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Fate of microplastics in agricultural soils amended with sewage sludge: Is surface water runoff a relevant environmental pathway?

Theresa Schell[1,2]*, Rachel Hurley[3], Nina T. Buenaventura[3], Pedro V. Mauri[4], Luca Nizzetto[3,5], Andreu Rico[1,6], Marco Vighi[1]

[1] IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Avenida Punto Com 2, 28805, Alcalá de Henares, Madrid, Spain

[2] University of Alcalá, Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Ctra. Madrid-Barcelona KM 33.600, 28871 Alcalá de Henares, Madrid, Spain

[3]Norwegian Institute for Water Research (NIVA), Gaustadelléen 21, 0349 Oslo, Norway

[4] Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA), Department of Agricultural and Environmental Research, Ctra. Madrid-Barcelona (N-II) KM. 38.200, 28805 Alcalá de Henares, Madrid, Spain

[5] Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Kamenice 753/5, Brno 62500, Czech Republic

[6] Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, c/ Catedrático José Beltrán 2, 46980, Paterna, Valencia, Spain

*Corresponding author; *email:* theresa.schell@imdea.org;

rachel.hurley@niva.no

nina.buenaventura@niva.no

pedro.mauri@madrid.org

luca.nizzetto@niva.no

andreu.rico@imdea.org

marco.vighi@imdea.org

Abstract

Sewage sludge used as agricultural fertilizer has been identified as an important source of microplastics (MPs) to the environment. However, the fate of MPs added to agricultural soils is largely unknown. This study investigated the fate of MPs in agricultural soils amended with sewage sludge and the role of surface water runoff as a mechanism driving their transfer to aquatic ecosystems. This was assessed using three experimental plots located in a semi-arid area of Central Spain, which were planted with barley. The experimental plots received the following treatments: (1) control or no sludge application; (2) historical sludge application, five years prior to the experiment; and (3) sludge application at the beginning of the experiment. MPs were analyzed in surface water runoff and in different soil layers to investigate transport and infiltration for one year. The sewage sludge used in our experiment contained 5,972-7,771 MPs/kg dw. Based on this, we estimated that about 16,000 MPs were added to the agricultural plot amended with sludge. As expected, the sludge application significantly increased the MP concentration in soils. The control plot contained low MP concentrations (31-120 MPs kg^{-1} dw), potentially originating from atmospheric deposition. The plot treated five years prior to the experiment contained 226-412 and 177-235 MPs kg^{-1} dw at the start and end of the experiment, respectively; while the recently treated plot contained 182-231 and 138-288 MPs kg^{-1} dw. Our study shows that MP concentrations remain relatively constant in agricultural soils and that the MP infiltration capacity is very low. Surface water runoff had a negligible influence on the export of MPs from agricultural soils, mobilizing only 0.2-0.4% of the MPs added with sludge. We conclude that, in semi-arid regions, agricultural soils can be considered as long-term accumulators of MPs.

Keywords: microplastic, sewage sludge, agroecosystems, water runoff, exposure assessment

1. Introduction

Wastewater treatment plants (WWTPs) are important pathways for microplastics (MPs) into the environment (Nizzetto et al., 2016; Ziajahromi et al., 2016). MPs are efficiently retained during wastewater treatment, mainly during mechanical treatment and sludge settling processes (Leslie et al., 2017; Murphy et al., 2016). It has been estimated that about 60 to 99% of MPs in WWTP inflow waters are ultimately transferred into sewage sludge, in which MP concentrations from around 1,000 to 186,700 MPs/kg dw have been reported (Gao et al., 2020). As sludge is rich in organic matter and nutrients, it is commonly used as an agricultural soil conditioner or fertilizer and has been shown to enhance soil properties by increasing the microbial activity and improving carbon and nitrogen mineralization processes, as well as some enzymatic functions (Roig and Mart, 2012). Around 35% of the sludge produced in Europe is applied on land (Table S1). In some countries, such as Spain, about 80% of the sludge produced by WWTPs is applied to agricultural soils, thus constituting a major environmental pathway for MPs (Rolsky et al., 2020). The relevance of sludge as a MP input source into soil ecosystems has been investigated recently (Corradini et al., 2019; Crossman et al., 2020; Van Den Berg et al., 2020; Zhang et al., 2020). These studies show that sludge applications increase the MP content in soils and that successive sludge applications can lead to considerable MP accumulation. Other sources of plastic pollution into agricultural soils include compost, plastic mulching, wastewater irrigation, road runoff, atmospheric deposition, and littering (Bläsing and Amelung, 2018; Corradini et al., 2019; Huang et al., 2020; Piehl et al., 2018; Zhang et al., 2020). Hence, also soils never treated with sludge can become contaminated by MPs.

