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Biosolids are commonly applied to agricultural soils, yet their role in microplastic (MP) contamination is not fully understood. This is critical to assess, as MPs in soil can potentially alter ecosystem function and soil structure. We compared MP abundance and composition in eleven biosolid-amended and nine nonamended fields in Southern Ontario. Biosolid-amended soils had over three times more MPs on average, with concentrations influenced by biosolid type and number of applications. Amendments also shifted MP composition, increasing textile-derived polyester fibres. These findings highlight biosolids as a major vector of MPs to terrestrial systems. Understanding this pathway is essential for generalizing MP pollution risks beyond aquatic environments and informing policies on land-based waste management and soil health protection.

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Comparing the Microplastic Content in Biosolid-Amended and Non-Amended Agricultural Soils.

Nicholas V. Letwin, Adam W. Gillespie, Joel D. Csajaghy, Yaryna M. Kudla, Moira M. Ijzerman, Ryan S. Prosser*

School of Environmental Sciences, University of Guelph, 50 Stone Rd E, Guelph, ON N1G4W2

* Corresponding author
Ryan S. Prosser
prosserr@uoguelph.ca

1 Abstract

Biosolids have been identified as a major source of microplastics (MP) to the environment. While they have been heavily studied, the impacts biosolids have following their amendment to agricultural soils on the MP content of these soils is poorly understood. Eleven biosolid-amended and nine nonamended agricultural fields in Southern Ontario were sampled to compare the MP content between them. Biosolid-amended fields averaged 2,441.82 ± 268.03 MP/kg, while nonamended fields averaged 775 ± 50.97 MP/kg. Additionally, MP abundance was correlated with the type of biosolid being applied, as fields that received one application of dewatered biosolids averaged 2,412.14 ± 174.81 MP/kg, whereas fields with a single application of liquid biosolid averaged 1,689.83 ± 225.81 MP/kg. However, differences in MP abundance were primarily dictated by differences in application rate between dewatered and liquid biosolids. In addition to increasing overall MP content, biosolid amendments influenced MP composition. Biosolid amendment led to an increased fibre content within soil, as biosolids are rich in textile fibres derived from the laundering process. As a result, biosolid-amended soils primarily contained polyester, while unamended soils primarily contained polypropylene. Quantifying and characterizing MP content in biosolid-amended fields, as well as understanding how it differs from unamended fields, is crucial for accurately assessing the risks microplastics pose to terrestrial ecosystems.

2 Introduction

The widespread use of plastic, coupled with poor waste management, has led to extensive pollution, making it a significant environmental concern. It is estimated that of all generated plastics, 9% is recycled, 12% is incinerated, and 79% is accumulated within landfills or the environment [1]. Furthermore, estimations indicate that plastic contamination in soil is 40 times greater than in water [2]. Microplastics (MPs, plastic particles <5mm) are primarily derived from the fragmentation of larger plastic materials [3], and are an emerging contaminant of concern.

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There has been an increased focus on the research of MPs as they are ubiquitous, resist degradation, and their interactions with other components in the environment are relatively unknown. Sources of MP pollution to soils are vast, with examples being improper waste disposal, plastic mulching, urban and road runoff, aerial deposition, wastewater irrigation, and land application of biosolids [4-6]. One of the main concerns of MPs are the risks they could pose to organisms through their uptake [7]. Recent findings indicate that MPs can reduce reproduction in soil biota, such as nematodes, earthworms, and springtails, and can also cause shifts in the soil microbiome [8]. Additionally, there is evidence to suggest that MPs can alter soil structure, nutrient availability, and soil fertility [9]. These risks associated with MPs are strongly influenced by their physicochemical properties such as variations in size, morphology, polymer type, and chemical additives [10]. As such, it is essential to characterize the types, amounts and shapes of MPs that may be accumulating in soil ecosystems.

Biosolids (treated sewage sludge) are a by-product of the wastewater treatment process. They are often used as an agricultural fertilizer amendment because they supply organic matter, nitrogen, and phosphorus to soils [11]. In Ontario, approximately 300,000 dry tons of biosolids are produced annually. Of this amount, about 40% are applied to agricultural land, 40% are deposited in landfills, and 20% are incinerated [12]. As biosolids are derived from human waste and urban wastewater, they may contain a number of contaminants, including metals, pharmaceuticals, personal care products, polycyclic aromatic hydrocarbons (PAHs), and per- and polyfluoroalkyl substances (PFAS) [13-17]. In some cases, these contaminants have been identified, and regulatory tools are in place to mitigate their transfer to agricultural lands [18]. Biosolids also have been identified as potentially containing a significant source of MPs, which could be transferred to agricultural soils [19]. Studies have shown that up to 99% of MPs that enter wastewater treatment plants (WWTPs) are removed from the effluent and deposited into biosolids [20, 21]. Current reported estimates of MPs within Canadian biosolids range from 8,000 to 1,350,000 MP/kg dry weight [5, 22, 23]. MPs in biosolids are predominantly sourced from personal care products and textile fibres released during laundering [24]. As a result, biosolids contain high levels of plastics commonly associated with textile fibres, such as polyester, polyacrylamide, and polyamide [23].

While biosolids have been identified as a major potential source of MPs in agroecosystems, there are conflicting results regarding whether MPs are incorporated into soils [5, 25]. In addition, it is uncertain if biosolids contribute more MPs than other fertilizers. Similar to biosolids, compost has been identified as a notable contributor of MPs to agricultural fields [26]. Inorganic fertilizers, including slow- or controlled-release fertilizers, are often coated with synthetic polymers such as urea-based resins and polyurethanes which gradually break down into MPs over time [27]. Additionally, manure may introduce MPs indirectly through contaminated feed, equipment, or handling tools [28]. Evidence suggests that runoff from biosolid-amended fields contains higher MP concentrations than runoff from manure-amended fields, yet there is limited research comparing MP concentrations within the soil itself [29]. Furthermore, the fate and accumulation of MPs in soils following biosolid application remains largely unknown. Previous studies looking at Ontario biosolid-amended soils reported that only 1-7% of the biosolid-derived MPs are retained within the surface levels of the soil, with the majority hypothesized being lost to wind erosion or surface runoff [5, 30]. In addition, natural processes such as bioturbation and water infiltration can move MPs to deeper soil horizons, removing them from topsoil [31]. Currently, the highest reported average MP concentration found in Canadian biosolid-amended soils is 6870 MP/kg dry

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weight [30]. Nevertheless, it is estimated that Ontario biosolids can deposit between 4.1×10^{11} and 1.3×10^{12} MP particles into agricultural fields per year [5]. Further MP loss may be attributed to movement into deeper soil layers via bioturbation or water infiltration, potentially reaching groundwater [32]. Furthermore, physical properties of an agricultural field such as slope and moisture redistribution are hypothesized to affect the long-term retention of MPs in the soil column [33, 34].

This study aimed to contribute to the understanding of MP presence within agroecosystems. The key objectives of this study were to (1) enumerate and characterize MP content within biosolid-amended soils, (2) compare the MP content in biosolid-amended soils with fields that have never received biosolid treatment, and (3) determine if the physical properties of an agricultural field can predict long-term MP retention.

