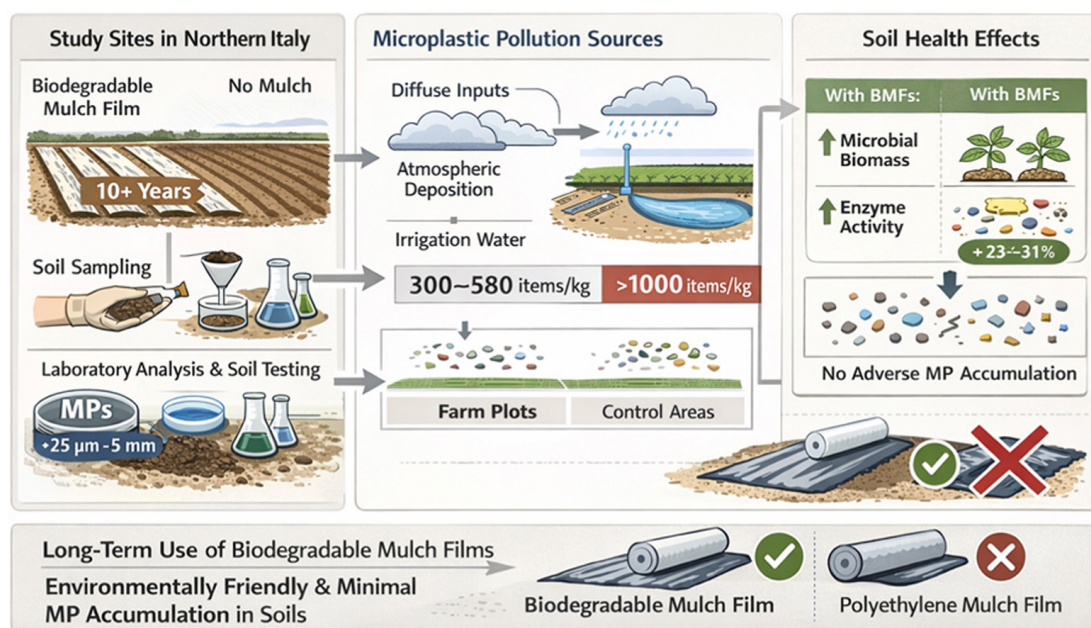


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Long-term biodegradable mulch films application in agricultural fields: effects on soil functionality and microplastic generation

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Abstract

The accumulation of microplastics (MPs) in agricultural soils has become a growing environmental concern, particularly due to the use of plastic mulch films. Biodegradable mulch films (BMFs) have been proposed as a sustainable alternative, yet long-term field evidence remains scarce. In this study, we evaluated MPs occurrence and soil functionality in two commercial farms in northern Italy where BMFs have been applied for over a decade. Soil samples were collected from plots with and without mulch, MPs (25 μm -5 mm) were isolated and chemically characterized, and soil biochemical parameters were assessed. The results revealed a diverse composition of plastics mainly not related to agricultural practices, suggesting external inputs from diffuse sources such as atmospheric deposition or irrigation water. Overall, MPs concentration over the two fields ranged between 300 and 580 MP items $\text{kg}_{\text{ds}}^{-1}$ with control surrounding areas reaching >1000 MP items $\text{kg}_{\text{ds}}^{-1}$. Notably, among the fragments spectrally compatible with biodegradable materials, their origin could not be directly linked to the applied mulch films and are therefore presumed to originate from external sources. From a biochemical point of view, BMFs application enhanced, on average, microbial biomass carbon (+27%) and enzymatic activity (+23% for β -glu, +13% for β -xyl, and +31% for PME), although these effects were strongly site-specific, with soil type emerging as the main driver of variability. Overall, our findings indicate that long-term use of BMFs does not lead to clear MP accumulation within the assessed size range nor adversely affect soil functionality, supporting their potential as an environmentally compatible alternative to conventional polyethylene mulches. Further research is nevertheless required to better understand their long-term transformation and environmental fate under field conditions.

1 Introduction

In the last decade a growing interest has been paid to the study of the generation and effects of microplastics (MP), which are defined as synthetic, insoluble solid particles or polymeric matrix, with regular or irregular shape and size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin (Frias and Nash, 2019). A large body of research on MPs has focused on marine environments (Ortega-Zamora et al., 2025), as well as wastewater (Edo et al., 2025) and atmospheric compartments (Casella et al., 2025). These studies reflect the growing scientific and societal interest in understanding the environmental fate of these pollutants, their potential impacts on ecosystems and agricultural systems, and their implications for human health (Casella et al., 2026).

During the recent years, driven by the direct connection between soil contamination and food production systems, increasing concern has also emerged regarding the presence of MPs in soils. MPs in soils originate from multiple sources, including agriculture, soil amendments such as compost or sewage sludge, and diffuse inputs like atmospheric deposition (Horton et al., 2017; Lofty et al., 2022; Pérez-Reverón et al., 2022). Reported soil plastic concentrations vary widely, ranging from 0.000001% to 0.3% by mass, equivalent to estimated loads of 50-250 kg ha^{-1} (FAO, 2025; Rashid et al., 2025). Such contamination is highly heterogeneous, complicating the identification of dominant sources across different environments (FAO, 2025).

The European Commission has introduced regulatory measures to reduce MPs emissions (Casella et al., 2024), including Regulation (EU) 2023/2055 under REACH, aligned with the EU Plastics Strategy (European Commission, 2018) and the Zero Pollution Action Plan (European Commission, 2021), while the EU Fertilizers Regulation (European Commission, 2019) sets limits for MPs in organic fertilizers and

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establishes biodegradability criteria for polymers and mulch films to mitigate soil contamination (Hechmi et al., 2024).

Agricultural plastics are a direct source of MPs. Different practices generate distinct types of contamination, including inputs from irrigation pipes, containers, and coated fertilizers. However, the long-term use of conventional mulch films (non-biodegradable) has been identified as one of the most significant contributors to increased MPs concentrations in agricultural soils (Huang et al., 2020; Sa'adu and Farsang, 2023). Although Biodegradable Mulch Film (BMF) are considered a promising solution to reduce the accumulation of plastics in soils, field evidence remains scarce, particularly from over medium to long periods. In fact, no data is available beyond four years (Xiong et al., 2025), despite the commercial use of BMF for over two decades. The BMF are intended to be tilled into the soil, thereby becoming a physical input (which is not easily identifiable) and an input of carbon (C), albeit a negligible one (Bandopadhyay et al., 2018; Xu et al., 2025). BMFs have been shown to increase microbial biomass and activity, while modifying microbial structure (Bandopadhyay et al., 2018). The microbial response to BMF incorporation into the soil can be studied by measuring the soil enzymatic activities. It is widely accepted that such activities reflect the degree of biochemical reactions in the soil and the state of nutrient cycling. Xu et al. (2025) highlight their value as rapid, sensitive soil health indicators. However, shifts in composition and enzymatic activity signal accelerated decomposition, potentially altering carbon and nitrogen cycles (Bandopadhyay et al., 2018).

Here, we investigate the presence of MPs and their effects on soil biochemistry and functionality in farmers' fields where commercial BMF (specifically Mater-Bi) have been used for more than 10 years. We hypothesized that: (i) a fraction of biodegradable plastics from the most recent mulch application (<1 year before sampling) would be detectable; (ii) a significant number of non-agricultural MPs would also be present; (iii) mulch application would enhance microbial biomass and activity; and (iv) soil responses would vary depending on farm location and soil type.

2 Materials and methods

2.1 Site description and sampling methods

During the spring of 2022, two Italian agricultural soils under active horticultural production were sampled. In April 2022, a sandy soil (Arenosol; (WRB-IUSS, 2015) was collected from an organic farm

(FARM 1) located near Ferrara, in the coastal area of the Emilia Romagna region (Figure 1). The study site is located within the Regional Park of the Po River, a protected area characterized by a temperate climate with annual precipitation of 500–700 mm, cold winters, and hot summers. In June 2022, a second soil was sampled from a farm (FARM 2) located in the province of Cuneo, Piedmont (Figure 1). This soil is classified as loamy (Luvisol; (WRB-IUSS, 2015) and is managed following integrated crop management guidelines. The region has a subcontinental climate influenced by the surrounding mountains, with cold winters, hot summers, and an average annual precipitation of approximately 950 mm.

Field selection and management information were defined in close collaboration with the farmers, who provided detailed records of crop rotations, fertilization, tillage operations, and mulch film use. This ensured comparable agronomic conditions between mulched and control fields while remaining representative of the inherent heterogeneity of the agricultural land (approximately 1 ha per field). Both farms had been using BMF (commercial Mater-Bi black films; 15 μm thickness) for a period longer than 10 years for the cultivation of annual vegetable crops. FARM 1 consistently used BMF for 15 years: one application per year. Nevertheless, the possibility of previous use of conventional films cannot be excluded. In contrast, FARM 2 had been using BMF for 13 years, although not on an annual basis, as crop rotations including corn or beans were introduced every two years. Prior to the adoption of this, cropping seasons alternated between no mulch use and the application of conventional non-biodegradable mulch films.

The samplings were conducted just before the new mulch film was applied on the soil, at the beginning of the vegetative season, such that residues from previous BMF applications could still be present. In FARM 1, the most recent BMF application occurred during the previous growing season at transplanting and was incorporated into the soil at the end of the crop cycle (August), approximately eight months prior to sampling. In FARM 2, the interval since the last BMF application was longer due to the biennial crop rotation. A single application of BMF corresponds to approximately 0.0063% of the soil mass, when expressed as the proportion of material added to 0.2 m^3 of soil (equivalent to 1.88 kg m^{-2} of mulch film in 300 kg of soil (Sforzini et al., 2016).

In each field (~ 1 ha), four composite samples were collected from randomly distributed subareas of approximately 25 m^2 . Each composite consisted of ten samples taken at a depth of 0.2 m avoiding border zones. In addition, CONTROL soil samples were collected in both farms, from adjacent fields where

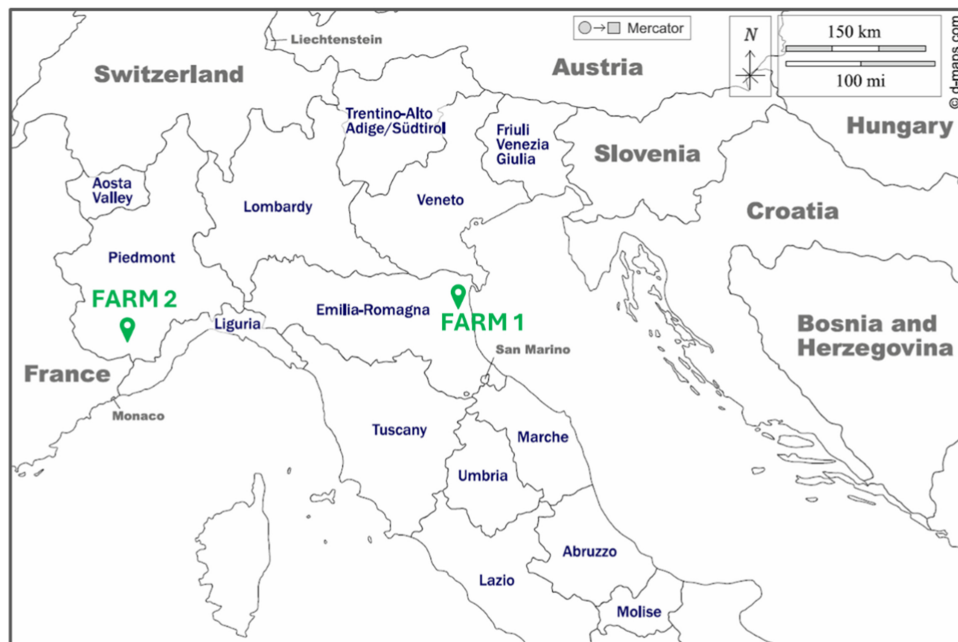


Figure 1: Map of Italy showing the locations of Field 1 in the Emilia Romagna region (near Ferrara) and Field 2 in the Piedmont region (near Cuneo).

neither BMF nor conventional plastic mulch films had been applied in the previous decade. During sampling, the presence of larger plastic debris (macroplastics; >5 mm (Ryan et al., 2019), was also assessed; however, both fields were visually clean, and no items were observed or collected. To prevent possible contamination, no plastic tools were used during sample collection or storage. Samples were then placed in metallized paper bags and subsequently divided into two sub-samples, one for MPs analysis and the other for soil functionality measurements.

2.2 Microplastic identification

A total of 16 soil samples were analyzed, consisting of four samples with mulch use and four control samples (without mulch) collected from each of the two farms. Initially, soil samples were vacuum dried at 60°C for 72 h to reduce moisture content before sieving. The dried samples were then separated using standard sieves into three size fractions to facilitate manipulation: >5 mm, >1 mm, and >25 μm (Figure S1, Supplementary Material), therefore, particles smaller than this threshold may not have been recovered and were therefore not considered in this study. Each fraction was weighed before further processing, with subsample masses adjusted according to particle size to facilitate MPs extraction: 15–20 g for the large fractions (1–5 mm), and 5 g for the small fraction (25 μm –1 mm), analyzing a minimum of three replicates per size in each composite sample (data in Supplementary Material).

The larger fractions (1–5 mm) were hydrated

overnight to eliminate agglomerates, filtered through a 375 μm stainless steel mesh to ensure that no material was lost during processing, and rinsed with ultrapure water to remove sand and liberate potential attached larger plastics. The small fraction (25 μm^{-1} mm) was first digested with hydrogen peroxide (H_2O_2 ; 20 mL of 33% solution per 5 g of sediment, Panreac) to eliminate excess organic matter. After digestion, these samples were vacuum-filtered through a 25 μm mesh. Subsequently, density separation was performed using zinc chloride (ZnCl_2 ; 100 mL), stirred for 10 min, and left to settle in tubes for 1 h. The supernatant was then filtered through a 25 μm stainless steel mesh and oven-dried at 60°C . The efficiency of both digestion and density separation for biodegradable and compostable polymers was previously tested and validated using the same protocol, demonstrating recovery efficiencies $>98\%$ and reliable FTIR identification of Mater-Bi and other EN-13432-certified bioplastics (Edo et al., 2022).

Samples were visualized and counted using a Leica KL300 stereo microscope. Infrared analysis (micro-FTIR; $\mu\text{-FTIR}$) was conducted on cleaned particles using a PerkinElmer Spotlight 200 Spectrum Two micro-FTIR spectrometer (20 scans, 8 cm^{-1} spectral resolution, $550\text{--}4000\text{ cm}^{-1}$ spectral range, apertures 50×50 and $20\times 100\text{ }\mu\text{m}$). The number of particles analyzed by $\mu\text{-FTIR}$, was statistically defined to ensure robust estimation of polymer proportions, following the recommendations of (Kedzierski et al., 2019; Cowger et al., 2024) targeting a maximum relative error of $\leq 5\%$.

Spectra were compared against existing reference databases (Aldrich Chemical Databases; Polymer Reference Databases or publications (De Frond et al., 2021)) and a custom-built laboratory database with more than 300 spectra using the OMNIC 9 software (Thermo Scientific). To ensure reliable polymer identification, commercially used Mater-Bi® BMF from the field were also included in the database (Figure S2, Supplementary Material). MPs concentrations were expressed per kilogram of dry soil (kg_{ds}).

2.3 Quality control on microplastic extraction

Throughout the entire process, strict contamination control measures were implemented to minimize external plastic contamination (Bhat et al., 2024). All laboratory surfaces were thoroughly cleaned prior to sample processing, and only cotton laboratory coats were worn. Plastic materials were strictly avoided or kept to a minimum; instead, glass and metal equipment were used. All reusable glassware was washed three times with Milli-Q water, rinsed with HPLC-grade methanol, and thermally treated prior to use (420°C) for at least 4 h to remove potential plastic residues. In addition, all working solutions were pre-filtered using pore sizes equal to or smaller than those employed for sample filtration (GFF; $1\ \mu\text{m}$, Whatman Cytiva) to minimize airborne or solution-borne MP contamination. New thermally treated filters were used for each analysis to reduce background contamination. Procedural blanks were included throughout the analytical workflow to monitor potential contamination. Airborne contamination controls were also assessed; blank-derived particle counts were subtracted from sample counts, and samples were discarded when blank contamination exceeded 10% of the corresponding sample particle count.

2.4 Soil analysis

Sixteen fresh soil samples were sieved at 4 mm, cleared from plant residues and debris, and divided into two aliquots: one air-dried for chemical determinations, one stored at 4°C for biochemical measurements. Indeed, to assess soil functionality after long-term BMF application in field, several soil parameters were determined in triplicate for each sample.

Carbon (C) and nitrogen (N) content in soil were measured by analyzing total organic carbon (TOC), total nitrogen (TN), and their ratio (C/N). Additionally, dissolved organic carbon (DOC), microbial biomass carbon (MBC), total dissolved nitrogen (TDN) and microbial biomass nitrogen (MBN) were quantified following standard protocols (Vance et al., 1987). These analyses were performed us-

ing an elemental analyzer for liquid samples (TOC-VCPH/CPN), with results expressed as mg kg^{-1} dry soil (ds).

Furthermore, four extracellular hydrolytic enzymatic activities involved in C, N, and P cycles were assessed fluorometrically using fluorogenic 4-methylumbelliferyl (MUF) conjugates: β -glucosidase (β -glu) and β -xylosidase (β -xyl) (involved in carbon cycling), N-acetyl- β -D-glucosaminidase (NAG) (involved in nitrogen cycling), and phosphomonoesterase (PME) (involved in phosphorus cycling (Giacometti et al., 2014)). For enzymatic activity measurements, 2 g of dry soil was homogenized in a sodium acetate buffer. A 100 μL aliquot of soil slurry was mixed with 100 μL of substrate solution in 96-well microplates. The microplates were incubated in the dark at 30°C , and fluorescence was measured ($\lambda_{excitation}$ 365 nm, $\lambda_{emission}$ 450 nm) using a microplate fluorometer (Infinite® 200, TECAN, Männedorf, Switzerland). Readings were taken immediately after plate setup and every 30 min for 3.5 h. Enzyme activities were expressed as $\text{nmol MUF g}_{ds}^{-1} \text{h}^{-1}$. Additionally, dehydrogenase activity (Dehy) was measured as an indicator of intracellular oxidative activity related to C cycling and microbial oxidative metabolism (Kumar, 2013). The analysis followed the method of (von Mersi and Schinner, 1991), in which 1 g of moist soil was incubated with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 37°C for 2 h. The reaction product (5-(4-iodophenyl)-1-(4-nitrophenyl)-3-phenylformazan; INTF) was quantified spectrophotometrically at 464 nm, and activity was expressed as $\mu\text{g INTF g}_{ds}^{-1}$.

To further assess microbial activity, two indices were calculated: (i) the Metabolic Index (MI), defined as the ratio between dehydrogenase activity and DOC, an indicator of soil metabolic activity in relation to carbon sources (Bastida et al., 2008), and (ii) the specific Enzyme Activities, calculated as the ratio between enzyme activity and MBC content, which serve as sensitive indicators of changes in soil microbial activity (Gil-Sotres et al., 2005; Trasar-Cepeda et al., 2008).

2.5. Data handling and statistical analysis

Polymer identification as mentioned, was carried out using the OMNIC software (Thermo Scientific). The comparison was performed applying a Pearson correlation threshold of 70% as the criterion for positive confirmation. This threshold was selected as a compromise between analytical feasibility and the inherent challenges associated with natural samples, particularly soils, where particles are often coated with organic matter or mineral residues that hinder

spectral clarity. In addition, this value is consistent with values commonly applied in previous MPs studies (Botterell et al., 2022; Hildebrandt et al., 2024). MPs concentrations (items per unit mass of soil) were calculated using a correction-factor approach (data in Supplementary Material). For each sampling site, the total number of visually suspected particles was recorded through the stereomicroscope (N_{count}) separating the different typologies and colours. A representative subsample was analyzed by μ -FTIR to determine the fraction of particles confirmed as plastics ($f_{plastics}$). This fraction was calculated as the ratio between positive particles identified in FTIR ($N_{positive}$) and the total number of particles analyzed ($N_{analyzed}$).

$$f_{plastic} = \frac{N_{positive}}{N_{analyze}} \quad (1)$$

The estimated number of microplastic particles for each type and colour was then obtained by multiplying N_{count} by $f_{plastic}$.

$$MP_{items} = N_{count} \times N_{plastic} \quad (2)$$

Finally, corrected particle counts were normalized by using the dry mass of soil processed (M_{soil}), yielding concentrations expressed as items per kilogram of soil, according to:

$$[MP] = \frac{\Sigma MP_{items}}{M_{soil}} \quad (3)$$

Statistical analyses of chemical and biochemical data were performed using the R statistical environment (www.r-project.org, v. 4.2.2). For chemical and biochemical parameters, principal component analysis (PCA) was performed in the R environment using the princomp function applied to the correlation matrix. Scores of the main principal components were subsequently analyzed using a split-plot ANOVA to identify the dominant discriminating factor (site or mulch) for each PC (Giacometti et al., 2021; McDaniel and Grandy, 2016). Farm and mulch use were considered as factors in the two-way analysis of variance (ANOVA). Assumptions of normality distribution and homogeneity of variance were verified; ANOVA was carried out for p -value < 0.05 . In case of significant differences, the HSD test was applied to separate the means (p -value < 0.05).

3 Results

3.1 Microplastics identification

In total, 700 particles with anthropogenic appearances were counted in soil samples (Figure S3, Supplementary Material). A random subset of 282 particles was then analyzed by infrared spectroscopy

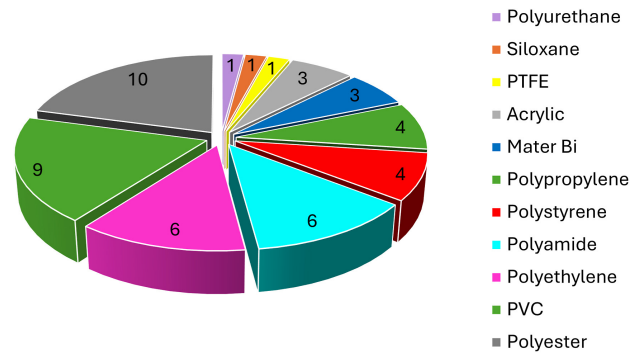


Figure 2: Polymers identified in the particle samples analyzed. Numbers correspond to the absolute frequency for the different polymers.

(including 26 particles from procedural controls). The particles selected were distributed as follows: in FARM 1, 65 particles in Mulch and 70 in the CONTROL plot were analyzed; and in FARM 2, 63 particles in Mulch and 58 in CONTROL were selected for FTIR analysis.

Despite their potential anthropogenic aspect, the analysis revealed that out of the 256 particles from samples, 48 particles (19%) were positively identified as 11 different types of plastic polymers, with polyester (PES), polyvinyl chloride (PVC), polyethylene (PE), and polyamide (Nylon 6-6) being the most abundant (Figure 2).

The remaining 208 particles did not yield positive polymer identification. Of these, 101 particles (39%) were identified as originating from natural materials (e.g. cotton, cellulose or animal remains), while the remaining particles (42%) could not be attributed to a specific anthropogenic origin, being consistent with natural inorganic materials or yielding spectra not represented in available databases, including potentially highly degraded polymers.

During the analyses, particular attention was given to 92 black particles morphologically consistent with the BMF used in the target plots (Figure S4, Supplementary Material). These particles were examined in detail, revealing that eight corresponded to plastic polymers, of which only two matched bioplastic spectra of the known film (Figure S5, Supplementary Material). Both were black fragments from FARM 2, collected in CONTROL areas without mulch application and likely originating from adjacent mulched plots (outside of the controlled area). The last particle showing a compatible infrared spectrum was a transparent fragment found in MULCH samples from FARM 1, whose colour differed from that of the commercial BMF applied there. MPs concentrations were calculated per kilogram of dry soil by extrapolating subsample data across each particle size class, typology, and sampling site are shown in Table 1.

Table 1. Concentration of microplastics per gram of soil (MP items $\text{kg}_{\text{ds}}^{-1}$) in the two locations (FARM 1 and FARM 2). Microplastics were divided according to their concentration in the soil classes 25 μm -1 mm and 1-5 mm.

Size	FARM 1				FARM 2			
	Mulch		Control		Mulch		Control	
	Average	Std.dev	Average	Std.dev	Average	Std.dev	Average	Std.dev
1-5 mm	146.1	35.8	820.7	661.2	44.2	16.9	25.8	7.7
25 μm -1 mm	439.6	131.8	571.8	167.6	257.3	92.5	107.0	45.4

Most MPs were found in the smallest size fraction, as expected from the fragmentation patterns of plastic particles. The larger fraction (1-5 mm) contained variable concentrations of MPs in Mulch and Control plots. In FARM 2, the Mulch plot had a higher concentration of MPs ($44.2 \pm 16.9 \text{ MPkg}_{\text{ds}}^{-1}$) compared to the CONTROL plot ($25.8 \pm 7.7 \text{ MPkg}_{\text{ds}}^{-1}$). In contrast, FARM 1 showed the opposite trend, with the CONTROL plot exhibiting a higher concentration ($820.7 \pm 661.2 \text{ MPkg}_{\text{ds}}^{-1}$) than the Mulch plot ($146.1 \pm 35.8 \text{ MPkg}_{\text{ds}}^{-1}$). When considering the smallest size fraction (25 μm -1 mm), MPs abundances increased by approximately 4-5 times, while maintaining the same overall trends.

3.2 Soil biochemical parameters

Principal component analysis (PCA) was performed on carbon and nitrogen pools (DOC, TDN, TOC, TN, MBC, and MBN), the CN ratio, the metabolic index (MI), dehydrogenase activity, and all specific enzymatic activities. The first two principal components explained 61% (PC1) and 15% (PC2) of the total variance (Table 2). An analysis of variance (ANOVA) was subsequently used to assess the influence of the discriminating factors, farm location and mulch application, on the principal components. The results showed that farm location significantly affected PC1 ($p < 0.001$), while mulch application significantly influenced both PC1 and PC2 ($p = 0.007$ and $p = 0.008$, respectively).

The PCA biplot (Figure 3) was constructed on the basis of the ANOVA results by plotting the first two principal components. The PCA revealed a clear separation between the two farm locations with FARM 2 characterized by higher microbial biomass, nitrogen pools, metabolic index, and most enzymatic activities. Along PC1, mulch application also emerged as a discriminant factor, with mulched plots exhibiting more positive scores. With respect to PC2, the mulch treatment showed evidence mainly within FARM 1, whereas this pattern was less pronounced in FARM 2.

Both farm location (i.e., soil type) and mulch application significantly affected TOC and TN content

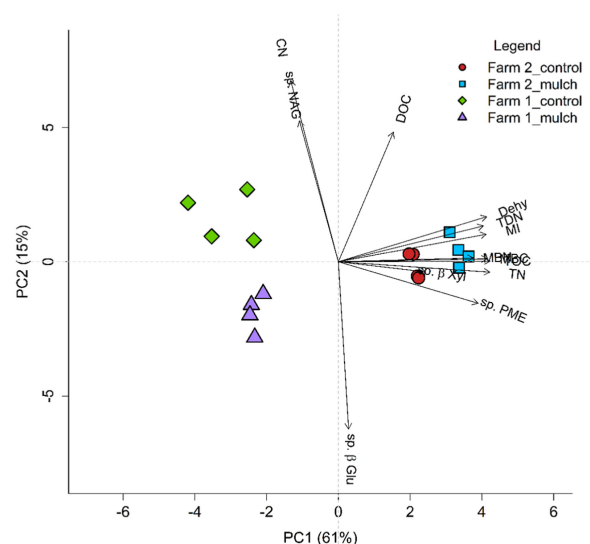


Figure 3: Principal component analysis plot including the soil C and N pools, the metabolic index, the dehydrogenase activity, and the specific soil enzymatic activities: dissolved C and N (DOC, TDN), microbial biomass C and N (MBC, MBN), total N (TN), total organic C (TOC), TOC to TN ration (CN), Dehydrogenase activity (Dehy), metabolic index (MI), specific activities of β -glucosidase (sp. β -Glu), β -xylosidase (sp. β -Xyl), N-acetyl- β -glucosaminidase (sp.NAG), and phosphomonoesterase (sp.PME). See Table 2 for PCA and ANOVA results.

(p -values ranging from 0.008 to < 0.001 ; Table 3). Higher TOC and TN values were measured in FARM 2 (+68% and +69% respectively) and in mulched plots (+17% TOC and +20% TN). In contrast, the C/N ratio was significantly higher in FARM 1 and in CONTROL plots (p -value = 0.046) with a significant Farm $\times$ Mulch interaction (p -value < 0.001), showing the highest values in FARM 1 CONTROL plots.

Dissolved and microbial C and N contents are reported in Figure 4. DOC values were relatively uniform within each farm location and only mulch application in FARM 1 determined a significant reduction (-24%; p -value = 0.036). The other three parameters, MBC, TDN and MBN, resulted significantly affected by farm location (p -value < 0.001), with higher values in FARM 2 (+64%, +55%, +62% respectively). Mulch application significantly increased MBC values in both farms, +22% in FARM 2 and +31% in FARM 1 (p -value < 0.001).

Table 2. Analysis of variance of results from the principal component analysis (PCA) with respect to farm location and mulch use. Significant comparisons are in bold.

ANOVA Parameter	PC1		PC2		PC3		PC4	
Prop. variance (%)	61		15		12		7	
ANOVA factor	F	p-value	F	p-value	F	p-value	F	p-value
Farm location (F)	743	<0.001	0.352	0.595	0.069	0.810	0.075	0.802
Mulch use (M)	15.7	0.007	15.4	0.008	0.399	0.551	1.33	0.293
F × M	0.653	0.459	27.7	0.002	0.054	0.824	1.00	0.356
Significant comparison	FARM 1 > FARM 2 Mulch > Control		Control > Mulch FARM 2_Ctrl = Farm 1_Mulch ≥ FARM 1_Ctrl ≥ FARM 2_Mulch		FARM 1 = FARM 2 Mulch = Control		FARM 1 = FARM 2 Mulch = Control	

All enzymatic activities were significantly higher in FARM 2 compared to FARM 1 (p -value <0.001), with an average increase of +74% on average (Figure 5). Mulch application and the Farm × Mulch interaction significantly affected only β -glucosidase, dehydrogenase, and phosphomonoesterase activities. Specifically, mulch application induced an increase in β -glucosidase activity (+45% in FARM 1) and phosphomonoesterase activity (+11% in Farm 2 and +51% in FARM 1). For the dehydrogenase activity, it is showed a site-specific result (p -value = 0.007), increasing in +19% in FARM 2 and decreasing in FARM 1 (-29%).

Specific enzymatic activities of β -xylosidase, phosphomonoesterase, and the metabolic index (MI) were significantly higher in FARM 2 than in FARM 1 (p -value = 0.019, <0.001, and <0.001 respectively; Table 4). Phosphomonoesterase and MI also showed significant differences and were increased by mulch application (+18% and +15%; p -value = 0.037 and 0.028, respectively). The MI showed significant differences with respect to the Farm × mulch interaction (p -value = 0.023), with the highest values measured in FARM 2 mulched samples. A similar interaction was found for the specific β -glucosidase activity (p -value = 0.007) which showed a high site-specific response with the higher values measured in FARM 2 (CONTROL) and FARM 1 (MULCH) plots.

4 Discussion

4.1 Microplastic identification

MPs contamination in agricultural soils has been increasingly reported across Europe, and the concentrations measured in this study are consistent with this growing body of evidence (Table S1, Supplementary Material). For instance, Sa'adu and Farsang (2022) reported a concentration of 225 ± 62 MP kg_{ds}⁻¹ in

greenhouse soils in Hungary, while Isari et al. (2021) observed values of 301 ± 140 MP kg_{ds}⁻¹ and 69 ± 38 MP kg_{ds}⁻¹ in greenhouse growing of watermelon and tomato respectively. This contamination was largely attributed to the use of conventional polyethylene mulch films. Conversely, lower concentrations have been reported when larger particle size thresholds were applied. One example was shown by (Piehl et al., 2018), who only considered MPs >1 mm and reported 0.34 ± 0.36 MP kg_{ds}⁻¹.

Beyond particle size, the extent of chemical characterization represents another major source of variability among MP studies. Full spectroscopic identification of all recovered particles is often not feasible due to technical constraints and limited access to instruments with automated mapping capabilities using LDIR or O-PTIR (Jia et al., 2022; Böke et al., 2022). This was also the case in the present study, where μ -FTIR analysis without automated surface mapping required the adoption of a representative subsampling strategy. While subsampling may introduce uncertainty (van Schothorst et al., 2021), recent statistical frameworks demonstrate that analyzing approximately 300-400 randomly selected particles provides robust polymer distribution estimates (95% confidence level, ≤5% relative error), even in heterogeneous environmental matrices (Cowger et al., 2024; Kedzierski et al., 2019). Accordingly, the number of particles analyzed here falls within the statistically robust range reported in the literature.

Building on the polymeric profiles identified, which are consistent with plastics commonly associated with agricultural environments (PES, PE, PVC, Nylon) and global production patterns (Houssini et al., 2025), this study specifically evaluated the presence of MPs derived from BMFs as a proxy for assessing long-term agricultural plastic accumulation in soils. The overall diversity of polymers detected, together with the absence of significant differences in

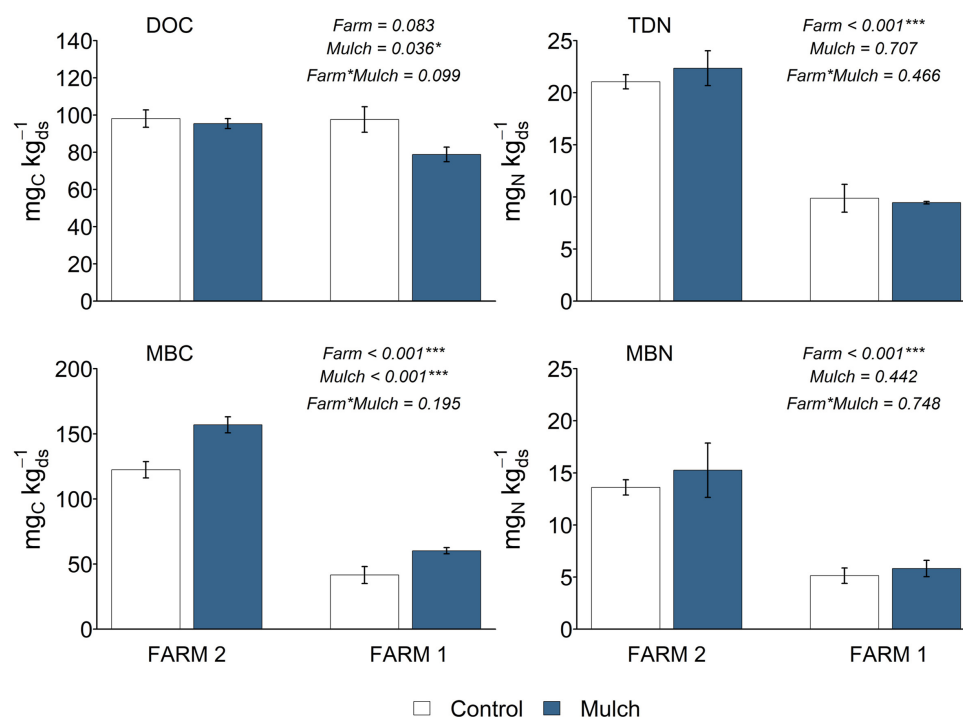


Figure 4: Marginal means of dissolved and microbial C (DOC and MBC) and N (TDN and MBN). Error bars represent the standard errors and the significant differences for Farm, Mulch, and Farm $\times$ Mulch are reported (p -value < 0.05).

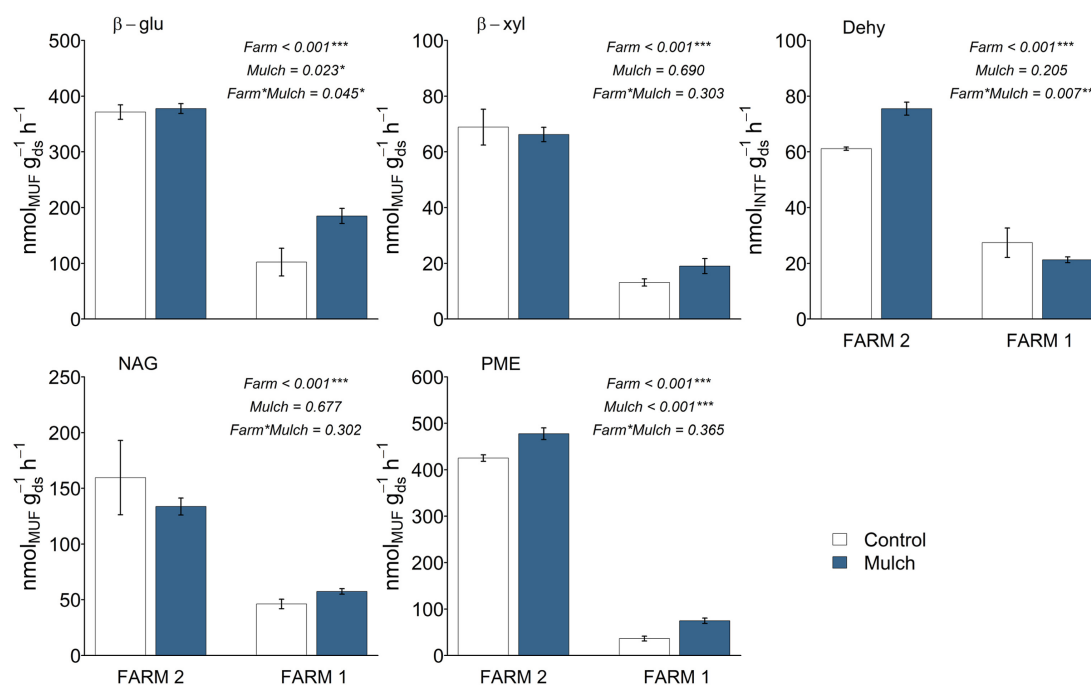


Figure 5: Marginal means of β -glucosidase (β -glu), β -xylosidase (β -xyl), Dehydrogenase (Dehy), N-Acetyl- β -glucosaminidase (NAG), and Phosphomonoesterase (PME) enzymatic activities. Error bars represent the standard errors and the significant differences for Farm, Mulch, and Farm $\times$ Mulch are reported (p -value < 0.05).

Table 3. Marginal means $\pm$ standard error of soil Total Organic C (TOC, $\text{g kg}_{\text{ds}}^{-1}$), Total N (TN, $\text{g kg}_{\text{ds}}^{-1}$), and ratio between TOC and TN (CN). Different lowercase letters indicate significant differences (p -value < 0.05) according to the HSD test.

	TOC	TN	CN
<i>Farm location</i>			
FARM 1	4.88 $\pm$ 0.55b	0.554 $\pm$ 0.05b	8.96 $\pm$ 0.19a
FARM 2	15.3 $\pm$ 0.53a	1.78 $\pm$ 0.06a	8.61 $\pm$ 0.09b
<i>p</i> -value	< 0.001	< 0.001	0.046
<i>Mulch use</i>			
Control	9.16 $\pm$ 0.58b	1.03 $\pm$ 0.05b	9.18 $\pm$ 0.17a
Mulch	11.0 $\pm$ 0.50a	1.29 $\pm$ 0.06a	8.39 $\pm$ 0.11b
<i>p</i> -value	0.008	< 0.001	< 0.001
<i>Farm $\times$ Mulch interaction</i>			
FARM 1 (Control)	4.11 $\pm$ 0.67	0.42 $\pm$ 0.06	9.76 $\pm$ 0.24a
FARM 1 (Mulch)	5.66 $\pm$ 0.43	0.69 $\pm$ 0.05	8.17 $\pm$ 0.13b
FARM 2 (Control)	14.2 $\pm$ 0.49	1.65 $\pm$ 0.05	8.60 $\pm$ 0.09b
FARM 2 (Mulch)	16.4 $\pm$ 0.57	1.90 $\pm$ 0.07	8.62 $\pm$ 0.09b
<i>p</i> -value	0.623	0.794	< 0.001

total biodegradable MP abundance between Mulch and Control plots, suggests that most of the identified plastics originated from diffuse, non-agricultural sources rather than from local farming practices. Potential inputs include atmospheric deposition and waterborne transport. In this context, both farms rely on irrigation water from nearby canals applied through drip irrigation systems. Additionally, FARM 1 is located close to a main road with regular traffic, which may represent a source of airborne MPs, whereas FARM 2 is not influenced by nearby major roads. These surrounding land-use characteristics may partly explain the differences observed in MP concentrations between sites (FARM 1 $>$ FARM 2). Similar diffuse inputs and site-specific factors have been widely reported as dominant drivers of MP occurrence in agricultural soils, often exceeding the contribution of on-site plastic use (Shcherbatyuk et al., 2025; Withana et al., 2025).

A focused analysis of black particles, considered most relevant for detecting mulch-related materials, further supports this interpretation. In CONTROL plots, conventional polymers accounted for 9.76% of the particles analyzed by μ -FTIR, while 4.88% showed spectral compatibility with biodegradable polymers, likely originating from diffuse sources. In contrast, in soils amended with BMFs, FARM 1 and FARM 2, only 2.17% of the analyzed particles were identified as conventional polymers, and no particles compatible with BMFs were detected. Overall, these results indicate a reduced occurrence of plastic particles in

BMF-amended soils, with the dominant contribution arising from conventional plastics of diffuse origin, in agreement with previous field studies comparing BMFs and polyethylene mulches (Schmidt et al., 2025).

Notably, the original Mater-Bi formulation used as BMFs, was detected only at very low levels, indicating a practical absence of BMF materials even after more than ten years of repeated application. This finding is consistent with field-based studies reporting that certified biodegradable mulch films, when properly incorporated into soil, can undergo effective degradation within relatively short timeframes without leaving persistent residues or inducing negative impacts on soil systems (Sintim et al., 2019; Yu et al., 2021b). This observation does not necessarily indicate the absence of degradation processes. Recent field studies have shown that BMFs can undergo substantial chemical and surface-level degradation in soil without measurable physical fragmentation into MPs. Graf et al. (2025), for example, reported that several BMFs exhibited clear degradation signals after 12 months of soil exposure, while no physical fragmentation was observed, regardless of meso- and macrofauna presence. From an analytical perspective, however, the present data do not allow the complete exclusion of the transformation of BMFs into secondary materials that fall outside the identification capability of μ -FTIR and Pearson correlation-based matching used.

Addressing this uncertainty would require the in-

Table 4. Marginal means $\pm$ standard error of the specific enzyme activities of β -glucosidase (β -glu, mmolMUF $\text{mg}_{\text{MBC}}^{-1} \text{h}^{-1}$), β -xylosidase (β -xyl, mmolMUF $\text{mg}_{\text{MBC}}^{-1} \text{h}^{-1}$), N-Acetyl- β -glycosaminidase (NAG, mmolMUF $\text{mg}_{\text{MBC}}^{-1} \text{h}^{-1}$), and Phosphomonoesterase (PME, mmolMUF $\text{mg}_{\text{MBC}}^{-1} \text{h}^{-1}$) and of the metabolic index (MI, mmolINTF $\text{mg}_{\text{MBC}}^{-1} \text{h}^{-1}$). Different lowercase letters indicate significant differences (p -value < 0.05) according to the HSD test.

	β -glu	β -xyl	NAG	PME	MI
<i>Farm location</i>					
FARM 1	2.72 $\pm$ 0.23	3.05 $\pm$ 0.48b	0.42 $\pm$ 0.07	3.46 $\pm$ 0.41b	0.27 $\pm$ 0.02b
FARM 2	2.74 $\pm$ 0.16	4.97 $\pm$ 0.82a	0.39 $\pm$ 0.06	6.78 $\pm$ 0.36a	0.71 $\pm$ 0.04a
<i>p</i> -value	0.913	0.019	0.863	< 0.001	< 0.001
<i>Mulch use</i>					
Control	2.71 $\pm$ 0.21	3.93 $\pm$ 0.57	0.48 $\pm$ 0.11	4.61 $\pm$ 0.55b	0.45 $\pm$ 0.03b
Mulch	2.75 $\pm$ 0.18	4.09 $\pm$ 0.72	0.33 $\pm$ 0.02	5.63 $\pm$ 0.22a	0.53 $\pm$ 0.02a
<i>p</i> -value	0.845	0.825	0.156	0.037	0.028
<i>Farm $\times$ Mulch</i>					
FARM 1 (Control)	2.36 $\pm$ 0.23b	2.70 $\pm$ 0.43	0.54 $\pm$ 0.13	2.88 $\pm$ 0.51	0.27 $\pm$ 0.04c
FARM 1 (Mulch)	3.08 $\pm$ 0.22a	3.39 $\pm$ 0.53	0.31 $\pm$ 0.01	4.04 $\pm$ 0.31	0.27 $\pm$ 0.01c
FARM 2 (Control)	3.06 $\pm$ 0.19a	5.15 $\pm$ 0.71	0.43 $\pm$ 0.09	6.34 $\pm$ 0.59	0.63 $\pm$ 0.03b
FARM 2 (Mulch)	2.42 $\pm$ 0.14b	4.78 $\pm$ 0.92	0.36 $\pm$ 0.03	7.23 $\pm$ 0.13	0.80 $\pm$ 0.04a
<i>p</i> -value	0.007	0.468	0.373	0.760	0.023

tegration of complementary techniques, such as thermogravimetric analysis (TGA) (Oliver et al., 2024), pyrolysis-GC/MS (De Falco et al., 2023), or thermal extraction-desorption GC/MS (Kittner et al., 2024), which are better suited for characterizing highly degraded polymers and low-molecular-weight transformation products. The application of such methods was beyond the scope of the present study but represents a clear priority for future research and follows previous recommendations (Campanale et al., 2024).

Recent modelling approaches have begun to elucidate degradation pathways and environmental fate of biodegradable plastics. Degli-Innocenti et al. (2022), using the same commercial Mater-Bi formulation investigated here as BMF, reported that although small particles may be generated during early stages of degradation, complete biodegradation occurs without long-term persistence. Similar conclusions were reached by Tosin et al. (2020), who showed that certified BMFs biodegrade at rates comparable to natural organic materials such as paper or dried leaves. However, other studies have highlighted that biodegradation rates of BMFs can be highly variable and strongly dependent on soil properties, temperature, moisture, and microbial community structure (Shao et al., 2026). For instance, Chinaglia et al. (2018) reported partial fragmentation and delayed mineralization of biodegradable polymers under certain soil conditions, while Ham et al. (2026) showed that biodegradable mulch films may persist longer in

cooler or less biologically active soils, with degradation proceeding more slowly than predicted by standardized laboratory tests. In contrast, polyethylene mulch films exhibit negligible degradation, with rates below 0.4% over 800 days, and are known to accumulate in soils when not fully removed or when too thin to be retrieved (Li et al., 2022; Mongil-Manso et al., 2023).

The focus on the 25 μm -5 mm size fraction reflects its relevance for accumulation in agricultural soils and its widespread use as an indicator of terrestrial plastic pollution (Büks and Kaupenjohann, 2020; Horton et al., 2017). While this approach does not capture macroplastics, nanoplastics or all potential degradation by-products, it provides a robust proxy for assessing long-term accumulation under real farming conditions. In this context, the strength of the present study lies in the long-term nature of the investigated sites, where BMFs have been applied for 10-13 years under contrasting soil textures and agronomic practices.

Considering crop rotations, agricultural management, and soil-dependent differences in microbial communities, biodegradation rates, and vertical transport processes, the absence of detectable BMF-derived fragments within the analyzed size range supports the 'steady-state' model proposed by Pechiari et al. (2024). According to this model, continuous inputs of biodegradable plastics result in only temporary persistence of material, followed by

progressive degradation rather than long-term accumulation in soil. Biodegradable mulch films may transiently generate micro- and nanoplastics during early degradation stages (Yu et al., 2021a). These particles are considered non-persistent, as they continue to degrade over time, but their potential off-site transport to air and water has been highlighted as a concern. This underscores the need for appropriate management practices and regulatory frameworks for biodegradable plastic use.

4.2 Future prospects

The results of this study should be interpreted within the methodological constraints applied to assess the long-term behaviour of BMFs in agricultural soils. The use of a density separation medium of 1.7 g cm^{-3} and a particle size threshold of $\geq 25 \text{ }\mu\text{m}$ limits the analysis to a specific fraction of biodegradable plastic residues, potentially excluding denser transformed products and/or highly degraded polymer fragments. As the primary objective of this study was to assess field-scale accumulation rather than to validate analytical methodologies, additional experimental verification, such as systematic evaluation of spectral changes across different degradation stages, was beyond of the scope of the present work but is a key driver for future investigations.

Another limitation inherent to the analysis of natural soil matrices is the relatively high proportion of unidentifiable particles. The combined effects of weathering, polymer blending, and the analytical limitations of μ -FTIR inevitably lead to conservative estimates of MPs occurrence. These findings should therefore be regarded as a starting point, highlighting the need for further research focused on the identification, characterization, and environmental fate of secondary degradation by-products derived from biodegradable materials.

Natural soil heterogeneity, including variations from loamy to sandy textures within and between fields, as considered in the present study, may influence MP retention, transport, and degradation dynamics, potentially requiring different BMF formulations adapted to specific soil conditions. As MPs detection, future studies employing higher-density separation media and complementary analytical approaches are therefore needed to better characterize biodegradable products across different matrixes and climatic conditions.

Extending analytical coverage to particle sizes below $25 \text{ }\mu\text{m}$ also represents a key step toward capturing the full degradation continuum of both conventional plastics and BMFs. Emerging techniques such as Laser Direct Infrared imaging (LDIR) (Jia et al., 2022) and Optical Photothermal Infrared (O-PTIR)

spectroscopy, capable to reach 500 nm (Böke et al., 2022), combined with high-retention membrane filtration protocols, offer promising opportunities to identify smaller MP fractions, nanoplastic precursors, and degradation by-products not addressed in the present study. Finally, while abundance-based metrics currently represent the most robust approach for assessing microplastics in soils, the use and development of complementary mass-based quantification methods would further improve understanding of BMF transformation and fragmentation processes.

4.3 Soil biochemical parameters

The analysis of the soil samples collected from the two fields did not reveal critical alterations in soil C and N contents or in the soil functionality indicators due to the long-term use of BMFs. Most experimental studies addressing the impacts of MPs on soils have relied on unrealistically high-test material concentrations, sometimes up to 10% of soil weight, largely exceeding typical agricultural inputs (Qi et al., 2020; Zhou et al., 2021). Under such conditions, conventional plastics have been shown to alter soil C and N cycling, microbial activity, bulk density, pH, and biodiversity, with negative consequences for plant growth and contaminant dynamics (Boots et al., 2019; de Souza Machado et al., 2018; Rodríguez-Seijo and Pereira, 2019; Wan et al., 2019; Zhang et al., 2022). In contrast, the samples in this study, based on over a decade of field-tested BMF use under realistic conditions (one application per cultivating season), revealed no changes in the dissolved pool of C and N, an increase in total organic carbon content (TOC), and microbial biomass carbon (MBC) content, and only slightly increased β -glucosidase and PME activities due to mulch incorporation. It can be argued that the increased MBC, β -glu and PME activities could be related to the ongoing degradation process, as previously also observed in other studies (Bandopadhyay et al., 2018; Mazzon et al., 2022; Xu et al., 2025). Indeed, in FARM 2 Mulch incorporation was associated with a significant increase also of the metabolic index (MI), which is an indicator of higher microbial metabolic efficiency and potentially improved carbon use efficiency.

Although mulch treatment was initially expected to be a major driver of variability, the observed differences were primarily associated with contrasting soil types, sandy (FARM 1) and loamy (FARM 2). Their contrasting textural characteristics influenced the biochemical properties measured such as enzymatic activities, microbial biomass C content and dissolved C content as observed by Mazzon et al. (2022). These results are in line with other studies performed in open fields with realistic biodegradable plastic doses

for more than one growing season (Bandopadhyay et al., 2020; Chu et al., 2023; Di Mola et al., 2021; Gao et al., 2023; Moreno and Moreno, 2008; Sintim et al., 2019). A dose-response has been proposed as a possible mechanism explaining several of the negative effects reported in literature for BMFs, including N immobilization, C priming effects, increased enzymatic activities, and reduced plant growth. Those responses are generally attributed to rapid variation in C/N caused by the introduction of a labile C source from biodegradable materials (Brown et al., 2023; Li et al., 2022; Meng et al., 2023). These findings indicate that, under the conditions tested, and within the assessed time frame, BMFs did not induce adverse effects on soil health indicators, in contrast to what has been reported for conventional plastics.

It is also important to note that in many publications the 'biodegradable mulch' definition refers to a wide range of materials, either commercial or experimental. In this study, a commercial Mater-Bi based BMF was used, which has been available on the market since the beginning of 2000. This material has been extensively evaluated in previous studies under field realistic application rates (Bandopadhyay et al., 2020; Di Mola et al., 2021; Kapanen et al., 2008) in laboratory studies assessing the ecotoxicity of these materials on different target organisms (Sforzini et al., 2016), and as a model material for biodegradation studies (Pecchiari et al., 2024). This makes our results robust and in line with already existing literature.

5. Conclusions

The results obtained from two commercial farms in northern Italy show that under field application dose (one mulching event per year or biannual) and standard agronomic practices, no clear accumulation of MPs derived from BMFs was observed within the particle size range assessed (25 μm -5 mm). Furthermore, no environmentally relevant differences in soil functionality were detected between BMF-amended soils and their respective control plots. This study provides long-term insights into the interaction between agricultural soils and biodegradable mulches, which can be summarized as follows:

- MPs contamination is widespread: The overall MP load was predominantly associated with conventional plastics from diffuse, non-agricultural sources rather than BMF application. Potential input pathways include atmospheric transport and irrigation water.

- The concentrations ranged between 300 and 500 MP items $\text{kg}_{\text{ds}}^{-1}$ across the two mulched fields and values up to three times higher in surrounding control areas.

- BMF-derived MPs were detected only at trace levels. Only very low amounts of MPs attributable to the applied BMFs were identified. Under the environmental conditions and agricultural practices examined, BMF use did not result in an increased risk of physical soil contamination.

- Potential formation of secondary by-products: Although no clear accumulation of BMF-derived MPs was observed, the possible formation of secondary degradation by-products cannot be excluded and warrants further investigation.

From a biochemical perspective, long-term BMF application did not induce adverse biochemical effects on soil functionality. While microbial biomass and activity increased in BMF-amended soils, these responses were strongly site-specific and primarily driven by soil type rather than BMF application itself. Overall, this study provides early long-term field evidence supporting the potential of BMFs as a viable alternative to polyethylene mulches. However, further long-term and multi-site field investigations are needed to fully elucidate their transformation pathways, by-product formation, and cumulative effects on soil systems.

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Supplementary Information

Long-term biodegradable mulch films application in agricultural fields: effects on soil functionality and microplastic generation

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Figure S3. Suspected anthropogenic particles observed under the microscope, including fibres, films, filaments, and fragments, showing a wide range of colours, shapes, and sizes.

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Table S1. Abundance of microplastics in agricultural soil in Europe.

Dataset. The data are available to download with DOI [10.5281/zenodo.18248389](https://doi.org/10.5281/zenodo.18248389)

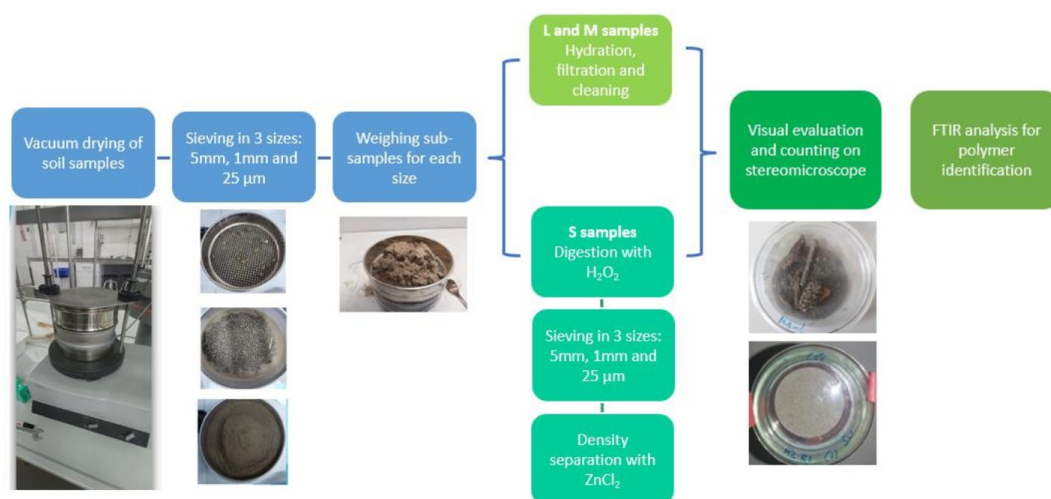


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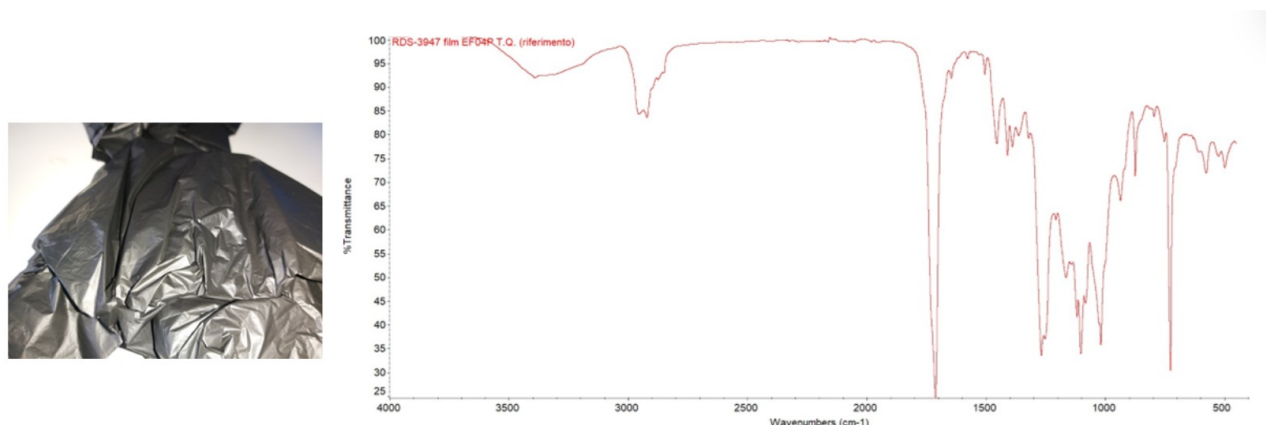


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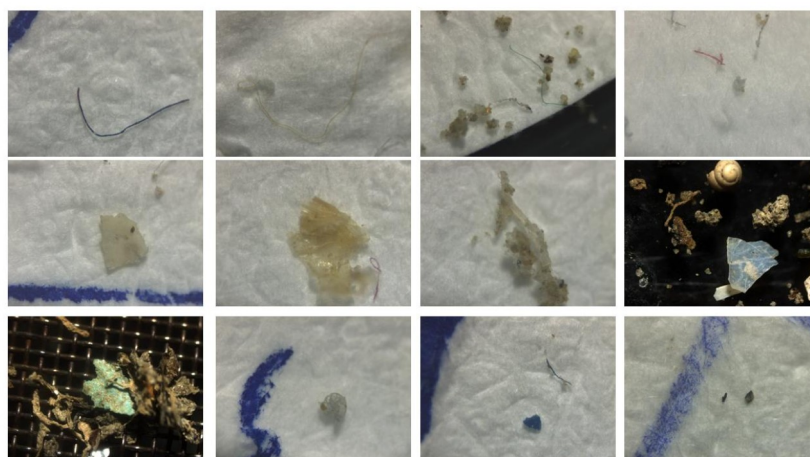


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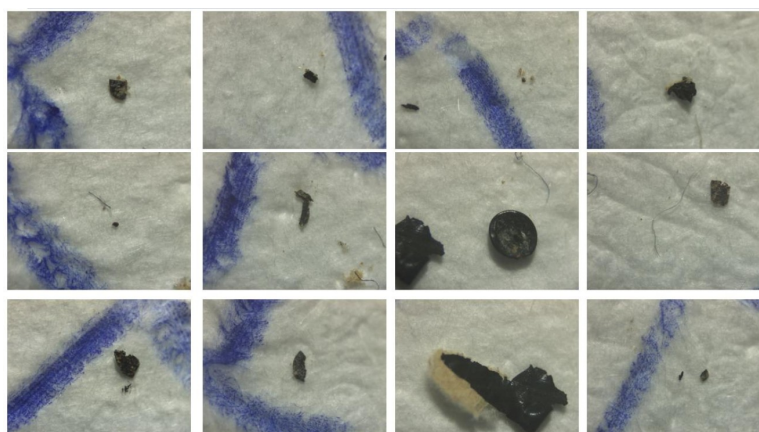


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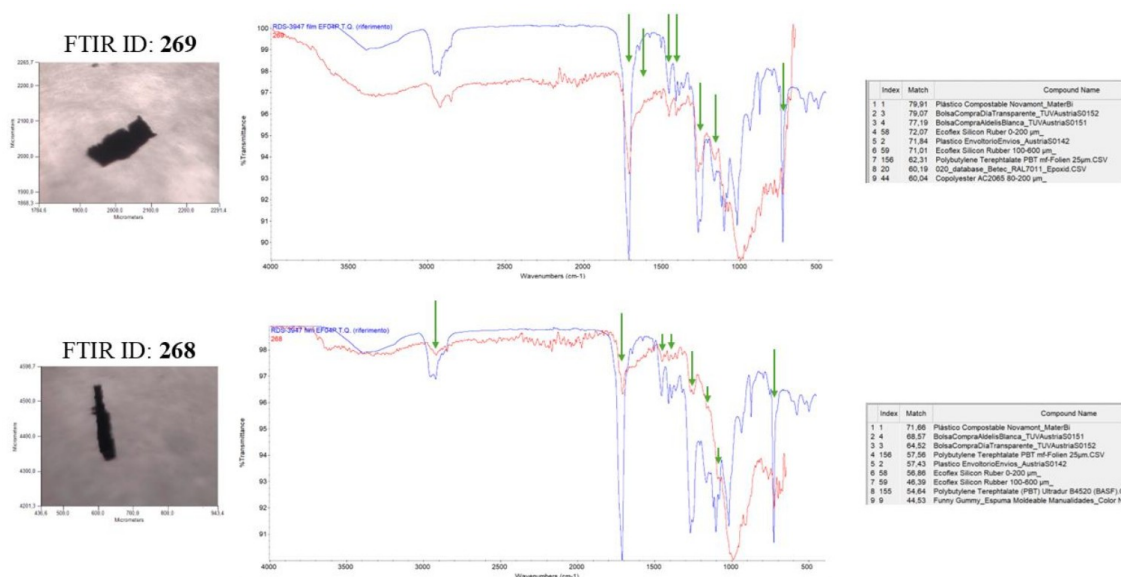


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Country	Plastic source	Soil type and crops	Abundance and composition	Reference
Hungary	Greenhouse	Arable land; tomatoes	225 ± 62 MPs/kg; PE, PVC, (PES, PP)	Sa'adu and Farsang, 2022
Germany	No plastic used	Arable land	206 macroplastics/ha and 0.34 ± 0.36 MPs/kg dry soil; PE, PS and PP	Piehl et al., 2018
Greece	Film	Greenhouse; watermelon and tomato	301 ± 140 (watermelon) and 69 ± 38 (tomato) MPs/kg; PE	Isari et al., 2021
Spain and The Netherlands	Mix	Mixed soil; broccoli, celery, watermelon	2242 ± 984 (Spain) and 888 ± 500 (The Netherlands) MPs/kg; PE, PP (only 5 plastics analyzed and only in Dutch samples)	Van Schothorst et al., 2021
Switzerland	Mulch	Drainage water; vegetables	10.5 ± 9.5 MPs/L; PA, PE, (SBR, PP)	Bigalke et al, 2022

PE: polyethylene; PP: polypropylene; PVC: polyvinyl chloride; PES: polyester; PA: polyamide; SBR: styrene-butadiene rubber.