To date, there is a paucity of information on the fate of MPs in agricultural soils. MP fate may depend on the polymer type, size, and morphology of the MPs. Smaller MPs, for instance, may be more readily transported into deeper soil layers (Liu et al., 2018; O'Connor et al., 2019). The fate of MPs in agricultural soils may also depend on the environmental conditions, soil characteristics and agricultural management (Kim et al., 2021; Zhang et al., 2020). Due to soil tillage and bioturbation, MPs may be translocated into deeper soil layers (Huerta Lwanga et al., 2017; Kim et al., 2021; Maaß et al., 2017; Rillig et al., 2017; Zhang et al., 2020), which can hinder photoinduced degradation and disintegration processes (De Souza Machado et al., 2018), thus increasing their persistence. For example, synthetic fibers have been shown to remain for more than 15 years in sludge-amended soils (Zubris and Richards, 2005). While there are several studies indicating that MPs are relatively persistent in soils, other studies point at environmental processes that contribute to their transport and dissipation, such as water runoff (Crossman et al., 2020; Kim et al., 2021). However, the efficiency of vertical and lateral water flows in mobilizing MPs from soil to freshwater ecosystems has not been studied empirically thus far (Schell et al., 2020). Obtaining this information is especially relevant to assess the environmental risks of MPs and to support appropriate sludge management.

Therefore, this study aimed to assess the fate of MPs applied with sewage sludge to agricultural soils and their transport to surface water ecosystems using experimental plots. We assessed to what degree MPs are retained by agricultural soils, their infiltration capacity into deeper soil horizons, and their capacity to be mobilized from agricultural soils due to transport via surface water runoff. Furthermore, this study provides first estimates of MP loads emitted from agricultural soils into freshwater ecosystems and discusses potential implications for sustainable sludge applications in European agriculture.

2. Material and Methods

2.1 Experimental set-up

This study was carried out at the Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA), which is located near Madrid (Central Spain). The area is characterized by a semi-arid climate, with an average yearly rainfall of 430 mm (data 1957-2000), mainly concentrated during spring and autumn. The experiment was set up on an agricultural field without historical sewage sludge application, which was assured by records of the experimental farm. Three agricultural plots ($2m^2$; Figure S1) with comparable soil characteristics, composition (Table S2), and a slope of 5% (facing south-east) were prepared. Each plot received a different treatment. Plot 1 served as a control (no sludge application). Plot 2 was prepared with soil from an adjacent field (located approximately 150 m north-west of the test plots) to which sludge (5 kg m^{-2} ww) was applied only in 2013 (i.e., five years prior to the start of the experiment). To prepare this plot, the upper 15 to 20 cm of sludge treated soil were removed using an excavator. During this process, the original soil structure was impaired; however, the transportation of soil to the experimental plot was required to allow planting the same crop and to ensure the same environmental conditions. Plot 3 was treated with sewage sludge immediately prior to the start of the experiment. The sludge was applied following standard agricultural practices in the region, using a dose of 5 kg m^{-2} ww (1.1 kg m^{-2} dw). After spreading it over the agricultural plot surface on October 30th 2017, it was left to dry for two days. The sludge was then manually incorporated into the soil using a hoe to a depth of approximately 10 cm. All plots were sown with barley (*Hordeum vulgare*, 350 seeds m^{-2}) on November 24, 2017. The barley was harvested on July 4, 2018. Subsequently, the soil was manually plowed, mimicking regular agricultural procedures for soil aeration and preparation for the next crop. The experiment was terminated on October 14, 2018. Details on the source and handling of the sludge used in Plot 3 are outlined in the Supporting Information (SI).

2.2 Soil sampling

The migration of MPs into deeper soil horizons was assessed by taking samples in all plots at the start and the end of the experimental period, producing soil samples for three different soil horizons: 0-5, 5-10, and 10-15 cm. The soil below 15 cm was very compacted, which hampered the sampling of deeper soil layers. At the start of the experiment, 5 samples of each soil horizon were taken with a stainless-steel core sampler (Ø 4 cm) with minimal disturbance of the experimental plots. Each sample contained about 50-100 g of soil. Since the soil samples taken at the start of the experiment showed a very heterogeneous MP distribution, the sample volume was increased for the sampling at the end of the experiment. Five samples of each soil horizon were taken with a metal shovel, which contained approximately 500 g each. Soil samples were transferred into glass jars and stored at room temperature until further analysis.

2.3 Runoff sampling

Surface water runoff was collected from the experimental plots during the 12 months of the experiment using runoff collectors modified after Pinson et al. (2004). The collectors were made entirely of metal. The runoff was collected through a metal pipe into a metal bucket (Figure S1), which was emptied following each rainfall event that generated runoff. The buckets used for runoff water collection were kept covered to avoid air contamination. Runoff volume from the plots was measured, preconcentrated by filtering it through a 20 µm plankton net and transferred into glass bottles. The buckets and nets were carefully rinsed with filtered (0.22 µm) Milli-Q water, which was also transferred into the bottles.

Runoff samples collected from the three plots after the first rain event were unfortunately lost during shipment to the NIVA MP laboratory (Norway). This unfortunate event was considered to be of critical importance for the results of the study since, in Plot 3, sludge was added recently, and MPs were most likely present on the soil surface where erosion would be maximal. For this reason, this part of the runoff experiment was repeated (for further details, see SI). During two rain events in March 2018 and one in October 2018, runoff unexpectedly exceeded the capacity of some of the collecting buckets. The overflow volume was monitored, and data were corrected accordingly.