3 Materials and Methods

3.1 Field Selection

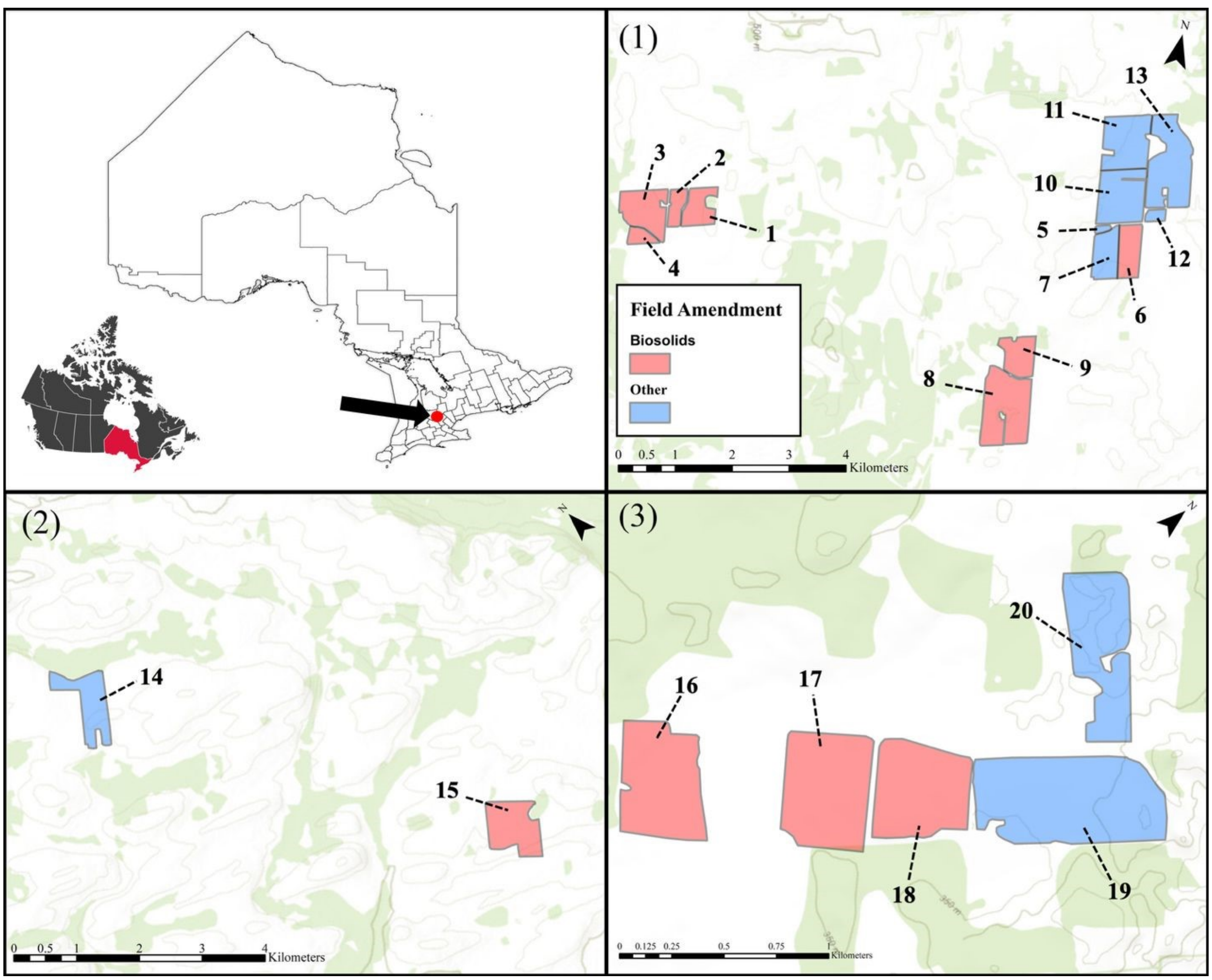

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Figure 1. Map of 20 agricultural fields across three locations in southern Ontario. Fields shown in red indicate areas that received biosolid amendments, while fields in blue represent non-amended sites.

This study was conducted in Wellington County, located in southern Ontario, Canada. This region experiences a humid continental climate with cold winters, warm summers and annual precipitation of 800 – 1000 mm [35]. Land use in predominantly agricultural, as Wellington County only covers 0.2% of the total land area of Ontario but provides 5% of the provinces field crops [36]. A 2021 census estimates a population of ~ 240,000 residents [37].

Twenty agricultural fields across three locations were selected for sampling (Figure 1). Fields 1 - 12 are in Location 1, Fields 14,15 are in Location 2, and Fields 16 - 20 are in Location 3. None of the twenty fields practice tilling. Fields 1, 2, and 5-10 are classified as silt loams, Fields 3, 4, 7, and 12 are loams, and Field 13 is a gravelly sandy loam. Furthermore, fields in Locations 2 and 3 are all classified as loam. Detailed information on cation exchange capacity, pH, particle size distribution, and levels of phosphorus, inorganic and organic carbon, organic matter, and heavy metals can be found in the supplementary information (Tables S1–S8). Eleven of these fields received biosolid amendments, while the remaining nine were fertilized with manure. All 20 fields are on a three-crop rotation of corn-soybeans-wheat. At the time of sampling, Field 15 had received four biosolid applications between 2015 and 2021. All other fields amended with biosolids had only one application, conducted between 2019 and 2021. Fields 1-9, and 15-18 were sampled in May 2022, while Fields 10-14, 19, and 20 were sampled in May 2023. A summary for each field can be found in Table 1.

Table 1. Field characteristics, biosolid application history, application type, and application rate.

Field	Amendment	Year(s) of Biosolid Application	Biosolid Type	Application Rate (m^3/hectare)	Field Size (hectare)	Crop Type
Field 1	Biosolids	2019	Dewatered	19.77	15.53	Corn
Field 2	Biosolids	2019	Dewatered	19.77	7.48	Corn
Field 3	Biosolids	2019	Dewatered	19.77	26.12	Corn
Field 4	Biosolids	2019	Dewatered	19.77	3.90	Corn
Field 5	Other	N/A	N/A	N/A	3.51	Corn
Field 6	Biosolids	2020	Dewatered	31.15	16.66	Corn
Field 7	Other	N/A	N/A	N/A	20.28	Corn
Field 8	Biosolids	2019	Dewatered	63.71	46.24	Corn
Field 9	Biosolids	2019	Dewatered	63.71	18.45	Corn
Field 10	Other	N/A	N/A	N/A	37.57	Corn
Field 11	Other	N/A	N/A	N/A	37.44	Corn
Field 12	Other	N/A	N/A	N/A	3.82	Soybeans
Field 13	Other	N/A	N/A	N/A	52.11	Corn
Field 14	Other	N/A	N/A	N/A	28.67	Corn
Field 15	Biosolids	2015, 2017, 2019,	Liquid	105.80 (2015), 95.16 (2017), 107.91 (2019),	31.54	Corn (2015), Soybeans (2017), Corn (2019),

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Field	Amendment	Year(s) of Biosolid Application	Biosolid Type	Application Rate (m^3/hectare)	Field Size (hectare)	Crop Type
		2021		100.66 (2021)		Corn (2021)
Field 16	Biosolids	2021	Dewatered, Liquid	8.15 80.13	9.70	Corn
Field 17	Biosolids	2021	Liquid	100.00 (± 5%)	10.56	Corn
Field 18	Biosolids	2021	Liquid	100.00 (± 5%)	9.64	Corn
Field 19	Other	N/A	N/A	N/A	18.00	Wheat
Field 20	Other	N/A	N/A	N/A	11.51	Wheat

3.2 Sample Collection

To eliminate potential bias from the horizontal movement of MP within an agricultural field, a conditioned Latin hypercube sampling approach was employed [38]. First, polygons representing each field were created in Google Earth and then overlayed onto digital elevation models (DEMs) provided by the Ontario Ministry of Natural Resources and Forestry within ArcMap 10.0 [39]. Using the "Extract by Mask" function, DEMs for each polygon were generated. Estimates of slope, LS-factor (a measure of slope length and steepness used to estimate soil erosion risk), and topographic wetness index were derived from these DEMs using the System for Automated Geoscientific Analyses (SAGA GIS). R packages "raster", "terra", and "clhs" were then used to generate 20 sampling points for each field, with DEM, slope, LS-factor, and topographic wetness index serving as covariates.

