-FTIR analysis and polymer identification

To identify polymers, micro-Fourier Transform Infrared Spectroscopy (µ-FTIR) was applied to each isolated item suspected to be MPs. Using a Nicolet In5 instrument and a line detector, analyses were carried out by adopting the reflection acquisition mode in a wavenumber range of 4000 – 550 cm⁻¹. A total of 128 scans

were taken for each spectrum, with a spectral resolution of 2 cm^{-1} . The optimal detection limit of the instrument was $20\text{ }\mu\text{m}$. Spectral acquisition, analysis and library research were performed using OMNIC Spectra software. At least four spectra were recorded for each suspected MP item. The IR absorbance was compared with spectra in the software database, recording the best match ($> 70\%$) of each final μ -FTIR spectrum with the library. Six items with a match in the range $60 - 70\%$ were also recorded as polymers due to the presence of all characteristic peaks.

Confirmed MP polymers were subsequently measured (Feret max diameter) using the imaging software ImageJ and classified according to shape and colour.

Quality control and assessment

Quality control precautions are crucial during sampling and laboratory analysis to prevent MP contamination from the environment, operators, instruments and reagents (Cowger et al., 2020; Winkler et al., 2020). In the field, to investigate potential MP contamination caused by surveyors, two aluminium foils per sample site ($n = 6$) were placed next to the detected pellets for the duration of sample collection and then folded and stored in the same way as pellets. To account for long-term atmospheric deposition, we also included two field blank samples per site, consisting of the upper part of the sediment ($5 \times 5\text{ cm}$) on which pellets had been ejected. As the sediment of site B consisted entirely of decayed/disintegrated pellets, this procedure was possible in sites A and C only. To account for potential airborne MP contamination in the laboratory, we also included two laboratory blanks per site. All blanks were treated and analysed in the same way as pellet samples. Furthermore, plastic-free materials, including non-synthetic clothing, were used during both field collection and laboratory analyses. In the laboratory, both working surfaces and equipment were cleaned using ethanol and white cellulose tissues after examining each pellet.

To evaluate the efficiency of the extraction and purification methodology, a mass recovery test was performed using either low or high-density polymers, as described in Winkler et al. (2022). Briefly, polystyrene (PS) standard pellets (INEOS Styrolution PS 124N/L, Frankfurt am Main, Germany) and polyethylene terephthalate (PET) from water bottles, cryomilled and sieved to a $60\text{ }\mu\text{m} - 2,000\text{ }\mu\text{m}$ fraction, were used. The mean (\pm SD) recovery rate was $98.2 \pm 1.0\%$ ($n = 3$) and $47.7 \pm 28.7\%$ ($n = 3$) for PS and PET fragments, respectively. Electron microscope analysis (Zeiss LEO 1430 SEM) on the integrity of plastic materials before and after all analytical steps confirmed that the impact on MPs of chemical digestion can be considered negligible.

Diet analysis

The identification of prey species and estimation of the number of prey items were based on the morphology of skulls, jaws and teeth, following the keys of Nappi (2001) and Amori et al. (2008).

Diet composition was expressed as frequency of occurrence ($F\% = \text{number of samples containing a specific prey item divided by the total number of pellets} \times 100$), relative frequency of occurrence ($rF\% = \text{number of occurrences of any prey item divided by the total number of items} \times 100$), estimated per cent volume ($V\% = \text{total estimated volume of each prey item as ingested divided by the number of pellets containing that item}$) and mean per cent mean volume ($mV\% = \text{total estimated volume of each prey item as ingested divided by the total number of pellets}$). The latter outlines the proportional contribution of each prey item to the overall diet (Kruuk and Parish, 1981).

To assess the relative importance of each prey item in terms of biomass, the 'live-weight equivalent' of small mammals was calculated using the average prey weights of individuals sampled in Lombardy region (Prigioni et al., 2001).

Statistical analysis

Analyses were performed using R 3.6.1. The relationship between the number of MP items and either the number of preyed specimens or total biomass per pellet was tested by Spearman's correlation, using the

“cor.test” function of the “corrplot” package (Wei et al., 2017). Using the “glmmTMB” package (Brooks et al., 2017), generalized linear mixed models (GLMM, family = Poisson) were run to explore the effects of prey type on MP ingestion, testing the number of MPs per pellet and the ‘live-weight equivalent’ of the four most predated species, to understand if there is a relationship between the presence of microplastics and the type of prey.

Raw frequency data of prey items and MPs were compared among sampling sites by the chi-squared test (χ^2).

Principal Components Analysis (PCA) was used to describe the main sources of variation in the barn owl diet in the three study sites. PCA was performed on an arcsine transformed 3 x N matrix, with the main food items (accounting for more than 95% of the overall diet), number and biomass of prey species and number of MPs in barn owl pellets as active variables, and sampling site as supplementary variable.

Results

Out of 228 items isolated from the 60 analysed pellets, 34 were confirmed as MPs by μ -FTIR analysis. The other items suspected to be MPs turned out to be other organic materials, mainly cellulose and cotton fibres. Confirmed MPs were detected in 20 pellets (33%), with 1 – 5 MPs per pellet. MPs were found in pellets from all sites: 12 MPs in 8 (40%) pellets from site A, 16 in 9 (45%) pellets from site B and 6 in 3 (15%) pellets from site C. The frequency of occurrence of contaminated pellets differed among sites; samples from site C were less contaminated (site C-site B, $\chi^2 = 4.29$, $P = 0.038$; site C-site A, $\chi^2 = 3.13$, $P = 0.076$). Polyester (PET, polyethylene terephthalate; Fig. 2A) was the most frequently recorded polymer (41.2%), followed by polyacrylonitrile (PAN, 26.5%; Fig. 2B), polyamide (PA, 14.7%), PE (8.8%), PP (5.9%) and PS (2.9%). No significant difference in the frequency of occurrence of polymers was recorded among the three sites ($\chi^2 = 0.9$ – 3.9 , 2 df, $P > 0.1$ for all comparisons). Most MPs were bright coloured (light blue 23.5%; red 17.6%; pink 8.8%; blue 8.8%; green 8.8%; yellow 2.9%), while the rest was black fibres (29.4%).

About 50% of ingested MPs were in the range 0.5 – 2.0 mm (Fig. 3). Microfibrils (mean length \pm SD = 2.4 \pm 2.2; min-max: 0.3 – 10.4 mm, diameter = 10 – 40 μ m) accounted for 88.2% of MPs ($n = 30$). Four fibres were longer than 5 mm and should be recorded as mesoplastics. The mean (\pm SD) size of irregularly shaped fragments ($n = 4$) was 0.71 \pm 0.64 mm. All the spectra of detected MPs are reported as supplementary materials.

No MP was detected in any blank sample, while four cotton fibres were found in as many samples (three field blank samples and one lab sample), probably shed from clothes.

Diet analysis allowed us to identify a total of 205 small mammals, belonging to nine species and three families: Muridae, Cricetidae and Soricidae (Tab. 2, Fig. 4). The small mammal species most commonly found in barn owl pellets was *Apodemus sylvaticus* ($n = 93$; mV% = 49.4), followed by *Crocidura leucodon* ($n = 47$; mV% = 12), *Microtus savii* ($n = 34$; mV% = 16.1) and *Rattus norvegicus* ($n = 12$; mV% = 17.3). The mean number of prey items (\pm SE) per pellet was 3.5 \pm 1.09 (min-max: 1 – 10), corresponding to a mean ingested biomass per pellet of 110 \pm 111.8 g (min-max: 10.5 – 569.5 g). Barn owl diet differed among sampling sites ($\chi^2 = 3.3$, 2 df, $P = 0.04$), particularly for omnivores, rats and house mice, which were rarely preyed on in site C with respect to the other two sites. There is no relationship between number of MPs and either the number of preyed specimens (Spearman correlation: $r = -0.19$, $P = 0.16$) or total biomass per pellet ($r = 0.03$, $P = 0.82$). The number of MP per pellets was inversely related to the total ‘live-weight equivalent’ of *Microtus savii* ($P = 0.01$), while no significant relationship was found with the other major prey species: *Crocidura leucodon*, *Apodemus sylvaticus*, *Rattus norvegicus*.