2.4 MP analysis

2.4.1 MP extraction

Surface water runoff samples contained both water and some soil material. The overlying water was carefully decanted, vacuum filtered onto Whatman GF/A (Ø 47 mm) filter papers and retained for analysis. The soil material was subjected to two freshwater density extractions (filtered (0.22 µm) RO water; ρ=1.0 g cm^{-3}) followed by two saturated NaI solution (ρ=>1.75 g cm^{-3}) extractions to isolate higher density plastic particles. The overlying liquid of each extraction step was filtered onto a new filter paper and retained for visual analysis and chemical characterization (see below).

Soil and sludge samples were dried at 50 °C for 48 hours and homogenized using a stainless-steel sample splitter (Haver RT; Haver & Boecker, Germany). Three 75 g subsamples were taken from each soil sample, with the exception of the control sample at 10-15 cm and the historical application at 10-15 cm for which a lower sub-sample amount was used, as the collected sample volume was not sufficient (Table S3). MPs were isolated from the soil using two density separation extractions using saturated NaI solution (ρ=>1.75 g cm^{-3}). Soil samples had a high organic content and required treatment with 30% (v/v) H_2O_2. After organic matter removal, the samples were filtered onto Whatman GF/A (Ø 47 mm) filters and retained for analysis.

For the sewage sludge, three 10 g subsamples were processed. The organic matter content was reduced by treating the samples with Fenton's reagent, followed by two freshwater density extractions (ρ=1.0 g cm^{-3}). Following this, two high density extractions were performed using a saturated NaI solution (ρ=>1.75 g cm^{-3}). Each density extract was filtered onto a separate Whatman GF/A filter paper. Full details of the MP extraction procedure for all sample types are outlined in the SI.

2.4.2 Quality assurance/quality control (QA/QC)

All sample processing was performed in the NIVA MP Laboratory, which is a positive pressure room with HEPA-filtered (class H13) air input. Several contamination reduction procedures are in place, including the use of natural fiber clothing and lab coats, removal of loose fibers using a lint roller upon entry to the laboratory, and regular removal of dust from all areas of the laboratory. All steps during which samples were exposed to the laboratory environment (e.g., during weighing or sample homogenization) were undertaken in a laminar flow cabinet inside the MP Laboratory, to further reduce the potential for contamination. Samples were kept covered at all other stages of processing. All laboratory water or solutions used in the sample processing were pre-filtered (0.22 µm for RO water, 1.2 µm for NaI and H_2O_2) prior to use. All containers were rinsed with filtered RO water three times before use, to remove any potential contamination.

A total of three blanks were included for each batch of samples that were processed. These were combined procedural, container, and solution blanks, that were treated to an identical sample processing procedure within each sample batch. All suspected MPs found in the blanks were treated to the same visual and chemical characterization as in the environmental samples. The method used in this study has previously been validated using spiked samples for a range of MP particle types in Hurley et al. (2018) and Crossman et al. (2020).

2.4.3. Visual analysis

All filter papers were first visually analyzed for MP particles following Lusher et al. (2020). Based on the inclusion of this visual analysis step, a lower size limit of detection of 50 µm was set for all the samples. Each filter was traversed at 20-50x magnification using a Nikon SMZ 745T stereomicroscope. All suspected MPs were photographed using an Infinity 1 camera and their long and short dimension were measured using the Infinity Analyze (v.6.5.4) software package, following calibration using a measurement standard. In addition, the depth axis of each particle was estimated to the nearest 25 µm. The particles were classified by size and shape (bead, fiber, film, fragment, glitter, granule). Beads were defined as spherical particles; fibers as textile fibers/threads with an elongated shape and cylindrical form indicating extrusion; films as particles with a thickness of 10 µm or below; fragments as irregularly shaped particles that derive from the breakdown of larger plastic items; glitter as particles that have a reflective layer embedded within plastic with a shape that is hexagonal or formerly hexagonal (in the case of broken glitter pieces); and granules as semi-rounded particles with similar depth and width dimensions. Additionally, a small number of fiber bundles that could not be untangled were recorded as such.

2.4.4. Chemical characterization

All suspected MP particles were further chemically characterized to confirm their plastic composition. Large MPs (>300 µm; excluding fibers) were analyzed using an Agilent Cary 630 ATR-FTIR equipped with a diamond crystal. Small MPs and all fibers (50-300 µm) were analyzed using a Perkin Elmer Spotlight 400 µFTIR in transmission mode. Each spectrum was compared to a series of commercial (PerkinElmer Polymer library, Agilent Polymer library), open source (Primpke et al., 2018), and in-house libraries and were manually verified to confirm the polymer type. In the soil samples, 6% of the particles were lost after visual inspection prior to chemical characterization, while transferring them to the diamond compression cell. Across the remaining particles, 85% of all suspected MP particles were confirmed by FTIR to be plastic and 15% as non-plastic particles. Therefore, 15% of the lost particles were randomly excluded as non-plastic particles. The datasets presented here represent the MP component only. Full details of the chemical characterization are outlined in the SI.

2.5 Particle mass estimation

Following physical and chemical characterization of MP particles, the mass of each particle was estimated. This utilized the three analyzed axes related to the size of the particle to provide an estimate of particle volume. The calculation used to assess volume differed for different morphologies: fibers were treated as cylinders; fragments, films, glitter and granules were treated as cuboids; beads were treated as spheres; and fiber bundles were treated as cuboids and then divided to reflect the estimated percentage of the cuboid that was taken up by fiber versus empty space. Particle volume was converted to mass using the density of the polymer of each particle, as identified by FTIR analysis. Polymer density was established from a literature search, where the most commonly reported densities for each polymer were used (Table S4). For particles lost prior to FTIR analysis a density of 1 g cm^{-3} was assumed.