A T-handled stainless steel soil augur with a tip that was 5 cm in diameter and 15 cm long was used to sample soil up to 30 cm in depth. Although MP concentrations are highest within the top 10 cm of soil, evidence suggests that approximately 70% of MPs within the soil column are found in the first 30 cm [40]. Upon site arrival, debris on the top of the soil was removed. To minimize MP contamination, soil samples were wrapped in aluminum foil immediately following collection. Samples were held at 4°C until further subsampling.

3.3 Microplastic Extraction

Each individual soil sample was transferred from aluminum foil to a stainless-steel mixing bowl and mixed to thoroughly until homogenized, resulting in 20 homogenized sampled per field. A 100g subsample was transferred to a 250mL glass beaker and dried in a drying oven set to 65°C. Following this, a 10g subsample of dried soil was placed into a 300mL glass beaker. Soil samples were digested using Fenton's reagent, with a 5:1:1 ratio of 30% w/w H_2O_2, 20 mmol/L iron (II) sulfate heptahydrate solution, and 20mmol/L protocatechuic acid solution. Digestions were conducted on shaker tables set to 175 revolutions per minute in a fume hood for 24 h. Afterward, the samples were returned to a drying oven set to 65°C to remove any residual H_2O_2. Once dried, density separation was performed by adding 100mL of 905g/L NaBr, a solution with a specific gravity of approximately 1.45 g/cm^3, to each sample. The samples were mixed for 5 min with a stir bar, then allowed to separate for 24 hours. Following separation, the top 50mL

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was transferred to a secondary beaker using a 10-mL stainless steel ladle. Vacuum filtration was then carried out using 0.45-µm Durapore™ polyvinylidene fluoride membrane filters.

To validate the method, a spike-recovery test was conducted, with seven replicates analyzed. MP recovery was assessed using white polyethylene spheres (962.75 ± 21.83µm), red polyester fibres (860.65 ± 129.65µm) and blue polypropylene fragments (508.82 ± 56.91µm). The white polyethylene spheres were purchased from Cospheric, while the red polyester fibres and blue polypropylene fragments were produced by freezing and bead milling their respective macroplastics. Ten of each MP was measured and added to 1 g of dried reference soil. The reference soil was a loam with approximately 2% organic carbon. Three method blanks were included in the spike-recovery test, and no contamination of the spiked MPs was observed. Results showed above 70% recovery for all three MPs (Table S9).

3.4 Microplastic Quantification

MPs were visually quantified using a Nikon® SMZ18 stereomicroscope paired with a Nikon® DS-Ri2 camera. Each particle was categorized by colour, size, and morphology (fragment, sphere, fibre, film, or foam). Size was determined by measuring the longest dimension of each MP. For fibres, length was measured as the distance between the two endpoints of the fibre. To simplify colour classification, similar shades were grouped together. Particles that appeared white, clear, or grey were classified as "clear." Those that appeared black or dark grey were grouped as "black." Finally, particles in shades of brown or orange were categorized as "orange."

3.5 Microplastic Characterization

MP polymers were identified using a Bruker LUMOS II FT-IR microscope. Individual MPs were transferred to a Thermo Scientific™ Micro Compression Cell™ with KBr windows and analyzed through transmission FT-IR spectroscopy with a mercury–cadmium–telluride detector. From each field, 50 MPs were analyzed, representing 5.64–51.00% (mean = 22.12%) of all individual MPs quantified per field. To accurately represent MP content, MPs were selected based on the calculated proportions of their morphology and colour found within the field. The resulting spectra were compared to the open-source Open Specy FT-IR spectral library [41], chosen for its extensive database of FT-IR spectra of weathered MPs. Environmental MP samples often show altered spectra due to surface degradation caused by factors such as bacterial digestion, heat, ozone, and UV radiation. As such, spectra from pristine plastics may not provide an exact match for weathered MPs [42]. Therefore, MPs were considered a positive match if they exhibited a similarity score of 60% or higher.

3.6 Quality Assurance and Quality Control

Plastic use was minimized as much as possible during sample processing and collection. Initially, soil samples were wrapped in aluminum foil during collection. While processing, samples were covered with an aluminum foil lid at all times, except during the addition of chemicals. Furthermore, hands, surfaces, and glassware were thoroughly rinsed with deionized (DI) water

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between samples. When handling samples, emphasis was placed on wearing bright-coloured, non-synthetic clothing to easily identify contaminated fibres.

Samples were processed in batches of 10, with one method blank included per batch, resulting in a total of 40 method blanks, and 400 soil samples. Method blanks followed the same procedures as the soil samples for collection, storage, and processing. MPs detected in the method blanks were subtracted from the final estimates of the 10 soil samples within their batch. Method blank analysis showed a range of 2-9 particles per filter (mean = 5.28), with 83.89% of them being fibres.

3.7 Data Analysis

Twenty samples and two method blanks were analyzed for each agricultural field. Results are reported in MPs per kilogram of soil (dry wt). Standard errors were calculated and reported for each sample.

The Shapiro-Wilk test and Bartlett's test were used to check assumptions of normality and homogeneity of variances. Once these assumptions were met, independent t-tests ($\alpha = 0.05$) were used to compare differences across amendments and biosolid types. An F-test was performed to compare variances of biosolid-amended and unamended soils.

To estimate MP load from biosolids in soil, the following equations were used:

$$V_{Biosolid} = Application\ Rate \times 1\ Hectare \tag{1}$$

Where $V_{Biosolid}$ is the volume of biosolid (m^3) spread on 1 hectare of field, and $Application\ Rate$ is the application rate denoted in Table 1 (m^3/ha).

$$M_{Biosolid} = V_{Biosolid} \times \rho_{Biosolid} \tag{2}$$

Where $M_{Biosolid}$ is the mass of biosolid spread on to 1 hectare of land (kg), and $\rho_{Biosolid}$ is the density of the biosolid, estimated to be 1000 kg/m^3 for dewatered biosolids [43], and 1040 kg/m^3 for liquid biosolids [44].

$$MP = [Biosolid] \times M_{Biosolids} \tag{3}$$

Where MP is the total number MPs spread onto 1 hectare of land, and $[Biosolid]$ is the concentration of MPs within biosolids (wet wt, MP/kg). Estimates of 8428 MP/kg for liquid biosolids, and 114,331 MP/kg were derived from Letwin et al., 2023 as fields in this study were amended with biosolids sourced from the same locations described in that work [23]. While concentrations may have varied over time, these values provide a reasonable estimate of the MP load associated with the biosolids applied.

