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Impact of Microplastics on Forest Soil Properties in Pollution Hotspots in Alluvial Plains of Large Rivers (Morava, Sava, and Danube) of Serbia

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Abstract: Plastic pollution has become a major environmental problem, while the products of its degradation, microplastics (MPs), appear everywhere on Earth. Data on MPs in agricultural soils have appeared lately, but a significant knowledge gap exists regarding forest soils. In Serbia, municipal waste is often dumped in forests, creating environmental problems that have not been documented. To explore the impact of waste dumping and MPs on forest fluvisols, we evaluated MPs from topsoils of three waste dumps and adequate visibly plastic non-contaminated forest sites located in alluviums of the largest rivers in Serbia. For assessing the influence of environmental factors on soil MPs, samples were taken in three forest vegetational seasons, in two years. The impact of MPs on soil structure, chemistry, and microbial respiration (MR) was examined. Undisturbed soil columns from uncontaminated sites with added known MP particles were used to estimate the dynamic of MP transfer through the topsoil. Large aggregate formation, soil coarse sand content, specific mass, porosity, and available P, but not MR were affected by contamination. Seasonal and annual environmental changes significantly influenced the behavior of MPs in forest luvisols. MPs effectively penetrated the deeper layers of soil columns within 3 months, with strong accumulation in the 0–10 cm layer.

Keywords: soil plastic pollution; waste dumps in forests; microbial respiration; MP soil vertical dynamic

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Academic Editors: Yuanying Peng, Xiaoyong Chen and Wende Yan

Received: 3 January 2025
Revised: 30 January 2025
Accepted: 6 February 2025
Published: 17 February 2025

Citation: Grujić, T.; Saljnikov, E.; Mutavdžić, D.; Jovković, M.; Stefanović, S.; Miladinović, V.; Krnjajić, S.; Belanović Simić, S.; Marjanović, Ž. Impact of Microplastics on Forest Soil Properties in Pollution Hotspots in Alluvial Plains of Large Rivers (Morava, Sava, and Danube) of Serbia. *Forests* **2025**, *16*, 363. https://doi.org/10.3390/f16020363

1. Introduction

Since its discovery, plastics have become an important part of human daily production and life [1]. The cumulative global production of plastics is expected to be 33 billion tons annually by 2050 [2]. However, only about 20% of plastics are recycled, while the remaining 80% end up in nature and eventually accumulate in soil, rivers, and ocean environments [3]. The plastic waste that accumulates in the environment is broken down into smaller fragments and particles under physical, chemical, or biological action, gradually forming microplastic (MP) particles that are <5 mm in size or amended with originally produced plastic particles of the same size [4–6]. Plastic waste is known for its stability and recalcitrance in the environment [2,6]; therefore, it is commonly assumed to be non-degradable. Plastic degradation is assumed to vary in the environment, ranging between 10 and more than 1000 years depending on the environmental condition, the type of plastic monomer, and the treatment applied to the plastic waste [7]. Nowadays, MPs causes numerous adverse effects on various ecosystems and living beings, from microorganisms to humans globally (summarized in [8]). Based on previous findings, MPs have been recognized as a major pollutant of anthropogenic origin on a global level, and the resolution of the United Nations Environmental Assembly to end plastic pollution by 2024 was adopted during the UNEA 5.2.

The danger of plastic waste became evident much earlier in water ecosystems [9]. However, in recent years the impact of MPs on terrestrial ecosystems has become a research hotspot. Recent findings on temperate terrestrial environments revealed the occurrence and persistence of MPs in soil, the effects of MPs on soil physical, chemical, and biological properties, and the toxicological effects on the growth, reproduction, survival, and immunity level of the soil biota [2,10–14]. Despite increasing research interest, there is a lack of sufficient information on the effects of MPs on different terrestrial ecosystems [10]. Moreover, most data on MPs in soils originate from the experimental conditions where the addition of known types and weights of non-aged plastic particles are often used [11,15,16], or from agricultural soils already highly disturbed by agricultural practice [17,18]. Still, the work on undisturbed soils like those in natural forests is negligible, especially in Europe, and we detected no previous data in Serbia [19]. On the other hand, forest soils have significantly different features than arable soils: undisturbed site-specific profiles, metabolism, structures that depend on vegetation cover (tree species), pedofauna activity, climate, and soil–water dynamics, as well as different exposure to the irradiation from the sun [19]. Forest soils have more or less pronounced organic surface layers, where the microbial biodegradation of organic matter (plant litter) starts, followed by the nutrient transformation and transport towards deeper mineral layers specific for every soil type [19]. MP particles as organic polymer-based substances are expected to significantly influence the forest topsoil and follow the same vertical path into deeper soil layers as other particles from organic layers [19,20].

Due to the specific soil water dynamic and fluvial influence of the river, alluvial forests are generally unique ecosystems that perform a high diversity of ecosystem services [21]. They influence groundwater preservation, flood risk, fluvial activities of the river, carbon sequestration, and soil erosion, serve as a source of biodiversity, and have socio-economic value not only as a wood source but also providing secondary forest products and recreational facilities [22]. Floodplain soils are generally recognized as very specific environments that are highly exposed to different pollution agents, including MP [23]. Constant sedimentation caused by the fluvial activities was recognized as one of the major factors of the MP accumulation especially in riparian zones [24]. Strong accumulation of the MPs was dated to the younger sediments (corresponding to the rise in plastic usage in 1970 ties), especially in topsoils [24]. Additionally, extreme flooding that has been detected in recent decades caused a widespread redistribution of sediments and pollutants across wide alluvial areas, with long-term effects on floodplain ecological, environmental, agricultural, and socio-economic features [24,25]. Therefore, these areas are among the most endangered ecosystems globally [26]. In natural conditions, occasional and seasonal floods renew the sediments full of nutrients, which, together with high water availability, establish very fertile soils that support high plant biodiversity and biomass production in floodplain forests [21]. The dynamic of soil water availability in such ecosystems strongly depends on the dominating climate. Due to the access to the groundwater, in Mediterranean and sub-Mediterranean zones, alluvial forests are more resistant to the summer high temperatures and drought and serve as shelters for diverse flora, fauna, and soil microorganisms. However, these soils are highly vulnerable to environmental changes, especially since they are constantly subject to the deposition of different pollutants (pesticides, excessive fertilizers, municipal waste, heavy metals, etc.) that can migrate into groundwater and end up in the rivers [27].

In Serbia, alluvial forests express a vegetation differentiation gradient caused by the intensity of the fluvial activities of the river and the depth of groundwater [28]. Most riparian zones of the large riverbanks are populated with *Populetum albae* on the sandy or clay fluvisoils, which are usually highly porous [29]. Further from the riverbank, less influenced by fluvial activity, mixed forests of *Quercus robur*, *Populus alba*, *Fraxinus angustifolia*, and *Ulmus* sp. are the dominating forest types, and soils are more developed and less leaky like humogley, semigley, and glay [28]. According to previous investigations, forest soils in alluviums of river Sava and its tributaries are usually formed by young calcareous sediments originating from the Dinaric Alps [28,29]. These soils experience distinct fluvial activities with the maximum in May/June during the melting of the snow in the high mountains and the highest in the year rainfall rates [28,30]. Soil processes are strongly dependent on the seasonality of environmental factors and the dynamic of soil water availability [29]. High soil pH and amounts of Ca and Mg cause low phosphorous availability, while high soil organic matter (SOM), N, and K availability imply high soil fertility [21,28,29]. Therefore, P is the limiting factor for plant growth in these soils. A significant clay percentage, large aggregates, and high porosity characterize alluvial fluvisols in the wider sedimentation zones of larger river plains in Serbia [28,29]. Additionally, these forests are the extremely rare habitats of the highly priced white Piedmont truffle (*Tuber magnatum* Pico), which increases their importance and economic potential on the global level [29]. Beside their high importance, alluvial forests are the most endangered forest type in the country, mostly because of centuries-long overexploitation of timber, urbanization, and industrialization—the transformation of forests into agricultural and industrial land. Nowadays, these forests are scattered remnants, mostly privately owned, and not managed sustainably—undesired timber harvesting is still a dominant risk factor that exposes these rare ecosystems to the effects of climate change. Furthermore, in recent decades, they have become targets for numerous illegal depositions of municipal waste. Due to the poor waste management in Serbia, local inhabitants consistently dump their undesired waste (including waste from the plasticulture production of vegetables, municipal waste, pesticide, fertilizer packaging, etc.) into forests or even rivers. The problem has become alarming. We are unaware of any published or available data regarding MP pollution that such dumping causes, or any other data on MPs in forest soils in wider areas of Southeast Europe. Also, waste dumps are very convenient spots for studying MP behavior in natural forest soils, since the enrichment of plastic particles is expected, and they can be efficiently compared to the places not directly exposed to MP pollution, especially in fluvisols exposed to sedimentation [29].