2.6 Statistical analyses

To test significant differences between soil MP concentrations at the start and end of the experiment, t-tests were applied. All soil data were checked for normality using Shapiro-Wilk's tests. To assess statistical differences between soil treatments, analysis of variance (ANOVA) was performed, followed by Tukey tests. Data not meeting requirements for parametric testing were tested using Kruskal-Wallis tests. All analyses were done using R for Mac version 4.0.3 (R Core Team, 2020). Statistically significant differences were assumed when the calculated p-value was <0.05.

3. Results and Discussion

3.1 MPs in sludge

Blanks from the sludge analysis (n = 3) contained only non-plastic particles (Table S5). The MP analysis of the sludge samples shows that the count-based concentration of MPs >50 µm in sludge was around 7000 MPs kg^{-1} dw (Table 1). Only a small numerical fraction (<3%) of plastics was >5000 µm in their longest dimension, which comprised almost 25% of the mass (Table S6). MPs in the sludge consisted of fragments (48-56%), of which most were <500 µm, and fibers (44-52%), of which most were >1000 µm (Figure S2; Table S7). No other shapes were observed. Fifteen different polymer types were identified in addition to a type of paint for which the specific polymer type could not be further determined. Fragments consisted mainly of polyethylene, followed by polypropylene, whilst fibers were mostly composed of polyester, followed by polypropylene and acrylic (Figure S3; Table S8).

3.2 MPs in soil

Blanks from the soil analysis (n = 18) contained mainly non-plastic particles and three MP particles (Table S9), hence the background contamination was judged to be negligible, and no data corrections were made. The control soil (Plot 1) contained on average 57 (31-84) MPs kg^{-1} at the start and 99 (79-120) MPs kg^{-1} at the end of the experiment. The historically treated plot (Plot 2) contained 330 (226-412) and 204 (177-235) MPs kg^{-1} and the recently treated plot (Plot 3) contained 215 (182-231) and

211 (138-288) MPs kg^{-1}, at the start and the end of the experiment, respectively (Figure 1A). These concentrations refer to MPs <5000 µm. Concentrations including plastic particles up to 12800 µm (maximum length measured) are shown in Table S10. At the start of the experiment, the concentrations were significantly higher in the plot that received the historical application (p=0.003) and the plot that received the recent application (p=0.04), compared to the control. No statistically significant differences were observed between the historical and recent application (p=0.12). At the start of the experiment, the total MP concentrations were not significantly different from the concentrations at the end of the experiment in any of the investigated plots, indicating losses resulting from runoff or other processes were likely small and that MP concentrations in soil are relatively stable over time. This is further supported by the similar concentrations measured in the historical and recently treated plot. In the control plot, the concentration slightly increased at the end of the experiment, but this could be an effect of the larger soil volume taken in the last sampling. In general, fibers were by far the most commonly observed MP type (44-91%) in soil, followed by fragments (4-44%; Figure S4; Table S11 and S12). Most fibers were >1000 µm in size, with exception of fibers in the upper layers (0-5 and 5-10 cm) of the historically treated plot at the start of the experiment, where a higher proportion of fibers <1000 µm was observed. Most other MP types were <500 µm, with almost no particles >1000 µm (Figure S2). A small fraction of particles >5000 µm were observed (Table S13 and S14). The presence of fibers in soil has previously been related to sewage sludge application (Corradini et al., 2019; Habib et al., 1998; Zubris and Richards, 2005). However, the current study shows that soil without historical sludge application can also contain MP fibers, which may originate from atmospheric deposition (Allen et al., 2019; Cai et al., 2017). The most common polymer types in the soil samples were polyester (22-53%) and acrylic (22-37%; Figure 1B; Table S15 and S16). A higher polymer diversity was observed in the soils amended with sludge compared to the control. At the start of the experiment 12 different polymer types were observed in total, and at the end of the experiment 20 were detected. Higher sample volumes taken at the end of

the experiment may be more representative of the polymer diversity and less affected by the heterogeneous distribution of MPs observed in the soil.

Regarding the vertical distribution in soil, MPs were found to be more abundant in the uppermost layer in all plots, both at the start and end of the experiment (Figure 1; Table S10). In the plot receiving the recent application, a trend of MP infiltration towards lower soil layers was observed, with a slight increase in the number of MP particles at the end of the experiment in the intermediate and bottom layers (Figure 1C). No clear influence of MP properties (i.e., size, shape, and polymer composition) on their vertical distribution in soil was observed. Soil properties likely impact the integration of MPs into soils and their lateral and vertical transport through them. The analyzed soils were fine textured with a high silt content, and low organic matter content (Table S2). Coarse textured soil with larger pore spaces may allow for a higher infiltration compared to the soils analyzed here. Crossman et al. (2020) − who monitored MP concentrations in soils with and without biosolid application in Ontario, Canada − observed that in a field with lower soil density, MPs abundance increased over time at deeper layers (10-15 cm) and MPs were better retained in this soil during heavy rainfall events, compared to MPs in soils with higher densities. Additionally, the MP size may affect the vertical movement. It has been shown using soil column experiments that smaller MPs have a greater movement potential, and that the migration depth significantly increases with an increasing number of wet-dry cycles (O' Connor et al. 2021). However, the smallest particle size (21 µm) investigated by these authors was below the size threshold considered in the current study.

Results for mass estimates show different patterns compared to those derived from the analysis of MP counts (Figure S5). Different replicates showed a higher variation based on mass, caused by a few large and therefore heavy fragments with an estimated mass of >0.1 mg. Following the estimation frame, the mass of a particle is fundamentally linked to the particle size (e.g., measured by the length along three main direction) through a cubic power law. This explains why a few rare, large particles can have a large influence on total mass estimates. This behaviour obviously also amplifies the uncertainty associated to the relatively low resolution of the sampling to consistently detect such rarer particles. For example, a very high mass-based concentration was observed at the end of the experiment in the recently treated plot, which was caused by one large polyethylene particle of 1 mg in the 10-15 cm soil layer.

Comparing MP contamination between studies reporting MP in estimated mass data and particle count is a useful exercise. Obtaining robust mass estimates is possible if sampling effort is properly sized, enabling consistent observations of rare large particles. Increased resolution can be achieved by increasing the size of individual soil samples or increasing the frequency and total number of observations. While the first case seems a more cost-efficient approach, processing large samples through multiple extraction, clean-up, particle detection and counting steps imposes serious analytical hindrances. A consolidated theoretical frame for optimizing sampling effort that simultaneously considers expectation of MP contamination characteristics and analytical limitations is not yet available. The results presented here provide some useful insights into the challenges and current limitations for MP assessment in soils, especially concerning the large heterogeneity of their spatial distribution (even when operating at relatively small scales) and their physical characteristics. Taking this into consideration, assessing the particle shape, size, and counts should be considered as a priority in soil MP assessment as impacts on both the soil properties and organisms are likely to depend more on these particle characteristics (Boots et al., 2019; De Souza Machado et al., 2019, 2018).

3.3 MPs in surface water runoff

Blanks from the runoff analysis did not contain MPs (Table S17). The observed annual precipitation during the study period (490 mm) was comparable with the long-term average annual precipitation for the study area (430 mm), and individual rainfall events within the study period were considered as representative for the study area too (Table S18).

From a visual inspection of the soils, it appeared that there was a slight difference in texture, which may have determined the observed difference in runoff generation between soil plots (Figure S6A;

Table S18). Furthermore, the recent addition of sludge as a soil amendment expectedly affects water infiltration rates and hydraulic properties of soil, contributing to a reduction in the volume of macropores that favour runoff (Mamedov et al., 2016). The repeat of the first runoff events of the study confirmed that these events did not yield a significantly higher MP mobilization compared to subsequent events and was therefore excluded from the data analysis (see SI section S3 for further details). During the runoff events, in which some runoff-collector buckets were overflowing due to unexpectedly high runoff formation (Table S18), the water loss appeared to be minimal for the control and the historically treated plot. Although the collector buckets were embedded in the soil and weighted down (Figure S1), the bucket from the recently treated plot had tipped to the side slightly during these three runoff events, causing additional water loss. During the last of these runoff events, runoff from the recently treated plot was completely lost. This is not expected to have substantially affected the results, as measured MP levels in runoff samples from events where overflowing occurred were comparable to those observed during runoff events capturing the total runoff volume (Table S18). For the two runoff events from the recently treated plot during which the total runoff was lost (first and last runoff event), data was corrected for the mass balance estimations.

MPs were mobilized by runoff from all plots, yet in very small numbers, as shown in Figure 2. The highest average MP concentration in runoff water was obtained from the recently treated plot and was 1.6 MPs L^{-1} (total MP number observed in runoff during the study period divided by total runoff volume collected). In this plot, the MP concentration in individual runoff events ranged between 0-14 MPs L^{-1}. Runoff water from the historically treated plot contained 0.5 MPs L^{-1} (0-0.7 MPs L^{-1}), and from the control plot 0.12 MPs L^{-1} (0-2.5 MPs L^{-1}; Table S18). The same pattern was observed based on mass concentration (Table S19).

In terms of MP mobilization from surface area, about 4 MPs m^{-2} were mobilized from the control plot over the period of one year, while 6.5 MPs m^{-2} were mobilized from the plot subject to historic sludge application. From the recently treated plot, 17.5 MPs m^{-2} were mobilized. Considering corrections applied to account for lost runoff, this value may be up to 31.5 MPs m^{-2} (Table S20). Despite similar soil MP concentrations in the recently and historically treated plots, the concentration and total number of MPs in runoff water was much lower in the historically treated plot than in the recently treated plot. Possible reasons for this may be that: i) MPs in recently treated soils are more mobile as they are less well incorporated into the soil matrix, and/or ii) particles with physical/chemical characteristics more prone to be mobilized by runoff (e.g., low density polymers, more regular morphologies) were lost from the soil in the historically treated plot well before the beginning of the experiment.

No direct relationship was found between the total MP count in the runoff and the rainfall volume (Figure S6B). However, there was a trend towards a positive correlation between the mass of soil exported via runoff and the number of MPs detected in the total runoff material (Figure 3, Figure S7). This suggests that there may be some similarities in the thresholds for the mobilization of both soil and MP particles in surface water runoff.

Fragments were the dominant particle type (62-88%) in runoff from all three plots. Fibers represented only 0-31% and were absent in the runoff from the control plot (Figure 2; Table 21). This indicates that fragments are more easily mobilized, while fibers are preferentially retained in the soil compartment. The increased fiber retention could be explained by their morphology, which may promote a more efficient entangling in soil aggregates compared to fragments. MPs in runoff were ≤3420 µm in size (Figure S3; Table S22). In total, 12 different polymer types were observed. Polyethylene (48%), polypropylene (18%) and polyester (16%) were the most common polymers in the runoff (Figure 2; Table S23). The prevalence of polyethylene and polypropylene, which are both less dense than water, may suggest that low density polymers are also preferentially mobilized.

3.4 Mass balance and upscaling

Based on the MP concentration in sludge and the mass of sludge added to the plot, about 16,000 particles or 164 mg were added to the recently treated experimental plot (Table 2). This includes plastic particles up to 12,800 µm (maximum particle length observed). According to the soil analysis results, approximately 80,000 MPs or 364 mg of MP were calculated to be present in the recently treated soil plot (2 m^2 with a depth of 15 cm) at the start of the experiment. Thus, the calculated MP count in the soil plot was twice as expected, considering the background contamination based on the MP presence in the control soil (22,000 MPs) and the MPs added by sludge (16,000 MPs). The calculated mass-based estimate, however, was found to be congruent with the MP mass added at the start of the experiment and the background contamination (Table 2).

In the current experiment, the MP concentration in soil did not significantly change over the period of one year. The persistence of MP in soil is reinforced by the very low losses measured through runoff. Based on count-based data, we estimate that about 0.2% (measured value) – 0.4% (value corrected for lost runoff events) of the MPs added by sewage sludge, and about 0.04-0.08 % the MPs measured in the soil, were exported via surface water runoff in one year. Regarding mass-based data, we estimate that 0.3-0.5% (2.3-4.1%; considering the mass of one silicone particle that influence the data greatly) of the MPs added by sludge to the soil, and 0.1-0.2% (1.0-1.8 %) of the MPs present in the soil, were exported by runoff. The mass-based export is higher as mainly fragments were transported by runoff, which tended to have a higher mass compared to fibers.

Based on the MP concentration observed in sewage sludge in the current study and the estimated sludge volume applied to agricultural soils in Spain (941.6 thousand tons; Eurostat, 2020), it can be estimated that 5.9×10^{12} - 7.5×10^{12} MPs or 5.0×10^{4} – 1.1×10^{5} kg MPs are added to soils annually in this country. This is a rough estimate, as observed MP concentrations in sludge vary greatly, not only between different WWTPs but also between different sampling events at the same WWTP (Schell et al., 2021).

Our results show that most of the MPs applied by sewage sludge are retained in the soil (>99% based on count-based data or >95% based on mass-based data). Thus, it can be assumed that soils act as important long-term stores of MPs, with repeated sludge applications leading to MP accumulation over time. The estimated annual runoff emissions from agricultural settings in Spain are in the range of 1.2 x 10^{10} – 3.0 x 10^{10} MPs (assuming the observed 0.2 – 0.4% export for count-based data) or 150 - 4455 kg MPs (considering the observed 0.3% - 4.1% export for mass-based data). It is important to note that not all MPs removed from soil by runoff will directly reach surface water bodies, as some may be transported to forested areas or to other agricultural fields instead. However, these estimations provide a first assessment of MP sources from agricultural soils treated with sewage sludge. Such estimations may vary notably depending on soil type, crop type, agricultural practices or geographic location, but are useful for future comparisons and MP source apportionment exercises at regional scales.

3.5 Critical remarks and lessons learned

We observed highly heterogeneous MP distributions in the experimental soils and a discrepancy between the measured count-based and estimated mass-based concentrations. Follow-up studies should increase the number of replicates and/or the sample volume to improve the representativeness of the results. Furthermore, the analysis of smaller MPs in environmental matrices is still challenging and time-consuming, while it is possible that in a relatively long-term study (e.g., one year or more), MPs undergo further fragmentation due to various biotic and abiotic agents (e.g., UV exposure, erosion, soil invertebrate ingestion; Ng et al., 2018). Therefore, small-sized MPs and nanoplastics may represent a currently non-quantifiable but important fraction, potentially hampering the mass balance. This highlights the need for more efficient analytical methods for small-sized MP determination in soil and other environmental matrices (Vighi et al., 2020).

Different MP types have been shown to negatively affect soil properties. They can lead to changes in soil hydrology (i.e., increase the water holding capacity) and microbial activity, which in turn can affect the crop performance (Boots et al., 2019; De Souza Machado et al., 2019, 2018). Furthermore, although research on the effects of MPs on soil organisms is still very limited, studies have indicated that inhibition in growth and reproduction, mortality, and internal injuries after ingestion may occur at high concentrations (Cao et al., 2017; Huerta Lwanga et al., 2016; Selonen et al., 2020; Song et al., 2019). Test concentrations in those studies were several orders of magnitude higher than maximum concentrations (500 MP kg^{-1} dw or 16 mg kg^{-1} dw) observed in the current study. However, as MPs accumulate in the soil over time (Corradini et al., 2019; Van Den Berg et al., 2020), repeated sludge applications as well as other input sources such as atmospheric deposition (Allen et al., 2019), wind erosion (Rezaei et al., 2019) and plastic mulching (Huang et al., 2020), may cause soil MP contamination to reach these levels in the future.

The sustainability of sludge application in agriculture has been debated due to its potential human and environmental health consequences. To date, sewage sludge must fulfill the quality requirements regulated under the European Sewage Sludge Directive (86/278/EEC) to be used as agricultural fertilizer. Thus far, MPs have not been included in this directive, but there is a currently undergoing initiative to evaluate whether certain contaminants of emerging concern, including MPs, should be regulated. To understand at what contamination level the presence of MPs outweighs the benefits of sludge application, further research is needed to assess the long-term persistence and infiltration of MPs in soils under different conditions, their potential mobilization into other environmental compartments (i.e., surface and groundwaters), and their ecological effects.

4. Conclusions

To our knowledge, this paper represents one of the first attempts to assess MP fluxes in agricultural soils empirically. The study shows that sludge application significantly increases MP content in soils and that those concentrations remain relatively stable over time. Mobilization of MPs, both into deeper soil layers and along surface water runoff, was very low during the period of one year under the environmental conditions tested in this study. Thus, we conclude that in semi-arid regions, surface water runoff has a negligible influence on the export of MPs from agricultural soils and that agricultural soils can be considered long-term MP accumulators.

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Table 1. *MPs in sludge applied to the runoff plot treated with sludge at the beginning of the experiment (n=3). Plastic particles >5000 µm that were observed were between 5000 and 6000 µm with one exception that was 11,750 µm.*

	Mean particle count in kg^{-1} dw (min-max)	**Mean estimated particle mass in mg kg^{-1} dw (min-max)**
Including plastics >5000 µm	7266 (6351-7956)	74.4 (53.6-115.4)
MPs 50- 5000 µm	7078 (5972-7771)	56.1 (52.1-60.6)

Table 2. *Calculated and measured input (by sludge) and output (runoff) of MPs (including small plastic particles up to 12,800* µm, which was the maximum particle length observed) *for count based (*#) and mass based (mg) *data per soil plot and per kg soil or L runoff from different soil treatments. Calculations are based on the concentration measured in soil and the approximated amount of soil in the runoff plot based on soil density and plot dimensions. The MP mass transported by runoff from the recently treated plot is shown with and without the mass of one silicone particle which determined most of the observed mass (3.18 mg) and thus strongly influenced the data.*

	MPs per soil plot or average concentration per kg soil or L runoff	**MPs added by sludge to soil plot**		**MPs at the start of the experiment**		**MPs at the end of the experiment**		**MPs transported by runoff water in one year**	
		#	mg	#	mg	#	mg	#	mg
Control	plot	-	-	22,200	215	37,100	44	8	0.071
	kg or L	-	-	62	0.57	103	0.12	0.12	0.001
Historical application	plot	unknown	unknown	121,600	277	73,350	174	13	0.074
	kg or L	unknown	unknown	338	0.76	208	0.46	0.54	0.003
Recent application	plot	16,000	164	80,000	364	79,100	935	35[a]-63[b]	0.52/3.7[a] - 0.84/6.67[b]
	kg or L	44	0.44	226	0.97	219	2.48	1.6	0.17/0.02

[a] *measured value*

[b] *corrected value considering lost runoff events (see Table S20 for further details)*

