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

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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 microfibres (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

Introduction

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

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

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

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

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

While plastic ingestion is usually assessed by analysing the stomach content of dead animals, minimally 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 gliacialis*), 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 µm. 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 (5x5 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 µm – 2,000 µm fraction, were used. The mean (± SD) recovery rate was 98.2 ± 1.0% (n = 3) and 47.7 ± 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% = number of samples containing a specific prey item divided by the total number of pellets x 100), relative frequency of occurrence (rF% = number of occurrences of any prey item divided by the total number of items x 100), estimated per cent volume (V% = 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% = 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, $\chi2 = 4.29$, $P = 0.038$; site C-site A, $\chi2 = 3.13$, $P = 0.076$). 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 (χ^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). Microfibres (mean length ± SD = 2.4 ± 2.2; min-max: 0.3 – 10.4 mm, diameter = 10 – 40 µm) accounted for 88.2% of MPs (n = 30). Four fibres were longer than 5 mm and should be recorded as mesoplastics. The mean (± SD) size of irregularly shaped fragments (n = 4) was 0.71 ± 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 (± SE) per pellet was 3.5 ± 1.09 (min-max: 1 – 10), corresponding to a mean ingested biomass per pellet of 110 ± 111.8 g (min-max: 10.5 – 569.5 g). Barn owl diet differed among sampling sites (χ^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 μm (the smallest item detected by the eye was 70 μm), 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/cm^3) and polyethylene terephthalate (PET, 1.37 – 1.45 g/cm^3), 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.

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Table 1. Area (m^2) 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

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
Sorex antinorii	3	22	2.5	0.7	11.6	0.6
Crocidura leucodon	47	493	19.7	10.7	30	12
Crocidura suaveolens	2	11	1.6	16.4	7.6	0.3
Microtus arvalis	1	30	0.8	0.3	9.5	0.2
Microtus savii	34	449	18.9	3.0	42	16.1
Rattus norvegicus	12	3354	9	3.0	94.4	17.3
Mus domesticus	5	80	4	1.0	22.3	1.9
Apodemus sylvaticus	93	2055	34.4	2.0	70.5	49.4
Micromys minutus	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	-	-

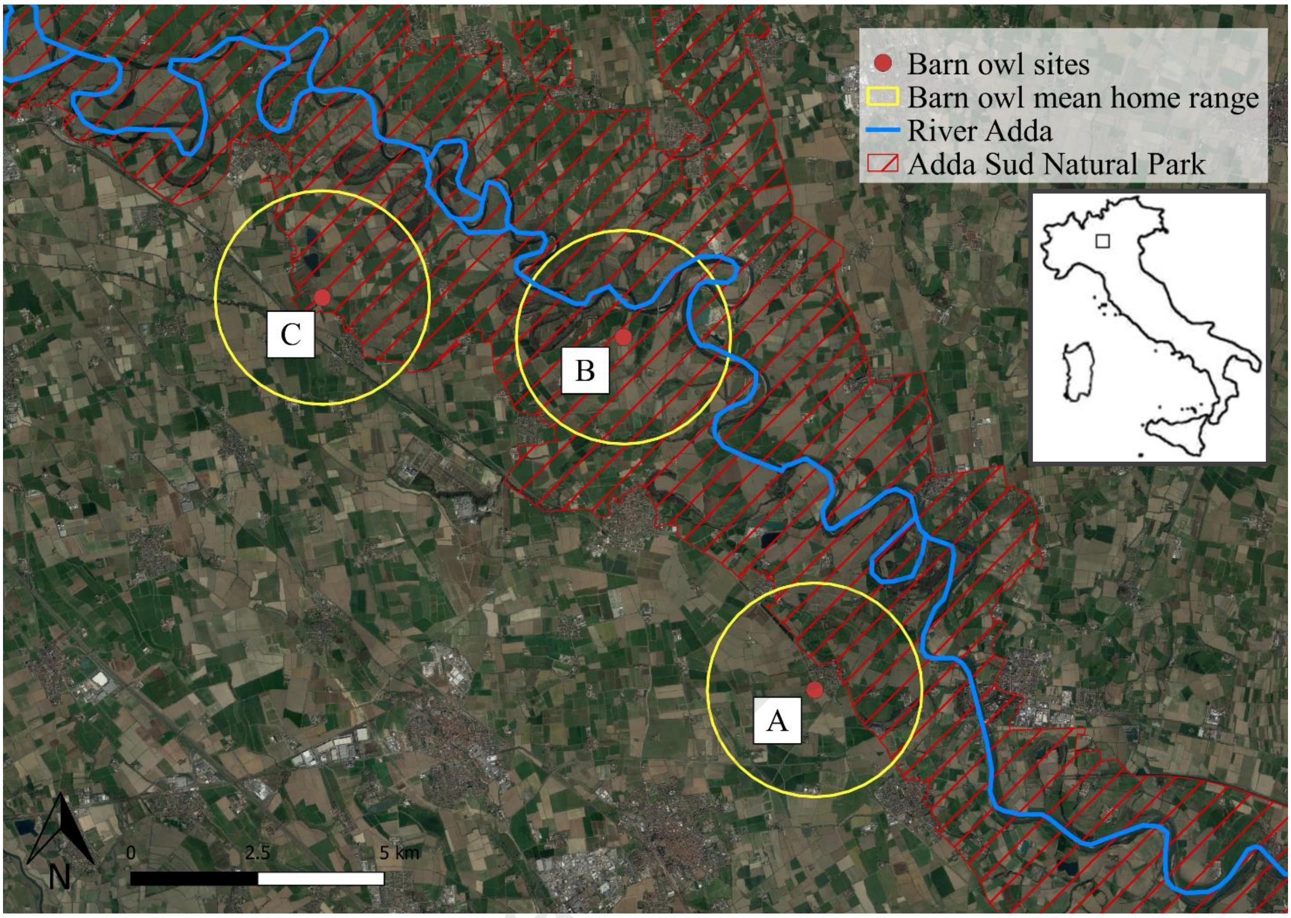

Figure 1. Study area, showing the location of the three barn owl's roosts and average home ranges (2 km radius buffer).

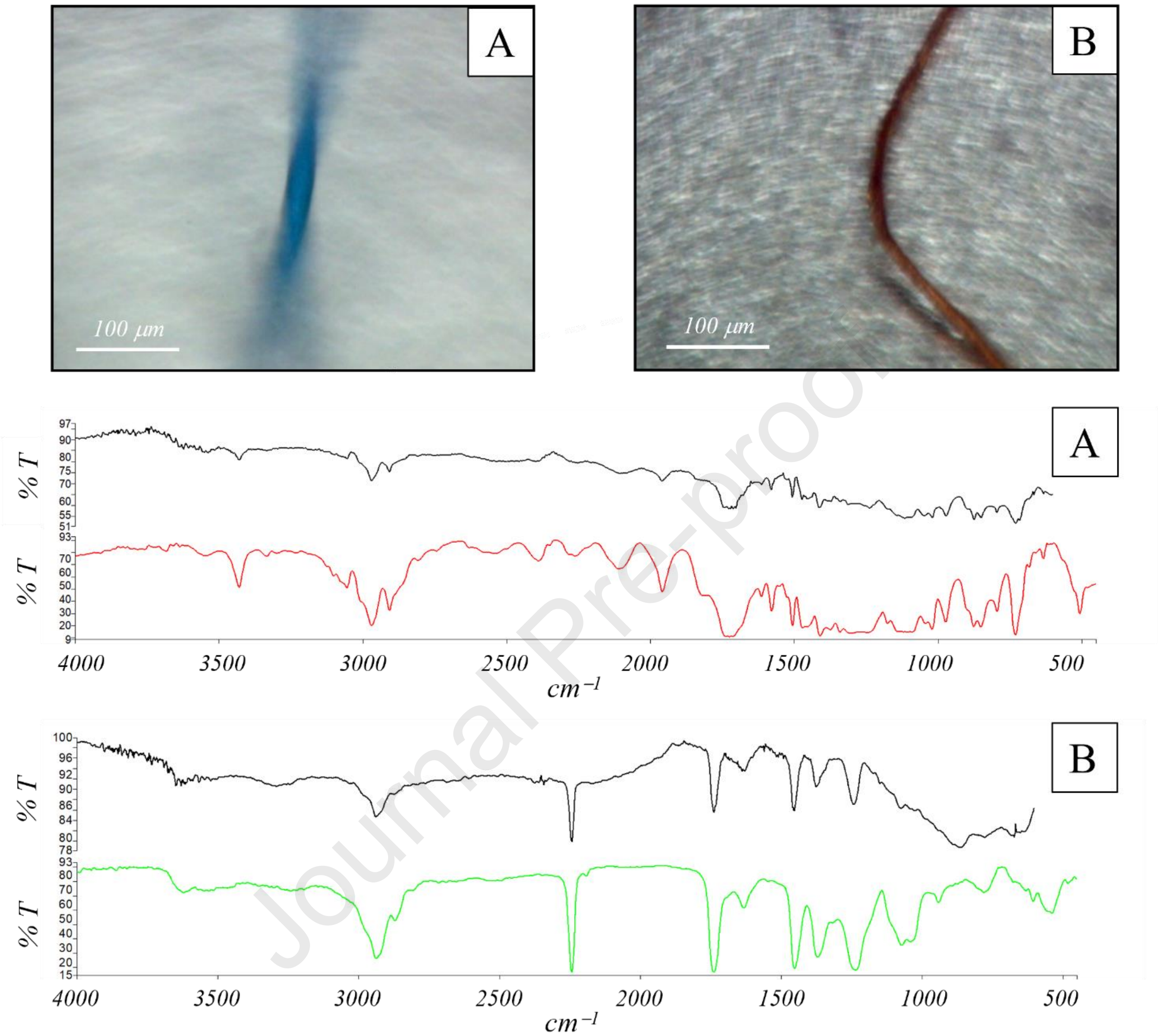

Fig. 2 Microscope images of two microplastics found in barn owl pellets and respective μ-FTIR spectra (black) with matches to reference polymer from the library (coloured spectra). A: polyester (PET) fibre (93% match); B: polyacrylonitrile (PAN) fibre (90% match). %T = % of transmittance; cm^{-1} = wavenumber.

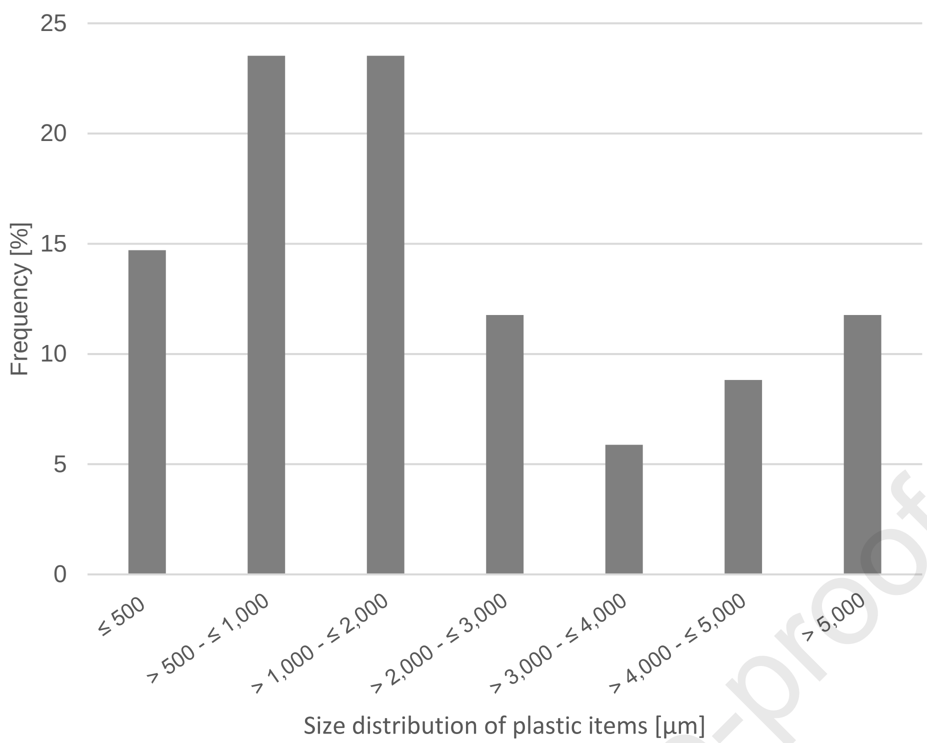

Figure 3. Size distribution of the plastic items recorded in barn owl pellets.

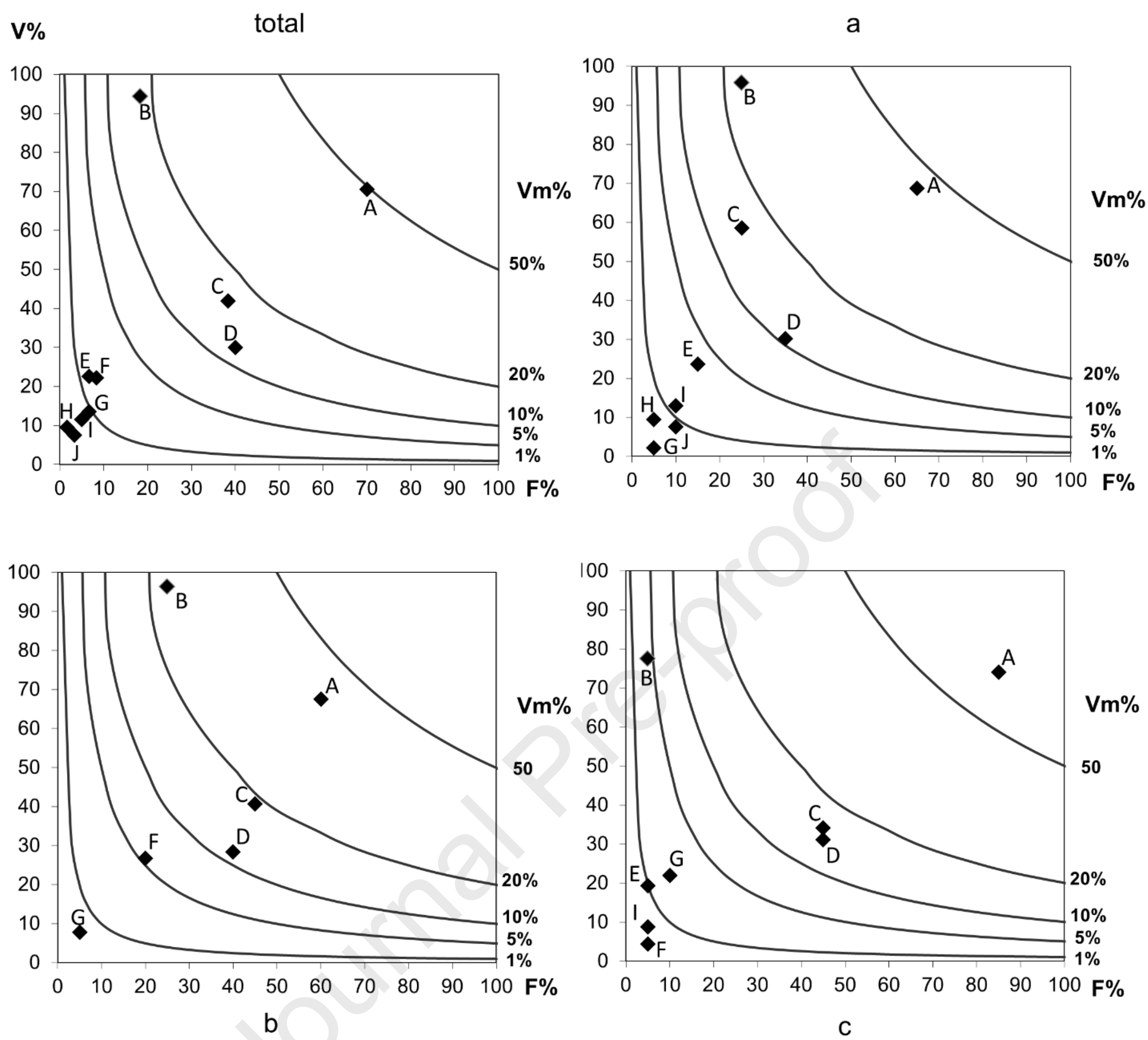

Figure 4. Estimated volume (V%) of main food categories, whenever eaten, vs. their frequency of occurrence (F%) for the overall diet of Barn owls and each of the three sites – a, b and c. Isopleths connect points of equal per cent mean volume in the diet (mV%). A = *Apodemus sylvaticus*; B = *Rattus 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.*

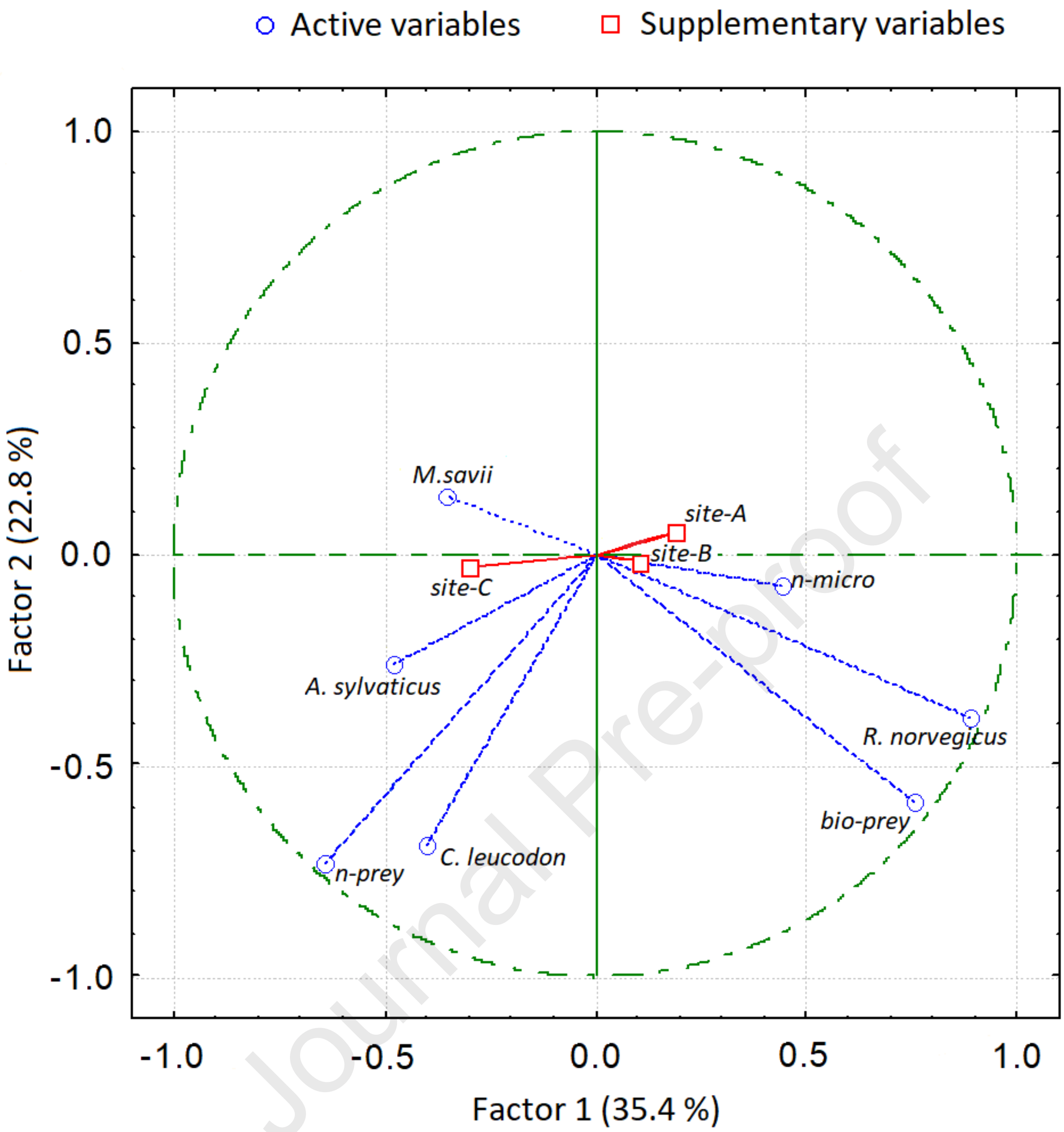

Figure 5. Plot of barn owl diet in relation to the first two PCA values extracted from biomass data of 205 small mammals preyed on in the three sites (A, B, C). Items are represented by lines that point towards the direction of maximum variation of each factor.

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: