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Changes in Soil Properties Under the Influence of Microplastics in Plastic and Open Field Production in Three Serbian Valleys

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Abstract: Crop production in plastic greenhouses is one of the major sources of plastic pollution worldwide. The main hypothesis of this study is that the regular use of mulch film in greenhouses leads to the cumulative accumulation of microplastic particles (MPs) in the soil, which ultimately leads to changes in the soil properties. Therefore, the main objective of this study was to identify the possible changes in the physical, chemical, and biological properties of soil in greenhouses in three regions of Serbia. The following chemical parameters were determined: electrical conductivity, soil acidity, cation exchange capacity (CEC), total carbon (TC) and nitrogen (TN) content, plant-available phosphorus and potassium content, and trace element content. The following physical parameters were determined: particle size distribution, volumetric mass, specific mass, and porosity; the biological parameters that were determined were microbial respiration and labile carbon. The obtained data were processed using network analysis (NA) to identify the complex relationships between MP content and soil parameters. The NA results support the main findings that the presence of microplastics leads to the destruction of soil structure, which reduces bulk density and increases soil porosity. A strong positive correlation of MPs with soil particles < 0.02 mm and a negative correlation with CEC were found. In the Danube Valley, soil respiration was 78% higher in the open ground compared to a plastic greenhouse. The results contribute to a better understanding of the influence of MPs on soil properties and its ecological functions.

Keywords: microplastics; plasticulture; mulching; labile carbon; soil properties; network analysis

1. Introduction

Pollution of arable soil comprises different elements and materials. Plastic residues, microplastics, and nanomaterials have come into focus in more recent years. Plastic greenhouses have quickly gained notoriety as a transformative and environmentally conscious approach to growing crops. In a world struggling with issues of food security and environmental impact, these structures have become a beacon of hope, offering a host of benefits beyond traditional farming methods. From increased yields to precisely controlled conditions, plastic greenhouses are poised to redefine the landscape of sustainable agriculture [1].

However, the widespread use of polyethylene mulch film (PEM) in agriculture creates significant problems due to its difficult degradability [2] and the resulting environmental impact. The removal and disposal of plastic mulch are labor-intensive and costly; therefore, plastic fragments of various dimensions are cumulatively deposited in the soil of plasticulture over years [3].

Numerous studies have not only proven the prevalence and persistence of MPs in soil but also shown that MPs affect the physical, chemical, and biological properties of soil, and also have a toxicological effect on the growth and development of plants and soil microorganisms [4–14]. In a meta-analysis of over 5000 observations, [15] found that MPs suppressed both plant growth and development and soil fauna. Moreover, MPs contributed to greenhouse gas emissions [15].

Plastic particles found in soil have large surface area and strong hydrophobicity [16] and therefore can serve as an acceptor and donor for microelements and nutrients. They can eventually transport and release potentially toxic elements (PTEs) originating from agrochemicals and fertilizers [12,17]. This is especially addressed to the aged plastic particles that can release PTEs and plastic monomers into the soil matrix [18] that can be consumed by organisms [11]. In addition, since MPs are organic polymers, they influence global CO2 production and therefore global climate by influencing soil microbial processes, plant growth, or litter decomposition [9,10].

Agricultural soils are highly prone to plastic pollution, mainly due to mulch degradation and the use of organic fertilizer [7,11,19,20]. Crop production in plastic greenhouses and plastic mulching are among the major sources of MPs in the environment worldwide. There are few published data on the impact of MPs on soils in Serbia [18]. Fertile alluvial plains in Serbia focus on vegetable production with growing plastic farming practices, while nearby forests and riverbanks have become sites of the wild dumping of plastic waste (Figure 1).

The river valleys of the Danube, Sava, and southern Morava rivers are mainly represented by fertile alluvial soils. Therefore, these regions intensively and extensively grow vegetable crops in greenhouses and in the open ground. The favorable climate allows for several harvests per year. The soils of the main river basins of Serbia (Danube, Sava, and Morava) were chosen as the main object of the study, since they are exposed to a high risk of plastic pollution. On the other hand, alluvial plains are the source of the MPs that end up in the rivers and further pollute riverine ecosystems.

This study is part of a larger project to study the microplastic pollution of alluvial soils in the valleys of the major rivers in Serbia. The main sampling locations were in natural forest ecosystems, which are periodically flooded, depositing various types of pollutants with river water on the riparian soils [21]. However, during reconnaissance of the area, wild dumps of waste plastic greenhouse coatings were noticed (Figure 1). These dumps were mainly noticed on the banks of nearby streams or nearby forest areas. The hypothesis was that soils intensively used in plastic farming are more susceptible to MP contamination than nearby open field soils and that an elevated MP content alters soil properties.

Figure 1. Wild plastic waste discharge at a forest stream near the sampling site at the Morava River basin, May 2022 (photo by E. Saljnikov).

The main objectives were (1) the assessment of the MPs in soils of three horticultural regions in Serbia; (2) the investigation of changes in soil properties; and (3) the determination of the effect of MPs on potentially mineralizable carbon.

2. Materials and Methods

2.1. Site Description and Sampling

The river valleys of the Danube, Sava, and southern Morava are mainly represented by fertile alluvial soils. In the studied areas, vegetable cultivation in open ground and film greenhouses is widely practiced. The crops growing in the greenhouses during the sampling season were tomatoes (*Solanum* L.) and different types of peppers (*Capsicum*) at all three sites. The crops grown in the open field were potato (*Solanum tuberosum*, L.) (Sava site), onion (*Allium cepa* L.) (Morava site), and corn (*Zea mays*, L.) (Danube site).

The sites are located in the towns of Jakovo (44°45′6.54″ N, 20°16′3.41″ E and 44°45′6.72″ N, 20°16′5.72″ E, Sava site), Smederevo (44°64′1.42″ N, 20°93′10.2″ E and 44°64′1.54″ N, 20°93′8.2″ E, Danube site), and Leskovac (43°40.6′00″ N; 21°08.4′55″ E and N 43°41.5′00″; 21°10.5′55″ E, southern Morava site) (Figure 2). The soils of the studied sites are alluvial loam (Sava), clay loam (Danube), and sandy loam (Morava).

For chemical analysis of the soil, a three-composite sample was formed from six subsamples collected from the greenhouses of the three locations in November 2022 and 2023 at a depth of 0–15 cm. Soils from the adjacent open fields were sampled to compare the content of MPs (Figure 3). For the analysis of microbial respiration, samples were taken three times a year (June, August, and November). For the physicochemical analysis, the samples taken in November were analyzed. The samples were immediately transported to the laboratory. Next, the soil samples for determining the chemical and physical properties were air-dried, ground through a sieve with a mesh size of <2 mm, and stored for further analysis. The samples for microbial respiration were immediately prepared for analysis. Overall, 72 samples from three sites and both treatments (greenhouse and open field) were analyzed in 2022 and 2023.

Figure 2. Studied locations: Sava River valley (Jakovo town); Danube River valley (Smederevo town); and south Morava River valley (Leskovac town), Serbia.

(**a**) (**b**)

Figure 3. Example of a sampling location: the greenhouse (**a**) and adjacent open field (**b**) in the Morava River Valley, 2022 (photo by E. Saljnikov).

The following fertilizers were added to the greenhouse soils in 2002 and 2023: at the beginning of the growing season, a standard nitrogen–phosphorus–potassium fertilizer (NPK 15:15:15) was applied at a dose of 50 kg/ha. After planting, a formula of NPK and microelements (MEs)—NPK 14/40/5 + 13% SO_3 + ME (B, Cu, Fe, Mo, Zn)—was applied to stimulate the growth and development of the root system in the form of an aqueous solution at a dose of 50 kg/ha. Later, during the period of intensive fruiting, an aqueous solution of NPK 15/5/35 + ME (B, Fe, Mo) was applied at a dose of 50 kg/ha. And finally, at the end of the growing season, an aqueous solution of NPK 8/16/42 + ME (B, Fe, Mn, Zn) at a dose of 50 kg/ha was applied for the improvement of the flavor and color of fruits. A standard fertilizer (NPK 15:15:15) was added to the open ground soil once during the growing season before planting crops.

2.2. Analytical Methods

The procedure of the determination of MPs is described in detail in our recent paper [21] that was the first part of the research project. The content of MPs < 5 mm in size was determined as follows: 30 g soil was treated with 50 mL of 1 M sodium pyrophosphate for the disaggregation of soil samples, 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). The samples were then transferred to tubes and centrifuged for 5 min at 1500 rcf (MSE Harrier 15/80 MSB080.CX1.5 MSE (UK) Limited, London, UK) using a sucrose solution as density separation media. The supernatant was then collected, air-dried, and treated with 30% H_2O_2 to digest organic matter. The undigested OM particles were removed manually using a stereomicroscope (Optech LFZ, Hermes Lab Systems, Bratislava, Slovakia). After the removal of non-plastic particles, the MPs were weighed on an analytical balance (AS 160/C/2, RADWAG, Radom, Poland).

Each soil sample was analyzed for the following chemical parameters: electrical conductivity, pH, CEC, total C, as well as the nutritional status of the soil (total C and N, P_2O_5, K_2O, and $CaCO_3$) and the concentrations of microelements (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn). The physical parameters assessed were particle size distribution, volumetric mass, specific mass, and porosity. The biological parameter of microbial respiration (CO_2 mg/kg/day) was measured via a long-term laboratory incubation.

Soil total carbon (TC) and nitrogen (TN) were measured on a CNS elemental analyzer (Model Vario EL III-ELEMENTAL Analysis systems, GmbH, Hanau, Germany) by dry combustion at 1150 °C [22,23]. Soil pH was determined with a glass electrode pH meter (Mettler-Toledo, Greinfensee, Switzerland) in 1 mol L^{-1} KCl 1:5 v/v and water [24]. 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 [25]. The content of inorganic carbon ($CaCO_3$) was determined volumetrically with a Scheibler calcimeter (Many Agrovet, Belgrade, Serbia) [26].

Available phosphorus and potassium were determined using the AL method described by [27]. Available phosphorus (P_2O_5) was analyzed spectrophotometrically (SHIMADZU CORPORATION UV-VIS spectrophotometer UV—160 A, Kyoto, Japan) and available potassium (K_2O) by flame emission photometry (HINOTEK FP6440 flame photometer, Ningbo, China) after color development with ammonium molybdate and stannous chloride. Soil Ca and Mg were extracted by ammonium acetate and determined with a SensAA dual Atomic Absorption Spectrophotometer (Dandenong, Australia).

Particle size distribution was determined by the International "B" pipette method [28]. Soil bulk density (BD) was measured by drying the soil cores at 105 °C to a constant weight [29]. Soil specific mass (particle density) was determined using a pycnometer with the water method [30]; porosity was obtained computationally from the bulk density and specific mass [31].

Microelements were determined on an iCAP 6300 ICP optical emission spectrometer (Thermo Electron Corporation, Cambridge, UK), after digestion with concentrated HNO_3 for the extraction of hot-acid-extractable forms. Merck standards were used for determinations on the ICP and SensAA Dual.

Soil microbial respiration was measured as CO_2 evolved due to organic C mineralization under controlled laboratory conditions (temperature 30 °C and soil moisture 50% WHC) at the following intervals: 3, 9, 16, 30, 44, 62, and 83 days for each treatment in four replications. The carbon dioxide released was trapped by 0.5 mol L^{-1} NaOH, and the amount of residual free NaOH was determined by titration with 0.05 mol L^{-1} HCl. The amount of carbon dioxide released for each incubation period was calculated from the difference between the amount of NaOH taken for carbon dioxide fixation and that determined by titration with HCl [32]. The labile organic carbon was calculated from the cumulative data on the sequential respiration during 83 days of incubation using a first-order kinetic model:

$$\text{Cmin} = C_0(1 - \exp^{-kt}) \quad (1)$$

where Cmin is the experimentally obtained value of mineralized C (mg kg^{-1}) during t (days), C_0 is the potentially mineralizable carbon (PMC) (mg kg^{-1}), and k is the nonlinear mineralization constant, i.e., the rate of mineralization (the amount that is mineralized per day) (d^{-1}).

2.3. Statistics

The data obtained were processed using a one-way blocked ANOVA and two-tailed paired T-test (SPSS version 16 software). Carbon mineralization potential and rate were obtained after the data were processed via SigmaPlot 12.3 version software, (Informer Technologies, Inc. New York, United States) using a Maximum to Rise nonlinear regression model.

To gain a deeper understanding of the complex relationships that microplastics establish in open field and greenhouse soils, we applied an advanced approach using network analysis (NA). This method allowed the discovery of complex and multidirectional relationships between the different variables studied, providing insight into the underlying processes and the intensity of their mutual interactions. Namely, NA includes nodes representing different soil indicators, connected by edges symbolizing positive or negative relationships between them. To address the uneven distribution of indicator values, a network model was developed using the extended Bayesian information criterion (EBIC) in combination with the graphical least absolute clustering and selection operator (gLASSO). The EBIC hyperparameter (γ) was set to 0.50, which controlled the sparsity of the model and determined the number of edges included in the final network representation. This choice of parameter allows for an optimal balance between precision and network complexity, ensuring that the model reflects key interactions without losing interpretability. Although network analysis allows the assessment of four key metrics of network centrality (closeness, betweenness, strength, and expected influence), in this study, we focused on expected influence (EI). This metric offers superior performance because it takes into account both negative and positive links, providing a more complete overview of the interrelationships in the network. This is crucial for understanding dynamic processes such as the migration of PTEs, the impact of microplastics on soil structure, and the disruption of nutrient cycling.

3. Results

3.1. Content of Microplastic Particles <5 mm

The distribution of MPs (<0.5 mm) in the studied soils are presented in Figure 4. There were significant differences between the MP content in the plastic greenhouse and open field soils within each site (JakPL and JakCON, SmePL and SmeCON, LesPL and LesCON, respectively) as well as between sites (Figure 4; Table 1). The weight of MPs did not follow a similar trend in the 2022 and 2023 years. In 2022, the difference between MP weight was greater than in 2023. The T-test results for the weight of MPs in 2022 and 2023 are given in Table 1.

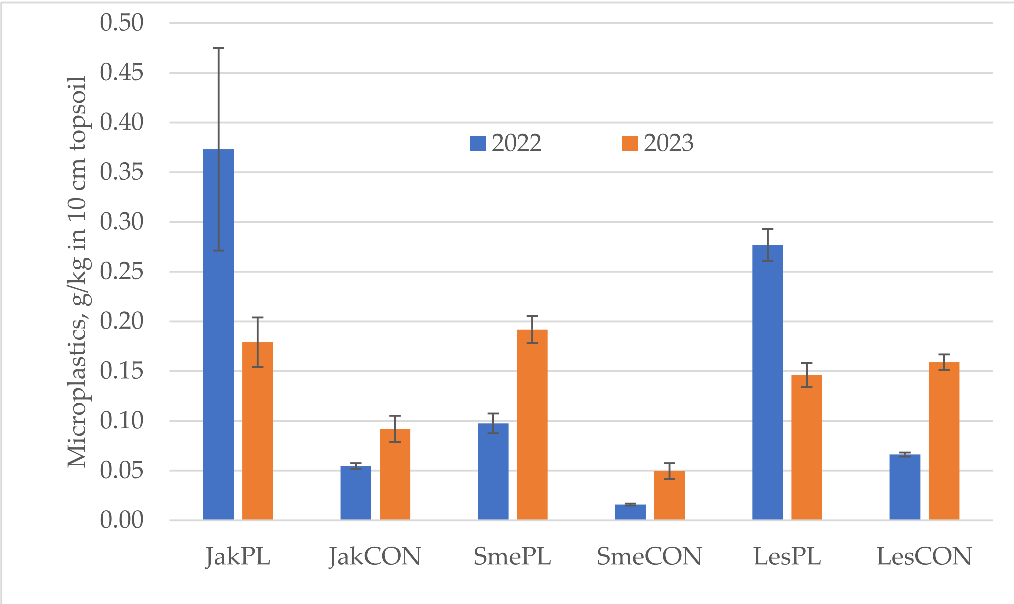

Figure 4. Content of microplastics (g/kg) in the soil of the greenhouse and open fields from three locations in Serbia, where JakPL and JakCON represent the greenhouse and open-field at the Sava River site, respectively; SmePL and SmeCON represent the greenhouse and open field at the Danube River site; and LesPL and LesCON represent the greenhouse and open field at the south Morava River site.

Table 1. Paired samples test for the content of MPs in plasticulture and open field soils in 2022 and 2023.

Pairs	Paired Differences					t	df	Sig. (2-Tailed)
	Mean	Std. Dev.	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
2022								
JakPL–JakCON	0.21856	0.21410	0.01470	0.07712	0.35999	3.043	8	0.006
SmePL–SmeCON	0.08156	0.03503	0.01168	0.05463	0.10848	6.984	8	0.000
LesPL–LesCON	0.21078	0.04437	0.01479	0.17667	0.24488	14.25	8	0.000
2023								
JakPL–JakCON	0.08700	0.04590	0.01874	0.03884	0.13516	4.643	5	0.006
SmePL–SmeCON	0.14250	0.02469	0.01008	0.11659	0.16841	14.14	5	0.000
LesPL–LesCON	0.20700	0.03324	0.01357	0.17212	0.24188	15.25	5	0.000

3.2. Soil Chemical Characteristics

The main soil chemical characteristics are given in Table 2. Among the three sites, soil from the Danube basin showed a higher electrical conductivity (EC), content of total N and organic C, content of plant-available phosphorus and potassium, and cation exchange capacity (CEC) (Table 2). A comparison of the soil chemical parameters of the plastic-polluted and open field sites showed that the values of EC were significantly higher in all soils from plasticulture compared to the open field. Other parameters, CEC, carbon, nitrogen, P_2O_5, and K_2O, were higher in the samples from the open field soils (Table 2) at all sites.

Table 2. Main soil chemical characteristics from the plastic greenhouse and open field sites at the Sava, Danube, and Morava River basins, Serbia.

Site	EC	pH		N tot.	C tot.	P_2O_5	K_2O	$CaCO_3$	CEC
	μS/cm	KCl	H_2O	%		mg/100 g		%	cmol/kg
				0–15 cm					
JakPL	509b	8.43a	8.79a	0.196a	2.66a	138a	24.3a	5.33a	19.3a
JakCON	295a	7.68a	8.22a	0.364b	4.05b	255b	177b	4.44a	29.5b
SmePL	1091d	7.45a	7.76a	0.368b	3.18b	177a	15.1a	0.44b	29.3b
SmeCON	775c	6.88ba	7.24a	0.670c	6.82c	290b	279c	0.44b	39.8b
LesPL	547b	7.20a	7.48a	0.131a	1.07d	94.5a	47.5a	n.d.	10.0c
LesCON	343a	7.71a	8.04a	0.250a	2.32a	271b	102d	0.89b	17.8a
LSD	**			*	*	**	**	*	*
SE	56.92	0.116	0.121	0.037	0.376	15.246	17.859	0.432	2.036
SD	278.83	0.566	0.594	0.179	1.842	74.69	87.493	2.117	9.974

Note: SE—Standard error; SD—Standard deviation; different letters within columns mean statistically significant difference; *, **—correlation is significant at the 0.05 and 0.01 levels, respectively

3.3. Content of Microelements

The content of all microelements was higher in the soil of the Danube basin compared to Sava and Morava (Table 3). The relative concentrations of the studied microelements in all soils were as follows: Mn > Zn > Ni > Cr > Pb > Cu > Co > As > Cd, which were below the background boundary according to the regulations of the Republic of Serbia [33], except Ni. The concentration of Ni in the soil from the Sava and Danube sites exceeded the maximum allowed concentration (MDK) of 50 mg/kg but was below the remediation value of 210 mg/kg.

The concentrations of microelements in the soils of the plasticulture and open field sites showed that for most of the elements, there was not a statistical difference. The exceptions were at the Danube site, where the concentrations of Cu, Pb, and Zn were higher in open field soil than those from the plastic greenhouse, and the Morava site, where the concentration of Zn was significantly higher in open field soil.

Table 3. Content of microelements in the soils from the plastic greenhouse and open field sites at the Sava, Danube, and Morava River basins, Serbia.

Site	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
					mg kg^{-1}				
				0–15 cm					
JakPL	8.12a	0.40a	11.7a	48.4a	20.4a	531a	69.7a	13.9a	73.0a
JakCON	8.20a	0.45a	12.0a	50.7a	27.6a	551a	70.3a	16.1a	115b

Table 3. *Cont.*

Site	As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
					mg kg^{-1}				
					0–15 cm				
SmePL	14.3b	0.82b	16.5b	80.6b	28.3a	580a	111b	71.0b	149b
SmeCON	14.8b	0.94b	17.0b	80.5b	49.0b	607a	112b	86.6b	257c
LesPL	6.02c	0.65c	11.9a	29.8c	21.6a	468a	22.4c	67.5b	117b
LesCON	5.69c	0.64c	11.2a	28.2c	25.4a	457a	22.7c	59.3b	135b
LSD	**	***	*	**	**		**	***	***
SE	0.773	0.04	0.511	4.453	2.002	12.123	7.618	5.799	11.95
SD	3.789	0.196	2.505	21.814	9.81	59.39	37.323	28.41	58.541

Note: SE—Standard error; SD—Standard deviation different letters within columns mean statistically significant difference; *, **, ***—correlation is significant at the 0.05, 0.01 and 0.001 levels respectively

3.4. Soil Physical Properties

The soil physical characteristics and particle size distribution are given in Table 4. There were no significant differences found between the plasticulture and open field soil. However, there was observed a clear tendency for higher porosity in the plasticulture soil of Jakovo and Smederevo. The Leskovac soil showed a high content of coarse fractions; therefore, the bulk density and porosity were not possible to determine.

Table 4. Soil physical characteristics from the plastic greenhouse and open field sites at the Sava, Danube, and Morava River basins, Serbia.

Site	Bulk Density	Specific Mass	Porosity	Particle Size Distribution, %					
				>0.2 mm	0.2–0.02 mm	0.02–0.002 mm	<0.002 mm	>0.02 mm	<0.02 mm
					0–15 cm				
JakPL	1.449	2.702	46.36	6.5	54.6	15.4	23.5	61.1	38.9
JakCON	1.449	2.609	44.46	5.0	51.9	17.3	25.8	56.9	43.1
SmePL	1.480	2.648	44.12	8.3	38.8	26.1	26.8	47.1	52.9
SmeCON	1.480	2.573	42.48	4.5	28.0	37.2	30.3	32.5	67.5
LesPL	n.d	2.740	n.d	27.1	48.1	11.9	12.9	75.2	24.8
LesCON	n.d	2.699	n.d	27.4	45.7	13.3	13.6	73.1	26.9
LSD			*	**			**		

Note: LSD—Least significant difference; *, **—correlation is significant at the 0.05 and 0.01 levels, respectively.

3.5. Potentially Mineralizable Carbon Measured via Sequential Soil Microbial Respiration

Organic carbon mineralization potentials (Cmin) and the rate of mineralization (k) are shown in Table 5. The amount of labile carbon was significantly higher in open field soils from the Jakovo and Smederevo locations (Sava and Danube river basins), and for Leskovac (Morava), there was no significant difference. At the Danube location, the soil from plasticulture showed 34.4% less labile carbon than the corresponding open field soil. The rate of carbon mineralization showed an opposite trend, being higher in plasticulture soils. Generally, comparing the location, Leskovac soils showed the smallest amount of labile carbon for both plastic greenhouse and open field plots.

The correlation analyses showed the significant effects of MPs on soil parameters, as shown in Table 6. Generally, in 2023, there was a greater effect of MPs on soil parameters. Among the soil physical parameters, only specific mass was affected by MPs in both years, with soil porosity being influenced in 2022. Among the chemical parameters, only the contents of P_2O_5 and K_2O were greatly affected by MPs in both years. Additionally, in 2023, the content of total N, total C, CEC, and labile C was significantly influenced by MPs. Among the mentioned parameters, only soil porosity and specific mass were positively correlated, while other parameters correlated negatively with MPs.

Table 5. Potentially mineralizable C (Cmin) and mineralization rate constant (k) of soils from the plastic greenhouse and open field sites at the Sava, Danube, and Morava River basins, Serbia.

Site	Respiration, CO_2, mg/kg	Cmin, mg/kg	Rate Constant, *k*	Rsqr	P	SEE
Jak PL	403.33	1865.67	0.030	0.948	<0.0001	113.25
JakCON	891.41	2012.59	0.021	0.981	<0.0001	76.68
SmePL	400.01	1807.13	0.016	0.976	<0.0001	70.51
SmeCON	366.90	2756.10	0.016	0.948	<0.0001	158.39
LesPL	419.853	1707.98	0.008	0.966	<0.0001	58.55
LesCON	969.61	1594.87	0.012	0.979	<0.0001	52.84
LSD		**				

Note: **—correlation is significant at the 0.01 level.

Table 6. Pearson correlation coefficients for MP content and soil parameters.

	MP22	Sig. (1-Tailed)	MP23	Sig. (1-Tailed)
MP22	1			
MP23	0.681	0.068	1	
Cmin	−0.425	0.2	−0.794 *	0.03
porosity	0.907 *	0.047	0.702	0.149
sp.mass	0.738 *	0.047	0.908 **	0.006
totN	−0.726	0.051	−0.853 *	0.015
totC	−0.625	0.092	−0.919 **	0.005
P_2O_5	−0.865 *	0.013	−0.876 *	0.011
K_2O	−0.856 *	0.015	−0.836 *	0.019
CEC	−0.686	0.066	−0.860 *	0.014

Note: *, **—correlation is significant at the 0.05 and 0.01 levels.

4. Discussion

4.1. Soil Chemical Characteristics

Soil pH was not sensitive to the MPs in the studied soils. Apparently, the effect of MPs on soil pH depends on many factors, such as the origin of MPs, their chemical composition, as well as their size, shape, and age [4,34–36]. Since we did not determine the nature of MPs in this study, their influence on soil pH remains to be elucidated in future, more detailed studies.

The higher content of mineral nitrogen, as well as available phosphorus and potassium, in the soil of open ground is explained, firstly, by the fact that the fertilizers that were applied in greenhouses and on open ground differ in their form and accessibility by crops. In greenhouses, the NPK fertilizer dissolved in an aqueous solution was applied for better plant uptake. This explains the higher removal of nutrients with the harvest in greenhouses [1,13,37]. Secondly, since in greenhouses plant residues are removed, and most plastic materials contain negligible concentrations of nitrogen and phosphorus [36,38], there was therefore no replenishment of the consumed nutrients.

The content of all microelements in the soil of the Danube basin was higher than in the Sava and Morava. The microelements were distributed as follows in all the studied soils: Mn > Zn > Ni > Cr > Pb > Cu > Co > As > Cd, and were below the background limits according to the regulations of the Republic of Serbia, except for Ni. The concentration of Ni in the soil from the Sava and Danube sites exceeded the maximum allowed concentration (MDK) of 50 mg/kg but was below the remediation value of 210 mg/kg. The high concentration of Ni in the Sava and Danube locations is explained by the parent rocks of an alluvial–diluvial origin that geochemically contain high concentrations of total Ni [33].

The concentrations of trace elements in the soils of the plastic-contaminated sites and open fields showed that there was no direct statistical difference for most elements. The exceptions were found at the Danube site, where the concentrations of Cu, Pb, and Zn were higher in the open field soil than in the plastic greenhouse soil, and the Morava site, where the concentration of Zn was significantly higher in the open field soil. There are a few possible reasons for this: one is an air deposition of these trace elements, as these soils are prone to air deposition from urban sources [18]; another reason is the indirect effect of MPs involving other soil parameters (see Section 4.5).

4.2. Soil Physical Properties

Greater soil porosity in plasticulture was somehow expected given the higher content of MPs. Because MPs typically have a lower density than soil particles, they are expected to reduce soil bulk density while increasing porosity [36]. However, there is no consensus about the effect of the presence of MPs on soil physical parameters. Ref. [8] found a moderate decrease in bulk density and porosity (4–6%). However, [39] did not find any changes in the bulk density of a clayey soil when 0.1% and 0.3% PES microfibers were present. Ref. [35] found that the presence of films, fibers, and foams/fragments can decrease soil bulk density, increase water holding capacity, and decrease soil aeration/porosity, respectively. Some studies found that MP fragments have the potential to bind soil particles together, forming larger aggregates [36,40]. Contrarily, [41] found that by increasing soil porosity and aeration followed by the acceleration of soil enzymatic activities, MPs can enhance water and nutrient availability for plants, thereby promoting the growth of belowground plant biomass.

The results obtained in this study and others assume that the presence of plastic particles may affect the physical parameters of soil in different ways [41]. There is a possibility that changes in the physical parameters of soil due to the presence of MPs may be positive or negative under certain conditions (e.g., soil texture; moisture and temperature regime; concentration, size, type, and age of MPs). MPs are subject to physical weathering, aging, and quality deterioration due to many factors, such as contrasting temperatures, solar radiation, and the freezing and melting of soil moisture [16]. In addition, agrochemicals applied to arable soil also induce the aging of plastic materials in soil [11]. These discrepancies in the influence of MPs on the physical properties of soils are explained by the heterogeneity of soil types, climatic and environmental conditions, parent rock, as well as the size, shape, and age of the MPs [4,34]. Moreover, the synergistic or antagonistic influence of these changes, together with other processes in the soil such as the adsorption and desorption of substances and elements, on soil parameters, as well as on plant growth and development, the assimilation of nutrients, and their toxic effect on soil flora and fauna, has not been elucidated yet. All the above-mentioned factors must be taken into account, and further detailed study of the effect of MPs and their behavior in the soil matrix is required.

4.3. Soil Organic Carbon

Soil is the medium for the growth, survival, and development of both above- and belowground organisms. Therefore, both the living part of soil and the dead organic substance (humus, soil organic matter) are important factors in maintaining the structure for the next generations of soil biota and plant roots. The biomass of terrestrial organisms accounts for 99.87% of the total planet biomass [42]. The transformation of soil organic carbon (mineralization and humification) is directly dependent on the activity of soil microorganisms, while the activity of soil microorganisms greatly depends on environmental factors, such as temperature and moisture, as well as on soil characteristics such as texture, the availability of oxygen, and others. Since fresh plant residues are an easily accessible source of energy and nutrients for soil microorganisms [43], the amount of plant residues remaining in the soil after harvest plays a major role in the accumulation of the stable soil organic matter (soil humus) and labile organic carbon (easily decomposable). Soil organic matter is a complex substance with a large surface area and a colloidal system that allows it to retain macro- and microelements, as well as various compounds. On the other hand, disaggregated particles of plastic materials also act as a donor for the absorption and retention of various compounds and substances.

The results showed that there was a statistically significant difference in organic carbon content between the greenhouse and open field soils at all three locations. Based on fundamental knowledge of soil organic matter accumulation, we suppose that the main reason for this difference is the lack of crop residues to be returned to the soil in the plastic greenhouses. After each season, a comprehensive disinfection of greenhouses is carried out, the main goal of which is to minimize the likelihood of the persistence of infections and pests. During the disinfection process, plant residues are completely removed. In contrast, growing outdoors allows crop residues to be returned back to the soil, thereby partially offsetting the loss of organic carbon due to decomposition.

4.4. Soil Labile Organic Carbon

Soil ecosystems contain a large variety of animals, macro-, meso-, and microfauna, as well as microorganisms that are spatially distributed in the soil and litter layers. They all contribute significantly to the transformation and distribution (including mixing and bioturbation) of soil organic matter (in particular in the litter and root layers) and thus to nutrient, carbon, and water cycling [44]. Roughly half of SOM is soil organic carbon (SOC), and roughly 5% of SOM is nitrogen in the topsoils of agricultural land. The largest proportion of SOM/SOC consists of microbial necromass [45].

Anthropogenic factors such as cultivation, the use of agrochemicals, mechanical disturbances, and plant biomass removal affect the diversity and number of soil fauna [45]. Since the majority of these organisms are aerobic, the amount of porous space, pore size distribution, surface area, and oxygen levels are also of crucial importance to their life cycles and activities. The assemblage and activity of soil microorganisms are greatly influenced by soil physical properties [40,46], porosity in particular [47]. Given the increased porosity and favorable temperature and moisture conditions in greenhouses, microorganisms were more active in their search for food and energy, thereby mineralizing organic carbon. Since all crop residues are removed from the greenhouses, the sources of labile organic carbon are also reduced. Consequently, the soil from the greenhouses showed significantly less labile carbon. In addition, the decrease in microbial activity in the soils from the greenhouses may be due to a direct effect on the organisms living in the soil via changing their habitat [20].

In this study, the lowest amount of labile carbon found in the Leskovac soil is due to the higher content of the sand fraction. Sandy soil promotes the mineralization of organic matter due to better aeration. Comparing the microbial activity in the soils from plastic greenhouses and their corresponding open fields, the soils from Danube and Morava showed significantly higher microbial activity when growing crops in the open field than in plastic greenhouses. The main reasons for this are (1) a lack of a labile carbon source in greenhouse soil such as crop residues and (2) a higher content of MPs that changed soil porosity in the plastic greenhouses.

Although the effect of MPs originating from plastic film residues on microbial soil respiration is not yet well understood [6,46], we can assume that MP pollution can inhibit microorganisms' activity and potentially diminish soil fertility. The findings of other research showed no consistency concerning the effect of plastic materials on labile carbon content. Some studies showed an increase in dissolved organic carbon (DOC) in the presence of high concentrations of certain types of MPs [48], while others did not find significant changes [49] or even a decrease in SOM [50].

Soil organic matter is a highly heterogeneous organomineral complex. The addition of polymers of a different chemical composition, shape, and size only complicates the assessment of the interaction between soil components, soil-dwelling organisms, plants, and MPs [38]. The effect of non-degradable and biodegradable MPs on labile OC, i.e., soil enzymatic activity, is still under discussion [51]. Currently, MP carbon is not considered to be a SOC [10], although available test methods cannot distinguish MP carbon from natural SOC [10]. According to [52], the determination of SOC using strong oxidants could result in the release of organic compounds from MPs, which are mistakenly considered to be SOC. It is therefore argued that MPs are disguised as soil carbon storage, leading to an overestimation of soil carbon stocks [53].

4.5. Network Visualization and Analysis

To enable a clearer and more intuitive interpretation of the network results, a graphical visualisation was created, which can be seen in Figures 5 and 6. The combination of the gLASSO method and the EBIC criteria enabled the construction of a robust network that provides precise information on the connection of indicators. The non-paranormal transformation further improved the model by fitting the data for analysis, allowing the detection of linear and nonlinear relationships between nodes. The resulting network illuminates the key points of interaction between microplastics and the indicators of physical and chemical soil properties, both under open field and plastic greenhouse conditions (Figures 5 and 6).

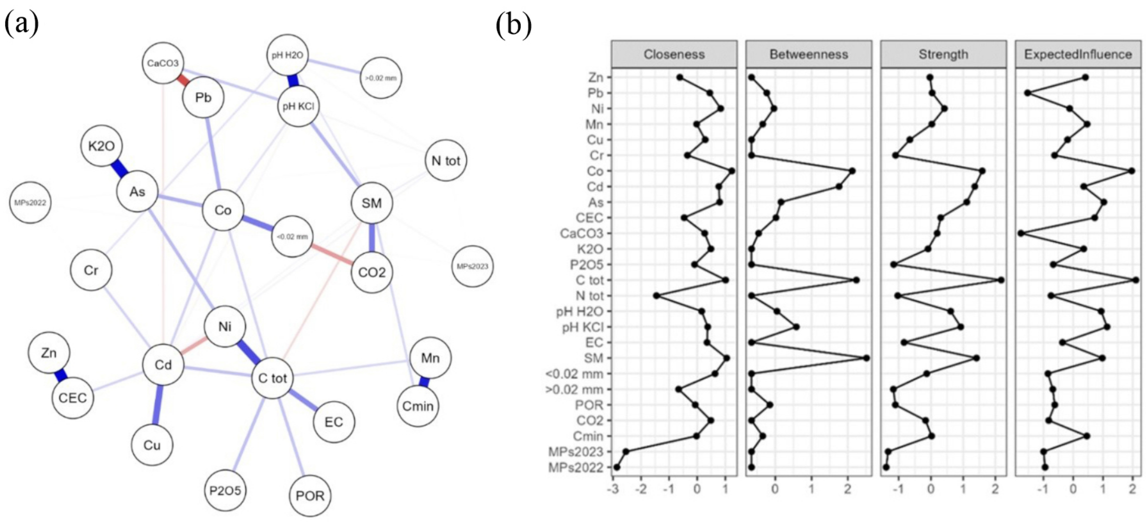

Figure 5. Network structure and centrality measure in open fields: (**a**) network structure of the soil indicator nodes; (**b**) centrality (strength) plot. Blue lines represent positive relationships; red lines indicate negative relationships. The variations in the intensity and thickness of the lines illustrate these relationships.

The network analysis of soils indicates the absence of significant nodes between microplastics (MPs) and other variables in the open field soils. The lowest recorded expected impact (EI) is for $CaCO_3$, whose only recorded relationship, a negative correlation with Pb, further reduces its significance. In contrast, Ctot and Co have the largest number of connections with other variables in the network, which gives them the strongest influence. In the greenhouses, the picture is significantly different (Figure 5). Microplastics from 2022 (MPs 2022) show a strong positive correlation with soil particles smaller than 0.02 mm, while negative correlations exist with CEC. A weaker negative relationship was observed with Cd, while weak positive correlations were noted with Co and soil pH. The low EI of microplastics may partly explain the weakness of these links. The network analysis indicates a relatively weak influence of microplastics on the chemical properties of soil under open-air crop production.

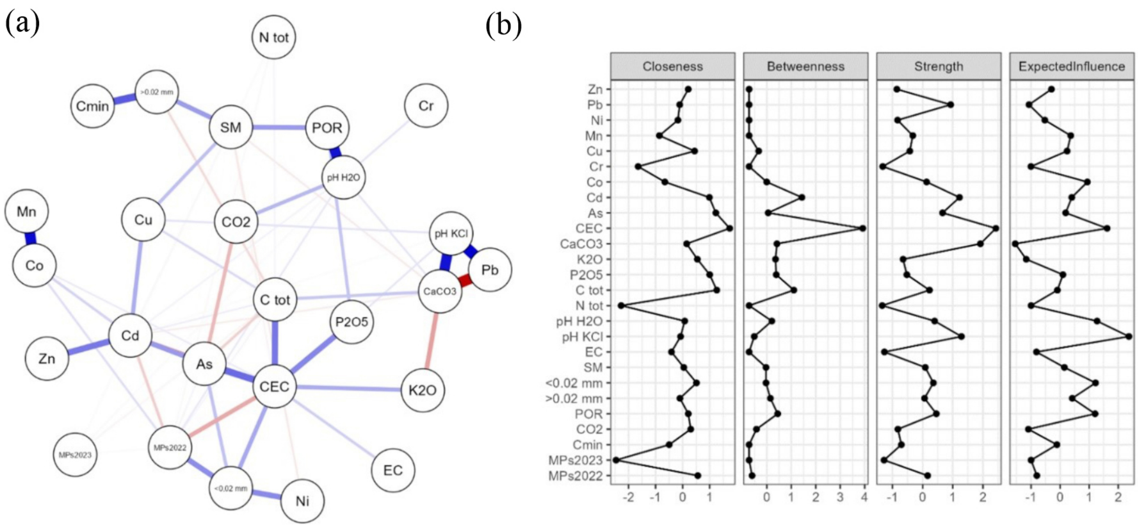

Figure 6. Network structure and centrality measure in greenhouses: (**a**) network structure of the soil indicator nodes; (**b**) centrality (strength) plot. Blue lines represent positive relationships; red lines indicate negative relationships. The variations in the intensity and thickness of the lines illustrate these relationships.

However, an increase in the concentration of microplastics in the greenhouses, confirmed by the T-test, causes changes related to the physical properties of the soil (Figure 6). For example, the increase in soil porosity in plasticulture allows the migration of Cd into deeper soil layers due to its natural solubility [54,55]. The negative correlation of microplastics with CEC indicates a reduced ability of the soil to adsorb metals. On the other hand, the weak positive correlation with Co may be the result of a higher content of this element in environmental plastics compared to other PTEs [56]. In comparing the soil in plasticulture with that of open fields, key correlations were noted between MPs and physical soil properties. The positive correlations were found with soil porosity, fine particle content (<0.02 mm), and soil pH, while negative correlations were found with total and mineral carbon, total nitrogen, available phosphorus and potassium, as well as CEC.

The NA outputs support the main findings that the presence of microplastics leads to the destruction of soil structure, which reduces bulk density [36,57]. An increase in the number of macropores (>30 μm) accelerates the leaching of PTEs from upper soil layers, despite the increased adsorption of these elements onto microplastic particles [58]. Increased soil aeration further raises the pH value [5]. Microplastics negatively affect the available phosphorus and potassium, as well as the C/N ratio, due to disruption of the nutrient cycle. This effect depends on the type of microplastic and specific soil properties [48].

Although many studies have shown the various effects of MPs on soil properties and functions, most of them were conducted using a defined concentration (mostly elevated concentrations) and known type of MP. As such, the MPs added to the soil in these studies were new, i.e., non-degraded. We assume that polymers naturally found in a soil matrix present there for decades and undergo physical disaggregation and chemical decomposition due to abiotic stress factors, thus contributing to the amount of MPs in the soil matrix of plastic greenhouses. During decomposition, MPs may impose some negative effects on soil-dwelling organisms and soil functional properties [59]. The obtained results proved that MPs in arable soil change certain soil properties. The vector, magnitude, and amplitude of MP influence on soil properties have yet to be studied. Moreover, the interactions of MPs with soil components that can act synergistically or antagonistically have not yet been studied. It is also necessary to study the threshold values of MP concentration for each soil parameter at which the natural functions of the soil are disrupted. It is important therefore to set a long-term observation of the fate and ecotoxicity of MPs in soil ecosystems, as well as their contribution to GHG emissions [60]. Furthermore, the introduction and accumulation of MPs in the terrestrial food web can lead to unforeseen health consequences, highlighting the necessity for further research in this regard [34,61–63].

5. Conclusions

Microplastic content and its effect on main soil characteristics were investigated in plasticulture and the open field. This study showed that MPs cause changes in soil physical, chemical, and biological properties. Among the physical properties, soil porosity and soil particles <0.2 mm in size showed significant changes due to the pollution of MPs. They positively correlated with MP concentration in the soil. Among the chemical properties, cation exchange capacity, total nitrogen and carbon contents, and plant-available phosphorus and potassium showed strong negative correlations with MPs. Soil labile organic carbon was also strongly negatively correlated with MP contamination. These soil parameters can be successfully used to assess the impact of MPs on soil health. The interpretation of some of the obtained results requires further, more in-depth, and long-term studies on the influence of MPs on soil parameters and functions.

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Abbreviations

The following abbreviations are used in this manuscript:

MPs	Microplastics
PMC	Potentially mineralizable carbon
NA	Network analysis
PTEs	Potentially toxic elements

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