The first two eigenvalues of PCA represented 58.2% of the initial variability. Sites A and B were linked to predation on *R. norvegicus*, resulting in high biomass content of pellets and MP occurrence, while site C was characterised by higher prey diversity (Fig. 5).

Discussion

Among terrestrial ecosystems, agricultural areas are particularly exposed to MP pollution (Campanale et al., 2022). Top predators occupying large home ranges may represent effective indicators for assessing MP bioconcentration and biomagnification and monitoring of environmental contamination at the landscape scale (Carlin et al., 2020).

Our results confirm the occurrence of MPs in the environment and suggest the transfer of these contaminants through the food chain. The adopted analytical methodology can be considered very conservative in terms of MP overestimation, as demonstrated by the low percentage of suspected plastic polymers confirmed by μ -FTIR analysis. This technique is essential for MP identification, as cotton, wool, and cellulose items can be easily mistaken for MPs by the eye (Lusher et al., 2020a, b; Primpke et al., 2017). Previous studies have recorded positive identification rates of putative MPs ranging from 20% to 98.33% (Lenz et al., 2015; Tagg et al., 2015; Ivleva et al., 2017; Winkler et al., 2022), highlighting the importance of supplementing visual identification with spectroscopic analysis. However, the high occurrence of non-plastic fragments in barn owl pellets further confirms the widespread transfer of anthropogenic materials through the food web.

Agricultural activities and traffic enhance the break-down of MP debris into small fragments, also generating nanoplastics (Wang et al., 2021). As our method of analysis allowed us to recover only a few particles in the range of 1 – 100 μ m (the smallest item detected by the eye was 70 μ m), due to the large number of hairs and organic material contained in every pellet, the recorded MP number was probably underestimated, especially for the smaller ones.

Consistently with previous studies (Carlin et al., 2020), in our study area fibres were by far the dominant contaminants, suggesting that, as recorded for freshwaters and oceans (Browne et al., 2011; Claessens et al., 2011; Hu et al., 2018), fibres are also widespread in terrestrial habitats. Blue and black were the most frequent colours of the MPs ingested by the barn owl, which, interestingly, agrees with the findings by Zhu et al. (2019), suggesting that small mammals may be particularly exposed to the ingestion of these MPs. As available data, recorded in the mid-west Pacific by Wang et al. (2020), indicate that white and transparent MPs may be the most spread in the environment, these results suggest that either blue and dark fragments are more attractive to small mammals (i.e. mistaken for food or used for insulating nests and unintentionally ingested), or transparent and pale-coloured fibres, being less visible under the stereomicroscope, may be under-recorded. Most detected fibres consisted of PET and PAN, polymers typically used for clothing and home textiles. This is an indication that in our study area, a major source of MP might be atmospheric deposition since the largest portion of MP in the atmosphere consists of fibres (Henry et al., 2019), and synthetic clothing has been reported as the largest source of airborne MP (mostly polyester; Dris et al., 2016). We acknowledge that contamination by dense polymers, such as polyvinyl chloride (PVC, 1.16 – 1.58 g/cm³) and polyethylene terephthalate (PET, 1.37 – 1.45 g/cm³), may have been underestimated using NaCl as floatation medium (Radford et al., 2021); on the other hand, this method is currently recommended by international guidelines due to its effectiveness and safety (Cutroneo et al., 2021). Based on recovery tests (recovery rate of PET fragments = 48%), we estimate that the number and occurrence of denser polymers ranged between 50 and 100%, depending on shape and density. However, to avoid overestimating MP contamination, we adopted a prudential approach and did not correct the concentrations of polymers with a low recovery rate.

Variation in MP contamination among sites could not be directly associated with the consumption of any species, possibly due to the small sample size. However, barn owl diet in the two sites where MP occurrence was the highest (A and B) was characterised by predation on rats and a significantly higher frequency of occurrence of synanthropic omnivores compared to site C. Although this greater consumption of rats by barn owls was not associated with the percent cover of urban areas (see Hindmarch and Elliott, 2015), it may be indicative of an intensification of land use and habitat degradation as a whole (Wells et al., 2014), exposing barn owl's prey to increased availability of plastic waste. Although we acknowledge that our results need to be tested by the analysis of larger numbers of samples, they suggest that barn owl prey may be a more effective indicator of exposure to contaminants than land cover *per se*.

Furthermore, the negative correlation between MP concentration and the biomass of strictly herbivorous Savi's vole in barn owl pellets indicates that, as recorded for other contaminants (Dimitrov et al., 2016; Komov et al., 2017), herbivorous rodents may be less exposed to MP ingestion than omnivorous (wood mouse and brown rat) and small insectivorous mammals.

Most studies on plastic ingestion by predators have been carried out by analysing one-off samples, such as the stomach contents of dead animals. However, this method is not free from bias due to the small sample size (Zhao et al., 2016) or both spatial and temporal heterogeneity (i.e., stomachs need to be collected over large areas or periods to get an adequate sampling size; Carlin et al., 2020), and poses ethical questions whenever animals are appositely euthanised (Sherlock et al., 2022). On the contrary, routinary pellet analysis may provide useful indications on lifetime exposure to contaminants. Barn owl pellets contain more undigested remains (hair) than fish specialist predators, making the identification of anthropogenic materials more time-consuming. However, once roosting sites have been mapped, pellets can be easily found in large numbers, as the habit of barn owls of roosting in man-made constructions enhances their long-term preservation.

Conclusions

This study represents the first assessment of MP occurrence in the diet of a terrestrial nocturnal raptor through the analysis of pellets. Barn owls' habits, including territoriality, roost fidelity and diet specialisation, make them suitable indicators of MP contamination in agricultural landscapes.

Diet data suggest that small mammals may transfer MPs to top predators through a strictly terrestrial food chain and substantiate the claim by a previous study on kingfisher diet (Winkler et al., 2020) that the analysis of pellets may be a promising, non-invasive and cost-effective tool for monitoring large areas and assessing MP contamination levels in terrestrial ecosystems. While land cover has been reported to be a good indicator of both air (Yang and Jiang, 2021) and water quality (Julian et al., 2017), our results suggest that MP abundance may depend on point sources (i.e., agricultural practices) which GIS-based models may overlook.

However, despite the rapidly growing body of available literature on the fate and effects of MPs in the environment, we are far from understanding the effects of this type of pollution on food webs (Baho et al., 2021). Hence, to better quantify concentrations and trends, it is pivotal to monitor these contaminants in different environmental matrices and develop cost-effective methodologies for MP detection at regional or national scales. We strongly suggest the need for multi-matrix monitoring protocols, including, for instance, the analysis of soils, earthworms, small mammals and vertebrate predators (faecal samples of carnivorous mammals and pellets of prey birds). Such studies may allow assessing contamination pathways, and the role played by feeding habits in determining exposure to MPs.

Acknowledgements We are grateful to Giulio Formenton (Parco Adda Sud) for helping with fieldwork. J. Lovejoy kindly revised the English language.

