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Preliminary Proposal for Standardizing the Protocol for the Determination of Microplastics' Influence on the CO_2 and/or CH_4 Emission in Agricultural Soils

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Abstract

Soil contamination by microplastics (MPs) is a global problem, exacerbated by the growing production of plastics and low levels of recycling. Considering that agricultural lands constitute a significant part of the land surface (37.7%), the study of the influence of MPs on the carbon cycle in such ecosystems is extremely important for understanding the global carbon balance. This work aims to develop a standardized protocol for determining the effects of microplastics (MPs) on the carbon cycle in agricultural soils. Differences in existing research protocols hinder comparability and limit conclusions about real-world impacts. A preliminary proposal for standardizing the protocol for the determination of MPs influence on the CO_2 and/or CH_4 emission in agricultural seeks to improve reproducibility and transparency in future studies. The protocol incorporates a wide variety of MPs characteristic in agricultural soils and allows experiments at realistic contamination levels, reflecting both current and projected future scenarios. Key recommendations include several points. Stringent contamination control during sample collection and preparation is of paramount importance. The selection of microplastic types and concentrations specific to agricultural environments is also recommended. Furthermore, maintaining consistent experiment durations is crucial, and the utilization of gas chromatography for analysis is highly desirable.

Keywords: microplastic pollution; climate-active gases; emission; cultivated soils; standardized method

1. Introduction

By 2050, global plastic production is projected to reach an impressive 33 billion tons per year [1]. The increase in production led to 400.1 million tons being produced in 2020, combined with the predominance of single-use plastic products (accounting for about 50% of the total production), low recycling rates, and the high resistance of polymers to degradation, results in a significant accumulation of plastic debris in the environment. Under the influence of environmental factors such as ultraviolet radiation, seawater, hydrolysis, soil alteration, and biological effects, large plastic wastes are fragmented to smaller particles. The term "microplastics" (MPs) was first used to describe the smallest plastic

particles in the ocean by Thompson et al. in 2004 [2]. Currently, MPs are defined as plastic fragments less than 5 mm in size [3]. MPs are ubiquitously found in various environmental media: soils, marine and fresh water, air, and tissues of living organisms, including animals and humans [4,5]. Soil, especially agricultural soils, are the main sinks of MPs. The concentration of MPs in soil varies in a wide range, from 0.03% to 6.70%, with urban and agricultural areas exposed to the most intensive pollution. The prevalence of MPs in agricultural soils worldwide is impressive: polyethylene concentrations in Japanese rice fields where controlled-release fertilizers (CRFs) have been used for more than 30 years reached 369 mg/kg, MPs concentrations in agricultural land in China range from 7100 to 42,960 particles kg^{-1}, dominated by polyethylene and polypropylene, in a fruit field 8 ± 25–540 ± 603 mg kg^{-1} [6], in a cotton field 70 ± 0.86–1724 ± 68.3 particles kg^{-1} [7], in agricultural fields in Chile 0.57–12.9 mg kg^{-1} [8], and a mean value of 46.88 p kg^{-1} in Germany [9].

MP accumulation affects physicochemical properties including bulk density, water dynamics, soil aeration, soil aggregate formation, pH, redox potential, nutrient levels, chemical diversity of dissolved organic matter (DOM), and soil microbiome, which in turn can influence biogeochemical processes, including carbon cycling, which is a key ecosystem function.

MPs are organic polymers containing almost 90% C [10] and, due to degradation and leaching, can promote soil organic carbon (SOC) accumulation, affect humification, and further polymerization of dissolved organic matter (DOM), indirectly affecting SOC stability. In addition, MPs are able to influence SOC formation by affecting the soil's microbial community structure, functional gene expression, and enzyme activity [11]. Even small fluctuations in the SOC pool, the largest active terrestrial carbon pool in the biosphere, can cause significant changes in atmospheric climate-active gas concentrations. An increase in atmospheric climate-active gas concentrations will further exacerbate climate warming. In turn, climate warming can promote soil carbon release, creating positive feedback to global warming [12]. Thus, the distribution and transformation of SOC have a profound impact on the global carbon balance and climate change.

Agricultural land, occupying 37.7% (4900 million ha) of the total land area (amounting to just over 13 billion ha) [13], which is simultaneously one of the places most polluted with MPs, has the potential to contribute meaningfully to changes in the carbon balance.

In recent years, there has been a rapid increase in the number of publications concerning the impact of MPs on soil carbon cycling and several reviews have been compiled, the authors of which analyzed the influence of MPs on the processes of carbon cycling in soils [10], the probability of MPs influence on the aggravation of global warming [14], the microbial mechanisms of carbon transformation in soils contaminated with MPs, as well as those devoted to the accumulation of MPs in agricultural soils [15], and include data on the influence of MP application on carbon cycling in soils of different ecosystems [16]. The number of experimental works is also increasing [17–34]. However, the results of the studies are heterogeneous; although most of the works noted the stimulating effect of MPs on the presence of CO_2 and/or CH_4 emissions, some studies showed the opposite [21,29] or a neutral result [29,31,33].

The incomparability and absence of detailed analysis regarding the influence of microplastic (MP) presence and accumulation in agricultural soils are attributable, on the one hand, to the immense variability in MP types, modifications (including particle size and nature), and morphologies (fibers, particles, granules, etc.), all of which exert varying effects. On the other hand, the problem is exacerbated by the divergent methodological approaches employed by various scientific groups, leading to inconsistencies

in the MP types and/or forms used, polymer concentrations, temperature regimes, and experiment durations.