To provide first insights on the influence of waste depositions and MPs on the quality of floodplain forest soils in Serbia (and wider), pollution hotspots in alluvial planes of large rivers (Danube, Sava, and Morava rivers) were chosen as the targets of the current investigation. Being on the border between rivers and land, fluvisols in *Populetum albae* are the most pollutant-exposed forest soil types since they are collection points of the gravitational waterways, including plastic waste from higher elevations and surrounding agricultural fields and flooding deposits [20]. We have recognized illegal waste dumps as the major hazard points for MP pollution, not only in the locations of depositions but also in the surrounding areas and river waters due to the fluvial and vertical groundwater movements. The main objectives of the current study were to provide the first data on the presence and influence of MP on pollution hotspots in forest alluvial soils in large alluvial plains of Serbia, determine the influence of waste depositions on forest soil parameters, determine the influence of seasonal environmental factors on MP dispersal in natural forest fluvisols, and determine the vertical distribution of MP in undisturbed natural topsoil columns as influenced by gravitational water.

The main hypotheses of our study were as follows: (1) MP presence in the forest soil will be strongly affected by waste depositions; (2) soils of the largest river (Danube) floodplain forests will be the most polluted due to the large river capture area and sedimentation introduced by other tributaries; (3) long-term depositions of plastic waste affect forest soil physical and chemical characteristics; (4) waste depositions and MPs affect soil MR; (5) seasonal changes in rainfall/soil water content and temperature will significantly affect MP distribution in topsoils; (6) due to the structure and intensive dynamics of surface water in alluvial forest zones, vertical MP movements through the undisturbed alluvial forest topsoil columns will be fast.

2. Materials and Methods

2.1. Site Description and Sampling

The research sites were in the alluvial plains of the three largest rivers in Serbia (Figure 1), the Danube (44°42′02.62″ N/21°01′43.12″ E) and its tributaries Sava (44°43′28.45″ N/20°18′10.28″ E) and Morava (43°37′06.50″ N/21°31′55.21″ E). The Danube sampling location was located after the Sava joint, on the wide Morava joint. Sava and Danube sites were positioned on the very edge of the Pannonian plain and exposed to the typical continental climate. According to the Hydrometeorological Institution of Serbia (https://www.hidmet.gov.rs/ (accessed on 23 November 2024)). the mean annual temperature (for the period 1961–1990) for the area is about 11 °C, while the highest mean temperatures are in July and August (about 21.1 °C) and the lowest in January (about −2.1 °C) with an average annual precipitation of 690 mm. In contrast, the Morava site was in Southern Serbia (near the Great Morava constituent South Morava) in the milder continental climate with a Mediterranean influence (mean annual temperature of 11.4 °C and average annual precipitation of 589 mm, while the highest mean temperatures are in July and August (about 22.3 °C) and the lowest in January (about 0.6 °C)). The climatic characteristics of the examined years are presented in Table S1. In Serbia, maximum rainfall is usually in May/June, when the seasonal floods and potential inundation caused by the river hydraulic maximum of the local rivers are common.

In the described locations, we detected waste dump sites in forests that were taken as “contaminated”, while locations within the same forests, at least 100 m away, that were visibly not exposed to the plastic waste were taken as “uncontaminated” (Table S2). The local inhabitants created Morava and Danube site waste depositions in the zones that were not directly influenced by the seasonal floods but may be inundated. Between November sampling in 2022 and June sampling in 2023, the waste was removed from the Morava site, leaving only debris in the soil. The Sava contaminated site was in the seasonal flooding zone and may have been created, at least partially, by the flooding deposits. After the inundation period in June/July 2023, the waste was still there but relocated a bit. The sites and waste dumps characteristics are presented in Table S2.

The forest litter layer (or layer of waste) and organic soil layers were removed, and composite samples (from 6 subsamples) were taken at 0–15 cm depth at each point. For detailed physical and chemical parameter analyses, soil samples were taken in November 2022 and 2023, at the end of vegetation seasons when a high level of soil metabolism is expected [28,29]. For microbial respiration and MP isolation, soil samples were taken in three forest vegetation seasons: June (maximum rainfall and soil water content, soil pores saturated, canopy developing), August (minimum rainfall and soil water content, topsoil highly aerated, full canopy), and November (increased rainfall after the summer drought, soil air-to-water ratio balanced, leaf fall—canopy strongly reduced) 2022, and 2023, to assess the influence of seasonality of environmental factors on these two parameters.

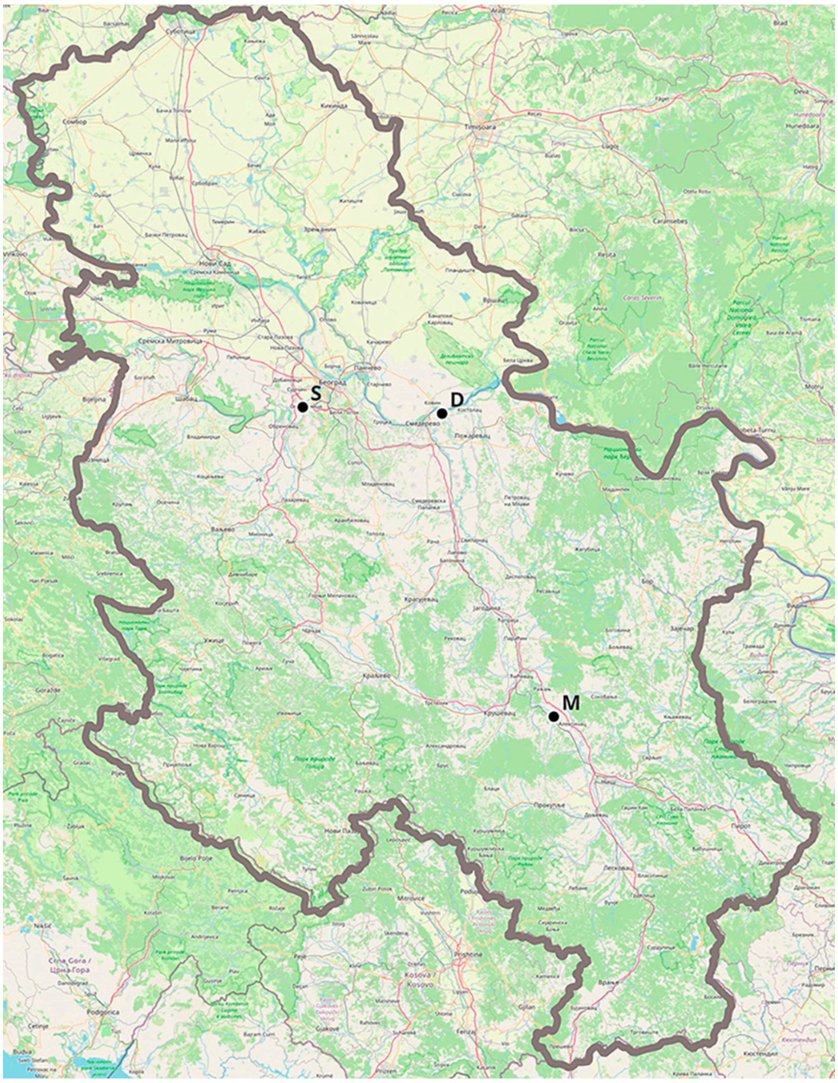


Figure 1. Sampling locations in the map of Serbia. For the visualization of sampling points, a thematic map was created using QGIS (version 3.34.10), a free and open-source Geographic Information System software, with OpenStreetMap 2.0, license (CC BY-SA 2.0). XYZ Tiles as the basemap. S—Sava sampling site; D—Danube sampling site; M—Morava sampling site.

2.2. Analytical Methods

Particle size distribution was determined by the pipette method according to the modified International "B" method [31]; bulk density was measured by drying the soil cores at 105 °C to a constant weight [32]; soil specific mass (particle density) was determined using a pycnometer with water method [33]; porosity was obtained computationally from bulk density and specific mass [34]; aggregate size distribution was determined using dry sieving method according to [35].

Soil pH was determined with a glass electrode pH meter (Mettler–Toledo, Greinfensee, Switzerland) (in 1 M KCl 1:5 v/v and water) [36]; the content of $CaCO_3$ was determined volumetrically [37]; available phosphorus (P_2O_5) was analyzed spectrophotometrically, and available potassium (K_2O) was analyzed by flame emission photometry, using the AL method [38], where K_2O was determined by flame emission photometry (HINOTEK FP6440 flame photometer, Ningbo, China and P_2O_5 by a spectrophotometer SHIMADZU CORPORATION UV-VIS spectrophotometer UV—160 A, Kyoto, Japan after color development with ammonium molybdate and stannous chloride. Total carbon content was determined after dry combustion on an elemental CNS analyzer Elementar Analysensysteme GmbH, Langenselbold, Germany [39]; total nitrogen content (N) was analyzed by dry combustion using the elemental CNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) [40]; soil organic matter (SOM) was calculated using $CaCO_3$ content and total C [41]; cation exchange capacity (CEC) was determined using the tube leaching method with 1 mol L^{-1} ammonium acetate and reading the Na^+ concentration in the resulting solution by atomic emission spectrometry [42]. Soil microbial respiration was measured as CO_2 evolved due to organic C mineralization under controlled laboratory conditions (temperature 28 °C and soil moisture 50% WHC) and trapped by 0.5 mol L^{-1} NaOH. The amount of residual free NaOH was determined by titration with 0.05 mol L^{-1} HCl [43]. Every sample was measured in triplicates.

2.3. MP Extraction from the Soil Samples

The content of MPs < 5 mm in size was determined as follows: 30 g of air-dried composite soil samples per site (in triplicates) was suspended in 500 mL of deionized water and 50 mL of 1 M sodium pyrophosphate, followed by wet sieving through a set of corresponding meshes for 5 min or until clear seepage (Retsch vibratory sieve shakers of the series AS 200 basic B, 2018. Amplitude 85, Haan, Germany), then transferred to tubes and centrifuged for 5 min at 1500 rcf (MSE Harrier 15/80 MSB080.CX1.5 MSE (UK) Limited, London, United Kingdom) using sucrose solution as density separation media to separate from the mineral part [44]. The remaining sample was treated with 30% H_2O_2 to digest organic matter, and undigested organic matter was removed manually using a stereomicroscope (Optech LFZ, San Prospero, Italy). After the removal of non-plastic particles, the remaining MP samples were weighed on an analytical balance (RADWAG AS 160/C/2, Radom, Poland).

2.4. Soil Columns

To estimate the dynamic of MP in explored soil environments, the 30 cm deep undisturbed soil columns with a 7 cm diameter were sampled at the uncontaminated sites in Sava, Danube, and Morava locations in triplicates. The freshly sampled columns were transferred to the laboratory and installed vertically, with the water-permeable mesh at the bottom. Known amounts of MP, 5 g of 1000–3000 μm-sized PVC, and 1.5 g of 500–1000 μm-sized PVC were applied on each column surface. The columns were kept at ambient temperature and watered with tap water once a week for 3 months. The amount of water applied on column surfaces was decided upon analyzing the five-year rainfall rates (2017–2021) at the research sites (https://www.hidmet.gov.rs/ (accessed on 25 April 2023)). It was calculated that approximately 845 mL of precipitation would fall on average on an area of 38.5 cm^2 (columns surface) in June, July, and August. At the end of the experiment, MPs that remained on soil column surfaces were collected, sieved through a 1000 μm sieve to separate size groups, and measured on an analytical balance (RADWAG AS 160/C/2, Radom, Poland) to assess the percentage of MPs that penetrated soil columns. MPs that penetrated soil columns were isolated from the matrix and quantified. The columns were divided into 3 segments by depth: 0–10 cm, 10–20 cm, and 20–30 cm, and MP was isolated from each depth and weighted to access the vertical distribution.

2.5. Statistics

Linear modeling, specifically categorical regression (CATREG), was used to assess the effects of soil contamination, location, and sampling year on the physico-chemical properties of soil. CATREG allows the analysis of relationships between categorical predictors and a dependent variable while accommodating nonlinear effects. Categorical regression quantifies categorical variables by assigning numerical values to the categories, resulting in an optimal linear regression model for the transformed variables. Encoding of categorical independent variables was carried out by creating one or more dummy variables (depending on the number of levels of the categorical variable). Each dummy variable only has the value 0 or 1. The linear model created in this way was used to evaluate the significance and relative importance of each independent variable. Model performance was assessed using adjusted R-squared values and statistical significance of predictors ($p < 0.05$). Linear modeling was conducted using IBM SPSS Statistics (version 25).

To examine the influence of the observed factors on respiration, total microplastics, percentage of applied MP that penetrated soil columns, and percentage distribution of isolated MP that penetrated soil columns by depth, an appropriate factorial analysis of variance with a balanced design was used. Duncan's test was used for post hoc comparisons. A significance level of 5% was used in all tests. The quantification of the linear relationship between soil parameters was evaluated by the Pearson correlation coefficient. All the graphs were constructed using the ggplot2 package in R version 4.4.2. [45].

3. Results

3.1. Characterization of the Forest Soils in Three Investigated Locations

According to field observations and laboratory data, the soils of the investigated sites were classified as clay loam Fluvisols (Calcic) (FAO/ISRIC/ISSS, 2006, Table S3). No significant differences in physical parameters were detected when uncontaminated sites were compared. When contaminated sites were compared with uncontaminated ones, the differences appeared obvious in parameters: specific mass (particle density), porosity, and texture fracture of "coarse sand". Contaminated sites had significantly higher specific mass (particle density), lower porosity, and higher percentages of coarse sand than uncontaminated sites (Table S1), confirmed by the linear regression model (Table 1). Concerning aggregate size distribution, no significant differences between any class of aggregates were detected except the largest one (larger than 10 mm, Table S4)—pollution strongly decreased large aggregate formation, which was confirmed by the linear regression model (Table 1). Analyses of the chemical soil parameters revealed that all fluvisols examined originate from the calcareous sediments, and they had high pH, were rich in organic C, total N, and available K, but very depleted with regard to available P (Table S5). However, the categorical linear regression model revealed only a significant increase in available P in polluted sites (Table 1), while no other parameter appeared to correlate significantly with waste deposition. Available potassium was not influenced by waste deposition but by environmental factors (year of sampling, Table 1). The linear regression model pointed out that the Morava location was different from the other two having higher percentages of course sand, large aggregates, and higher availability of potassium than in the other two.

Table 1. A summary of the results of a categorical regression model was performed by presenting a table of key statistics that highlights the relationships between predictors (location, contamination, and year of sampling) and the soil parameters in contaminated and uncontaminated sites in alluvial forests in Serbia. Model performance was assessed using adjusted R-squared values and statistical significance of predictors ($p < 0.05$).