References

- Acampora, H., Berrow, S., Newton, S., O'Connor, I., 2017a. Presence of plastic litter in pellets from Great Cormorant (*Phalacrocorax carbo*) in Ireland. *Mar. Pollut.* 117(1-2), 512-514.
- Acampora, H., Newton, S., O'Connor, I., 2017b. Opportunistic sampling to quantify plastics in the diet of unfledged Black Legged Kittiwakes (*Rissa tridactyla*), Northern Fulmars (*Fulmarus glacialis*) and Great Cormorants (*Phalacrocorax carbo*). *Mar. Pollut.* 119(2), 171-174.
- Almroth, B.M.C., Åström, L., Roslund, S., Petersson, H., Johansson, M., Persson, N.K., 2018. Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment. *Environ. Sci. Pollut. Res.* 25(2), 1191-1199.

- Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. *Environ. Pollut.* 223, 223e229.
- Amori, G., Contoli, L., Nappi, A., 2008. Mammalia II: Erinaceomorpha, Soricomorpha, Lagomorpha, Rodentia. Vol. XLIV. Fauna d'Italia. Edizioni Calderini, Il Sole-24 Ore, Milano.
- Avio, C. G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization characterisation of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. *Mar. Environ.* 111, 18-26.
- Baho D.L., Bundschuh M., Fütter M.N., 2021. Microplastics in terrestrial ecosystems: Moving beyond the state of the art to minimize the risk of ecological surprise. *Glob. Change Biol.* 27, 3969–3986.
- Bakir, A., Desender, M., Wilkinson, T., Van Hoytema, N., Amos, R., Airahui, S., Graham, J., Maes, T., 2020. Occurrence and abundance of meso and microplastics in sediment, surface waters, and marine biota from the South Pacific region. *Mar. Pollut. Bull.* 160, 111572.
- Balestrieri, A., Gazzola, A., Formenton, G., Canova, L., 2019. Long-term impact of agricultural practices on the diversity of small mammal communities: a case study based on owl pellets. *Environ. Monit. Assess.* 191(12), 1-14.
- Bläsing, M., Amelung, W., 2018. Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.* 612, 422-435.
- Boerger, C. M., Lattin, G. L., Moore, S. L., Moore, C. J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Mar. Pollut.* 60(12), 2275-2278.
- Bosè, M., Guidali, F., 2001. Seasonal and geographic differences in the diet of the barn owl in an agro-system in northern Italy. *J. Raptor Res.* 35, 240–246.
- Bour, A., Avio, C. G., Gorbi, S., Regoli, F., Hylland, K., 2018. Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level. *Environ. Pollut.* 243, 1217-1225.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. “glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling.” *R. J.* 9(2), 378–400.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* 45(21), 9175–9179.
- Büks, F., Kaupenjohann, M., 2020. Global concentrations of microplastics in soils—a review. *Soil* 6(2), 649-662.
- Cai, H., Chen, M., Chen, Q., Du, F., Liu, J., Shi, H., 2020. Microplastic quantification affected by structure and pore size of filters. *Chemosphere* 257, 127198.
- Campanale, C., Galafassi, S., Savino, I., Massarelli, C., Ancona, V., Volta, P., Uricchio, V.F., 2022. Microplastics pollution in the terrestrial environments: Poorly known diffuse sources and implications for plants. *Sci. Total Environ.*, 805, 150431.
- Carlin, J., Craig, C., Little, S., Donnelly, M., Fox, D., Zhai, L., Walters, L., 2020. Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA. *Environ. Pollut.* 264, 114633.
- Carr, S. A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* 91, 174-182.
- Chae, Y., An, Y.J., 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. *Environ. Pollut.* 240, 387–395.
- Chen, Y., Leng, Y., Liu, X., Wang, J., 2020. Microplastic pollution in vegetable farmlands of suburb Wuhan, central China. *Environ. Pollut.* 257, 113449.

- Choy, C.A., Drazen, J.C., 2013. Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. *Mar. Ecol. Prog. Ser.* 485, 155-163.
- Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull.* 62(10), 2199e2204.
- Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* 62 (12), 2588e2597.
- Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Sci. Total Environ.* 671, 411-420.
- Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C., Athey, S.N., Lynch, J.M., De Frond, H., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020. Reporting guidelines to increase the reproducibility and comparability of research on microplastics. *Appl. Spectrosc.* 74(9), 1066-1077.
- Crawford, C.B., Quinn, B., 2017. *Microplastic Pollutants*. Elsevier Science Publishing Co Inc.
- Crossman, J., Hurley, R.R., Futter, M., Nizzetto, L., 2020. Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. *Sci. Total Environ.* 724, 138334.
- Cutroneo, L., Reboa, A., Geneselli, I., Capello, M., 2021. Considerations on salts used for density separation in the extraction of microplastics from sediments. *Mar. Pollut. Bull.* 166, 112216.
- de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Chang. Biol.* 24, 1405-1416.
- de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Gorlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. *Environ. Sci. Technol.* 53, 6044–6052.
- D'Souza, J.M., Windsor, F.M., Santillo, D., Ormerod, S.J., 2020. Food web transfer of plastics to an apex riverine predator. *Glob. Chang. Biol.* 26, 3846–3857.
- Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Sci. Rep.* 7, 46687.
- Dimitrov, H.A., Mitkovska, V.I., Tzekov, V.D., Chassovnikarova, T.G., 2016. Bioaccumulation of Cadmium and Lead in Rodent Species from the Region of Lead-Zinc Smelting Factory–Plovdiv (South Bulgaria). *Ecol. Balk.* 8(1), 9-18.
- Donohue, M.J., Masura, J., Gelatt, T., Ream, R., Baker, J. D., Faulhaber, K., Lerner, D.T., 2019. Evaluating exposure of northern fur seals, *Callorhinus ursinus*, to microplastic pollution through fecal analysis. *Mar. Pollut. Bull.* 138, 213-221.
- Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? *Mar. Pollut. Bull.* 104(1-2), 290-293.
- English, M.D., Robertson, G. J., Avery-Gomm, S., Pirie-Hay, D., Roul, S., Ryan, P. C., Mallory, M. L., 2015. Plastic and metal ingestion in three species of coastal waterfowl wintering in Atlantic Canada. *Mar. Pollut. Bull.* 98(1-2), 349-353.