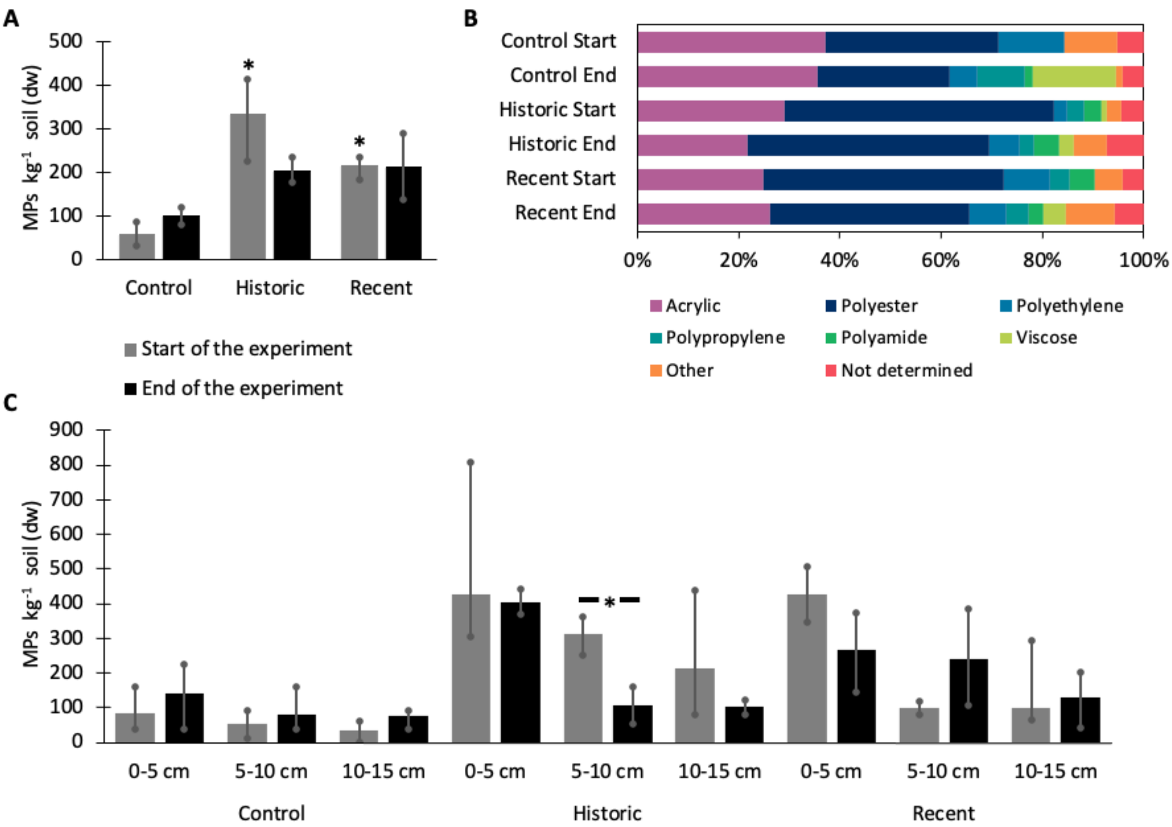

Figure 1. MPs detected in soils, subjected to different sludge treatments, at the start and the end of the experiment reported by A) mean, min and max (error bars) concentration (MP kg^{-1}) per experimental plot; B) polymer type observed at the start of the experiment (Start) and the end of the experiment (End); and C) mean, min and max (error bars) concentration (MP kg^{-1}) according to different soil sample depth (0-5 cm; 5-10 cm and 10-15 cm). Statistically significant differences compared to the control are indicated by asterisks (*), while significant differences between the start and the end of the experiment are indicated by asterisks in between dashes (-*-).

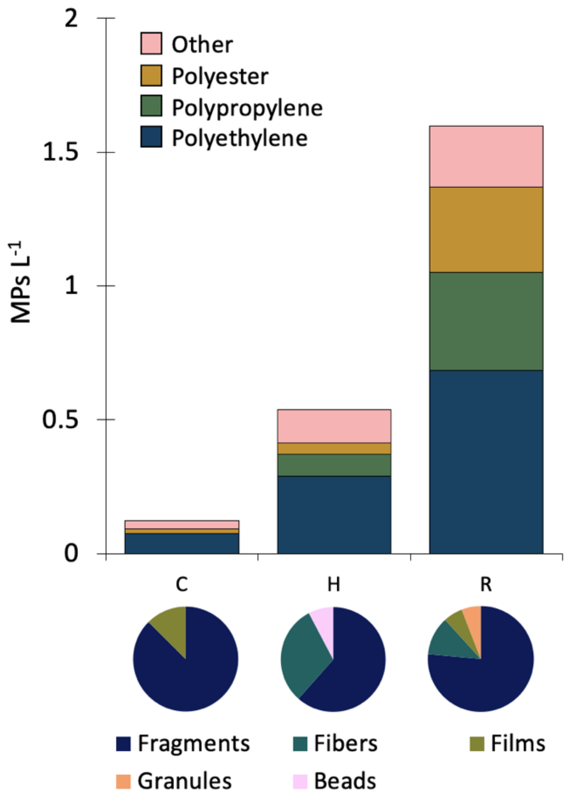

Figure 2. *MP concentration (MPs L^{-1}) in the runoff water from the different sludge application treatments (C= control; H = historic application; R = recent application) aggregated for the whole experimental period (one year). The pie charts show the distribution of particle shapes.*

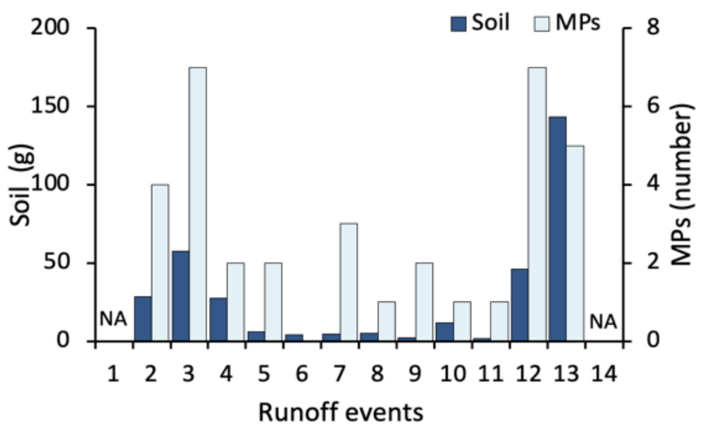

Figure 3. *Total mass of soil material transported by runoff into the collector bucket (grams) and number of MPs detected in the runoff from the recently treated plot. NA in indicates that runoff occurred but the measurement was lost.*

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