	Soil Parameters	Factor's Category	Regression Coefficient	R^2 (Adj)
Physical parameters	Specific mass (particle density)	Intercept (Danube + C)	2.596	0.396
		Morava (vs. Danube)	−0.060 [ns]	
		Sava (vs. Danube)	−0.048 [ns]	
		UNC (vs. C)	0.068 *	
	Porosity	Intercept (Danube + C)	60.074	0.285
		Morava (vs. Danube)	0.447 [ns]	
		Sava (vs. Danube)	0.095 [ns]	
		UNC (vs. C)	1.032 *	
	Coarse sand	Intercept (Danube + C)	3.60	0.719
		Morava (vs. Danube)	7.45 **	
		Sava (vs. Danube)	0.03 [ns]	
		UNC (vs. C)	−4.32 **	
	Aggregate size > 10 mm	Intercept (Danube + C)	11.29	0.855
		Morava (vs. Danube)	8.88 **	
		Sava (vs. Danube)	−0.93 [ns]	
		UNC (vs. C)	13.63 **	
Chemical parameters	P_2O_5	Intercept (Danube + C)	3.31 [ns]	0.784
		Morava (vs. Danube)	0.21 [ns]	
		Sava (vs. Danube)	−1.73 **	
		UNC (vs. C)	−1.14 **	
	K_2O	Intercept (Danube + 2022)	63.69 [ns]	0.778
		Morava (vs. Danube)	18.79 **	
		Sava (vs. Danube)	−8.36 [ns]	
		2023 (vs. 2022)	−14.36 **	

[ns]—nonsignificant; *—difference is significant at 0.05 level; **—difference is significant at 0.01 level.

3.2. Evaluation of the Influence of Waste Depositions on Forest Soil Microbial Respiration

Exposure to waste deposits did not significantly affect MR in all experimental sites, in general, but the seasonal and annual changes in environmental conditions did (Figure 2). Respiration in June was significantly lower than in August and November in both years (in the Sava site sampling was not possible in June 2023 since the site was inundated, below approximately 0.5–1 m of water, for a few weeks). No significant differences in MR were detected between the three locations in contaminated soils. In contrast, in the Sava uncontaminated site, it was statistically higher in November than in the other two sites in both years.

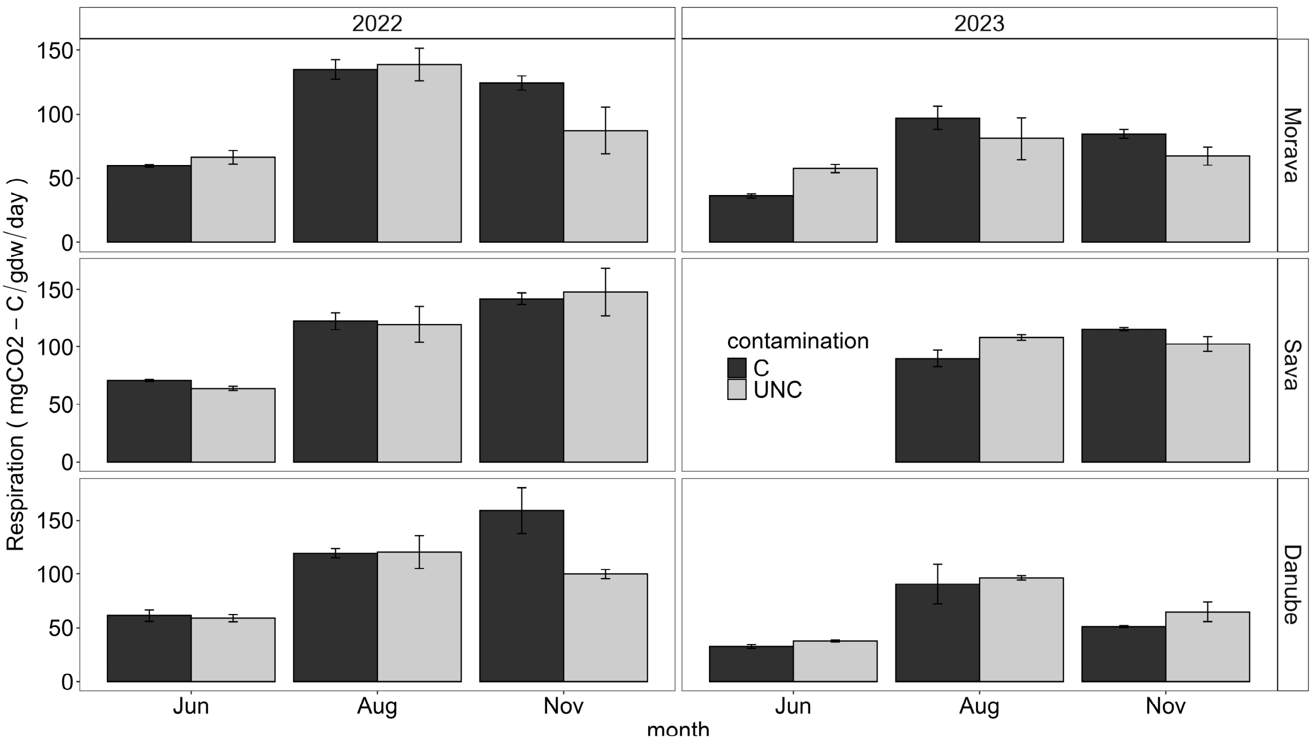


Figure 2. Rates of microbial respiration in different years/seasons in contaminated and uncontaminated sites. Factorial analysis of variance with a balanced design was used, with Duncan's test applied for post hoc comparisons (significance level of 5%).

Categorical regression analyses confirmed that environmental factors influenced MR, but no significant correlation was detected with waste deposition or measured MP amounts (Table 2).

Table 2. Summary results of the categorical regression analysis examining the effect of location, year, and month on microbial respiration. Model performance was assessed using adjusted R-squared values and statistical significance of predictors ($p < 0.05$).

Factor's Category	Estimated Regression Coefficient	R^2 (Adj)
Intercept (Sava + June + 2023)	78 **	0.746
Site [Morava]	−12 **	
Site [Danube]	−15.2 **	
Year [2023]	−33.0 **	
Month [Aug]	57.1 **	
Month [Nov]	51.1 **	

**—difference is significant at 0.01 level.

To check if nutrient availability was correlated with soil MR, we applied correlation analysis between MR rates and nutrient contents in soils in November 2022/2023, since we had available values for nutrients only for November samples. No statistically significant influence of total nitrogen (r = 0.245), available phosphorus (r = −0.334), and potassium (r = 0.051) on microbial respiration was detected.

3.3. Evaluation of Extracted MP on Forest Soil Parameters in Three Locations

Amounts of MP extracted from all samples are presented in Figure 3. The percentage of MP particles varied from 0.02%–0.60% *w*/*w* on contaminated sites and 0.003%–0.06% *w*/*w* at uncontaminated.

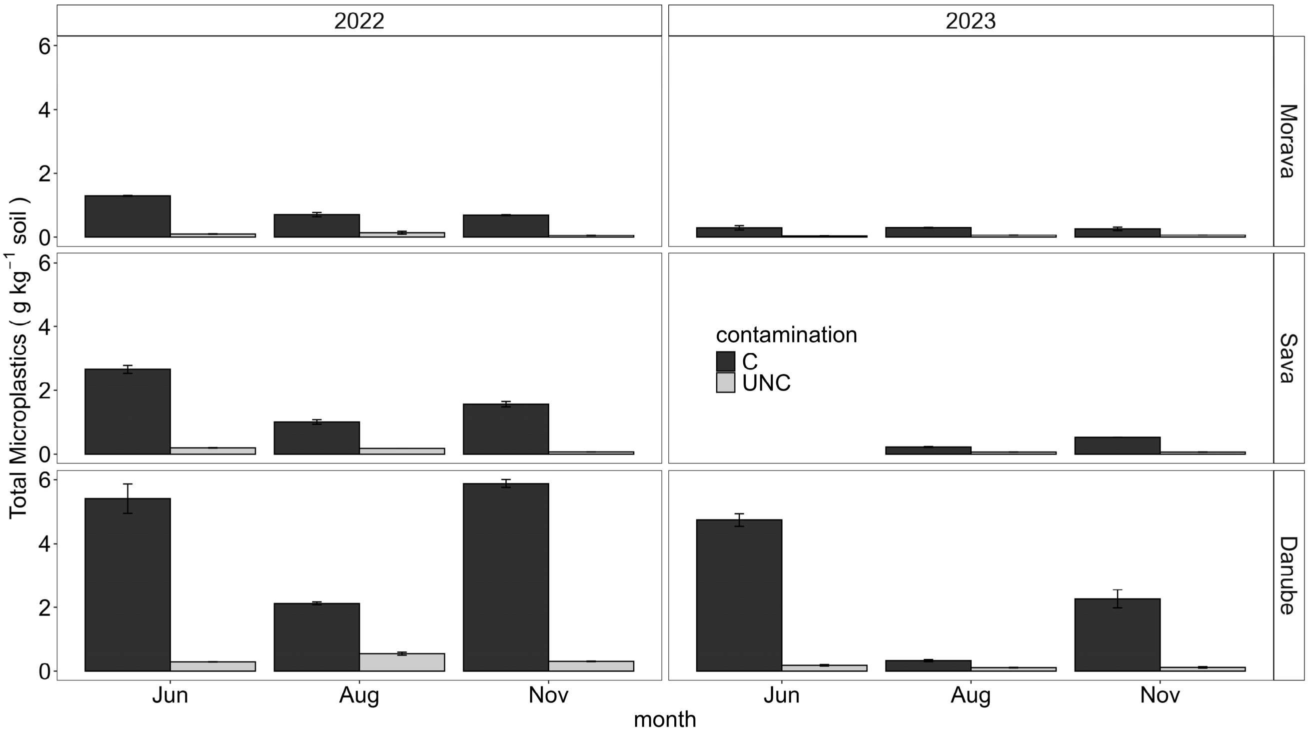
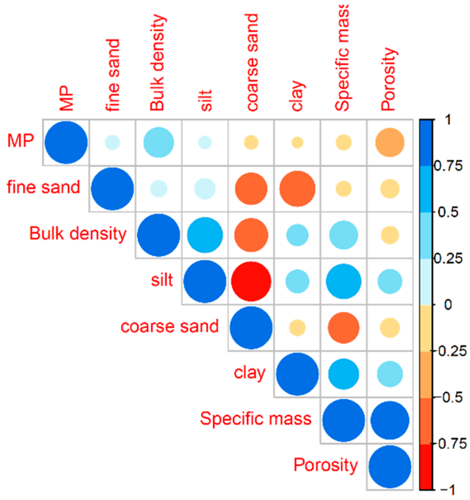


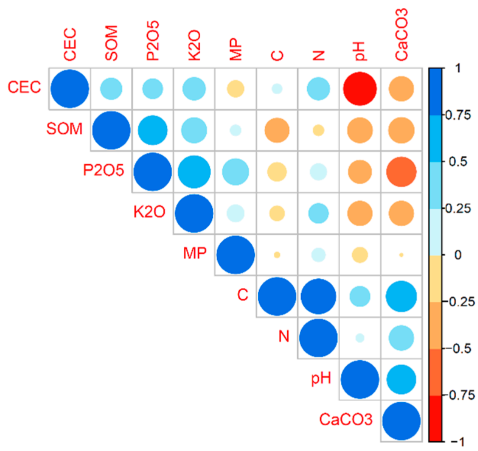
Figure 3. The amounts of MP extracted from contaminated and uncontaminated soil samples in 2022 and 2023, in three forest vegetation seasons (June, August, and November) in selected locations of Morava, Sava, and Danube alluviums.

The MP amounts extracted from the soil samples in two consequent years revealed an obvious pattern—MP amounts in uncontaminated sites were significantly lower than in contaminated sites in all cases (Figure 2). Even though uncontaminated sites were not visibly exposed to plastic waste, some quantities of MP were detected. The lowest amount of MP was detected in an uncontaminated site near the Sava River. The highest amount (app 10× larger than in the other two) of MP in the uncontaminated site was detected near the Danube—at the site located after the joining of Sava and Morava. The trend was a bit different in contaminated sites; the lowest amounts of MP were detected in the Morava site in 2022, and after the waste was removed from this site, this trend was even stronger in 2023 (Figure 3). However, significantly smaller amounts were isolated from all sites in 2023 than in 2022, especially in the Sava plain where the seasonal flood in late May left sites inundated for a few weeks in 2023. In active waste deposition in the Danube site, the significantly highest amounts of MP were detected in all cases (Figure 3).

Pearson correlation coefficient, which incorporated all samples/cases, revealed a significant positive correlation between measured amounts of MPs isolated from soil samples and bulk density and available P, whereas a strong negative correlation was detected with porosity (Figure 4). Other interesting correlations detected in this analysis revealed that available P was negatively correlated with $CaCO_3$ and positively with SOM. The correlation between isolated amounts of MP and microbial respiration was not statistically significant (p value -0.007) and is not presented.



(**a**)



(**b**)

Figure 4. Visualization of correlations between physical (**a**) and chemical (**b**) soil parameters and isolated MP amounts according to the Pearson correlation coefficient. The circle's size equals the correlation level, while the intensity of blue corresponds to the positive and the intensity of red to the negative correlation.

3.4. Vertical Distribution of MPs Detected in Undisturbed Soil Columns

The experiment set for estimating the approximate transport speed of MPs through the actual soil profile of its first 30 cm revealed that app. 40%–60% of particles with a size of 1000–3000 µm and 65%–75% of particles with a size of 500–1000 µm of the total amount of added MPs penetrated the soil in three months, only by the action of gravitation flow (Figure 5). A significantly highest amount of MPs penetrated in columns taken from the Morava site and the smallest in the Sava site in surface layers; significantly higher amounts of smaller particles penetrated in all columns (Figure 5).

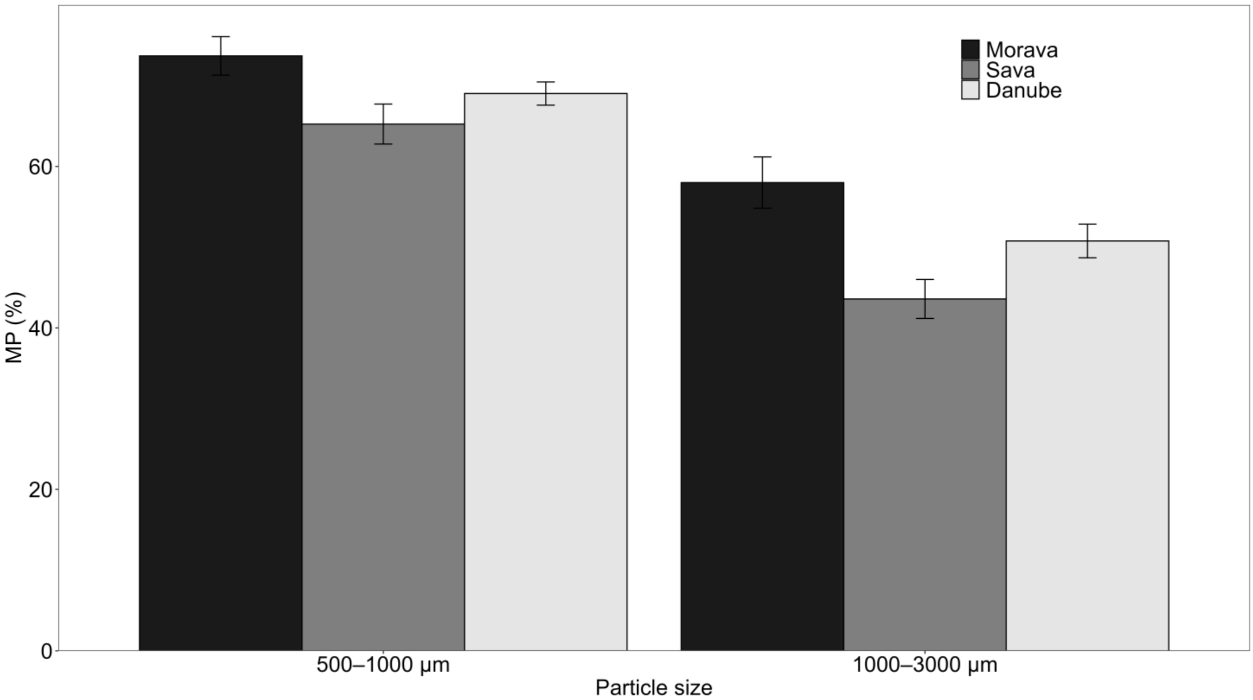


Figure 5. Percentage of total applied MPs that penetrated soil columns measured after 3 months from application. Soil columns were taken at three investigated uncontaminated sites. Applied on each column surface was either 5 g of 1000–3000 µm sized-PVC or 1.5 g of 500–1000 µm-sized PVC.