- Esselink H., van der Geld F.M., Jager L.P., Posthuma-Trumpie G.A., Zoun P.E.F., Baars A.J., 1995. Biomonitoring heavy metals using the barn owl (*Tyto alba guttata*): sources of variation especially relating to body condition. Arch. Environ. Contam. Toxicol. 28, 471-486.
- Evangelidou, N., Grythe, H., Klimont, Z., Heyes, C., Eckhardt, S., Lopez-Aparicio, S., Stohl, A., 2020. Atmospheric transport is a major pathway of microplastics to remote regions. Nat. Commun. 11(1), 1-11.
- Frey C., Sonnay C., Dreiss A., Roulin A., 2011. Habitat, breeding performance, diet and individual age in Swiss Barn Owls (*Tyto alba*). J. Ornithol. 152: 279–290.
- Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3(7), e1700782.
- Gil-Delgado, J. A., Guijarro, D., Gosálvez, R. U., López-Iborra, G.M., Ponz, A., Velasco, A., 2017. Presence of plastic particles in waterbirds faeces collected in Spanish lakes. Environ. Pollut. 220, 732-736.
- Guilhermino, L., Vieira, L. R., Ribeiro, D., Tavares, A.S., Cardoso, V., Alves, A., Almeida, J.M., 2018. Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*. Sci. Total Environ. 622, 1131-1142.
- Hammer, S., Nager, R.G., Johnson, P.C.D., Furness, R.W., Provencher, J.F., 2016. Plastic debris in great skua (*Stercorarius skua*) pellets corresponds to seabird prey species. Mar. Pollut. Bull. 103(1-2), 206-210.
- Han, M., Niu, X., Tang, M., Zhang, B.T., Wang, G., Yue, W., Kong, X., Zhu, J., 2020. Distribution of microplastics in surface water of the lower Yellow River near estuary. Sci. Total Environ. 707, 135601.
- Hashmi, M.Z. (Ed.), 2022. Microplastic Pollution: Environmental Occurrence and Treatment Technologies. Springer Nature.
- Heisler, L.M., Somers, C. M., Poulin, R. G., 2016. Owl pellets: a more effective alternative to conventional trapping for broad-scale studies of small mammal communities. Methods Ecol. Evol. 7, 96–103.
- Henry, B., Laitala, K., Klepp, I. G., 2019. Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. 652, 483-494.
- Hernandez, L. M., Yousefi, N., Tufenkji, N., 2017. Are there nanoplastics in your personal care products? Environ. Sci. Technol. Lett. 4(7), 280-285.
- Hindmarch, S., Elliott, J. E., 2015. A specialist in the city: the diet of Barn Owls along a rural to urban gradient. Urban Ecosyst. 18(2), 477–488.
- Holland, E. R., Mallory, M. L., Shutler, D., 2016. Plastics and other anthropogenic debris in freshwater birds from Canada. Sci. Total Environ. 571, 251-258.
- Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127-141.
- Hu, L., Chernick, M., Hinton, D.E., Shi, H., 2018. Microplastics in small waterbodies and tadpoles from Yangtze River Delta, China. Environ. Sci. Technol. 52, 8885–8893.
- Huang, Y., Liu, Q., Jia, W., Yan, C., Wang, J., 2020. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ. Pollut. 260, 114096.
- Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environ. Sci. and Technol. 50(5), 2685-2691.
- Hurley, R. R., Nizzetto, L., 2018. Fate and occurrence of micro (nano) plastics in soils: Knowledge gaps and possible risks. Curr. Opin. Environ. Sci. Health. 1, 6-11.

- 487 Iannilli, V., Pasquali, V., Setini, A., Corami, F., 2019. First evidence of microplastics ingestion in benthic
488 amphipods from Svalbard. *Environ. Res.* 179, 108811.
- 489 Ivleva, N.P., Wiesheu, A.C., Niessner, R., 2017. Microplastic in aquatic ecosystems. *Angew. Chem. Int. Ed.*
490 56(7), 1720-1739.
- 491 Janžekovič, F., Klenovšek, T., 2020. The biogeography of diet diversity of barn owls on Mediterranean islands.
492 *J. Biogeogr.* 47, 2353-2361.
- 493 Julian, J. P., Beurs, K. M. D., Owsley, B., Davies-Colley, R. J., Ausseil, A.-G.E., 2017. River water quality changes
494 in New Zealand over 26 years: Response to land use intensity. *Hydrol. Earth Syst. Sci.* 21, 1149–1171.
- 495 Kawecki, D., Nowack, B., 2019. Polymer-specific modeling of the environmental emissions of seven
496 commodity plastics as macro-and microplastics. *Environ. Sci. Technol.* 53(16), 9664-9676.
- 497 Kole, P.J., Löhr, A.J., Van Belleghem, F.G., Ragas, A.M., 2017. Wear and tear of tyres: a stealthy source of
498 microplastics in the environment. *Int. J. Environ. Res. Public Health* 14(10), 1265.
- 499 Komov, V.T., Ivanova, E.S., Poddubnaya, N. Y., Gremyachikh, V.A., 2017. Mercury in soil, earthworms and
500 organs of voles *Myodes glareolus* and shrew *Sorex araneus* in the vicinity of an industrial complex in
501 Northwest Russia (Cherepovets). *Environ. Monit. Assess.* 189(3), 104.
- 502 Kruuk H, Parish T., 1981. Feeding specialization specialisation of the European badger *Meles meles* in
503 Scotland. *J. Animal. Ecol.* 773–788.
- 504 Lahive, E., Walton, A., Horton, A.A., Spurgeon, D.J., Svendsen, C., 2019. Microplastic particles reduce
505 reproduction in the terrestrial worm *Enchytraeus crypticus* in a soil exposure. *Environ. Pollut.* 255, 113174.
- 506 Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M., Nielsen, T.G., 2015. A critical assessment of visual
507 identification of marine microplastic using Raman spectroscopy for analysis improvement. *Mar. Pollut. Bull.*
508 100(1), 82-91.
- 509 Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X., He, D., 2018. Microplastic and
510 meso plastic pollution in farmland soils in suburbs of Shanghai. China. *Environ. Pollut.* 242, 855–862.
- 511 Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., Soudant, P., 2017. Interactions
512 between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation.
513 *Environ. Pollut.* 228, 454-463.
- 514 Love, R.A., Webon, C., Glue, D.E., Harris, S., 2000. Changes in the food of British barn owls (*Tyto alba*) between
515 1974 and 1997. *Mammal Rev.* 30, 107–129.
- 516 Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and
517 hepatic lipid metabolism disorder in mice. *Sci. Total Environ.* 631, 449-458.
- 518 Lusher, A. L., Hernandez-Milian, G., Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in
519 cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. *Environ.*
520 *Pollut.* 232, 467-476.
- 521 Lusher, A. L., Mchugh, M., Thompson, R. C., 2013. Occurrence of microplastics in the gastrointestinal tract of
522 pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* 67(1-2), 94-99.
- 523 Lusher, A.L., Brâte, I.L.N., Munno, K., Hurley, R. R., Welden, N.A., 2020. Is it or isn't it: the importance of visual
524 classification in microplastic characterization. *Appl. Spectrosc.* 74(9), 1139-1153.
- 525 Lusher, A.L., Munno, K., Hermabessiere, L., Carr, S., 2020. Isolation and extraction of microplastics from
526 environmental samples: an evaluation of practical approaches and recommendations for further
527 harmonization. *Appl. Spectrosc.* 74(9), 1049-1065.