At the same time, taking into account the growing volumes of soil contamination with the above-mentioned material, further clarification and development of ideas about the influence of MPs on the carbon cycle in agricultural ecosystems remains relevant.

This study aims to propose a standardized protocol, based on a critical review of the existing literature, to assess the effects of microplastic contamination on CO_2 and CH_4 emissions from agricultural soils, in order to enhance the comparability and reproducibility of future experiments.

2. Targeted Review of Methodologies

2.1. Materials and Methods

The search and selection of the relevant peer-reviewed literature were conducted by two researchers in August 2025, using two online publication databases: Google Scholar and ScienceDirect. The selection was limited to data published since 2010 in English, German, and Russian, and included empirical or review studies that involved different laboratory-based experimental procedures for the determination of CO_2/CH_4 emissions from agricultural soils with the addition of MPs. Results of studies in German and Russian have also been published in English in international journals; therefore, we used these manuscripts as a primary source. The following keywords were used to identify relevant articles: "microplastic", "agricultural soil", "emission activity", "CO_2 emission", "CH_4 emission". Potentially relevant articles were read in full.

2.2. Review of Approaches Used to Determine CO_2 and/or CH_4 Emissions in Agricultural Soils

Various methods and their modifications have been used to determine the effect of MPs addition on CO_2/CH_4 emissions from agricultural soils (Table 1).

Table 1. Comparison of several methods used in previous studies regarding CO_2 emissions from agricultural topsoils under MPs' influence (NS = Not Stated).

Type of MPs	Soil Description	Concentration, % *w/w*	Incubation Time	Method of GHG Detection	Results of Experiments	Reference
FPP	Coastal wetland	0.2, 2	60 days	Gas chromatography	Elevation in emissions	[27]
PET, PVC	Albic Luvisol	0.4	40 days	FTIR spectroscopy and special flow systems	Elevation in emissions	[22]
LDPE, PBAT	Sandy loam, cropland	0.1, 1	NS	Gas chromatography	LDPE—no effect, PBAT—elevation of emissions	[25]
PLA, PP, LDPE	Surface soils from a rice-paddy field	0.5, 1, 1.5	41 days	Gas chromatography	Reduction in emissions	[23]
PBC	Topsoil, agricultural site	0.021	360 days	Closed chamber titration	Reduction in emissions in short-term	[21]
PE, PVC, PBAT, PLA	Surface silty soils	NS	21 days	Fluorescence excitation–emission matrix spectroscopy, ultrahigh-resolution FTIR mass spectrometry	Elevation in emissions	[30]

Table 1. *Cont.*

Type of MPs	Soil Description	Concentration, % *w/w*	Incubation Time	Method of GHG Detection	Results of Experiments	Reference
PE	Chernozems, Ferrasols, Luvisols	NS	56 days	Gas chromatography	Elevation in emissions in Ferrasols, No effect in Chernozems and Luvisols	[33]
LDPE	Loamy sand soils	NS	287 days	Fluorescence measurement of hydrolytic enzymes, involved in carbon cycling	Elevation in emissions	[35]
PE	NS	0.8	15 weeks	Closed chamber titration	Elevation in emissions	[36]
PE	Topsoil from greenhouses	NS	90 days	Closed chamber titration	Reduction in emissions	[37]
PET	NS	0.4–7	60 days	Infrared gas analyzer	Neutral	[38]
PET	Alluvial paddy soils	0.2	60–120 days	Gas chromatography	Reduction in emissions	[39]
Conventional and biodegradable	Various	Various	>30 days	NS	Elevation in emissions	[40]

The most common method of preparation involves sampling topsoil (0–20 cm), air-drying, and sieving through a 2 mm sieve with further storage at 25 °C [17]. However, sometimes samples are taken from the whole soil profile to understand the effects of the vertical migration of elements and differences in CO_2/CH_4 emissions between different soil layers. In addition, before and after sieving, samples can be stored at an air temperature of 4 °C [20].

The types of MPs and their sizes can vary essentially between experiments. Rauscher et al. [25] pointed out the importance of particle size as CO_2 emissions into soil were higher when small particle MPs were added compared to large particle MPs [25]. Different shapes of MPs were also observed in different studies; these included round shaped particles [26,27], fibers [22,29], and fragments [28]. The study of Zhang et al. [41] showed that the concentration of MPs can change the experimental results: while the addition of 1.5 wt% *w/w* PLA increased soil carbon uptake; a higher climate-active gases emission was observed at 0.5 wt% *w/w* concentration. The biggest challenge for researchers is to determine the correct concentration to model low-, medium-, and high-levels of pollution with MPs. Chen et al. [27] added 0.25% *w/w* MPs as a widespread concentration in soil, 2% *w/w* as an elevated concentration, and 7% *w/w* to create highly contaminated conditions. Moreover, a study by Chen et al. [17] showed that a low concentration of MPs (0.2 wt%) had no effect on climate-active gas emissions, while a higher concentration (2 wt%) showed a significant increase in emissions.

An incubation experiment is considered the standard for determining GHG emissions in the laboratory, and the duration of incubation can vary considerably. A short-term experiment is one type of conventional incubation period that is used to determine early peaks in GHG emissions. Chen et al. [27] conducted a 30-day incubation with five sampling events during this period, while Barili et al. [21] argued that a longer incubation duration is

necessary to properly estimate GHG emissions in microplastic-affected soils and conducted 360 days of incubation with sampling at specific time periods.

Despite all the similarities in sampling, storage, and incubation experiments, the choice of method for the determination of GHGs is one of the most important steps. The measurement of hydrolytic enzymes involved in carbon cycling were used to first determine GHG emissions from microbial biomass [35]. Nowadays, the most common method is gas chromatography, which determines the concentration of a specific GHG in an air sample. It has been used in various studies and may become the standard in this field [23,27,33]. FTIR spectroscopy and special flow systems are also used in some studies [10,30], but this method requires expensive equipment. Finally, the closed chamber titration method is also used by various laboratories because of its low cost and ease of application [21].

Having identified the best practices, taking into account the availability, repeatability in different laboratories, and accuracy of the methods, as well as recreating the experiments in the natural conditions characteristic of agricultural soil as much as possible in (including temperature, moisture content, types, and concentrations of MPs), we developed a preliminary proposal for standardizing the protocol for the determination of microplastics' influence on the CO_2 and/or CH_4 emissions in agricultural soils.

3. Results

We have developed a step-by-step protocol for assessing the influence of microplastics on CO_2/CH_4 emissions in agricultural soils, which includes the use of standardized materials and procedures aimed at enhancing the reproducibility and comparability of microplastic (MP) studies across different laboratories and regions.

Acknowledging the importance of MPs' polymer type for their environmental impact, this protocol emphasizes the standardized characterization and selection of MPs for experiments. It recommends the use of ecologically relevant MP types and concentrations, thereby addressing a common limitation of existing studies that often employ unrealistic MP exposure scenarios. The protocol recommends the use of new specifically engineered MP particles of the above types for laboratory experiments; however, if it is not possible for researchers to use specially produced MPs, it is possible to obtain the required MPs by shredding the mulch film from an appropriate polymer.

The protocol also specifies incubation conditions, measurement duration, and analytical methods (gas chromatography) optimized to detect MP-induced changes in soil respiration and methanogenesis.

For flexible adaptation among different research groups and laboratories, we prepared two protocol variants: a more straightforward and cost-effective option (Figure 1), and a detailed version that employs a more expensive and less accessible, but most accurate, gas chromatography method at the concluding stage (Figure 2).

Currently, the protocol is a preliminary proposal based on the existing literature, a comparison of available research methodologies, and a thorough analysis of entry pathways and accumulation patterns specific to agricultural areas (see Section 4). The next step will be an experimental validation of the protocol, rigorously testing its feasibility and sensitivity.
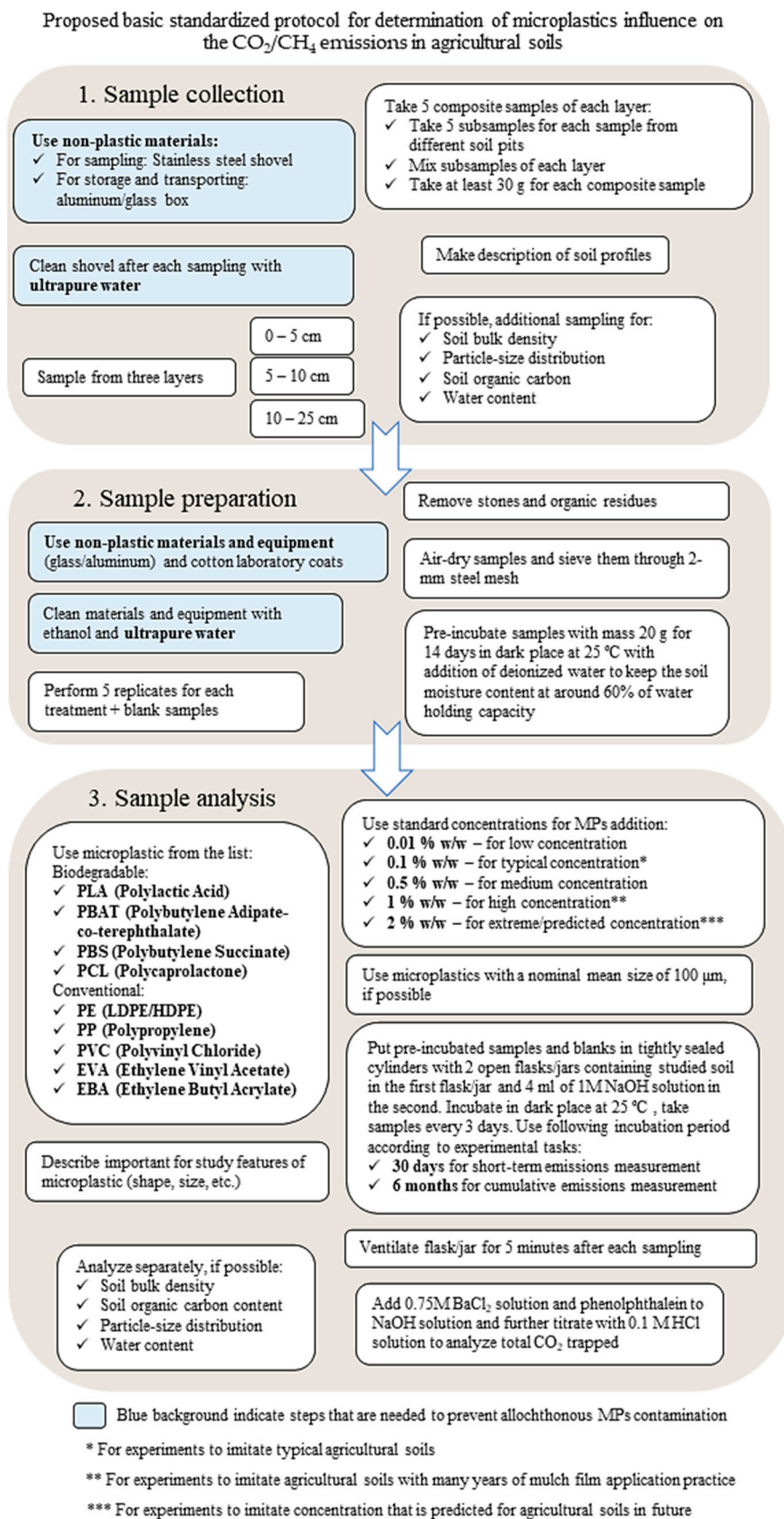
Proposed basic standardized protocol for determination of microplastics influence on the CO_2/CH_4 emissions in agricultural soils

1. Sample collection

Use non-plastic materials:
✓ For sampling: Stainless steel shovel
✓ For storage and transporting: aluminum/glass box

Clean shovel after each sampling with **ultrapure water**

Sample from three layers
| 0 – 5 cm |
| 5 – 10 cm |
| 10 – 25 cm |

Take 5 composite samples of each layer:
✓ Take 5 subsamples for each sample from different soil pits
✓ Mix subsamples of each layer
✓ Take at least 30 g for each composite sample

Make description of soil profiles

If possible, additional sampling for:
✓ Soil bulk density
✓ Particle-size distribution
✓ Soil organic carbon
✓ Water content

2. Sample preparation

Use non-plastic materials and equipment (glass/aluminum) and cotton laboratory coats

Clean materials and equipment with ethanol and **ultrapure water**

Perform 5 replicates for each treatment + blank samples

Remove stones and organic residues

Air-dry samples and sieve them through 2-mm steel mesh

Pre-incubate samples with mass 20 g for 14 days in dark place at 25 °C with addition of deionized water to keep the soil moisture content at around 60% of water holding capacity

3. Sample analysis

Use microplastic from the list:
Biodegradable:
✓ PLA (Polylactic Acid)
✓ PBAT (Polybutylene Adipate-co-terephthalate)
✓ PBS (Polybutylene Succinate)
✓ PCL (Polycaprolactone)
Conventional:
✓ PE (LDPE/HDPE)
✓ PP (Polypropylene)
✓ PVC (Polyvinyl Chloride)
✓ EVA (Ethylene Vinyl Acetate)
✓ EBA (Ethylene Butyl Acrylate)

Describe important for study features of microplastic (shape, size, etc.)

Analyze separately, if possible:
✓ Soil bulk density
✓ Soil organic carbon content
✓ Particle-size distribution
✓ Water content

Use standard concentrations for MPs addition:
✓ 0.01 % w/w – for low concentration
✓ 0.1 % w/w – for typical concentration*
✓ 0.5 % w/w – for medium concentration
✓ 1 % w/w – for high concentration**
✓ 2 % w/w – for extreme/predicted concentration***

Use microplastics with a nominal mean size of 100 μm, if possible

Put pre-incubated samples and blanks in tightly sealed cylinders with 2 open flasks/jars containing studied soil in the first flask/jar and 4 ml of 1M NaOH solution in the second. Incubate in dark place at 25 °C, take samples every 3 days. Use following incubation period according to experimental tasks:
✓ **30 days** for short-term emissions measurement
✓ **6 months** for cumulative emissions measurement

Ventilate flask/jar for 5 minutes after each sampling

Add 0.75M $BaCl_2$ solution and phenolphthalein to NaOH solution and further titrate with 0.1 M HCl solution to analyze total CO_2 trapped

Blue background indicate steps that are needed to prevent allochthonous MPs contamination

* For experiments to imitate typical agricultural soils

** For experiments to imitate agricultural soils with many years of mulch film application practice

*** For experiments to imitate concentration that is predicted for agricultural soils in future

Figure 1. Basic standardized protocol for the determination of MPs' influence on the carbon cycle in agricultural soils (more straightforward and cost-effective option).
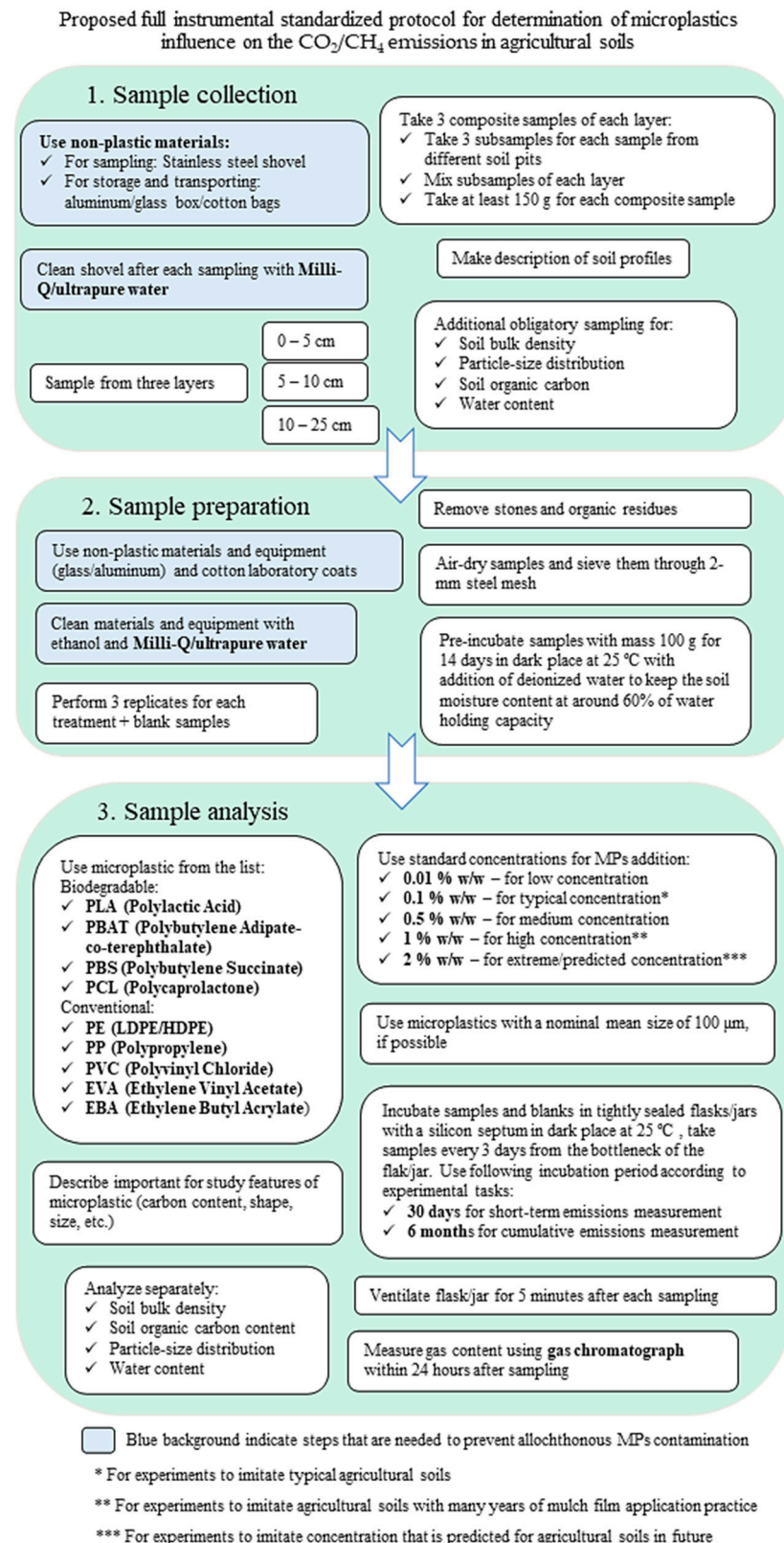
Proposed full instrumental standardized protocol for determination of microplastics
influence on the CO_2/CH_4 emissions in agricultural soils

1. Sample collection

Use non-plastic materials:
- ✓ For sampling: Stainless steel shovel
- ✓ For storage and transporting: aluminum/glass box/cotton bags

Take 3 composite samples of each layer:
- ✓ Take 3 subsamples for each sample from different soil pits
- ✓ Mix subsamples of each layer
- ✓ Take at least 150 g for each composite sample

Clean shovel after each sampling with **Milli-Q/ultrapure water**

Make description of soil profiles

Sample from three layers

| 0 – 5 cm |
| 5 – 10 cm |
| 10 – 25 cm |

Additional obligatory sampling for:
- ✓ Soil bulk density
- ✓ Particle-size distribution
- ✓ Soil organic carbon
- ✓ Water content

2. Sample preparation

Use non-plastic materials and equipment (glass/aluminum) and cotton laboratory coats

Clean materials and equipment with ethanol and **Milli-Q/ultrapure water**

Perform 3 replicates for each treatment + blank samples

Remove stones and organic residues

Air-dry samples and sieve them through 2-mm steel mesh

Pre-incubate samples with mass 100 g for 14 days in dark place at 25 °C with addition of deionized water to keep the soil moisture content at around 60% of water holding capacity

3. Sample analysis

Use microplastic from the list:
Biodegradable:
- ✓ PLA (Polylactic Acid)
- ✓ PBAT (Polybutylene Adipate-co-terephthalate)
- ✓ PBS (Polybutylene Succinate)
- ✓ PCL (Polycaprolactone)

Conventional:
- ✓ PE (LDPE/HDPE)
- ✓ PP (Polypropylene)
- ✓ PVC (Polyvinyl Chloride)
- ✓ EVA (Ethylene Vinyl Acetate)
- ✓ EBA (Ethylene Butyl Acrylate)

Use standard concentrations for MPs addition:
- ✓ 0.01 % w/w – for low concentration
- ✓ 0.1 % w/w – for typical concentration*
- ✓ 0.5 % w/w – for medium concentration
- ✓ 1 % w/w – for high concentration**
- ✓ 2 % w/w – for extreme/predicted concentration***

Use microplastics with a nominal mean size of 100 μm, if possible

Incubate samples and blanks in tightly sealed flasks/jars with a silicon septum in dark place at 25 °C , take samples every 3 days from the bottleneck of the flak/jar. Use following incubation period according to experimental tasks:
- ✓ **30 days** for short-term emissions measurement
- ✓ **6 months** for cumulative emissions measurement

Describe important for study features of microplastic (carbon content, shape, size, etc.)

Analyze separately:
- ✓ Soil bulk density
- ✓ Soil organic carbon content
- ✓ Particle-size distribution
- ✓ Water content

Ventilate flask/jar for 5 minutes after each sampling

Measure gas content using **gas chromatograph** within 24 hours after sampling

☐ Blue background indicate steps that are needed to prevent allochthonous MPs contamination

* For experiments to imitate typical agricultural soils

** For experiments to imitate agricultural soils with many years of mulch film application practice

*** For experiments to imitate concentration that is predicted for agricultural soils in future

Figure 2. Full instrumental standardized protocol for determination of MPs' influence on the carbon cycle in agricultural soils (more expensive and less accessible, but the most accurate).

4. Discussion

4.1. Objectives of the Standardization of the Methodology

The main objectives in the work to standardize the approach to determine the effect of MP addition on CO_2/CH_4 emissions from agricultural soils under laboratory conditions were as follows:

(1) The identification of key types of added polymers specific to agricultural soils;
(2) The determination of applied concentrations comparable to those actually detected in soil under natural conditions, also including the predicted future level of pollution;
(3) The determination of uniform experimental conditions and the selection of a uniform method of CO_2 and/or CH_4 emission determination;
(4) To ensure qualitative control of MP pollution from outside.

4.2. Determination of Types and Concentrations of MPs and Experimental Conditions

4.2.1. Sources of Contamination of Agricultural Soils with MPs

To solve the first two problems, we analyzed sources of MPs (Figure 3). MPs penetrate agricultural soils in different ways, the main ones are as follows:

(a) The application of sewage sludge;
(b) The use of compost;
(c) The use of controlled-release fertilizers (CRF), plant protection products using capsule suspension (CSPs), and seed film;
(d) The application of mulch film.
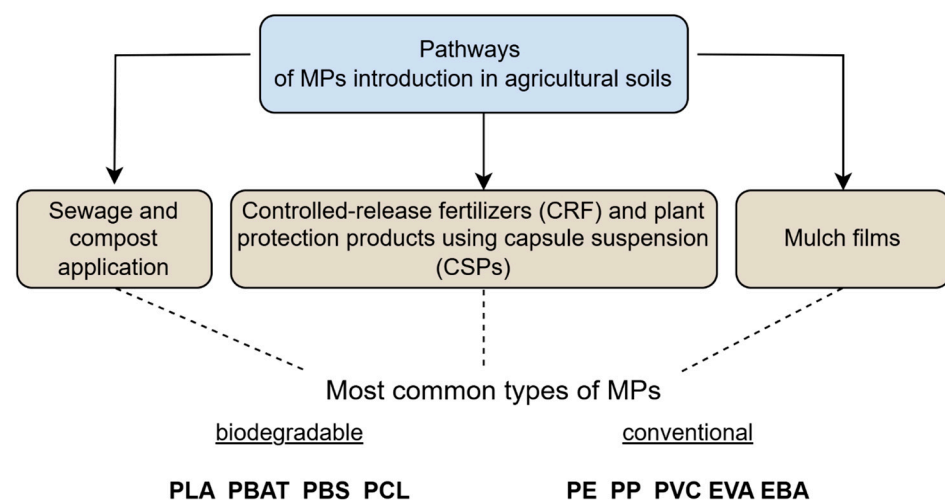
Figure 3. Main sources of MP pollution in agricultural soils and the prevailing types of MPs.

(a) Sewage sludge.

The widespread use of sewage sludge (SW) as a fertilizer in agricultural soils is due to its high content of organic matter and macro- and microelements that help to increase soil fertility and functionality [42]. However, this method of waste utilization is simultaneously a source of ingestion of significant amounts of MPs. Despite the high efficiency of modern treatment facilities in removing MPs from incoming wastewater, a significant amount of these particles still overcome filtration systems and remain in the effluent, and the concentrations can vary from units to hundreds of thousands of particles per unit volume. Annual inputs of MPs to agricultural land in North America through SW applications range from 44 to 300 kilotons, while in Europe this can be as high as 63 to 430 kilotons, and concentrations in Europe range from 1 to $4 \times 1 \times 3$ particles per kilogram of dry OSW [8]. Moreover, a study of archival soils showed MPs from sewage sludge persist in soils at a

constant level for 25 years. An analysis of the polymer composition of MPs in SW and soils revealed the predominance of polyester (PE) in the form of microfibers.

(b) Compost utilization.

The composting of livestock manure is considered an effective way to recycle excess manure for agricultural purposes. Recycling organic waste by composting and applying it as fertilizer on agricultural land is an environmentally friendly measure to return trace elements, nutrients, and humus to soils. But most of the livestock and poultry manure is contaminated with MF. Possible sources of these MPs include the following:

- The extensive use of plastic products in poultry and livestock production (feed packaging bags, feed transportation pipelines, etc.);
- The ingestion of MPs contaminated feed by livestock and poultry, which subsequently excrete MF in their feces;
- MPs from the environment can penetrate manure during composting and transportation.

MPs are found in compost products in concentrations of 14–895 particles kg^{-1} [43]. Zhang et al., 2023 confirmed the pathway of MP transfer to soils through compost, specifying that the contribution of organic composts to the accumulation of MF in soils can be up to 43–76% of the amount of MPs and significantly increase with increasing time and rate of compost application, and the number and size of MPs decrease with increasing soil depth [41,44].

Confirmation of the introduction of the mentioned part of MPs through compost application is the similarity of forms and types of polymers in the studied soils and composts—PE and PP fragments and fibers prevailed in the currently available studies.

(c) The use of controlled-release fertilizers (CRFs), plant protection products using capsule suspension (CSPs) and seed film coating.

The agricultural industry has recently introduced slow-release fertilizers (SRFs) and controlled release fertilizers (CRFs) to address inefficiencies and environmental concerns associated with conventional fertilizer methods. SRFs and CRFs provide higher nutrient levels than conventional fertilizers and deliver nutrients gradually, resulting in higher yields and improved fertilizer efficiency [45]. The development of CRFs started in the 1960s and initially sulfur and PE were used as coating materials, but the limitation of sulfur coatings in nutrient release led to the development of various polymeric materials, natural coating agents, multifunctional superadsorbing materials, and even nanocomposites [46]. Now the main polymers for the production of coatings are as follows:

- For CSPs (Capsule Suspension Products): polyurethane (PU), polylactic acid (PLA), ethylcellulose (EC), and styrene-acrylate (SA) copolymers;
- For CRFs: PE, PP, ethylene-vinyl acetate (EVA), sulfur-coated (S-polymers), polycaprolactone (PCL), polylactic acid (PLA), and hydrogels polyacrylamide (PAM).

Over the past two decades, there has been a 200% increase in the use of SRFs and CRFs: increasing from 500,000 metric tons [43] in 1995–1996 to 1.5 million metric tons in 2018 [47]. CRFs accounted for 2.3% of total fertilizer use worldwide [48], with a projected compound annual growth rate of 6.5% from 2021 to 2030. Concerns are emerging about the long-term impact of their plastic coatings on soil health. Microcapsules remaining after nutrient release from PC-CRFs can be considered MPs if they are less than 5 mm in diameter, and additionally they can degrade to smaller MPs.

(d) Application of mulching film.

Plastic film mulching (PFM) is widely used to cover agricultural land in many regions of the world, especially in arid and semi-arid areas, because of its positive effects by increasing soil temperature, improving moisture retention, reducing damage from weeds

and pests, expanding crop area, and hence increasing crop yields. Moreover, long-term plastic film mulching (LFM) [49] is a common practice and the use continues to increase. The global consumption of mulch film is about 8 million tons per year and already about 20 million hectares of arable land worldwide is covered with plastic, with China accounting for the largest share (∼90%) [50]. In particular, the use of PFM in China has rapidly increased from 0.38 million tons in 1992 to 1.32 million tons in 2022, accounting for 75% of the global use. However, extracting the film from the soil at the end of the growing season remains difficult. The difficulty of collecting, recycling, and reusing films means that they are often incorporated into the soil during plowing. Over time, residual plastic materials on farmland undergo weathering and fragmentation to form MF and accumulate in the soil.

According to the data obtained through a soil survey in China, the prevalence of MPs in mulched soil (754 ± 477 pc kg^{-1}) was significantly higher than that in non-mulched soil (376 ± 149 pc kg^{-1}), indicating that plastic film mulching could contribute half of the MPs to soils. And the prevalence of MPs was significantly positively related to the duration of time of application of plastic film mulching. The percentage of microplastics < 0.5 mm in mulched soil (50.9%) was significantly lower than that in non-mulched soil (62.2%) [8]

PE, especially LDPE, is the most widely used polymer in the production of agricultural mulch films [50]. Its dominance in the mulch films market is attributed to the characteristics of this polymer such as puncture resistance, tensile strength, and ability to withstand the effects of solar radiation and low temperatures. But besides LDPE and other PE variants (HDPE, LLDPE), polyvinyl chloride (PVC), ethylene vinyl acetate (EVA), and ethylene butyl acrylate copolymers (EBA) are also predominantly used for mulch production.

As a potential alternative to conventional non-biodegradable mulch films, many different biodegradable mulch films [51] mainly made of PBAT are on the market: polyhydroxyalkanoate (PHA), PLA, PCL, poly (propylene carbonate) (PPC), PVA, poly (butylene succinate) (PBS). They are produced from either renewable resources (bio-based) or fossil fuels (fossil-based). PBAT is one of the common fossil-based polymers that is highly flexible, abrasion- and tear-resistant, and water and oil resistant. This material is biodegradable in soil and compostable, certified according to European standard specifications, but its degradation in soil is estimated to be moderately low, with the polymer remaining in soil for up to 1–3 years [51]. The addition of biodegradable MPs to soil results in enhanced SOC turnover by increasing microbial and enzymatic activity. A four-year field study by Huang et al., 2022 [51] found that SOC was higher when mulched with biodegradable film than when mulched with polyethylene film, and a three-year field study by Wang et al., 2024 determined that the SOC content of large and small macroaggregates increased after continuous use of biodegradable MPs compared to polyethylene [49].

4.2.2. Typical and Limit Concentrations of MPs in Soils

MP concentrations show considerable variability, ranging from a few particles/kg in regions with low inputs to more than 80,000 particles/kg in areas with long-standing mulching practices. Experimental concentrations used in many studies from 0.01% (m/m) to 7% (m/m) are well above typical levels in the environment. A percentage of 1% by weight corresponds to 10,000 mg MPs per kilogram of soil, which is much higher than typical MP concentrations currently found in agricultural soils. They typically vary from a few hundred to a few thousand particles per kilogram, corresponding to much lower mass concentrations, often below 0.1% (m/m) [52]. Although, in regions where plastic mulching is used, concentrations can be as high as 1% or higher, for example, according to Griffin-Lahue et al. biodegradable plastic mulch takes about 5 years to fully biodegrade. Re-

peated annual applications and biodegradation rates can result in maximum concentrations ranging from 0.23% by weight to 0.43% by weight [53].

Based on the data presented above, we recommend adhering to more realistic concentrations for the natural environment when conducting experiments and considering 0.1% as an upper limit for typical concentrations (Figures 1 and 2). However, plastic pollution is increasing, especially in areas with the intensive use of plastics and high concentrations may become more common; moreover, further studies are needed to determine the threshold concentrations at which MPs significantly change the physical characteristics of the soil, so that concentrations of about 1–2% may remain relevant for a number of experiments.

4.2.3. Determination of Incubation Time

For agricultural soils with possible practical use, the best choice of incubation period is 60 days because it captures both the initial and stabilized microbial and soil respiration responses to microplastic amendments, balancing robust effect detection with practical experiment management [20,25]. Most studies and meta-analyses use this period, as it provides reliable, comparable results without the confounding factors of longer-term instability [54].

4.2.4. Determination of Types and Concentrations of MPs

An important step in the experiments is the method of determination of gases. Gas chromatography (GC) is the gold standard for measuring greenhouse gas emissions in soil experiments due to its high sensitivity, selectivity, and ability to quantify multiple gases (CO_2, CH_4, N_2O) simultaneously [55]. GC provides precise, repeatable results and can detect even low concentrations, making it ideal for detailed kinetic studies and comparative analyses. However, GC has notable limitations: it requires expensive, specialized equipment, skilled operators, regular calibration and maintenance. Sample preparation and handling are critical for achieving of correct results. Moreover, the method is less suited for continuous, real-time monitoring without automated sampling systems. In contrast, FTIR spectroscopy allows for the rapid, non-destructive, and often real-time measurement of CO_2, but is less specific for other gases and can be affected by water vapor or overlapping absorption bands [20]. The closed-chamber titration method is simple and low-cost, suitable for cumulative CO_2 measurement, but lacks the ability to distinguish between gases, offers poor time resolution, and is prone to errors from incomplete absorption or interfering substances [37]. Thus, while GC is the most accurate and versatile, its complexity and cost limit accessibility compared to other methods.

4.3. Prevention of External Contamination

The contamination of MP samples during collection, preparation, and analysis can alter the results of the study. Therefore, measures to limit contamination are necessary to obtain accurate experimental results. MPs can enter the samples from the surface of plastic objects, water, and air. We recommend using non-plastic objects as containers for soil samples, laboratory utensils, and researchers' clothing at each stage wherever possible. Ensure that samples remain covered whenever possible under experimental conditions. Also, distilled water should be used throughout the experiment to clean the equipment, researchers' hands, and application according to the experimental conditions (Figures 1 and 2).

5. Conclusions

The use of the proposed standardized protocol will help to improve the comparability, reproducibility, and transparency of studies. In addition, the presented protocol allows us to include a wide variety of MP types characteristic of agricultural soils and allows us to

conduct experiments both at target MPs concentrations based on current levels of pollution and at future, expectedly higher levels.

Based on the processed data, we have standardized the laboratory protocol for emission determination in MP-contaminated agricultural soils and offer the following recommendations (full piercing—Figures 1 and 2):

1. Sample collection
   - Comply with external contamination control methods: use non-plastic materials for sample collection and transport (metal scoop, glass/metal boxes or for weight reduction and compactness cotton bags; rinse equipment with distilled water).

2. Sample preparation
   - Observe methods to control external contamination (non-plastic laboratory equipment where possible, cotton lab coats, use distilled water to moisten soils).

3. Sample analysis
   - Use specific, agricultural soil-specific MP types (see Figures 1 and 2) for a complete list of common and biodegradable options);
   - Adhere to current and/or predicted concentrations that are realistic for agricultural soils;
   - Use a temperature of 25 °C for the incubation of samples that is easily achievable, maintainable, and common in agricultural areas;
   - Adhere to a uniform duration of the experiment (30 days for short-term emissions measurement and 6 months for cumulative effect assessments);
   - Select a gas chromatography method for determining the results.

The emergence of new studies remains vital in view of growing volumes of pollution of agricultural soils with MPs. The development of research standards for further clarification and development of ideas on the impact of MPs on carbon cycling in other ecosystems also seems to be expedient in the future.

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Abbreviations

MPs	Microplastics
OC	Organic carbon
SOC	Soil organic carbon
SOM	Soil organic matter
DOM	Dissolved organic matter
CRFs	Controlled-release fertilizers
CSPs	Capsule suspensions
LDPE	Low-density polyethylene
HDPE	High-density polyethylene
SW	Sewage sludge

PE	Polyethylene
PLA	Polylactic acid
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PP	Polypropylene
PET	Polyethylene terephthalate
HBC	Hydrochar
PU	Polyurethane
PPC	Polyvinyl alcohol
EC	Ethylcellulose
SA	Copolymers (Styrene-Acrylate)
EVA	Ethylene-Vinyl Acetate
EBA	Ethylene butyl acrylate
PCL	Polycaprolactone
PVA	Polybutylene succinate

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