The majority of penetrated MP particles remained in the first 10 cm of soil columns (more than 90% in all cases, not presented for better visibility in Figure 6, and already visible in Figure 5), while a small percentage of particles reached deeper layers (Figure 6). In the 10–20 cm layer, a similar trend as in the previous figure was detected concerning sites, for particles 1000–3000 µm, but no significant differences were detected for smaller particles. The significantly smallest amounts of MP reached the deepest layer, with significantly higher amounts of smaller particles, but no significant difference was detected between sites (Figure 6).

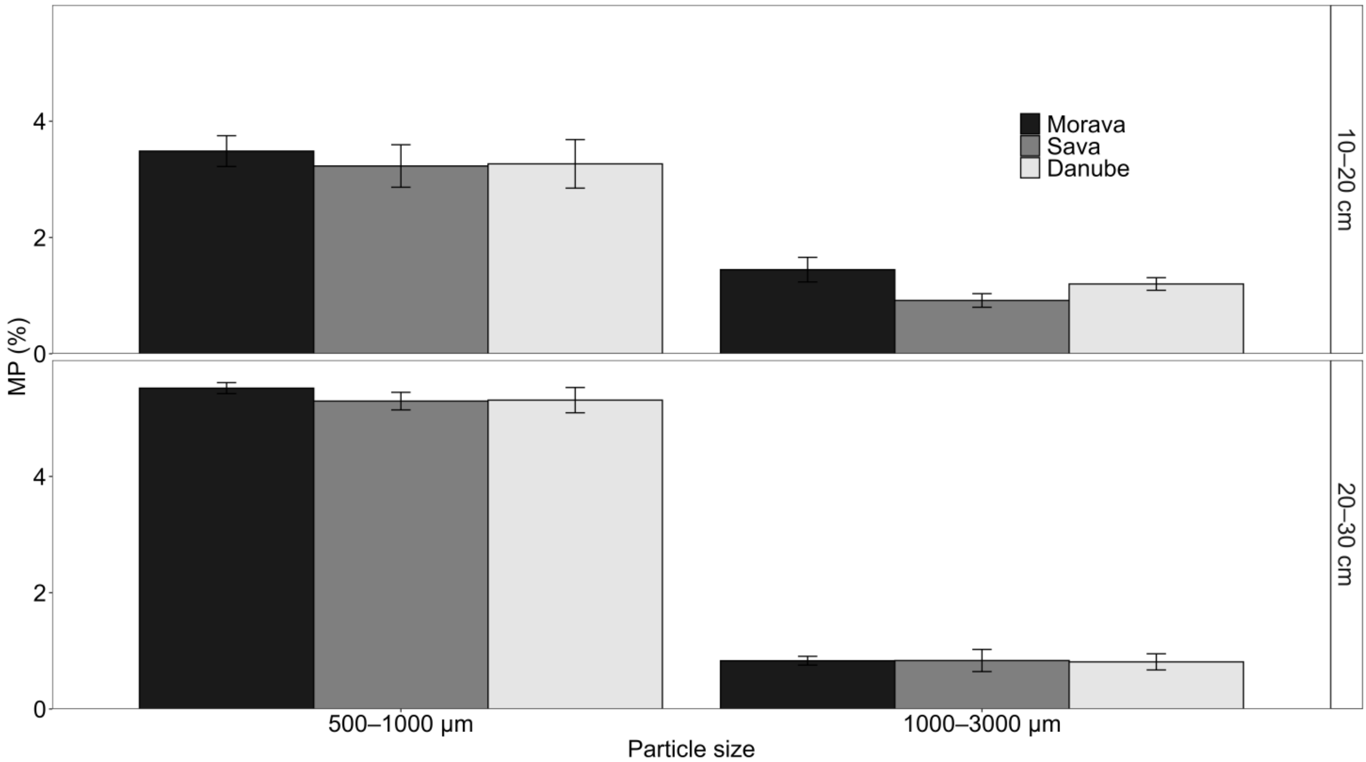


Figure 6. Percentage distribution of isolated MP that penetrated soil columns by depth in three months after the addition of known MP particles. Only deeper layers were presented to reach better visibility—more than 90% of particles that penetrated the soil columns remained in layers 0–10 cm.

4. Discussion

Our results provide evidence that sites where municipal waste was piled in the alluvial forest for a longer time were indeed the sites where large amounts of MPs were released from the discarded plastic objects into the soil. The amounts of detected MP were the highest under the largest waste dump (Figure 3). Also, we proved that waste depositions affected soil structure and porosity, as well as available P, but not soil microbial respiration. Environmental factors, presumably soil water movements, strongly influenced the MP distribution in the surface soil layers.

4.1. The Influence of Waste Depositions on Forest Soil Properties

Previous publications revealed that MP may alter soil physiochemical properties, thereby impacting its structure, water-holding capacity, bulk density, soil aggregation, and soil water distribution [11–14]. Our results are in line with previous findings—the parameters that appeared correlated with waste deposition were specific mass (particle density), porosity, coarse sand content, and large aggregate formation (Table 1). Similar results were obtained with a correlation of the exact MP amount with soil parameters in all soil samples (Figure 4), implying that MP is the factor that affects these parameters.

The percentage of texture particles larger than 0.2 mm corresponding to the coarse sand was significantly higher, while porosity was significantly lower in contaminated soils compared to uncontaminated ones in all research locations (Table 1), which is in line with the existing literature [46]. It was proposed that MP particles clog and occupy the pores [47], thereby disrupting the water–air regime, and changing water retention capacity [46,48–50], water flow [51], and movement by altering the proportion of macro- and micropores [47,50,52,53], increasing water repellence due to their hydrophobic nature [54–56], and affecting evaporation [57,58]. The linear regression model showed no difference in bulk density between contaminated and uncontaminated sites, but there was a significant difference in particle density (specific mass, Table 1). We can suppose that MP particles may pile within soil pores leading over time to a decrease in total porosity.

Fluvisols usually do not have a distinct structure that would originate from longer pedogenetic soil maturation, which is difficult in frequent flooding conditions [59]. However, the high content of clay and organic matter in examined forest soils enabled the formation of large aggregates (Table S4). The percentage of the largest aggregates was significantly lower in contaminated soils, supporting previous findings in which MPs negatively affected aggregate stability [50,57,58,60–62]. A laboratory study conducted on sandy loam Dystric Cambisol by Sepehrnia et al. (2024) [50] demonstrated that MPs were incorporated into aggregates after 90 days of incubation. Nonetheless, their results also showed that MPs significantly decreased aggregate stability since they lacks adhesive functions, thereby lowering the aggregate destruction point. They reported formations of surface cracks on the aggregates containing MP particles, attributed to the weak bonds between MP and minerals, which can potentially cause aggregate destruction at low physical impacts [50]. Similar outcomes were observed by other researchers [63,64].

Previous reports showed that MP enrichment reduced the bulk density of the soils in experimental conditions [11,57]. However, correlation analysis revealed significant positive correlations between MP amounts and bulk density (Figure 4), even though the categorical regression model detected no significant difference in bulk density between contaminated and uncontaminated sites (Table 1). This controversy can be explained by the fact that the highest MP amounts were isolated at both Danube sites in comparison to other respective sites, since this location was a sediment accumulation point and the waste pile was the largest, but it also happens to have the highest bulk density out of the three locations (Table S3).

Existing literature indicated that MPs affect various soil physical, chemical, and biological parameters that were not affected in our study [2,10–13,20]. However, most results from previous publications originate from experimental conditions where high amounts of unaged MP particles were used. In our contribution, we deal with realistic concentrations that were extracted from the natural soil samples. Therefore, the reason for why our results designate only some of the examined parameters as significantly affected by MP enrichment might be the low MP concentrations detected in the investigated natural forest soils. Recently, the questions of what MP concentrations can affect soil parameters and whether there are any significant impacts of concentrations detected from environmental samples have been raised [65]. Several studies highlighted the dependency of MP influence on soil type, polymer type, size, shape, observed parameters, etc. [65,66], but they all agree that there is a clear trend of increasing effect on soil parameters with increasing MP concentrations [42,43,50,58,65,66]. For example, [67] reported that no changes were observed in the bulk density of sandy soil at a concentration of 0.5% w/w of polyethylene fragments, but it was detected at higher concentrations of 1 and 2% w/w. [65] documented significant changes in silt loam soil parameters including bulk density at concentrations greater than 0.5% w/w and 2% w/w depending on polymer type and shape. Other studies have reported different threshold levels in experimental conditions depending on various factors (soil type, polymer, size, shape, observed parameter) namely 0.1% *w*/*w*, 0.3% *w*/*w* [55], 0.4% *w*/*w* [11], 0.5% *w*/*w* [62,66], 1% *w*/*w* [67], and 2% *w*/*w* [11,67]. Within our research, the concentrations of isolated MP were between 0.02%–0.60% *w*/*w* on contaminated sites and 0.003%–0.06% *w*/*w* at uncontaminated sites. Scheuer and Bigalke [68] reported MP concentrations of 0.006%–0.06% *w*/*w* in the Swiss floodplain area, which agrees with our results for uncontaminated soils (Figure 3). Fuller and Gautam [69] found concentrations between 0.03%–6.75% *w*/*w* and [70] of 0.016%–1.6% *w*/*w* in samples from historical plastic production industrial areas in Australia and roadsides in Germany, respectively, in agreement with our contaminated sites. Nevertheless, much lower MP pollution levels have been reported outside of industrial areas, landfills, and roadsides [65] even with intense agricultural production that can introduce MP into the soil, where reported MP concentrations vary between 0.001%–0.04% *w*/*w* [55,60,71,72].

Considering soil chemical properties, only available P appeared significantly higher in contaminated sites. Since MP presence did not change the pH or SOM of contaminated soils (Table S5), an increase in available P cannot be attributed to these expected factors (Figure 4). High concentrations of MPs could enhance phosphorus availability by supplying additional carbon sources, creating selective niches for the soil microbial community, and boosting phosphatase activity [73,74]. Jamtsho et al. [75] examined the effects of MPs in riparian soils in Bhutan and reported a moderate positive correlation between MPs and available P, which is in line with our results. However, the authors emphasize that other human activities could also lead to an increase in available phosphorus, which also applies to our research. The most probable explanation would be that P may be a constituent of the additives that were part of plastics and were somehow released into the soil. Investigated waste piles were made of very different items, and it is impossible to determine the exact origin of elevated available P with the MP enrichment. If containing P-based additives, MP particles could also attract certain P-solubilizing microorganisms, which can release P from its insoluble forms [76]. Further investigation is needed to test this hypothesis. For example, Yan et al. [77] observed a positive correlation between MP and available P and attributed it to plasticizer di (2-ethylhexyl) phthalate (DEHP), which is commonly used in plastic production [78].

Nevertheless, reports on MP effects on available phosphorus have been quite inconsistent. Jiang et al. [79] conducted an in situ study on the impact of MP on nutrient dynamics and found no significant influence of MP presence on phosphorus flux regardless of the MP concentrations (0.5%–0.05%) and size. Some studies showed a negative correlation between MP and available phosphorus at higher MP concentrations [80–83]. The data were mostly obtained through laboratory testing using a wide range of MP concentrations., e.g., [81,83] applied MP concentrations of 0.5%–5.0% and 0.25%–2.0%, and they reported greater reductions in available P with increased MP concentrations. Zhang et al. [84] observed that nonbiodegradable MPs significantly decrease soil P availability even at low, environmentally relevant concentrations of 0.01%, emphasizing the impact of long-term exposure. On the contrary, some studies indicate that biodegradable MP has more negative responses on soil available P than nonbiodegradable MP, and they also report reductions or even gradual elimination of negative effects of MPs on available P over time [85]. This can be attributed to temporal changes in soil microbial activity and phosphatase levels [86,87]. The polymer backbone of microplastics (MPs) breaks down into smaller oligomers over time, which are readily utilized by microorganisms, thereby enhancing phosphatase activity and facilitating the mineralization of organic phosphate [88]. In investigated forest soils, available P is naturally very low, and elevated P may even have a positive effect on plant growth and cause changes in soil metabolism and microbial communities.

4.2. The Influence of Waste Deposition on Soil Microbial Respiration in Investigated Sites

Contrary to many previous experiments [89,90], our data did not show any correlation between rates of MR and extracted MP amounts (Table 2). However, most data on the influence of MP on soil MR originate from the disturbed soil samples in experimental conditions and high MP concentrations. Here, we worked with natural soil samples and in situ present MP amounts. Highly porous and aggregated soils under environmental conditions like those used in our study could be expected to have very different rates of MR, much closer to reality, than soils from controlled experimental conditions. Indeed, meta-analyses of 1980 papers revealed that soil microbial respiration was unaffected when MP concentrations were below 5% [91], and detected amounts in this study were far below this limit. Also, no correlation was detected between MR rates in November in both years (samples used for detailed soil analyses) and available nutrients in the soil. From our previous investigation, we know that in November strong nitrification processes are activated in alluvial forest soils in Serbia due to the high availability of organic matter and good water/aeration ratio [29]. Still, the high total N measured here may not be a good indicator of total MR—detailed analyses of microbial communities would provide better insight into the soil processes [92]. Our results imply that soil MR is strongly connected to the aeration level—it was highest in the August samples when the aeration reached maximum since topsoil pores are filled with air, not water (Figure 2, Table 2). This parameter does not distinguish the respiration of different microbial groups of organisms. It is possible that even though plastic is an organic polymer, it is considered resistant to biodegradation and does not support the increase in microbial respiration, in general, but may change the functional structure of the microbial communities [92]. The data presented here cannot estimate a specific influence of MPs on microbial communities. The only evident fact rising from our results is that microbial activity strongly depends on environmental factors, probably soil water content and temperature [93].

4.3. The Distribution of MP Amounts in Investigated Sites

The largest amounts of MPs in uncontaminated sites were detected in the Danube location (Figure 3), confirming our hypothesis that sediments deposited in the largest river alluvium are probably enriched by MP particles brought by all its tributaries over long periods [24]. Contaminated sites cannot be comparable because of the unknown nature and history of the waste. Since standing plastic waste is fragmented by different factors, mainly exposure to UV light and other climatic factors [94], it was expected that the highest concentrations of MP would be detected in the largest waste pile, which was confirmed by our data (Figure 3). UV light is the most often mentioned factor that causes the physical degradation of plastic waste in nature [94]. In the present study, the lowest amounts of MPs were detected in soils sampled in August, even if the irradiation and temperatures are the highest at this time of the year (Figure 3). Forest canopies protect from direct sun irradiation, so we assume that physical degradation by UV light in forest ecosystems may be slower [19]. Indeed, the amounts of MP in contaminated sites slightly increase in autumn when the leaf-fall exposes waste to the sunlight in these broadleaved forests (Figure 3). Additionally, rainfall increases significantly in Autumn in Serbia after a dry Summer [28], which could be the factor of MP penetration into the soils (Figure 5). However, low temperatures, and possibly freezing–thaw effects during the winter, as well as exposure to the UV light before the canopy forms, may have caused increased degradation of plastic materials, resulting in the detection of the highest amounts of MPs in spring in all sites (Figure 3). Also, the gravitational water movements may be slower in winter due to the low temperatures causing inert snow cover, possible freezing of the topsoil, or lack of groundwater movements [95,96], which could cause accumulation of the MPs in the topsoil in spring. The waste removal (after the November 2022 sampling) caused a significant drop of MP particles in the Morava contaminated site, supporting our hypothesis of the increased deposition of MPs during the winter in forest ecosystems in continental climates. In the location of Sava, since the flooding wave may have removed some waste from the experimental sites and the water layer may have prevented aerobic processes, as well as the consideration of the influence of UV light and high temperatures (Figure 3), amounts of MP in the samples from August and November were significantly lower than those in 2022. The fact that the MP amounts in the contaminated site of the Danube remained high in both years implies that MP input in large waste deposits will remain high no matter what environmental conditions may be present.

4.4. The Dynamic of the MP Through the Soil Columns

Experiments with undisturbed soil columns in this study showed a relatively high level of mobility of MPs in the topsoils from research locations, which may explain why MPs could not be accumulated at hotspot sites at higher concentrations in the long term and why the deviations were recorded between samplings. The transfer of MPs in porous media like soil is influenced by the nature of MP particles, the nature of the porous system (soil), and the nature of flow [96]. It should be noted that this experiment was performed on a specific soil type and only assessed vertical migration under the influence of gravitating water. Gravitational and capillary water movements between layers are often highly complex in alluvial soils due to potentially different properties of sediments, which could be amplified in conditions of high groundwater levels and frequent flooding followed by rapid and deep drainage [97]. A study indicates that MP vertical migration could be more prominent in frequent wetting–drying cycles [98], which is more pronounced in riparian zones and enhanced by extreme weather events that occur as one of the effects of climate change [99]. This is why we can only expect higher mobility of MPs at research sites in natural conditions compared to conditions within our experimental setup. The destiny of MP particles in deeper soil layers is not known—some of them may remain in different soil layers for long periods, while some may eventually end up in the groundwater and be taken by the hydrological processes transferred to the river [100,101]. In natural riparian zones, MP was detected in deep soil layers, but even in fluvisols where the dynamic of soil water is strong, the majority of MP was detected in the first 30–40 cm [24], which corresponds to our column height.

Alluvial forest soils in Serbia have been surprisingly little explored, even though this forest type is highly endangered and strongly exposed to pollutants including plastic of different origins [102]. Data on forest soil parameters in alluviums of the Danube and the Morava are very scarce, but in this experiment, they appeared to be very similar to soils in the Sava basin and in line with the known literature data [28,29] (Tables S3–S5). High porosity detected in the surface layers of all investigated sites is usually good for plants and soil communities in alluvial forests because it enables drainage in water-excessive periods and provides good aeration, which is why the organic matter turnover is quite fast [28] (Table S5). In such soils, where the risk of exposure to excessive water is persistent, aeration is extremely important because it enables soil processes to produce enough available nutrients through biological cycles performed by microorganisms. However, high porosity also supports the penetration of MPs into the surface soil layers (Figure 5, [97]). In clay-rich sediments characteristic of the areas of the quiet flow of the big rivers in the wide alluvial plains, porosity decreases with depth, which also slows down the gravitational transfer of the MP particles (Figure 6, [24]). Additionally, the groundwater level may vary significantly during the season in luvisols, and upward transfer cannot be excluded. This is exactly the case in the Sava location, where factors other than rainfall influenced MP distribution

(Figure 3, [24]). We cannot estimate the exact water movement pattern in this site in 2023; it is uncertain whether there was a flood wave or whether it was simply groundwater uprising, but it seems that MP particles were somehow washed out from surface soils sampled after the inundation event (Figure 3). Since the inundation causes low oxygen conditions, the fall of the biological activity in the Sava site soils is expected (Figure 2, [90]).

At higher concentrations than those detected in our study, MPs appeared to affect soil organisms, especially various species of annelids and nematodes [103] or arthropods [104]. According to the mentioned studies, the concentrations of MP presented here may not impose direct risk for soil fauna but can generate additional stress for organisms dependent on the soil matrix. However, these potential effects on organisms should be further investigated, especially in natural conditions and different soil types, since soil physico-chemical parameters may have a significant effect on target organisms as well.

5. Conclusions

To our knowledge, the results presented here are the first reports on MP in natural forest soils, as well as on the influence of waste depositions on forest soils in Serbia and wider. Our results showed that waste depositions in alluvial forests significantly influence soil structure and, consequently, soil processes. However, the amount of MPs that can be detected in natural forest soils even in the pollution hotspots are much lower than those examined in experimental conditions. Even in places that do not appear to be exposed to plastic waste, some amount of MPs can be detected because fluvisols are extremely imposed to the variable dynamic of soil water content and potential, including horizontal groundwater movements through porous sediments [24]. Once they penetrate the soil, MP particles may be incorporated into soil aggregates, destroy their stability cause pore clogging, or be transported vertically into the deeper soil layers [24]. From there, they may end up in rivers where their dispersal becomes a further problem. MP release from discarded plastic items to the soil strongly depends on environmental factors like irradiation exposure, temperature, rainfall intensity, and water stagnation. In forest soils, all of these parameters are influenced by the tree canopy, the bulky root systems, the dynamic of organic matter turnover, and soil organisms' activity. It seems that, in general, soil microbial activity is not influenced by MPs in the reported concentrations, but the nature of microbial processes cannot be estimated from parameters determined here. The input of MPs into the alluvial forest soils is obviously enlarged by waste dumping and mitigated by waste removal, implying that the problem of MP pollution in alluvial forest soils is easily solvable, with the involvement of local communities and authorities. It is important to educate the citizens about the danger of dumping waste in the forests and rivers, which impacts the environment, living organisms, and health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16020363/s1. Table S1. Temperature and precipitation averages in site areas from 2019-2023 according to the Hydrometeorological Institution of Serbia; Table S2. Summary of site coordinates, sample acronym, and waste dumps descriptions; Table S3. Physical characterization of the soils from Morava, Sava, and Danube alluviums experimental sites; Table S4. Aggregate size distribution of the soils from Morava, Sava, and Danube alluviums experimental sites; Table S5. Chemical characterization of the soils from Morava, Sava, and Danube alluviums experimental sites.

Author Contributions: Conceptualization, Ž.M. and E.S.; methodology, T.G., E.S., Ž.M. and D.M.; software D.M.; validation, Ž.M. and S.B.S.; formal analysis, T.G., E.S., M.J., S.S. and V.M.; investigation Ž.M., T.G., E.S., M.J., S.S. and V.M.; resources, Ž.M. and S.K.; data curation T.G. and Ž.M.; writing—original draft preparation, Ž.M. and T.G.; writing—review and editing, S.B.S., E.S. and S.K.; visualization, D.M.; supervision, Ž.M., E.S. and S.B.S.; project administration, Ž.M. and S.K.;

funding acquisition, Ž.M. and S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Fund of the Republic of Serbia, grant No. 7742318, and the Evaluation of the Microplastics in the Soils of Serbia (EMIPLAST). S.o.S., T.G., E.S., V.M., and M.J. were funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, grant No. 451-03-136/2025-03/200011.

Data Availability Statement: Data are contained within the article or Supplementary Materials.

Acknowledgments: We are grateful to the heads and administrative employees in all institutions—members of the consortium of EMIPLAST SoS—for all the support during the project's duration. We are also grateful to Lana Kukobat for constructing the QGIS map of the sampling locations.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MP	Microplastic
MR	Microbial respiration

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