- 528 Lv, W., Zhou, W., Lu, S., Huang, W., Yuan, Q., Tian, M., Lv, W., He, D., 2019. Microplastic pollution in rice-fish
529 co-culture system: a report of three farmland stations in Shanghai, China. *Sci. Total Environ.* 652, 1209–
530 1218.
- 531 Lwanga, E.H., Vega, J. M., Quej, V.K., de los Angeles Chi, J., del Cid, L S., Chi, C., Escalona Segura, G., Gertsen,
532 H., Salánki, T., van der Ploeg, M., Koelmans, A. A., Geissen, V., 2017. Field evidence for transfer of plastic
533 debris along a terrestrial food chain. *Sci. Rep.* 7(1), 1-7.
- 534 Massa, C., Gabelli, F., Cueto G.R., 2015. Using GPS tracking to determine movement patterns and foraging
535 habitat selection of the common barn-owl (*Tyto alba*). *Hornero* 30, 7-12.
- 536 Masura, J., Baker, J., Foster, G., Arthur, C., and Herring, C (technical editor). 2015. Laboratory methods for
537 the analysis of microplastics in the marine environment: recommendations for quantifying synthetic
538 particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48.
- 539 Meek, W.R., Burman, P.J., Sparks, T.H., Nowakowski, M., Burman, N.J., 2012. The use of barn owl *Tyto alba*
540 pellets to assess population change in small mammals. *Bird Study* 59, 166–174.
- 541 Moller, J.N., Loder, M.G.J., Laforsch, C., 2020. Finding microplastics in soils: a review of analytical methods.
542 *Environ. Sci. Technol.* 54, 2078–2090.
- 543 Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing
544 machines: Effects of fabric type and washing conditions. *Mar. Pollut. Bull.* 112(1-2), 39-45.
- 545 Nappi, A., 2011. L'analisi delle borre degli uccelli: metodiche, applicazioni e informazioni. Un lavoro
546 monografico. *Picus* 37, 106-120.
- 547 Newton, I., Willie, I., Freestone, P., 1990. Rodenticides in British barn owl. *Environ. Pollut.* 68: 101–117.
- 548 Nizzetto, L., Langaas, S., Futter, M., 2016. Do microplastics spill on to farm soils? *Nature* 537(7621), 488.
- 549 Perpiña Castillo, C., Kavalov, B., Diogo, V., Jacobs-Crisioni, C., Batista e Silva, F., Baranzelli, C., Lavalley, C., 2018.
550 Trends in the EU agricultural land within 2015-2030. JRC113717, European Commission.
- 551 Pico, Y., Alfarhan, A., Barcelo, D., 2019. Nano-and microplastic analysis: Focus on their occurrence in
552 freshwater ecosystems and remediation technologies. *TrAC* 113, 409-425.
- 553 Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to
554 microplastics: an overview on possible human health effects. *Sci. Total Environ.* 702, 134455.
- 555 Prigioni, C., Cantini, M., Zilio, A., 2001. Atlante dei mammiferi della Lombardia. Regione Lombardia e
556 Università degli Studi di Pavia.
- 557 Primpke, S., Lorenz, C., Rascher-Friesenhausen, R., Gerdts, G., 2017. An automated approach for
558 microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis. *Anal. Methods* 9(9),
559 1499-1511.
- 560 Radford, F., Zapata-Restrepo, L. M., Horton, A. A., Hudson, M. D., Shaw, P.J., Williams, I. D., 2021. Developing
561 a systematic method for extraction of microplastics in soils. *Anal. Methods* 13(14), 1695-1705.
- 562 Reynolds, C., Ryan, P. G., 2018. Micro-plastic ingestion by waterbirds from contaminated wetlands in South
563 Africa. *Mar. Pollut. Bull.* 126, 330-333.
- 564 Rodriguez-Seijo, A., Lourenço, J., Rocha-Santos, T. A. P., Da Costa, J., Duarte, A. C., Vala, H., Pereira, R., 2017.
565 Histopathological and molecular effects of microplastics in *Eisenia andrei* Bouché. *Environ. Pollut.* 220, 495-
566 503.
- 567 Rogival, D., Scheirs, J., Blust, R., 2007. Transfer and accumulation of metals in a soil–diet–wood mouse food
568 chain along a metal pollution gradient. *Environ. Pollut.* 145, 516–528.
- 569 Roulin, A., 2016. Shrews and moles are less often captured by European Barn Owls *Tyto alba* nowadays than
570 150 years ago. *Bird Study* 63, 559-563.

- 571 Roulin, A., 2020. Barn Owls, Evolution and Ecology. Cambridge University Press, Cambridge.
- 572 Ryan, P. G., Fraser, M. W., 1988. The use of Great Skua pellets as indicators of plastic pollution in seabirds.
573 Emu 88(1), 16-19.
- 574 Sherlock, C., Fernie, K.J., Munno, K., Provencher, J., Rochman, C., 2022. The potential of aerial insectivores
575 for monitoring microplastics in terrestrial environments. Sci. Total Environ. 807, 150453.
- 576 Silva-Cavalcanti, J. S., Silva, J. D. B., de França, E. J., de Araújo, M. C. B., Gusmão, F., 2017. Microplastics
577 ingestion by a common tropical freshwater fishing resource. Environ. Pollut. 221, 218-226.
- 578 Smith, P.N., Cobb, G. P., Godard-Codding, C., Hoff, D., McMurry, S.T., Rainwater, T.R., Reynolds, K.D., 2007.
579 Contaminant exposure in terrestrial vertebrates. Environ. Pollut. 150, 41–64.
- 580 So, M.W.K., Vorsatz, L.D., Cannicci, S., Not, C., 2022. Fate of plastic in the environment: From macro to nano
581 by macrofauna, Environ. Pollut. 300, 118920
- 582 Sorensen R.M., Jovanović B., 2021. From nanoplastic to microplastic: A bibliometric analysis on the presence
583 of plastic particles in the environment. Mar. Pollut. Bull. 163: 111926.
- 584 Tagg, A. S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in
585 wastewater using focal plane array-based reflectance micro-FT-IR imaging. Anal. Chem. 87(12), 6032-6040.
- 586 Taylor, I.R., 1994. Barn owls. Predator–prey relationships and conservation. Cambridge Univ, Press,
587 Cambridge.
- 588 Thompson, R.C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., Russell A.E.,
589 2004. Lost at sea: Where is all the plastic? Science 304(5672), 838.
- 590 Van Schothorst, B., Beriot, N., Huerta Lwanga, E., Geissen, V., 2021. Sources of Light Density Microplastic
591 Related to Two Agricultural Practices: The Use of Compost and Plastic Mulch. Environments 8(4), 36.
- 592 Walkinshaw C., Lindeque P.K., Thompson R., Tolhurst T., Cole M. (2020). Microplastics and seafood: lower
593 trophic organisms at highest risk of contamination. Ecotoxicology and Environmental Safety, 190 , 110066.
- 594 Wang, S., Chen, H., Zhou, X., Tian, Y., Lin, C., Wang, W., Zhou, K., Zhang, Y., Lin, H., 2020. Microplastic
595 abundance, distribution and composition in the midwest Pacific Ocean. Environ. Pollut. 264, 114125.
- 596 Wang, J., Coffin, S., Sun, C., Schlenk, D., Gan, J., 2019. Negligible effects of microplastics on animal fitness and
597 HOC bioaccumulation in earthworm *Eisenia fetida* in soil. Environ. Pollut. 249, 776-784.
- 598 Wang, J., Li, J., Liu, S., Li, H., Chen, X., Peng, C., Zhang, P., Liu, X., 2021. Distinct microplastic distributions in
599 soils of different land-use types: a case study of Chinese farmlands. Environ. Pollut. 269, 116199.
- 600 Wang, L., Li, P., Zhang, Q., Wu, W. M., Luo, J., Hou, D., 2021. Modeling the conditional fragmentation-induced
601 microplastic distribution. Environ. Sci. and Technol. 55(9), 6012-6021.
- 602 Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., and Zemla, J., 2017. Package ‘corrplot’. Stat. 56, 316-324.
- 603 Wells, K., Lakim, M.B., O’Hara, R.B., 2014. Shifts from native to invasive small mammals across gradients from
604 tropical forest to urban habitat in Borneo. Biodiver. Conserv. 23: 2289–2303.
- 605 Wiggin, K.J., Holland, E.B., 2019. Validation and application of cost and time effective methods for the
606 detection of 3–500 µm sized microplastics in the urban marine and estuarine environments surrounding
607 Long Beach, California. Mar. Pollut. Bull. 143, 152-162.
- 608 Winkler, A., Nesi, A., Antonioli, D., Laus, M., Santo, N., Parolini, M., Tremolada, P., 2020. Occurrence of
609 microplastics in pellets from the common kingfisher (*Alcedo atthis*) along the Ticino River, North Italy.
610 Environ. Sci. Pollut. Res. 27(33), 41731-41739.

- 611 Winkler, A., Antonioli, D., Masseroni, A., Chiarcos, R., Laus, M., Tremolada, P., 2022. Following the fate of
612 microplastic in four abiotic and biotic matrices along the Ticino River (North Italy). *Sci. Total Environ.*
613 153638.
- 614 Xu, C., Zhang, B., Gu, C., Shen, C., Yin, S., Aamir, M., Li, F., 2020. Are we underestimating the sources of
615 microplastic pollution in terrestrial environment? *J. Hazard.* 400, 123228.
- 616 Yang, Y. F., Chen, C. Y., Lu, T. H., Liao, C. M., 2019. Toxicity-based toxicokinetic/toxicodynamic assessment for
617 bioaccumulation of polystyrene microplastics in mice. *J. Hazard. Mater.* 366, 703-713.
- 618 Yang, W., Jiang, X., 2021. Evaluating the influence of land use and land cover change on fine particulate
619 matter. *Sci. Rep.* 11, 17612.
- 620 Yang, L., Zhang, Y., Kang, S., Wang, Z., Wu, C., 2021. Microplastics in soil: A review on methods, occurrence,
621 sources, and potential risk. *Sci. Total Environ.* 780, 146546.
- 622 Zhao, S., Zhu, L., Li, D., 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not
623 only plastics but also natural fibers. *Sci. Total Environ.* 550, 1110-1115.
- 624 Zhou, Y., Wang, J., Zou, M., Jia, Z., Zhou, S., Li, Y., 2020. Microplastics in soils: A review of methods,
625 occurrence, fate, transport, ecological and environmental risks. *Sci. Total Environ.* 748, 141368.
- 626 Zhu, C., Li, D., Sun, Y., Zheng, X., Peng, X., Zheng, K., Hu, B., Luo, X., Mai, B., 2019. Plastic debris in marine
627 birds from an island located in the South China Sea. *Mar. Pollut. Bull.* 149, 110566.

Table 1. Area (m²) and percent cover of main land use categories in the three barn owl home ranges (2 km radius buffers).

Land use	Site A		Site B		Site C	
	area (m ²)	%	area (m ²)	%	area (m ²)	%
Woods	41385	0.30	51429	0.39	120189	0.88
Riparian woods	252736	1.81	1357771	10.32	601100	4.39
Poplar plantations	35633	0.25	2186334	16.62	362671	2.65
Grassland	1463494	10.5	967158	7.35	1088976	7.95
Arable crops	11076944	79.2	7877578	59.90	10514799	76.8
Orticultural crops and orchards	22704	0.16	0	0.00	11015	0.08
Abandoned areas	70639	0.51	171997	1.31	117265	0.86
Urban areas (residential areas and productive settlements)	1018302	7.28	539876	4.10	876755	6.40
TOTAL	13981837	100	13152142	100	13692770	100

630

631

632

633

634

635

Table 2. Barn owls' diet, expressed as number (N) and biomass (BM) of prey species and both in terms of frequency of occurrence (F%, rF%) and volume (V%, mV%).

Food items	N	BM (g)	F%	rF%	V%	mV%
Small mammals	205	6599	100	100	100	100
<i>Sorex antinorii</i>	3	22	2.5	0.7	11.6	0.6
<i>Crocidura leucodon</i>	47	493	19.7	10.7	30	12
<i>Crocidura suaveolens</i>	2	11	1.6	16.4	7.6	0.3
<i>Microtus arvalis</i>	1	30	0.8	0.3	9.5	0.2
<i>Microtus savii</i>	34	449	18.9	3.0	42	16.1
<i>Rattus norvegicus</i>	12	3354	9	3.0	94.4	17.3
<i>Mus domesticus</i>	5	80	4	1.0	22.3	1.9
<i>Apodemus sylvaticus</i>	93	2055	34.4	2.0	70.5	49.4
<i>Micromys minutus</i>	4	28	3.3	34.6	13.5	0.9
Unidentified rodents	4	76	3.3	3.4	22.6	1.5
Birds	3	-	5	2.5	-	-

638

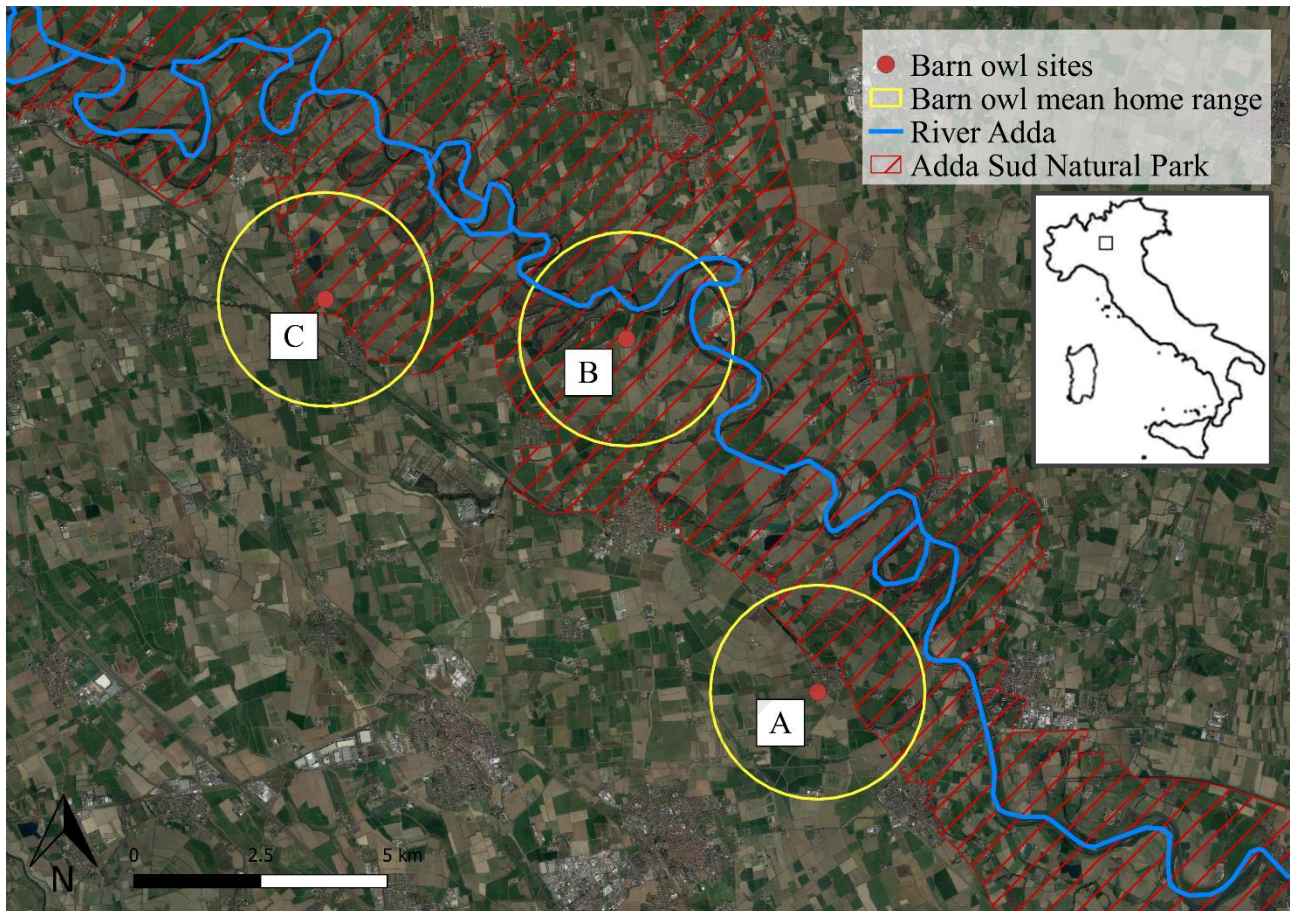
639

640

641

642

643



644

645

646

647

648

649

Figure 1. Study area, showing the location of the three barn owl's roosts and average home ranges (2 km radius buffer).