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$$M_{Soil} = V_{Soil} \times \rho_{Soil} \quad (4)$$

Where M_{Soil} represents the mass of soil used to estimate MP concentration. V_{Soil} is the volume of soil within one hectare of land, determined to be 3,000 m^3, given that the samples in this study were taken to a depth of 0.3m. Finally, ρ_{Soil} is the density of soil, estimated to be 1,550 kg/m^3 for loams, and 1,450 kg/m^3 for silt loams [45]. However, changes in organic matter from amendments can alter bulk density, and the use of fixed soil density values represents a limitation of this approach [46].

$$[Soil] = \frac{MP}{M_{Soil}} \quad (5)$$

Where $[Soil]$ is the estimated concentration of MP added to soil via the application of biosolids (expressed as number of particles per kilogram of soil, MP/kg).

To create a predictive model of MP size, the data was grouped into size classes with 25μm intervals. The midpoint of each class was calculated as the average of the lower and upper bounds. Due to uncertainties in quantifying MPs within the smallest size ranges, any MPs smaller than 50μm were excluded from the analysis. A series of regression models, including Poisson, Negative Binomial, and Log-Normal were fitted to examine the relationship between the midpoints of the size classes and the frequency of occurrences. Model fit was assessed using Akaike Information Criterion (AIC) and deviance scores. Additionally, visual inspection of the models was conducted to evaluate their effectiveness.

The data for this study is available on the University of Guelph's Research Data Repository at: https://doi.org/10.5683/SP3/HEVKKP

4 Results and Discussion

4.1 Microplastic Concentrations

MP concentrations within biosolid-amended fields ranged from 1,450 ± 226 MP/kg to 3,610 ± 684 MP/kg, with an average of 2,442 ± 268 MP/kg. These levels are slightly elevated compared to global concentrations of MPs in biosolid-amended soils, where most studies report values between 532 MP/kg and 2,263 MP/kg [47]. In addition, MP concentrations in fields amended with Canadian biosolids exhibited considerable variability, ranging from 187 to 6,870 MP/kg [5, 30]. MP levels in biosolid-amended soils can be influenced by a number of factors, including biosolid application rate, number of applications, biosolid type, and biosolid source [48]. Furthermore, environmental factors such as temperature and precipitation can affect the transport, retention, and breakdown of MPs within soil [33, 49]. Additionally, soil properties such as texture, organic matter content, and porosity play a role by influencing MP movement and aggregation [34, 50]. All fields chosen for this study received biosolids sourced from the same region, suggesting that MP concentrations in these biosolids may be higher than global averages. However, MP loading in

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the soils could vary depending on the application rate, number of applications, and cumulative amendments, highlighting the difficulties of comparing MP concentrations across studies. Furthermore, variations in extraction methods make it challenging to compare MP concentrations across studies globally [51]. Nonetheless, the findings of this study are consistent with estimates of MP concentrations within Canada and worldwide.

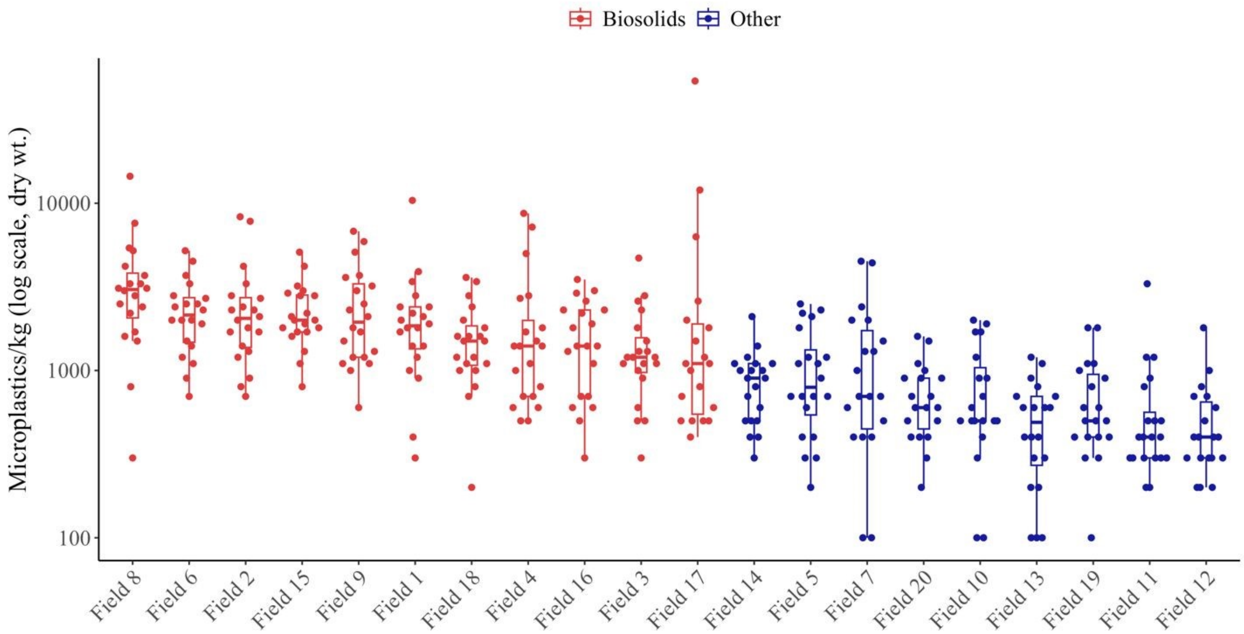

Figure 2. Microplastic concentrations in 11 biosolid-amended fields (red) and 9 unamended fields (blue), ordered by decreasing median concentration. The box represents the interquartile range (IQR), with the lower and upper edges corresponding to the first (Q1) and third quartiles (Q3), respectively. The horizontal line inside the box indicates the median (Q2). The whiskers extend to the minimum and maximum values within 1.5 times the IQR.

Of the twenty fields analyzed, the eleven with the highest MP concentrations were all biosolid-amended (Figure 2). MP concentrations within fields that have never received biosolids ranged from 490 ± 87 MP/kg to 1,250 ± 287 MP/kg, with an average of 775 ± 50 MP/kg. An independent t-test ($\alpha = 0.05$) comparing the average MP concentrations between biosolid-amended and non-amended soils revealed significant differences across locations 1, 2, and 3 ($p < 0.05$). These results suggest that biosolid amendments significantly increase the average MP concentrations within agricultural soils.

Individual samples from biosolid-amended fields varied widely, from 0 to 53,800 MP/kg, with the highest concentration (53,800 MP/kg) far exceeding the next highest value (14,500 MP/kg), while non-amended fields had a more limited range from 0 to 4,500 MP/kg. Additionally, an F-test showcased a significant difference in the variance between biosolid-amended and non-amended fields ($F_{(219,179)} = 33.79$, $p < 2.2e-16$). This suggests that the distribution of biosolid-derived MPs within the soil matrix is likely to exhibit significant variability. This is likely due to the heterogeneity of the composition of biosolids, as well as inconsistencies during the spreading process [48, 52]. In contrast, when comparing variances between fields that received one application

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of dewatered biosolid and those that received one application of liquid biosolid, no significant difference was observed ($F_{(139,58)} = 1.42$, $p = 0.129$), indicating that the type of biosolid applied does not appear to substantially influence variability in MP concentrations. MPs are hydrophobic particles with large surface areas, so they tend to interact with one another [53]. This, combined with their interactions with organic matter, can lead to pockets of increased MP density within biosolids [6]. This will further contribute to the uneven distribution of MPs in biosolid-amended fields.

While it is clear that biosolids introduce MPs to the soil matrix, factors such as the type of biosolid and the frequency of application can influence MP concentrations within fields (Figure 3).

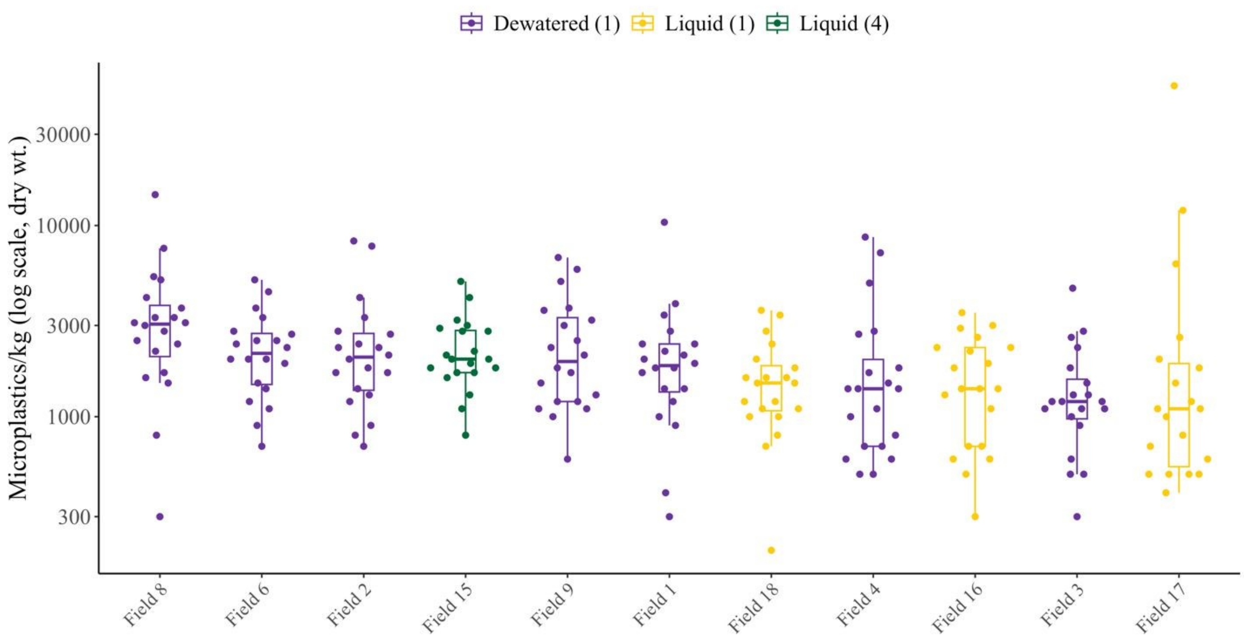

Figure 3. Microplastic concentrations across 11 fields showcasing different types of biosolids applied, ordered by decreasing median concentration. Numbers in parentheses indicate the number of biosolid applications (Liquid (1) is one application of liquid biosolids, while Liquid (4) is four applications of liquid biosolids). The box represents the interquartile range (IQR), with the lower and upper edges corresponding to the first (Q1) and third quartiles (Q3), respectively. The horizontal line inside the box indicates the median (Q2). The whiskers

For example, fields with a single application of dewatered biosolid had an average MP concentration of 2,412 ± 175 MP/kg, whereas fields with a single application of liquid biosolid averaged 1,690 ± 226 MP/kg. An independent t-test ($\alpha = 0.05$) comparing these two groups revealed a significant difference in MP concentrations ($p = 0.0126$). Although the application rate of liquid biosolid (80.13–100.00 m³/hectare) was higher than that of dewatered biosolid (19.77–63.71 m³/hectare), this did not correspond to higher MP levels (Table 1). Liquid biosolids have a solids content of 2–7%, while dewatered biosolids exhibit a broader range, with solids content between approximately 20–80% [54, 55]. Evidence suggests that MPs are primarily retained within the solids portion of biosolids, indicating minimal loss of MPs during the dewatering process [56]. As a result, MP density is much greater in dewatered biosolids. While the application

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rate of liquid biosolid is greater, it is not high enough to offset the MP content of dewatered biosolids, resulting in increased MP levels in fields spread with dewatered biosolids.

Field 15, which was amended with liquid biosolid, exhibited an average MP concentration of 2,300.00 ± 232 MP/kg. This elevated concentration is likely due to the field receiving a total of four applications (i.e., 2015, 2017, 2019, 2021) (Table 1). While this concentration was not statistically different from fields that received a single biosolid amendment ($p = 0.0645$), Field 15 has a higher MP concentration compared to fields that received a single application of liquid biosolid (Fields 16, 17, and 18) (Figure 3). Previous evidence suggests that successive applications of biosolids contribute to an elevated accumulation of MPs [57]. However, while all four of these fields were spread with liquid biosolids from the same source and had similar application rates, the observed MP concentrations in Field 15 are not proportionally higher. This provides evidence that there is a partial loss or redistribution of MPs in the time following application.

Estimations of MP load introduced through biosolid application vary widely based on factors such as application rate, biosolid type, and biosolid density (Eq. 1-5). For example, Fields 16, 17, and 18, which were amended with liquid biosolids, are estimated to have received 151–188 MP/kg of soil (Table S26). In contrast, Fields 1–4, 6, 8, and 9, which received dewatered biosolids, are estimated to have received 438–1674 MP/kg of soil, with large variations being reliant on the application rate (Table S26). Filed 15, which received four applications of liquid biosolid, is estimated to have additional MP concentrations in soil of 771 MP/kg. With these estimations, four applications of liquid biosolids (application rate of approx. 100 m^3/ha) are equal to one singular application of dewatered biosolid at an application rate of 29.33 m^3/ha. Furthermore, the estimated MP load from biosolid applications highlights that differences between amended and non-amended fields reflect the addition of biosolids rather than external factors (Figure 2).

4.2 Microplastic Morphology

MPs in biosolid-amended fields were 46.36 ± 3.83% fragments, 49.50 ± 4.11% fibres, 2.19 ± 0.33% films, 1.42 ± 0.04% foams, and 1.87 ± 0.35% spheres (n = 5596). In contrast, MPs in unamended fields were 67.42 ± 2.63% fragments, 29.55 ± 2.40% fibres, 1.88 ± 0.54 % films, 0.94 ± 0.34% foams, and 0.21 ± 0.21% spheres (n = 1174, Table S11). Some key differences between these fields include an increase in fibre content in biosolid-amended fields ($p = 0.000715$), along with a decrease in fragments ($p = 0.000295$) (Figure 4).

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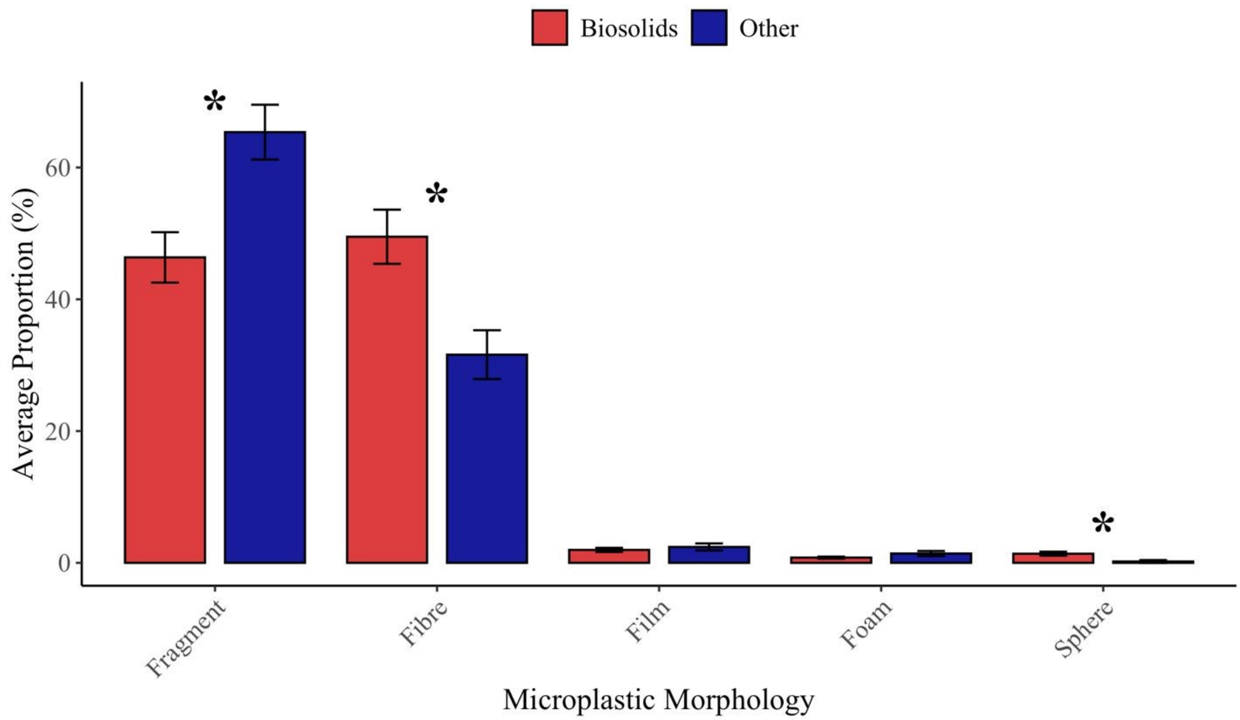

Figure 4. Mean microplastic morphology percentages for each morphology for biosolid-amended and unamended fields. Error bars represent calculated standard errors for each morphology. An asterisk (*) denotes a significant difference between morphology abundance ($\alpha = 0.05$).

While the reduction in biosolid fragment % is significant, it is likely a consequence of the increased fibre content introduced into the soil through biosolid application. It is widely documented that fibres are the most common MP found within biosolids globally, however, there are constraints in interpreting these patterns, as microplastics tend to lose their distinct morphologies as particle size decreases [58]. Large proportions of synthetic fibres such as polyester, polyacrylamide, and nylon are deposited in biosolids via the laundering of textile materials [59]. The fibre content in biosolids is so great that the detectability of synthetic fibres can serve as a reliable indicator of historical biosolid amendment in agricultural soils [60]. In addition to fibres, spheres showed a significant increase ($p = 0.00477$) in biosolid amended fields. Spheres are a type of primary MP, manufactured to a small size rather than resulting from the breakdown of larger plastic items. Notably, of the nine unamended fields, eight contained no spheres at all. The majority of spheres found in biosolids are derived from microbeads used in personal care products such as exfoliating scrubs, toothpaste, and cosmetics [61]. As of 2018, microbeads were classified as a Schedule 1 toxic substance under the Canadian Environmental Protection Act, 1999 [62], leading to a ban on their manufacture and use in personal care products. With the exception of Field 15, all biosolid-amended fields received biosolids generated between 2019 and 2021, suggesting that the microbeads detected in these fields are possibly from personal care products. However, as time progresses, it is expected that the abundance of microbeads in newly amended fields will decline due to compliance with the ban .

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While this study found that the majority of MPs in biosolid-amended soils are fibres, there is considerable variation in the literature. For example, Ziajahromi et al. [25] examined the MP content in two biosolid-amended fields in Queensland, Australia, where 1 to 3 applications had been made to specific areas, and found that only 15-30% of the MPs were fibres. In contrast, Adhikari et al. [48] investigated a biosolid-amended field in Washington, USA that had received 6 applications and identified that greater than 50% of the detected MPs were fibres. Furthermore, a study by Corradini et al. [57] which sampled 31 fields in Mellipillia County, Chile, with 1 to 5 biosolid applications per field, revealed a staggering 97% abundance of fibres. This inconsistency across studies highlights the numerous confounding variables involved in assessing MP concentrations in biosolid-amended soils. Factors such as the type, source, application frequency, and application rate of biosolids, along with variations in extraction methods and potential personal biases, all could potentially contribute to the wide discrepancies observed between studies.

Classification of MP morphology is crucial for the understanding of risk associated with MPs. Within soil, different MP morphologies have been shown to influence various soil properties, such as aggregate formation, bulk density, aeration, and microbial activity [63]. Furthermore, the potential toxicity of MPs to soil biota is closely tied to their surface area [64]. MP morphologies with larger surface areas pose a greater risk due to their increased capacity to interact with other pollutants, including metals, organic pollutants, and antibiotics [65].

4.3 Microplastic Size

Of the 6,770 particles categorized in this study, 6,759 were analyzed for size, while 11 particles were excluded because they were fibres that were entangled or overlapping, making accurate measurement impossible. Following analysis, it was determined that detected MP sizes ranged from 10.81 – 4,909.65µm, with an average size of 442.88 ± 6.55µm, and a median size of 240.13 µm. A well-established trend emerged showing an increase in MP frequency as particle size decreased [66, 67]. Specifically, 57.5% of all MPs identified were smaller than 300µm (Table S17). This trend was consistent across different MP morphologies, with fragments (76.5%), films (57.8%), foams (74%), and spheres (100%) showing similar patterns (Table S14). However, only 28.0% of fibres fell below 300µm. Fibres dominated the larger MPs, comprising 89.7% of MPs above 500µm (Tables S14, S15). Interestingly, 90.3% of all MPs below 100µm were considered fragments (Table S15). This is likely due to the challenges associated with visually quantifying particles at smaller sizes, as determining MP morphology becomes increasingly difficult. This often leads to a higher inclination to classify most MPs as fragments. Additionally, identifying MPs smaller than 50µm proved challenging, which likely contributed to an underestimation of MPs within this size range. Finally, there was no clear difference in MP size between biosolid-amended and unamended fields (Table S16).

The trend of increased agricultural soil MP frequency at smaller sizes is seen widely across the literature [68-71]. The degradation of MPs is controlled by factors such as UV radiation, heat, microbial activity, and physical abrasions [42]. As these processes break MPs into smaller pieces, their frequency within smaller size classes increases. Research shows that smaller MPs are more likely to undergo both horizontal and vertical movement within soil [33]. For example, they are more likely to penetrate deeper into the soil column, raising the risk of entering groundwater [72].

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In agricultural fields, smaller MPs are more prone to migration during rainfall events, increasing their likelihood of reaching freshwater systems [33]. They are also more susceptible to wind, which enhances their potential for long-term transport [73]. Additionally, smaller MPs are more likely to be ingested by soil biota, potentially leading to further horizontal or vertical movement through bioturbation, as well as adverse effects on the organisms that ingest them [74].

Due to challenges in accurately quantifying very small MPs, a Poisson regression model (Pseudo $R^2 = 0.93$) was applied to estimate the total MPs within the smallest size classes (<25µm and 26–50µm) (Figure 5).

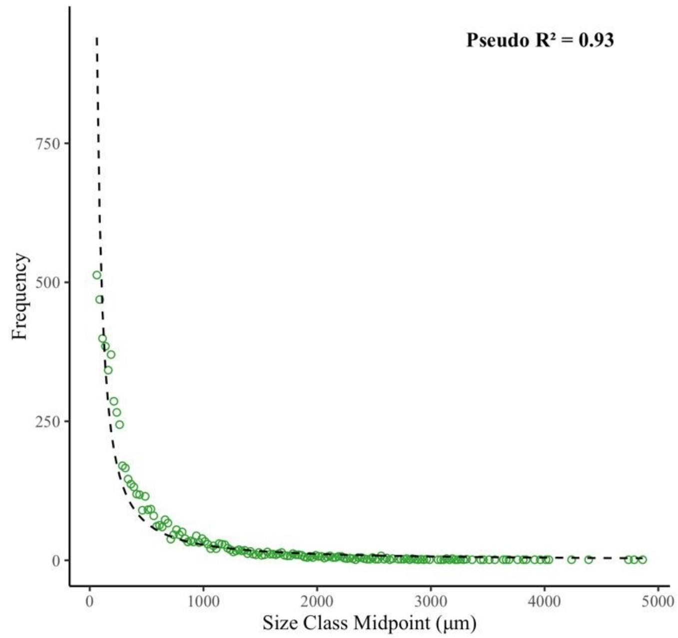

Figure 5. Poisson regression model fit to the frequency of microplastic size classes. The points represent the observed frequency for each size class midpoint (µm, n=6,759), while the dashed black line indicates the fitted Poisson regression model. The pseudo R^2 value (0.93) is displayed in the top right corner.

This model was used under the assumption that MP frequency continuously increases as size decreases. The observed values for these size classes were 80 MPs (<25 µm) and 361 MPs (26–50 µm). However, according to the predictive model, the estimated counts were significantly higher, with 7,260 MPs expected in the <25µm range and 1,799 MPs in the 26–50µm range. It is important to note that these predictions represent the total MPs counted across all 20 agricultural fields. Estimates for individual fields would be considerably lower. Nonetheless, this model highlights the potential for error in visually quantifying MPs as the size decreases, suggesting that the actual MP counts in this study are likely much higher than reported.

4.4 Microplastic Polymers

A total of 1,000 particles were identified using FT-IR microscopy, including 550 from biosolid-amended fields and 450 from non-amended fields. In biosolid-amended fields, 63.1 ± 3.21% of particles were confirmed plastic (specific identifications are below), while 9.60 ± 1.60% were

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organic matter, 13.5 ± 2.89% were natural or semi-synthetic fibres, 10.0 ± 2.07% were minerals, and 5.78 ± 1.65% were inconclusive. Similarly, in non-amended fields, 58.4 ± 1.79% of particles were confirmed plastic, with 17.1 ± 2.19% classified as organic matter, 8.00 ± 1.20% as natural or semi-synthetic fibres, 10.2 ± 1.71% as minerals, and 6.22 ± 1.08% as inconclusive (Table S18). Additionally, no individual field had a plastic confirmation rate lower than 50%. Difficulties arose during FT-IR analysis as particles had to be large enough to transfer from the sample to a compression cell. Additionally, the Bruker LUMOS II FT-IR microscope used had a sensitivity of ~100 μm. These two factors made analysis of particles below 100 μm extremely difficult.

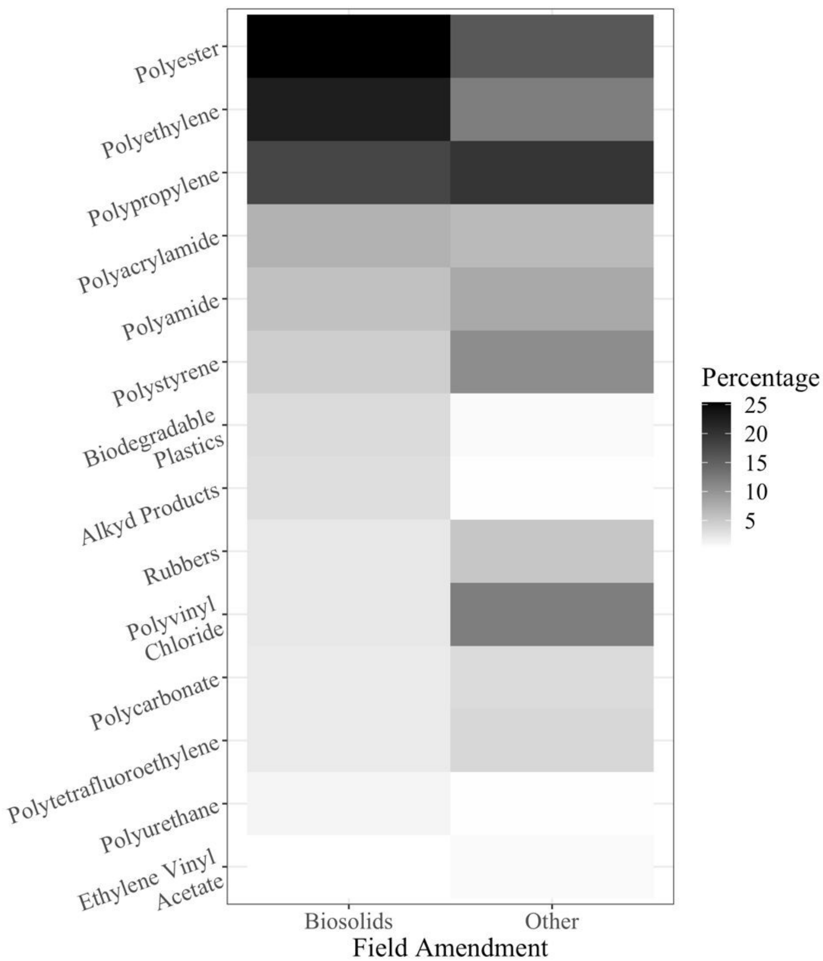

Figure 6. Heatmap highlighting the percentage (%) of each positively identified microplastic across field amendments (n = 347, n = 263 for "Biosolids" and "Other", respectively). The "Rubbers" category includes epichlorohydrin rubber (ECO), ethylene propylene rubber (EPR), nitrile butadiene rubber (NBR), silicone rubber, and fluoroelastomer (FKM). The "Biodegradable Plastics" category includes cellulose acetate, cellulose propionate, ethyl cellulose, polybutylene succinate (PBS), polylactic acid (PLA), and polyvinyl alcohol (PVA). "Alkyd Products" refer to alkyd resins or paints.

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The most prominent MPs found in biosolid-amended fields included polyester (25.3%), polyethylene (22.4%), and polypropylene (17.9%) (Tables S22-S24, Figure 6). These polymers dominated the composition of microplastics in biosolids, which is consistent with global trends observed in various studies on the MP content of biosolids [23, 29, 58, 75]. In contrast, the dominant microplastics in non-amended fields were polypropylene (19.8%), polyester (16.0%), polyvinyl chloride (12.2%), polyethylene (12.2%), and polystyrene (10.6%) (Tables S22-S24, Figure 6). The majority of polyvinyl chloride particles were identified in Fields 10-13, which are all owned and cultivated by the same farmer. This suggests that previous agricultural practices or land use may have contributed to the elevated levels of polyvinyl chloride observed in these fields, making them unique compared to the others. A possible explanation could be the usage of plastic tubing for drainage, as drainage tile is primarily composed of high density polyethylene or polyvinyl chloride [76]. While some overlap with the biosolid-amended fields exists, the relative abundances differ, particularly with a higher prevalence of polyester and polyethylene in biosolid-amended soils. These differences are likely as a result of the high prevalence of polyester and polyethylene within biosolids, further providing evidence that the MPs being applied to soils through biosolids are incorporated into the soil matrix.

4.5 Field Characteristics and Microplastic Retention

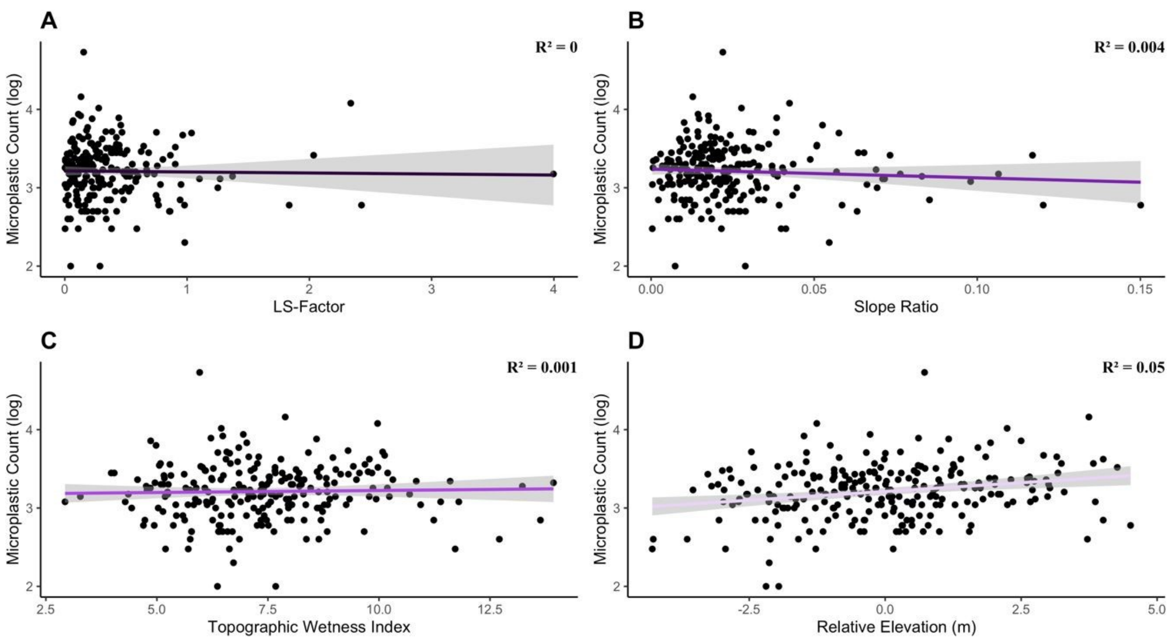

Figure 7. Relationships between log-transformed microplastic counts and field characteristics in biosolid-amended agricultural fields. Panels (A) – (D) show the relationships with LS-factor, slope ratio, topographic wetness index, and relative elevation, respectively.

Analysis of microplastic (MP) counts in relation to the physical characteristics of each biosolid-amended field revealed that LS-factor ($R^2 = 0$), slope ($R^2 = 0.004$), topographic wetness index ($R^2 = 0.001$), and elevation ($R^2 = 0.05$) were not reliable factors for predicting MP deposition (Figure 7). The data for this analysis were separated between biosolid-amended and unamended fields, as biosolid application was found to be a strong confounding variable in MP

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concentration. Similarly, analysis of physical characteristics in unamended fields showed no predictive relationship, with LS-factor (R^2 = 0.001), slope (R^2 = 0.001), topographic wetness index (R^2 = 0.003), and elevation (R^2 = 0) also demonstrating no capability for predicting MP deposition (Figure S1).

While these factors were hypothesized to influence the movement of MPs within agricultural soils, the data provides little support for this hypothesis. Some studies suggest that microplastic abundance may be inversely correlated with soil moisture content, and slope, LS-factor, and elevation all play a role in water redistribution [77]. Heerey et al. [78] examined MP concentrations along slopes within biosolid-amended fields by measuring MP levels at different transects, finding that MP abundance remained relatively unchanged throughout the slope. Additionally, it is important to note that the movement of MPs within soil is largely dependent on soil texture [79]. Of the 20 fields sampled in this study, 13 are classified as silty loams, while the remaining 7 are classified as loams. This subtle difference in soil texture could influence the long-term movement and behavior of MPs within these fields. It is also important to note that 10 of the 11 biosolid-amended fields received their first application within 3 years of sampling, meaning these factors may become more reliable predictors over time as MPs have more opportunity to travel within the field.

Although no significant relationship was found between the four factors (elevation, LS-factor, slope, and topographic wetness index) and MP counts, we feel using Latin hypercube sampling was still a valuable approach. This method reduces bias by avoiding over/under-sampling specific areas and ensured that many possible environmental conditions were considered. Additionally, this approach allows for further exploration of other physical characteristics of fields, such as land cover, proximity to water sources, and soil permeability, which may more effectively influence microplastic movement and deposition. In addition, further analysis on incorporating factors such as time since amendment and spatially resolved soil chemistry (e.g., organic carbon and cation exchange capacity) may help to better explain within-field dispersion patterns and provide additional insight into the mechanisms driving MP distribution.

5 Conclusion

This study provides further evidence that biosolid applications significantly increase MP concentrations in agricultural soils. On average, MP levels in biosolid-amended fields were 3.15 times higher than in non-amended fields, with considerable variability observed both within and between fields. The influence of biosolid type was also evident, with dewatered biosolids contributing significantly higher MP loads compared to liquid biosolids, despite differences in application rates. Furthermore, MP morphology in biosolid-amended soils was distinct from that in unamended soils, with fibres dominating in biosolid-treated fields. The high fibre content aligns with previous research, highlighting the role of biosolids as a key pathway for synthetic fibres into the soil. In turn, this led to a shift in the distribution of MP polymers found within biosolid-amended fields, as polyester and polyethylene were more abundant. Additionally, while MPs were detected across a broad size range, smaller MPs were more prevalent, which is consistent with trends observed in other MP studies. Finally, MP retention showed to be

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unaffected by topographical variation. However, this could be subject to change as more time passes. Overall, this study contributes to the growing body of evidence on MP contamination in agricultural soils, reinforcing the impact of biosolids as a primary source of MPs. Future research should focus on long-term monitoring of MP accumulation and the potential ecological impacts of MPs in soil ecosystems.

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Data availability statement

Data for this article are available at the University of Guelph's Research Data Repository at https://doi.org/10.5683/SP3/HEVKKP