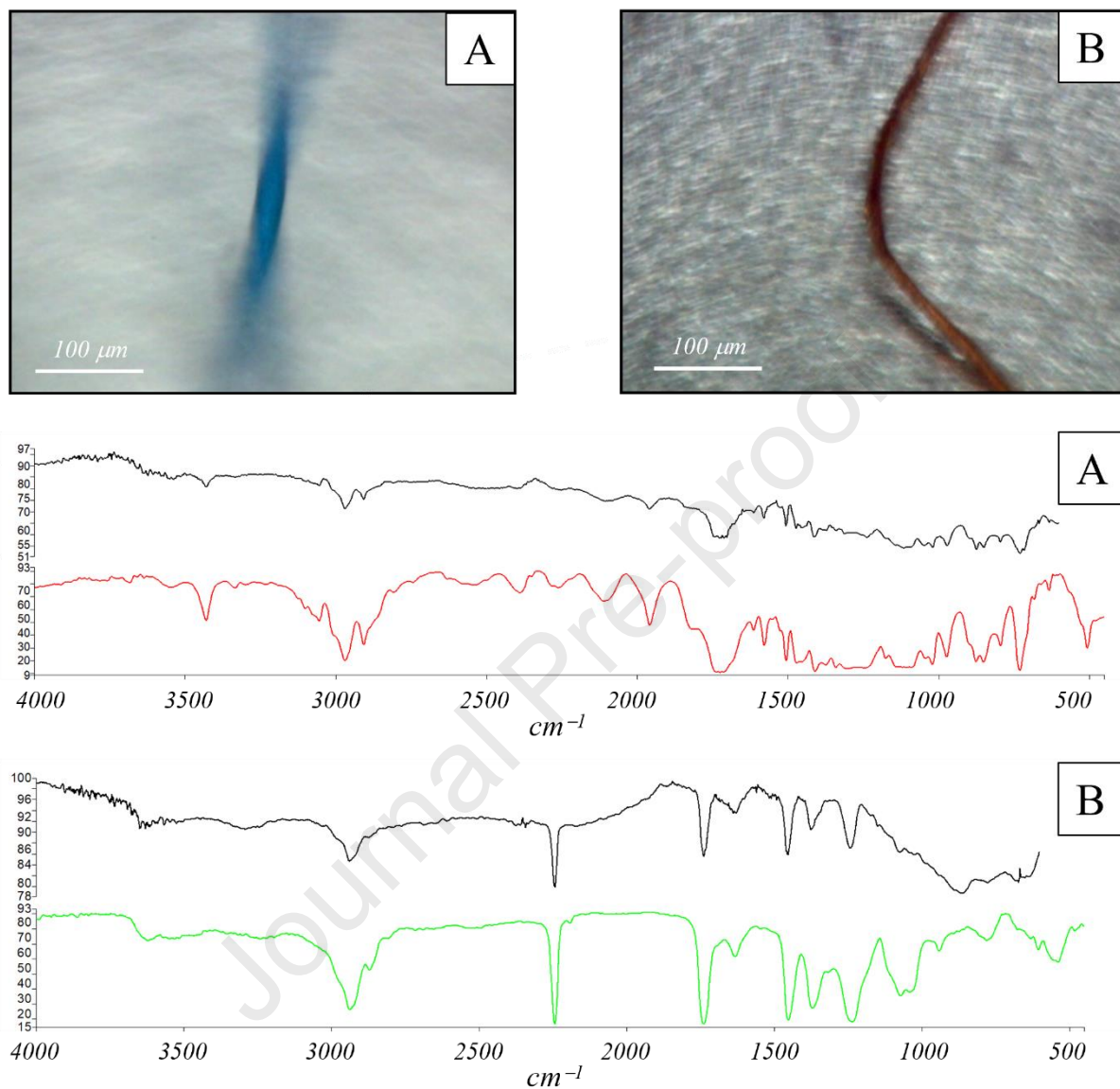
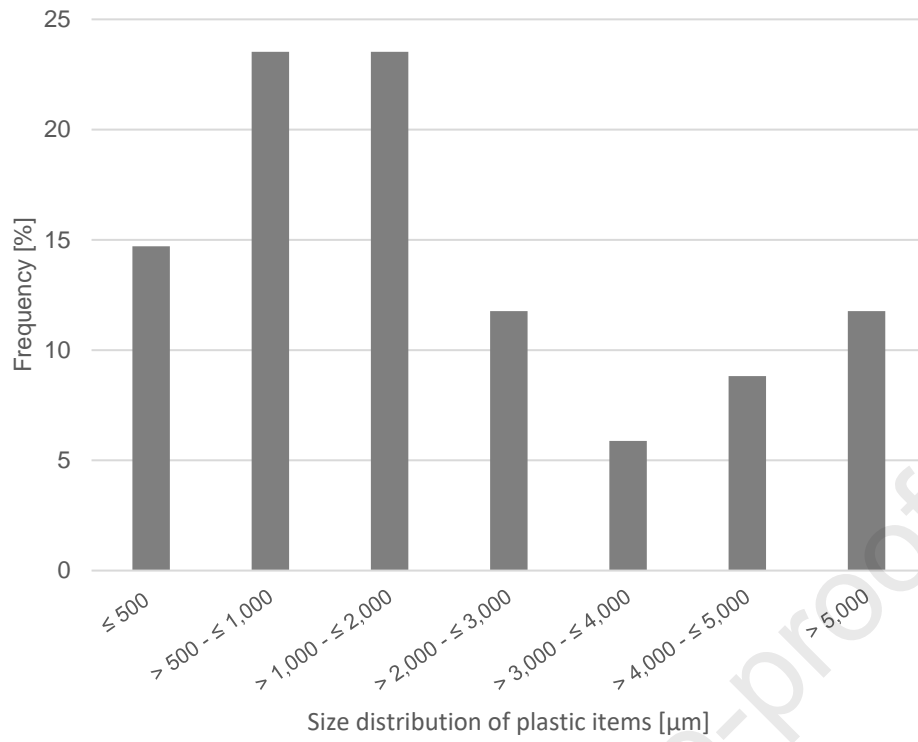


Fig. 2 Microscope images of two microplastics found in barn owl pellets and respective μ -FTIR spectra (black) with matches to reference polymer from the library (coloured spectra). A: polyester (PET) fibre (93% match); B: polyacrylonitrile (PAN) fibre (90% match). %T = % of transmittance; cm^{-1} = wavenumber.



661
662 Figure 3. Size distribution of the plastic items recorded in barn owl pellets.
663

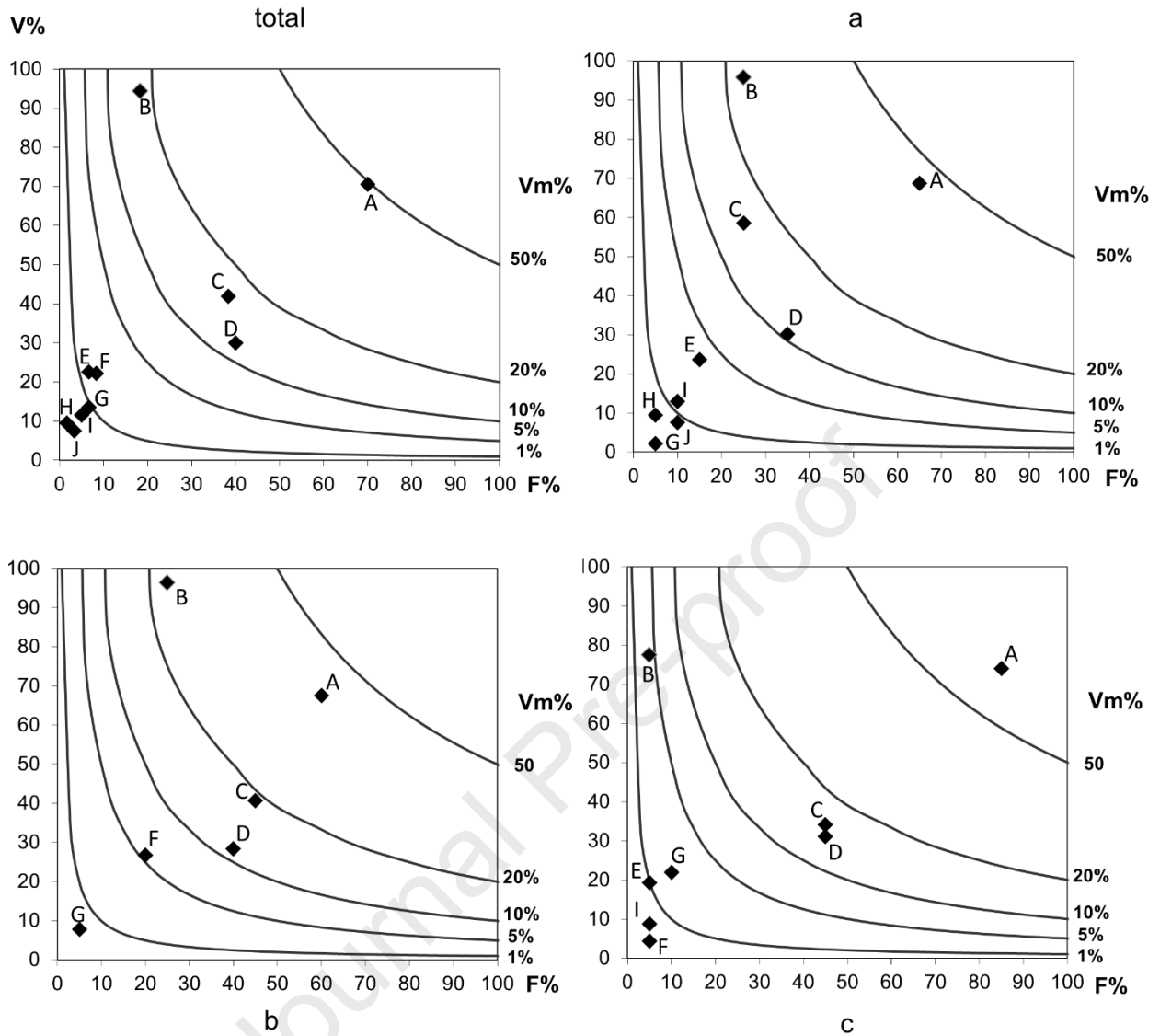
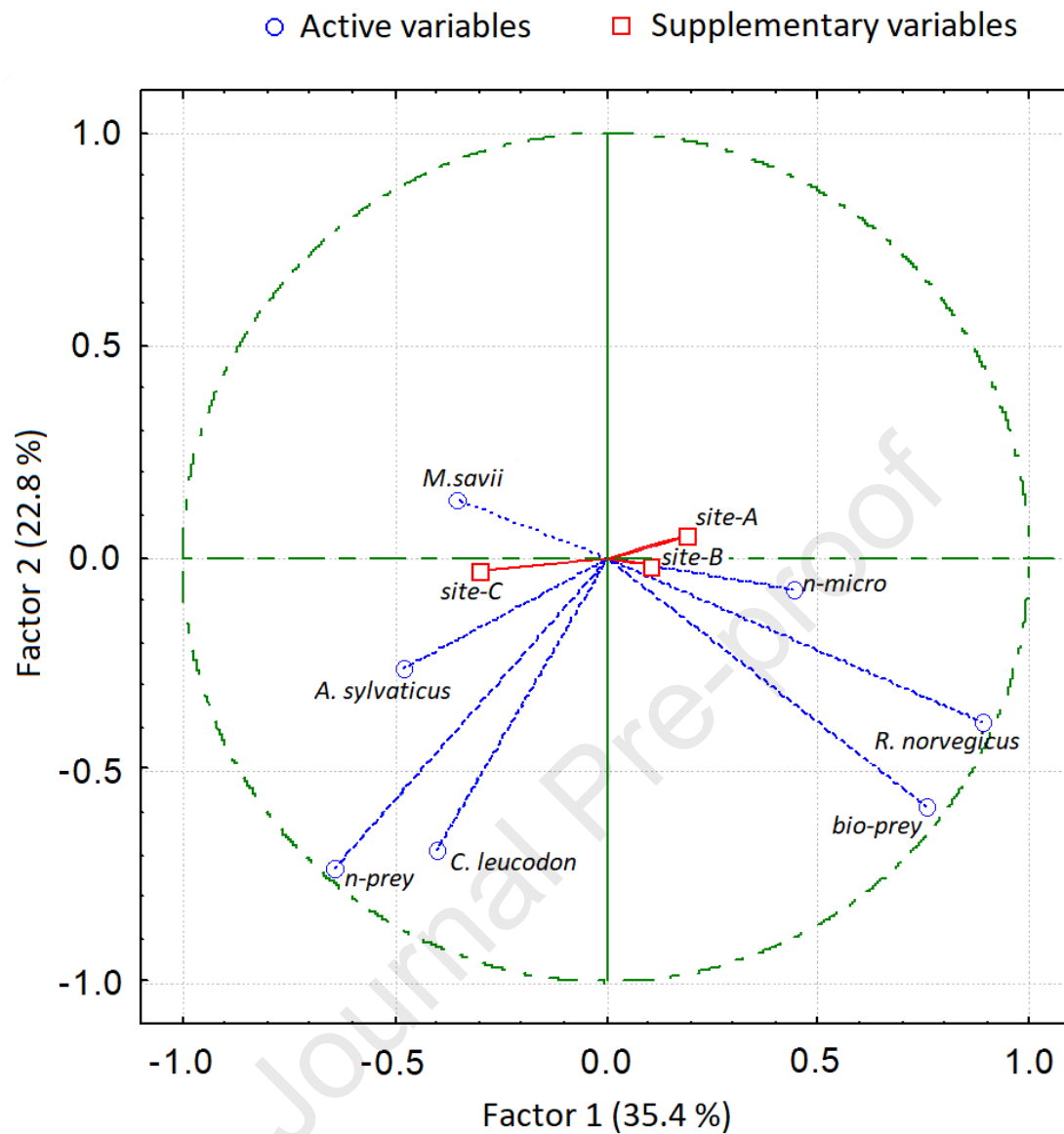


Figure 4. Estimated volume (V%) of main food categories, whenever eaten, vs. their frequency of occurrence (F%) for the overall diet of Barn owls and each of the three sites – a, b and c. Isopleths connect points of equal per cent mean volume in the diet (mV%). A = *Apodemus sylvaticus*; B = *Rattus norvegicus*; C = *Microtus savii*; D = *Crocidura leucodon*; E = unidentified rodents; F = *Mus domesticus*; G = *Micromys minutus*; H = *Microtus arvalis*; I = *Sorex antinorii*; J = *Crocidura suaveolens*.



671

672 Figure 5. Plot of barn owl diet in relation to the first two PCA values extracted from biomass data of 205 small
 673 mammals preyed on in the three sites (A, B, C). Items are represented by lines that point towards the
 674 direction of maximum variation of each factor.

Highlights

- Barn owls are suitable indicators of MP contamination of terrestrial habitats
- Pellet analysis may be a cost-effective and non-invasive method for monitoring MPs
- Our results suggest the transfer of MPs through the food chain
- Predation on synanthropic rodents increases the ingestion of MPs by barn owls

Declaration of interests

☒ 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: